V01

Mast cell-mediated allergic inflammation is impaired in the absence of cutaneous nerves

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While several lines of evidence suggest that mast cells (MCs) contribute to cutaneous neurogenic inflammation, the role of skin nerves in MC-mediated allergic inflammation remains unclear. To test whether MC-dependent immediate hypersensitivity reactions are altered in the absence of nerves, we made use of a recently described murine model of unilateral cutaneous surgical denervation. Dorsal cutaneous nerves T3-12 of adult C57BL/6 mice were exposed (both sides) and removed (right side only) under a dissection microscope. Immunohistochemistry for PGP 9.5 (panneuronal marker), substance P and CGRP (neuropeptides), and tyrosine hydroxylase (marker for adrenergic nerves) on day 10 after denervation showed that cutaneous innervation was completely absent in denervated skin areas and normal in contralateral sham-denervated back skin. Successful and selective denervation was confirmed by functional testing for pinch sensitivity. Passive cutaneous anaphylaxis was induced in surgically denervated and sham-denervated back skin of mice by i.c. injection of IgE-anti-DNP and subsequent i.v. application of DNP. MC-mediated inflammation was assessed by quantification of the extravasation of Evans Blue from the vasculature into inflamed skin. Extravasation of Evans Blue was significantly reduced in denervated skin areas as compared to sham-denervated skin (28.1 ± 1.8 vs. 42.3 ± 2.4 microg/mg, $p<0.005$). The reduction of inflammation in denervated skin was not associated with a 1) drop in MC numbers, 2) reduced MC size, or 3) redistribution of cutaneous MCs as assessed by quantitative histomorphometry. Reduced inflammation in the absence of nerves was correlated with impaired degranulation of MC in denervated skin, suggesting that skin nerves contribute to immediate hypersensitivity reactions, e.g. by releasing neuropeptides that modulate MC releasability. These findings implicate sensory nerves as potential effector cells in the context of MC-mediated allergic inflammation.

V02

ADAM sheddases cleave the ectodomain of collagen XVII

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Collagen XVII/BP 180 is a structural component of the hemidesmosomes. It exists in two forms, as a 180 kDa type II transmembrane protein and as a soluble 120 kDa form, which corresponds to the extracellular domain and is presumably released from the cell surface through furin-mediated processing. In this work, we identified more than one enzyme, that catalyzes this process. Time chase experiments with biotinylated keratinocytes showed that the soluble ectodomain was stable in the medium for more than 48 hours. The use of domain specific antibodies demonstrates that the authentic shedding product contains at least a part of the NC16A domain and the C-terminus of the collagen XVII molecule. Collagen XVII-shedding of cultured keratinocytes was enhanced by phorbol esters and inhibited by phenanthroline, sheddase-targeting hydroxamates and TIMP-3, but not by serine protease inhibitors, TIMP-1, TIMP-2 and a selective gelatinase inhibitor. The keratinocyte-derived MMP-2, MMP-9 and MT1-MMP were excluded, since they cleaved purified collagen XVII to a non-physiological fragment and MMP-2 and MT1-MMP-deficient cells showed normal collagen XVII-shedding. Another candidates are the prototype sheddase TACE, ADAM-10 and ADAM-9. RT-PCR analysis and immunoblotting demonstrate that all three ADAMs were expressed and activated in keratinocytes. IF-staining of human skin revealed that TACE- and especially ADAM-9-immunoreactivity were restricted to basal keratinocytes and therefore in neighborhood to collagen XVII. Transfection of HaCaT cells with cDNA of these three ADAMs led to a dose dependent increase of ectodomain-shedding, with a concomitant decrease of full-length collagen XVII. In concert, TACE-deficient cells showed significantly reduced collagen XVII-shedding. These results demonstrate that ADAMs catalyze the collagen XVII shedding and thereby contribute to the regulation of keratinocyte adhesion and migration in physiological and pathological processes.

V03

K5/Cre-Lox-mediated VEGF A deletion retards angiogenesis-dependent processes in the skin

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Vascular endothelial cell growth factor (VEGF) is a major inducer of angiogenesis. Since in the skin, epidermal keratinocytes (KC), are an important source of VEGF, we wished to investigate the contribution of KC-derived VEGF to angiogenesis-dependent processes in this organ. We have inactivated VEGF specifically in mouse cells that express keratin 5, using a Cre/Lox-P system. Deletion of exon 3 of VEGF A in genomic DNA from K5 expressing tissues was demonstrated by PCR and Southern blotting, and cultured epidermal keratinocytes produced no detectable VEGF. Depilation-induced anagen resulted in sparser hair regrowth and fewer hair follicles in mutant mice, and wound healing was retarded compared to controls. Anti-CD31 staining of wounds demonstrated a reduced microvessel density immediately below the regenerating keratinocytes. A complete carcinogenesis protocol using dimethyl benzanthracene (DMBA) induced epidermal tumors significantly earlier in control mice compared to mutants. We thus show that KC-derived VEGF plays an important role in hair regrowth, and epidermal wound healing, while lack of VEGF in the epidermis retards chemically-induced tumor formation.

V04

Autoantibodies to Type VII Collagen From Patients with Epidermolysis Bullosa Acquisita Mediate Fcγ-Dependent Neutrophil Recruitment and Induce Subepidermal Blisters in Cryosections of Human Skin

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Epidermolysis bullosa acquisita (EBA) is an autoimmune subepidermal blistering disease associated with autoantibodies to type VII collagen, the main constituent of anchoring fibrils. The actual pathogenic relevance of autoantibodies to this autoantigen, however, has not yet been demonstrated. To elucidate the blister-inducing potential of autoantibodies to type VII collagen, we used an *in vitro* model involving cryosections of normal human skin incubated with patients' autoantibodies and leukocytes from healthy donors. Sera from 13 out of 15 EBA patients, in contrast to healthy control sera, induced dermal-epidermal separation of the skin sections. Blister formation was dependent on the recruitment of neutrophils to the dermal-epidermal junction (DEJ). To confirm these findings, we purified IgG from serum by protein G affinity chromatography. Again, patients' IgG induced blisters, while IgG from healthy controls did not. Subsequently, we affinity purified patients' autoantibodies against a recombinant form of the non-collagenous 1 domain of type VII collagen (NC1), expressed in baculovirus-infected insect cells and covalently coupled to an agarose matrix. Patients' autoantibodies eluted from the NC1 column retained their blister-inducing capacity, while IgG that was depleted of reactivity to NC1 lost its ability to recruit neutrophils and to induce blisters. Monoclonal antibody LH7.2 directed to NC1, in contrast to monoclonal antibody to type IV collagen, also induced subepidermal splits in the cryosections. Finally, autoantibodies from EBA patients, specific to recombinant NC1, were subjected to pepsin digestion. This resulted in $F(ab')_2$ fragments, lacking the effector (Fc) portion, that still reacted with the DEJ but failed to induce blisters. For the first time, we demonstrate the capacity of autoantibodies to type VII collagen from patients with EBA to trigger an immune complex-mediated inflammation leading to blister formation in cryosections of human skin. This experimental model should greatly facilitate the further dissection of the autoimmune response in EBA.

V05

Isolation and characterization of a novel human antibiotic peptide secreted by mucous sweat gland cells

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Antimicrobial peptides are an important component of innate immunity in many species. Here we describe the isolation of a gene named Dermcidin (DCD) encoding an antimicrobial peptide with broad spectrum activity and with no homology to other known antimicrobial peptides. The transcript of the gene is 458 bp long and encodes an open reading frame of 110 amino acid residues. The gene consists of 5 exons and 4 introns and is located on chromosome 12q13. DCD has a very restrictive expression pattern as the gene is only expressed in human skin and was not detected in any of 50 analyzed human tissues of different origins nor in human foreskin, fibroblasts, keratinocytes, melanocytes and melanoma cell lines. Nevertheless, expression of DCD can be induced by oxidative stress, LPS and TNF-alpha in melanoma cell lines, but not in keratinocytes, fibroblasts or melanocytes. Protein expression analysis revealed that in human skin DCD is specifically and constitutively expressed in sweat glands, secreted into the sweat and transported to the epidermal surface. In sweat a proteolytically processed 47mer peptide is generated which has antimicrobial activity against a variety of pathogenic microorganisms in a dose-dependent manner. The activity of the peptide is maintained over a broad pH range and in high salt concentrations resembling the conditions in human sweat. This indicates that sweat plays a role in the regulation of the human skin flora through the presence of an antimicrobial peptide.

V06

Cathepsin D regulation of involucrin expression is mediated by transglutaminase 1

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Increased expression of the lysosomal aspartat protease cathepsin D has been reported in melanoma, in squamous cell carcinoma, in wound healing, in psoriasis, and during epidermal differentiation. We recently found that cathepsin D is targeted by acid sphingomyelinase derived ceramide and regulates involucrin expression. The aim of the present study was to explore if transglutaminase 1 is involved in this regulation process. Epidermal transglutaminase 1 is a key enzyme in the processing of the cornified envelope proteins and catalyzes the formation of intermolecular epsilon-(gamma-glutamyl) lysine bonds. In the present study, we topically applied monodansylcadaverine, an inhibitor of transglutaminase 1 activity, to wild type hairless mice. In cathepsin D-/--mice, generated by gene targeting, we determined epidermal transglutaminase 1 activity and protein expression by a specific enzyme assay and by Western blot analysis using a specific antibody suitable for the mouse system, respectively. Topical application of monodansylcadaverine significantly impaired epidermal differentiation. In homozygous cathepsin D-/--mice epidermal transglutaminase 1 enzyme activity was severely reduced (-72%) compared to wild type animals. In heterozygous cathepsin D+/--mice enzyme activity was still significantly decreased (-48%). Western blot analyses showed that processing of the precursor to mature forms of transglutaminase 1 was reduced in cathepsin D-/- and even in +/--mice. Transglutaminase 1 deficiency in cathepsin D-/--mice, leading to reduced involucrin expression, resulted in dryness of the skin and epidermal hyperkeratosis. Similarly, dry skin and hyperkeratosis are signs of human lamellar ichthyosis. In this disease mutations in the transglutaminase 1 gene, leading to decreased enzyme activity and decreased involucrin expression are known. In summary, we found that cathepsin D regulation of involucrin expression during epidermal differentiation is mediated by transglutaminase 1. We propose a signal transduction pathway in which acid sphingomyelinase derived ceramide regulates cathepsin D activity, which in term stimulates transglutaminase 1 activity for the processing of involucrin.

V07

Functional specificity and diversity of the cyclin-dependent kinase inhibitors $p27^{KIP1}$ and $p21^{CIP1}$ in T cell anergy

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The molecular events governing the induction and maintanance of T cell anergy are poorly understood. Recent evidence points to a central role of basal cell cycle regulators, particularly the cyclin-dependent kinase (CDK) inhibitors, $p27^{KIP1}$ and $p21^{CIP1}$, in the regulation of anergy. Specifically, $p27^{KIP1}$ was implied as an anergy factor because (i) $p27^{KIP1}$ protein levels were not downregulated in anergic cells in contrast to productively stimulated cells, (ii) overexpression of $p27^{KIP1}$ in T cells in vitro mimicked an anergy-like G_1 cell cycle arrest, and (iii) the association of $p27^{KIP1}$ with the c-Jun co-activator JAB1 could be involved in the decreased transactivation of the *IL-2* gene, a hallmark of anergic cells. Furthermore and unexpectedly, $p21^{CIP1-/-}$ mice developed a lupus-like syndrom with age. To further rigorously evaluate the requirement of $p27^{KIP1}$ and $p21^{CIP1}$ for T cell anergy, purified $CD4^+$ T cells from $p27^{KIP1-/-}$ and $p21^{CIP1-/-}$ mice were compared with corresponding wild-type cells. Phenotypically, $p27^{KIP1-/-}$ T cells show an increased fraction of $CD25^+$ cells before stimulation, a shortened cell cycle, and an accelerated response to stimulation, while $p21^{CIP1-/-}$ T cells closely resemble wild-type cells. In sharp contrast, the induction and maintainance of proliferative quiescence appears unaltered, indicating that neither $p27^{KIP1}$ nor $p21^{CIP1}$ are required for this response. Strikingly, $p27^{KIP1-/-}$ T cells exhibit resistance to anergy induction as demonstrated by multiple parameters including increased cell duplication, increased BrdU incorporation in S phase, CD25 upregulation, synthesis of CDK4/cyclin D3 complexes, and retinoblastoma protein (RB) hyperphosphorylation. In comparison, wild-type and $p21^{CIP1-/-}$ T cells become anergic as judged by these criteria, and $p27^{KIP1+/-}$ cells have an intermediate phenotype. Therefore, $p27^{KIP1}$ seems to act as a specific and possibly essential regulator of T cell anergy rather than a general cell cycle regulator of T cell proliferation. The role of $p21^{CIP1}$, however, appears to be non-essential in this scenario. Further analyses are aimed to characterize the specific molecular pathways involved as well as the *in vivo* phenotypes of the respective mice.

V08

IL-4 and IL-4 receptor alpha chain independent IgE synthesis in an allergy model in mice

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IL-4 and IL-13 are key regulators in atopic disorders, and both signal through the receptor chain IL-4Rα. IL-4 and IL-13 are also the only cytokines known to induce class switching to IgE. We sought to compare allergen specific IgE responses and allergic reactivity of wild type mice with IL-4-/- and IL-4Rα-/- mice, which lack both IL-4 and IL-13 functions.

BALB/c wild type, IL-4-/- and IL-4Rα-/- mice were weekly immunized with Ovalbumin intranasally or twice intraperitoneally. In addition, groups of wild type and knockout mice were treated with an IL-4/IL-13 inhibitor during allergic sensitization, which has been shown to block IL-4 and IL-13 effects completely *in vitro*. After 6 weeks specific antibody titers were measured by ELISA, bronchoalveolar lavage fluids and lung tissue were analyzed cytologically and histologically. Allergic reactivity was determined by active cutaneous anaphylaxis and anaphylactic shock.

Wild type mice immunized intranasally or intraperitoneally showed high titers of specific IgE 3 and 6 weeks after primary sensitization, resulting in cutaneous anaphylaxis and anaphylactic shock upon challenge. Intranasal sensitization resulted in airway eosinophilia and goblet cell metaplasia as well. In contrast, IL-4-/- and IL-4Rα-/- mice showed no specific IgE after 3 weeks, but produced significant titers after 6 weeks. At this time cutaneous anaphylaxis and anaphylactic shock could be induced similar as in wild type mice, but lung pathology was absent. In wild type mice the inhibition of the IL-4/IL-13 receptor system resulted in a dose dependent reduction of OVA specific IgEs, however, residual IgE synthesis could be detected after 6 weeks. In contrast, knockout mice, which were treated with the IL-4/IL-13 inhibitor showed no further reduction of specific IgEs in comparison to untreated knockout mice.

We conclude that upon long term allergen exposure alternative switch mechanisms independent of IL-4 and IL-4Rα may induce IgE, but not asthma-like lung pathology. This may be relevant for the development of allergic disease in men, since long term allergen exposure is a frequent condition during allergic sensitization.

V09

Bone Morphogenetic Protein-1 (BMP-1) Processes Procollagen VII to Mature Collagen VII

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Collagen VII is the major structural component of the anchoring fibrils which ensure the cohesion between the epidermis and the dermis. This collagen is secreted by keratinocytes into the extracellular matrix as a precursor, procollagen VII. During polymerization and maturation of the anchoring fibrils, two procollagen VII monomers form an antiparallel homodimer with an overlapping C-terminus, and a C-terminal propeptide is removed. However, the mechanisms of anchoring fibril maturation, including the cleavage site and the enzyme processing procollagen VII have remained elusive. Genetic evidence has suggested the involvement of BMP-1 in this process, since a naturally occurring deletion in the COL7A1 gene, 8523del14, which eliminates a putative cleavage site for BMP-1, prevents processing of procollagen VII in the skin. Here we show that recombinant BMP-1 cleaves full-length human procollagen VII in vitro, yielding a cleavage product of the same size as mature collagen VII in the dermis. In order to determine the cleavage site, a truncated recombinant procollagen VII containing an intact C-terminus was produced. This miniprocollagen VII was cleaved by both BMP-1 and by mammalian tolloid-like 1 (mTLL-1), another protease of the same enzyme family. N-terminal sequencing showed that miniprocollagen VII was processed at the predicted site. Analysis of collagen VII in the skin of BMP-1 deficient mouse embryos demonstrated that procollagen VII was processed to the same extent as in wild type embryos. This suggests that in situ the collagen VII precursor can be cleaved by at least two metalloproteases including BMP-1 and mTLL-1. The cleavage of the keratinocyte-derived procollagen VII by fibroblast-derived enzymes presents a novel mechanism of regulation of anchoring fibril formation and an intriguing manifestation of epithelial-mesenchymal interactions.

V10

Crossing of female keratin K14-Cre mice with male manganese superoxide dismutase floxed mice yields a complete MnSOD knockout mouse with a severe phenotype

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In order to investigate the role of manganese superoxide dismutase (MnSOD) and UV-generated reactive oxygen species (ROS) in photoaging and photocarcinogenesis, we have set out to generate a skin-specific MnSOD knockout mouse using the Cre/loxP system. Unexpectedly, crossing of female mice carrying the Cre transgene under the control of the keratin K14 promotor with male MnSOD-floxed mice yielded not an epidermis-specific but a complete MnSOD knockout mutant (MnSOD-/-). Our hypothesis that temporary K14 promotor activity in oocytes of female K14-Cre mice is responsible for transient Cre expression with subsequent ubiquitous deletion of the loxP-flanked MnSOD gene right after fertilization, could be confirmed by immunohistochemistry and RT-PCR for K14 expression in oocytes. These findings are of general interest for the generation of epidermis-specific knockout mice, independent of the nature of the targeted gene. Characterizing these accidentally generated MnSOD-/- mice revealed some overlap, but also distinct differences, when compared to the earlier published MnSOD-deficient mouse models. Most striking common features were decreased size, motor disturbances and early mortality between day 6 and 10 after birth. Using light and transmission electron microscopy, we observed changes in heart, liver, gut, skin and brain in 3 and 6 days old MnSOD-/- mice and, in contrast to earlier published MnSOD-deficient mouse models, also in 10 days old heterozygous MnSOD+/- mice. Changes include dilated cardiomyopathy with a striking loss of myofilaments and of typical myofilament organization in cardiomyocytes. Swollen mitochondria with enlarged cristae, loss of cell organells, increased numbers of cytoplasmic vacuoles and lipid droplets were also observed. Immunohistochemical detection of 8-isoprostane, a central indicator of ROS-mediated cell damage, revealed substantial accumulation of this compound in all investigated tissues 3 days after birth in MnSOD-/-, and 10 days after birth in MnSOD+/- mice. These data strongly underline the impact of MnSOD gene dosage on intramitochondrial ROS-detoxification pathways, cell and organ fate.

V11

Targeting LFA-1 binding as a therapy for atopic dermatitis

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Atopic dermatitis (AD), a chronic inflammatory skin disease, is considered to be the cutaneous manifestation of atopy and induced by activation of Th2 cells that migrated to the skin. We demonstrated that CCR4 is the major chemokine receptor expressed on AD derived Th2 cells and established a Th2 cell migration model in SCID mice with previously transplanted human skin. Injection of the CCR4 ligand macrophage derived chemokine (MDC/CCL22) into these SCID mice mimicked AD inflammation since it was efficiently presented by human dermal endothelial cells and recruited AD derived Th2 cells into human skin *in vivo*. This experimental protocol was used to investigate the role of the integrin LFA-1 in the process of skin homing of human Th2 cells. LFA-1 is important during the establishment of the immunological synapse required for Th cell activation and in the process of leukocyte migration, where chemokines activate LFA-1 for the necessary firm adhesion of Th cells to ICAM-1 expressing endothelium. These dual actions of LFA-1 may be involved in the pathogenesis of AD and therefore represent a potential target to treat the disease. Indeed, anti-LFA-1 mAbs blocked specific cytokine production during the activation of memory Th2 cells and abrogated CCL22 mediated firm adhesion of AD derived Th2 cells in an *in vitro* adhesion assay. Moreover, CCL22 induced migration of AD derived Th2 cells to human skin grafts on SCID mice was also inhibited by mAbs to LFA-1. These *in vitro* and *in vivo* experimental systems were used to evaluate low molecular weight compounds antagonising LFA-1. These antagonistic compounds blocked both, antigen specific Th2 cell cytokine production *in vitro* and Th2 cell migration to human skin *in vivo*. Thus, targeting LFA-1 binding promises to be an effective new therapy for AD.

V12

Di-Acylated-Ureas: A Novel Family of Proinflammatory and Fungus-specific Pathogen-Associated Lipids

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The survival of multicellular organisms depends on their ability to recognize invading microbial pathogens and to induce appropriate host defense reactions. Pathogen-associated molecules (PAMs) signal the presence of pathogens and induce innate immune responses to eliminate the infectious agents.
Formylated methionyl-peptides represent prokaryote-specific PAMs that are powerful chemoattractants for neutrophils signalling to the host the presence of infecting bacteria and leading to neutrophil-rich pustule and pus formation.
Similarily fungal infection can lead to neutrophil infiltration and inflammation suggesting that fungi might also release neutrophil-chemotactic PAMs.
We previously identified unstable lipid-like leukocyte attractants (LILAs) in dermatophytes, that differed from known chemotactic factors in both chemical and biological properties. Here we show, that also yeasts, but not bacteria or human cells produce these LILAs.
Purification and rigorous chemical and physicochemical analyses of these shortlived compounds (halflife-time < 1h under physiological conditions), which include 1H-NMR-COSY-analyses and ESI-orthogonal-accelerated-quadrupole-time-of-flight mass spectrometry, led to the structure proposal, that these fungal lipids are di-acylated ureas, a family of lipids not known so far as natural compounds.
By chemical synthesis we could prove our structure hypothesis and found synthetic LILA to be identical with natural, yeast-derived LILA with N,N`-di-palmitoleoyl-urea (C16:1DAU) being the most potent chemotactic and activating compound (ED50: < 1 nM). Apart from their chemotactic and secretory functions-activating properties, DAUs induce in human monocytes, like bacterial LPS, release of proinflammatory cytokines like TNF-α, IL-1ß and IL-8. Furthermore these DAUs induce in primary keratinocytes and HaCat keratinocytes the production of TNF, IL-1 as well as the chemokines IL-8 and RANTES. Therefore DAUs represent a novel class of proinflammatory lipids, that, apart from leukotactic properties also induce proinflammatory cytokines in monocytes and skin keratinocytes. Their isolation exclusively from fungi suggests that members of this lipid family are fungus-specific PAMs that may alert the human innate immune system to the presence of fungi.

V13

Photoprotection by cholesterol (Chol): Analysis of its role in Ultraviolet (UV) A radiation-induced gene expression in human keratinocytes (KC).

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UVA radiation-induced signal transduction in human KC was previously shown to result from the formation of ceramide from cell membrane sphingomyelin. Ceramide then served as a second messenger and induced the release of cytochrome C from mitochondria, which by means of a redox regulation activated transcription factor AP-2 and thereby caused an increased transcriptional expression of UVA-inducible, proinflammatory genes such as ICAM-1. Cell membrane lipids thus play a pivotal role in controlling UVA radiation-induced biological effects in human KC. It is now generally believed that signal transducing cell membrane lipids are organized in "rafts" or microdomains, which, in addition to sphingoymelin/ceramide also contain chol. In the present study we have therefore analyzed the role of chol in UVA radiation-induced ICAM-1 expression in longterm cultured, normal human KC. As previously reported, exposure of KC to sublethal doses of UVA (30 J/cm2) radiation increased ICAM-1 mRNA and protein expression in a biphasic manner with a first maximum (4-fold) between 0.5 to 4 hr, and a second more sustained peak (5-fold) at 24 to 48 hr. This biphasic increase in ICAM-1 expression could be mimicked in unirradiated cells, in which chol synthesis was inhibited upon treatment with lovastatin, a specific hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase inhibitor. Also, extraction of chol from the cell membrane of unirradiated KC through treatment of cells with cyclodextrin strongly (14-fold) induced ICAM-1 expression in a monophasic manner. Lowering of cell membrane chol levels thus led to increased expression of a UVA-inducible gene indicating a regulator function for chol. Accordingly, cyclodextrin treatment of UVA-irradiated KC resulted in a 2-fold enhancement of UVA radiation-induced ICAM-1 upregulation at 24 to 48, but not at 1 to 4 hr. This effect was specific because cyclodextrin did not increase IFN-gamma or TNF alpha-induced ICAM-1 expression. Even more important, incubation of KC with chol prior to UVA irradiation completely abolished at all time points their capacity to mount a UVA response by upregulating ICAM-1 expression. These studies demonstrate that in addition to ceramide, chol has an important role in UVA radiation-induced signal transduction in human KC. The capacity of chol to prevent UVA radiation-induced gene expression indicates its previously unrecognized potential as a photoprotective molecule.

V14

BIA-1: A cytoplasmic protein, interacting with integrin α-1 tail, regulating synthesis of collagen I

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Interaction of fibroblasts with extracellular matrix (ECM) proteins influences a large number of cellular functions. The contact with most ECM proteins is mediated by integrin receptors of the β1 family, and binding of the integrin cytoplasmic domains to intracellular proteins is thought to initiate signal transduction. Our studies address two questions: what is the contribution of individual integrin α-chains? And, in view of our previous results linking the α1β1 integrin to regulation of collagen synthesis: which intracellular proteins bind to the cytodomain of α1 integrin? A yeast-two hybrid screen was performed, resulting in identification of proteins which bind to the conserved membrane-proximal region, and others which bind either to several or exclusively to individual integrin cytodomains.

Of the specific binding partners for α1 integrin, one was further analyzed. Termed BIA-1, it represents an as yet unknown protein which displays the structural organization characteristic of the "B30.2" protein family: RING finger, zinc-binding and coiled-coil domains, and a C-terminal B30.2 box.

To study the biological function of BIA-1 and its possible role in modulating collagen synthesis, fibroblasts were engineered to either overexpress BIA-1 or expression was reduced by transfecting antisense oligodeoxynucleotides. In both approaches, BIA-1 and collagen mRNA levels were regulated in opposite directions, i.e. low BIA-1 levels correlated with high collagen synthesis. These results were confirmed by culturing untransfected primary skin fibroblasts under conditions of well-characterized modulation of collagen synthesis.

These results strongly support a role for BIA-1 in initiating the signaling cascade elicited by activation of α1β1 integrin receptors and regulating collagen synthesis.

KV01

Activation of eotaxin gene expression in dermal fibroblasts (and A549 cells) by IL-4 or TNFα depends on intact eotaxin promoter recognition sites for both Stat6 as well as NFκB nuclear factors

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Eosinophils in addition to being important effector cells against helminths are involved in tissue damage characteristic for allergic and atopic skin diseases. Eosinophil-specific chemoattractants like eotaxin-1-3 but also MCP-3, MCP-4 and RANTES are believed to be responsible for tissue-accumulation of eosinophils. To understand the unique regulation of eotaxin-1 gene expression we investigated the activation of this gene in cultured human dermal fibroblasts and A549 lung epithelial cells by analysing nuclear extracts of dermal fibroblast and A549 cells for NFκB and Stat6 binding to the overlapping Stat6/NFκB region of the eotaxin-1 promoter and performed luciferase reporter gene assays using eotaxin-1 promoter reporter constructs.

Reporter gene assays showed that the selected 125 bp eotaxin promoter region containing the NFκB/Stat-6 recognition site was sufficient to mediate IL-4, IL-13 or TNFα-induced transcription as well as enhanced transcription upon stimulation with a combination of TNFα and IL-4. Nuclear extracts of IL-4, TNFα or IL-4+TNFα treated cells showed Stat6, NFκB-p65 (but not NFκB-p50) or Stat6 + NFκB-p65 binding respectively to eotaxin-1 promoter DNA. Reporter constructs defective in one of the 2 recognition sites (leaving either an intact STAT6 or NFκB recognition site) did not mediate IL-4 or TNFα induced transcription. These results underscore that the combination of either IL4 or IL13 with TNFα is the major stimulus for eotaxin-1 dependent eosinopil accumulation and may point to the involvement of additional nuclear factors interacting with the STAT6/NFκB recognition site.

KV02

Promoter polymorphisms of the genes encoding tumor necrosis factor-α and interleukin-1β are associated with different subtypes of psoriasis characterized by early and late disease onset

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The psoriatic inflammatory process is characterized by an overexpression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β compared to a relative deficiency of anti-inflammatory factors such as IL-10 and the IL-1 receptor antagonist (IL-1Ra). Gene polymorphisms that affect cytokine production may contribute to the disease-associated cytokine imbalance and influence susceptibility to psoriasis. Here, we investigated the relationship between polymorphisms in the genes encoding for TNF-α (G-238A, G-308A), IL-1b (C-511T, T+3953C), and IL-1Ra (intron 2) and cytokine production in peripheral blood mononuclear cells of healthy donors, and analyzed the distribution of these polymorphisms in patients with psoriasis vulgaris (n = 231) and healthy controls (n = 345). Carriage of TNFA-238 allele 2 (-238*A) was associated with increased production of TNF- α in response to lipopolysaccharide in vitro, and with early onset disease (< 40 y), especially in male patients with psoriasis (32% vs 7% in male controls; odds ratio = 6.78, 95% confidence interval = [3.18 - 15.15], $p_{adjusted} = 2 \times 10^{-7}$). Carriage of the IL1B-511*1 (-511*C) homozygous genotype was associated with increased production of IL-1Ra in response to lipopolysaccharide and IL-10, and with late onset psoriasis (≥ 40 y; 61% vs 44% in controls; odds ratio = 2.04, 95% confidence interval = [1.19 - 3.53], $p_{adjusted} = 0.0419$). These findings indicate that gene polymorphisms associated with altered cytokine responses *in vitro* may modify age of onset of psoriasis. They also provide further evidence that patients with early and late onset psoriasis differ in their genetic background.

KV03

Interleukin-10 Knockout Mice are relatively resistant to the induction of Alopecia areata

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Alopecia areata occurs spontaneously in 20% of C3H/HeJ mice, but it can be induced in all unaffected C3H/HeJ mice or other histocompatible strains by grafting of AA-affected mouse skin. Because IL-10 expression is increased in successfully DCP-treated scalp from patients with AA, we wanted to determine whether the absence of IL-10 results in an increased susceptibility to the development of AA. For this purpose we we tried to induced AA in 20 C3H/HeJBir-Il10-/- mice by skin grafting and as controls we grafted aa-affected skin onto 20 so far unaffected C3H/HeJ mice.
After an observation period of 12 weeks, FACS-analysis for a wide panel of surface markers and cytokines was performed on skin infiltrating leucocytes and cells from skin draining lymph nodes. Surprisingly, only 7/20 C3H/HeJBir-Il10-/- mice developed limited AA and 13/20 C3H/HeJBir-Il10-/- mice did not develop any hair loss and no mice developed extensive AA. By contrast, 19/20 controls developed AA. FACS-analysis revealed elevated levels of monocytes and dendritic cells in the skin and of dendritic cells in lymph nodes of Il-10 deficient mice compared to controls, but a similar level of monocytes in lymph nodes of both groups. IL-10-/- mice had increased levels of IFN-γ and TNF-α in lymph nodes and decreased levels of IL-6 in skin.CD25, CD28, CD40, CD80 and CD86 were increased in skin of IL-10-/- mice and CD44s, CD44v3, CD44v6, CD44v7 and CD44v10 were increased in both, skin and lymph nodes of IL-10-/- mice.
In summary we have surprisingly found, that IL-10 deficient mice are relatively resistant to the induction of AA, despite their elevated levels of proinflammatory cytokines and adhesion molecules. This results are inline with our findings that AA in C3H/HEJ-mice is not treatable with IL-10 injections. Whether the decreased levels of IL-6 could be responsible for the resistance to AA induction has to be investigated in further studies.

KV04

Terminal B cell differentiation is skewed by deregulated interleukin-6 secretion in β2 integrin deficient mice

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The β2 integrin family (CD11/CD18) of leukocyte adhesion molecules plays a key role in inflammatory cell-cell and cell-matrix interactions. Absence of the common β chain (CD18) simultaneously affects all heterodimeric β2 integrins, and leads to leukocyte adhesion deficiency type 1 (LAD1) in humans. Mice with a CD18 null mutation suffer from recurrent bacterial infections, impaired wound healing and skin ulcers closely resembling human LAD1.Previous findings in CD18-/- mice have demonstrated a highly impaired terminal B cell differentiation including plasmacytosis, leading to a 10- to 20-fold elevated serum IgG, and light chain amyloid deposits. Since interleukin-6 (IL-6) is a potent enhancer of plasma cell formation and immunoglobulin secretion, we assessed IL-6 serum levels of CD18-/- and wild-type (WT) mice kept under conventional, barrier facility or specific pathogen-free (SPF) conditions. We detected an up to 20-fold increase in IL-6 serum concentrations in CD18-/- mice compared to WT controls when kept under conventional or barrier facility conditions, respectively ($p<0.001$). Under SPF conditions, no significant differences in terms of IL-6 serum levels were found between CD18-/- and WT mice ($p=0.5$). However, histological alterations like plasmacytosis, abnormal plasma cells and amyloid deposits as well as hypergammaglobulinemia were still detectable. To analyze the role of IL-6 in these pathological alterations, we established a CD18-/- IL-6-/- double-deficient mouse mutant. In these mice, serum IgG levels were normal, and in histology, abnormal plasma cells and plasmacytosis were no longer detectable. The CD18-/- IL-6-/- double-deficient mouse model thus demonstrates that IL-6 is responsible for parts of the phenotype seen in the CD18-/- mouse mutants. It may be of interest to closer examine human LAD1 patients and search for pathological changes possibly induced via hyperproduction of IL-6.

KV05

Interleukin-10 Is Required For Induction of Low Zone Tolerance to Contact Allergens

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The induction of hapten-specific low zone tolerance (LZT) by epicutaneous application of low doses of contact allergens is mediated by $CD8^+$ suppressor T cells (Tc2) that secrete immunosuppressive cytokines such as IL-4 and IL-10. Here, we have used IL-10-deficient (IL-10 -/-) mice and normal wild type (+/+) animals to better characterize the role of IL-10 in the induction of LZT. IL10-/- mice and +/+ mice were treated epicutaneously with tolerizing doses of the contact allergen TNCB (0.45 or 4.5 µg per site, 5x per mouse). Subsequently, contact hypersensitivity reactions (CHS) to TNCB were induced in all mice and quantified by measuring ear swelling. Tolerization resulted in robust LZT in +/+ mice, as reflected by a pronounced inhibition of CHS (-87±15%), but failed to induce LZT in IL-10 -/- mice (-20±5% inhibition of CHS, $p< 0.01$). Reconstitution of IL-10 -/- mice with IL-10 during tolerization completely restored LZT in these mice, indicating that normal LZT is IL-10-dependent. In addition, lymph node cells (LNC) or purified T cells of tolerized +/+ mice and IL-10-reconstituted IL-10 -/- mice showed markedly reduced antigen-specific proliferation as compared to LNC or T cells derived from tolerized IL-10-/- animals. To identify potential mechanisms of IL-10-dependent induction of tolerance, LZT was assessed in +/+ mice that had received LNC obtained from tolerized IL-10 -/- mice or +/+ mice. Interestingly, adoptive transfer of LNC derived from tolerized +/+ mice induced strong LZT in naive mice (-83±20% inhibition of CHS), whereas LNC obtained from tolerized IL-10-/- mice failed to do so (-29±13% inhibition of CHS), indicating that the induction of LZT requires the presence of IL-10 at sites of antigen presentation and lymphocyte activation. To test whether LZT in normal mice may be enhanced by IL-10, C57BL/6 mice were treated systemically with IL-10 during tolerization (i.p., 3x 100 ng/day for 10 days). Notably, such treatment significantly increased LZT as compared to vehicle-injected control animals. These data show: 1) that the induction of LZT is IL-10-dependent, 2) that IL-10 facilitates the induction of LZT, at least in part, via effects on lymphocytes, and 3) that tolerance can be enhanced by treatment with IL-10. Our findings indicate that IL-10 is a critical player in the induction of tolerance to contact allergens.

KV06

Autoreactive T- and B-cells of patients with bullous pemphigoid (BP) preferentially target epitopes of the NH2- and COOH-terminus of the extracellular domain (ECD) of BP180 (BPAG2)

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BP is the most frequent autoimmune bullous skin disorder in adults and represents a model of antibody (Ab)-mediated autoimmunity. Ab against the hemidesmosomal adhesion molecule BP180 seem to be critical for the loss of dermoepidermal adhesion. Our investigations were therefore aimed at characterizing autoaggressive T- and B-cell responses against BP180 utilizing several recombinants of the ECD of BP180. In particular, we investigated the correlation of T cell and B cell reactivity based on our hypothesis that in individual BP patients, BP180-reactive T cells may target the same epitopes as subsequently produced auto-Ab. A total of 20 BP patients were studied, all of whom had IgG reactive with the ECD of BP180. In 14/20 patients, autoreactive T cells recognized epitopes within the NH2-terminal region, while T cells from 10/20 patients reacted specifically with the COOH-terminus of BP180. In several patients (8/20), additional T cell epitopes were located throughout the ECD of BP180. Correlating auto-Ab profiles with T cell reactivity, we found that 85% of patients with NH2-terminal reactivity had also IgG directed against the NC16A domain and 60% of patients with COOH-terminal T cell reactivity had auto-Ab targeting this region of the ECD of BP180. In 3/20 patients, the pattern of T and B cell reactivity matched exactly in that it was directed to the same regions within the ECD of BP180. Furthermore, we identified an immunodominant peptide in the NC16A domain of the ECD of BP180 which was recognized in association with HLA-DRB1*1301 by an autoreactive CD4+ T helper cell clone. These results are in line with recent findings from our laboratory demonstrating that 80% of 116 BP sera showed IgG reactivity against the NH2-terminus and/or 45% against the COOH-terminal end of the ECD of BP180. Our observations suggest that T cell epitopes of BP180 may be similar or identical to the regions that are recognized by auto-Ab. The identification of immunodominant T cell peptides will hopefully facilitate immunomodulatory strategies in BP.

KV07

Interleukin-2 is a major stimulus for Interferon-gamma production by dendritic cells

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A characteristic feature of dendritic cells (DC) is the process of maturation by which DC develop potent T cell stimulatory capacities. During maturation DC strongly upregulate surface expression of MHC class -I, -II and various costimulatory molecules as well as CD25, the alpha-chain of the IL-2 receptor. The function of the IL-2R on mature DC, however, has for a long time remained unknown. Only recently Fukao et al. (Eur.J.Immunol. 2000) reported that IL-2 costimulates with IL-12 the production of IFN-gamma by spleen DC in C57/BL6 mice.
We have reevaluated the issue of IFN-gamma production in epidermal Langerhans cells (LC), bone-marrow derived DC (BMDC) and sorted subpopulations of $CD11c^{high}$ spleen DC from Balb/C and C57/BL6 mice. Stimulation with IL-12, IL-18 and IL-2 or combinations of these never induced IFN-gamma in LC, BMDC and CD4+ spleen DC. Among the CD4- spleen DC, both CD8+ and CD8-4- DC produced comparable amounts of IFN-gamma and this result was confirmed using $Rag\text{-}2^{-/-}$ mice and by intracellular staining of DC. Although highest yields of IFN-gamma production in CD4- spleen DC were seen by a combination of IL-12 and IL-18, IL-2 alone was sufficient to induce significant amounts of IFN-gamma, which only partially could be blocked by mAb to IL-12 and IL-18. Analysis of spleen DC from $CD25^{-/-}$ mice revealed that IL-2 induction of IFN-gamma requires expression of the IL-2R alpha-chain. Finally, IL-2 as a stimulus for IFN-gamma production could be confirmed in cytokine cocktail matured human monocyte derived DC.
In aggregate these data suggest that 1) IL-2 induction of IFN-gamma in DC involves signals other than IL-12 and IL-18 2) point to an important feedback loop mechanism during cognate interaction of cytokine producing DC [IL-12, IFN-gamma] and T cells [IL-2] and 3) imply that IL-2 immunotherapy may bias towards Th1 responses via inducing IFN-gamma production by DC.

KV08

Selective Glucocorticoid Receptor Agonist (SEGRA)

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Glucocorticoids (GC) are widely used in anti-inflammatory therapy. They exert their activity by binding to and activation of a specific receptor, the glucocorticoid receptor (GR). Upon activation the cytoplasmic GR may translocate into the nucleus, dimerize and act as a transcription factor binding to a specific DNA-sequence in the promoter of certain target genes (GR-DNA-interaction). Alternatively, it may interact, possibly as a monomer, with other transcription factors, like NFκB or AP-1, thus inhibiting their biological activity (GR-protein-interaction). Many of the anti-inflammatory activities of GCs are exerted by their suppressing activity of certain target genes via a GR-protein-interaction. This could be shown by the anti-inflammatory activity of GCs in transgenic mice carrying a dimerization-incompetent GR. In contrast, several side effects of GCs seem to be mediated by GR-DNA-interactions and subsequent transactivation of target genes. Therefore, our aim was to identify compounds which preferentially induce transrepression with a weaker induction of transactivation. Here, we show a SEGRA compound that is a representative of a novel anti-inflammatory principle aiming at the predominant induction of transrepressing versus transactivating effects. The potency in transrepression of our compound is clearly better than that of prednisolone whereas transactivation efficacy is markedly lower. The SEGRA compound reaches full inhibition of inflammatory effects in the croton oil induced ear inflammation model at higher concentrations, i.e. its maximal anti-inflammatory efficacy is comparable to glucocorticoids of moderate potency. The compound shows topical side effects clearly lower than prednisolone and almost no systemic side effects after topical high-dose treatment for a long period of time. Thus, the selective SEGRA compound represents a useful novel therapeutic modality which may complement existing therapeutic principles for topical and systemic treatment of inflammatory diseases.

KV09

Cysteine proteases play an important role for the invasion of melanoma cells into dermal extracellular matrix

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Local invasion of melanoma cells into the dermal connective tissue is the first step in the complex process of metastasis. Recently, we have developed an improved culture model that allows the detailed study of melanoma cell invasion *in vitro*. In this model high (BLM) or low (530) invasive melanoma cells were seeded on the dermal side of dead deepidermized dermis and these composits were cultured for 14 days at the air/liquid interface. The high invasive cells rapidly invade the tissue, whereas the low invasive cells do not enter the tissue. As it is well known that proteolytic alteration of the extracellular matrix is a key event in tumor invasion, we investigated the expression and activity of different proteases. Immunohistochemistry of this melanoma composits reveals the presence of different members of the matrix metalloprotease (MMP) family and the lysosomal cysteine protease cathepsin B. To characterize the proteolytic activity of the two different cell lines, we performed in-situ gelatin zymography of cryosections under different environmental conditions. Areas of gelatinolysis were only detected in composits cultured with high invasive BLM cells, but not with 530 cells. Interestingly, in-situ zymograms incubated with an acidic buffer, which favours cysteine protease activity show enhanced gelatinolysis compared to zymograms incubated with neutral buffer, which favours MMP activity. The gelatinolytic activity at acidic conditions is inhibited by E-64 and Leupeptin, both inhibitors of cysteine proteases, but not by EDTA, an inhibitor of MMPs. To further prove the role of cysteine proteases in the invasive process, BLM composits were cultured in presence of leupeptin revealing a marked reduction in the number of invading melanoma cells in the deeper layers of the composit. As cysteine proteases like cathepsin B are able to degrade different components of the extracellular matrix, these results indicate an important role for cysteine proteases in the invasive process.

KV10

Targeting human melanoma cells in-vitro and in-vivo by the Fas signaling system.

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Fas (CD95, Apo-1)-signaling triggers apoptosis and has been recognized as a powerful tool against cancer cells when using agonistic antibodies. However, considerable side effects resulted from systemic treatment with Fas-antibodies. The hypothesis of a tumor counter attack against immune cells due to Fas ligand expression has been discussed conversely for malignant melanoma. Our data clearly show that melanoma cell lines in-vitro do express Fas receptor (FasR) mRNA whereas Fas ligand (FasL) was not expressed. Applying a doxycyclin-inducible expression system, we demonstrate that melanoma cells respond with a strong increase of apoptosis (2 to 10-fold) after transient expression of FasL, whereas FasR expression by itself did not induce apoptosis in 4 tetracyclin-regulatable melanoma cell lines, established and investigated. However, sensitivity to the Fas signal (agonistic antibody CH-11) was significantly enhanced after transient FasR expression. In cell clones stably transfected with FasL, apoptosis could be triggered by addition of doxycyclin to the growth medium. Activation of the signal cascade downstream of the Fas receptor was confirmed in the stable transfected cell clones by Western blots for caspase-8 , Bid and caspase-3. Soluble FasL was released from stable transfected cells after induction, as conditioned medium itself was also able to kill recipient cells, therefore opening the possibility of a bystander effect in-vivo. Finally in-vivo, stable transfected, doxycyclin-inducible cells were tumorigenic in nude mice when the animals were grown without doxyxyclin, but failed to develop any tumor when mice were treated with doxycyclin for FasL induction. Furthermore, when tumors had already developed under permissive conditions and treatment was started, stable disease was the result or further tumor growth was significantly inhibited.
Our data do not support the often discussed hypothesis of a tumor counter attack by FasL expression for malignant melanoma, rather Fas ligand turned out as a powerful tool against melanoma cells in-vitro and in the mouse model, also when using gene transfection techniques. Side effects in-vivo may be overcome by applying melanoma cell-specific gene targeting (tyrosinase promoter); experiments are just in preparation.

KV11

Evidence for a role of Langerhans cell-derived interleukin-16 in atopic dermatitis

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Background: The factors controlling infiltration of inflammatory cells into atopic dermatitis (AD) lesions remain to be fully explored. Recently, epidermal cells in lesional AD were reported to contain increased mRNA levels of IL-16, a cytokine that induces chemotactic responses in CD4+ T cells, monocytes and eosinophils. The cellular sources of IL-16, however, were not further explored.
Objectives: To determine the expression of IL-16 in epidermal cells in normal skin and AD, and to investigate whether Langerhans cell-derived IL-16 may contribute to the initiation of atopic eczema.
Methods: The cutaneous expression of IL-16 was investigated by *in situ* hybridization and immunohistochemistry. Expression of IL-16 was also investigated in freshly isolated Langerhans cells and in keratinocytes by intracellular cytokine staining, quantitative real-time reverse transcription-PCR, and ELISA.
Results: Low levels of IL-16 mRNA, but no stored IL-16 protein, were detected in keratinocytes and Langerhans cells isolated from normal skin. Synthesis, storage and secretion of IL-16 could be induced in Langerhans cells, but not keratinocytes, by activation with phorbol ester and ionomycin. In normal skin (n=10) neither keratinocytes nor Langerhans cells expressed IL-16. In contrast, IL-16 was contained in approximately 40% of CD1a-positive Langerhans cells in active AD (n=16). IL-16 expression in Langerhans cells in AD correlated with the number of infiltrating CD4+ cells (r = .72, P = .0017) and was completely downregulated parallel to the clinical response of AD lesions to topical treatment with FK506.
Conclusion: Langerhans cell-derived IL-16 may participate in the recruitment and activation of inflammatory cells in atopic dermatitis.

KV12

Mal de Meleda (MDM) is caused by mutations in the gene for SLURP-1

in patients from Turkey, Palestine, and the United Arab Emirates

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Mal de Meleda (MDM), or keratosis palmoplantaris transgrediens of Siemens, is a hereditary skin disorder characterized by diffuse palmoplantar keratoderma (PPK) and transgressive keratosis with an onset in early infancy before one year of age. There is no associated involvement of other organs, however, a rather broad spectrum of clinical presentations with other optional and variable features is characteristic of MDM. Some patients show a marked progression of most symptoms of the disease. MDM was first described in patients from the isle of Mljet (Meleda) in Croatia. A locus for MDM was found on chromosome 8q24-qter, and recently, mutations in the *ARS (component B)-81/s* gene were identified in patients with MDM. We have shown lately that a very similar phenotype of transgressive PPK is not linked to the interval on chromosome 8q in several families from the United Arab Emirates. Here we analysed further families with transgressive PPK. In a large Palestinian pedigree with multiple consanguinity, patients were homozygous for a new mutation, 1764G>A, in *ARS (component B)-81/s*, which substitutes an arginine for a conserved glycine residue. Another mutation affecting the same nucleotide, 1764G>C, results in the same amino acid exchange. Moreover, a third new mutation, 578A>C, was seen in the patients from a consanguineous Emirati family, which alters the translation initiation codon to a leucine codon. These findings show that the MDM type of transgressive PPK may be caused by SLURP-1 mutations in patients from various origins. A founder effect is supposed to be responsible for MDM on Mljet, however, here we demonstrate allelic heterogeneity for mutations in the gene for SLURP-1.

KV13

Dichotomy of autoreactive T helper (Th) 1 and 2 cell responses to desmogleins in patients with pemphigus and healthy individuals.

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are mediated by autoantibodies (Ab) against the desmosomal glycoproteins desmoglein 3 (Dsg3) and Dsg1, respectively. Previous studies showed that Ab belong to the Th1-regulated IgG1 and Th2-regulated IgG4 subtypes and autoreactive Th1 and Th2 cells have been identified in active PV. Since autoreactive T cells are critical for the induction and regulation of Ab production, the aim of this study was to quantitate Th1 and Th2 cell reactivity of PV patients and healthy controls against Dsg3/Dsg1. Peripheral Dsg-specific T cell clones of Th1 (IFN-γg) and Th2 (IL-4) type were quantitated and subcloned by MACS secretion assay. We investigated a total of 15 patients (PV=13; PF=2) and 13 healthy individuals. All pemphigus patients showed IgG1 and IgG4 reactivity to Dsg1 (PF) or Dsg3 (PV) by ELISA. Both, Dsg-autoreactive Th1 and Th2 cells were isolated from patients with acute (n=5), chronic (n=3), and remittent disease (n=7). The frequency of Dsg-reactive Th2 cells was constant in disease (3.6±0.6/105) and remission (2.8±0.4/105) while Dsg-reactive Th1 cells were detected at a significantly higher frequency (32.0±3.5/105) in chronic pemphigus compared to acute (7.9±1.8 /105) or remittent (5.0±3.5/105) disease. By MACS secretion analysis, 9/13 healthy donors did not exhibit Th1 or Th2 reactivity against Dsg3. In contrast, 4 healthy carriers of the PV-prevalent HLA class II alleles, HLA-DRß1*0402 and HLA-DQß1*0503, showed an exclusive Th1 cell response against Dsg3 which was detectable at frequencies (6.3±1.8/105) similar to those in the patients. Our findings strongly suggest that the presence of Dsg-reactive Th2 cells is restricted to patients with pemphigus which supports the idea that pemphigus is a Th2-mediated autoimmune disorder.

KV14

Repetitive injections of dendritic cells matured with TNF-α induce antigen-specific protection of mice from autoimmunity

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Mature dendritic cells (DC) are believed to induce T cell immunity, whereas immature DC induce T cell tolerance. Here we describe that injections of DC matured with TNF-α (TNF/DC) induce antigen-specific protection from experimental autoimmune encephalomyelitis (EAE) in mice. Maturation by TNF-α induced high levels of MHC II and costimulatory molecules on DC, but they remained weak producers of cytokines. One injection of TNF/DC pulsed with auto-antigenic peptide ameliorated the disease score of EAE. This could not be observed with immature DC or DC matured with LPS plus anti-CD40. Three consecutive injections of peptide-pulsed TNF/DC derived from wild type led to the induction of peptide-specific exclusively IL-10-producing T cells and complete protection from EAE. Blocking of IL-10 in vivo could only partially restore the susceptibility to EAE, suggesting an important but not exclusive role of IL-10 for EAE prevention. Importantly, the protection was peptide-specific since TNF/DC pulsed with unrelated peptide could not prevent EAE. In conclusion, this study describes that stimulation by TNF-α results in incompletely matured DC (semi-mature DC) which induce peptide-specific IL-10-producing regulatory T cells in vivo and prevent EAE.

KV15

The DEC-205 receptor mediates presentation of soluble protein antigens on MHC class I products of dendriric cells in vivo

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Langerhans cells express a unique antigen receptor, DEC-205, that mediates endocytosis. Recently we have shown that antigens endocytosed in vitro via DEC-205 are effectively presented to CD4+ T cells. We expanded our studies and asked whether DEC-205 mediated uptake could be exploited for targeting of antigens to Dendritic cells (DC) in vivo. By injecting anti-DEC-205 antibodies as surrogate ligand we could show that anti-DEC Abs targeted to lymphnode (LN) DCs and were detectable in intracellular compartments. Other DC reactive Abs such as anti-MHC class II Abs or IgG control failed to become endocytosed. Further characterization of the DEC-205+ compartments by immunofluorescence revealed only little colocalization with MHC-II. Instead abundance of MHC-I products were detectable in these vesicles. To investigate whether these compartments give rise to active MHC-peptide complexes we coupled the model antigen Ovalbumin (OVA) to anti-DEC and control Abs. After s.c. injection of these conjugates into mice, CD11c+ LN cells were prepared and presentation of OVA derived peptides was accessed by coculture with MHC-I-restricted OT-1 and MHC-II-restricted OT-2 T cells. Here we show, that anti-DEC: OVA conjugates in general were more potent to induce presentation of OVA to both types of T cells as compared to soluble OVA or anti-MHC, or anti LAMP-1 coupled OVA. Strikingly, the anti DEC coupled OVA was up to 100-fold more potent in inducing MHC-I restricted OT-1 proliferation than the controls. This effect was TAP dependent. Thus these data show, that exogenous antigens can be effectively targeted to LN DC in vivo via DEC-205 resulting in potent presentation of MHC-I peptide complexes.

KV16

Fumaric acid ester an antipsoriatic drug abolishes the capacity of T cells to induce Th1-mediated autoimmune disease

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Psoriasis is an inflammatory T cell mediated autoimmune disease dominated by interferon γ (IFN-γ) producing type 1 T cells infiltrating skin lesions. Predominant Th1 responses are also found in the peripheral blood. Therapy with monomethylfumarate (MMF) is effective for psoriasis in a large number of patients. In order to understand the underlying mechanisms we compared the *in vitro* and *in vivo* effects of fumaric acid esters (FAE) on human and mouse T cells. Eighteen patients were treated with FAE according to standard regimen. The dose of FAE was increased individually, serum and PBMC were analyzed at regular intervals. Leukocyte counts and relative distribution of subsets remained largely unaffected, except for an increase of eosinophils. Serum IgE levels were not influenced. In humans, intracellular cytokine analysis of freshly isolated T cells showed a significant suppression of the Interferon-γ/Interleukin 4 ratio of $CD4^+$ T cells, starting at about 3 weeks of treatment. Importantly, the peripheral suppression of the IFN-γ/IL-4 ratio of $CD4^+$ cells was accompanied by a marked decrease of PASI (>70%). Similarly, Th1 responses were deviated into a Th2-phenotype when cells were stimulated in vitro with FAE. To determine whether this deviation of Th1 into Th2 responses might be causally related to the improvement, we investigated the effect of MMF on the capacity of autoreactive Th cells to transfer autoimmune diseases in experimental mice. Feeding MMF delayed the onset and decreased the severity of experimental autoimmune encephalomyelitis (EAE) in transgenic mice. As observed in human T cells, MMF prevented Th1-development and skewed MBP-specific Th cells toward a Th2 phenotype *in vitro* and *in vivo*. Thus, myelin basic protein (MBP)-specific T cells from mice fed with MMF aquired a Th2 phenotype. Importantly, these cells as well as MBP-specific T cells generated *in vitro* in the presence of MMF were unable to cause EAE following adoptive transfer into naive mice. Thus our data prove that MMF has a direct effect on the pathogenecity of autoreactive T cells and strongly suggest that it also inhibits the disease inducing capacity of disease inducing T cells in psoriasis.

KV17

Interleukin-3 modifies the capacity of human monocyte-derived dendritic cells to induce helper T cell responses: Shift towards a TH_2 cytokine secretion pattern

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Human monocyte-derived dendritic cells (DC1) induce TH1 differentiation, whereas dendritic cells (DC2) derived from plasmacytoid cells induce TH_2 differentiation. We wondered whether human mono-cyte-derived dendritic cells cultured in different cytokine microenvironments might display different TH-inducing patterns. The standard method to generate dendritic cells from monocytes is culture with GM-CSF+IL-4; standard approach to obtain DC2 is culture in the presence of IL-3. Human CD14+ monocytes (pDC1) express not only GM-CSF-receptors (CD116) but also IL-3-receptors (CD123), less than DC2 precursors, though. Therefore, we cultured dendritic cells with IL-3 (i.e., instead of GM-CSF) and IL-4. Maturation of both subpopulations (i.e., control GM-CSF+IL-4 DCs and experimental IL-3+IL-4 DCs) was induced with a cytokine cocktail of TNFα, IL-1β, IL-6 and PGE2. Yields of these two populations were almost identical; they showed similar morphology (*veils*) and pheno-type. Only CD1a was differentially expressed: *GM-CSF cells* expressed high levels whereas *IL-3 cells* were negative. Also, the immunostimulatory capacity in the mixed leukocyte reaction was equal. When these two mature dendritic cell populations were stimulated with CD40 ligand, *IL-3 cells* secreted significantly less IL-12 than *GM-CSF cells*. In co-culture experiments with naive allogeneic CD4+ T cells these *IL-3 cells* induced T cells to produce significantly more IL-5 and IL-4 and less IFNγ as compared to stimulation with conventional *GM-CSF cells*. These results indicate that a different cytokine environ-ment during differentiation of pDC1 can change the nature of dendritic cells from a TH_1-inducing antigen-presenting cell to a more TH_2-inducing antigen-presenting cell.

KV18

Removal of UV-induced DNA damage by interleukin-12 is mediated via nucleotide excision repair

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It was recently shown that the immunomodulatory cytokine interleukin 12 (IL-12) protects keratinocytes from UV-induced apoptosis both i*n vitro*and i*n vivo*. Inhibition of apoptosis by IL-12 was associated with a significant reduction in UV-induced cyclobutane pyrimidine dimers as demonstrated i*n vitro* by Southwestern dot blot analysis and *in vivo* by immunohistochemistry. The reduction of DNA damage by IL-12 was not due to filtering since immediately after UV exposure the amounts of DNA lesions were the same irrespective whether IL-12 was applied or not. IL-12 did also not induce cell cycle arrest which may enable more efficient removal of DNA lesions. In addition, IL-12 did only protect from UV- but not from gamma irradiation-induced apoptosis.

Since the vast majority of UV-induced DNA lesions are removed by nucleotide excision repair (NER) these data suggest that IL-12 might induce NER. To prove the link between IL-12 and NER, Xpa knock out mice were used. These animals are deficient in NER since they lack the Xpa gene, a crucial component of the NER. Intracutaneous injection of IL-12 into wild type mice before UV exposure significantly reduced the number of apoptotic keratinocytes, while it did not suppress sunburn cell formation in Xpa knock out mice. In addition, UV-induced pyrimidine dimers were significantly reduced by IL-12 in peripheral blood mononuclear cells of healthy volunteers upon UV exposure in vitro, while IL-12 had no affect on the amounts of pyrimidine dimers in cells of a Xeroderma pigmentosum patient. Taken together, these data demonstrate that IL-12 inhibits UV-induced apoptosis through reduction of DNA damage via induction of NER. This is the first demonstration that NER can be regulated by a
cytokine.

KV19

Loss of the melanoma suppressor gene locus CDKN2a confers a reduced and error-prone DNA repair and a UVB-hypersensitivity to cells from CDKN2a knockout mice

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The melanoma suppressor gene locus CDKN2a encodes two cell cycle inhibitors, $p16^{INK4a}$ and $p14^{ARF}$. UV-irradiation of cells usually leads to an upregulation of p16 and a subsequent cell cycle arrest. These effects are lost in $CDKN2a^{-/-}$ cells. Recently, we described a hypermutable repair of UV-induced DNA damage in melanoma lines with loss of p16 function, as compared to p16-intact melanoma lines. In order to exclude that this difference was due to differences in the genetic background of these cell lines, and to elucidate further the link between p16 and DNA repair, we compared the repair of DNA photoproducts in mouse embryonal fibroblasts from CDKN2a-knockout mice and their $CDKN2a^{+/+}$ littermates. We used the shuttle vector plasmid pYZ289, damaged with various doses of UVB, and transfected into the two different host cell lines. With plasmids damaged with 10 to 50 mJ/cm^2 UVB, we saw a significant, 3.7- to 10.5-fold reduced plasmid survival and a 7.1- to 8.9-fold increased mutation frequency in the CDKN2a-deficient cells (n=5, $p<0.01$). This indicates a profoundly reduced repair efficiency and a reduced repair fidelity. Since in this DNA repair assay, the plasmid is irradiated and damaged prior to transfection, the difference in DNA repair appears to be due to a direct influence of CDKN2a-gene products on DNA repair. It cannot be the result of a different effect on cell cycle regulation after UV-exposure, because that could only be expected, if the whole cells were irradiated. In order to exclude that the two cell lines reacted differently to the trauma of transfection, we performed a cell cycle FACS-analysis after transfection of undamaged and damaged plasmid. No cell cycle arrest and no differences between the two cell lines were found. It remains unclear, which of the two CDKN2a-gene products is responsible for the effect on DNA repair. FACS-analysis of cells 24 hours after irradiation with 50 mJ/cm^2 UVB showed a G2/M-phase arrest with the CDKN2a-intact cells and pronounced apoptosis with the CDKN2a-deficient cells. This UVB-hypersensitivity of the CDKN2a-deficient cells can be interpreted to be the result of an uncontrolled progression of the cell cycle with unrepaired DNA damage. Here we suggest that the impaired DNA repair might also be contributing to this UVB-hypersensitivity.

KV20

High invasive melanoma cells induce MMP-1 synthesis and activation in fibroblasts by a cytokine-mediated mechanism.

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Proteolytic degradation of the extracellular environment by matrix metalloproteinases (MMPs) has been shown to play an important role during tumor invasion and metastasis.
In previous studies we could show that increased proteolysis of matrix is localised at the border of tumour cells and the adjacent connective tissue suggesting a crucial role of tumour-stroma interaction in the regulation of synthesis and activation of MMPs.
In the present study we analysed the molecular mechanisms involved in the induction and activation of MMPs upon interaction of melanoma cells with stromal fibroblasts.
Herein we used cocultures of high, BLM, and low, IF6, invasive melanoma cells in direct or indirect (Transwell) cell contact with normal human dermal fibroblasts.
In contrast to melanoma cells of low invasive capability, which did not alter MMP synthesis in stromal fibroblasts, the high invasive melanoma cells strongly induced production of MMP-1 mRNA and protein in both co-culture systems suggesting that diffusible proteins e.g. cytokines mediate this induction. In addition, MMP-1 was also activated in co-cultures of fibroblast and BLM cells.
Therefore the role of IL-1 and IL-6 known to be strong inducers of MMP-1 synthesis was then investigated. Treatment with recombinant IL-1a resulted in an induction of MMP-1 which was similar to the co-culture systems while stimulation with recombinant IL-6 displayed a lower induction of MMP-1 in fibroblast. In addition the melanoma cells mediated induction and activation of MMP-1 by fibroblasts was dose dependently abolished in the presence of recombinant human IL-1 receptor antagonist whereas neutralisation with anti IL-6 receptor antibodies had no effect.
These data suggest that the induction and activation of MMP-1 observed in fibroblasts cultured in direct or indirect contact with the high invasive melanoma cells may be mediated to some extent by the soluble factor IL-1a. However, we cannot exclude the involvement of additional factors since treatment with IL1a was not, per se, sufficient to induce the activation of proMMP-1.

KV21

cDNA CLONING AND RECOMBINANT PROTEIN EXPRESSION OF A NOVEL HIGH MOLECULAR BIRCH POLLEN ALLERGEN

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Recombinant allergens are important for a patient-tailored component-resolved diagnosis (CRD) and therapy (CRT) of Type I allergy. Worlwide, birch pollen is a predominant allergen source. Several birch pollen allergens (Bet v 1- Bet v 7) have been characterized, so far. Allergens of on high molecular allergen complex have failed to be characterized so far. In attempt to isolate further cDNAs coding for crossreactive plant allergens of the high allergen complex, we screened a birch pollen cDNA library with serum IgE from a patient allergic to various plant pollens and plant-derived food. A partial cDNA clone coding for a novel birch pollen allergen was obtained and, by oligonucleotide screening of the original and one further library, additional cDNAs were isolated. A full length clone was expressed as recombinant protein and further characterized. The complete double-stranded sequence of the 2012 basepair-long cDNA revealed an open reading frame of 1782 base pairs coding for a novel birch pollen allergen with a deduced molecular weight of 65.3 kDa and a pI of 6.6. The amino acid sequence showed significant sequence homology with pectinesterases from various plant species (Citrus sinensis, Phaseolus vulgaris and Medicago sativa). A rabbit antiserum, raised against the C-terminal portion expressed as β-galactosidase fusion protein, cross-reacted with high molecular weight moieties (40-60 kDa) present in tree, timothy grass and mugwort pollens as well as peanut, celery and apple extracts. The frequency of recognition among birch pollen allergic patients determined by IgE-ELISA was about 20%. Our data indicate that the newly characterized high molecular birch pollen allergen represents a highly crossreactive allergen, which may be used to define a group of patients allergic to pollen, fruits, vegetables and spices.

KV22

Human *TERT*-promoter fragments as tumor specific E1A-targets for gene therapy with transformation-defective E1A-derivatives

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Multifunctional adenovirus E1A exhibits strong antioncogenic activity in human tumor cells. Since it is also able to participate in transformation of rodent and human cells, the therapeutic use of wild-type E1A harbors the principal risk of enhancing tumor malignancy. This prompted us to construct E1A 13S cDNA-derived mutants that retained a strong tumor-suppressive activity but are unable to transform baby mouse kidney cells in cooperation with E1B. Among the derivatives of E1A proteins, the Ad12 $E1A_{Spm2}$ mutant with a small deletion in Ad12 E1A unique sequences had the strongest antioncogenic properties. For gene therapeutic purposes we develop a tumor specific E1A-expression system: Since E1A is a strong activator of transcription for different genes, we examined promoters from several genes for gene expression in human tumor cell lines. We found a distinct activation of transcription by E1A-proteins from core promoters of the human Telomerase Reverse Transcriptase (*hTERT*) gene. The highest transactivation of transcription was observed with the del-208 and the del-149 constructs of the *TERT* promoter, the latter lacking the c-Myc-binding E-Box element. Of note, the E1A-construct with the highest antitumor activity, Ad12 $E1A_{Spm2}$, was a highly efficient activator of transcription from the *TERT*-promoter constructs in all tumor cell lines examined. $E1A_{Spm2}$-enhanced transcription from the del-149 construct was higher than from the pGL3-control plasmid (SV40 promoter). Transfection efficiency-corrected transcriptional activation by $E1A_{Spm2}$ from all *TERT* promoter constructs was negligible in primary human foreskin fibroblasts. Taken together, we present data recommending expression of transformation defective antioncogenic E1A from *hTERT* core promoters for transcriptionally targeted antitumor gene therapy.

KV23

Enhancement of acquired T cell memory and effector function in vivo by overexpression of IL-15 in murine keratinocytes.

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IL-15 plays an important role in the control of T- and NK cell homeostasis. Although transcriptionally expressed in multiple tissues and cell types, including keratinocytes, IL-15 is very poorly translated and secreted, which impairs investigations on the role of IL-15 in immunity. To study the effects of IL-15 on cutaneous T cell-mediated immune responses, we have generated transgenic (tg) mice which overexpress IL-15 in basal keratinocytes under control of the keratin 14 promoter. Modifications to the cDNA included removing upstream AUG's that impede translation, replacing the inefficiently translated and secreted endogenous IL-15 signal peptide with the CD33 signal peptide, and stabilizing the COOH terminus of the mature protein with a FLAG epitope tag. IL-15tg mice are healthy, breed normally and show a uniform transgene expression in their basal keratinocytes. IL-15tg mice have normal numbers of epidermal Langerhans cells, but greatly reduced numbers of Thy-1+ dendritic epidermal T cells. However, wild-type (WT) and IL-15tg mice displayed equal numbers of CD3+ epidermal T cells (DETC), suggesting that either Thy-1 expression was lost on DETC or a different subset of intraepithelial T cells may have developed in tg mice. To investigate the effects of IL-15 on contact hypersensitivity (CHS) responses, WT and IL-15tg mice were immunized and ear-challenged with DNFB. IL-15tg mice developed a significantly enhanced CHS response compared to WT controls. Since IL-15 has been shown to regulate memory phenotype T cells, DNFB immunized/challenged mice were re-challenged on the ears after six weeks. Interestingly, IL-15tg mice demonstrated a significantly increased and prolonged ear swelling response compared to WT mice. 96 h after re-challenge memory phenotype (CD44, CD122/IL-2Rβ) and effector (CD43) phenotype CD8+ T cells of these mice were evaluated by two-color flow cytometry. Notably, re-challenged IL-15tg mice demonstrated markably increased numbers of CD8+CD44+, CD8+CD122+ memory T cells as well as CD8+CD43+ effector T cells indicating augmented T cell activation by IL-15. These data demonstrate that extracellular (or secreted) IL-15 enhances (cutaneous) immune responses and is involved in prolonging and maintaining T cell memory.

KV24

Oligosaccharides of Hyaluronan activate Dendritic Cells via the Toll-like Receptor 4

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Low molecular weight fragmentation products of the polysaccharide hyaluronic acid (sHA) produced during inflammation have been shown to be potent activators of immunocompetent cells such as dendritic cells (DC) and macrophages. Here we report that sHA induces maturation of DC via the Toll-like-receptor-4 (TLR4), a receptor complex associated with innate immunity and host defense against bacterial infection. Bone marrow-derived DC from C3H/HeJ and C57BL/10ScCr mice carrying mutant TLR4-alleles were non-responsive to sHA-induced phenotypic and functional maturation. Conversely, DC from TLR2-deficient mice were still susceptible to sHA. In accordance, addition of an anti-TLR4 mAb to human monocyte-derived DC blocked sHA-induced TNFα production. Western-blot analysis revealed that sHA treatment resulted in distinct phosphorylation of p38/p42/44 MAP-kinases and nuclear translocation of NF-κB, all components of the TLR4 signalling pathway. Blockade of this pathway by specific inhibitors completely abrogated the sHA-induced DC-maturation. Finally, intravenous injection of sHA induced DC emigration from the peripheral blood and their maturation in the spleen, again depending on the expression of TLR-4. In conclusion, this is the first report that polysaccharide degradation products of the extracellular matrix produced during inflammation might serve as an endogenous ligand for the TLR4 complex on DC.

KV25

Smad7 binds the transcriptional co-activator p300 via the C-terminal domain and represses the expression of extracellular matrix genes in dermal fibroblasts

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Smad7 is an inhibitory Smad which acts as a negative regulator of transforming growth factor-β (TGF-β) signaling. Smad7 stably interacts with activated TGF-β type I receptor and interferes with the phosphorylation of receptor-regulated Smads. Recently it has been shown that Smad7 can be located in the nucleus, suggesting a direct role on gene transcription. To explore potential functions of Smad7 in the nucleus, we ectopically expressed Smad7 in dermal fibroblasts, together with various promoter/reporter gene constructs. In addition to blocking TGF-β-mediated Smad-specific gene transcription, Smad7 was also found to directly inhibit the acitvity of the minimal SV40 promoter, which contains a TATA box. Similarly, Smad7 was found to repress the activity of the human COL1A2 promoter, which also contains a TATA box. The latter does not seem to play a role in mediating Smad7 effect, as the activity of the human COL7A1 promoter, which is TATA box less, was also repressed by ectopic expression of full length Smad7. Smad7-driven repression of the minimal SV40 promoter could be antagonized by cotransfecting the transcriptional coactivator p300. Using a mammalian two-hybrid system we identified a direct interaction between Smad7 and p300. Using various deletion constructs for p300, we identified the region between amino acids 1732-2414 as binding Smad7. This region corresponds to the CH3 domain of p300, which is capable of binding members of the basal transcription machinery such as the TATA-box-binding protein (TBP), and TFIIB. However, no interaction of Smad7 with the TFIID complex was observed in a mammalian two-hybrid system. Using deletion constructs for Smad7, we identified the C-terminal part as binding p300. We propose a model in which Smad7 binds the CH3 domain of p300, thereby preventing the binding of TBP to p300 and subsequent gene transactivation. This mechanism may be responsible for the inhibitory effect of Smad7 on some extracellular matrix genes in dermal fibroblasts.

KV26

Proteasome Inhibitor PS519 Reduces Delayed-Type Hypersensitivity Response via Inhibition of Carbohydrate Determinant Synthesis of Cutaneous Lymphocyte-Associated Antigen (CLA) and T-cell Rolling.

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The first step in the process of T-cell recruitment to skin is mediated through the interaction of cutaneous lymphocyte-associated antigen (CLA) and its ligand E-selectin, a central prerequisite for delayed-type hypersensitivity (DTH) responses. In the set of glycosyltransferases involved in the synthesis of CLA α1,3-fucosyltransferase VII (FucT-VII) and β1,4-galactosyltransferase I (β4GalT-I) are believed to be mainly responsible for the regulation of its expression on T-cells. Since at least FucT-VII has a putative NF-κB binding site we addressed the question if the proteasome inhibitor PS519 suppresses CLA expression, T-cell rolling and DTH. PBMCs from healthy volunteers were stimulated with the superantigen TSST-1, a known inducer of CLA expression on T-cells, in the absence or presence of nontoxic concentrations of PS519 (1-10µg/ml). PS519 blocked the activation of NF-κB as visualized by EMSA and markedly reduced the expression of FucT-VII as determined by RT-PCR. A parallel quantitative determination of the backbone (PSGL-1) and the set of glycosyltransferases involved in the synthesis of CLA (C2GnT, β4GalT-I, FucT-IV, FucT-VII) by real-time PCR (ABIPrism7700) revealed a 10-fold decrease of FucTVII and to a smaller extend of C2GnT and β4GalT-I upon PS519 treatment (5µg/ml). Concomitantly, CLA showed a constant inhibition by PS519 up to 7 days (38,8±13,6 vs. 5,8±3,1, n=5, mean±SD) as did CD15s expression and E-selectin binding. Using intravital microscopy with fluorescently labeled human T-cells injected retrogradely into the right carotid artery of mice we observed a significantly decreased in vivo rolling of PS-519 treated T-cells in the left ear postcapillary venules (16,9±5,7 vs. 1,8±2,6%, n=3/13, $p<0.001$). The functional relevance of these findings could be further corroborated in a DTH model were a significant decrease of ear swelling could be observed in 1 mg/KGbw i.v. treated BALB/c mice (161±37 vs. 92±46µm, n=130, mean±SD). We conclude that the inhibition of NF-κB through PS519 reduces the expression of FucT-VII in T-cells leading to decreased CLA expression with less rolling in skin vessels and reduced DTH response in vivo.

KV27

Antitumor effects of CD4+ T cells specific for a colon carcinoma

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Adoptive transfer of strongly polarized Th1 cells can eradicate non-immunogenic A20 lymphomas in Balb/c mice. To test, whether Th1-therapy is limited to MHC class II-expressing tumors, we examined the protection generated by Th1 cells against the CT26 colon carcinoma cell line, transfected with the human epithelial cell adhesion molecule (huEpCAM). EpCAM-specific Th cell lines were generated by stimulating CD4+ cells derived from lymph nodes and spleens of immunized BALB/c mice with EpCAM protein and antigen presenting cells in the presence of CpG-1668 for either 3 or 8 weeks. The cytokine phenotype of outgrowing T cells was determined following in vitro stimulation with EpCAM and APC by analyzing of the interleukin-4 (IL-4) or interferon-γ (IFN-γ) content in the culture supernatant. For in vivo experiments, 10 BALB/c mice received a lethal CT26-EpCAM tumor dose ($1x10^5$ cells) together with CD4+ T cells ($5x10^5$ cells) i.v. After 3 weeks of culture, CD4+ cells released high amounts of both IL-4 and IFN-γ, showing that the cells were of the Th0 phenotype. However, after another 5 weeks cells produced still large amounts of IFN-γ while they had lost their capacity to produce IL-4. Adoptive transfer of EpCAM-specific Th0 cells did not prevent tumor growth, while the Th1 cells provided efficient protection. Based on these results, we tested the protective capacity of EpCAM-specific Th cell lines, derived from IL-4-/- mice. Again, Th1 cells, from immunized IL-4-/- mice, protected 50% of mice against a lethal dose of CT26-EpCAM cells, Th0 cells from wild type repeatedly failed to protect against CT26-EpCAM tumor cells. Thus, protection provided by CT26-EpCAM specific Th cells against the tumor growth, seems to depend on a highly polarized Th1 phenotype and is rapidly abolished by the simultaneous production of IL-4.

KV28

Activation of the IκB kinase (IKK) complex is both essential and sufficient for expression of proinflammatory genes in human primary endothelial cells

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Induction of endothelial chemokines and adhesion molecules by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) critically depends on activation of the transcription factor NF-κB. However, a detailed analysis regarding contribution of the different NF-κB upstream components to endothelial activation has not been performed yet. We employed a retroviral transfection approach to stably express transdominant (TD) mutants of IκBα, IκBβ or IκBε and dominant negative (dn) versions of IKK1 or IKK2 as well as a constitutively active version of IKK2 in human endothelial cells. TD IκBα, IκBβ and IκBε were not degraded upon TNF-α exposure and each prevented NF-κB activation. The IκB mutants almost completely inhibited induction of monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin expression by TNF-α. In addition, adhesion of peripheral blood monocytes to TNF-α-exposed endothelium was almost completely abolished after stable expression of dn IKK2 or TD IκBα. In contrast, induction of gene expression via interferon-γ-dependent pathways was not affected at all. Expression of dn IKK2 completely blocked TNF-α-induced upregulation whereas dn IKK1 only partially inhibited expression of these genes. Importantly, expression of constitutively active IKK2 was sufficient to drive full expression of all chemokines and adhesion molecules in the absence of cytokine. We conclude that the IKK/IκB/NF-κB pathway is crucial and sufficient for the proinflammatory activation of human endothelium.

KV29

Restoration of energy metabolism protects from induction of photoaging-associated mitochondrial (mt) DNA deletion.

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Deletions of mitochondrial (mt) DNA are involved in ultraviolet (UV)-induced photoaging. MtDNA only encodes for genes of the respiratory chain responsible for generation of energy intermediates (ATP) It has been hypothesized that the most frequent mutation of mtDNA, the so called common deletion, induced by reactive oxygen species (ROS) leads to a decreased pool of energy equivalents. The cell is then believed to increase respiratory chain activity leading to the generation of more ROS. Evidence for the vicious cycle: ROS - mtDNA deletions – energy reduction - respiratory chain upregulation – ROS – mtDNA deletions, has been elusive, however. In the present study it has therefore been assessed whether restoration of energy levels in cells undergoing chronic UVA exposure could prevent induction of the common deletion.

While cellular ATP levels are volatile, phosphocreatine is a more stable energy equivalent in the cell. Therefore, employing a semiquantitative nested-PCR assay we investigated whether the generation of the common deletion by UVA and subsequent functional changes (oxygen consumption, MMP-1 induction) could be inhibited by coincubation of human dermal fibroblasts with the energy precursor creatine. Lipid peroxidation assays and absorbance spectrometry revealed that creatine has no antioxidative or UV absorbing capacities. However, coincubation of cells with creatine led to a dose dependent decrease of UVA-induced levels of the common deletion. Most interestingly, creatine coincubation not only reduced mtDNA mutagenesis but also normalized mitochondrial oxygen consumption and MMP-1 induction indicating a protective effect of creatine from mtDNA mutagenesis and subsequent functional changes.

These results provide direct evidence for the existence of a vicious cycle in which mtDNA mutations lead to reduced energy levels, an increase of ROS and thereby to new mtDNA mutations. Furthermore, application of energy equivalents may represent a new and innovative way to protect the skin from UV-induced photoaging.

KV30

The cTAGE family: CTCL-associated antigens with tumor-specific splicing

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We recently identified cTAGE-1 as the first tumor-specific cancer-testis-antigen (CTA) for cutaneous T-cell lymphoma (CTCL) (PNAS (2001) 98: 629). Further studies have now demonstrated that cTAGE-1 is a 3´- prime splicing variant of a longer gene, named cTAGE-2. Moreover, by screening a testis cDNA library we could identify 4 new homologous cDNAs (cTAGE-3 to -6). Genomic alignment revealed four different chromosomes for the identified clones: Chromosomes 18p11.2 (cTAGE-1/-2), 13 (cTAGE-3), 7q33-35 (cTAGE-4), and 14 (cTAGE-5 /-6). The gene localized on chromosome 14 contains 26 exons giving rise to the differently spliced cTAGE-5 and cTAGE-6, as well as MGEA/MEA-6 and -11. Differential RT-PCR expression analysis revealed, that cTAGE-1 could be detected only in testis and 30% of the CTCL-tumor tissues tested (n=23), whereas cTAGE-2 was found in 63% percent of control tissues, but none of the tested CTCL tumor tissues. Similarly, cTAGE-5 is a CTA (37% positive CTCL tissues, n=19, controls negative), while cTAGE-6 could be found in a significant number (20%) of control tissues by RT-PCR. We could detect reactive antibodies in the sera of CTCL-patients against recombinant cTAGE-1, cTAGE-3, cTAGE-4 and cTAGE-5, but not against cTAGE-6, while only 1/10 healthy donor sera reacted against cTAGE-3. Expression analysis on protein level will have to prove the value as targets of all cTAGE-genes for immune-therapy.

KV31

No pigmentation abnormalities in MIA deficient mice

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MIA is a small soluble protein secreted from malignant melanoma cells and from chondrocytes. Recent evidence identified MIA as the prototype of a small family of extracellular proteins adopting an SH3 domain-like fold. It is thought that interaction between MIA and specific epitopes in extracellular matrix protein regulate attachment of tumor cells and chondrocytes. In order to study the consequences of MIA deficiency in vivo we generated mice with a targeted gene disruption. Complete absence of MIA mRNA and protein expression was demonstrated by reverse transcriptase, Western blot analysis and ELISA measurements of whole embryo extracts. MIA-/- mice were viable, developed normally and histological examination of the organs by means of light microscopy revealed no major abnormalities. In skin the number and distribution of melanocytes was unchanged. In contrast, electron microscopic studies of cartilage composition revealed subtle defects in collagen fiber density, diameter and arrangement as well as changes in number and morphology of chondrocytic microvilli. Taken together, our data indicate that MIA is essentially required for formation of the highly ordered ultrastructural fiber architecture in cartilage and may have a role in regulating chondrocyte matrix interactions.

KV32

Studies to Improve the Efficiency of Desensitization

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The prevalence of allergies is increasing. Today around 20% of the population produce IgE to otherwise harmless substances such as pollen or insect venom. Desensitization leads to efficient relief but its wide spread use is prohibited by the fact that desensitization regimens stretch over 3-5 years involving 30-80 subcutaneous allergen injections with significant side effects. The therapeutic mechanism is thought to be the induction of allergen specific blocking IgG. We compared the efficiency of different routes of desensitization in mouse models and found that injection of only 1% of the allergen dose directly into a lymph node induces 100-times higher IgG titres when compared to subcutaneous injection. Allergen injection into the lymph node induced stronger Th1 responses than subcutaneous injection. A clinical pilot study was performed on 12 patients with grade III-IV allergy to bee venom. These patients would normally be desensitized with 60-80 subcutaneous injections of 100mg bee venom over 5 years. Instead, patients received only 3 intralymphatic injections of 10mg bee venom within 4 weeks. Injections into the subcutaneous lymph nodes of the inguinal area were painless and no side effects were observed. The increases in bee venom specific IgG1 and IgG4 titres were comparable to conventional subcutaneous desensitization. Patients were also protected against bee sting challenges. Thus, intralymphatic desensitization allows to reduce the allergen dose and the number of injections, making desensitization as easy as any other vaccination.

KV33

Streptococcal infection and the HLA-system in psoriasis

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The association between group A streptococcal infections and the development or exacerbation of psoriasis is well established. According to age of onset, HLA-haplotype, and family history, two major types of psoriasis are being distinguished. Type 1/2 with disease onset before/after the age of 40y, positive/negative family history and association with HLA-Cw6, B13 and B57/no HLA-association. Further classification linking age of onset with HLA-allele-pattern allows differentiation in 4 subtypes (type Ia/b = early onset without/with typical HLA alleles, type IIa/b = late onset without/with typical HLA alleles). This study compared the incidence for positive streptococcal and staphylococcal infection parameters in 96 HLA-typed patients classified according to the above mentioned criteria. Evidence of microbial infection was assessed by nasopahryngeal swabbing and standard methods of bacteria isolation, determination of anti-streptolysine-O (ASLO)- and anti-desoxyribonuclease B (ADNase-B)-serum antibody titres (normal range < 200 IU/ml each) resp. anti-staphylolysin-titre (ASTA, normal range < 2 IU/ml). Evidence of group A streptococcal infection was found exclusively in type I psoriasis patients. The difference compared to type II patients was significant ($p = 0.004$). Irrespective of the age of disease onset, psoriasis patients with the typical HLA antigens showed a similar prevalence of streptococcal infection (type Ib 45%, type IIb 46%), whereas signs of streptococcal infection appeared to be slightly less frequent in type Ia patients (29%), and they were completely missing in type IIa patients ($p = 0.002$ type Ib vs. IIa; $p = 0.006$ type IIb vs. IIa; $p = 0.04$ type Ia vs. IIa). Only 2 out of 19 healthy HLA-Cw6, B13 or B57 positive controls revealed positive streptococcal infection parameters. In contrast, assessed parameters for Staphylococcus aureus (Table 1) did not differ significantly between type I and II or type Ia/b and type IIa/b patients.Our findings clearly demonstrate that susceptibility to streptococcal infection distinguishes two clinically and genetically defined types of psoriasis, implying distinct inherited immune response patterns to streptococcal antigens as a key to understanding psoriasis pathogenesis.

KV34

THE PROCESSING OF A NEW MAGE-3 EPITOPE PRESENTED BY HLA-B*4001 REQUIRES THE IMMUNOPROTEASOME

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By stimulating human CD8+ T lymphocytes with autologous dendritic cells infected with an adenovirus encoding MAGE-3, we obtained a CTL clone that recognized the new MAGE-3 epitope AELVHFLL, which is presented by HLA-B*4001 molecules. This CTL clone recognized MAGE-3- positive tumor cells only when they were treated for several days with IFN-γ. Since this treatment is known to induce the exchange of three catalytic subunits of the proteasome to form the immunoproteasome, our results suggested that the processing of this MAGE-3 epitope required the immunoproteasome. Transfection experiments demonstrated that the substitution of β5i (LMP7) for β5 is necessary and sufficient for producing the epitope, whereas a mutated form of β5i (LMP7) lacking the catalytical active site was ineffective. In vitro digestions of a long precursor peptide showed that the immunoproteasome was more efficient to produce the proper C-terminus and N-terminus of the antigenic peptide, whereas the standard proteasome was more efficient to introduce cleavages that destroy the antigenic peptide. This is the first example of a tumor-specific antigen produced exclusively by the immunoproteasome.

KV35

Resistance in experimental leishmaniasis is associated with early expression of the Th1-inducing cytokine osteopontin

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In experimental leishmaniasis, susceptibility in BALB/c mice is associated with a Th2 cell response, and resistance in C57Bl/6 mice with a Th1 cell response. The elaboration of the Th1 or Th2 cells depends on the early cytokine milieu. While there are early differences in IL-4 expression, so far none of the Th1-inducing cytokines has been shown to differ significantly between both strains. To identify potential factors and cells contributing to Th cell polarization we used Differential Display-PCR and Northern Blot analysis to study i) differences in the gene expression of bone marrow derived macrophages (BMDM) from both strains infected with L.major, and ii) gene expression in the early cutaneous infiltrate. One of the genes we investigated more closely was osteopontin (Opn), recently described as a Th1-associated cytokine derived from macrophages (MΦ). MΦ from both mouse strains expressed high levels of Opn mRNA, but there were no differences between un- and IFN-y stimulated BMDM derived from C57/Bl6 and BALB/c mice. In contrast to IL12, Opn transcript levels were not reduced after phagocytosis of L. major. We subsequently analysed Opn expression by Northern blot in the infiltrate of infected mice. Surprisingly we now detected a striking difference as early as 1 day after infection with approximately 3-4 fold higher Opn transcript levels in C57/Bl6 than in BALB/c mice. Since MΦ expressed similar amounts of Opn in vitro we compared numbers of infiltrating MΦ in vivo. Immunohistochemistry revealed only a slightly higher percentage of MΦ in C57Bl/6 within 1d after infection. We then performed in situ hybridization in order to detect the cells expressing Opn in vivo. We detected Opn expression by only few infiltrating cells (likely MΦ). However, there was a marked expression of Opn transcripts by epidermal cells in infected feet. The expression of Opn by keratinocytes would be of eminent relevance in sight of the high number of epidermal cells at the site of infection. As Opn is known to stimulate the secretion of IL12 it could directly overcome the IL12 suppression by L.major. Our results thus introduce i) a Th1-inducing cytokine which presents with significant differences very early in leishmaniasis and ii) epidermal cells as possible participants in influencing the Th1 response.

KV36

Containment of skin infections with Pseudomonas aeruginosa is impaired in the absence of mast cells

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We and others have previously shown that mast cells (MC) are essential for initiating efficient innate immune responses in murine sepsis. Since effective host-defense is especially called for in the skin, an organ frequently targeted by bacterial pathogens, we asked whether MC are involved in controlling bacterial skin infections. Genetically MC-deficient $Kit^{W}/Kit^{W\text{-}v}$-mice and normal +/+ litter mates (*Kit*+/+) were subjected to infections with *Pseudomonas aeruginosa* (PA) by s.c. injection of ~$8x10^6$ colony forming units (0.1 ml, shaved lower back). Developing skin lesions characterized by infiltration and subsequent necrosis were markedly larger (up to 3 fold as assessed by planimetric analysis) in $Kit^{W}/Kit^{W\text{-}v}$-mice than in *Kit*+/+ mice at all time points studied (every 2-6h, for 72h). *Kit*+/+ skin lesion size reached a maximum at 14h after infection (0.7 ± 0.2 cm^2 vs. 1.4 ± 0.7 cm^2 in $Kit^{W}/Kit^{W\text{-}v}$), while lesions in $Kit^{W}/Kit^{W\text{-}v}$-mice continued to increase for 36h following infection (1.6 ± 0.6 cm^2 vs. 0.6 ± 0.1 cm^2 in *Kit*+/+ at 36h). Interestingly, sites of PA injection showed markedly more extensively degranulated MC (36±8%) and significantly less normal non degranulated MC (53±12%) than vehicle-injected skin (extensively degranulated MC: 4±2%, normal MC: 90±2%, $p<0.005$). Most notably, virtually all $Kit^{W}/Kit^{W\text{-}v}$-mice, but no *Kit*+/+ mouse, exhibited piloerection, a sign of systemic infection, as soon as 12h and up to 50h after PA-injection, indicating that containment of PA is impaired in MC-deficient skin. This finding prompted us to monitor clinical symptoms of sepsis with the help of a clinical disease score, grading the degree of spontaneous activity, social behavior and flight reaction. Clinical disease after injection of PA in $Kit^{W}/Kit^{W\text{-}v}$-mice started earlier and was more severe than in *Kit*+/+ mice ($Kit^{W}/Kit^{W\text{-}v}$: 1.8 ± 0.2 vs. *Kit*+/+: 0.7 ± 0.3 at 2h, $p<0.05$). Clinical disease score values in *Kit*+/+ mice, but not in $Kit^{W}/Kit^{W\text{-}v}$ mice, started to drop 12h after infection (0.5 ± 0.2 vs. 1.7 ± 0.3 in $Kit^{W}/Kit^{W\text{-}v}$ at 14h, $p=0.02$), indicating that recovery from PA-induced morbidity is impaired in MC-deficient mice. Our data suggest that activated MC control skin lesion size and promote containment of bacteria at sites of skin infections. These findings extend the view of MC as salient sentinels in the context of innate immune responses against bacteria.

KV37

CD8alpha+ and CD4+ splenic dendritic cells show differential capacities to prime CD8 T cells in vitro and in vivo

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The dendritic cell (DC) system encompasses subpopulations of DC that differ in phenotype and immunstimulatory properties. Mouse spleen contains CD8alpha+ DC which are potent inducers of in vivo TH1 responses, whereas Th2 responses are predominantly induced by spleen CD8alpha- DC (Maldonado et al 1999. J.Exp.Med.).
We have recently found that spleen CD8alpha- DC can further be subdivided in CD4+ DC and CD4-/CD8alpha- DC. In vitro, CD8alpha+ DC primed only weak allogeneic responses in naive or activated CD8 T cells, while CD4-/CD8alpha- DC and CD4+ DC were strong inducers of allogeneic CD8 T cell proliferation. Given the inability of CD8alpha+ DC to stimulate CD8 T cells in vitro, we were interested in determining the ability of the various DC subsets to stimulate minor histocompatibility CTL responses using H-Y male-specific antigens as a model. Male DC were sorted by FACS into CD4-/CD8alpha-, CD4+ DC and CD8alpha+ subsets and 1-2 x 10^5 injected intravenously into female C57BL/6 mice. After in vitro restimulation with H-Y+ male splenocytes, the levels of CTL priming were determined by killing of male Con A blasts using the JAM assay. Surprisingly, CD4-/CD8alpha- and CD8alpha+ DC were highly efficient stimulators of CTL, while CD4+ DC generated several-fold weaker CTL responses and failed to prime mice in several experiments.
In summary, CD8alpha expression labels DC unable to stimulate CD8 T cells in vitro. In contrast, CD4 expression is a marker for DC with low in vivo CTL priming capacity. The superior CTL priming capacity of CD4- DC in vivo might be due to production of IL-12 and/or IFN-gamma, whereas CD4+ DC do not have this ability. Moreover, the inability of CD8alpha DC to stimulate CD8 T cells is limited to certain in vitro assays that lack enhancing signals present during in vivo CD8alpha+ DC: CD8 T cell interactions.

KV38

Identification of syndecan-1, the major heparan sulfate proteoglycan (HSPG) on keratinocytes, as a primary receptor for human papillomavirus type 16.

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The human papillomaviruses (HPV) are associated mostly with benign epithelial proliferations, but high-risk genital types, in particular HPV16 are the main cause for cervical and other anogenital malignancies. We have reported previously that alpha-6 integrin, which has been proposed as a cellular papillomavirus receptor, is not required for high-risk HPV16 virus-like particles (VLP) binding to their natural host cells, human keratinocytes. In contrast, pretreatment of HPV16 VLP with high molecular weight heparin inhibits cell binding in a dose-dependent manner confirming recent reports that cell surface heparan sulfate (HS) proteoglycans (HSPG) act as attachment structure for HPV capsids. To characterize the heparan sulfate carrying core protein, keratinocytes were treated with heparinase I or phosphatidyl inositol-specific phospholipase C (PI-PLC). Removal of cell surface HS by heparinase I inhibited VLP binding in a dose dependent manner, whereas treatment with PI-PLC had no effect. These results suggested that transmembrane syndecans, rather than glycosyl phosphatidyl inositol (GPI)-anchor linked glypicans, act as the VLP-binding structure. In addition, a direct physical interaction of syndecan-1 and HPV16 VLP was demonstrated by co-immunoprecipitation of VLP and cell membrane extracts using HPV16- and syndecan-1 specific monoclonal antibodies. These findings for the first time identify syndecan-1, the major HSPG on keratinocytes, as a primary receptor for genital HPV.

KV39

Depletion of CD25+ CD4+ T cells enhances the IFNα -induced CD8+ T cell-dependent immune defense of B16 melanoma cells in C57BL/6 mice

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Recombinant IFNα is currently used for the adjuvant treatment of patients with melanoma. Understanding its mechanism of action is a prerequisite for the development of more effective therapeutic strategies. In the B16 melanoma model of C57BL/6 mice we found that retroviral transduction of melanoma cells with cDNA encoding murine IFNα2 (B16-IFNα) resulting in constitutive production of IFNα in the tumor microenvironement significantly delayed tumor establishment in vivo. B16-IFNα grew only a little slower than parental B16 melanoma cells in T cell-deficient nude mice, indicating that T cells participated in the IFNα-induced tumor immune defense. Injection of a cytotoxic anti-CD8 mAb enhanced growth of B16-IFNα in immunocompetent mice, suggesting an important role for CD8+ T-cells. Surprisingly, injection of a cytotoxic anti-CD4 mAb further delayed growth of B16-IFNα, implying that CD4+ T-cells downregulated the IFNα-induced tumor immune defense. Furthermore, injection of a cytotoxic anti-CD25 mAb prevented growth of B16-IFNα in most mice. These tumorfree mice rejected a subsequent challenge with parental B16 melanoma cells, indicating the induction of long-lasting protective tumor immunity. Taken together, these results suggest that elimination of regulatory CD25+ CD4+ T cells is able to enhance the IFNα-induced CD8+ T cell-dependent immune defense of B16 melanoma cells. Our findings support the development of novel strategies for the immunotherapy of melanoma using IFNα in combination with elimination of regulatory T-cells.

KV40

Melanoma induction in human skin by combined effects of growth factors and UVB

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Deleterious effects of ultraviolet (UV) radiation from sunlight have been associated with melanoma induction, however, the molecular mechanisms are unknown and additional carcinogenic factors have been supposed. An imbalance of growth factor production can cause uncontrolled proliferation of melanocytes, which may represent the first critical step of melanoma development. We have recently established a human skin graft/SCID mouse model, in which dermal overexpression of basic fibroblast growth factor (bFGF) combined with exposure to UVB led to melanocytic changes including lentiginous melanoma. Subsequent studies including microarray analyses now suggest that additional melanocytic mitogens, i.e., endothelin-3 (ET-3) and stem cell factor (SCF) may be involved in melanomagenesis. The dermal expression of bFGF, ET-3, and SCF via adenoviral vectors in human skin grafts combined with UVB irradiations led to human nevus- and melanoma-like lesions in only 3-4 weeks. The lesions consisted of melanocytic nests throughout the epidermis and dermoepidermal interface and stained positively for S100 and HMB45. Pagetoid growth indicated malignant transformation of the melanocytes, which could only be induced by a combination of the three growth factors and UVB, but not by each factor alone. These data provide new evidence that melanocytes that are activated by a homeostatic imbalance of their immediate environment are highly susceptible to the carcinogenic effects of UV radiation in contrast to quiescent normal melanocytes in human skin. Immunohistochemical studies as well as gene expression and mutational DNA analyses on the microdissected experimental lesions are now expected to shed more light on the molecular events of UV-induced melanoma development in humans.

KV41

Hepatocyte growth factor (HGF/SF) confers phosphorylation of Sp1 transcription factor: a likely mechanism of paracrine upregulation of vascular endothelial growth factor (VEGF/VPF) gene expression by cultured keratinocytes

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Keratinocyte-derived expression of VEGF/VPF as a key angiogenic molecule is subject to regulation by autocrine expression of transforming growth factor-alpha (TGF-α), or by mesenchymal cell-derived HGF/SF in a paracrine fashion, contributing to angiogenic responses during wound healing, skin inflammation and cutaneous neoplasia. As a paramount control point of VEGF/VPF gene expression, transcriptional activation induced by TGF-α requires, in addition to constitutive binding of Sp1-like proteins, inducible AP-2-dependent DNA binding to a core GC-rich promoter region between bp -88 and -65. On the contrary, paracrine HGF/SF-mediated VEGF/VPF transcription by epidermal keratinocytes occurs regardless of any additional inducible binding activity, although the same response region is mandatory (bp -88/-65). HGF/SF does not favour preferable nuclear translocation of Sp1 or Sp3 protein. In addition, no evidence for a HGF/SF-mediated shift in relative binding of different Sp1-like factors (e.g., Sp1, Sp3) to the response region or diverse effects of overexpression of the respective nuclear factors on promoter activity as a potential mechanism of induced VEGF/VPF gene transcription were found (as derived from competitive/mutational EMSA analyses and from VEGF/VPF reporter gene assays along with co-expression of Sp1 and/or Sp3 factors). However, HGF/SF-induced VEGF/VPF gene transcription was demonstrated to depend on distinct signaling pathways, including p42/p44 MAP and PI-3 kinase as well as PKC-ζ (as shown by usage of explicit inhibitors or by specific antisense oligonucleotides in VEGF/VPF reporter gene assays). Thus, we next hypothesized that biochemical modification of Sp1-like factors may present a molecular mechanism by which HGF/SF induced VEGF/VPF gene transcription. Lysates of HGF/SF-treated HaCaT cells were immunoprecipitated by anti-Sp1 antibody, and were consecutively probed with a panel of antibodies directed against phosphorylated serines and threonines. These studies for the first time show that HGF/SF is capable of conferring phosphorylation of Sp1 transcription factor, an event occurring within 10 min. Hence, our findings reveal a likely mechanism of paracrine HGF/SF-induced VEGF/VPF upregulation, thereby offering a potential basis for therapeutic strategies to modulate skin VEGF/VPF expression in angiogenic responses.

P001

Intradermal application of bone-marrow derived Dendritic Cells "instructs" CD8+ T cells for homing to inflamed skin

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Application of antigens through the skin results in the activation of T cells which home to inflamed skin. In the mouse, these skin-homing T cells have been shown to express ligands for E- and P-selectin as well as the chemokine receptor CCR4. At present it is not known how these T cells are "instructed" to become skin-homing T cells. A role for the site of priming and/or the specific antigen presenting cell is discussed.

In our experiments, we adoptively transferred TCR transgenic CD8+ P14 T cells specific for the LCMV p33 peptide into C57BL/6 mice. These T cells were then primed in vivo by intradermal or intravenous injection of p33-pulsed bone marrow-derived Dendritic Cells (DC). Skin inflammation was induced at the same time by elicitation of CHS with the hapten trinitrochlorobenzene (TNCB). Homing of P14 T cells to inflamed skin was then analysed by preparation of ear sheets 24 h after ear challenge with TNCB and phenotypic analysis of the emigrated T cells 24 h later. Blood levels of P14 T cells were similar in mice after intradermal or intravenous injection at the time of the analysis.

We found that 2-10 times more transgenic P14 T cells emigrated from inflamed skin when the p33-pulsed DC were injected intradermally as compared to intravenous injection. The expression of adhesion molecules and chemokine receptors on these T cells is currently under investigation.

Our results may be relevant to the design of DC based immunotherapeutic strategies with respect to the targeting of T cells to specific tissues.

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P002

Cytogenetic Findings in Primary Cutaneous B-Cell Lymphomas

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Cytogenetic aberrations play an important rule in the characterisation of biological, histopathological and clinical subgroups of B-cell lymphomas. The identification of chromosomal aberrations reulted in the detection of putative oncogenes like bcl-2 and tumor suppressor genes like p56. Moreover non-random aberrations showed to be of prognostic significance in nodal B-cell lymphomas.
Surprisingly until now there are no cytogenetic data on primary cutaneous B-cell lymphomas (CBCLs) available. By comperative genomic hybridisation (CGH) we studied 21 CBCLs and for comparison 5 primary nodal follicular lymphomas. Chromosomal aberrations were detected in 16 of 21 CBCLs. Gain of chromosome 19 was present in 5 of 8 large B-cell lymphomas of the leg but in none of the 11 follicular center cell lymphomas investigated. The aberration pattern of the cutaneous follicular center cell lymphomas was similar to aberrations found in the nodal counterpart. Further investigations will show, whether these findings are of relevance for the pathogenesis of CBCLs contributing to the well known different clinical behaviour of the different subgroups.

P003

Attributable risk estimate of atopic skin diathesis in explaining occupational skin disease

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Objectives: Various studies have proven a higher proportion of atopic workers with occupational skin disease (OSD), but no systematic epidemiology study has ever attempted to estimate the contribution of atopic skin diathesis (ASD) to the total number of OSD cases. We used the concept of attributable risk (AR) for quantification of the impact of ASD on OSD occurrence in a working population.

Methods: We analysed data of our population-based register study of OSDs in Northern Bavaria (BKH-N), Germany [1]. The investigation is based on 3,730 workers with a stated OSD registered between 1990 and 1999. Workers diagnosed as having ASD had a personal history of flexural involvement, visible flexural eczematous or lichenified dermatitis, and/or an atopy score according to Diepgen ≥ 10. To calculate the AR we assumed an ASD prevalence of 20% in the total population.

Results: Of the 3,730 workers analysed, 37% (n=1,366) presented an ASD showing that workers with ASD who developed OSD were over-represented compared to the assumed 20% for the total population. Those with an ASD developed their OSD at a younger age implying earlier working life and were more susceptible to ICD than ACD. We found that 21.6% (95% CI 19.4-23.7) of OSD cases within 24 risk occupational groups may be ascribable to ASD. Our results illustrate a potential impact of ASD on OSD in the context of preventive strategies, primarily in food preparation workers (pastry cooks, bakers, cooks), florists, and health care workers.

Conclusions: We conclude that ASD makes a worker more likely to develop OSD, particularly in occupations involving intense exposure to wet work and skin irritating chemicals. The AR of ASD is a suitable measure for providing an estimate of the expected proportion of OSD which may be preventable by achieving successful preventive measures. We suggest heightened efforts in terms of proper medical advice at pre-employment examination and regular follow-up and counselling of workers with ASD to support them in avoiding OSD.

1. Dickel H, Kuss O, Blesius CR, Schmidt A, Diepgen TL (2001) Occupational skin diseases in Northern Bavaria between 1990 and 1999: a population-based study. Br J Dermatol 145:453-462

P004

Lipid mediators from pollen lead to activation and migration of neutrophils

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Under natural exposure condition pollen act as allergen carriers and belong to the most common causative agents to induce allergy. Recently we demonstrated that pollen rapidly release eicosanoid-like substances preceding the allergen liberation. The objective of the current investigation was to characterize substances released from pollen grains upon contact with the aqueous phase. Furthermore we aimed to identify the rapid responses induced in polymorphonclear granulocytes (PMN) in order to specify the biological relevance of the identified substances. HPLC-analysis of aqueous and lipid extracts from pollen was performed. PMNs were exposed to the aqueous phase or lipid subfractions from pollen and pure substances. Effects were tested by transwell migration, surface expression of CD11b and calcium mobilization. HPLC-analysis of pollen extracts revealed that predominantly monohydroxylated products derived from linoleic-acid and linolenic-acid were found. Notably, PMN show significant migration to aqueous extracts from pollen. Further investigations revealed that this migratory capacity could be attributed to lipid-derived mediators isolated from pollen. Notably, aqueous as well as lipid extracts lead to mobilization of calcium, surface expression of β2-integrins on PMN. Functional experiments with either pure monohydroxylated products of the linoleic acid, and with subfractions of pollen extracts separated by HPLC showed in comparison to the aqueous pollen extracts reduced but significant activity in the functional experiments performed. In summary, Pollen release lipid-mediators activating cells of the innate immune systems. We speculate that pollen release these substances within the humid milieu of mucosal surfaces and may such act as potent adjuvant on the elicitation phase of allergic reactivity, thus leading to an aggravation of the allergic disease. Supported partially by a grant of BayStMLU (76b-8733 3-1998/2-12)

P005

Cytolysis of CD4+ T cells as the mechanism for CD8+ effector T cell dominance in contact hypersensitivity

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Chemical haptens like trinitrophenyl (TNP) simultaneously generate immunogenic T cell determinants for CD4+ and CD8+ T cells by haptenization of MHC bound peptides, or proteins which are then processed to haptenated peptides. It is therefore still unclear why CD8+ T cells rather than CD4+ T cells are the crucial effector cells in many allergic reactions to haptens. Using the murine model of contact hypersensitivity (CHS) to the hapten TNP we could show a clear predominance of CD8+ effector T cells when mice were sensitized by i.d. injection of TNP-modified DC or skin painted with the hapten. The predominance of cytotoxic, IFN-? producing Tc1 type CD8+ T effector cells could also be demonstrated in an in vitro T cell priming system using DC as APC. We have now shown that CD4+ T cells acquire TNP-peptide/MHC class I complexes from the DC and are killed by CD8+ effector T cells in a Perforin- and Fas/FasL-dependent manner. Since CD8+ T cells also acquire antigen from antigen presenting cells, we currently analyse whether fratricide of CD8+ T cells also occurs. This could be one factor explaining the fast downregulation of the inflammatory response. Our findings show new roles for cytotoxic effector T cells in skin inflammation.

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P006

UVB-irradiated dendritic cells (DC) induce non-proliferating, regulatory type T cells (TC)

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It is well established that low-dose UVB-radiation (UVBR) inhibits the APC function of murine Langerhans cells in vivo and converts them from immunogenic to tolerogenic APC. Recently, we have shown that UVB-irradiated murine bone marrow-derived DC (UVB-DC) suppressed proliferation of naive and primed TC, but tolerized primed TC only.

To examine the underlying mechanism for these differences, naive $OVA_{323-339}$-peptide-specific, TCR-transgenic TC from DO11.10 mice were analyzed following coculture with unirradiated DC or UVB-DC. First, we found UVB-DC to inhibit OVA-specific TC-proliferation UVB dose- and antigen dose-dependently. Analysis of TC cocultured with both, unirradiated and UVB-DC, revealed an activated TC-phenotype with increased expression of CD25 and CD69 by FACS. Supernatants harvested from cocultures with UVB-DC showed reduced levels of IFN-γ, IL-2 and IL-4 , but not TGF-β, compared to unirradiated DC as determined by ELISA. Furthermore, these TC did not proliferate upon restimulation. Interestingly, addition of these non-proliferating TC to cocultures of naive TC and freshly prepared unirradiated DC inhibited TC-proliferation depending on the number of added non-proliferating TC. Also, in supernatants increased levels of TGF-β were found. Therefore our data indicate that UVB-DC propagate TC with a regulatory function. Since regulatory TC are characterized by enhanced TGF-β secretion and increased CTLA-4 expression, we currently investigate the role of CTLA-4 phenotypically and functionally.

In conclusion, we have shown UVB-DC to inhibit proliferation of naive OVA-specific TC. These TC, exhibiting an activated phenotype and increased TGF-β production, suppress proliferation of naive TC cocultured with unirradiated DC. These results suggest that UVB-DC induce non-proliferating, regulatory type TC.

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P007

Development of ELISA-based assays for detecting autoallergic IgE class antibodies against thyroid antigens

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Autoimmune responses in diseases such as Hashimotos thyroiditis are characterized by auto-antibodies (Abs) of various Ig-classes, including IgE. We have previously shown that such IgE-auto-Abs, namely IgE-anti-thyroid peroxidase (TPO), 1) can degranulate mast cells after stimulation with TPO and 2) are detectable in a subgroup of patients with chronic urticaria (CU). Here, we report two ELISA-based techniques for detecting IgE auto-Abs against TPO. We first employed an IgE capture-immunoprecipitation assay using radio-labelled TPO to identify 13 IgE-anti-TPO positive sera (from 52 CU patients tested). To test whether IgE-anti-TPO in these sera can be detected by direct ELISA, we 1) used plate bound human TPO, 2) incubated with IgE-anti-TPO+ serum, and 3) added horseradish peroxidase (HRP)-labelled goat-IgG-anti-IgE. While monoclonal recombinant IgE-anti-TPO (positive control) gave a strong positive signal in this assay, none of the sera did, presumably because of epitope masking by IgG-anti-TPO. Therefore, multiple steps of purification were performed prior to direct ELISA-detection: Sera of CU patients were subjected to ammonium sulfate precipitation for protein concentration, followed by ultrafiltration (cut off membrane: MW<100 kD) to eliminate small molecules. In addition, interfering IgG-Abs were removed via Protein-G affinity column (FPLC). These pre-purification steps allowed for detection of IgE-anti-TPO by direct ELISA, though with weak signals (maximum OD: 0.2), possibly due to a loss of IgE during purification. However, this technique is costly and labor-intensive. Thus, we established a "capture-sandwich" ELISA: CU sera were incubated with purified goat-anti-IgE (Fc region covalently plate bound) before adding human TPO, mouse-IgG-anti-TPO and HRP-labelled donkey-anti-mouse-IgG. This assay gave strong signals for IgE-anti-TPO+ sera (maximum OD: unpurified sera = 0.6, pre-purified IgE-fractions = 1.3). Out of 126 patients with CU, 19%, 7%, and 5.5% exhibited IgE-anti-TPO levels that were >2-fold, >3-fold, and >4-fold increased, respectively, as compared to baseline. This capture-sandwich ELISA may be well suited to screen for autoallergic antibodies in patients with autoimmune diseases.

P008

A Shift Towards a CD28-Positive Tc1-Subtype Participates in Perforin-Depletion of $CD8^{hi+}$ Cytotoxic T Cells in Patients with Exacerbated Atopic Dermatitis

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Perforin (Perf)-based cytotoxicity regulates T cell homeostasis, immunoglobulin production, $T_{h1/h2}$-balance and the clearance of viral infections. We recently demonstrated a profound defect of the Perf-system in atopic patients, namely a Perf-hyperreleasability in $CD8^{hi+}$ cytotoxic T lymphocytes (CTLs) and, in addition, a marked numerical reduction of $Perf^{+}$ $CD8^{hi+}$ CTLs [*J Allergy Clin Immunol 2001: 107:878-86*]. Now, these cells were further characterized by analyzing their Perf-load and subtype in 8 patients with exacerbated atopic dermatits (AD) and 8 sex and age matched healthy controls (Ctr). Monoclonal antibodies (mAB) used were anti-Perf(FITC), -CD8(PE, cy5), -CCR5(PE), -CD26(cy5), -CD28(cy5) and an isotype control for the Perf-mAB. Ficoll isolated peripheral mononuclear cells were fixed with formaldehyde, permeabilized with saponin, triple-stained and analyzed in a FACScan.(1) The $Perf^{+}$ portion of $CD8^{hi+}$ AD-CTLs was reduced (AD 25±18%, Ctr 41±14%, $p<0.05$). This was significant in all $CD8^{hi+}$ subtypes investigated: $Perf^{+}$ portion of cells double-positive for $CD8^{hi+}$ and a) $CD28^{+}$ AD 9±7%, Ctr 35±9%; b) $CD26^{+}$ AD 10±7%, Ctr 38±22%; c) $CCR5^{+}$ AD 37±20%, Ctr 70±16%, $p< 0.01$. (2) In addition, $Perf^{+}$ $CD8^{hi+}$ AD-CTLs contained significantly less Perf-granules as compared to Ctr (mean fluorescence intensity for Perf: AD: 59 ± 24; Ctr: 105 ± 43, $p<0.05$). (3) There was a significant decrease of the CD28-negative (AD 32±11%, Ctr 56±18, $p<0.05$) and an increase of the $CD28^{+}$ $CD8^{hi+}$ subtype in AD-patients (AD 69±21%, Ctr 43±17, $p<0.05$). (4) AD-CTLs showed a significant increase of the $CD26^{+}$ subpopulation (AD 59±10%, Ctr 37±12%, $p<0,005$). This increase was also seen in $CD8^{hi+}$ $CCR5^{+}$ cells but did not reach significance. $CD28^{+}$ cells are known to express lower amounts of Perf per cell ($CD28^{+}$ $Perf^{lo+}$) as compared to the $CD28^{-}$ subtype ($CD28^{-}$ $Perf^{hi+}$). Thus, the decrease in perforin-load of $CD8^{hi+}$ AD-CTLs may be attributed in part to the shift from $CD28^{-}$ $Perf^{hi+}$ towards a $CD28^{+}$ $Perf^{lo+}$ CTLs. However, this does not explain the numerical reduction of $Perf^{+}$ CTLs in every CTL-subtype, even in $CD28^{+}$ $Perf^{lo+}$ cells. The pathophysiological role of $CD28^{+}Perf^{lo+}$ T_{c1}cells ($CD8^{hi+}$,$CD26^{+}$, $CCR5^{+}$) and of $CD28^{+}$ $Perf^{lo+}$ T_{c2}/T_{c0} cells in AD remains to be elucidated.

P009

The mediator secretion pattern of dendritic cells from atopic individuals can be reversed to that of non-atopics.

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The pattern of soluble mediators released by human blood-derived dendritic cells (DC) obtained from atopic donors has been shown to differ from that observed in non-atopics. Thus far, in 18/19 age- and sex-matched comparitive experiments, atopic DC showed a significant higher secretion of the chemokine MCP-1 and a tendency to secrete more IL-10 than non-atopics. Further experiments characterizing atopic and non-atopic DC showed no additional differences for the expression/secretion of IL-12, TNFα and its type I receptor, IL-1β and IL-1 receptor type I, IL-6 and its receptors, GM-CSF and its receptor, CD86, CD83, prostaglandin E2, and thromboxane. Although by far not resembling a complete list of biologically relevant molecules fully defining properties of DC, these molecules are obviously not relevant for differences in atopic versus non-atopic DC. On the other hand, these observations are suggestive for the relative importance of the production of, in particular, MCP-1 in atopic DC. In attempts to analyze enhanced MCP-1 production and to "correct" this atopic feature of DC, atopic and non-atopic DC were kept in culture in the presence of different biologic response modifiers putatively related to the atopic status. In both DC phenotypes, a 24 h pretreatment prior to allergen- or endotoxin-challenge with IL-10 lead to enhanced MCP-1 production, whereas IFNα decreased MCP-1 production moderately, IL-12 had no effect. Addition of neutralizing anti-IL-10 antibodies, however, was able to decrease MCP-1 secretion selectively in atopic DC, resulting in production rates similar to those in non-atopics. These data indicate that 1) DC from atopics differ from those of non-atopics with regard to, at least, MCP-1 production, 2) IL-10 might be important for the atopic status of DC, and that 3) atopic DC can be reversed to a non-atopic phenotype, probably suitable for therapeutic strategies in atopic diseases.

P010

Monocyte derived dendritic cells of normal and nickel allergic individuals show different phenotype changes upon nickel exposure

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Upon stimulation with nickel antigen-specific T-cell proliferation in vitro can be observed in PBMC of nickel allergic individuals. Here we examined whether exposure to nickel in vitro would induce different phenotype changes in monocyte-derived dendritic cells depending on the allergic/non allergic status of the blood donor. Monocytes were isolated from PBMC of 10 nickel allergic patients and 10 non allergic individuals by magnetic cell sorting. To induce dendritic cells, the monocytes were then cultured for 6 days in the presence of GM-CSF and IL4. Subsequently cells were incubated for 2 days with medium alone or stimulated with LPS or $NiSO_4$ (10^{-5}M). To assess DC-typical surface markers and accessory molecules immunostaining was performed with antibodies to MHCI, MHCII, CD80, CD83, CD86 and CD40 followed by flow cytometry. When comparing $NiSO_4$-stimulated DCs from allergic and non allergic patients as compared to medium control the most evident differences were a marked upregulation of CD 83 (mean, allergic vs non allergic: +30,1%/-1,6%) and of CD 40 (+17%/-17%). In contrast, stimulation with LPS led to similar increase of the tested surface markers in both groups. In parallel PBMC of the nickel allergics and the controls were stimulated by PHA, TT and $NiSO_4$ (10^{-4}M, 10^{-5}M, 10^{-6}M). By analysis of 3H-Thymidin incorporation specific reactivity to $NiSO_4$ was restricted to PBMC of the nickel allergic patients. Thus, altered DC reactivity to nickel could contribute to enhanced immunoreactivity in nickel allergic individuals.

P011

UV light induces apoptosis in human immature, but not in skin mast cells

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Diverse pruritic, mast cell associated cutaneous diseases are known to respond to UV treatment. We have therefore investigated the effect of UV-light on the induction of apoptosis in human mast cells. Cells of the immature human leukemic mast cell line HMC-1 were exposed to single doses of UVB (0-100 mJ/cm^2), of UVA1 (0-25 J/cm^2), or UVA1 after preincubation with 8-methoxypsoralen (PUVA1, 0-5 J/cm^2). Mature human dermal mast cells obtained from cosmetic breast surgery were challenged with single doses of 100 mJ/cm^2 UVB, 25 J/cm^2 UVA1 and 5 J/cm^2 PUVA1. In addition repetitive low dose irradiation with of UVB (5 mJ/cm^2 ,3x/wkx2wks) and single high dose UVB irradiation up to 500 mJ/cm^2 was performed. After 3, 6, 12, 24 and 48 hrs the percentage of apoptotic and dead cells was measured and morphological features of apoptosis were assessed by electron microscopy. Biochemical aspects like activity of effector caspase 3, changes in mitochondrial membrane potential (DY) or release of cytochrome c were studied. In addition the regulation of p53 and PARP cleavage during the first 24 hrs were examined in nuclear extracts on Western blot analysis. HMC-1 cells showed a time- and dose-dependent induction of apoptosis. A maximum of 50% apoptotic cells was observed 12 hrs after UVB irradiation compared to 50% to PUVA and 35% to UVA1. The activation of Caspase-3 activation followed the dose- and time-course of apoptosis. Increased nuclear expression of p53 and PARP cleavage could be demonstrated as well, but changes in mitochondrial membrane potential could not be obserevd. However, mature skin mast cells could not be induced to go into apoptosis in any of the conditions studied. Resistance to apoptosis in normal mast cells may explain the long-lived nature of these cells, in line with their recently demonstrated important function in immune defense. Possibly, in vivo effects of UV-light treatment affect primarily immature mast cell precursors.

P012

The vitamin D system in cutaneous malignancies

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Using conventional RT-PCR with total RNA from normal skin (NS) and basal cell carcinomas (BCCs), we identified splice variants of 25-hydroxyvitamin D-1a-hydroxylase (1a-OHase) in both tissues. Using real time PCR (LightCycler), we have analyzed BCCs, squamous cell carcinomas (SCCs) and NS for mRNA expression of vitamin D receptor (VDR) and of the main enzymes involved in the synthesis and metabolism of calcitriol (vitamin D-25-hydroxylase [25-OHase], 1a-OHase, 1,25-dihydroxyvitamin D-24-hydroxylase [24-OHase]). Interestingly, RNA for VDR (BCCs: median 16,54; SCCs: median 37,4; NS: median 0) and 25-OHase (BCCs: median 0,17; SCCs: median 32,52; NS: median 0) was upregulated in BCCs and SCCs as compared to NS. Additionally, we have analyzed immunohistochemically expression of VDR in malignant melanoma (MM), acquired melanocytic nevi (MN), SCCs and BCCs. Intensity of VDR immunoreactivity was increased in MM as compared to MN as well as in SCCs and BCCs as compared to normal skin (NS). However, staining did not correlate with histological type or grading of skin tumors. Comparing VDR-staining with staining for apoptotic cells (TUNEL), Ki-67, cytokeratin 10 and transglutaminase K, no correlation was found. Our findings indicate that (i) MM, SCCs and BCCs may be considered as potential targets for prevention or therapy with new vitamin D analogs that exert little or no calcemic side effects (ii) VDR expression is not exclusively regulated by the proliferative activity or by the differentiation of these tumor cells, but by additional, unknown mechanisms (iii) synthesis and metabolism of vitamin D metabolites may regulate growth of BCCs and SCCs (iv) pharmacological modulation of vitamin D synthesis or metabolism may be a target for treatment of SCCs. The function of alternative transcripts of 1a-OHase that we here describe for the first time in BCCs and NS and its effect on activity level has to be investigated in future experiments.

P013

Regulation of IL-12 production by activated skin infiltrating T-lymphocytes

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The production of IL-12 by monocytes/macrophages is tightly regulated. Depending on the activation state of the APC a number of stimuli can either enhance or inhibit the production of IL-12. We have previously shown that preincubation with a *second signal* such as LPS or ligands of the TNF receptor superfamily before the priming signal IFNγ leads to an almost complete suppression of IL-12 on the protein and mRNA level. A likewise suppression is also observable using autologous activated T-cells (but not resting T-cells) before the stimulation procedure with IFNγ is started. We could show that the IL-12 inhibiton by activated T-cells (either mitogen-, antigen- (TT) or allergen (grass or birch pollen) stimulated) is dose-dependent and not due to endogenous production of known IL-12 antagonists (i.e. IL-10, IL-4, PGE2). Interestingly activated T-cells derived from human eczematous skin were also very effective IL-12 suppressors if given before a priming signal. As $CD40L^+$ T-cells are present in early eczematous skin lesions shown by immunohistochemical staining this phenomenon may contribute the Th2 like cytokine milieu found in early eczematous skin lesions. As to the underlying mechanism of the IL-12 downregulation, experiments performed in the presence of MEK1/2 inhibitors and western blot analysis of p42/44 MAP-kinase provide evidence that activation of ERK plays an important role. A better understanding of the mechanisms regulating IL-12 production in eczematous skin lesions are likely to be important as this Th1 skewing cytokine plays an important role in the chronification of atopic as well as contact dermatitis lesions.

P014

Wasp venom-induced IL-5, IL-10 and IFN-gamma secretion from human PBMC from patients with wasp venom allergy is diversly inhibited byloratadine, desloratadine, fexofenadine and cimetidine

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It has been reported, that certain histamine 1 receptor (H1R) antagonists can inhibit cytokine release of specific and unspecific stimulated PBMC. In this study we investigated whether loratadine, desloratadine, fexofenadine and cimetidine can modify the cytokine release of PBMC´s from 30 wasp-allergic patients following in vitro stimulation with wasp venom. The cells were cultured in the presence of commercially available wasp venom at the concentration of 1 µg/ml with and without different H1R antagonists at concentrations of 1 µg/ml and 10 µg/ml. ELISPOT-culture plates were used coated with monoclonal antibodies against IFN-gamma, IL-5 and IL-10. After 5 days the assay was stopped and the spots, each representing a specific cytokine releasing cell were counted using an automatic BioReader counting system. Coincubation with desloratadine significantly ($p<0,05$) reduced thenumber of IFN-γ, IL-5 and IL-10 secreting cells. The prodrug loratadine still significantly decreased ($p<0,05$) IL-5 secretion whereas fexofenadine and cimetidine did not modulate cytokine secretion of the PBMC of the patients.

P015

Prevalence of mould and candida allergies in patients with chronic urticaria

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We and others have previously reported that levels of total IgE are increased in patients with chronic urticaria (CU), a frequent and disabling dermatosis characterised by *allergic* skin symptoms such as weals and itch. However, relevant sensitisations to type I allergens are rarely detected in CU patients by routine prick testing for standard allergens. Here, we have asked whether CU patients show increased rates of positive skin reactivity and increased serum levels of specific IgE to mould and Candida allergens. 100 CU patients, of which 52% exhibited increased total serum IgE (> 120 U/ml), were subjected to intracutaneous skin testing for seven ubiquitous moulds, including Alternaria alternata, Aspergillus fumigatus, Mucor mucedo, Fusarium roseum, Cladosporium herbarum, Penicillium notatum, and Aureobasidium pullulans as well as Candida albicans (CA) and specific IgE was determined with Pharmacia CAP System. Skin reactivity to one or more moulds was observed in 49% of CU patient as compared to 34% in healthy controls (n = 30). Increased specific serum IgE to mould allergens was only seen in 7% of CU patients (3% in healthy controls), suggesting that mould allergies are not increased in CU patients. In addition, CU patients did not show significantly increased rates of skin reactivity to CA (CU: 51 % vs. control: 39%). However, increased specific IgE-anti-CA could only be detected in CU patients (13%) but not in healthy controls. These data indicate that 1) positive skin test reactions to moulds and CA do not require the presence of increased serum levels of specific IgE. 2) CU is not associated with significantly increased rates of skin reactivity to moulds and CA. 3) Increased CA-specific IgE can be found in one out of 10 CU patients. Our findings suggest that skin symptoms in some CU patients may represent type I immediate hypersensitivity reactions to Candida albicans.

P016

Signaling pathways in ceramide-mediated activation of the transcription factor AP-1

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Sphingolipids, known as structural elements of the cell membrane take also part in cell signaling. The term sphingomyelin cycle was coined to describe this novel pathway of signal transduction. Ceramide, a product of agonist-stimulated sphingomyelin hydrolysis is a intracellular mediator of the sphingomyelin cycle and was shown to induce apoptosis, cell differentiation and to reduce cell proliferation. To date several agonist of sphingomyelin cycle have been described including tumor necrosis factor α (TNFα), IFN-γ, IL-1 and 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$). Previously, we could show that TNFα induces sphingomyelin hydrolysis in primary human keratinocytes and HaCaT keratinocytes resulting in an increase of intracellular ceramide. To exert its biological effects, ceramide transduce the intracellular signals by activating protein kinase cascades leading to transcription factor activation. The nuclear transcription factor activator protein 1 (AP-1) is known as a target in ceramide induced signaling pathway. In keratinocytes AP-1 is involved in the regulation of differentiation and epidermal gene expression. Therefore we studied the role of ceramide in AP-1 activation of HaCaT keratinocytes.Using luciferase reportergene assays we could show that TNF α (555 ng/ml) and C_2-ceramide (5 and 30 µM) activate AP-1 in a time dependent manner. For ceramide action the extracellular signal-regulated kinase (ERK) and the c-Jun (JNK) kinase pathway are described. In the present study we report that TNFα (100 ng/ml) and C_2-ceramide (5 and 30 µM) induce c-Raf threonine phosphorylation. Using antibodies against activated ERK1/ERK2 we could show increased phosphorylation following treatment of cells with both stimuli. Additional incubation with MEK-1 specific inhibitor PD 98059 (10 µM) caused a decreased phosphorylation of ERK1/ERK2. In contrast, we could still determine increased AP-1 reportergene activity using C_2-ceramide and MEK-1 specific inhibitor PD 98059 (5 µM and 10 µM). Using JNK/c-jun/AP-1 inhibitor curcumin (20 µM) alone or together with PD 98059 no AP-1 reportergene activity could be determined for ceramide treated cells. Our results suggest that for ceramide-mediated activation of AP-1 in HaCaT-keratinocytes the JNK kinase cascade is preferred.

P017

NEUROTROPHINS ACT AS REGULATORS OF ADULT HUMAN HAIR GROWTH

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Neurotrophins such as brain derived neurotrophic factor (BDNF) are known to mediate neuronal cell survival as well as to guide and sustain neuron development and differentiation. BDNF is produced by peripheral targets such as the hair follicle epithelium. However, the high affinity receptor for BDNF tyrosine kinase B (TrkB), is also found on cutaneous epithelial and mesenchymal cells. In murine hair follicle cycling, BDNF and TrkB distribution varies hair cycle dependently, and BDNF signaling appears to play an important role in catagen development. However, studies on the role of BDNF/TrkB human hair growth are missing to date. Here, we present immunohistochemical evidence for BDNF and TrkB expression in human anagen scalp hair follicles. By standard immunofluorescence, BDNF expression was strongest in the proximal inner root sheath and in the dermal papilla. TrkB-immunoreactivity, in contrast, was strongest in the basal layer of the hair follicle ostium, positive in the hair matrix and proximal outer root sheath, and negative in the dermal papilla. This distribution suggests mesenchymal-epithelial cross-talk, exchanged between the BDNF-excreting dermal papilla and the TrkB+ hair matrix. In cultured human scalp skin anagen hair follicles BDNF (5-50 ng/ml) decreased hair shaft elongation over a culture period of 10 days. Histomorphometric analysis showed that hair follicles treated with 50 ng/ml BDNF had entered a catagen-like stage, while the majority of control hair follicles remained in anagen VI. These results suggest the existence of complex pilo-neural signaling networks also within the human hair follicle, where TrkB-mediated-signalling may act as one important switch between growth and regression.

P018

The collagen cross-link hisitdino-hydroxymerodesmosine is an artifact

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An appropriate cross-linking of collagen molecules is an essential prerequisite for proper biomechanical function of connective tissues. The cross-link histidino-hydroxymerodesmosine (HHMD) has been described as a tetrafunctional cross-link derived from lysine-aldehyde pathway and was suggested as a major cross-link in skin. An increase in the concentration of HHMD has been shown for sclerotic skin in localized scleroderma and keloids, while a decrease has been reported for lipodermatosclerosis. However, there is no convincing evidence that HHMD is formed under in situ conditions. In this study, we analysed the cross-link pattern of skin and other HHMD-containing tissues by different analytical procedures.

Human tissues (normal skin, keloids, palmar aponeurosis) and collagens synthesized by cell culture (normal fibroblast) underwent various treatments for cross-link analysis. Untreated tissues were reduced by borohydride and then digested by collagenase or firstly digested by collagenase and then reduced by borohydride. After hydrolisation cross-links were analysed with an amino acid analyser.

Cross-link analysis yielded in all tissue samples similar levels of reducible and non-reducible cross-links except HHMD, which was solely found in the samples which were reduced prior to collagenase digestion. HHMD could also be induced in collagen from cell culture.

Our study demonstrate, that HHMD is not present in tissues in situ. HHMD is an artifact which is generated during reduction of tissues by borohydride. The analysis of HHMD may be indicative for the content of the intramolecular aldol condensation product (ACP), which plays no role in the mechanical properties of the fibre.

P019

Non homogeneous distribution of topically applied compounds

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The investigation of the distribution of active components, e. g. drugs, cosmetics and UV filters used in sunscreen products, within the horny layer is an important prerequisite for the determination of their efficiency and safety. The aim of these investigations was to study the light protection properties of UV sunscreen filter substances relative to the homogeneity of distribution on and in the stratum corneum. The minimal erythema dose was investigated on one hand by classical application of 2 mg/cm^2 sunscreen product directly to the skin of volunteers. On the other hand the same amount was introduced into an optical cell, which was positioned to the skin of the same volunteer. The minimal erythema dose through the optical cell was measured. First, the minimal erythema dose was determined on the untreated skin on the back of the volunteer. Secondly, 2 mg/cm^2 of a commercial sunscreen product were applied to the same volunteer. An optical cell containing the vehicle (2 mg/cm^2) without UV filter substances - placebo - was positioned upon the treated skin area. The minimal erythema dose was determined for the optical cell / skin setting. Thirdly, the sunscreen was distributed homogeneously in the optical cell (2 mg/cm^2) whereas the placebo emulsion was applied to the skin. The minimal erythema dose was determined again for this setting. Significant differences were found in the two different approaches: The minimal erythema dose was higher when ever sunscreen products were placed in the optical cell. These differences were even higher when the sunscreen substances were presented in a dissolved form in the optical cell to the skin. Additional measurements performed by laser scanning microscopy demonstrated that the sunscreen product was distributed homogeneously in the optical cell while furrows and wrinkles lead to a non-homogeneous distribution of the sunscreen on the skin.

P020

APOPTOSIS IN SPERMATOZOA: KEY ENZYMES OF DIFFERNENT PATHWAYS ARE READY TO BE USED

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IntroductionAssisted reproduction (AR) realizes fertilization of oocytes using a single sperm, selected up to now just by supramolecular properties. It has been assumed that ejaculated spermatozoa partly undergo apoptosis which cannot be detected by conventional methods. Caspases are key enzymes of apoptosis when activated (aCP). It was investigated if subpopulations of spermatozoa with aCP can be removed using the externalization of phosphatidylserine (PS) of apoptotic cells.MethodsNative sperm samples from healthy donors (n=60) were investigated in 2 sperm fractions separated by magnetic cell sorting (MACS®) in dependence on bound annexin-microbeads (ANMB) to PS. The separation effect was monitored by detection of ultrastructural changes by electron microscopy (ELMI, n=3). The initiation and execution of apoptosis by means of aCP 8, 9, 1 & 3 were supervised by FACS (CaspaTag™) and fluorescence microscopy (n=40). Inactive procaspases (pCP) and aCP were detected by Western blots (WB, n=20).ResultsSpermatozoa contain to a similar amount aCP 8, 9, 1 & 3 (% Spermatozoa, X±SEM, p>0.05): aCP8 16.0±1.2; aCP9 14.9±2.1; aCP1 13.8±2.0; aCP3: 18.1±2.4. They are mainly localized at the region behind the acrosome and within the midpiece. High power magnification of ELMI showed that microbeads were bound exclusively on cells within the AN-MB positive fraction (ANMB+) and not within the ANMB negative fraction (ANMB-). The passage through the magnetic field reduced percentage of sperms containing aCP within the ANMB-: aCP8 10.3±0.7; aCP9 7.4±1.0; aCP1 9.3±1.9; CP3: 13.2±2.1 (p<0.01). ANMB+ sperm were significantly enriched concerning aCP compared to the unseparated fraction and to ANMB-: aCP8 65.3±2.2; aCP9 68.3±1.8; aCP1 48.1±1.8; CP3: 50.8±3.1 (p<0.01). In comparison to aCP8 & 9 the aCP3 & 1 were less found in ANMB+ spermatozoa (p<0.01). WB analyses of both fractions confirmed the presence and quantity of reduction of aCP after MACS separation.ConclusionSpermatozoa contain CP 8, 9, 1 & 3 which become activated in subpopulations. Cells with aCP can highly efficient depleted by annexin MACS. The separation effect was confirmed by ELMI and WB. In conclusion we advise to extract spermatozoa with externalized PS out of samples planned to use for AR to prevent reduced pregnancy rates.

P021

Diagnostic Tissue Elements in Melanocytic Skin Tumors in Automated Image Analysis

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In tissue counter analysis, digital images are divided into subregions (“elements”), and the digital information in each element is used for statistical analysis.
In this study we assessed the morphologic details of tissue elements, which have turned out to be of diagnostic significance in the discrimination of benign common nevi and malignant melanoma.
After creation of a data set based on a total of 12.000 cellular elements obtained from 100 benign common nevi and 100 malignant melanomas, CART (Classification and Regression Tree) analysis was performed in order to differentiate between cellular elements of nevi and melanoma. In a second step, the slides were re-evaluated by the decision tree and cellular elements suggestive either for benign common nevi or for malignant melanoma were highlighted on zoomed images of the whole sections and the individual elements were displayed in galleries.
8 groups of elements (so-called terminal nodes) seemed to indicate benign common nevi, whereas 7 terminal nodes were suggestive for malignant melanoma. The elements of nodes suggestive for benign nevi largely contained nevus cells with amphiphilic cytoplasm, intermingled with fibrillary material, while the elements of the nodes suggestive for malignant lesions often showed hyperchromatism, perinuclear halos, heavy pigmentation, or a lymphohistiocytic infiltrate.
In conclusion, tissue counter analysis automatically detects tissue elements for diagnostic discrimination, which are in accordance with morphologic criteria used in conventional histopathology.

P022

Expression of E-, N-, and P-cadherin, and a-, b-, and g-catenin in melanocytic nevi, UV-irradiated melanocytic nevi, and malignant melanoma

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Changes in cadherin and catenin expression may impair keratinocyte control over melanocyte proliferation and function. In melanoma, preliminary in vitro data suggest that downregulation of E-cadherin and ββ-catenin together with upregulation of N-cadherin might represent important pathogenic steps. Few reports describe the expression of cadherins and catenins in a full range of benign and malignant excised melanocytic tumours. In this study, we report the immunohistochemical expression pattern of E-, N-, and P-cadherin, and αα-, ββ-, and γγ-catenin in melanocytic nevi, UV-irradiated melanocytic nevi, and malignant melanoma. In nevi, E-cadherin is expressed in the majority of junctional melanocytic cell nests. Upper dermal melanocytic cells generally stain weaker, and deeper dermal cells are completely negative. N-cadherin tends to be negative or only very weakly positive in junctional melanocytic cells. In dermal melanocytic cells, we found weak to moderate expression in a subgroup of nevi, whereas others were negative. P-cadherin is strongly expressed in junctional melanocytic cells and expression decreases in deeper dermally located cells. ββ-catenin in most of the nevi is found strongly positive in junctional and dermal nests, and only in some nevi expression tended to decrease in the reticular dermis. αα- and γγ-catenin expression in our study with few exceptions showed a pattern similar to that of b-catenin. In melanocytic nevi that had been irradiated with UVB light one week prior to excision, epidermal E- and P-cadherin expression was weaker in the irradiated half. However, melanocytic cells showed no clear difference in cadherin and catenin expression between irradiated and non-irradiated half. In malignant melanoma samples, E- and P-cadherin as well as catenins showed a moderate to strong expression in junctional and early invasive dermal tumor cells. In the majority of the samples, this expression decreased in deeper invasive tumor cells. Immunoreactivity of N-cadherin was weaker than that observed for E- and P-cadherin, but was markedly stronger compared to benign melanocytic nevi, especially in junctional and upper dermal tumor cells. The results are discussed regarding their relevance for melanocytic tumor progression and the impact of UV-light on epidermal cells.

P023

GRAYING, HAIR CYCLING AND MELANOCYTE FUNCTION – AN ENIGMANTIC BALANCE WAITING TO BE DISSECTED

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Melanin-production in the hair bulb is the strictly anagen-coupled, a capacity progressively lost with age. Possible mechanisms leading to cessation of follicular melanin-production range from keratinocyte failure to accept melanosomes to exhaustion of a follicular melanocyte stem cell pool. In this pilot investigation we studied the morphology and growth characteristics of pigmented versus white human anagen hair follicles by immunohistochemistry to tyrosinase- (active melanocytes), trp-1- (differentiated melanocytes), NKI-beteb- (melanosomes), Ki67- (proliferation) and TUNEL- (apoptosis) and in culture. In graying hair bulbs, melanocytes gradually decreased in number, appeared to round up and loose contact with the basement membrane, but retained tyrosinase, trp-1 and NKI-beteb expression. Completely white hair follicles were devoid of tyrosinase-, trp-1-, or NKI-beteb-immunoreactivity in the hair follicle pigmentary unit, but retained some NKI-beteb expressing cells in the outer root sheath. Melanocytes in pigmented to white anagen VI hair bulbs were never Ki67+ or TUNEL+. Interestingly, neither morphology nor growth rates differed between pigmented versus non-pigmented hair follicles cultured over 8 days. When cultured follicles were treated with H2O2, the response ranged from necrosis of the entire organ (10-2 M) to no response at all (10-5 to -9 M). Between 10-2,5 to 10-4 M H2O2 hair bulb cells displayed varying degrees of vacuolization of mainly melanocytes (by routine H&E) as well as TUNEL/NKI-beteb+ melanocytes, which exited the hair bulb via the medulla of the hair shaft in large pigmented clumps two days after treatment. Melanocytes thus may disappear from human hair bulbs by apoptosis, perhaps due to excessive oxidative stress.

P024

Mast cell numbers in human skin increase with distance of skin layers and anatomical sites from the body center

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Mast cells (MC), important effector cells in inflammatory reactions to pathogens and allergens, are known to accumulate in organs that border our environment, including the skin. However, little is known about how MC are distributed within such organs and what factors control the distribution of MC. Therefore, we have performed extensive histomorphometric entire body surface mapping of MC populations in normal human skin. Seven defined skin layers (subepidermal to subcutaneous) of 15 distinct anatomical sites from 150 individual donors were analysed with respect to MC numbers and distribution by quantitative histomorphometry: 1) thorax, 2) abdomen, 3) lower and 4) upper back, 5) lower and 6) upper arm, 7) lower and 8) upper leg, 9) hand, 10) foot, 11) chin, 12) nose, 13) cheek, 14) forehead, and 15) back of head. In all skin sites, regardless of age and sex, MC numbers were found to 1) be lowest in the most proximal skin layer, 2) increase with distance from the subcutis, and 3) peak in most distal skin layers (subepidermal = 9.3 ± 0.4 MC/MF, subcutaneous = 0.9 ± 0.1 MC/MF). No differences in MC numbers and distribution were found comparing skin obtained from male or female donors (n: f = 87, m = 63). In contrast to reports from the older literature, MC numbers were not found to differ in young and old individuals (ages 10 - 86). Most notably, skin MC numbers were highest in the most distal anatomical sites we analysed (head, foot, and hand; maximum: nose = 9.4 ± 0.9 MC/MF) and lowest in skin sites close to the body center (minimum: abdomen = 2.9 ± 0.3 MC/MF). Arm and leg skin sites exhibited intermediate MC numbers (upper arm: 3.7 ± 0.2 MC/MF, lower arm: 4.9 ± 0.3 MC/MF). These data show that MC numbers in human skin increase with distance of skin layer and anatomical site from the body center. Possible explanations of these unexpected findings include a skin layer- and site-specific gradients of factors that induce MC-proliferation and/or -recruitment, either 1) due to increased exposure to external factors (UV, trauma) or 2) because of the unique anatomy of distal skin-layers and -sites (vasculature, innervation). Our data also suggest that MCs are ?needed" at the host/environment-interface, possibly to contain infections by invading microorganisms.

P025

Pathways of chemotherapy-induced alopecia: New criteria for the diagnosis of hair follicle dystrophy.

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Hair follicles damaged by chemotherapeutic agents such as cyclophosphamide (CYP) undergo two distinct pathways of dystrophy which are characterized by specific morphological abnormalities. Light follicular damage leads to dystrophic anagen, mainly normal catagen and telogen as well as slow follicular recovery. More severe follicular damage leads to dystrophic catagen, abnormally short telogen and fast follicular recovery. In order to recognise these distinct pathways of chemotherapy-induced alopecia, it is critically important to be able to identify and accurately classify the distinct stages of hair follicle dystrophy. We, therefore, have developed a set of pragmatic classification criteria for chemotherapy-induced hair follicle dystrophy, using the C57BL/6J mouse model of CYP-induced alopecia. We suggest to divide murine hair follicle dystrophy into eight distinct stages: healthy anagen VI (HA), light dystrophic anagen VI (LDA), moderately dystrophic anagen VI (MDA), severe dystrophic anagen VI (SDA), early, mid and late dystrophic catagen (DC), dystrophic telogen (DT). The basic classification criteria include: the structure and pigmentation of the hair shaft (continuous shaft and normal pigmentation during HA and LDA; broken shaft and disrupted pigmentation during SDA, DC and DT), the location and volume of ectopic melanin granules, the location, volume and shape of the dermal papilla (large and oval during HA and LDA; swollen during SDA; round and compacted during DC and DT), abnormal widening of the hair canal (during DC, DT). In addition, the following immunohistochemical markers aid in dystrophy classification: the number of TUNEL+ keratinocytes in the bulb as a marker for the level of dystrophy-associated apoptosis (HA: no; LDA >3; MDA >5, DC >10), the immunoreactivity for neural cell-adhesion molecule (NCAM) and alkaline phosphatase activity as a marker for the level of damage to the DP, broadly increased immunoreactivity for ICE (caspase-1), Bax, Fas/Apo-1, p53, p55 TNFR , p75 NTR and a decrease of bcl-2 immunoreactivity.

These staging parameters not only offer a useful tool for the diagnosis of two distinct pathways of chemotherapy-induced alopecia, but also for the highly standardised screening of drug-treated murine skin for even discrete forms of hair follicle dystrophy.

P026

Immunohistochemical Analysis of DNA Mismatch Repair Proteins in Skin Tumors as a Screening Test for the Inherited Cancer Predisposition Syndrome HNPCC

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A subtype of Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is identical to Muir-Torre syndrome (MTS). In these cases, MTS is caused by germline mutations in the DNA mismatch repair genes *MSH2* or *MLH1*, and both internal and skin tumors show characteristic microsatellite instability (MSI). The aim of the present study was to determine whether immunohistochemical examination of MSH2 or MLH1 protein expression in MTS-related skin tumors can be used as a diagnostic screening tool to identify patients with HNPCC.
In the present study 24 skin lesions from 13 patients (19 sebaceous gland tumors, 1 sebaceous hyperplasia, 3 keratoacanthomas, and 1 squamous cell carcinoma) were tested immunohistochemically with antibodies against the DNA mismatch repair proteins MSH2 and MLH1. 18 of these tumors were from 8 MTS patients with known *MSH2*germline mutations, 2 tumors from one MTS patient with a germline mutation in *MLH1,* and 4 microsatellite stable sporadic skin tumors served as controls. One sample had to be excluded due to lack of immunoreactivity. All four microsatellite stable tumors expressed both DNA repair proteins. In 15 of the tumors from *MSH2*germline mutation carriers a loss of MSH2 protein expression was observed, 1 tumor showed reduced MSH2 protein expression, 1 tumor displayed positive immunoreactivity to the MSH2 protein. Both tumors of the *MLH1*germline mutation carrier showed loss of the MLH1 protein.
In conclusion, our findings demonstrate that immunohistochemical testing of MTS-related skin tumors is a reliable screening method with a high predictive value for the diagnosis of the inherited cancer predisposition syndrome HNPCC.

P027

A new K1 mutation results in epidermolytic hyperkeratosis with involvement of the palms and soles

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Epidermolytic hyperkeratosis (EHK) is a generalized, hyperkeratotic, occasionally blistering skin disease variably involving the palms and soles. This autosomal dominant inherited disease is caused by mutations in the K1 or K10 genes, which are expressed in the suprabasal layers of the epidermis, resulting in an abnormal keratin-intermediate filament cytoskeleton. Here we describe a 24-year-old man with generalized EHK involving the palms and soles. Histology showed massive hyperkeratosis and cytolysis of the suprabasal layers. By electron microscopy perinuclear intermediate filament clumps in the keratinocytes were detected. To identify the genetic defect DNA was extracted from blood lymphocytes of the patient and his clinically unaffected non-consanguinous parents. In the patient a heterozygous point mutation (CTC to TTC) was identified in exon 1 of the K1 gene by bi-directional sequence analysis, confirmed by NmuCl restriction enzyme analysis and was not detected in the unaffected parents, indicating a spontaneous mutation. This mutation affects residue 187 of the K1 protein (residue 7 of the 1A domain) with a predicted change from a highly conserved hydrophobic leucine to phenylalanine of an alpha-helical heptad repeat. The homologous mutation (L187F, also called L7F) in basal keratin genes K5 or K14 causes epidermolysis bullosa simplex. To further examine the molecular mechanism underlying the disease mRNA was extracted from a skin biopsy. K1-mRNA levels detected by RT-PCR were similar to that from normal skin, indicating that the mutation did not affect the amounts of steady state mRNA. In addition, immunohistochemistry for K1 (and K10) showed abnormal clumping in suprabasal keratinocytes, in contrast to homogenous staining observed with normal skin. Thus we propose that the severe EHK-phenotype in our patient results from a dominant negative effect of mutant L187F K1 on K10, the second suprabasal partner- keratin and also on normal K1 expressed from the second allele. These results are useful for genetic counseling, prenatal diagnosis and for studying molecular structure and function relationship.

P028

Interleukin-10 promoter region polymorphism IL-10.G and early onset psoriasis: Evidence for a protective, but not for a susceptible effect

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Interleukin-10 is believed to play a major role in the pathophysiology of psoriasis. The IL-10 promoter region contains a highly polymorphic microsatellite (IL-10.G) and in a recent case-control study the IL-10.G13 (144bp) allele was found to be associated with early onset psoriasis having a susceptible effect. We wanted to evaluate the importance of the IL-10.G promoter polymorphism for the genetics of psoriasis and decided to follow a genetic approach analysing allele transmission in families and comparing the results to those previously obtained for the tn62-marker on chromosome 6p21 (PSORS1). We therefore studied 199 nuclear families (trios) comprising 628 individuals and genotyped the IL-10.G marker. Allele transmission was evaluated using the family based association test (FBAT) and using the reconstruction-combined transmission/disequilibrium test (RC-TDT) to correct for exact p-values. The G13 allele (144 bp) had a frequency of 0.24 and was present in 129 families clearly showing an even transmission: 66 transmitted vs. 63 untransmitted alleles (FBAT p=0.73). In contrast, the GDB allele 11 (134 bp) with a frequency of 0.03 was present in 21 families and was found to show preferential non-transmission: 5 transmitted vs. 16 untransmitted alleles (FBAT p=0.016). In the RC-TDT an exact p-value of 0.027 was calculated. For comparison in the HLA-linked tn62-marker we obtained for allele 4 an exact p-value of 6.2x10-7 corresponding to 55 transmitted vs. 2 untransmitted alleles in the same study group. In conclusion, we failed to confirm the susceptible effect of the G13 allele, but provide first evidence for a protective effect of the GDB allele 11. Our results suggest that the IL-10.G polymorphism is not a major locus, but rather acts as a disease modifying gene or is in transmission disequilibrium with a modifying locus.

P029

Effects of balneophototherapy on psoriasis analysed by cDNA arrays

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For assessing the mechanism of response to balneophototherapy in psoriasis we analysed the changes of cytokine profiles using Panorama cDNA arrays (Sigma-Genosys, Cambridge, UK) comprising a comprehensive collection of 350 human genes encoding human cytokines, receptors and related factors.
Punch biopsy specimen of 6 mm in diameter were taken from the centre of a psoriatic lesion of seven patients before and after balneophototherapy. Treatment consisted of 35 treatment sessions of synchronous application of narrow band UVB irradiation (311nm) and bathing in Dead Sea salt solution completed within three months. The epidermal compartment of biopsy specimens was separated from the dermis using the salt-split method. Epidermal cells were lysed and total RNA isolated. Synthesis and amplification of double-stranded cDNA was performed according to the SMART protocol (Clontech,Heidelberg, Germany) under real time PCR conditions. The purified double-stranded cDNA was labelled radioactively with alpha 33P-deoxycytidine triphosphate and hybridised to Panorama arrays. Specific signals were evaluated by phosphorimaging and AIDA array analysis software (Raytest, Berlin, Germany).
As most important finding eleven genes were found upregulated in the majority of the seven patients. Specifically upregulated were the cell surface protein CD28 antigen ligand 2, the integrins alpha-6 and alpha-8, insulin-like growth factor II, insulin-like growth factor-binding protein 5, ephrin B1 and ephrin receptor EphA4, platelet-derived growth factor receptor alpha, interleukin 1-alpha and interleukin 3 receptor alpha, and the orphan receptor G-protein-coupled receptor 15.
Among those CD28 antigen ligand 2 seems to be of particular interest because it is known as an important modulator of T-cells entertaining psoriasis. Our study comprehensively displays complex changes of gene expression in the epidermal compartment, which may exhibit a pivotal role in suppression of psoriasis induced by balneophototherapy.

P030

First recessive case of epidermolytic hyperkeratosis - a functional "knockout" of human keratin 10

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Epidermolytic hyperkeratosis (EHK) is generally inherited as an autosomal dominant trait and caused by mutations in keratin 1 and 10. In the here described family two children from consanguineous clinically unaffected parents exhibited typical clinical features of severe EHK such as generalized exfoliative scaling erythroderma. Palms and soles were not affected. The diagnosis was confirmed by electron microscopy demonstrating abundant keratin tonofilament aggregates in the suprabasal epidermal layers in both affected children. On ultrastructural evaluation of a maternal skin biopsy this pathognomonic keratin clumping could only be detected in one single keratinocyte. Sequence analysis of PCR amplified DNA fragments from both affected children using specific primers for keratin genes 1 and 10 revealed a homozygous CAA to TAA base pair exchange at position 1276 in exon 6 of the keratin 10 gene. Both parents were heterozygous carriers of this mutation. The mutation results in a premature stop codon (Q426X) 27 amino acid residues before the end of the 2B rod domain of keratin 10. Immunohistochemistry applying a specific polyclonal antibody against the C-terminus of the K10 protein failed to detect K10 in a skin biopsy of one of the affected children. Semiquantitative rT-PCR showed significantly reduced K10 mRNA levels. These results suggest that the mutant K10 mRNA is mostly degraded due to nonsense mediated RNA decay and is not or only partially translated in a stable truncated K10 protein. In the heterozygous parents this defect might be compensated by the remaining unaffected K10 allele. The composition of the keratin clumps seen by EM is still under investigation. It remains of interest if the phenotype of this functional knockout of the human K10 gene will improve with age as typically observed in autosomal dominant EHK patients.

P031

Absence of mutations from typical sites of the ABCC6-gene in a child with pseudoxanthoma elasticum associated Moya-Moya-syndrome

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Pseudoxanthoma elasticum (PXE) is a heritable disorder characterised by dermal, ocular, and vascular lesions that result from accumulation of morphologically abnormal and mineralised elastic fibers in these tissues. The cause of PXE is currently assigned to a spectrum of mutations in the ATP-binding cassette subfamily C member 6 (ABCC6). The gene is composed of 31 exons and located on chromosome 16p13.1. Typically, mutations of the exons 16, 24, 27, 28, 30 in PXE patients have been described in the literature. In the propositus, a 2-year-old girl, in addition to typical skin lesions the involvement of the cranial vessels resulted in relapsing CNS-apoplexy presenting with the angiographic picture of Moya-Moya-syndrome. This is characterised by a cloudy-foggy angiographic appearance of reactive neovascularisation in brain areas affected by insults due to vascular obliteration. These severe clinical findings prompted us to sequence the known frequently mutated sites of the ABCC6-gene in the index patient and her parents. The intron-exon structure of the gene, which has not been previously published, was established by using the BLAST-algorithm (N.C.B.I., USA), comparing the cDNA (genbank acc.: NM_01171) sequence with the genomic sequence information of the publicly accessible BAC-clone (human chromosome 16 BAC clone CIT987SK-A-962B4; genbank acc.: U91318). Primers were designed for the aforementioned exons and PCR-dye-sequencing was performed in both directions twice. No mutation was found, but we observed yet undescribed polymorphisms in exon 27 and 30, in both parents and the affected child (Exon 27 base 3826 G/A Arg->Gln, 3841 G/A Arg->Lys, G/A 3887 G/A Lys, 3900 G/A Glu->Lys, 3902 G/A Glu->Lys, Exon 30 4276 G/A Arg->Gln). These findings increase the probability, that by sequencing the remaining exons we may find a mutation other than the previously described ones. Chances are that this particularly severe vascular involvement in early childhood represents a special subset in the spectrum of PXE. Further molecular analysis of this index case may also help to elucidate, which functional domains of the ABCC6-gene correlate with pronounced vascular damage in PXE.

P032

Demystification of Chester porphyria: A nonsense mutation in the porphobilinogen deaminase gene.

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The porphyrias are disorders arising from predominantly inherited catalytic deficiencies of one of the eight enzymes along the heme biosynthetic pathway. All genes encoding these enzymes have been cloned and several mutations underlying the different types of porphyrias have been reported. The diagnosis of porphyria is traditionally made on the basis of clinical symptoms, characteristic biochemical findings, and spezific enzyme essays. In some cases however, these diagnostic tools reveal overlapping findings, indicating the existence of dual porphyrias with two enzymes of heme biosynthesis being simultaneously deficient. Recently, it was reported that the so called Chester porphyria shows attributes of both variegate porphyria and acute intermittent porphyria. Linkage analysis revealed a novel locus on chromosome 11 for the underlying genetic defect in this disease. For the first time, the data suggested that a gene might be involved in the pathogenesis of porphyria, which does not encode one of the known enzymes of heme biosynthesis. After excluding two candidate genes within the originally published linkage interval, we refined the linkage analysis and subsequently identified a nonsense mutation in exon 9 of the porphobilinogen deaminase gene, which harbors the mutations causing acute intermittent porphyria, as the underlying genetic defect in Chester porphyria. However, we could not detect any mutation in the coding or the promotor region of the protoporphyrinogen oxidase gene that is mutated in variegate porphyria. The results of our molecular genetic studies indicate that Chester porphyria is neither a dual porphyria, nor a separate type of porphyria, and also exclude the possibility that a hitherto unknown gene-enzyme-system is involved in the pathogenesis of these disorders.

P033

Loss of the second nuclear localisation signal in DNA polymerase η is sufficient to cause XP-V. A. Gratchev[1], E. Bohnert[1], P. Strein[1], B. Schleider[1], S. Goerdt[1]

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Xeroderma pigmentosum variant (XP-V) is an inherited disorder which is associated with an increased incidence of sunlight-induced skin cancers. In contrast to other XP subsets, XP-V cells show no defect in nucleotid excision repair; however, they are defective in replication after UV irradiation. Recently, it was found that mutations of DNA polymerase η are responsible for the development of the XP-V phenotype. Here, we report the molecular analysis of DNA polymerase η from a 45 years old, female patient of Italian origin (Sicily), who was diagnosed for XPV in 1980, when the first malignant tumours developed. In the meantime, malignant skin tumours appear about 3-4 times a year, comprising basal cell carcinomas, squamous cell carcinomas. Treatment includes surgical excision and radiotherapy. The presence of other affected members in the patient´s family was consistent with an autosomal recessive disorder. Molecular analysis revealed a novel molecular defect, i.e. a deletion of a 176bp fragment between positions 1234 and 1409 in the XP-V cDNA of the patient. This corresponds to a deletion of a 1.2 kb of genomic sequence containing one exon of the XP-V gene. The same genomic deletion was detected in a heterozygous fasion in the nonaffected children of the patient. This deletion causes a frameshift which results in a truncated protein of 389 AA. Surprisingly, the deletion does not influence the conserved functional domains of DNA polymerase η, but does solely cause the loss of the second nuclear localization signal. This is the longest truncated DNA polymerase η protein described to date to be involved in the development of an abnormal phenotype. The detailed analysis of this mutated variant will facilitate the understanding of various aspects of DNA polymerase ? biochemistry and function.

P034

High-Level and Persistent Transgene Expression by Topical Selection for Long-Term Repopulating Human Keratinocytes Transduced with the Multidrug Resistance (MDR) Gene

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Gene therapy can be defined as the introduction of new genetic material into the cells of an individual to achieve a therapeutic benefit to the individual. However, the in vivo expression of a desired transgene is often lost over time thus hampering successful clinical applications. Possible reasons could be low transduction efficiency among stem cells or transcription silencing of the transgene. In skin, one approach to ensure long-term expression would be to topically select for keratinocytes (KC) expressing both a desired gene and a linked selectable gene, such as the multi-drug resistance (MDR) gene. To test feasibility and to examine possible mechanisms of transgene expression loss, primary human KC were transduced with the MDR retroviral vector pHaMDR1/A (transduction efficiency approximately 50% by flow cytometry), grafted onto nude mice, and subsequently treated by topically applying colchicine. At different time points, grafts were harvested and assessed for both level of MDR transgene expression (by immunohistochemistry and flow cytometry) and level of MDR provirus integration (by quantitative real-time polymerase chain reaction (PCR)). Without colchicine treatment, the percentage of MDR-expressing human KC showed a continual decline (13+/-4% and 6+/-4% at 8 and 16 weeks after grafting, respectively) while grafts treated with colchicine cream (200 µg/gm) had significantly increased percentages of MDR-expressing KC (50+/-24% and 44+/-15%, respectively). Furthermore, MDR-expression levels, as measured by mean fluorescence intensity, were markedly enhanced in colchicine treated grafts (21.4 compared to 10.6 in controls). Interestingly, quantitative real-time PCR performed on grafts harvested 16 weeks (about 5 epidermal turn-over cycles) after grafting showed almost twofold increased levels of MDR-proviral integration in colchicine selected grafts when compared with controls. These results demonstrate that topical selection of MDR-transduced KC results in increased, persistent transgene expression levels in skin, probably by enriching for KC stem cells expressing the selectable gene. This approach suggests that successful clinical skin gene therapy applications are possible.

P035

α-Melanocyte Stimulating Hormone (α-MSH) modulates immune responses in allergen-induced airway inflammation

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Besides its roles in pigmentation and behavioural processes the neuropeptide α-MSH has been shown to be a potent immunomodulator. α-MSH inhibits induction and elicitation phase of contact hypersensitivity and induces hapten-specific tolerance in vivo. It has been shown that α-MSH reduces the expression of co-stimulatory molecules by dendritic cells and induces the expression of IL-10 by monocytes. Moreover, α-MSH decreases the secretion of IgE by B-cells in the presence of monocytes. In a murine model of allergy and asthma it was studied whether α-MSH also has a role in airway inflammation. Mice were sensitised to Ovalbumin (OVA) or treated with PBS i.p. (days 1, 14 and 21), following an OVA aerosol challenge days 26 and 27. The broncho-alveolar-lavage fluid (BAL) of OVA-treated animals contained significantly reduced α-MSH amounts, indicating a role of α-MSH in airway inflammation. In further experiments mice were sensitised and challenged in the presence or absence of α-MSH. α-MSH and OVA-treated animals showed reduced OVA specific serum IgE, and reduced eosinophil counts in BALs, while BAL IL-10 concentrations were increased. In further experiments using IL-10 knock-out mice this α-MSH effect was not detectable, suggesting that α-MSH activity crucially involves the induction of IL-10. This is the first time that a function for an anti-inflammatory neuropeptide in allergic airway inflammation has been reported These findings indicate that α-MSH has an important role in airway inflammation and may be useful for the treatment of allergen induced asthma.

P036

The streptococcal exotoxin streptolysin O triggers the activation of mast cells

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In murine models of acute inflammation, mast cells have recently been discovered as prominent part of the innate immune system which is due to the existence of alternative pathways of mast cell activation independently of IgE. Streptolysin O (SLO) is produced as exotoxin by Streptococci like Streptococcus pyogenes, an important gram-positive pathogen responsible for a range of human diseases like pharyngitis, impetigo, the life threatening streptococcal toxic shock syndrome and necrotizing fasciitis. After binding to cell membranes, SLO protein monomers oligomerize to form transmembrane pores whose diameter can reach 35 nm, thereby exerting its cell and tissue-destructive activity. Herein we report that, at sublethal doses, SLO activates primary murine bone marrow-derived mast cells (BMMC) to degranulate and to rapidly induce or enhance the production of cytokine mRNAs for IL-4, IL-6, GM-CSF and TNF-α. Since it has been shown that mast cell-derived TNF-α , upon the early recruitment of neutrophils, plays a critical protective role in murine models of acute inflammation, we analysed the production of this cytokine in more detail. The maximum release of TNF-α into the medium is reached at about 4 hrs after stimulation with SLO and the production is decreased upon the inhibition of protein kinase C and the MAP-kinase p38, which is strongly induced by SLO. Therefore, we hypothesize that the activation of mast cells by SLO might combat streptococcal infections and might even prevent their clinical manifestation.

P037

IL-3Rα+ myeloid dendritic cells and mast cells develop simultaneously from different bone marrow precursors in cultures with IL-3

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GM-CSF is the major cytokine for in vitro myeloid dendritic cell (DC) development, while IL-3 promotes DC development from IL-3R-expressing plasmacytoid precursor cells. Here, we investigated the development of DC from murine bone marrow (BM) cells cultured with IL-3. Such liquid BM cultures are established to develop exclusively mast cells (MC) after 4-6 weeks. We found that after 8-10 days also about 10-30% DC can be detected. Phenotypical and functional comparison with BM-DC generated with GM-CSF revealed a high similarity in the expressioin of DC markers, MHC II and costimulatory molecules, endocytosis, maturation potential, IL-12 production and antigen presentation in vitro and in vivo. However, IL-3/DC expressed high levels of IL-3R. Using ER-MP12 (CD31) and ER-MP20 (Ly-6C) antibodies to sort BM monocytes, we observed that the development of IL-3/DC or GM-CSF/DC from these cells is identical. Taken together, DC generated from BM with IL-3 are clearly myeloid and, thus, different from plasmacytoid DC. However, MC did not develop from BM monocytes cultured with IL-3, indicating that IL-3 acts on different early precursors for DC and MC. Moreover, once established, MC cultures could not be converted to DC or stimulated to express MHC molecules. In summary, we describe and characterize DC generated from BM precursors with IL-3 and their relation to the MC lineage.

P038

Identification and Partial Purification of Factors derived from Supernatants of Gram-Positive Bacterial Strains that are Capable of Eliciting an Innate Immune Response.

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Human epithelial cells are well equipped with an array of antimicrobial substances that function to control the growth of a range of potentially pathogenic miroorganisms that occur on body surfaces. While some of these antimicrobial agents are constitutively expressed, others are induced by exogenous stimuli. It has already been established that keratinocytes are capable of recognising specific chemical factors released by gram-negative bacteria such as Pseudomonas aeruginosa and initiating an innate immune response against them. The objective of this work was to determine the identity of the chemical factors that are responsible for eliciting an innate immune response against the gram-positive bacteria Staphylococcus aureus (SA).
Preliminary results from investigations to determine the effect of utilising bacterial growth conditions that favour biofilm formation have confirmed that such conditions are most effective in inducing an innate immune response in epithelial cells. The application of crude supernatants from a selection of SA (ATCC) strains grown under such conditions to cultured primary keratinocytes was found to result in an elevation of the mRNA levels of a number of proinflammatory cytokines including IL-1β, IL-6, IL-8, IP-10, TNF-α and RANTES. Furthermore, RT-PCR analysis revealed that transcription of human beta defensin-2 (hBD-2) was also strongly induced. An initial chromatographic separation performed utilizing a Resourse Q anion exchange column revealed that at least three distinct factors are present in these crude culture supernatants that are capable of eliciting the induction of peptide antibiotics and cytokines. Further chromatographic separations of the fractions identified as containing inducing activity are now being undertaken so as to enable their identification utilizing tandem mass spectroscopy.
These findings demonstrate that human skin keratinocytes are capable of recognising and responding to specific chemical factors released by gram-positive bacteria. Efforts will now be directed at identifying these factors and elucidating the processes by which epithelial cells recognise them.

P039

Role of β2 integrins in dendritic cell - T cell interactions

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The β2 integrin LFA-1 has important functions in cell-cell adhesion, transendothelial migration and activation of T cells during antigen presentation. However, it has been unclear whether LFA-1 acts as an adhesion molecule, costimulatory molecule, or whether it enhances the signal transduced by T cell receptor (TCR) ligation. Therefore, it was the aim of this study to determine the relevance of β2 integrins for antigen presentation. Since both, APC and T cells, co-express β2 integrins as well as their receptors, ICAM-1/-2/-3, dendritic cells (DC) or T cells that lack β2 integrins were generated from CD18-deficient mice and DC-T cell interactions were investigated. Whereas DC from CD18ko and control mice were equally capable of inducing proliferation of allogeneic T cells, T cells from CD18ko mice exhibited a decreased capacity to proliferate in mixed lymphocyte responses, indicating that β2 integrin expression on T cells, but not on DC, plays an important role for T cell activation. Immature, but not mature, DC from CD18ko mice failed to cluster allogeneic T cells, suggesting that binding of β2 integrins on DC to ICAM on T cells facilitates DC-T cell adhesion, but that other ICAM-ligands are induced during DC maturation. Moreover, T cells from β2 integrin-deficient mice failed to cluster allogeneic immature or mature DC, suggesting that LFA-1 expression on T cells is required for DC-T cell clustering. Likewise, DC-T cell contact, as determined by time-lapse video microscopy, was independent of β2 integrin expression by DC, but strongly dependent on LFA-1 expression on T cells. To distinguish between effects of β2 integrins on the TCR-mediated signal (signal 1) vs. costimulation (signal 2), stimulating antibodies were added to the MLR cultures to optimize signal 1 (anti-CD3) and/or signal 2 (anti-CD28+anti-CD40). Enhancement of costimulation did not restore the proliferative capacity of CD18ko T cells, whereas crosslinking of CD3 potently enhanced the proliferative potential of CD18ko T cells, suggesting that β2 integrins play a role in augmenting the TCR-mediated signal, but do not mediate costimulation. Taken together, expression of the β2 integrins on T cells, but not on DC, is essential for antigen presentation. Here, β2 integrins augment TCR-mediated signals but do not act as costimulatory molecules.

P040

Suppression of MHC Class I can lead to T cell-dependent immunity

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MHC downregulation is considered as a major tumor escape mechanism. However, in some tumors suppression MHC I may result in natural killer (NK) cell mediated cytotoxicity and elimination of adoptively transferred tumors. To analyze the mechanisms underlying T cell-mediated tumor rejection more precisely, we generated either random MHC I-low variants of the murine A20 lymphoma or inhibited MHC I-expression by introducing the CMV m6 gene into the tumor cells. In both variants H-2D expression was reduced to about 20% of wild-type cells. MHC I-low A20 cells failed to present peptide antigens to $CD8^+$ T cells and were poorly lysed by cytotoxic T cells. However, when injected into mice, the tumor cells failed to grow in normal BALB/c mice. Importantly, MHC I-low A20 cells readily grew in nude and SCID mice. This excluded an intrinsic growth defect and showed that NK cells alone did not cope with this tumor cell line. Further analysis with deletion experiments and secondary challenges showed that 1) rejection of MHC I-low A20 cells critically required a close interaction between NK and $CD8^+$ T cells and 2) that rejection of MHC I-low A20 cell induced a T cell dependent memory response against the wild type tumor. Thus, suppression of MHC class I on A20 cells abolished tumorigenicity of this B cell lymphoma. Rejection of A20 MHC class I-low lymphoma cells was dependent on NK and on $CD8^+$ cells. MHC class I-deficient A20 cells induced longlasting, T cell-dependent immunity against wildtype tumor. Since suppression of MHC is considered as an escape mechanism used by viruses and tumors, our data will further our understanding on how innate and adaptive systems cooperate in the defense against this important evasion mechanism..

P041

Targeting keratinocyte apoptosis in the treatment of atopic dermatitis and allergic contact dermatitis

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Activation and skin-selective homing of T cells, and effector functions in the skin, represent sequential events in the pathogenesis of atopic dermatitis and allergic contact dermatitis. T cell-mediated keratinocyte (KC) apoptosis plays a key pathogenic role in the formation of eczema (J. Clin. Invest. 2000;106:25; J. Invest. Dermatol. 2001 in press). IFN-γ released from activated T cells upregulates Fas on KC, which renders them susceptible to apoptosis. T cell-mediated, Fas-induced KC apoptosis in a KC -T cell coculture system serve as an *in vitro* model of eczema. We tested in this model, whether immunomodulatory agents (dexamethasone, cyclosporin A, rapamycine, FK506, IVIG, and theophylline) are able to inhibit apoptosis of KC. Additionally, skin biopsies from untreated and successfully treated eczema were evaluated for KC apoptosis. Dexamethasone, cyclosporin A, FK506, rapamycine, and IVIG are inhibitors of KC apoptosis induced by activated T cells. This effect is mediated by two major mechanisms directed on T cells or KC. T cell activation was mainly inhibited by dexamethasone, FK506, cyclosporin A, and rapamycine. Interestingly, high dose dexamethasone and IVIG directly inhibited Fas-mediated KC apoptosis. *In vivo*, KC apoptosis was significantly reduced following successful topical treatment of eczematous lesions with glucocorticoids and FK506. These results demonstrate mechanisms of action of current treatment approaches and open a future for more focused therapeutic applications.

P042

The high-affinity IgE receptor (FceRI) induces the tryptophan degradation pathway involved in the control of T cell responses

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The high affinity receptor for IgE (FceRI) is suspected to play a pivotal role in the pathophysiology of atopic disorders such as atopic dermatitis. In search for differentially regulated genes by FceRI, a genebank of receptor stimulated and nonstimulated monocytes was established. By means of suppression subtractive hybridization, we identified kynurenine 3-monooxygenase and subsequently indoleamine 2,3-dioxygenase (IDO) to be overexpressed in FceRI-activated monocytes. IDO is the rate-limiting enzyme in the catabolism of the essential aminoacid tryptophan. We show that FceRI-activated monocytes, besides their production of proinflammatory cytokines, acquire the ability to suppress T cell proliferation in vitro via the degradation of tryptophan. The inhibition of IDO with the 1-methyl analogue of tryptophan prevented FceRI-mediated T cell suppression. We speculate that the expression of IDO by FceRI+ antigen-presenting cells in vivo allows these cells to counterregulate inflammatory T cell responses in atopic disorders by inhibiting T cell proliferation.

P043

Correlation between Interferon-gamma and inhibin B in seminal plasma of men

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Introduction: The role of the cell-mediated immunity in male infertility is still far from clear. The function of interferon-gamma, a secretory product of activated T cells and natural killer cells, is unclear. Interferon-gamma exerts a variety of biologic effects such as the inhibition of cell growth and the activation of the immune system. In normal individuals, the spleen, kidney, liver, peripheral blood leukocytes and placenta all produce a low level of interferon, which has physiologic roles. In contrast, interferons are associated with disease such as the viral infection. The expression of interferon-gamma and interferon-alpha in the rat testis has been reported. Therefore, we measured the seminal plasma level of interferon-gamma in donors and patients.
Material and Methods: The presence of interferon-gamma was investigated in seminal plasmaof 136 patients and 47 donors as control, using a specific enzyme-linked immunosorbent assay, in order to study its role in male infertility (ELISA by Bender MedSystemsTM, Wien, Austra, distribution by Biozol,Germany). Statistical analysis was performed using analysis of variance (ANOVA).
Results: Interferon-gamma was present in similar levels in the seminal plasma of patients (3.25±10.57 pg/mL) and of the control group (2.86 ±4.83 pg/mL). There was no significant difference between the group of patients and the control group (p= 0.8). Moreover, the correlation between interferon-gamma and spermcount, polymorphonuclear granulocyte elastase and inhibin B was evaluated. No significant correlations were observed between the levels of interferon-gamma in seminal plasma and the serum level of inhibin B.
Conclusions: In this study, the levels of interferon-gamma in the seminal plasma of patients and donors have been measured. Recently, the expression of IFN-alpha and gamma were reported in the rat testis. Peritubular and Sertoli cells are able to produce IFN-alpha protein only after viral stimulation. IFN-alpha1 mRNA is expressed in meiotic pachytene spermatocytes in early spermatids and Sertoli cells. In contrast, IFN-gamma protein was consistently detected in PHA stimulated early spermatid-conditioned medium, but not in non-stimulated medium. IFN-gamma mRNA was also found in early spermatids. At present, the function of IFNs in the testis and in the seminal plasma cannot be completely understood.

P044

A polymorphism in the human cytotoxic T-lymphocyte antigen 4 gene alters T-cell activation

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The cytotoxic T-lymphocyte antigen 4 (CTLA4) is an important modifier of T-cell activation with downregulatory properties upon B7 engagement. An allelic polymorphism in exon 1 of the CTLA4 gene coding for the peptide leader sequence of CTLA4 was recently described. This polymorphism was detected in association with several autoimmune diseases. In this study we investigated the functional impact of the CTLA4 exon1 +49 A/G dimorphism on T-cell activation and subcellular localization. We examined the T-cell response from healthy donors either homozygous for A or G at position +49 exon1. Under suboptimal stimulation conditions we found a greater proliferative response of cells from donors homozygous for G at position +49. FACS analysis of CTLA4 expression revealed a reduced upregulation of CTLA4 from G/G donors upon T cell activation, if compared to wild type. Intracellular CTLA4 distribution demonstrated qualitative different staining patterns between the two genotypes using confocal fluorescence microscopy. Our results suggest that the G allele at position +49 exon1 affects the CTLA4 driven downregulation of T-cell activiation and may be an important factor in the pathogenesis of autoimmune diseases.

P045

The Extracellular Domain of CD83 inhibits Dendritic Cell-mediated T-cell Stimulation and binds to a Ligand on Dendritic cells

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CD83 is an Ig superfamily member that is upregulated during the maturation of dendritic cells (DC). It has been widely used as a marker for mature DC, but its function is still unknown. To approach its potential functional role, we have expressed the extracellular Ig domain of human CD83 (hCD83ext) as a soluble protein. hCD83ext interferes with DC maturation downmodulating the expression of CD80 and CD83, while no phenotypical effects were observed on T cells. In addition, hCD83ext inhibits DC dependent allogeneic and peptide-specific T-cell proliferation in a concentration dependent manner in vitro. Furthermore, we show that immature as well as mature DC bind to CD83. Since CD83 binds a ligand also expressed on immature DC, which do not express CD83, indicates that binding is not a homophilic interaction. This is the first report regarding functional aspects of CD83 and the binding of CD83 to DC.

P046

Human skin keratinocytes express Toll-like receptors: evidence for a role in infection and psoriasis

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The ability to sense the presence of pathogenic organisms is part of the active role of human keratinocytes in mounting an innate immune response resulting in the release of antimicrobial peptides which kill invading microorganisms. To explain our findings of epithelial peptide antibiotic beta-defensins 2 and 3 (hBD-2, hBD-3)-upregulation after contact with microorganisms, we speculated about a role of keratinocyte-derived pathogen recognition receptors. Using semi-quantitative and real-time RT-PCR with gene specific primers we recently could show constitutive mRNA expression of human Toll-like receptors (hTLRs) 1-6 in cultured primary human keratinocytes (NHK). Except for hTLR1 (no constitutive mRNA expression) the same constitutive hTLR expression pattern was found in HaCaT keratinocytes. Interestingly, upon incubation with heat inactivated *Pseudomonas aeruginosa* or *Staphylococcus aureus*, as well as with supernatants of bacterial cultures, we found upregulated expression of mRNA encoding Toll-like receptors hTLR2 and hTLR4 in NHK and HaCaT cells. While hTLR2 mRNA upregulation appeared most prominent in NHK, hTLR4 mRNA upregulation was most prominent in HaCaT cells. The observed extend of mRNA upregulation varied between different *Pseudomonas* or *Staphylococcus* strains and correlated with hBD-3 upregulation. Immuno-histochemical experiments with hTLR2 specific antibodies show granular basal and suprabasal hTLR2 specific immunoreactivity in the epidermis of normal skin and increased immunoreactivity in the parakeratotic layers of psoriatic skin. Upregulation of constitutive basal expression of hTLR2 and hTLR4 (and possibly other pathogen recognition receptors) may contribute to the activation and maintenance of innate immunity in infected skin. Increased hTLR2 expression together with increased expression of antimicrobial peptides may explain the low level of infections seen in psoriatic skin.

P047

T cells link streptococcal angina and psoriasis

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Psoriasis is considered as a disorder of abnormal keratinocyte proliferation induced by antigen-specific activation of T lymphocytes. Still unresolved, however, is the question of how streptococcal angina which is a major trigger of first psoriasis onset may contribute to lesional psoriatic T cell activation.
We compared the TCR usage in skin lesions and tonsils of two psoriasis patients with recurrent psoriasis exacerbations induced by streptococcal tonsillitis. Following tonsillectomy and taking of a lesional biopsy the TCR ß-chain repertoire was amplified by PCR using 26 different BV gene specific primers together with a BC primer. PCR products were analyzed by fragment length analysis, cloning and sequencing of TCR cDNA.
By this approach, clonally expanded TCR rearrangements were detected in skin lesions of both patients. Strikingly, these particular TCR rearrangements were also identified within the tonsils. When tonsilar T cells were sorted according to the expression of the cutaneous lymphocyte-associated antigen, CLA, the clonal TCR rearrangements from psoriasis lesions could be selectively assigned to the CLA-positive T cell fraction.
Together these results demonstrate that the same T cell clones are present in psoriatic skin lesions and tonsillar tissue of psoriasis patients with recurrent angina due to Streptococcus pyogenes. They suggest that T cells activated during streptococcal tonsillitis are primed to enter the skin where they become activated and induce psoriatic inflammation. Our data thus suggest that T cells may constitute the link between streptococcal throat infection and manifestation of psoriasis.

P048

Onset of alopecia areata in C3H/HeJ mice involves transient, high expression of CD44 variant isoforms

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Standard CD44s and CD44 isoforms are adhesion molecules required for leukocyte extravasation during inflammatory processes. Alopecia areata (AA), an autoimmune disease of anagen hair follicles, can be induced in normal haired C3H/HeJ mice by grafting AA-affected skin. However, AA onset can be retarded using anti-CD44v10 monoclonal antibody. Thus the expression of CD44s and isoforms in AA may be important for understanding disease development and defining therapeutic interventions. By flow cytometry, we examined the expression levels of CD44 and variant isoforms at 2, 6, and 12 weeks post grafting as compared to sham grafted mice, normal haired mice and chronic AA affected mice. In addition, mice were examined using a panel of anti- cytokine and cell surface molecule antibodies.
Leukocytes in the skin of mice 2 weeks after transplanting AA affected skin highly expressed CD44v3, CD44v6, CD44v7 and CD44v10 as compared to sham grafted control mice. By 12 weeks, expression of CD44 isoforms had returned to normal. With chronic AA expression, CD44s and CD44v6 were slightly elevated, but expression of CD44v3 was reduced. In contrast, in the draining lymph nodes CD44v3, CD44v6, and to a lesser degree CD44v7 and CD44v10 were reduced from 2 weeks post grafting onwards. Expression of cytokines IL-2, IL-4, IL-6, IL-10 and IL-12 was elevated in mice receiving AA affected skin at all times whereas sham grafted mice exhibited only transient increases post surgery. AA development was associated with a recruitment of CD4+ and CD8+ cells.
Taken together, expression of CD44 variant isoforms appears most important for the migration of leukocytes during the initial onset of AA, but is less significant in maintenance of the disease state, that is characterized by high cytokine production levels and an increased number of CD4+ and CD8+ cells. Modulation of CD44 variant isoforms other than CD44v10 during the initial events in AA pathogenesis may inhibit disease development.

P049

Alopecia areata in C3H/HeJ mice is associated with low CD4+/CD25+ suppressor cell activity

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Alopecia areata (AA) is regarded as an autoimmune disease of anagen stage hair follicles. CD4+/CD25+ cells are known to be important for T cell homeostasis and may act as suppressors of immune system activity. Thus, it was of interest whether the presence and frequency of these regulatory T cells was abnormal in AA-affected C3H/HeJ mice.
Cells derived from skin and draining lymph nodes of age matched female mice, 4 per group, were analyzed by flow cytometry and immunohistology by 2 separate analyses. Mice grafted with AA-affected skin were examined 2 weeks post grafting along with mice with chronic AA expression as compared to equivalent sham grafted and normal haired mice.
CD4+/CD25+ cells were present in significantly reduced numbers in mice grafted with AA-affected skin both at 2 weeks after grafting and in chronic AA-affected mice as compared to controls. In association, CD40 and CTLA-4 expression was reduced. The phenomenon was not seen in draining lymph nodes, where expression of CD25 was slightly elevated in AA-affected mice. Immunohistology revealed intense graft infiltration of CD4+ cells and moderate CD8+ cell presence 2 weeks after grafting AA-affected skin while sham grafted mice demonstrated mild CD4+ cell inflammation in association with scar tissue at the periphery of the graft. Inflammation distant from the graft was not apparent. Chronic AA-affected mice exhibited CD4+, CD8+, macrophage, and dendritic cell infiltration of anagen stage hair follicles adjacent and distant to the skin graft site.
Previously it has been demonstrated that autoimmune reactions in the absence of CD40 are characterized by a significant decrease in the number of CD4+/CD25+ regulatory T cells. It also is known that CD4+/CD25+ cells signal via CTLA-4. Thus, the low level of CD40 and CTLA-4 as well as of CD4+/CD25+ cells in the skin of AA-affected mice all are in support of AA as an autoimmune disease on the basis of an altered regulation of immune cell homeostasis.

P 050

Analysis for TNP-groups in proteins of dendritic cells after stimulation with TNCB

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Strong contact sensitizers are able to couple to cellular proteins. This is important for the initiation of signaling mechanisms and finally formation of immunogenic peptide-hapten conjugates. Thiol- and amino-groups in cysteine and lysine residues are thought to be important target structures for haptens. We studied the binding of TNCB to monocyte-derived DC by flow cytometry, immuncytochemistry and Western blot analysis. Using a technique for membrane permeabilization it could be demonstrated that the majority of TNCB binds to intracellular proteins. Combined Western blot analysis for TNP-labeled and tyrosine-phosphorylated proteins showed a dense pattern of TNP-labeled protein band, some of them sharing their molecular weight with distinct tyrosine-phosphorylated molecules. Stimulation in the presence of cysteine blocked the binding of TNCB to DC as detected by flow cytometry and Western Blot analysis. In addition tyrosine phosphorylation was completely prevented by cysteine. The signal strength and degree of separation of TNP-labeled protein bands should be sufficient to perform mass spectrometric analysis. Analysis of both TNP-labeled and tyrosine-phosphorylated proteins will help to identify relevant proteins involved in the initial phase of activation. The capacity of compounds with free thiol-groups to block the binding of TNCB to DC and consequently their activation underlines the central role of thiol-groups as target structures for contact sensitizers. Further work attempted to uncover the starting points of the signaling cascades has to take into account this fact and should focus on proteins with thiol-groups at functionally important positions.

P051

The *E.coli*-Selective Antimicrobial Protein Psoriasin (S100A7) kills *E. coli* by Depletion of Zinc^{2+}-Ions

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In order to explain, why human skin is unexpectedly very resistant towards infection by *E. coli* we analysed extracts of stratum corneum derived from healthy individuals for the presence of *E. coli*-selective peptide antibiotics. Recently, we could identify the S100-protein psoriasin as a major *E. coli*-directed antimicrobial protein, indicating that psoriasin has a role as an innate defence molecule.

As revealed by chromatographic analyses and electrospray-time-of-flight-mass-spectrometry we found several microheterogenous psoriasin variants. Apart from posttranslational modifications we speculated that this microheterogeneity could result from the occurrence of divalent cation-containing psoriasin species, because psoriasin has been reported to contain two Ca^{2+}-binding sites. Furthermore, ultrastructural analyses of *E. coli* treated with psoriasin revealed, in contrast to most of the known antimicrobial peptides, the absence of perforation of the bacterial membrane, indicating an alternative killing mechanism for this novel antimicrobial protein.
In the light of previous observations that *Candida albicans* is killed by the calprotectin complex (comprised of two subunits of the S100-proteins MRP-8 and -14) in a Zn^{2+}-ion-dependent fashion, we hypothesised that psoriasin possibly affects the bacterial availability of divalent ions. Therefore we pretreated purified natural psoriasin (10µg/ml) with various divalent cations and subsequently investigated divalent-cation-saturated psoriasin for its antimicrobial activity against *E. coli*.
Whereas incubation with Mg^{2+}, Fe^{2+} and Mn^{2+} did not influence the antimicrobial activity, a dose-dependent inhibition was observed, when psoriasin was treated with Zn^{2+} (ID_{50}: 2,5-5µM).

This finding strongly indicates that psoriasin kills *E. coli*by depleting Zn^{2+}-ions, which are essential for many cellular functions including transcriptional regulation.

P052

Gene gun immunization of C57BL/6 mice with cDNA encoding a melanocytic self antigen linked to foreign helper sequences breaks peripheral T-cell tolerance associated with autoimmune-mediated destruction of melanocytes and subsequent development of vitiligo

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Mechanisms maintaining peripheral tolerance to self antigens prevent induction of autoimmunity, presumably because self antigens are not able to stimulate a CD4 T-helper response. Using the melanosomal enzyme tyrosinase-related protein 2 (TRP2) expressed by melanocytes as a model self antigen, we investigated whether linkage with a foreign immunogenic protein providing strong CD4 helper sequences would be able to circumvent T cell tolerance. We found that gene gun immunization of mice with cDNA encoding a fusion protein between enhanced green fluorescent protein (EGFP) from jellyfish and aa30-517 of autologous murine TRP2 (EGFP.mTRP2) resulted in vitiligo-like fur depigmentation as a sign of autoimmune-mediated destruction of melanocytes. Furthermore, immunization with pCMV-EGFP.mTRP2 but not with pCMV-EGFP or pCMV-mTRP2 alone was able to stimulate TRP2-specific T cells, suggesting that linkage of foreign helper sequences circumvented peripheral T cell toerance. To further analyze the mechanism of the autoimmune-mediated destruction of melanocytes, we constructed expression plasmids with C-terminal deletions of mTRP2. Mice immunized with cDNA encoding EGFP.mTRP2(aa30-188) developed fur depigmentation, while mice immunized with cDNA encoding EGFP.mTRP2(aa30-179) did not. We are currently also immunizing mice with cDNA encoding EGFP.mTRP2(aa180-188). Our results strongly suggest that T cells recognizing the CTL-defined H2-Kb-binding peptide aa180-188 of TRP2 are critically required for autoimmune-mediated destruction of melanocytes. Our results also imply that melanoma vaccines designed to stimulate T cells specific for melanocytic self antigens should incoporate linkage of immunogenic helper sequences in order to break peripheral tolerance.

P053

IMPORTANCE OF EPITHELIAL EXPRESSION OF MRP8 AND MRP14, FOR THE INITIAL PHASE OF SYSTEMIC ONSET JUVENILE RHEUMATOID ARTHRITIS (STILL´S DISEASE)

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The initial phase of systemic onset juvenile rheumatoid arthritis (SOJRA) is characterized not only by involvement of joints and systemic organs, but also by a specific cutaneous exanthema. Analysing skin biopsies of SOJRA-patients by immuohiostochemistry we found beside slight infiltration of leukocytes, epithelial activation is an initial phenomenon in this disease. Activation of keratinocytes was demonstrated by expression of two inflammatory activation markers, MRP8 (S100A8) and MRP14 (S100A9), two calcium binding proteins, which encompassed large areas of the epidermis.
MRP8 and MRP14 could also been shown to be secreted by keratinocytes activated in cell culture experiments. Subsequently serum concentrations of both proteins were analysed by ELISA. Serum levels were markedly increased and correlated tightly with disease activity of SOJRA. They were more specific than the common inflammation markers such as CRP and were not raised to the same extent as in other systemic inflammations, e.g. sepsis or leukemia. On endothelial monolayers in vitro MRP8 and MRP14 directly induced disruption of adherence junctions (transendothelial resistance, Ω x cm2), a process involved in diapedesis.
These findings indicate a novel, proinflammatory role of epithelial cells in this systemic autoimmune disease.

P054

Serum levels of the chemokine IP-10 in inflammatory skin diseases

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Chemokines have been shown to be involved in both homeostatic regulation and inflammatory tissue reactions. Apparently, their main function can be seen in focussing and amplifying the local cellular response by activating or attracting both resident and migratory cells. The chemokine IP-10 (interferon γ induced peptid 10kD) has been described well before the definition of the chemokine family. T cell activating properties as well as its involvement in the psoriatic tissue reaction at local level indicate an important role in the chronic dermal inflammatory processes. The question if such processes are reflected by elevated IP-10 levels in the blood circulation is addressed in this study by using a specific and sensitive IP-10 ELISA. It could be established by using two monoclonal antibodies raised in the laboratory. Serum levels of healthy volunteers (n=40), patients with psoriasis vulgaris (n=40), generalized eczema (n=10) , erysipelas (n=12) and herpes zoster (n=10) were evaluated. In all disease groups IP-10 levels were increased compared to non-detectable levels in the healthy group. Highest levels were found in psoriasis (mean 0.58ng/ml) and even more in erysipelas (mean 2.1 ng/ml). In psoriasis no correlation to disease activity as represented by PASI could be found nor to therapeutic responses. Accordingly, IP-10 seems to be a marker for inflammatory processes of the skin, however rather indiscriminative in respect to their extent and pathogenesis. This is in contrast to IL-8 and RANTES blood levels which could not be found elevated in psoriasis. Accordingly, chemokines are differentially released into the circulation under dermal inflammatory conditions and if biologically active may play a differential role in systemic inflammatory responses.

P055

Aggregation of antigen-specific T cells at the inoculation site of mature dendritic cells

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Cellular immune responses are initiated by direct interaction of naive T cells with professional antigen presenting cells, i.e., dendritic cells. In general, this interaction takes place in secondary lymphoid organs to which both naive T cells as well as mature dendritic cells preferentially home. However, this physiological scenario differs substantially from therapeutic dendritic cell-based vaccinations used to treat human cancer. In fact, only a small fraction of intradermally injected cells migrate to the draining lymph node and the majority of mature dendritic cells remain at the site of inoculation. These sites are characterized by a distinct oligoclonal T cell infiltrate comprising both L-Selectin+/CD45RA+ and L-Selectin+/CD45RA- cells. PNAd+ blood vessels represent possible entry channels for naive and central memory T cells, the former are likely attracted by DC-CK1 produced by the injected DC. In situ staining with multimeric peptide/MHC class I complexes revealed that clonally expanded T cells recognize peptide epitopes presented by the injected dendritic cells. The specificity of these stainings was confirmed by a series of controls like using irrelevant peptide/MHC class I complexes or applying the method to non-HLA matching tissue. In summary, the fraction of DC not migrating to secondary lymphatic tissue after therapeutic inoculation nevertheless seem to be involved in a specific immune modulation.

P056

Cytotoxic T-cell responses to the apoptosis inhibitor protein Survivin: a potential universal cancer antigen

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Survivin is a recently identified member of the family of inhibitor of apoptosis proteins and is overexpressed in most human cancers but not in normal tissue. An inhibition of its function results in increased apoptosis. Therefore, survivin may serve as a advantageous target for therapeutic CTL responses, if survivin derived peptides are able to elicit such responses. To this end, we detected specific T-cell reactivity against epiptopes deduced from survivin in peripheral blood from chronic lymphatic leukemia patients and in tumor-infiltrated lymph nodes from melanoma patients by ELISPOT analysis. CTL responses against two survivin-deduced peptide epitopes were detected in three of six melanoma patients and three of four chronic lymphatic leukemia patients after one *in vitro* antigen stimulation of the PBL. In contrast, no T-cell reactivity was detected in peripheral blood lymphocytes from six healthy controls. Moreover, we observed - *in situ* as well as *ex vivo* - even spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted epitopes in breast cancer, leukemia and melanoma patients. In addition, survivin-reactive T cells isolated by magnetic beads coated with MHC/survivin peptide complexes were cytotoxic against HLA-matched tumors of different tissue types. Thus, survivin may serve as a widely applicable target for anticancer immunotherapy.

P057

Engagement of the high affinity IgE receptor stimulates the production of IL-16 in Langerhans cell-like dendritic cells from atopic donors

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Preferential uptake and presentation of IgE-bound allergens by epidermal Langerhans cells (LC) *via* the high affinity IgE receptor, FcεRI, is regarded as an important mechanism in the induction of cutaneous inflammation in atopic dermatitis (AD). However, the signals controlling infiltration of inflammatory cells into AD lesions remain to be fully explored. Here, we show that activation of monocyte-derived LC-like dendritic cells (LLDC) through engagement of FcεRI induces the expression of IL-16, a chemoattractant factor for dendritic cells, CD4+ T cells, and eosinophils. We found that ligation of FcεRI on LLDCs derived from atopic dermatitis patients, that express high levels of FcεRI, increases IL-16 mRNA expression and storage of intracellular IL-16 protein, and enhances the secretion of mature IL-16 in a bi-phasic manner. An early release of IL-16 (peak at 4 h) is independent of protein synthesis, while a more delayed release (peak at 12 h) requires protein synthesis and occurs subsequent to the induction of IL-16 mRNA and intracellular accumulation of pro-IL-16. There was evidence that LLDCs use caspase-1 to process IL-16, as inhibiton of caspase-1, but not of caspase-3, partially prevented the release of IL-16 in response to ligation of FcεRI. In an *in vivo* model of IgE-dependent LC activation, the atopy patch test, positive skin reactions were also associated with the induction of IL-16 in epidermal dendritic cells. These data indicate that IL-16 released from LCs after allergen-mediated activation through FcεRI may link IgE-driven and cellular inflammatory responses in atopic skin inflammation.

P058

The effectiveness of antigen-presentation by Dendritic cells to CD4+ T-helper cells but not CD8+ cytotoxic T-cells is depending on the maturational state.

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Dendritic cells as potent antigen presenting cells are able to induce both MHC class I dependent CD8+ cytotoxic T-cell responses as well as MHC class II dependent CD4+ T-helper cell responses. Here, we wanted to determine, whether the maturational state of the DC play a role during the early cell-cell contacts leading to induction of T-cell proliferation. TCR-transgenic CD4+ or CD8+ T-cells were used from either DO 11.10 or P14 mice and co-incubated with autologous bone marrow derived DC in a time-lapse video microscopy system. We found that DC can interact with T-cells by both short lived interactions of 3-5 min or steady state interactions. The ratio of short lived versus steady state interactions was not affected by the state of DC-maturation or peptide concentrations. When looking at Ca^{2+} influx in T-cells during DC-interactions in this system, we found significant differences between CD4+ and CD8+ T-cells. Whereas Ca^{2+} influx in CD4+ T-cells was depending on peptide concentrations and the state of DC maturation, Ca^{2+} influx in CD8+ T-cells was found to only depend on peptide concentrations, but not on the maturational stage of the DC. Since DC-maturation is accompanied with upregulation of MHC and costimulatory molecules, T-cell proliferation assay were performed. Interestingly, both CD4+ and CD8+ T-cells showed a clear dependence on both peptide concentrations and the maturational stage of the DC suggesting that Ca^{2+} signalling in CD8+ T-cells is independent of the level of costimulatory and MHC molecules on the surface of DC.

P059

Pathways of Signal Transduction In Anergic $CD4^+$ T Cells Induced by IL-10-Treated Human Dendritic Cells

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Previous results demonstrated that human IL-10-treated dendritic cells (DC) induce a state of antigen-specific anergy in various populations of naive and activated $CD4^+$ T lymphocytes. Anergic T cells are characterized by reduced production of IL-2 and an G_1 cell cycle arrest. In this study we investigated signal transduction pathways of the anergic T cells. After restimulation with coated anti-CD3Ab lysates of anergic and control T cell (stimulated with mature DC) were prepared. Subsequently, immunoprecipitation, SDS gel electropherisis, western blot analysis and in vitro kinase assays were performed. In our system of anergy induction by IL-10-treated DC we observed an inhibited TCR-induced activity of CD3-zeta-chain und of the protein tyrosine kinase ZAP-70. Compared to control T cells an enhanced expression and phosphorylation of the Src kinase $p59^{fyn}$ was observed. Furthermore, in vitro kinase assays revealed a markedly reduced activity of the MAP kinases JNK1/2 and ERK1/2. These results confirmed data obtained in other models of anergy induction, e.g. using altered peptides ligands or activation of T cells without costimulation. In contrast, in our model of anergy induction an increased expression and phosphorylation of the Src kinase $p56^{lck}$ was observed, which is involved in positive and negative regulation of T cells. Furthermore, in vitro kinase assays of the MAP kinase p38 demonstrated a markedly enhanced and sustained activity after restimulation. Our results demonstrate two new mechanisms of signal transduction pathways in anergic T cells. Further investigations will be performed analyzing whether these activation processes directly inhibit the signal transduction or induce the production of negatively regulating molecules.

P060

Expression of histamine receptor types 1, 2 and 4, but not type 3, on human mast cells

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Histamine is a ubiquitously distributed biogenic amine that is stored abundantly in and rapidly released from tissue mast cells and blood basophils. Besides its well known functions like increased vascular permeability and smooth muscle contraction in target organs of anaphylactic reactions, recently numerous additional functions have been reported. Thus, histamine is involved in adhesion, growth and mediator release of diverse cell types. In addition, there is ample evidence that histamine regulates mast cell mediator secretion via histamine receptors. Their nature is however as yet highly controversial. We have therefore examined human leukemic (HMC-1) and normal skin mast cells for mRNA expression of all four currently known histamine receptors by RT-PCR. Furthermore, we have investigated the receptor protein expression on HMC-1 cells by histamine binding and its displacement using fluorescent and radiolabeled histamine as such or after preincubation of cells with H1, H2 and H3 antagonists. Signal transduction in HMC-1 cells was also explored by measuring histamine induced increases of cAMP and IP3. In both types of mast cells, mRNA for H1, H2 and H4, but not for H3 receptors could be demonstrated by RT-PCR. Binding of radioactively labeled histamine to unstimulated HMC-1 cells was markedly inhibited by preincubation with cold histamine, slightly less so with an H2 and H3 antagonist, and only to a minor extent with an H1 antagonist. H2 receptor antagonists inhibited histamine induced rise of cAMP in resting HMC-1 cells, whereas an H1 receptor antagonist was instead enhancing. The lack of H3 receptor mRNA expression in mast cells seems to conflict with the histamine displacement achieved by the H3 antagonist in HMC-1 cells. However, these findings are in agreement with recent studies showing cross reactivity of H3 inhibitors with H4 receptors. The binding of H3 antagonists can therefore be interpreted as evidence for the presence of H4 receptor protein on mast cells. The data presented here show that human leukemic and cutaneous mast cells express histamine H1, H2 and H4 receptors at mRNA, protein and partly also at functional level. They should provide a basis for the better understanding of mechanisms governing histamine induced mast cell functions.

P061

Similar morphodynamic interaction of macrophages or dendritic cells with antigen specific T cells does not result in equal capacity for T cell activation

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T cell activation is dependent on the physical interaction between naive T cells and antigen presenting cells (APC) carrying MHC associated peptides. We have previously shown that the interactions between dendritic cells (DC) and TCR transgenic T cells within 3-D collagen lattices are highly dynamic, repetitive and short-lived, albeit leading to effective T cell activation. Since DC are the most effective APC, we were interested whether less potent APC, such as macrophages (Mφ) show different morphodynamic parameters during T cell interaction. Murine bone marrow was differentiated into Mφ. Cells of the same source were cultured for 8 days in the presence of GM-CSF and IL-4 adding CD40-L on day six to obtain mature DC. TCR-transgenic naive T cells from DO11.10/RC17 RAG -/- mice specific for a peptide of chicken ovalbumin were freshly prepared by negative enrichment. M φ and DC were loaded with OVA-peptide over night. Peptide-loaded or -unloaded APC were mixed with naive T cells and embedded within a 3-D collagen matrix. Cell-cell interactions within this matrix were observed by time-lapse video-microscopy. The morphology of Mφ and DC was comparable in 3-D collagen lattices, although DC exhibited more surface dynamics. As seen earlier, interaction times between T cells and DC were approx. 15-30 minutes, independent on the presence of antigen and despite effective and specific activation of the T cells. Interestingly, time, frequency, morphology and antigen-dependence of contacts between T cells and Mφ were indistinguishable from those with DC. However, Mφ in contrast to DC were virtually unable to induce measurable T cell activation. Thus, the morphodynamic parameters of T cell activation can not explain the fundamental differences in antigen presentation between DC and Mφ. Consequently, DC must be able to provide more stimulating signals within the same time frame to incoming T cells without being more "sticky" than Mφ. Therefore, we speculate, that parameters like the stability of surface MHC-peptide complexes and the general composition of T cell DC contact planes might explain the extraordinary presentation capability of DC. We are now analyzing the interaction between T cells and B cells, which represent another major APC in the cellular immune network.

P062

Interaction of Dendritic Cells with Cutaneous T-Cell Lymphoma Cells: Implications for DC based Lymphoma Immunotherapy

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of skin neoplasms that originate from T lymphocytes. Cutaneous Dendritic Cells (DC) are supposed to play a role in the control of this usually slowly progressing disease as suggested by the infiltration of non-tumoral, CTCL specific T-cells in cutaneous plaques of Mykosis fungoides (MF) lesions. The presented study analysis the direct effects of CTCL cells in different stages e.g. apoptotic (induced by UV-B irradiation) or necrotic (generated by repetitive freeze thawing cycles) CTCL cell material on the function and phenotype of immature DC. Furthermore, we tested different preparations of CTCL cells for the loading of DC in order to probe the development of DC based immunotherapies for late stage CTCL. Surprisingly, both viable and apoptotic CTCL cells were phagocytosed by immature monocyte derived DC generated from leukapheresises of healthy donors. Only unphysiological high concentrations of either apoptotic or necrotic CTCL cells (5:1) but not 1:1 ratios induced DC maturation to a certain degree as determined by FACS analysis of surface expression of DC maturation markers, increased induction of allo-proliferation in mixed leukocyte reactions and synthesis of TNF-α and IL-6. Loading of immature DC with apoptotic CTCL cells, necrotic CTCL cell material and lysate (generated by removing cellular components from the necrotic cell fraction) at a ratio of 1:1 together with the simultaneous induction of DC maturation by adding TNF-,α IL-1,β IL-6 and PGE_2 resulted in phenotypically mature DC. Unexpectedly, DC loaded with apoptotic CTCL had a reduced T-cell stimulatory capacity despite the expression of costimulatory- and MHC-molecules comparable to unloaded DC. Cryopreservation of loaded and matured DC was feasible without significant loss of DC viability or surface marker expression. These findings provide evidence for a role of DC in the regulation of CTCL specific CD4+ and CD8+ T cells detectable in MF lesions. For the therapeutic induction of an anti CTCL immunity in patients by using CTCL cell-loaded DC CTCL lysate is the most promising agent, while apoptotic CTCL cells inhibit mature DC function.

P063

SELECTIVE IMMUNOMODULATION WITH ALEFACEPT IMPROVES CLINICAL AND QUALITY OF LIFE OUTCOMES IN PSORIASIS (From the Alefacept Study Group)

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Alefacept (human LFA-3/IgG1) is a selective immunomodulator that binds CD2 on T cells and FcgammaRI/RIII on accessory cells, resulting in a reversible reduction of memory-effector T cells (CD4+CD45RO+ and CD8+CD45RO+), key pathogenic mediators of psoriasis. Two, multicenter, randomized, placebo-controlled phase III trials evaluated efficacy, safety, and quality of life (QOL) outcomes of once-weekly alefacept by intramuscular (IM; 10 or 15 mg) or intravenous (IV; 7.5 mg by 30-second bolus) injection in patients with moderate to severe chronic plaque psoriasis. Patients in the IM study (n=507) received one of the 2 alefacept doses or placebo weekly for 12 weeks and were followed for 12 weeks after the last dose. Patients in the IV study (n=553) were randomized to one of 3 cohorts, in which they received 2 treatment courses; each course consisted of a 12-week treatment phase and a 12-week follow-up phase. Cohort 1 received alefacept during both courses; Cohort 2 received alefacept followed by placebo; Cohort 3 received placebo followed by alefacept. Clinical efficacy was measured by change in PASI from baseline and by physician global assessment (PGA). Safety assessments included adverse event reports, laboratory tests, and monitoring of infections. QOL was prospectively assessed with 2 dermatology-specific measures: the Dermatology Life Quality Index (DLQI) and the Dermatology Quality of Life Scales (DQOLS). Alefacept was well tolerated after IM and IV dosing. Infections were uncomplicated, not opportunistic, and responded to usual treatments. No rapid flare or rebound of disease occurred after therapy was withdrawn. Dermatology QOL measures were significantly improved after both 7.5 mg IV and 15 mg IM ($P<0001$ vs placebo) 2 weeks after the last dose. The significant improvement in QOL for responders, as defined by a more than 50% and more than 75% PASI improvement, was sustained for more than 12 weeks after the last alefacept dose by either route of administration. The selective immunomodulatory effect of alefacept, by IV and IM administration, produced significant clinical efficacy, was well tolerated, and improved patient-reported quality of life.

P064

ALEFACEPT SELECTIVELY REDUCES MEMORY-EFFECTOR (CD45RO+) T CELLS: A MECHANISM FOR IMPROVING CLINICAL SYMPTOMS OF PSORIASIS WITHOUT IMPAIRING IMMUNE FUNCTION (The Alefacept Study Group)

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Alefacept is a fully human fusion protein consisting of the first extracellular domain of LFA-3 fused to the hinge, CH2, and CH3 sequences of IgG1. The LFA-3 segment binds CD2 on the surface of T cells, while the Fc portion of IgG1 binds FcgammaRI and FcgammaRIII on accessory cells (eg, natural killer cells and macrophages). These actions cause inhibition of T-cell activation and proliferation and stimulation of selective T-cell apoptosis. CD2 expression is higher on memory-effector than naive (CD45RA+) T cells, and memory-effector T cells contain the precursors that migrate from the blood and mediate disease activity in the skin. In vitro and in vivo studies demonstrated that alefacept produces a selective and reversible reduction in memory-effector (CD4+CD45RO+ and CD8+CD45RO+) T cells in both the skin and circulation. Keratome biopsies from psoriatic patients treated with alefacept revealed decreased density of activated T cells (CD3+CD69+, CD25+). Importantly, changes in the density of activated and IFNgamma-producing T cells were correlated with clinical improvements, as measured by reductions in PASI. In the randomized phase II and III clinical trials, the selectivity of alefacept for memory-effector T cells, with relative sparing of naive T cells, was repeatedly demonstrated. Typically, maximal decreases in memory-effector T cells in the circulation during weekly therapy with alefacept plateau out at approximately -55 to -65% of baseline. Even after repeated courses of alefacept therapy, further decreases beyond this nadir were not observed. In phase II trials, the overall reduction in memory-effector T cells correlated with clinical improvement. Changes in this T-cell subset over the first 4 weeks of therapy also were predictive of a good clinical response. The clinical responses following alefacept therapy were durable, lasting up to 18 months (median, 10 months) with no additional therapy. This finding suggests that alefacept is a disease-remittive therapy. The highly selective action of alefacept against memory-effector T cells, key pathogenic mediators of psoriasis, provides effective therapy for psoriasis with long-lasting disease remissions while maintaining normal immune function.

P065

Lack of HLA class I molecule expression on melanoma cells UKRV-Mel-2 caused by an extensive deletion in the beta2-microglobulin gene

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The induction of tumor-specific cytotoxic T cell (CTL) responses is a major goal of melanoma immunotherapy. However, downregulation or even total loss of HLA-class I molecule expression enables the tumor cells to escape HLA-restricted CTL effector activity. The molecular nature of these escape mechanisms has to be characterized in detail in order to develop immunotherapies which circumvent tumor defense strategies. UKRV-Mel-2, a melanoma cell line established from a pleural effusion sample, exhibits a lack in the surface presentation of HLA-class I molecules. In contrast to Interferon-gamma treatment, which could not revert this cellular phenotype, transient transfection of tumor cells with a beta2-microglobulin expression plasmid induced surface presentation of all HLA-class I molecules as verified by FACS analysis. This led to the assumption that a mutation in the beta2-microglobulin gene might be casual for the HLA-class I negative tumor cell phenotype. In order to define the molecular mechanism we first analysed beta2-microglobulin expression at the RNA level. Total RNA from UKRV-Mel-2 and the positive control cell line Hela was isolated followed by RT-PCR analysis. In contrast to the control, no beta2-microglobulin specific cDNA fragment was detectable in case of UKRV-Mel-2. Therefore we carried out PCR analysis on total genomic DNA. Different primer pairs were chosen for synthesis of overlapping PCR fragments covering the exons and the exon/intron transitions of the beta2-microglobulin gene. By fragment length analysis we detected an extensive deletion in a PCR product encompassing exon I and the neighbouring intron regions. Cloning and sequence analysis of the PCR product revealed a 498 base pair deletion which has not been described so far including the whole exon I region. Sequence data also indicated that the second beta2-microglobulin gene might be lost which has to be verified by LOH analysis.

P066

Human CD4+CD25+ regulatory T cells induce an infectious tolerance of conventional CD4+ T cells

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It was recently shown that human CD4+CD25+ regulatory T cells can be isolated from the peripheral blood or alternatively induced in vitro by repetitive stimulation of naive CD4+ T cells with immature dendritic cells. Both T cell populations, freshly isolated or differentiated in vitro, inhibit the proliferation and cytokine production of conventional CD4+ T cells in a dose- and contact-dependent manner. We report, that the functional activity of such regulatory T cells requires protein neo-synthesis and the stimulation of the T cell receptor. However, once activated by anti-CD3 antibodies or allogeneic dendritic cells the CD4+CD25+ regulatory T cells suppress the proliferation of conventional CD4+ T cells even after fixation with PFA and extensive washing, showing that their suppressive properties are completely independent of soluble factors and mediated by fixation-resistant molecules. In addition, using HLA-mismatched donors we determined that conventional CD4+ T cells anergized by primary CD4+CD25+ regulatory T cells are able to exert suppressive activity by themselves. They inhibit the proliferation of freshly isolated CD4+ T cells and Th1 cells in a dose-dependent manner. Yet, in contrast to the primary CD4+CD25+ regulatory T cells the inhibitory capacity of such anergized CD4+ T cells is completely abolished after fixation with PFA. In addition, such anergic T cells also inhibit the activation of freshly isolated CD4+ T cells after separation of both T cell populations using transwell chambers. Thus, in contrast to the CD4+CD25+ T cells, this result indicates that the inhibitory properties of the anergized T cells are cell contact-independent. Collectively, these data show that human CD4+CD25+ regulatory T cells induce tolerance by a cell contact-dependent anergization of cocultured CD4+ T cells which afterwards themselves show a contact-independent suppressive activity towards freshly isolated CD4+ T cells and Th1 cells.

P067

DURATION OF RESPONSE TO ALEFACEPT THERAPY IN PATIENTS WITH CHRONIC PLAQUE PSORIASIS (The Alefacept Study Group)

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Psoriasis is a T-cell-mediated immune disorder with a fluctuating course of remissions and exacerbations. Currently available systemic therapies for psoriasis fail to provide long-lasting remissions and are associated with safety concerns, including immunosuppression and organ toxicity. Alefacept (human LFA-3/IgG1 fusion protein) binds CD2-expressing CD4+ and CD8+ memory-effector (CD45RO+) T cells, inhibiting T-cell activation and proliferation. Alefacept simultaneously induces apoptosis of memory-effector T cells by binding of the Fc portion to FcgammaR+ accessory cells (eg, natural killer cells, macrophages). This mechanism of action is similar to that of PUVA, which nearly ablates tissue-infiltrating lymphocytes in the skin and has been considered disease-remittive. Thus, the duration of response following completion of alefacept therapy has been examined in clinical trials. In a phase II trial, patients with moderate to severe psoriasis were randomized to alefacept 7.5 mg by 30-second i.v. bolus or placebo once weekly for 12 weeks. Patients were followed for an additional 12 weeks postdosing, after which they were eligible for retreatment with alefacept in an open-label trial when their disease progressed. Duration of response was measured as the time from the last alefacept dose in the phase II trial until alefacept retreatment was initiated in the open-label trial. In the phase II trial, 19 patients achieved -clear- or -almost clear- by PGA 2 weeks after the last dose of alefacept. In 84% of these patients (16/19), the -clear- or -almost clear- response was maintained through the 12-week follow-up, plus an additional 12 patients improved during this period of no treatment and achieved -clear- or -almost clear-. Of these 28 patients, 26 were followed in the open-label trial and did not require further systemic treatment for a median of 10 months (range, 6 to 18 months). No rebound phenomenon or rapid flares of disease were reported after discontinuation of alefacept. Duration of response data are also being collected from 2 ongoing phase III clinical trials and will be presented. Over 1000 patients with moderate to severe psoriasis were enrolled in the phase III trials evaluating alefacept by i.v. and i.m. administration. The selective immunomodulatory effect of alefacept on memory-effector T cells appears to produce long-lasting remissions in plaque psoriasis patients

P068

Phenotypical and functional characterization of CD4+CD25+ regulatory T cells isolated from peripheral blood of patients with progressing melanoma or autoimmune diseases

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CD4+CD25+ regulatory T cells (Treg) play an important role in the regulation of immune responses. In mice, the depletion of these cells in vivo resulted in various autoimmune diseases. In contrast, elimination of Treg induced potent anti-tumor immunity in tumor-bearing mice. Therefore, we investigated whether patients with autoimmune diseases or progressing melanoma showed an alteration of Treg in the peripheral blood. We report that the number of regulatory CD4+CD25+ T cells in PBMCs of patients with progressing melanoma is significantly higher than in normal controls (8,5 % vs. 4,5 %). Even if the additional marker for regulatory T cells CD45RO was added to the staining, the difference in regulatory cell numbers remained detectable. In contrast, in patients with autoimmune diseases we could not detect any significant alterations in cell numbers compared to controls (5,3 % vs. 4,5 %). Simultaneous staining of extracellular CD25 and intracellular CTLA-4 revealed that intracellular CTLA-4 expression was strongest in regulatory T cells from melanoma patients (4,3 %), while no significant difference was detectable in the expression pattern of autoimmune patients and control PBMCs (1,1 % and 1 % respectively). We further investigated, whether the functional properties of CD4+CD25+ Treg from melanoma or autoimmune disease patients were altered in comparison to normal volunteers. Therefore, we analyzed the proliferative and suppressive capacities of isolated Treg of these three groups. After polyclonal activation using anti-CD3 mAb, Treg of all subgroups showed comparably suppressed proliferative capacity. Furthermore we detected no significant difference between the suppressive capacities of the CD4+CD25+ Treg isolated from patients with autoimmune diseases, progressing melanoma or normal controls. These data suggest, that the peripheral blood of patients with progressing melanoma contains an increased number of Treg. However, no functional differences between Treg from patients with autoimmune diseases or progressing melanoma compared with normal volunteers were observed.

P069

Expression of T cell receptor mimic peptides by dendritic cells prevents T cell activation in vitro and in vivo

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A 8-amino acid peptide encoding a sequence of the transmembrane region of the T cell receptor ? chain (mimic peptide) is able to block T cell activation by preventing the assembly of an functional intact T cell receptor (TCR).
We could show that addition of this peptide to MLR reactions in vitro blocks T cell proliferation. Moreover, in vivo we could show that topical application of this peptide onto psoriatic or eczematic skin resulted in improvement of the skin diseases. However, if the peptide is applied systemically in animal models, a generalized immunosuppression could be observed. Our aim was therefore to develop a system where the mimic peptide is released locally at the site of T-cell activation. Since DC are the most potent T cell stimulatory cells in vivo, we decided to genetically modify DC in a way that the peptide is released upon contact with T cells. Therefore we cloned the DNS sequence encoding for the peptide and generated recombinant Adenoviruses to transduce DC. In initial experiments we could show, that transduced DC-cellines release the peptide into the culture supernatant, resulting in reduced T cell proliferation in MLR assays. When transduced DC were pulsed with Ovalbumin and injected into mice bearing transgenic OVA specific T cells, these DCs failed to induce T cell proliferation. Thus these data show that DC expressing the the mimic peptide are able to prevent T cell activation in vivo.

P070

Granulocyte colony-stimulating factor-induced psoriasiform dermatitis resembles psoriasis with regard to abnormal cytokine expression and epidermal dysfunction

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Aberrant expression of cytokines is a pathogenetic hallmark of psoriasis, including overexpression of Th1-type cytokines and activation of the IL-8/CXCR2 pathway. The role of other cytokines remains to be established. Interestingly, development of psoriasiform skin lesions has been reported after administration of granulocyte colony-stimulating factor (G-CSF). Here, we investigated whether these skin lesions immunologically resemble psoriasis. A 56-y-old man with no history of psoriasis developed psoriasiform skin lesions after G-CSF therapy and peripheral blood stem cell transplantation for high-malignant B-cell lymphoma. Histologically, these lesions resembled psoriasis with acanthosis, hyperparakeratosis and predominantly perivascular lymphocytic infiltration in the upper corium. There were no known aggravating factors for psoriasis except G-CSF. The cutaneous cytokine profile (TNF-α, IFN-γ, TGF-β, IL-10, IL-12p40, IL-8, CXCR2) was determined by quantitative real-time RT-PCR and compared to that in active psoriasis (n=8) and normal skin (n=7). The expression of molecular markers of epidermal dysfunction was investigated by immunohistochemistry. Similar to the findings in psoriasis, G-CSF-induced psoriasiform skin lesions contained increased amounts of TNF-α, IL-12p40, and IL-8, but no IL-10. Levels of TGF-β were also comparable to those in active psoriasis, but largely reduced compared to those in normal skin (by a factor five). Moreover, immunohistology revealed an epidermal infiltration of CD8+ T cells associated with an aberrant epidermal expression of cytokeratin-16 and Ki-67. Keratinocytes in these areas were also positive for ICAM-1 and MHC class II molecules, indicating activation of non-professional APC functions, an important feature of psoriatic epidermal pathology. Therefore, G-CSF, otherwise implicated in the induction of Th2-type immune responses, may cause a psoriasiforme dermatitis that resembles psoriasis with regard to a predominant Th1-type cytokine pattern, activation of the IL-8 pathway and development of epidermal dysfunction. As G-CSF is also a product of activated keratinocytes, these findings may indicate a role for G-CSF in the pathogenesis of psoriasis.

P071

Tumor immunotherapy using dendritic cells pulsed with tumor antigen, tumor-derived RNA and IFNγ

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Due to their potent antigen presenting capacity, Dendritic cells (DC) are excellent tools to investigate the induction of T-cell mediated immune responses to specific tumor antigens and hold promise for development of effective tumor immunotherapy. In an attempt to improve DC-based tumor immunotherapy, we investigated various sources of tumor antigen, the route of DC application, as well as the effect of interferon (IFN)γ activation of DC on their efficacy to induce tumor immunity. For this purpose, murine bone marrow-derived DC were cultured in GM-CSF and IL-4 and subsequently activated with CD40L, since previous experiments indicated that these culture conditions yielded DC with optimal immunostimulatory capacity. DC were pulsed either with a soluble protein extract from the poorly immunogenic murine squamous cell carcinoma cell line, KLN205, and/or with tumor-derived RNA and injected subcutaneously into tumor bearing DBA/2 mice two times at weekly intervals. Vaccination with DC, incubated with protein tumor antigen (TA) alone or pulsed with tumor-derived RNA alone, resulted in a modest reduction of tumor growth. In contrast, tumor antigen loading of DC with TA plus RNA resulted in statistically significant reduction of tumor growth (p=0.05). Surprisingly, the intravenous route of DC application was at least as efficient for tumor vaccination as subcutaneous DC administration. IFNγ is known as a potent TH1-inducing agent and appears to act both on APC as well as on T cells. Treatment of tumor-bearing mice with DC that were activated by IFNγ, in addition to CD40L, induced a significant further reduction of tumor growth when compared to DC activated with CD40L alone (p=0.02). These data indicate that the immunostimulatory capacity of adoptively transferred DC can be further enhanced by modification of the DC stimulation and tumor antigen loading procedures, which may result in more efficient tumor immunotherapy protocols.

P072

Differential expression of inhibitory or activating CD94/NKG2 subtypes on MART-1-reactive T Cells in vitiligo *versus* melanoma

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A number of tumor rejection antigens recognized by tumor infiltrating lymphocytes (TIL) in melanoma belong to the group of melanocyte differentiation antigens that are also expressed by normal melanocytes. Hence, at least a set of antigenic determinants of the self have not induced self-tolerance, but furnish target structures for immune responses directed against tumors. The notion that targets involved in cancer regression comprise normal differentiation antigens is stressed by the association between vitiligo-like leukoderma, due to destruction of normal melanocytes, and melanoma regression, due to destruction of cancer cells. Selection and activation of T cells is tightly regulated by their antigen-specific receptor and co-receptors so that responses to self are largely avoided. By T-cell receptor clonotypic mapping and staining with tetrameric HLA-peptide complexes, we demonstrated the presence of same, over-represented T cells directed against the melanocyte differentiation antigen MART-1 in the areas of destruction of both neoplastic and normal melanocytic cells. These self-reactive T cells expressed CD94/NKG2 MHC class I specific C-Type lectin-like receptors. Since this family of receptors includes both activating and inhibitory isoforms, we performed a detailed analysis, which revealed the exclusive presence of the inhibitory NKG2-A/B receptors in the vitiligo-like leukoderma, whereas both the inhibitory receptors and the activating NKG2-C/E isoforms were present within the tumor. Thus, our data suggest an additional mechanism involved in the control of T-cell responses to self-antigens based on the differential expression of killer inhibitory receptors.

P073

Synthesis of Th1-stimulating cytokines in human dendritic cells upon infection by Listeria monocytogenes

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Dendritic cells (DCs), the major antigen-presenting cells, need to be activated in order to initiate an immune response. Here, we describe the immunostimulatory effects caused by infection with Listeria monocytogenes or by treatment with listerial lipoteichoic acid (LTA) in human DCs. Both stimuli caused upregulation of costimulatory molecules, induced T-cell proliferative responses and secretion of cytokines in vitro. While infection of DCs induced release of both, IL-12 and IL-18, by L. monocytogenes, purified LTA yielded a higher stimulation of IL-18 release than infection by whole bacteria but did almost not induced IL-12 production. Combined with IL-12, the both cytokines provide antitumoral effects in murine models. The release of Th1-stimulating cytokine upon infection of DCs suggest that these intracellular bacteria may be a valuable tool for subunit vaccine delivery by L. monocytogenes.

P074

Loading of Dendritic Cells with whole melanoma cell preparations

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For the application of Dendritic cells (DC) as cellular vaccines in anti-melanoma trials, efficient presentation of tumoral antigen is crucial. Loading of immature DC with necrotic melanoma cell, tumor cell lysates or apoptotic melanoma cells might be advantageous over peptide pulsing in the generation of anti-melanoma T-cells. This study describes optimal loading parameters such as DC-tumor cell ratio, longevity of the loading procedure, timing of the maturation and cryopreservation of the loaded DC. Furthermore, we compare different loading strategies in their ability to generate anti-melanoma immunity and demonstrate the applicability of allogeneic melanoma cell lines as source for DC loading. Uptake studies were performed with FITC-Ovalbumin and melanoma cell material generated from a melanoma cell line. Surface expression of relevant markers was measured by FACS. Antigen expression was determined by using an Ab detecting MAGE-1 antigen in the HLA-A1 context. We found that at least 1 mg/10^6 DC of tumor protein is needed for high intracellular detection of FITC-labeled Ovalbumin. 24 hours of antigen loading are necessary to allow for the efficient uptake of fluid protein antigen. The simultaneous addition of maturation inducing cytokines did not reduce the uptake of antigen. Both, lysate and necrotic tumor cells but not apoptotic cells, induce a dose dependant but incomplete maturation in immature DC. However, the number of recoverable DC was lowered when higher tumor cell concentrations were applied. Cryopreservation of loaded and matured DC was feasible. In general, peptide pulsing led to a strong yet transient MAGE-1 Ag presentation, while tumor cell loading methods led to a weak but sustained MHC-I restricted MAGE-1 presentation. When allogeneic melanoma lines were used to load DC, similar anti melanoma CTL reactivies were achieved as compared to autologous melanoma cell preparations as shown in ELISPOT and Cr release assays.

P075

Effects of Anti-TNF-alpha Therapy on the Expression of Proinflammatory Cytokines, Chemokines and Adhesion Molecules in Psoriatic Arthritis

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Anti-TNF-α therapy by a chimeric monoclonal antibody (Infliximab®) has been shown to be successful in the treatment of skin lesions as well as arthritis in patients with psoriatic arthritis. In this study we investigate the immunological events in psoriatic skin lesions and serum before and after anti-TNF- α therapy in 6 patients suffering from severe psoriatic arthritis resistent to methotraxate. Biopsies from lesional skin were taken before and 10 weeks after treatment. Immunohistochistry revealed a marked decrease in expression of TNF-α, HLA-DR, CD3, CD15, ICAM-1, and LFA-1. TNF-α and CD15 positive cells could not be detected any more in recovering lesions after treatment and CD3 positive cells were markedly reduced. By semiquantitative RT-PCR we analysed the m-RNA expression of IL-8, IL-20, TNF-R (TNFR p60 and TNFR p80), IL-1R I and IL-1R II, as well as ICAM-2. Before therapy m-RNA for IL-8, IL-20, TNFR p60, TNFR p80, IL-1R II, and ICAM-2 were detected in lesional skin. The m-RNA expression of IL-8 and IL-20 completely disappeared after therapy. This effect on IL-8 expression is of paticular interest because of the well estabished role of IL-8 as a neutrophil attracting chemokine in psoriasis. Loss of IL-20 m-RNA further supports the current hypothesis that this newly described IL-10 related cytokine plays a critical role in inflammatory skin disorders. The expression of TNFR p60 was reduced after therapy but the m-RNA expression of TNFR p80 and ICAM-2 remained unchanged. Serum CRP levels decreased dramatically in all patients. In four patients we found decreased serum levels of soluble CD25, whereas serum levels of IL-8 and IL-18 did not change significantly after Infliximab therapy. These data suggest that the clinical response of anti-TNF-α therapy in patients with psoriasis arthritis may be caused by the inhibitory effect on the production of proinflammatory cytokines as well as on the decreased expression of adhesion molecules.

P076

Treatment of cutaneous leishmaniasis with proinflammatory IL-1α: Regulation of the quality of an acute inflammatory infiltrate may influence development of characteristic Th cell responses.

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We have shown previously that *Leishmania major*-infected dendritic cells (DC) release IL-12 and efficiently vaccinate against cutaneous leishmaniasis. *In vivo*, however, skin-DC from genetically susceptible BALB/c mice do not induce Th1 responses that can control disease. We subsequently found that skin-APC from BALB/c mice produce significantly less IL-1α/β upon stimulation when compared to resistant C57BL/6 mice. BALB/c mice also developed significantly smaller skin lesions and decreased parasite burdens if IL-1α was administered locally early in high dose infections. Additional studies now revealed that restimulation of lymph node cells from IL-1α-treated mice with *Leishmania*-lysates led to increased production of Th1 cytokines (IFNγ), and reduced IL-4 levels. In standard high dose infections, we found that IL-1α (or IL-1β) treatment was as efficient as administration of Th1-promoting IL-12. Continued treatment with IL-1α for several weeks was not additionally beneficial. In infections that more closely model natural parasite transmission with low dose inocula (10^3 parasites), IL-1α treatment was only successful if administered for the initial 14 d post infection, whereas the original treatment protocol (d1-3 only) was without effect. Therefore, IL-1α treatment was therapeutic when given coincident with the onset of the initial inflammatory response induced by the parasites (which is delayed in low dose infections). This prompted us to investigate the effects of IL-1α on the local inflammatory infiltrate. We found that IL-1α induced a shift from a PMN-predominant inflammatory infiltrate towards increased recruitment of cells of the macrophage (MΦ) lineage. Depletion of Gr-1^+ inflammatory cells in *L. major*-infected BALB/c by mAb-injection abrogated the ability of IL-1α to down modulate IL-4 production. Taken together, our data suggest that transient local administration of IL-1α regulates the quality of an early inflammatory response leading to increased MΦ numbers in *Leishmania* lesions, influences Th1 development and attenuates disease progression in susceptible BALB/c mice.

P077

CD14 expression in human sebocytes and IL8 regulation by lipopolysaccharides, phorbol myristate acetate and linoleic acid

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We have previously reported that lipopolysaccharides (LPS) stimulate IL8 secretion by human sebocytes but do not influence IL1alpha expression at the mRNA and protein levels. Moreover, we have shown that linoleic acid (LA) reduces IL8 secretion. These results implicate the expression of CD14 molecules in human sebocytes and a competition of the proinflammatory activity of LPS through LA. Although CD14 expression was previously only determined in inflammatory cells, current reports have also postulated CD14 expression in keratinocytes. Therefore, we investigated the CD14 expression in SZ95 sebocytes by TaqMan PCR, FACS analysis and by fluorospectrometry. The secretion of human soluble CD14 (sCD14) was examined by ELISA. HL60 promyelocytes served as control. While CD14 was detected on the surface and in the cytoplasm of HL60 cells, SZ95 sebocytes expressed CD14 nearly exclusively in their cytoplasm and were able to secrete sCD14 into the medium. CD14 expression was enhanced 2.6-fold in HL60 cells by phorbol myristate acetate (PMA), but not by LPS and LA. LPS, PMA and LA did not alter the extracellular CD14 expression on SZ95 sebocytes, but LPS and PMA reduced markedly its intracellular expression. Surprisingly, no compound were able to modify sCD14 after 24 h incubation. These results were confirmed on adherent sebocytes by the absence of CD14 on the cell surface and the presence of intracellular CD14. Interestingly, both LPS and PMA enhanced IL8 expression in SZ95 sebocytes, while LA (10-5 M) was unable to inhibit the stimulatory LPS effect. In contrast, IL1alpha expression in SZ95 sebocytes was decreased by LPS and PMA, while LA did not alter the inhibitory LPS effect. PMA, but not LPS and LA, stimulated total sebaceous lipids in the sebocytes. In conclusion, LPS and PMA are likely to serve as proinflammatory mediators in human sebocytes inducing CD14-dependent and independent IL8 expression. The latter may be mediated by the PMA-enhanced total sebaceous lipids. LA was unable to antagonize the proinflammatory activity of LPS.

P078

Early mononuclear phagocyte recruitment to sites of cutaneous granuloma formation is dependent on neutrophil influx induced by mast cell-derived TNFalpha.

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We have recently shown that early inflammatory cell influx in murine cutaneous granulomas (CGs) is 1) associated with mast cell (MC) activation *in situ* and 2) impaired in the absence of MC. To assess whether MC recruit neutrophils and macrophages to CGs by release of TNFalpha, we have characterized polyacrylamide gel (PAG)-induced inflammatory cell recruitment in the absence of MC-derived TNFalpha. Genetically MC-deficient $Kit^{W}/Kit^{W\text{-}v}$-mice were reconstituted intradermally with 10^5 connective tissue type MC derived from normal *Kit*+/+ mice (*Kit*+/+*MC*->$Kit^{W}/Kit^{W\text{-}v}$), TNFalpha -/- mice (TNFalpha-/-MC->$Kit^{W}/Kit^{W\text{-}v}$) or TNFalpha +/+ mice (TNFalpha +/+MC->$Kit^{W}/Kit^{W\text{-}v}$). All mice were injected subcutaneously with PAG (Biogel P-100, 0.5 ml, back skin) and infiltrating cells were recovered from developing CGs for FACS analysis 12h after induction. CGs in $Kit^{W}/Kit^{W\text{-}v}$-mice contained significantly less neutrophils and macrophages (7.4 and 0.3 x10^6 cells/CG) as compared to *Kit*+/+ mice (20.5 and 0.9 x10^6 cells/CG) or *Kit*+/+MC->$Kit^{W}/Kit^{W\text{-}v}$ (29.3 and 1.2 x10^6 cells/CG). Interestingly, reconstitution with TNFalpha-deficient MC did not normalize inflammatory cell influx in $Kit^{W}/Kit^{W\text{-}v}$-mice (10.5 and 0.6 x10^6 cells/CG), while TNFalpha +/+MC->$Kit^{W}/Kit^{W\text{-}v}$ exhibited neutrophil and macrophage numbers comparable to those in *Kit*+/+ mice (34.8 and 2.6 x10^6 cells/CG). Furthermore, depletion of neutrophils by antibody treatment before induction of CGs completely inhibited MC-dependent macrophage influx. These findings indicate that MC are critical initiators of CG formation, since macrophage recruitment to CGs is dependent on early neutrophil influx induced by TNFalpha released from MC.

P079

Dendritic cells and macrophages from resistant or susceptible mice take up *Leishmania major* by different pathways and require different T-cell derived co-signals for IL-12 release.

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Skin-macrophages (MΦ) and dendritic cells (DC) are infected sequentially in cutaneous leishmaniasis and play distinct roles in the inflammatory and immune response initiated by *Leishmania major*. We have reported previously that *L. major*-infected DC - unlike MΦ - release IL-12 and effectively vaccinate against leishmaniasis *in vivo*. To further study differences in DC and MΦ infectability with and reactivity to *L. major*, we analysed parasite effects on bone marrow-derived DC (BMDC) compared to skin MΦ generated either from *Leishmania*-susceptible (BALB/c) or -resistant (C57BL/6) mice. Similar to results described previously using Langerhans cell-like fetal skin-derived DC, BMDC preferentially phagocytosed obligate intracellular *L. major* amastigotes, rather than promastigotes in a concentration- and time-dependent fashion. At parasite/cell ratios of 3:1, uptake of *L. major* reached its plateau at 12 hrs (26±6% of total cells with amastigotes, 8±2% with promastigotes). In contrast, phagocytosis of both *L. major* life forms by MΦ was significantly faster and reached a maximum at 4 hrs (62±12% and 53±8%, respectively) suggesting differences in the receptors employed for *Leishmania* uptake by these two APC populations. The percentage of infected BMDC and MΦ generated from susceptible or resistant mice was similar. BMDC and not MΦ were activated upon infection with *L. major* amastigotes and released significant amounts of IL-12 (272±109 and 442±155 pg IL-12p40/18 h/2x10^5 cells for BALB/c and C57BL/6 BMDC, respectively). Surprisingly, in BALB/c IL-12 release by BMDC was further enhanced by stimulation with anti-CD40 mAb only, whereas C57BL/6 cells showed increased IL-12 production when subjected to additional IFNγ stimulation or anti-CD40. In summary, our data suggest that differences between various APC populations with regard to receptors for uptake of *Leishmania* contribute to the delayed appearance of IL-12 and DC infected with *L. major in vivo*. They additionally highlight that DC from different mouse strains require different T-cell derived cofactors for efficient IL-12 production and development of Th1-dependent protective immunity

P080

Proteinase-activated Receptor-2 Stimulates Cytokine Release, Cell Adhesion Molecule Expression and Activation of Nuclear Transcription Factor kappa B in Human Dermal Microvascular Endothelial Cells.

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Proteinase-activated receptor-2 (PAR-2) belongs to a new G protein-coupled receptor subfamily activated by various serine proteases. PAR-2 has been demonstrated to play a role during inflammation of many tissues including the skin. PAR-2 is expressed by endothelial cells and regulates cutaneous inflammation in vivo. However, the underlying mechanisms of PAR-2 activation in the skin and the effects on microvascular endothelial cells (HDMEC) are still unknown. Therefore we tested whether HDMEC express functional PAR-2 and whether agonists of PAR-2 regulate inflammatory responses in these cells. Ca-mobilisation studies revealed that PAR-2 is functional in HDMEC. IL-6 and IL-8 were upregulated in a dose- and time-dependent manner as detected by RT PCR or ELISA indicating a role of PAR-2 in stimulating HDMEC. Electrophoretic mobility shift assays revealed PAR2-induced activation of NFkB with a maximum after 1h. In conclusion, agonists of PAR-2 upregulate IL-6 and IL-8 expression and release in HDMEC. Thus, PAR-2 may play an important role in cutaneous inflammation by mediating inflammatory responses on dermal microvascular endothelial cells probably via activation of NFkB.

P081

Dendritic cells, Apoptosis and Interleukin-10 in Mycosis Fungoides and their Relevance for the anti-Tumor Immune Response

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Mycosis fungoides (MF) is a slowly progressing cutaneous T-cell lymphoma. Skin infiltrates of MF comprise tumor cells and particularly in the early disease stage a predominant bystander infiltrate of CD4 and CD8 lymphocytes (TIL). An effective anti-tumor immune response has been shown by isolating tumor specific T-cell clones from MF patients. Dendritic cells (DC), the most efficient antigen presenting cells are supposed to be crucial for the induction of a potent anti-tumor immune response. The presence of DC in skin infiltrates of MF is well established, but further characterization has yet to be performed. By novel markers specific for immature and mature DC we characterized the DC in situ, which we found in high number interspersed between the lymphocytic skin infiltrates of MF. In patch/plaque-stage MF (PS), the immature DC are mostly lag/langerin+ Langerhans cells (LC). In the epidermis of PS LC predominate over fully mature DC characterized by the strong expression of CD83, DC-lamp as well as co-stimulatory molecules and MHC II. In the upper dermis of PS mature DC predominate over LC. In tumor stage MF (TS) equal numbers of immature (CD1a+, CD1c+) and mature DC (CD83+, DC-lamp+) are densely interspersed between the infiltrate, but immature DC mostly lack lag or langerin expression. Since apoptotic tumor cells are a major source for tumor antigens processed and presented by DC (cross presentation) we analyzed double stainings for apoptotic cells (TUNEL stain) and immature DC. Immature DC with incorporated apoptotic cells were found rarely in PS but increasingly in TS. Phagocytosis of apoptotic tumor cells with consecutive presentation will induce immunity if the DC mature, but tolerance if maturation does not occur. The production of IL-10, which blocks DC maturation, by MF tumor cells is acknowledged. By triple stainings in situ we could now show, that strongly IL-10+ cells frequently surround immature DC, some of them with incorporated apoptotic cells. The latter condition was again mainly seen in TS, possibly explaining disease progression. Further in vitro studies are now required illuming the role of DC for the anti-tumor immune response in MF.

P082

Expression of the chemotactic cytokines Interleukin-16 and Lymphotactin in distinct clinical stages of Mycosis fungoides

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Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma. Initially, it presents with eczematous patches, progressing to plaque and finally tumour lesions. Immunohistochemically, these lesions are dominated by a CD4+ lymphocytic infiltrate. Only a small fraction of these cells represent the malignant clone, the majority being inflammatory cells or so-called innocent bystanders. We hypothesized that locally produced chemoattractants such as IL-16 and Lymphotactin are involved in the formation and perpetuation of the infiltrate. IL-16 is a soluble ligand to the CD4 molecule with chemotactic properties for CD4+ T cells. The expression of IL-16 protein was analyzed by immunohistochemistry in normal skin and patch, plaque and tumour stage lesions of MF. The presence of IL-16 mRNA in the epidermis and dermis of clinically distinct stages of CTCL was investigated by in-situ hybridization and quantitative competitive RT-PCR. Lymphotactin represents a new member of the chemokines and is chemotactic for lymphocytes. Similarly, its expression was investigated on protein and mRNA level. By quantitative competitive RT-PCR, we quantitated the expression of both cytokines in different stage lesions. To quantitate mRNA expression per T-cell, we also established a quantitative RT-PCR for CD3. Expression of both cytokines increased with advanced disease. However, on a CD3+-cell basis, the expression of IL-16 mRNA was similar in all disease stages, whereas Lymphotactin mRNA expression was most pronounced in plaque stage disease and considerably lower in patch and tumour stage. We conclude that both cytokines are present in MF lesions in considerable quantities. However, IL-16 seems to be constitutively expressed, whereas Lymphotactin expression appears to be tightly regulated. Both cytokines might be involved in the formation, perpetuation and progression of the respective lesions, although they may serve different roles.

P083

Upregulation of integrin subunits α1, α2 and α3 on epidermal T-cells in psoriatic lesions - implications for tissue-specific T cell localization

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Adhesion to resident cells and ECM components is crucial for tissue-specific T cell localization in inflammatory skin disorders, e.g. psoriasis. These interactions are mediated in part by β1(CD29)-integrins. In contrast to extravasion and migration through ECM, we know little about the role of β1-integrins on epidermal T cells (ETC).Here, ETC from psoriatic epidermis were compared by two-color FACS with peripheral blood T cells (PBL) from the same psoriatic donors (n=9) or healthy volunteers (n=8). While the β1 chain was expressed by most ETC and PBL (ETC: 96,81±2,15%, PBL: 98,9±0,92), several α-chains known to associate with β1 showed considerable differences: CD49a (α1; ETC: 37,75±9,71%, PBL: 1,0±1,0%), CD49b (α2; ETC: 35,89±8,26%, PBL: 1,0±0,73%) and CD49c (α3; ETC: 85,72±6,84%, PBL: 21,9±22,66) were significantly upregulated in ETC. In contrast, CD49d (α4; ETC: 68,16±10,04%, PBL: 88,3±4,47%) and CD49f (α6; ETC: 56,17±5,98%, PBL: 72,3±11,2) were moderately but consistently reduced. Thus, the integrin α-repertoire of ETC was skewed towards α1 , α2 and α3, while α4 and α6 were reduced as compared to PBL. Interestingly, PBL isolated from psoriatic donors and healthy volunteers showed no significant differences regarding integrin α-chain expression, suggesting in situ-induction of the differential expression pattern in psoriatic skin. We then sought to approach the functional relevance of integrin α1 (CD49a) for epidermal T cell localization. TGFβ1 induced expression of α1 on both CD4+ (~30%) and CD8+ T cells (>50%). These cells were therefore used in antibody-mediated functional blocking studies. In the presence of 1 mM Mg^{2+}, T cells and magnetically separated subsets (CD3+, CD4+, CD8+) adhered well to a matrix of cultured keratinocytes (static adhesion assays) or frozen sections of psoriatic skin (modified Stamper-Woodruff assays). However, when T cells, cultured keratinocytes or tissue sections were incubated with antibodies directed against the β1, α1, or α5 subunits, T cell binding to epidermal keratinocytes was not significantly altered. Thus, while a role of integrin α1(CD49a)β1 for adhesive interactions between T cells and keratinocytes could not be directly demonstrated *in vitro*, it is conceivable that overexpression of this integrin or the α2 and α3 chains contribute to tissue-specific epidermal T cell localization *in vivo*.

P084

MHC CLASS II^+, DEC-205^+ DENDRITIC EPIDERMAL DONOR LEUKOCYTES APPEAR AFTER THE GRAFTING OF FETAL SKIN TO SCID MICE

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In mice initial colonization of the epidermis with $ADPase^+$, $CD45^+$, MHC class II^-, DEC-205^- dendritic leukocytes takes place around fetal day 16/17. A dramatic numerical increment in MHC class II^+, $ADPase^+$ dendritically-shaped cells occurs after birth. To better understand the role of the skin in Langerhans cell (LC) maturation, we transplanted full-thickness grafts from body skin of day 18 fetal C3H (I-Ek/I-Ak) mice onto full-thickness wound beds of C57BL/6-scid (I-Ab) mice. At various intervals after transplantation the skin grafts were removed, epidermal sheets were prepared and analyzed for the presence and density of donor and host MHC class II^+ dendritic leukocytes. As opposed to the uniform absence of MHC class II^+ dendritic epidermal leukocytes at the time of grafting, examination of the grafts three days after transplantation revealed a few dendritic leukocytes of donor (I-Ek: 56/mm^2) and host (I-Ab: 24/mm^2) origin. One to two weeks post-grafting the density of both MHC class II populations had dramatically increased (I-Ek: 1594/mm^2; I-Ab: 1523/mm^2). These cells mostly appeared in clusters, suggesting proliferation. Double-staining of epidermal specimens revealed an almost uniform expression of donor MHC class II^+ cells for DEC-205. Collectively, our data show that donor MHC class II^+, DEC-205^+ cells appear in the epidermis and imply that either the postnatal epidermal microenvironment induces MHC class II and DEC-205 expression in MHC class II^-, DEC-205^- dendritic epidermal leukocytes or, alternatively, that they derive and further mature from dermal precursors. It remains to be determined whether these cells are true LC or represent another subset of dendritic cells that is only present during the fetal period.

P085

Differential priming of dendritic cell maturation by inflammatory cytokines or infectious agents

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It is well accepted that, after having captured antigens, human epidermal Langerhans cells (LC) migrate to the regional lymph node for presentation of the processed antigens to T-cells. During this migration LC experience a profound phenotypic and functional maturation which is accompanied by the emergence or upregulation of CD83 as well as CD80, CD86 respectively. The exact time kinetics of this process are currently not known. In vitro, the maturation can be induced by the addition of various stimuli like TNF-a, a mix of TNF-a, IL-1 and IL-6, LPS or poly I/C.
Culture of CD34+ hematopoietic precursor cells in the presence of GM-CSF, TNF-a, SCF, FLT-3L and TGF-b gives rise to immature dendritic cells (DC). A significant amount of these cells can be identified as LC by the expression of Langerin, intermediate levels of MHC II and low levels of CD86. We exposed these cells to different maturation stimuli and analyzed in details their phenotype and functional capacity. In time course experiments we could show that after stimulation a rapid upregulation of maturation markers such as CD83, CD86, CD80 and MHCII appeared within two hours. Treatment of the cells with poly I/C or LPS led to a further increase of these markers until up to 6-12 hours after stimulation, CD83 expression was back to pre-stimulation levels after 24 hours. By contrast, monocyte-derived dendritic cells (MoDC)(generated in the presence of GM-CSF and IL-4) matured under poly I/C maintained their mature phenotype for at least 72 hours. Treatment of immature CD34-derived DC with cytokine mix increased their level of maturation markers for one to two days, followed by a slow decline. 30 minutes after TNF-a treatment, biosynthesis of MHCII molecules was stimulated as judged by immunoprecipitation. The in vitro generated LC were potent inducers of an allogeneic MLR under all stimulation conditions with increase of their potency after maturation. In conclusion, maturation kinetics of LC induced by inflammatory cytokines are different from those observed with stimuli from infectious agents like LPS or poly I/C. This may have functional consequences for the initiation of an immune response.

P086

Induction of human β-defensin gene expression in keratinocytes is downregulated by retinoic acid

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It has been shown that human skin is able to mount a fast response against invading harmful bacteria by the release of inducible peptide antibiotics such as the human β-defensins (hBDs), small cationic antimicrobial peptides. To date four hBDs are known. Recently human β-defensin-3 (hBD-3) has been isolated from psoriatic skin and very recently the fourth member of the human β-defensin family hBD-4 has been discovered by screening the human genome database. Since nothing is known about the participation of hBD-4 in the innate immunity of human skin and to get more insight into gene regulation of hBDs in human skin, we analyzed the gene expression of these novel peptide antibiotics in primary keratinocytes using real-time RT-PCR, *in-situ* hybridization and Luciferase gene reporter assays. Here we demonstrate for the first time that hBD-4 mRNA is expressed in human keratinocytes. Weak mRNA expression of hBD-4 and hBD-3 in keratinocytes was induced by a virulent form of *Ps. aeruginosa*. Furthermore we found that INF-γ selectively induces hBD-3 but not other hBDs. *In-situ* hybridization revealed hBD-3 mRNA expression in epidermis particular in the suprabasal terminal cell layer indicating that β-defensin gene expression is increased during differentiation. Indeed we found that gene expression of hBD-3 and hBD-4 in keratinocytes was strongly increased by high Ca^{2+} concentrations as well as by phorbol-myristate-acetate (PMA), which both promote keratinocyte differentiation. We next investigated whether all-trans-retinoic acid, a known inhibitor of PMA mediated gene induction, influences PMA induced β-defensin expression in human keratinocytes. Indeed we observed strong (> 90%) reduction of hBD-3 and hBD-4 gene expression in PMA stimulated keratinocytes when preincubated for 24 h with all-trans-retinoic acid. Furthermore induction of hBD-3 by INF-γ and induction of hBD-2, -3 and -4 by *Ps. aeruginosa* was nearly completely abolished upon pretreatment of the keratinocytes with all-trans-retinoic acid. In addition induction of β-defensin gene expression in keratinocytes treated with Ca^{2+} was completely abolished by pretreatment with all-trans-retinoic acid. We conclude that all-trans-retinoic acid is a potent inhibitor of β-defensin gene expression induction in keratinocytes and might downregulate the innate chemical defense system of human skin.

P087

Detection of candidate genes for melanoma growth control using a soluble Fas (sFas) transgenic mouse model.

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The phenomenon that tumor cells themselves express Fas ligand (FasL) and induce apoptosis of tumor-infiltrating immune cells was recently termed "tumor counterattack". Evidence was provided that this mechanism might also be active in malignant melanoma and might promote tumor growth. To further investigate this mechanism and address the question of molecules involved in local tumor growth we generated a soluble Fas transgenic mouse where the soluble Fas gene is under the control of the lymphocyte-specific Lck promoter. These mice have no particular phenotype but show a relative resistance to Fas-induced apoptosis of lymphocytes. We presumed that sFas might interfere with the melanoma cell-derived counterattack of B16 melanoma cells and might exert protective effects. This might enable tumor-infiltrating immune cells to further attack the tumor and leed to reduced growth or even tumor rejection. Indeed, local tumor growth after s.c. injection of B16 melanoma cells was significantly reduced in sFas transgenic mice compared with normal control mice (C57BL/6), arguing for a protective role of sFas in vivo. To identify target genes involved in tumor growth control in sFas mice we analysed the gene expression pattern of tumors showing different growth characteristics. mRNA expression profiling was carried out using commercially available oligonucleotide DNA microarrays. By this approach we were able to detect new genes which may play a role for melanoma growth. Interestingly, the function of one of these target genes was closely connected to apoptosis regulation. The presented data demonstrate that the sFas transgenic mouse model may serve as a model system to unveil growth and apoptosis-related genes in malignant melanoma and might open therapeutic perspectives for melanoma patients.

P088

INDUCTION OF SPECIFIC IMMUNE RESPONSES BY POLYCATION-BASED VACCINES

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Injection of tumor antigens together with cationic polyaminoacids (e.g., poly-L-arginine) has been shown to protect animals against tumor challenge. The goal of this study was to unravel the mechanism(s) underlying this phenomenon. For this purpose, we used β-galactosidase (βgal) as a surrogate antigen. BALB/c mice were injected i.d. on days 0 and 14 with βgal complexed to pArg (thereafter referred to as protein vaccine (PV)) and inoculated on day 24 with βgal-expressing RENCA cells. PV treatment protected 8/8 animals against RENCAlacZ, but none of 5 against the parental RENCA cells. Antigen-specific protection was found in only 3/8 βgal recipients. None of the negative control animals (naive, pArg recipients) but 7/8 mice that had received βgal pDNA (pos. contr.) were able to reject the RENCAlacZ inoculum. As for the tumor protection assay, PV injection was superior to βgal administration in its ability to induce specific Abs as well as IFNγ-producing T cells recognizing the immunodominant class I-restricted βgal epitope. T cell depletion studies demonstrated that the protective effect is critically dependent on CD8+ but not on CD4+ T cells. Priming of CD8+ T lymphocytes required the presence of CD4+ cells when mice were immunized with βgal pDNA but not when the PV was used. To learn more about the fate of the PV, injection sites were removed at 1.5 h, 1 and 11 days after injection and thus treated animals were analyzed for specific immunity on day 12. Results obtained showed that removal of the i-site as early as 1.5 h after PV application does not affect the elicitation of specific T cells. Draining lymph nodes were found to contain βgal protein already 20 min and cells displaying the immunodominant, class I-restricted peptide epitope 24 h after PV treatment. This was followed by the appearance of antigen-specific T cells on day 5. Together, these data demonstrate that the co-administration of pArg and βgal triggers a protective and specific CD8 T cell response independent of CD4+ T helper lymphocytes and the prolonged presence of the i-site. It remains to be seen whether this is due to the ability of polycations to act on lymphocytes, the APCs involved or both.

P089

Relationship between autoantibody (Ab) subtypes and the presence of mucosal lesions in European patients with pemphigus vulgaris (PV) by a novel ELISA with recombinant desmoglein 3 (Dsg3)

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IgG against the desmosomal adhesion molecule, Dsg3, plays a major role in the pathogenesis of PV, a potentially life-threatening autoimmune bullous skin disorder. Specifically, it has been shown that anti-Dsg3 IgG is responsible for loss of adhesion of the mucosal epidermis. Previous studies showed that patients with PV may exhibit Ab reactivity of different subclasses against Dsg3. We developed an ELISA system with the eukaryotic recombinant protein PVhis (extracellular portion of Dsg3) to investigate Ab reactivity against Dsg3 of sera from European PV patients. Sera of 85 patients with PV, 16 patients with pemphigus foliaceus (PF) and 71 healthy controls were investigated by this ELISA for IgG, IgG1, IgG4, IgA, and IgE reactivity against Dsg3. PV patients were classified as having active (n=78) or remittent disease (no blisters for more than 3 months, n=7). From PV patients with active disease 49/78 (63%) of the sera showed IgG reactivity against Dsg3 and the ELISA exhibited a specificity of 97.6%. Patients with active disease had significantly higher titres of Dsg3-reactive IgG than patients in remission (P=0,026). However, there was no relationship between disease activity and distinct Ab subtypes. Even though IgG4 was the major subtype and was detected in 45/78 (58%) and IgG1 was detected in 31/78 (40%) of the sera, neither the titres of Dsg3-reactive IgG1 nor IgG4 could be explained by the occurrence and severity of mucosal lesions. By ELISA, none of the patients sera contained Dsg3-reactive IgE, and only rarely IgA (5/78 sera; 6.4%). These observations strongly suggest that total IgG levels are related with the presence of mucosal lesions but that no single Ig subtype can be detected for disease activity in PV. Due to its high specificity and sensitivity, the present Dsg3-ELISA is very useful to identify and monitor patients with PV based on their autoantibody profile.

P090

Induction of anti-tumor cytotoxic T lymphocytes in humans using new peptide epitopes found by computer based algorithms

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Antitumor vaccines are based on weakly immunogenic specific antigens admixed with adjuvants in order to elicit, restore or augment immune response against residual or metastatic tumor cells. Cellular cytotoxicity is considered to play a major role in eliminating tumor cells. Activation of cellular toxicity requires at least three synergistic signals. Presentation of specific tumor antigens on MHC class I (HLA), costimulatory signal and propagation signal of cytokines.
HLA-restricted specific antigens recognized by cytotoxic T cells can be identified using pattern recognition algorithms. The use of a trained artificial neural network (ANN) to predict peptides binding to distinct HLA, which then can be recognized by T cells, allows a prediction of T cell epitopes for CD8+ cells.
We used an ANN to predict HLA-A*0201-binding epitopes for known tumor-associated antigens (CATD, GP100, MAGE-1, Tyrosinase). Additional filter and optimization steps resulted in 10 epitope sequences and 10 variants of these epitopes optimized for HLA binding, which were then tested for biological activity.
Two epitopes of these couples of GP100 and MAGE-1 showed the ability to activate T cells from melanoma patient-derived PBMC as investigated with an intracellular IFN-? assay. Additionally, one optimized epitope from the GP100 sequence but not its native counterpart showed reactivity with T cells from CTCL patient-derived PBMC. This fact indicates that the optimized epitope can activate a different pool of T cells than the native epitop.
Because of the speed and efficiency of our ANN and biological test system, vaccination with these predicted epitopes becomes a feasible and powerful tool in immunotherapy.

P091

Stress-triggered hair growth-inhibition in mice can be counteracted by topical minoxidil

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Stress has been shown to upregulate hair follicle apoptosis and inhibit keratinocyte proliferation in a murine model. Activated macrophages and mast cells are involved in the pathways of stress induced hair follicle apoptosis, thus resulting in hair loss. These novel data indicated the existence of a brain-hair follicle axis (BHA). Current drug treatment approaches of hair loss include the use of regrowth stimulators such as topical minoxidil, whereas the mechanism by which minoxidil prevents hair loss are still hypothetical. The aim of the present study was therefore to investigate the effect of topical minoxidil application on keratinocyte proliferation, apoptosis and immune cells in our murine stress triggered hair loss model. Female CBA/J mice were depilated and randomized in 2 groups, control (n=20) and stress (n=20). The 2 groups of mice were further divided in 2 subgroups and either treated daily with 5% solution containing minoxidil or solution without minoxidil. The stress group was exposed to ultrasonic stress 14 days after depilation. All mice were sacrificed 16 days after depilation. We observed that sonic stress significantly increased the number of hair follicles containing apoptotic cells around the bulge region in mice, and inhibited intrafollicular keratinocyte proliferation in situ. This effect could be significantly abrogated by minoxidil treatment. Sonic stress also increased the number of activated perifollicular macrophage clusters and degranulated mast cells. These stress-induced immune changes could also be prevented by minoxidil application. We conclude that stress-triggered hair growth-inhibition in mice can be effectively counteracted by topical minoxidil treatment.

P092

Inhibition of cytokine production and IgE synthesis by peroxisome proliferator activated receptors (PPAR´s) in peripheral blood cells from patients with atopic dermatitis

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PPAR´s act at the molecular level by regulating genes through ligand-dependent transcription activation and/or repression. Studies of PPAR expression in different tissues pointed out the role of these receptors in inflammatory processes. In the present study we analysed the role of PPARα and PPARγ on cytokine production and IgE synthesis in peripheral mononuclear cells (PBMC) from AD patients in vitro. PBMC´s were isolated by ficoll hypaque separation and were cultured (106 cells/ml) for 2 and 10 days in vitro in the presence of PPARα (ZK 219766) at 10^{-8}-10^{-6}M or PPARγ (ciglitazone) at 10^{-5}-$2*10^{-5}$M. For the analysis of the cytokines cells were concomittantly stimulated with PMA/Ionomycin (20 ng/ml/$2*10^{-7}$M). ELISA assays were performed from the supernatants to measure different cytokines (2 d) and the basal IgE production (10 d) respectively. Supernatants from PBMC´s stimulated with PMA/Ionomycin revealed modest inhibition of IL-2, IL-4, IL-6, TNFα and IFNγ production by PPARγ, but not or to a lesser extent by PPARα. A significant and dose dependent inhibiton in basal IgE synthesis from PBMC´s of AD donors was observed in the presence of PPARα (up to 47%) and PPARγ (57%) at the highest concentrations used. To identify the relevant cell population involved in inhibition of IgE production we used isolated B cells co-cultured with and without T-cells and monocytes. Our data show that monocytes significantly contributed to the decrease of IgE production by both PPAR´s. Analysis of cytokine levels from these co-cultures with and without PPAR ligands demonstrate ciglitazone ($2*10^{-5}$ M) as a potent inhibitor of IL-2, but also IL-6 synthesis. Taken together our data show that PPAR´s inhibit cytokine production and IgE synthesis of PBMC from allergic donors in vitro. Their potential therapeutic role for the treatment of this inflammatory skin disease is therefore worth to be analysed in an appropiate in vivo model. However, the manner and degree by which the different cell populations interact with IgE regulation in the presence of PPAR´s will need to be clarified.

P093

Immature Langerhans Cells can be enriched from human Epidermis using mAb anti-CD1c

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Several different protocols exist to isolate human Langerhans cells (LCs) from skin. Here we compared three methods with regard to viability, recovery, differentiation state and expenditure. Members of the CD1 family, the non-classical MHCI molecules CD1a - e are strongly expressed on DCs. Because CD1a sorted LCs showed also high expression of CD1c we could compare phenotype of CD1a vs. CD1c sorted cells vs. migrated cells of same donor: i) Stripes of fresh whole breast skin were incubated for 24 hours 37°C in RPMI/10%FCS and all migrated cells were collected. ii) epidermal sheets were digested with dispase and trypsin. LCs were isolated by CD1a or iii) CD1c magnetic activated cell-sorting. The viability (PI exclusion) was significantly higher in detached LCs than in migrated cells (80 vs. 60% viable cells). Splitting experiments with skin from one patient revealed 5x 107 migrated cells, 7x 10^5 and 1,3x 10^6 cells isolated with CD1a and CD1c, the mean recovery was $4x10^5$ LCs/10g skin (n=20) for CD1a. CD1c enriched 1,9x more CD1a/DR positive cells from the same amount starting skin than sorting with CD1a. CD1a/CD1c$^+$ cells represented the same distinct population of immature LCs concerning expression of CD40, CD80, CD83, CD86 and HLA-DR. Migrated cells were a non-homogeneous population with just 6 and 18% (stained with CD1c and CD1a) LCs at all, DR was present on 8-19 %, CD86 was expressed with 25% by less cells than it was shown by CD1-LCs (70%) and CD83 was slightly upregulated. In conclusion we could demonstrate that migrated cells have a decreased viability and constitute of several epidermal cell populations (keratinocytes, melanocytes, Merkel cells) that unspecifically leave the skin samples after incubation in medium. Magnetic cell sorting with anti-CD1a isolates LCs with no CD80 but CD86 expression. CD1c sorting is very effective because it isolates a cell population with same phenotype like CD1a sorted cells. But CD1c reveals a higher gain because the loss of cells on the second enrichment column was lower. This may be due to different membrane trafficking kinetics of CD1a and CD1c during isolation process as indicated by electron microscopy.

P094

Fast maturation of isolated human Langerhans Cells to CD83+ Cells by crosslinking with CD40 Ligand

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We have established a protocol for isolating and culturing ultrapure and viable human Langerhans cells (LCs). After four hours of isolation costimulatory CD40 and CD86 were already present on 80 and 70% of the cells, no expression of CD40L and CD80 could be detected on the surface of isolated LCs. For over two weeks all these cells had high expression of CD1a and HLA-DR. While CD40 and CD86 decreased, the maturation associated antigen CD83 was constantly low expressed during this period. According to common protocols in vitro generated immature DCs mature by MCM, TNF-α or additional IL-1β, IL-6 and PGE2. On freshly isolated LCs, all these stimuli, as well as IFN-γ or trimer of CD40L failed to induce CD83. Only when cocultured with hCD40L-transfected mouse L-cells (TRAP, adherent 1:2, TRAP:LC), or with J558L-cells (non-adherent 2:1) LCs strongly started to elevate surface expression of CD83. After 24 hours CD1a+ cells were sorted again and analysed for coexpression of HLA-DR and CD83. When activated with adherent TRAP-cells 94% of starting LCs became mature CD83-expressing cells. Also crosslinking with CD40L on J558L-cells induced activation in 66% of all LCs. Wild-type L-cells showed no, stimulation with 1 µg/ml LPS only weak upregulation of CD83 on LCs. In following experiments mature LCs were proven for expression of costimulatory B7.2 (CD86) and chemokine receptor CCR7. CCR7 was not present on freshly isolated LCs and de novo expression was induced by TRAP-stimulation after 24 hours. A subpopulation of isolated LCs already showed CD86 on the surface but levels of both molecules could significantly be upregulated after 3 days of crosslinking CD40 on LCs. These results suggest that isolated immature LCs become CD83 high expressing cells after cell mediated CD40 ligation but partly differ from monocyte/CD34+ cells generated immature DCs/LCs in their phenotype and potency to mature.

P095

Diffuse T cell activation in vivo, despite cellular immunodeficiency and impaired T cell activation in vitro, in CD18-deficient mice

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β2 integrins are important for transendothelial migration of leukocytes as well as for T cell activation during antigen presentation. To detemine the relevance of these molecules for the homeostasis of the immune system in vivo, we investigated the T cell immunophenotype of CD18 deficient mice, which lack all functional β2 integrins. Peripheral blood, lymph nodes and spleens of these mice were investigated for their cellular composition and for the activation state of T cells, using flow cytometry. Increasing with age, CD18-deficient mice develop profound hypercellularity of the lymphatic organs and an abnormal cellular composition. At a median age of approx. 4 weeks, we observed the appearance of an abnormal cell type that bears activation markers but was negative for T cell, B cell, NK cell, macrophage/dendritic cell and granulocyte surface markers. By electron microscopy, these abnormal cells (approx. 30% of all spleen or lymph node cells) had features of plasma cells, although so far we could not detect intracellular immunoglobulins by flow cytometry. However, due to the profound hyperimmunoglobulinaemia in these mice, we suspect these cells to be plasma cells. Interestingly, as soon as these abnormal cells appear, the number of activated T cells ($CD44^{high}$, $CD62L^{low}$) increased dramatically, both in spleens and lymph nodes as well as in peripheral blood. In addition, the percentage of $CD8^+$ T cells decreased by >80%. Thus, CD18 deficient mice exhibit generalized T cell activation and loss of $CD8^+$ T cells. In light of our previous observation that T cells from CD18 deficient mice exhibit greatly impaired capacity to proliferate upon antigenic stimulation, and that CD18 deficient mice are clinically immunodeficient, this observation was unexpected. We therefore speculate that β2 integrins provide an important, as yet unrecognized negative feedback loop that prevents uncontrolled plasma cell activation, leading to activation of $CD4^+$ T cells and disappearance of $CD8^+$ T cells.

P096

T Cell Mediated Immune Responses Require TNF Receptor 1 Signalling on Vascular Endothelial Cells

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TNF is a critically involved in the regulation of innate and T cell mediated immune responses and plays a central role in protective and harmful delayed-type hypersensitivity reactions (DTHR). However the role of TNFR1 in the induction and effector phase of DTHR remains controversial. To address this question we sensitized and challenged gene-targeted mutant $TNFR1^{-/-}$ mice with TNCB to induce and elicit contact hypersensitivity reactions (CHSR). These mice developed 60% lower ear swelling responses than wild-type mice, histology and determination of myeloperoxidase activity revealed that $TNFR1^{-/-}$ mice had strongly reduced neutrophil infiltrates and tissue destruction. To investigate whether reduced inflammation in $TNFR1^{-/-}$ mice is the consequence of a defect in T cell priming we examined hapten specific T cell responses No differences were found between $CD4^+$ T cells from $TNFR1^{-/-}$ and wild-type mice, analyzing T cell proliferation and IFN-γ producing T cell precursor frequency directly ex vivo. We next examined whether reduced ear swelling was secondary to impaired T cell functions. However hapten specific Th1 and Tc1 cells from $TNFR1^{-/-}$ mice were fully capable in transferring CHSR into WT mice. On the contrary hapten specific Th1 and Tc1 cells from WT mice failed to elicit normal CHSR in $TNFR1^{-/-}$ mice, suggesting that the defect residues inside the tissue. Indeed during early phases of CHSR mRNA expression of I-CAM, V-CAM and P-selectin was strongly suppressed in ears from $TNFR1^{-/-}$ mice. Our data show that inefficient DTHR in $TNFR1^{-/-}$ mice was not due to defective T cell responses but was associated with inappropriate expression of adhesion molecules. These data may help to elucidate the mode of neutrophil recruitment during T cell dependent immune responses such as rheumatoid arthritis or psoriasis.

P097

Reduced Skin Inflammation by Systemic Treatment of Mice with a New Vitamin D Analog Effective at Non-Hypercalcemic Dosages

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Recently, we identified a new Vitamin D derivative which exhibits potent biological activity in vitro and in vivo. This compound with structural modifications concerning the chemical side chain of Vitamin D has been extensively characterized in comparison to 1 alpha,25-dihydroxyvitamin D3 (calcitriol). Firstly, binding affinity of this Vitamin D analog to the Vitamin D receptor is 3-fold lower than for calcitriol. Secondly, other than calcitriol this compound failed to induce Vitamin D-induced differentiation of human promyelocytic leukemia cell line HL60 to monocytes but was capable to antagonize the action of calcitriol. Thirdly, this analog causes inhibition of proliferation of lymphocytes in mixed lymphocyte cultures but with lower efficacy and potency than calcitriol. Thus, with regard to the in vitro profile the Vitamin D derivative differs to some extent from calcitriol. In vivo, as analyzed in a T cell dependent allergic contact dermatitis model in mice, the Vitamin D derivative and calcitriol inhibit the delayed type hypersensitivity reaction (edema formation, cell infiltration) when applied systemically around sensitization phase. Interestingly, whereas both compounds exert potent immunosuppressive activity in vivo only the new Vitamin D derivative is efficacious also at nonhypercalcemic doses. In conclusion, this data indicates that this Vitamin D derivative has a clear therapeutic advantage compared to calcitriol and it might serve as a potential candidate for systemic treatment of inflammatory skin diseases and other immunological disorders.

P098

ULTRAVIOLET A IRRADIATION FAVOURS EXPRESSION AND SUBSTRATE SUPPLY FOR THE INDUCIBLE NITRIC OXIDE SYNTHASE IN NORMAL HUMAN SKIN

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Expression of the inducible nitric oxide synthase (iNOS) has been demonstrated in keratinocytes and in the dermal vasculature of psoriatic skin, but has also been shown to represent a normal time-limited response after ultraviolet (UV) irradiation. Thus, this response appears to be a regulating signal of local skin functions during inflammatory reactions following various environmental challenges. The enzyme activity of iNOS can be modulated by arginase 1 (inducible isoform, ARG1) and arginase 2 (constitutive isoform, ARG2), because these enzymes compete for the common substrate L-arginine. In addition, iNOS activity also depends on substrate supply provided by the cationic amino acid transporters CAT-1 (constitutive isoform) and CAT-2B (inducible isoform). To determine the in vivo enzyme activity of iNOS in human skin after UVA irradiation, short-term organ cultures of normal human skin, obtained from mammoplasty skin specimens, were UVA-irradiated and / or cytokine-challenged, and then analyzed for the expression of iNOS-specific mRNA as well as mRNA specific for ARG1, ARG2, CAT-1 and CAT-2B. Here we report that UVA irradiation of skin organ cultures leads to the induction of iNOS mRNA expression, which is paralleled by an increase in CAT-1 and CAT-2B mRNA expression. In contrast, expression of the inducible-type ARG1, which we found constitutively expressed in normal human skin, was strongly downregulated by UVA irradiation, whereas expression of the constitutive-type ARG2 was not affected. When iNOS was induced by cytokine-challenge, similar increases in CAT-1 and CAT-2B mRNA formation were demonstrated with a significant decrease in ARG1 mRNA expression. Remarkably, UVA irradiation of cytokine-activated skin organ cultures further increased these effects. Because we had previously demonstrated that cutaneous iNOS expression plays an important role in protecting against UVA-induced cytotoxicity, membrane damage and induction of apoptosis, the present data indicate that UVA irradiation leads to increases in the substrate supply for iNOS via augmented L-arginine transport and reduced substrate competition by downregulating ARG1 expression. Collectively, these findings argue in favour of increased iNOS activity following UVA irradiation of human skin.

P099

Preferential induction of cellular immunity by DNA vaccination transcriptionally targeting dendritic cells

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A crucial step in vaccination strategies is the mode of antigen delivery. A successful approach is transfection of skin with antigen-encoding plasmid DNA using the gene gun. Resident dendritic cells (DC), which are outstanding in activation of naive T cells, were shown to be pivotal for elicitation of strong cellular and humoral immune responses against the encoded antigen. Here we describe DNA vaccination transcriptionally targeting cutaneous DC with the promoter of the murine actin-bundling protein fascin. Using the reporter gene enhanced green fluorescent protein (EGFP) we demonstrate that the fascin promoter restricts antigen-expression to mature DC. Importantly, we show that selective expression of EGFP by a small number of skin-derived DC induces EGFP-specific CD8+ T cells as effectively as expression of EGFP in all skin cells with the CMV promoter. However, immunization with the fascin promoter stimulated significantly lower levels of EGFP-specific antibodies when compared with the CMV promoter. Thus, our DC-targeted DNA vaccination approach induces a qualitatively distinct, predominantly cellular immune response. This might offer new insights into the mechanisms underlying DNA vaccination and provides new opportunities for immunotherapy.

P100

The clinical phenotype and course of bullous pemphigoid (BP) relates toautoantibody reactivity against distinct regions of the ectodomain (ECD) of BP180

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The NC16A domain and the COOH-terminal region of the ECD of BP180 have been identified as preferential targets of autoantibodies (Ab) in clinical variants of BP, the most frequent autoimmune bullous skindisorder. To investigate epitope and subclass specificity of Ab in a large cohort of BP sera, we developed a novel ELISA utilizing baculovirus-derived recombinants of the NH_2- and COOH-terminus of the ECD of BP180. Sera of BP patients (n=116) and controls (n=100) were assayed for anti-BP180 IgG reactivity by this ELISA. 93/116 (80%) and54/116 (47%) BP sera recognized the N- and C-terminus of the ECD of BP180, respectively. The ELISA was highly sensitive since 108/116 (93%) BP sera reacted with either N- or C-terminus and exhibited a specificity of 98%. Ab levels of 95 BP sera to the N-terminus but not to the C-terminal end reflected disease activity since NC16A-reactive IgG levels were significantly higher in extensive BP than in limited orremittent BP (multivariate analysis, $p<0.005$). Patients with IgG reactive with both, the N- and C-terminus of BP180 developed morefrequently mucosal lesions (Fisher´s exact probability test, $p<0.05$). Ab against the N-terminus of the BP180 ectodomain showed a significant predominance of the IgG1 class, while a dual IgG1 and IgG4 response to this region was related to a more severe skin involvement ($p<0.01$). IgA and IgE against the N-terminus (4/116; 6/116) and C-terminus (3/116;2/116) were rarely detected and not associated with a distinct phenotypeor disease activity. These findings strongly suggest that Ab against the N-terminus of BP180 are directly related with disease activity and maythus be critical in the pathogenesis of BP. Ab against NH_2- and COOH-terminus seem to have a synergistic effect with regard to the development of mucosal lesions. This novel BP180-ELISA is not only a highly sensitive and specific tool for rapid diagnosis of BP but also provides a useful parameter to evaluate the clinical course of BP.

P101

Characterization and visualization of T-T-cell interactions during the differentiation of antigen-specific T-cells*in vivo*

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Upon antigenic stimulation, naive $CD4^+$ T lymphcytes proliferate and differentiate into functionally distinct phenotypes, two extreme statuses being the IFN-g producing T helper cell type 1 (Th1) or the IL-4 producing Th2 phenotype. The mechanism driving Th2 differentiation remains enigmatic. To determine whether surrounding memory T cells could influence the differentiation of naive $CD4^+$ T cells we asked whether polarized $CD4^+$ Th2 cells, expressing a TCR specific for the ovalbumin-peptide (OVA; DO 11.10) could influence the differentiation of naive T helper cells expressing a transgenic TCR specific for HA peptide. Both transgenic T helper cells were transferred into BALB/c mice. Mice were challenged with either one or both peptides (OVA/HA) in the presence of CFA, an adjuvant which is known to have strong Th1 inducing properties. Migration and T cell differentiation were followed by histology, FACS and cytokine analysis. As expected, naive HA-specific T cells developed an IL-4 deficient Th1 phenotype when stimulated in the absence of OVA Th2 cells. However, these naive HA-specific T cells developed into an IL-4 producing Th2 phenotype when OVA-specific Th2 cells were activated concurrently in the same lymph node. Importantly OVA-Th2 cells did not attenuate Th1 development of HA-specific T cells when present either in a resting status or when stimulated at a distant site. FACS analysis and immunohistology unraveled that naive T cells and Th2 cells homed with similar dynamics to draining lymph nodes and both cell types were in close vicinity inside T cell areas. The finding that activated Th2 cells can contaminate the differentiation of naive T cells may explain the spreading of Th2 immunology usually occurring in allergic diseases.

P102

Isolation and Characterization of Dermal Lymphatic and Blood Endothelial Cells Reveal Stable and Functionally Specialized Cell Lineages

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A plexus of lymphatic vessels guides interstitial fluid, passenger leukocytes, and tumor cells toward regional lymph nodes. Microvascular endothelial cells (ECs) of lymph channels (LECs) are difficult to distinguish from those of blood vessels (BECs) because both express a similar set of markers, such as CD31, CD34, podocalyxin, von Willebrand factor (vWF), etc. Analysis of the specific properties of LECs was hampered so far by lack of tools to isolate LECs. Recently, the 38-kD mucoprotein podoplanin was found to be expressed by microvascular LECs but not BECs in vivo. Here we isolated for the first time podoplanin+ LECs and podoplanin- BECs from dermal cell suspensions by multicolor flow cytometry. Both EC types were propagated and stably expressed VE-cadherin, CD31, and vWF. Molecules selectively displayed by LECs in vivo, i.e., podoplanin, the hyaluronate receptor LYVE-1, and the vascular endothelial cell growth factor (VEGF)-C receptor, fms-like tyrosine kinase 4 (Flt-4)/VEGFR-3, were strongly expressed by expanded LECs, but not BECs. Conversely, BECs but not LECs expressed VEGF-C. LECs as well as BECs formed junctional contacts with similar molecular composition and ultrastructural features. Nevertheless, the two EC types assembled in vitro in vascular tubes in a strictly homotypic fashion. This EC specialization extends to the secretion of biologically relevant chemotactic factors: LECs, but not BECs, constitutively secrete the CC chemokine receptor (CCR)7 ligand secondary lymphoid tissue chemokine (SLC)/CCL21 at their basal side, while both subsets, upon activation, release macrophage inflammatory protein (MIP)-3αa/CCL20 apically. These results demonstrate that LECs and BECs constitute stable and specialized EC lineages equipped with the potential to navigate leukocytes and, perhaps also, tumor cells into and out of the tissues.

P103

Loss of functional activity of a MART-1-reactive Vβ14 positive T cell clone persisting in peripheral blood: a longitudinal study.

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Dendritic cell vaccination using melanoma antigens has been demonstrated to provide clinical benefits to patients with stage IV melanoma. However, it still is a matter of debate why the disease progresses clinically despite an induced specific T cell response. In this study we monitored a HLA-2^+ patient who was vaccinated with a modified MART-1 peptide. To this end, we enumerated vaccine elicited MART-1 reactive T cell numbers by HLA-A2/MART-1_{26-35} tetramer staining in peripheral blood over a period of 12 months. The number of MART-1-reactive T cells was found to be constant over time and was not correlated with the course of disease. Indeed, reactive T cells were present even at the stage of catastrophic disease progression. However, functional characterization of MART-1 reactive cells in peripheral blood by ELISPOT assay revealed a decline in the number of Interferon-γ-producing cells in response to MART-1 peptides at that time. To scrutinize this discrepancy we isolated MART-1_{26-35}-reactive T cells by means of tetramer-coated magnetic beads and subjected this population to T cell receptor clonotype mapping. Two T cell clones positive for Vβ2 and Vβ14 were found to be persisting during the observation period. Intracellular FACS-staining analyses of the $V\beta14^+$ T cell population showed a bright expression of Interferon-γ and IL-2 after maximal stimulation with PMA/ionomycin at times of disease control and a decrease of expression during tumour progression. This observation demonstrates the functional changes of specific T cell clones over the course of the neoplastic disease, i.e., the initial capacity to produce relevant cytokines and the subsequent loss of this ability. Thus, therapeutic vaccinations against melanoma may overcome this limitation by continuously inducing new T cell responses.

P104

Murine Langerhans cells on their way to the draining lymph node, visualized by a novel antibody against Langerin (CD207).

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Dendritic cells are professional antigen presenting cells that initiate primary immunity. Migration from sites of antigen uptake to lymphoid organs is crucial for the generation of immune responses. We investigated the pathways of dendritic cell migration in human and murine skin explant cultures by tracing Langerhans cells from the epidermis to the draining lymph nodes. We selectively visualize Langerhans cells on their way through the tissues with a new monoclonal antibody against a murine type II lectin, called Langerin (CD207) that is associated with Birbeck granules and is thus specific for Langerhans cells. We report the following findings: (1) In situ, fresh and activated (i.e., by 48h skin explant culture) Langerhans cells express Langerin in the epidermis and on their way through the lymphatic vessels in the dermis. (2) Emigrated (into culture medium) and isolated (by trypsinization) Langerhans cells showed intracellular expression of Langerin but reduced it upon prolonged culture periods. (3) Only few Langerin-expressing cells could be found in the skin-draining lymph nodes but their numbers increased clearly after application of the contact sensitizer TNCB. (4) In lymph node sections we observed Langerin-positive cells localized in the T cell areas, more in auricular and less in inguinal lymph nodes. This is the first time, that Langerhans cells can be directly traced from the epidermis to the T cell area of the lymph node by immunohistochemistry, and that the specific contribution of Langerhans cells (as opposed to skin dendritic cells in general) can be studied.

P105

Tolerance to nickel: oral administration of nickel induces a high frequency of anergic T cells with persistent suppressor activity

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We adapted our mouse model of allergic contact hypersensitivity to nickel (Ni) for the study of tolerance. Sensitization in this model is achieved by the administration of Ni ions with H_2O_2; Ni ions alone are unable to prime naive T cells, but can restimulate primed ones. A 4-week course of oral or i.p. administration of 10 mM $NiCl_2$ to naive mice induced tolerance, preventing the induction of hypersensitivity for at least 20 wks. When splenic T cells of orally tolerized donors, even after a treatment-free interval of 20 wks, were transferred to naive recipients, as with lymph node cells (LNC), they specifically prevented sensitization of the recipients. The LNC of such donors proved to be anergic, because upon in vivo sensitization with $NiCl_2$ in H_2O_2 and in vitro restimulation with $NiCl_2$, they failed to show the enhanced proliferation and IL-2 production as seen with LNC of mice not tolerized prior to sensitization. As few as 100 bulk T cells, consisting of both CD4+ and CD8+ cells, and antigen presenting cells, respectively, were able to transfer tolerance to Ni. A hypothesis is provided to account for this extraordinarily high frequency of Ni-reactive, suppressive T cells; it takes into account that Ni ions fail to act as classical haptens, but form versatile, unstable metal-protein and metal-peptide complexes. Furthermore, a powerful amplification mechanism, such as infectious tolerance, must operate which allows but a few donor T cells to tolerize the recipient.

P106

Immunsuppressive agents regulate proliferation and survival of human mast cells

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New immunosuppressive agents such as rapamycin, cyclosporin A, and tacrolimus are increasingly used for the treatment of cutaneous diseases. However, the effect of these substances on different inflammatory cells is not fully understood. In the present study, the effect of rapamycin, cyclosporin A, and tacrolimus on proliferation and viability of human mast cells was investigated and compared to dexamethason. Proliferation of primary cord blood-derived mast cells (CBMC) and the human mast cell line HMC-1 was measured by microscopic counting of the cells and incorporation of BrdU. To assess the viability of mast cells, the uptake of PI and binding of annexin-V was investigated by flow cytometry. In addition, the effect of immunosuppressive agents on the expression of Kit, the receptor of the growth factor SCF, was studied by flow cytometric analysis. Rapamycin, tacrolimus, and dexamethason inhibited proliferation of HMC-1 cells in a dose- and time-dependent manner (0.01-1 ug/ml, 48-96 h). In contrast, cyclosporin A failed to regulate proliferation of HMC-1 cells. In CBMC, rapamycin and dexamethason induced apoptosis after 48 h. Apoptosis of CBMC in response to deprivation of SCF was enhanced by rapamycin (1 ug/ml) after 48 and 72 h. Rapamycin also inhibited the expression of Kit on CBMC after 24 and 48 h. Thus, immunosuppressive agents are able to reduce proliferation and viability of human mast cells and may therefore potentially be used for the treatment of mast cell disorders.

P107

TNFα ind**uce**s a**pop**tosis o**f hum**an i**mma**t**ur**e **m**ast ce**l**ls

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TNFα binds to two distinct cell surface receptors, TNFαR-I (p55) and TNFαR-II (p75). Depending on the expression level and the balance between the two receptors, they can both trigger diverse cellular responses via binding to specific intracellular proteins, including proliferation, differentiation or apoptosis. TNFαR-I preferentially activates apoptotic pathways, whereas TNFαR-II rather promotes survival by inhibition of apotosis. Since TNFα has been shown to induce mediator release from mast cells, we hypothesized that TNFα might also regulate survival of human mast cells. Using FACS analysis, we examined the expression of TNFαR-I and TNFαR-II on the human mast cell lines HMC-1 and KU-812 as well as on cord blood-derived mast cells (CBMC) cultured for 8-12 weeks with SCF. Viability and apoptosis of mast cells was assessed by FACS analysis of PI uptake and staining of annexin-V, respectively. HMC-1 and KU-812 cells both expressed high levels of TNFαR-I, while CBMC failed to express TNFαR-I. Expression of TNFαR-II was found only on KU-812 cells, but not on HMC-1 cells or CBMC. Incubation with TNFα reduced viability of HMC-1 cells in a dose- and time-dependent manner (5-500 ng/ml, 24-72 h). In addition, HMC-1 cells were also found to undergo apoptosis in response to TNFα. In contrast, TNFα failed to affect survival of KU-812 cells and CBMC. Inhibition of protein synthesis by actinomycin D (1-5 ng/ml) enhanced TNFα-mediated apoptosis of HMC-1 cells, but did not render KU-812 cells or CBMC susceptible to apoptosis induced by TNFα, as described for other cell types. Thus, immature mast cells, as exemplified by HMC-1 and KU-812 cells and as found in mastocytosis, express TNFαR-I and are able to undergo apoptosis in response to TNFα, providing also a mechanism for suicidal elimination of mast cells. Expression of TNFαR-II, as found on KU-812 cells, appears to counteract TNFαR-I-mediated apoptosis.

P108

Selective expression of C5aR by M-DC8+ human blood dendritic cells facilitates C5a-induced recruitment in a SCID mouse model

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With the help of the monoclonal antibody (mAb) M-DC8 we previously identified a population of CD16-expressing human blood dendritic cells (DCs). In blood, M-DC8+ cells circulate at a significantly higher frequency as two CD16-negative DC populations ($CD11^+$ and $CD11^-$) referred to as DC1 and DC2 (M-DC8 1.1%; DC1 0.6%; DC2 0.2%; among PBMC, n=20). When staining sections of normal human skin for M-DC8-expression a small population of dermal DCs could be identified, while $CD1a^+$ Langerhans cells stained negative. Comparative phenotyping of blood DCs revealed that M-$DC8^+$ cells share most cell surface marker with either DC1 or DC2. However, M-$DC8^+$ cells stand out among blood DCs by selective expression of the receptor for the anaphylatoxin C5a (C5aR) suggesting a novel mode of DC recruitment. To study the C5a-induced migration in an in vivo model, purified M-$DC8^+$ DCs ($1x10^7$) were injected i.v. into SCID mice that at the same time received 10 µg of hrC5a intraperitoneally. Four hours later 4.5 to 7 x 10^5 M-$DC8^+$ cells could be recovered from the site of C5a-challenge. While immature upon injection, M-$DC8^+$ cells migrating into the peritoneum revealed a phenotype of highly mature cells as compared with cells cultured in parallel. Taken together we document a C5a-dependent recruitment of a major population of native human blood DCs. Responsiveness to C5a is characteristic for cells engaged in the first line of immune defense and may indicate a particular role of M-$DC8^+$ DCs in inflammation.

P109

The T cell receptor as tumor-specific antigen in cutaneous T cell lymphoma

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If appropriately processed and assembled with MHC molecules, T cell receptor (TCR) peptides could be relevant tumor-specific antigens in T cell neoplasias such as in cutaneous T cell lymphoma (CTCL). Previously, we have shown that (i) the TCRα- and β- chains of the malignant clones of six CTCL patients contain MHC class I-binding peptides within their complementarity-defining, variable, and framework regions and that (ii) TCR peptide-specific cytotoxic T cell precursors are present in the peripheral blood of these patients. It remains to be seen whether and which TCR peptide-MHC class I complexes are generated by the proteolytic machinery of this T cell tumor. Here we used purified βα-TCR-expressing CTCL cells of two patients (patient 1: Vα21-Jα50-Cα, Vβ17-Dβ1.1-Jβ1.4-Cβ1, patient 2: Vα13-Jα24-Cα, Vβ13-Dβ-Jβ2.1-Cβ1) as targets for TCR-peptide-specific T cell lines (TCL). TCR peptides were selected on the basis of the predicted MHC class I-binding ability and on the predicted likelihood of their generation by the proteasome. In one case, tumor cells were lysed with high efficacy by TCL directed at the Vα13/1-peptide, and less efficiently by TCL directed against one CDR3β, and one Cβ-derived peptide. In the other case, the tumor cells were attacked by by TCL directed against the Vα21-, one CDR3α and the Cα -peptide. In all tested cases, tumor cell lysis was blocked by anti-CD8, rather than anti-CD4 mAbs, by cold target cells pulsed with the relevant peptide, and by target cell incubation with the proteasome inhibitor Z-L3VS. Importantly, the search for proteolytically generated peptides by the prediction algorithm for proteasomal cleavage, PAPRoC, were largely consistent with our experimental results. In summary, these findings confirm that TCR chains contain multiple immunogenic epitopes that can be recognized by anti-idiotypic T cells. Our data further demonstrate that such peptides can be generated and presented by CTCL cells and suggest that a two-tailed approach combining algorithms for the prediction of MHC class I binding affinities and of proteasomal cleavage sites within the TCR-chains is useful to identify those clonotypic TCR-derived moieties that serve as relevant immunogens for anti-tumor vaccination.

P110

Expression, regulation and function of the interleukin 18 receptor on human dendritic cells of skin and blood

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Interleukin 18 (IL-18) is secreted by a variety of cells such as epithelial cells, macrophages and activated dendritic cells (DC). It potently induces -in concert with IL-12- interferon gamma production in T-cells and NK-cells. We analyzed the expression ofI L-18 receptor (IL-18R) on human dendritic cells (DC) and functional effects of IL-18 on human monocyte derived dendritic cells (MoDC). IL-18R is expressed on human dendritic cells from skin, peripheral blood and on MoDC. Preincubation of MoDC with interferon gamma upregulated IL-18R expression on MoDC as determined by flowcytometry and quantitative real-time RT-PCR. Stimulation of immature and interferon gamma pretreated MoDC with IL-18 resulted in moderate upregulation of MHC class II, CD54, CD83 and CD86, but not CD40 and CD80. IL-18 stimulation of MoDC did not induce IL-12 production and did not increase the stimulatory capacity of MoDC in coculture experiments with T-cells. However, IL-18 stimulation increased F-actin polymerisation in MoDC as an indirect measurement for chemotaxis. The chemotactic effect of IL-18 on MoDC was confirmed in Boyden chamber chemotaxis assays. In conclusion, IL-18R is expressed on human DC and upregulated by interferon gamma. IL-18 appears to have a chemotactic effect on DC rather than immunological effects and might represent a mechanism to attract DC to areas of inflammation such as in psoriasis.

P111

Association of the -174 bp promotor polymorphism of the human interleukin 6 gene with elevated serum IgE levels in atopic disease

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The pluripotent cytokine interleukin 6 (IL-6) has strong impact on the development of an acute phase inflammatory reaction. Specific and non-specific immune mechanisms including the autoantibody response are regulated in part by IL-6. Rincón et al. (J Exp Med, 1997) gave first rise of the notion that IL-6 of antigen-presenting cells leads to coactivation of CD4+ T helper type 2 (Th2) specific responses. Furthermore, an allelic polymorphism (G to C transition) was described within the promoter region of the human IL-6 regulatory region in position -174 bp in close vicinity to a CRE/B specific recognition motif for transcription factors.We have analysed this polymorphism by restriction with the endonuclease NlaIII after amplification of the IL-6 promoter region by PCR (5´-Primer: 5´-TTGTCAAGACATGCCAAGTGCT-3´; 3´-Primer: 5´-GCCTCAGAGAGACATCTCCAGTTCC-3´). 83 patients with atopic diseases and 79 healthy individuals were screened. Serum IgE levels were determined by FEIA. The genotype frequency in atopic patients with elevated serum IgE levels (>100 U/l) was increased for the rare allele 1 (46%) compared to atopic patients with normal IgE (<100 U/l) (33%). Moreover, a strong association was found when genotype and allele frequencies of patients with IgE levels >500 U/l were compared (n=20; 50%; 0.75) with patients (IgE<100 U/l) (n=39; 33%; 0.63, $p<0.05$) and healthy controls (27%; 0.53, $p<0.05$) (Fisher´s and Yates´ corrections). Allele 1 of the -174 bp IL-6 promoter RFLP shows an significant association to higher serum IgE levels in patients with allergic diseases. This genetic polymorphism is a putative marker for a strong Th2 response and elevated serum IgE levels and could be directly involved in the regulatory differences of IL-6 by involving a functional transcription factor binding site.

P112

Towards a diagnostic chip for genetic susceptibility of autoimmune bullous diseases

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The bullous autoimmune diseases pemphigus vulgaris (PV), foliaceus (PF) and bullous pemphigoid (BP) are characterized by a rise of an autoantibody response against specialized adhesion molecules of keratinocytes leading to chronic attacks of blistering. Although immunologic, cellular and molecular mechanisms of these rare diseases are well studied only very little is known on the underlying genetic background leading to clinical manifestation and sometimes fatal outcome. Therefore, a multicenter effort was necessary to merge epidemiological information on risk and trigger factors with genetic studies on allelic polymorphisms focussing on the genes of target molecules of the autoimmune response (desmoglein 1, Martel et al. 2001), human leukocyte antigens (HLA-DR, -DQ) and immunomodulatory factors like cytokines (IL-1, IL-6, TNF-α, TNF-β) and their receptors. Genomic DNA of 133 BP, 33 PV patients and 192 healthy individuals was isolated and PCR-based RFLP and SSCP analyses were performed. As BP is a candidate Th2 disease and thus, putative associations to allelic polymorphisms of IL-4 the IL-4 receptor α gene (IL-4Rα) could squench a Th1 versus a Th2 response, results on the $Q_{576}R$ IL-4Rα polymorphism is reported here. No significant associations of the phenotype and allele frequencies for the rare allele RR which can lead to enhanced receptor activity were found for BP (2%, 0.21) or PV (30%, 0.23) compared to healthy controls (26%, 0.19). When stratified for epidemiological data no allelic differences to gender, age and for example albumin serum levels, which was a significant risk for lethality in BP, could be found. Interstingly, the systemic corticosteroid dose at dismission from hospital was twice as high in carriers of allele R compared to allele Q in PV patients. There is a plethora of allelic polymorphisms in genes relevant for the development of a divergent specific immune response. Only molecular epidemiology can provide a multivaluable genetic testing for risk factors of bullous autoimmune diseases in the future.

P113

Immunomodulation by Interleukin-10 Therapy Decreases the Incidence of Relapse and Prolongs the Relapse-free Interval in Psoriasis

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The ability of Interleukin-10 therapy to reduce the severity of exacerbated psoriasis has been demonstrated recently. Considering the immunobiological properties of this cytokine we investigated the effects of long-term Interleukin-10 application on the immune system and duration of psoriasis remission. We performed a placebo-controlled, double-blind, phase II trial using Interleukin-10 in patients with chronic plaque psoriasis in remission. Patients received subcutaneous injections with either Interleukin-10 (10 µg/kg body weight; n=7) or placebo (n=10) 3 times per week until relapse or study termination after 4 month. The treatment was well tolerated. In the placebo group almost all patients (90 %) showed a relapse during the observation period. In contrast to this, only 2 out of 7 patients (28.6 %) relapsed in the Interleukin-10-treated group. Kaplan Meier analysis revealed a significantly lower relapse incidence in the Interleukin-10 than in the placebo group ($p=0.02$). The mean relapse-free interval time was 101.6±12.6 days in the Interleukin-10 group in comparison to 66.4±10.4 days in the placebo group. Immunological activity of Interleukin-10 application was indicated by an increase in soluble Interleukin-2 receptor plasma levels and higher *ex vivo* Interleukin-4 secretion capacities. Remarkably, a significant negative correlation was demonstrated between the IL-4 secretion capacity and Psoriasis Area and Severity Index score ($r=-0.36$, $p<0.01$). Our data suggest that Interleukin-10 therapy is immunological effective, decreases the incidence of relapse and prolongs the disease-free interval in psoriasis. Its value should be further determined in larger trials and for the prevention of re-exacerbation of other inflammatory disorders with a similar immunological profile.

P114

Evidence for genetic predisposition to successful immunosuppressive treatment of aplastic anemia: role of TNF-α

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The role of autoimmunity is debated in aplastic anemia because of the efficacy of immunosuppressive therapy by corticosteroids or cyclosporin A in a large portion of partients. T cell activation, apoptosis, high levels of interferon-γ and overproduction of TNF-α by bone marrow and peripheral blood derived cells is thought to be of major importance. We have studied the role of the -308 bp TNF-α promoter/enhancer polymorphism with severity as well as the response to immunosuppressive therapy in 56 patients with aplastic anemia (9 severe, 29 severe, 18 non-severe). Five patients had antithymocyte globulin (ATG)+methylprednisolone (MP), 38 had ATG+MP+cyclosporine A (CsA), one patient had CsA+G-CSF, two patients had bone marrow transplantation and one remained untreated. Therapeutical response was classified as partial or complete by standard criteria, genomic DNA was isolated and the -308 TNF-α alleles were typed by the SSCP method. Response to immunosuppressive therapy at 3 months was significantly more frequent among patients who were heterozygotes or homozygotes of the -308 TNF-α polymorphism (chi-square test responder vs. non-responder: 11/14, 79% versus 14/39, 36%: p=0.006) while the overall distribution in aplastic anemia patients did not differ from 117 healthy control individuals. An association of the rare allele TNF2 with response to immunosuppressive treatment further supports the view that genetic factors might play a role in the pathophysiology and therapeutical response of aplastic anemia. Further autoimmune diseases involving the skin like SLE, SCLE, PM-Scl-overlap syndrome and others are associated to TNF2. We are encouraged by the data presented here to investigate the severity and response rates to immunosuppressive treatment in further autoimmune diseases using molecular epidemiology.

P115

Differential influences on skin inflammation models in mice deficient of α (1,3) Fucosyltransferase VII

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Synthesis of cutaneous lymphocyte-associated antigen (CLA), which plays a pivotal part in skin homing of memory T cells via CLA/E- and P-selectin binding, is strongly dependent on α (1,3) fucosyltransferase VII (FucTVII) activity. Although deficiency of leukocyte adhesion and leukocyte recruitment in acute cutaneous inflammation has been demonstrated in FucTVII -/- mice, the effects on other more chronic immune-mediated models of skin inflammation have not been completely characterized. Sensitization and challenge with DNFB in ears of wildtype mice induced a significant induction of edema and granulocyte infiltration as assessed by ear weight, peroxidase/elastase activity and skin histology. In contrast, mice deficient of FucTVII developed no edema in response to DNFB challenge. Moreover, no cutaneous infiltration of granulocytes /neutrophils was observed in FucTVII -/- mice post challenge. Interestingly, differential effects on inflammatory parameters were seen in the model of ovalbumin-induced late phase reaction (LPR). A significant post challenge increase in neutrophil infiltration seen in wildtype was decreased in FucTVII -/- mice. However, deficiency of FucTVII did not influence the induction of total granulocyte infiltration and IL-4 secretion in this Th2-type skin inflammation model. The data point toward a more pronounced inhibition of neutrophils within the granulocyte population with no impact on eosinophils, which play a strong pathogenic role in this model. These observations demonstrate a central role of FucTVII activity/CLA-positive T cells and neutrophils in the Th1-dominant delayed type hypersensitivity/contact hypersensitivity reaction with a minor role in the Th2-dominant ovalbumin-induced LPR.

P116

Jessner´s lymphocytic infiltration of the skin: A cytotoxic T-cell pseudolymphoma?

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Jessner´s lymphocytic infiltration of the skin is a clinically benign, recurrent condition ressembling cutaneous B-cell lymphoma rather than T-cell lymphoma. In previous studies, lymphocytic infiltration of the skin has been postulated to be derived from B and CD4+ T helper cells, respectively. In order to clarify this question and to reevaluate its designation as pseudolymphoma, we here performed immunohistochemical analyses and molecular studies of skin biopsies and peripheral blood samples of 34 patients with Jessner´s lymphocytic infiltration of the skin. For determination of T-cell receptor γ-chain gene rearrangement, polymerase chain reaction and GeneScan analysis (TCRγ-PCR-GSA) were used. As previously shown by us, TCRγ-PCR-GSA is a highly sensitive and specific method.
The immunohistochemical studies showed a clear predominance of T-cell receptor α/β + T cells (average 79% cells). In about three quarters of the patients (23/30 cases), the T-cell infiltrates were mostly composed of CD8-positive cells (70%). In the remaining quarter (7/30) only 28% of the T-cells were CD8-positive including one case with less than 5% CD8-positive T-cells in the infiltrate. CD20+positive B-cells were identified in only 11.5% of the infiltrates and only 4.8% of the cells stained positive for the proliferation marker Mib-1. TCRγ-PCR-GSA analysis demonstrated an oligoclonal/polyclonal T-cell population in 9/26, a pseudo-monoclonal infiltrate in 16/26 and a monoclonal pattern in 1/26 patients. In the peripheral blood, TCRγ-PCR-GSA showed a polyclonal result in 10/14 patients, in 1/14 cases a pseudo-monoclonal result and a monoclonal T-cell population in 3/14 patients. No monoclonal T-cell population identical to a skin clone could be detected by this highly sensitive technique, in the peripheral blood.
These results demonstrate that Jessner´s lymphocytic infiltration of the skin is dominated by CD8+ T-cells in most cases which do not show clonal dominance. In analogy to well recognised monoclonal gammopathy of undetermined significance in B-cell lymphoproliferations the findings of T-cell monoclonality in the skin (1/26) and in the peripheral blood (3/14) were interpreted with caution, i.e. as T-cell clonality of undetermined significance.

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The duration of anagen phase, telogen rate and rate of hair loss of human scalp hair has high interindividual variation under physiological conditions

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To date there has been no reliable data on the physiological duration of the anagen phase of human scalp hair. Existing studies have suggested 16 to over 20 months and 19 to 21 months but it appears these figures are too low. On the other hand, there is no experimental evidence for the 4-6 years quoted internationally in textbooks and literature. In our study we determined the anagen phase of 21 normal German subjects with healthy hair (20 females and one male, aged 8-52). Prior condition was the subjects had let their hair grow to its maximum possible length. Maximal hair length (63.6±19.5 cm) and average rate of growth (0.390±0.034 mm/day) were measured, and the individual duration of the anagen phase determined (4.8±1.5 years). Therefore based on the 95% tolerance interval the physiological duration of the anagen phase in German (female) population with healthy hair varies from 1.6-8 years. The high degree of interindividual variation has a direct effect on the telogen rate and rate of hair loss. Using this 95% tolerance interval, the physiological telogen rate in the trichogram of German (female) population with healthy hair lies between 3 and 16% and the physiological rate of hair loss varies from 34-185 per day. Inside these wide margins each individual has his or her own normal values for anagen duration, telogen rate and rate of hair loss. If, in any one individual patient, the anagen phase is shorter than his personal norm, than a pathological increase in telogen rate and in rate of hair loss follows. When this occurs, the values for this particular patient are pathological even if the anagen duration, the rate of telogen and the rate of hair loss are all within the limit of the 95% tolerance interval. For example: if the anagen duration is reduced from 6.2 to 3.3 years, than the telogen rate increases from 4.2% to 7.5% and the rate of hair loss from 44/day to 83/day. If there is an individual disposition to androgenetic alopecia, this must lead to alopecia in the long term. In conclusion, one cannot interpret any individual patient´s observed rate of telogen and rate of hair loss adequately (provided these values lie inside the 95% tolerance interval) without prior knowledge of his personal normal values.

P118

Regional lymph node metastasis in melanoma patients is associated with elevated serum levels of fibroblast growth factor and endostatin

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The growth of malignant primary tumors and their metastases is dependent on the process of angiogenesis. Beyond a critical size of approximately 2 mm in diameter the supply of the malignant cells with oxygen and nutritients by diffusion is not sufficient and new vessels need to be formed before further growth can occur. The dependency of tumor growth from the formation of new vessels has been demonstrated for a variety of different neoplasias.
In tissue specimens of malignant melanoma induced angiogenesis is frequently observed, even in early stages of the disease. Furthermore, increased vascularisation of primary melanomas was shown to represent a prognostic marker for poor outcome.
Angiogenesis is controlled by a number of soluble factors secreted by the tumor cells, which might be inducing (e.g. VEGF, bFGF) or inhibiting (e.g. angiostatin, endostatin).
To investigate whether these angiogenic and antiangiogenic factors might be useful as serum markers for progressive melanoma, we measured serum levels of VEGF, bFGF and endostatin in 160 patients representing different stages of the disease.
Similar to the tumor marker S-100 all three factors were significantly increased in stage IV (AJCC) disease. However, in stage III disease only bFGF (14,2 ng/ml) and endostatin (40,4 ng/ml) were significantly increased compared to normal control levels (6,8 ng/ml or 21,1 ng/ml, repectively), whereas serum S-100 protein and VEGF were only slightly elevated.
This pilot study suggests that serum levels of factors related to tumor angiogenesis might be useful to identify patients with regional lymph node metastasis. In comparison to the classical melanoma marker S-100, which is only sporadically increased in stage III disease, these factors might allow earlier detection of progressive disease.

P119

Thyroidectomy results in the reduction of autoallergic IgE-anti-TPO antibodies and clinical symptoms in patients with chronic urticaria

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We and others have previously shown that one in four patients with chronic urticaria (CU) exhibits increased serum levels of IgG class autoantibodies against thyroid antigens such as thyreoglobulin and/or thyroid peroxidase (TPO). Since most IgG-anti-TPO+ CU-patients also show functional IgE class antibodies against TPO that induce mast cell degranulation *in vitro*, we propose that CU may represent an immediate hypersensitivity reaction against self. To test whether reducing serum levels of autoallergic IgE-anti-TPO can alter the clinical severity of CU, we have monitored serum IgG-anti-TPO, serum IgE-anti-TPO, and clinical symptoms in two IgE- and IgG-anti-TPO+ CU patients subjected to thyroidectomy. Blood samples were obtained from CU patients before and at defined timepoints following thyroidectomy and serum IgG-anti-TPO levels were determined by standard ELISA. For quantification of serum IgE-anti-TPO, IgE-fractions were prepared by ammonium-sulfate precipitation followed by ultrafiltration and protein-G-affinitiy purification to eliminate interfering IgG. Pre-thyroidectomy ELISA-based detection of serum IgE-Ab bound to recombinant human TPO confirmed the presence of IgE-anti-TPO as assessed by an IgE capture-immunoprecipitation assay using radio-labelled TPO. Serum IgG-anti-TPO levels dropped rapidly to 55% by week 2 after thyroidectomy and continued to decrease for ten weeks following surgery (pre-thyoidectomy: >1000 IU/ml vs. week 10: 186 IU/ml). Interestingly, IgE-anti-TPO serum levels also declined continuously and markedly by 56% during the first two weeks following thyroidectomy, showing a 64% decrease in week 10 after surgery (5.92 U/ml vs. 2.12 U/ml). Most notably, this drop in IgE-anti-TPO following thyroidectomy was associated with a pronounced reduction of clinical symptoms as assessed by scoring CU activity (weal numbers and itch), i.e. the number of weals decreased from > 100 per day before thyroidectomy to five weals per day in week 10 after surgery. These data provide proof of principle that reducing levels of autoallergic antibodies and/or autoallergens can result in the clinical improvement of CU. Our findings support the view that autoallergy is a relevant pathomechanism in some patients with CU.

P120

Regulation of hair growth by activin and follistatin

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Members of TGFβ/BMP family are involved in the control of hair follicle morphogenesis and cycling. The activity of several members of this family (activin, BMP-2, 4, 7) is usually antagonized by follistatin. Since activin-follistatin interaction has been shown to play an important role in development and functions of many organs, we have explored its role in hair follicle morphogenesis and/or cycling. Compared to wild type controls, both activinβA transgenic mice and follistatin knockout mice showed a significant retardation of hair follicle morphogenesis. The follistatin null mutation mutation also significantly reduced keratinocyte proliferation in stage 1-2 hair follicles. In embryonic skin organ culture, recombinant follistatin accelerated hair follicle development, and this effect was inhibited by the addition of recombinant activin A. In situ hybridization revealed that follistatin is expressed in outer root sheath cells, hair matrix cells and basal cell layer of the epidermis in E18.5 mouse skin. In organ culture of human anagen VI scalp hair follicles, recombinant follistatin inhibited hair shaft elongation and induced hair follicle regression (catagen). These results implicate an important function of activin and follistatin in the control of hair growth.

P121

INDICATIONS TO A ROLE FOR KIT-LIGAND (SCF) IN EPITHELIAL CELL BIOLOGY

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In mammalian skin, Kit-ligand (SCF) regulates maintenance and proliferation of mast cells and melanocytes expressing the cognate Kit-tyrosinekinase receptor (Kit). To clarify whether Kit/SCF signaling also plays a role in epithelial growth control, we investigated Kit and SCF immunoreactivity (IR) during C57BL/6 mouse hair follicle cycling. We found that telogen skin (only melanogenically inactive melanocytes) displayed few clustered Kit-IR cells in the secondary hair germ. As expected, anagen hair follicles showed strong Kit-IR in the follicle pigmentary unit above the dermal papilla. However, an additional cobble stone-like immunoreactivity pattern of a large, heterogeneous group of cells with apparent epithelial morphology was observed in the most proximal hair bulb. These cells were negative for standard melanocyte-markers. By electronmicroscopy they had characteristics of immature epithelial cells but not of melanocytes, containing tonofilaments, desmosomes, and hemidesmosones, and lacking melanosomes, and some where even mitotic. Comparable groups of Kit-IR cells were detectable in the white hair bulbs lacking melanocytes (C57BL/6-mice treated with Kit-neutralizing antibody, Sl/Sld mice lacking melanocytes due to an SCF defect). The proximal epithelial hair bulb expressed substantial SCF immunoreactivity during anagen, which corresponded to high levels of SCF mRNA (semi-quantitative RT-PCR) and SCF protein (Western blot) in anagen skin homogenates. This raises the possibility of autocrine Kit/SCF signaling by matrix keratinocytes. Mice unable to respond to SCF stimulation (Kit-negative W/Wv) showed a very discrete, but significant retardation of induced anagen development. These results suggest that SCF may be a growth/maintenance factor for selected hair follicle keratinocyte subpopulations.

P122

Corticotropin releasing hormone and human sebocytes

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A hypothalamic-pituitary-adrenal axis (HPA) equivalent pathway has been proposed to function in mammalian skin in response to dyshomeostatic stimuli. Sebaceous glands express receptors for alpha-melanocyte-stimulating hormone (alpha-MSH), vasoactive intestinal polypeptide, neuropeptide Y, and calcitonin gene-related peptide (CGRP). alpha-MSH and CGRP are not produced by human sebocytes but regulate their cytokine synthesis in a paracrine manner. Because corticotropin releasing hormone (CRH) is the most proximal element of the HPA axis and acts as the central coordinator of the neuroendocrine responses to stress, we investigated the mRNA levels of CRH and CRH receptors (CRH-R) in SZ95 sebocytes and their regulation by CRH itself and by several hormones using TaqMan RT-PCR. Cell necrosis was detected by the LDH releasing assay, IL8 synthesis by ELISA and lipid synthesis by the nile red fluorescence assay. CRH mRNA was neither expressed nor induced by CRH itself or the hormones tested. In contrast, CRH-R1 and CRH-R2 mRNA were detectable in SZ95 sebocytes. CRH-R1 was the predominant type (CRH-R1 / CRH-R2 ratio=200 at 6 h). CRH-R1 levels significantly increased at 24 h (24 h / 6 h ratio=2). CRH did not regulate CRH-R expression. Testosterone at a high concentration (10^{-7} M) annulled CRH-R1 and CRH-R2 mRNA expression, especially at 24 h. GH (10^{-7} M) annulled CRH-R1 and increased CRH-R2 mRNA levels at 24 h. Dehydroepiandrosterone, insulin-like growth factor-I and 17beta-estradiol barely influenced CRH-R mRNA levels. All hormones tested induced no cytotoxic effect. CRH did not affect spontaneous IL8 secretion by sebocytes but significantly inhibited IL-1alpha-enhanced IL8 secretion in a dose-dependent manner. CRH did not exhibit a marked effect on sebocyte lipid synthesis, while IL-1alpha stimulated synthesis of neutral and polar lipids. In conclusion, CRH is a paracrine hormone for human sebocytes with a homeostatic anti-inflammatory activity. The low CRH-R1 mRNA levels in testosterone and growth hormone-treated sebocytes correlate with those found in telogen hair follicles and may indicate a dysregulatory pro-inflammatory effect of these hormones.

P123

Decreased activity of membrane bound neutral sphingomyelinase correlates with impaired epidermal differentiation in atopic dermatitis

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A defect in permeability barrier function, leading to the penetration of environmental allergens into skin is crucially involved in the pathogenesis of atopic dermatitis. This process induces immunological reactions leading to inflammation. Previous studies proposed that the decreased level of stratum corneum ceramides may be the cause for the defect in permeability barrier function in atopic dermatitis. We recently found that acid sphingomyelinase (A-SMase) localized in the endosomal epidermal lamellar bodies generates specific ceramides for permeability barrier function, whereas membrane localized neutral sphingomyelinase (N-SMase) generates ceramides from sphingomyelin for signaling in epidermal differentiation. The aims of the present study were to determine: 1) whether A- and N-SMase activities; 2) epidermal differentiation; 3) and/or epidermal proliferation are impaired in atopic dermatitis. By using specific enzyme assays we found unchanged A-SMase activities in lesional and non-lesional skin of patients with atopic dermatitis compared to healthy controls. In contrast, N-SMase activity was reduced by 63% ($p<0.025$, n=10) in non-lesional and by 85% ($p<0.005$, n=10) in lesional skin. As a marker of epidermal differentiation a premature expression of involucrin, was shown by immunocytochemistry in atopic dermatis lesional skin. The epidermal expression of the differentiation associated keratins K10 was reduced, whereas the expression of the basal keratin K5 was increased in diseased skin. Epidermal proliferation showed a four fold increase in lesional skin and was inversely correlated to differentiation. In summary, endosomal localized A-SMase is unchanged in atopic dermatitis, but we found a severely reduced membrane bound N-SMase activity which correlates with a changes in the expression of differentiation related proteins. Impaired regulation of epidermal differentiation by N-SMase derived ceramides, influencing permeability barrier function, may be of importance for the pathogenesis of atopic dermatitis.

P124

Neurotrophins as members of the cytokine network in the skin – role in pathogenesis of Atopic Dermatitis

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Neurotrophins (NT) are a family of structurally and functionally related polypeptides with up to 50 % of amino acid homology. They were originally described as regulators of neuronal proliferation, differentiation, survivial and function. It was previously shown that NT are also expressed in the skin. Atopic Dermatitis (AD) is known as an inflammatory skin disease with increased mast cell numbers, influenced also by the nervous system. We investigated the expression of NT-3, NT-4, NGF, BDNF and the NT receptors TrkA, TrkB, TrkC and p75 in normal skin (n = 13) and in the skin of patients with AD (n = 27) by immunohistochemistry. The serum levels of NT were detected by ELISA and mast cell numbers in the skin with toluidine blue staining and using tryptase- and chymase-specific antibodies. The NT mRNA expression is demonstrated by RT-PCR. We found an increased protein expression of TrkA, TrkB and p75 and a changed staining pattern for TrkC in the skin of patients with AD compared to normal skin. NT-3, NT-4 and NGF were also found to be increased, whereas BDNF expression was clearly decreased. The serum levels of all NT were significantly higher in patients with AD compared to normal persons. Skin mast cells are able to produce NT-3, NT-4, NGF and BDNF and to express the high affinity NT receptors TrkA, TrkB and TrkC, but not p75. Therefore, higher mast cell numbers are possibly one reason for increased NT and NT receptor expression in the skin and the serum of patients with AD. In addition, NT are potential mediators between mast cells and nerves during inflammatory processes.

P125

Stromal cell-derived factor (SDF) in human skin wound healing: simultaneous up and down regulation of SDF at different sites of the wound and induction of IL-4 in mast cells in vitro

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During wound healing the expression patterns of
chemokines tightly regulate the spatial and temporal infiltration of invading leukocyte subsets. Using a human skin wound repair model and an tissue array approach, we have tested additional chemokines (n=18). We discovered macrophage derived chemokine MDC, hemofiltrate CC chemokine HCC-1 and Exodus-2(6Ckine), Exodus-3 (MIP-3ß) and stromal derived factor SDF which were differentially expressed during wound repair. MDC was expressed by macrophages and Exodus-2 was strongly expressed by both macrophages and endothelial cells (EC) around the fibrous stroma. HCC-1 and Exodus-2 were found to be constitutively expressed in EC of adjacent normal skin whereas SDF was expressed both in resident cells (EC and fibroblasts) and passenger cells (monocytes/Macrophages). After wounding expression of Exodus-2 and in particular SDF were upregulated in the foci of strong inflammation around the developing fibrous stroma, whereas specific mRNA was not detectable in the neostroma until day 10 for Exodus-2 and even 49 days for SDF. Constitutive expression of HCC-1 was both downregulated around and within the fibrous stroma until day 10. Therefore, these chemokines contribute to infiltration of lymphocytes and monocytes/macrophages during wound healing. The prominent bimodal expression profile of SDF prompted us to further investigate its regulation and function in vitro. In vitro SDF was also constitutively expressed in skin endothelial cells and fibroblasts and addition of TNF?and/or IFN led to a dose dependant down-regulation. Most notably, addition of SDF to mast cells which carry the corresponding SDF-receptor CXCR4 induced synthesis of IL-4. This indicates that the chemokine SDF influences wound repair besides direct stimulation of resident cells and passenger cells carrying the corresponding CXC receptor 4 (CXCR4) also indirectly by stimulating e.g. fibroblasts via mast cells produced IL-4.
Our in vivo data have completed the set of chemokines being involved in wound healing and support the concept that a differential expression (up- and downregulation) of multiple chemokines with chemotactic and angiogenic (SDF) or angiostatic (Exodus-2) properties plays an important role in wound healing.

P126

RNase 7 in inflammatory skin diseases - detection of immunoreactivity using murine monoclonal antibodies

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Regarding constant exposure of the human skin to diverse microbial organisms, the incidence of clinically manifest infections is low. Apparently potent mechanisms are operative to protect the skin from such microbial attack. In this respect recent studies have demonstrated different antimicrobial proteins which are either constitutively expressed or are induced during infection. Among these, a novel 18-20 kD cationic protein with broad activity against staphylococcus aureus, pseudomonas aeruginosa, propionibacterium acnes and candida albicans could be characterized and defined as RNase7. Balb/c mice were immunized with natural protein purified from psoriatic scales. Several monoclonal antibodies could be raised one of which produced distinct epidermal reactivity in acetone fixed cryostat sections as well as in formaldehyde fixed, paraffin embedded sections of human skin. Whereas in normal skin all epidermal layers were found positive, immunoreactivity is shifted to upper layers below and within parakeratotic layers around microabscesses in chronic plaque type psoriasis. A similar pattern is found in eczema whereas diffuse epidermal staining is found in folliculitis. Surprisingly, dermal vasculature is stained as well. These data may supplement RNA detection in keratinocytes and add further evidence to the importance of antimicrobial peptides in skin disorders other than overt infectious diseases.

P127

A revival of urine PCR for the diagnosis of Lyme Borreliosis

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Laboratory methods for Lyme borreliosis (LB) are still a matter of controversy. Clinical diagnosis of the various clinical manifestations can often be confirmed serologically. Due to the fact that the value of this method is limited, the polymerase chain reaction (PCR) was chosen again for the detection of LB from urine. This method had been performed 5-10 years ago but left because of technical problems.
Methods: 121 serial urine samples were investigated from 36 erythema migrans (EM) patients before and up to 1 year after treatment. A culture of B. burgdorferi (Bb) served as positive, water as negative control. Urine was centrifuged at different speeds of rotations. Extraction of DNA from urine pellets was not successful below 14000 x g. Different DNA-extraction procedures were compared: alcaline lysis, chelex, puregene-, geneclean- and quiagen-kit, extraction with magnetic beads. Finally, extraction by DNAzol was done from urine pellets using a guanidine-detergent lysing solution. Nested PCR using flagellin primers was then performed and PCR products were visualised on agarose gels at 276 KD. Results were confirmed by sequencing or hybridisation of the amplicons.
Results: Only after DNAzol extraction, patient`s urine samples were positive. Typical bands were detected in 21/29 (72,4%) of EM patients before, 32/44 (72,7%) 3 weeks, 9/19 (47,4%) 3 months, 1/13 (7,7%) 6 months , 1/16 (6,3%) 1 year after treatment, whereas the negative controls and urines of 20 healthy persons were all negative. Sequencing identified flagellin from Bb in all 10 investigated amplification products. By hybridisation, B.afzelii was identified in 52/67 of the investigated samples. Freezing of urine at -80°C did not influence sensitivity. However, freezing of extracted samples reduced sensitivity drastically.
Conclusion: This PCR method showed a substantially better sensitivity than serological methods in EM patients. Follow-up of Bb-DNA excretion pattern gives the basis of the valuability of urine PCR. This method may be of importance in future for the diagnosis of unclear, unrecognised borrelia infections and diseases with a possible relation to LB. Furthermore, the need for antibiotic treatment could be better controlled by the use of this method.

P128

Biofilms of *Pseudomonas aeruginosa*: A new approach for understanding *P. aeruginosa* skin infections

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The gram-negative bacterium *Pseudomonas aeruginosa* (PA) is an ubiquitous opportunistic human pathogen which causes a variety of epithelial infections. The success of *P. aeruginosa* to colonize epithelia is due to its broad spectrum of virulence factors including multiple cell-associated factors which are induced under stress conditions. The ability of these virulent pathogens to form so-called biofilms as the consequence of density quorum-sensing is of high relevance for its pathogenity. It is well known that biofilms are highly resistent against several antibiotics, thus, the mucoid (biofilm-forming) bacteria represents the infectious and dangerous form of PA.
Therefore the aim of this study was to analyse whether human keratinocytes are capable of distinguishing between highly pathogenic biofilm-forming *P. aeruginosa* and non-biofilm forming *P. aeruginosa*.
We investigated different cultures of PA incubated under stress conditions and evaluated their ability to induce the production of inducible antimicrobial peptides (hBD-2, hBD-3) and proinflammatory cytokines (like IL-8) in primary human keratinocytes. The mRNA-expression in keratinocytes was measured by using RT-PCR and Realtime-PCR (LightCycler). We found that biofilms were formed under static growth conditions with low oxygen and starving conditions. These bacteria as well as supernatants of them showed a highly increased induction of hBD-2 and hBD-3 peptides and proinflammatory cytokines such as IL-1β, TNFα and IL-8 compared to non-biofilm bacteria. Stimulation experiments with PA culture supernatants revealed that highly pathogenic biofilm-forming *P. aeruginosa* release activities which are able to induce cytokines and/or antimicrobial peptides specifically. RP-HPLC of both the lipid-like and hydrophilic antimicrobial peptide- and cytokine- inducing activity revealed elution in single fractions indicating a panel of different inducing bacterial molecules. A molecular characterization of these molecules is currently in progress.
In conclusion, our findings support the hypothesis that human skin keratinocytes recognise highly pathogenic biofilm-forming microorganisms such as *P. aeruginosa* bacteria via different pathogen-associated molecules to mount an epithelial defense reaction by producing either antimicrobial peptides or proinflammatory cytokines to recruit proffesional phagocytes.

P129

Tissue sources for molecular diagnosis of Treponema pallidum infection.

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Introduction. *Treponema pallidum* (TP) infection may be difficult to diagnose in certain conditions, such as HIV infection, neurolues, neonates or treatment failure. Therefore, we investigated which blood or tissue samples can be used for molecular detection of *T. pallidum* DNA (TP DNA). Serum (Se), peripheral blood mononuclear cells (PBMC), granulocytes (Gr), sperm (Sp), lymph node (LN) aspirates, and skin rash biopsies (SR) were obtained from patients with suspect or diagnosed *T. pallidum* monoinfection or HIV coinfection.
Materials and methods. Biological samples were obtained from 15 patients. 12 suffered from early lues (L) (L I: 2; L II: 7; L latens (L lat): 3). 3 patients were suspected of active L: VDRL-, TPHA-, IgG-FTA-ABS positive, 19S-IgM-FTA-ABS negative or borderline positive. DNA was extracted by using DNAzol®BD reagent from the samples (Se, PBMC, Gr, SP, LN, SR). Presence of TP was determined by nested PCR using primer sets for a TpN47 DNA fragment according to standard techniques. The PCR product was analysed by ethidium bromide staining, and by Southern followed by specific hybridization with autoradiography.
Results. TP DNA was detected in Se (L I, L II but not in L lat); in PBMC (L II, L lat, but not in L I); in Gr (L II, L lat, but not in L I); in SP (L I, L II, but not in L lat); in SR (L II); in LN (L II, L lat). The TP DNA was also detectable in patients with serological suspected lues in Se and PBMC.
Conclusion. Se and PBMC appear as the best sourses for detection of TP DNA. Our data demonstrate that TP specific PCR is a sensitive method for detection of TP infection. It may be particular useful in conditions with questionable infection.

P130

The Neuropeptides VIP and PACAP Upregulate Expression and Release of Cytokines and Cell Adhesion Molecules in Human Microvascular Endothelial Cells via VPAC type 1 receptor.

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Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP) belong to the same superfamily of neuropeptides which exert their effects by activating G protein-coupled receptors defined as VIP/PACAP receptors (VPAC-R). So far, three receptor subtypes exist. Because VIP and PACAP appear to play a crucial role in cutaneous inflammation and vasoregulation we examined expression and biological effects of these peptides in human microvascular endothelial cells (HDMEC, HMEC-1). Moreover, we determined which VPAC receptor subtypes are expressed by HDMEC cells. We detected expression of PACAP and VPAC type 1 receptor at RNA- and protein level by RT PCR and immunohistochemistry indicating an autocrine regulatory mechanism. cAMP assays revealed VPAC-1 receptor to be functional in these cells. RT PCR showed upregulation of IL-6 and IL-8 in a time- and dose-dependent manner. ELISA experiments confirmed release of IL-6 and IL-8 by HDMEC cells. We also investigated cell adhesion molecule expression after stimulation with VIP and PACAP. ICAM-1 mRNA was upregulated at 3 and 6 h after treatment with VIP and PACAP while VCAM was only upregulated maximally at 6 h after PACAP stimulation indicating diverse regulation of cell adhesion molecules by VPAC receptors in human endothelial cells. Immunoreactivity for VPAC-1R was enhanced in microvascular endothelial cells of patients with atopic dermatitis and urticaria indicating upregulation of this receptor in endothelial cells during cutaneous inflammation. In summary, VIP and PACAP may play an important role in cutaneous neurogenic inflammation by activating VPAC-1 receptor on dermal microvascular endothelial cells.

P131

Aromatase and oxidative 3a-hydroxysteroid dehydrogenases are present in human hair follicles and regulate intrafollicular DHT levels

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Dihydrotestosterone (DHT) is the most important trigger of androgen-dependent hair growth. Without sufficient DHT levels neither androgenetic hair loss nor beard growth can be observed. The intrafollicular concentration of DHT is mainly regulated by steroid 5a-reductase which metabolizes testosterone to DHT. However, recent data from other tissues revealed that DHT levels can also be modulated via the action of aromatase or oxidative 3a-hydroxysteroid dehydrogenases (3HSD). We therefore addressed the questions whether aromatase and 3HSD are present in human hair follicles and whether their enzymatic activity can be modulated. By the use of immunohistochemistry, quantitative analysis of steroids with HPLC and direct measurement of enzyme activity with liquid szintillation counting we were able to detect aromatase mainly in the epithelial parts of the hair follicle, whereas 3aHSD was mainly expressed in the dermal papilla. In addition, we were able to show that aromatase activity in isolated intact human hair follicles is modulated by 17a-estradiol. In comparison to the controls, we noticed a concentration- and time-dependent increase of aromatase activity in 17a-estradiol-incubated female hair follicles (e.g. 24h: 1nM = + 18%, 100nM = + 25%, 1uM =+ 57%; 24h: 1nM = +18%, 48h: 1nM = +25%). Moreover, we noticed that the dermal papilla is able to convert the weak androgens 3a-androstanediol and androsterone back to DHT via 3aHSD. In summary our ex vivo results suggest that aromatase and 3aHSD are present in the hair follicle and act antagonistically. In theory, the aromatase pathway may diminish the amount of intrafollicular testosterone available for conversion to DHT, whereas 3aHSD adds additional DHT. The action of aromatase may therefore inhibit, whereas 3aHSD may accellerate, DHT-dependent hair growth or loss. In conclusion, our results suggest that increasing aromatase or inhibiting 3HSD activity are novel approaches to stop the development or progression of DHT-mediated processes of hair growth such as androgenetic alopecia.

P132

Inhibition of Basophil Chemotaxis and p38 MAP Kinase Phosphorylation Due to Ambroxol.

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Previous studies have shown that ambroxol abrogates IgE-dependent mediator secretion from human skin mast cells and basophils, which may explain the anti-inflammatory properties of this drug observed in animal models of antigen-induced allergic reactions. Our aims in the present study were to investigate the effects of ambroxol on both the phosphorylation of key signals involved in IgE-dependent basophil activation and chemotaxis induced by RANTES. Ambroxol (10-100 µM) strikingly reduced p38 MAP kinase activity caused by anti-IgE stimulation of basophils and reduced the RANTES-induced migration of these cells, in Boyden Chambers, by over 55 % at 100 M. In contrast, N-acetylcystein (100 M 1mM), which is also currently used clinically as a mucolytic agent, had no inhibitory effect in the above experiments. The inhibition of p38 MAP kinase activity by ambroxol demonstrates that this drug specifically modulates basophil signal transduction rather than non-specific physico-chemical actions. We have recently shown that p38 MAP kinase crucially regulates the release of both histamine, leukotrienes and cytokines from basophils. By targeting signals which activate this enzyme may provide an explanation as to the mode of action of ambroxol regarding blockade of mediator release. The kinase is also known to control cytoskeletal rearrangement thus indicating why ambroxol also inhibits basophil migration. Since ambroxol reduces both the migration and activity of basophils this drug may have therapeutic relevance in the treatment of allergic inflammation.

P133

Induction of apoptosis in human T-cells by fumaric acid esters

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Psoriasis is a chronic inflammatory and hyperproliferative skin disorder in which T-cells play a prominent role. There is evidence for (auto)-antigen-specific activation of T-cells in psoriasis. Treatment strategies targeting activated T-cells have been found to be highly effective.
Fumaric acid esters (FAE) are used for systemic therapy of psoriasis for over 40 years. Several studies have shown that T-cells are a main target for the action of FAE. Induction of interleukins 4 and 5 as well as inhibition of proliferation are among the mechanisms of different FAE.
We have previuosly shown that dimethylfumarate (DMF) induced apoptosis in human monocyte-derived dendritic cells. The aim of the present study was to investigate the effect of various FAE on the induction of apoptosis in human unstimulated and stimulated T-cells.
Purified human T-cells were obtained from healthy donors by Ficoll-paque density centrifugation followed by negative selection using magnetic beads (MACS). T-cells were co-cultured with various concentrations of either methylhydrogenfumarate (MHF), methylhydrogenfumarate-calciumsalt (CaMF), DMF, a respective DMSO-control or medium alone for 48 hours. Cells were stimulated with interleukin 2 (IL-2, 200 U/ml), solid-phase bound OKT3, IL-2 and OKT3, or were left unstimulated in medium alone. Thereafter apoptosis was measured using labelling with Apo 2.7 and by using the TUNEL-assay by flow cytometry.
The results of our study showed, that MHF, CaMF, and DMF induced apoptosis in unstimulated T-cells at high concentrations (50 µg/ml) In stimulated T-cells apoptosis was induced by DMF already at a dose of 10 µg/ml whereas MHF and CaMF only induced apoptosis at high concentrations (50 µg/ml).
Our data demonstrate that induction of apoptosis preferentially in stimulated human T-cells by DMF, the main ingredient of the marketed FAE-mixture, is a possible mechanism of action of this compound in psoriasis.

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Spatial Distribution of 8-Methoxypsoralen (8-MOP) Penetration into Human Skin after Systemic or Topical Administration

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Although systemic and topical PUVA-therapy is a dermatological standard therapy little is known about the time course and distribution of 8-MOP penetration. We previously determined the time course of 8-MOP penetration into skin by microdialysis. However, microdialysis does not allow the determination of spatial distribution of a drug. Therefore we now assessed 8-MOP concentrations in horizontal epidermal and dermal skin sections after oral or topical 8-MOP.
4 mm punch biopsies of patients undergoing oral (n = 4), bath (n = 3) or cream (n = 4) PUVA therapy were taken at different time points (30 to 180 minutes) after 8-MOP application. The frozen biopsies were sliced in parallel to the skin surface: the first five 50 µm slices were localized within the epidermis and the subsequent seven 250 µm sections corresponded to papillary dermis. Liquid chromatography coupled with tandem mass spectrometry was employed for the determination of 8-MOP concentrations in skin sections.
After topical application epidermal 8-MOP concentrations were significantly higher than those after oral 8-MOP (p=0.05). Oppositely, concentrations in papillary dermis were significantly higher after oral 8-MOP. Epidermal concentrations following cream or bath were highest at the end of the application period. In contrast, epidermal and dermal concentrations remained relatively constant between one and 3h after oral 8-MOP.
The time courses of 8-MOP concentrations confirms the data obtained by microdialysis and correspond with the time courses of photosensitivity. Furthermore, on the basis of these data we suggest that topical 8-MOP may be superior for dermatoses where the pathology is localized in the epidermis while in sclerosing diseases which mainly affect the dermis oral PUVA might be superior due to higher dermal concentrations.

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EFFECT OF CURCUMA LONGA EXTRACT ON THE EXPRESSION OF PROINFLAMMATORY CYTOKINES

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Curcuma longa is a medicinally active plant and spice. In the form of the herbal powder turmeric, it has been used as an anti-inflammatory remedy in Asiatic medicine. With regard to psoriasis, we tested the effect of a hydro-alcoholic extract of Curcuma longa rhizome on the UV induced expression of IL-6 and IL-8, at the protein and mRNA level in cultures of human keratinocytes (HaCaT) using enzyme immuno assays and a RT competitive multiplex PCR. After incubation of the keratinocytes with 5-50µg/ml Curcuma extract for 24 h the UV induced secretion of IL-6 and IL-8 was similarly suppressed as with betamethasone-17-valerate. These results corresponded with an inhibition of the UV induced enhancement of IL-6 and IL-8 mRNA. In order to check, whether the observed suppression of interleukin secretion is the result of general cell damage, the incorporation rates of BrdU and 14-C-amino acids were tested. The cell proliferation rate and the rate of protein synthesis were only slightly affected at high concentrations (50µg/ml) of Curcuma longa extract. In conclusion, the down regulation of IL-6 and IL-8 would contribute to an anti-inflammatory response. In addition IL-6 and IL-8 are considered to be autocrine and/or paracrine growth factors for keratinocytes. Inhibition would therefore reduce possible trigger factors of psoriatic keratinocyte hyperproliferation.

P136

Skin-expressed genes regulated by retinoids: CYP26, psoriasin (RIS-1) and CRABP-II

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CYP26 (cytochrome P450RAI), psoriasin (RIS-1) and CRABP-II are skin-expressed genes specifically regulated by retinoids. CYP26 encodes for the all-*trans*-retinoic acid (atRA)-4-hydroxylase which catalyzes atRA metabolism to biologically less active metabolites. Psoriasin is inducible after local skin exposition to atRA. CRABP-II (cellular retinoid acid-binding protein II) is an early marker of retinoid activity in the skin. It works as a transport protein and probably mediates retinoic effects. Aim of this study was to examine the expression of the above mentioned genes and their regulation by retinoids in the skin and in cultured skin cells. Expression at the mRNA level was examined in cells treated with atRA, 13-*cis*-retinoic acid and in untreated control by RT-PCR and Northern blotting. Additionally, we used in situ hybridisation to localize these genes in untreated cells and in the skin. RT-PCR detected CYP26 mRNA in keratinocytes, preputial fibroblasts and SZ95 sebocytes but not in preputial melanocytes, endothelial cells and HLA-60 cells. Psoriasin mRNA was found in keratinocytes, preputial fibroblasts and SZ95 sebocytes. CRABP-II could be shown by RT-PCR analysis in all examined cell types. The northern blotting analysis showed psoriasin mRNA expression in preputial keratinocytes and preputial fibroblasts. Interestingly, facial fibroblasts did not express detectable psoriasin mRNA levels. The in situ hybridisation localized psoriasin mRNA in epidermal and follicular keratinocytes of the stratum granulosum. These findings indicate the dominant role of epithelial skin cells as major targets of local retinoid treatment.

P137

Differential modulation by retinoids of CD14 expression in sebocytes

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Sebocytes are known to regulate androgen homeostasis within the skin, and may play a central role in the regulation of inflammation and immune functions following skin infection. We have previously suggested that the SZ95 sebaceous gland cell line expresses cytoplasmic CD14 but not upon the cell surface. Retinoids are efficacious in the treatment of acne through their inhibition of sebaceous gland activity. We address here a potential mechanism of retinoid action on SZ95 sebocytes through a possible modulation of CD14 expression. We examined the action of the retinoids, retinol, all-*trans*-retinoic acid (atRA) and 13-*cis*-retinoic acid (13cRA) upon CD14 mRNA expression after acute (1 h) and prolonged (24 h) exposure. Changes in CD14 expression were compared to the action of hydrocortisone, using the TaqMan quantitative RT-PCR method. 13cRA suppressed CD14 expression, upon acute, but not prolonged incubation (n=3, $p<0.05$), while hydrocortisone suppressed expression at both time points. Preliminary data suggest that retinol increases CD14 expression dramatically following acute and prolonged incubation, whereas tretinoin was without significant effect. These data may explain the reported anti-inflammatory activity of 13cRA. Future research will concentrate upon the role of CD14 in the immune-inflammatory response of human sebocytes.

P138

Oxidized eicosanoids in UV-irradiated human skin and HaCaT-cultures after administration of anti-inflammatory drugs usingmicrodialysis technique

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UV-irradiation of the human skin leads to induction of oxidative stress and inflammation mediated by reactive oxygen radicals, lipid peroxidation, liberation of arachidonic acid from membrane phospholipids and formation of prostaglandins and leucotrienes. Therefore, we investigated lipid mediators, such as 8-epi-PGF2a, 9a11a-PGF2a, HETEs and LTB4 in the dermal interstitial fluid obtained in vivo by cutaneous microdialysis technique and in vitro from keratinocyte (HaCaT) cultures after UV-irradiation and application of diclofenac, a nonsteroidal anti-inflammatory drug. Defined areas on the volar forearm of 10 healthy volunteers were exposed to UVB irradiation (20-60 mJ/cm^2). After 24 h, microdialysis membranes were cutaneously inserted beneath the irriadiated area and diclofenac was administered topically. The probes were perfused with isotonic saline solution and microdialysate samples were collected at 20 min intervals over up to 6 hours. Analysis of oxidized arachidonic acid derivatives using sensitive NICI-GC-MS showed enhanced amounts of 2-, 3-, 5-,12- and 15-HETE, LTB4, 8-epi-PGF2a and 9a11a-PGF2a in the picomol range after UV irradiation, which were suppressed by topical diclofenac. Further investigations may show whether these new findings may also be relevant to validate therapeutical strategies for other inflammatory skin conditions.

P139

Triple-helix formation at the ICAM-1 gene in living cells

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At certain sequences, the DNA doublehelix can be bound by oligonucleotides. The resulting triplehelix may interfere with gene transcription. We have previously designed a triplex-forming oligonucleotide (TFO) for a target sequence within the transcribed portion of the human ICAM-1 gene, which specifically inhibited ICAM-1 surface expression when it was transfeced into the keratinocyte-derived cell line A431. The demonstration of TFO binding to its ICAM-1 target sequence is an important experimental affirmation that the observed biologic effect (inhibition of ICAM-1 expression) is indeed due to sequence specific interactions. Using restriction enzyme protection assays, TFO binding was demonstrated on isolated DNA stretches containing the ICAM-1 target sequence. In order to demonstrate TFO binding in settings approaching the situation in vivo, e.g., when the target sequence is contained within whole genomic DNA or even in chromosomally organized nucleosomal DNA, a novel sensitive biotin/streptavidin-based magnetic capture assay was developed. In this assay the TFO was biotinylated allowing capture of DNA with streptavidin-coated magnetic beads after triplex-formation. Captured DNA was quantitated by conventional and by realtime PCR (Light cycler). Triplex-formation was demonstrated when TFO was reacted with purified whole genomic DNA and also with DNA extracted from TFO-exposed cell nuclei freshly prepared from A431 cells. Furthermore, triplex-formation was detected even in living cells after TFO was transfected into A431 cells and subsequently cellular DNA was analyzed by magnetic capture/realtime PCR for triplex formation. The triplex formation appeared to be sequence specific, because two different scrambled control oligonucleotides showed only background signals. Collectively, the data demonstrate that TFO has access to its ICAM-1 target sequence and forms a triplex within the chromosomal/nucleosomal context of living cells. The results support the view that TFOs may be used as specific anti-gene drugs in cutaneous anti-inflammatory or anti-cancer strategies.

P140

Resistance to TRAIL- and Fas-induced apoptosis is mediated by different mechanisms in human melanoma cells

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Human melanoma cells respond differently to the death inducing ligands TRAIL and Fas-ligand (FasL). While A375 cells rapidly undergo apoptosis when treated with TRAIL or FasL, IGR37 and Skmel30 cells show cross-resistance to TRAIL and FasL even upon application of high doses. FACS analysis revealed that ligand-sensitive A375 cells express the respective signaling receptors TRAIL-R1, TRAIL-R2 and Fas. Resistant IGR37 cells also expressed TRAIL-R1, TRAIL-R2 and Fas, while Skmel30 cells did not bare TRAIL-R1 but expressed TRAIL-R2 and Fas. This excludes that the resistance is due to a defect in the expression of the death signaling receptors. TRAIL resistance is also not due to the expression of the non-signaling and thus antiapoptotic decoy receptors TRAIL-R3 and R4 since they were not expressed on the tested human melanoma cells. TRAIL-R3 could be detected only in permeabilized cells, which indicates that this receptor might be retained intracellularly. TRAIL and Fas are supposed to use the same signaling pathway of which the formation of the death inducing signaling complex (DISC) is a critical event. IGR37 and Skmel30 coexpressed both FADD and caspase-8, the two most crucial components of the DISC, indicating that apoptosis resistance is not due a loss of either one of these components. Pretreatment of cells with cycloheximide rendered resistant cells highly sensitive to TRAIL, but failed to reverse resistance to FasL. Reversion of apoptosis resistance by cycloheximide is attributed to a decreased expression of anti-apoptotic proteins, like Flip (Flice inhibitory protein) or IAPs (inhibitors of apoptosis). However, the TRAIL-sensitizing effect of cycloheximide was not due to reduced levels of Flip, since cells costimulated with cycloheximide and TRAIL did not reveal an altered expression of Flip. In addition, preexposure of apoptosis-resistant cells to sublethal doses of ultraviolet B radiation (UVB) rendered cells susceptible to TRAIL, but again not to FasL. Although the mechanism underlying the diverse effect of cycloheximide and UVB, respectively, on the resistance against TRAIL and FasL of melanoma cells remains to be determined, the data indicate that, in contrast to the current view, differences in the signaling pathway of TRAIL-and FasL-mediated apoptosis may exist.

P141

DNA damage, death receptor activation and reactive oxygen species contribute to UVB-apoptosis in an essential but independent way

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Nuclear DNA damage as well as direct activation of death receptors like CD95 by UVB radiation play a major role in UVB-induced apoptosis. Accordingly, it was found that either removal of DNA damage via induction of DNA repair (by addition of the repair enzyme photolayse) or inhibition of cell death receptor activation (by irradiating cells at low temperature) resulted in partial reduction of UVB-mediated apoptosis. Combination of both strategies caused a further reduction of cell death, though some residual apoptosis was still observed. The fact that under these conditions UV-induced apoptosis was drastically but not completely reduced indicates that at least a third independent pathway may be involved as well. Since reactive oxygen species (ROS) can cause apoptosis and since UVB might induce ROS, the free radical scavenger pyrollidene-dithiocarbamate (PDTC) was used. PDTC prevented UVB-induced apoptosis partially, H2O2-induced cell death completely, while it had no effect on CD95-mediated apoptosis. The same was observed for cytochrome c release from the mitochondria into the cytoplasm, another important event during apoptosis. Activation of CD95 does not only trigger caspase-8 but also cleaves the proapoptotic bid protein which ultimately causes cytochrome c release from the mitochondria. Bid cleavage was observed upon treatment of cells with UVB or an agonistic anti-CD95-antibody, but not upon H2O2 exposure, indicating that H2O2 uses a different signaling pathway. Time course experiments revealed that cytochrome c release can only be suppressed by early application of PDTC, indicating that ROS formation is the cause but not the consequence of damage to the mitochondria. The fact that PDTC did not inhibit CD95-mediated apoptosis and did not affect UV-induced DNA-damage indicated that ROS generated during UV-irradiation may directly trigger cytochrome c release from the mitochondria and thereby contribute to apoptosis formation. Accordingly, complete inhibition of apoptosis was observed when in addition to removal of DNA damage via photoreactivation and blockade of death receptor signalling by the caspase-8 inhibitor zIETD, PDTC was added before UV exposure. This indicates that DNA damage, death receptor activation and ROS formation contribute to UVB-induced apoptosis in an essential but independent way.

P142a

PO2 MEASUREMENTS FOLLOWING CONTINUOUS VS. FRACTIONATED 5-AMINOLEVULINIC ACID (ALA)-PDT IN VIVO

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Photodynamic therapy (PDT) is oxygen dependent. Thus, a fast, non-invasive setup was developed using optical oxygen-sensors to measure pO2 in tumor and normal tissue following PDT with 5-aminolevulinic acid (ALA) in vivo. Amelanotic melanomas (A-Mel-3) were implanted in the dorsal skinfold chamber of Syrian Golden hamsters (n = 30). For systemical application ALA (500 mg kg^{-1}) was injected i.v. 150 min prior to irradiation by an incoherent lamp (λ_{em} = 570-700 nm). Irradiation parameters were changed using either a light dose of 10 vs. 100 J/cm^2 or a fluence of 25 vs. 100 mW/cm^2. The light dose was given continuously (100%) or fractionated (20%, 15 min break, 80%). MAP, paO_2 and hematocrit were determined for each animal. Prior to, during and 15 min, 1 h, 2 h and 24 h after PDT tissue $pO_2$was measured. Using this experimental setup images were recorded showing the two-dimensional oxygen distribution of the entire chamber with regard to the blood vessels. The pO_2 in normal and tumor tissue was reduced only after high dose PDT (100 mW/cm^2 and 100 J/cm^2). Continuos irradiation with either a high fluence/low dose or low fluence/high dose did hardly reduce the tissue pO_2. However, fractionation of the light dose (20 J/cm^2; 15 min break; 80 J/cm^2) significantly reduced the tissue pO_2 in tumor tissue independent from the fluence. Continuous irradiation did not yield complete anoxia explaining the failure of ALA-PDT to induce complete remission. However, fractionation of the light dose might overcome this dilemma.

P142b

The microvascular effects are more pronounced following PDT with topical as compared with systemical application of 5-aminolevulinic acid (ALA) in vivo

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Application of ALA to cells and tissues results in the formation of protoporphyrin IX which is used clinically as a photosensitizer for photodynamic therapy (PDT) of benign and malignant skin lesions. However, previous investigations showed only few tumor remissions after PDT with systemically applied ALA. Thus, the microcirculatory effects of PDT using i.v. or topically applied ALA were compared within the same tumor model in vivo.

Amelanotic melanomas (A-Mel-3) were implanted in the dorsal skinfold chamber of Syrian Golden hamsters (n=30). For systemical application ALA (500 mg kg^{-1}) was injected i.v. 150 min prior to irradiation by an incoherent lamp (λem = 590 - 700 nm, 100 mW cm^{-2}, 10 J cm^{-2} or 100 J cm^{-2}). For topical application 3 % ALA in aqua dest. with 4 h incubation time was used. Microcirculatory parameters were assessed by intravital microscopy prior to, 30 min, 2 h and 24 h after PDT. FITC-labelled erythrocytes were injected to determine red blood cell velocity (RBCV) and functional vessel density (FVD). 24 h after PDT the chamber tissue was taken for histology and immunhistochemistry to assess the degree of tissue destruction.

Directly after high dose-PDT (100 J cm^{-2}) RBCV and FVD were reduced independent of the route of administration. However, 2 h after PDT with systemically applied ALA RBCV and FVD increased again in normal tissue and tumor periphery. In contrast, topically applied ALA induced complete vascular stasis up to 24 h. Low dose-PDT (10 J cm^{-2}) did not lead to any significant changes of microvascular perfusion.

These results indicate that topical ALA-PDT induces a more pronounced microvascular damage as compared with systemical ALA-PDT. Therefore it is unlikely to obtain a clinical benefit by systemic ALA-PDT.

P143

Modulation of base excision repair alters mutation formation by UVA1, but not by UVB

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UVA might play an important role in photocarcinogenesis. Oxidative DNA base modifications have been suggested to be responsible for its mutagenic and carcinogenic properties, but the exact nature of DNA lesions underlying UVA-mutagenesis and carcinogenesis continues to be disputed. Oxidative DNA base lesions are primarily processed by base excision repair (BER), in which a DNA-glycosylase removes the damaged DNA base and leaves an apyrimidinic/apurinic AP-site for further processing. Recently, we reported that human fibroblasts, in which BER is blocked by methoxyamine (MX), are hypersensitive to UVA1, but not to UVB. This indicated a link between UVA-induced oxidative DNA damage and UVA-cytotoxicity. Since mutation formation is a much more relevant end point for carcinogenesis than cytotoxicity, we were interested, whether modulation of BER also alters UVA-mutagenesis. Toward that goal, we used the HPRT-mutagenesis assay with primary human fibroblasts, pretreated with 5 mM MX or diluent, and irradiated with 20 J/cm^2 UVA1, 20 mJ/cm^2 UVB, or sham. MX binds to AP-sites and blocks progression of BER beyond the initial removal of the damaged base. HPRT metabolizes 6-thioguanine (6-TG) to a cytotoxic nucleotide. The HPRT-assay measures the frequency of spontaneous or induced hprt-mutations by selection for 6-TG-resistance. In two independent experiments with cells from two different donors, UVA-irradiation increased the mutation frequency 235 and 410 fold over the background to 41 and 70.4 mutants per 1 million colony forming cells (CFC). Pretreatment with MX reduced this mutation frequency to 7 and 12.8 mutants/1 million CFC. UVB-induced mutation frequencies were 59- and 110-fold over background, and were not significantly altered by pretreatment with MX. This demonstrates that BER (of presumably oxidative DNA damage) contributes significantly, at least about 80 %, to UVA1-mutagenesis, but not to UVB-mutagenesis. UVB-induced mutagenesis is thought to be determined by nucleotide excision repair of pyrimidine dimers, which is not inhibited by MX. The finding that blocking BER at a stage after removal of the damaged base did not increase, but decrease UVA-mutagenesis suggests that UVA-induced mutations are not formed during replication of a damaged DNA template, as it is known for UVB-induced pyrimidine dimers, but rather during its repair.

P144

UVB-induced conversion of 7-dehydrocholesterol to 1α,25-dihydroxyvitamin D_3 (calcitriol) in cultured keratinocytes is upregulated by tumor necrosis factor-α

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UVB radiation induces both the conversion of 7-dehydrocholesterol (7-DHC) to calcitriol (1α,25$(OH)_2D_3$) and the release of tumor necrosis factor-α (TNF-α) in human keratinocytes. Here we investigated (i) the role of TNF-α in the regulation of UVB-induced synthesis of calcitriol in human keratinocytes and (ii) the effect of the wavelength of UVB on the generation of calcitriol and TNF-α. Keratinocytes were incubated in culture medium (KBM) containing 1% (w/v) bovine serum albumin with 25 µM 7-DHC and irradiated with a HI-monochromator (Dermolum) at 290, 300, 310 and 320 nm and UV-doses between 7.5 and 45.0 mJ/cm^2. The UVB-induced formation of both calcitriol and TNF-α in cultured cells was wavelength-, time- and dose-dependent and reached maximum levels 24 h after irradiation at 300 nm and dosis of 30 mJ/cm^2. Irradiation at 310 nm generated no TNF-α and only small amounts of calcitriol. After irradiation at 320 nm and in unirradiated cultures (controls) neither calcitriol nor TNF-α were detectable. Production of calcitriol was reduced by 30 percent if a neutralizing polyclonal antibody directed against human TNF-α was added to cultures immediately after UVB-irradiation. Our results indicate that the processes of UVB-induced generation of calcitriol and of TNF-α are related and that TNF-α acts as a positive regulator in the UVB-induced synthesis of calcitriol in keratinocytes.

P145

In vivo evidence for a link between photoaging and oxidative stress

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Exposure of human skin to UV irradiation, the major factor that affects the structure and function of skin, induces a spectrum of well-documented acute and chronic responses. The mechanisms which lead to these effects are not yet fully understood. Oxidative stress is discussed as one of the major contributors, but convincing in vivo evidence is still lacking. We hypothesized, that acute and chronic UV-exposures induce oxidative stress and protein oxidation in human skin. For investigations of chronic UV-exposure, biopsies were taken from patients with histologically confirmed solar elastosis (n=12), from non-UV-exposed sites of age-matched controls (n=12), and young healthy subjects (< 30 years, n=12). To evaluate the influence of acute UV-exposure, buttock skin of 12 healthy subjects was irradiated repetitively on 10 days with a solar simulator and compared intra-individually to non-UV-treated contralateral sites. As a marker of ROS-mediated protein oxidation, carbonyl groups were visualized in frozen sections by dinitrophenylhydrazine (DNPH) and anti-DNP-antibody incubation. After immunohistochemical staining, sections were analyzed by densitometric image analysis. Using an immunoblotting technique, protein oxidation was investigated in tissue lysates of photoaged skin as well as in cell lysates of UVA/UVB-irradiated or hydrogen peroxide treated fibroblasts and keratinocytes. Statistical analysis was performed using ANOVA. In photoaged skin, an accumulation of oxidatively modified proteins was found specifically within the upper dermis, whereas both, age-matched and young controls, displayed significantly lower immunoreactivity for protein oxidation. Intriguingly, in acute UV-exposed skin there was a significantly higher level of oxidative protein modifications within the outermost layers of the stratum corneum and in the papillary dermis. Exposures of keratinocytes and fibroblasts to UVB/UVA irradiation and hydrogen peroxide treatment lead to a dose-dependent protein oxidation, thus, confirming in vivo results. In conclusion, the correlation between photoaging and protein oxidation was demonstrated for the first time in vivo. Furthermore, we present conclusive evidence for an involvement of oxidative stress in barrier perturbation following acute UV-exposure.

P146

UV-induced downregulation of desmoplakin in human keratinocytes in vitro and in vivo

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Desmosomes are specialized domains of the plasma membrane that play a fundamental role in cell adhesion. The desmosomal plaque protein desmoplakin (Dsp) is regulated by a variety of signalling pathways, many of which are also sensitive to UVB and UVA. We hypothesized that the expression of Dsp in human keratinocytes is influenced by acute and repeated UVB irradiation. To assess this, HaCaT cells were UVB exposed to single doses of 5, 15, and 30 mJ/cm^2 or, on 4 consecutive days, to 5 and 15 mJ/cm^2 UVB, respectively. After 2, 4, 8, and 16 hours, cells were immunostained using double labeling immunofluorescence and analyzed by densitometric image analysis. After both single and repeated exposures to UVB, a time and dose dependent downregulation of Dsp was observed, exhibiting peaks after 12 hours, at doses starting at 15 mJ/cm^2 for single exposures and 5 mJ/cm^2 for repeated exposures. In contrast, vinculin expression, used as internal control, remained unchanged. Western blot analysis confirmed the downregulation of Dsp, also exhibiting a maximum decrease after 12 hours at doses of 15 and 30 mJ/cm^2, while vinculin levels remained unchanged. To verify whether UV-induced downregulation of Dsp occurs also in human skin in vivo, buttock test sites of human volunteers (n=14) were repetitively irradiated on 10 consecutive days using a solar simulator. Thereafter, frozen sections from irradiated and sham-irradiated contralateral sites were immunostained for Dsp. Compared to non irradiated skin a significantly weaker expression of Dsp was found in the overall epidermis of UV exposed skin sites ($p< 0.01$). This is the first in vivo report of UV-induced downregulation of Dsp in human epidermis. UV-dependent regulation of desmosomal plaque proteins may account for the impairment of epidermal cell adhesion and barrier function observed in photoaggravated skin disorders. Further studies are presently undertaken evaluating whether the responsible signalling pathways involve protein kinase C.

P147

Differential gene expression profiling in individuals with deficient and proficient DNA repair as a predictive tool for individual skin cancer risk

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Xeroderma pigmentosum (XP) is a disorder characterized by defective repair of ultraviolet (UV)-induced DNA damage comprising 7 complementation groups (A to G). Patients with XP have a highly increased skin cancer risk with the clinical phenotype varying according to the specific compl. group. The highest skin cancer risk is found in group A, an intermediate risk in group D and the lowest risk in group F. The high skin cancer risk is partly due to defective DNA repair, but other pathways also play a role (immune surveillance).Available test systems assessing the risk to develop skin cancer thus far only detected gross differences between single parameters. In order to increase the sensitivity and predictive power of a test system, employing DNA-array technology, we compared differential mRNA expression in fibroblasts from XP patients of the above complementation groups with a high, intermediate and low skin cancer risk after UVB irradiation. A dose dependent tendency from mild to severe was detected in 17 genes with 11 genes involved in functionally relevant pathways (DNA repair, cell-cycle, apoptosis, transcription, matrix-degradation). Thus it is possible to use DNA array technology to identify distinct patterns of UV-induced gene expression correlating with the skin cancer risk in XP patients. We were therefore next interested to see if this approach could also be used to determine the skin cancer risk in DNA repair proficient individuals which nevertheless exhibited an increased skin cancer risk. Gene patterns previously identified in XP patients could also be observed in patients with >2 skin tumours and an age <35 yrs. Expression levels of genes were lower than in XP cells but higher than in age matched normal cells.Thus it is possible to correlate the skin cancer risk with the complementation groups in XP patients, thereby identifying a limited number of genes with possible functional relevance for photocarcinogenesis. Furthermore this data could be transferred into the DNA repair proficient background and may provide a predictive test system to determine the skin cancer risk of clinically normal individuals.

P148

Bcl-2 protects against UVB, but not PUVA induced keratinocyte apoptosis independent of reactive oxygen species (ROS) generation

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An important response of keratinocytes to UVB or PUVA irradiation is the induction of apoptosis. In order to understand this cellular response of keratinocytes, we compared UVB, PUVA and UVA irradiated keratinocytes for activation of apoptosis signalling pathways. UVB and PUVA, but not UVA irradiation induced keratinocyte apoptosis in vitro. The activation of caspases 8/3 was detectable as soon as 3 h after UVB-irradiation, but only 12h after PUVA irradiation. Moreover, UVB and PUVA activated mitochondrial apoptotic pathways with different kinetics. Mitochondrial transmembrane potential (MTP) decreased 6-8 h after UVB, whereas PUVA mediated MTP loss started 12-14 h postirradiation, indicating the involvement of apoptosis pathways in a kinetically variant manner. The point of no return of cell death in UV induced keratinocyte apoptosis is not well defined. Bcl-2 is known to interfere with mitochondrial apoptotic pathways. It was suggested that Bcl-2 might act as antioxidant by blocking the production of reactive oxygen species (ROS). In keratinocytes, Bcl-2 overexpression protected against UVB induced MTP loss and caspase activation. Consequently, Bcl-2 also protected clonogenic survival following UVB irradiation. Intracellular ROS levels were increased by UVB, but unaffected by the expression level of Bcl-2. In marked contrast, Bcl-2 delayed PUVA induced MTP loss and caspase activation, but failed to protect clonogenic survival. Interestingly, similar levels of ROS were produced by UVA alone and PUVA irrespective of the expression of Bcl-2, suggesting that ROS generation does not play a major role in PUVA induced activation of apoptosis signalling. Although caspase activation was detectable in UVB and PUVA induced cell death, the pancaspase inhibitor ZVAD-fmk only delayed MTP loss, but did not protect clonogenic survival. We conclude that distinct pathways independent of caspases are employed to exert the cell death program in PUVA and UVB induced apoptosis. While PUVA-induced cell death overcomes Bcl-2 protected mitochondrial pathways of apoptosis, UVB induced apoptosis is efficiently inhibited by Bcl-2. Our data suggest that PUVA-induced DNA damage or other signals, rather than ROS generation, play the key role for PUVA induced apoptosis.

P149

DETECTION OF SINGLET OXYGEN IN VITRO.

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Singlet oxygen is an important mediator in UVA1, PUVA or photodynamic therapy. Singlet oxygen is generated by transferring energy of a photoactivated molecule (endogenous or exogenous) to oxygen. Usually, singlet oxygen is detected either by its chemical products (e.g. lipid peroxidation) or by using specific quenchers such as sodium azide. However, these are indirect methods and yield frequently no unequivocal results. A direct and non-invasive detection of singlet oxygen is the time-resolved measurement of its luminescence at 1270 nm. The decay time of the luminescence is determined by the cellular environment of singlet oxygen. However, there are no reports in the literature regarding the detection of singlet oxygen luminescence in living cells due to its extremely weak signal and an estimated decay time of less than 0.5 µs. In order to overcome this problem, a new photomultiplier (Hamamatsu, RR5509-42) was used as a detector of the luminescence showing a high sensitivity in the near-infrared spectral range. HT-29 and HaCaT cells were incubated with hematoporphyrin (5 µg/ml) for 1.5 h or 24 h. The subcellular localization of the sensitizer molecule were investigated using fluorescence microscopy. For luminescence detection the cells were suspended in water at a concentration of 7 Mio. cells /ml. The irradiation of the cell suspensions was performed using a frequency-doubled, q-switched Nd:YAG (λ=532 nm).Fluorescence images showed that the subcellular localization of the sensitizer was the plasma membrane or the mitochondria when using an incubation time of 1.5 or 24 h, respectively. The decay times of singlet oxygen was 12 ± 3 µs (1.5 h) or 15 ± 3 µs (24 h), which are clearly longer than 0.5 µs estimated for cells. The decay time of singlet oxygen in water is 3.5 µs but 12 µs in fatty acids such as phosphatidylcholine. These results provide evidence that the luminescence measured is due to singlet oxygen located in the plasma membrane (1.5 h) or in other membranes of the mitochondria.This is the first report regarding the detection of singlet oxygen in living cells. Thus, further investigations using the setup will elucidate the initial photooxidative reactions in dermatological phototherapy.

P150

Wavelength dependent UV-induced depletion of vitamin E and generation of a highly sensitive lipid photooxidation product in human sebum

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At the outermost surface of the stratum corneum, skin surface lipids (SSL) are first-inline-targets of natural UVB and UVA radiation. Recently, we have reported that human sebum and SSL contain substantial amounts of vitamin E, a powerful lipophilic antioxidant which protects lipids from oxidative Stress. We hypothesized that UVA-and UVB-irradiation deplete human sebum vitamin E and induce photooxidation of SLL. Samples of sebum were collected from facial skin of 15 healthy volunteers using sebutapes and immediately irradiated with different doses of UVB and UVA. Then, lipids were extracted from the tapes and analyzed by HPLC using electrochemical detection of vitamin E and in-line UV detection for screening of squalene and photooxidation products. To test possible protective effects, sunscreens and antioxidants (Vitamins E and C) were topically applied prior to UV-irradiation. Upon UVB and UVA exposure, a dose dependent depletion of human SSL-Vitamin E was observed. Intriguingly, a dose dependent generation of an unidentified sebum lipid photooxidation product (USLPP) was detected. USLPP appeared even after small physiological, suberythematogenic doses of UVA (5 J/m^2) and UVB (30 mJ /cm^2). While the UVB induced depletion of sebum vitamin E may be due to its absorption maximum at 293 nm (direct photooxidation), the even more sensitive UVA induced depletion of vitamin E and the formation of USLPP strongly points to an involvement of indirect mechanisms mediated by reactive oxygen species (ROS). Accordingly, UVA-induced USLPP generation was strongly inhibited by antioxidants. Squalene was sensitively depleted by UVA and was not affected by UVB. In conclusion, both, vitamin E depletion and USLPP formation in human sebum were demonstrated to be a very sensitive markers for UVB- and UVA-induced oxidative stress in skin. Current investigations focus on the identification of USLPP using LC-MS and NMR analysis as well as on the biological relevance of USLPP.

P151

Endothelin-1 mediates UVB-induced skin inflammation by activation of mast cells

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We have recently shown that UVB-induced skin inflammation in mice is mast cell (MC)-dependent. Given that UVB reportedly does not induce direct MC degranulation, as shown by UVB irradiation of isolated MC in vitro, it remains unclear how MC are activated by UVB. Here, we asked whether endothelin-1 (ET-1), a potent activator of peritoneal MC that is rapidly upregulated in UVB irradiated skin, is involved in UVB-induced activation of cutaneous MC. To test whether ET-1 can induce degranulation of cutaneous MC, ear skin of adult C57BL/6 mice was injected with ET-1 (10^{-6}M, 0.02ml, left ears) or vehicle (right ears) and the extent of MC degranulation was assessed by quantitative histomorphometry. ET-1-treated ears exhibited significantly more extensively degranulated MC and far less normal not degranulated MC than vehicle-treated ears (extensively degranulated MC: 36 ± 8% vs. 2 ± 1%, p<0.005). To check whether ET-1 can induce direct degranulation of skin MC, crude (~5% MC) and purified (>95% MC) skin MC suspensions were stimulated with ET-1 (10^{-6}M - 10^{-10}M) and MC degranulation was assessed by measuring serotonin release. ET-1 induced a strong and dose-dependent degranulation of MC that was independent of the absence or presence of other cutaneous cell populations (10^{-6}M: 56 ± 4%, 10^{-7}M: 56 ± 5%, 10^{-8}M: 40 ± 4%, 10^{-9}M: 12 ± 2%, 10^{-10}M: 1 ± 1%; for purified MC). ET-1-induced degranulation of isolated skin MC is receptor-mediated, since pre-incubation of MC with the ETA/ETB-receptor antagonist PD 142893 completely inhibited degranulation of ET-1-stimulated MC. To examine whether ET-1 contributes to UVB-induced MC degranulation, we pretreated mice with the selective ETA-receptor antagonist BQ-123 and quantified the swelling of UVB-irradiated ears, a response we have previously shown to be MC-dependent. UVB-induced (500mJ/cm^2) ear swelling in BQ-123 pretreated ears was markedly reduced as compared to vehicle pretreated ears (58 ± 7 microm vs. 7 ± 6 microm at 18h, p<0.01), indicating that ET-1 is required for the induction of inflammation after UVB-irradiation. Our findings suggest the following chain of events in UVB-induced murine cutaneous inflammation: UVB 1) upregulates the expression of ET-1, which 2) binds to ETA-receptors on MC and 3) degranulates MC, which 4) results in the release of proinflammatory MC-mediators that 5) induce skin inflammation.

P152

Single PUVA treatment of dermal human fibroblast result in a long lasting increase in mitochondria- and NADPH oxidase-derived reactive oxygen species(ROS) - Implication for premature replicative senescence

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Side effects of PUVA treatment involve premature aging and skin cancer. We have previously shown that following a single PUVA treatment (50ng/ml 8-methoxypsoralen and 90kJ/m^2 UVA irradiation) human dermal fibroblasts undergo long-term growth arrest (>90 days). Although the growth arrest is transient, PUVA-treated fibroblasts retain a memory of the preceding PUVA damage as in their regrowing state they reached the stationary phase of replicative senescence at a much lower cumulative population doubling (CPD) compared to mock-treated fibroblasts of the same strain. In order to analyze underlying mechanisms, we addressed the questions (1) whether enhanced and persistent oxidative stress develops post PUVA-treatment and if so of what subcellular origin (2) whether the ROS activated cell cycle control gene p16(INK4a), known to be involved in senescence, is up-regulated and (3) whether there is evidence for a ROS-dependent telomere shortening involved in the retained memory of the preceding oxidative damage post PUVA treatment. Using the ROS sensitive bioprobe dichlorodihydrofluorescein diacetate (DCF), we found a moderate 2-fold increase in ROS levels at 24h post PUVA treatment which time-dependently increased to 5-fold at 1 week and >20-fold 6 weeks post PUVA treatment compared to mock-treated fibroblasts. Using mitochondria depleted Rho-low fibroblasts and two different NADPH oxidase inhibitors (AEBSF and DPI), we found that both mitochondria and NADPH oxidase(s) contribute to the dramatically enhanced intracellular ROS levels at 3 and 6 weeks after PUVA treatment. The observed high intracellular ROS levels result in a long-lasting up-regulation of p16(INK4a) as well as oxidative telomere shortening in PUVA-treated fibroblast after regrowth compared to mock-treated fibroblasts of exactly the same CPD. Collectively, these data indicate that after a single PUVA treatment long-lasting oxidative stress from endogenous sources with subsequent oxidative telomere shortening is likely to be responsible for the premature replicative snenscence of fibroblasts.

P153

Overexpression of phospholipid hydroperoxide glutathione peroxidase in human dermal fibroblasts abrogates the UVA-induced expression of matrix-metalloprotease-1 by suppression of phospholipid hydroperoxide-mediated NFκB activation

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Phospholipid hydroperoxide glutathione peroxidase (PHGPx) reveals high specific activity in reducing phospholipid hydroperoxides (PCOOHs) and, thus, may play a crucial role in protecting the skin against UV-induced damage. As matrix-degrading metalloproteases (MMPs) promote UV-triggered detrimental long-term effects like cancer formation and premature skin damage, we here addressed the role of PHGPx in the protection against UV-induced expression of MMP-1/interstitial collagenase. For this purpose we have stably transfected the human dermal fibroblast cell line 1306 with a eukaryotic expression vector containing the human PHGPx cDNA. Overexpression led to a 5-fold increase in PHGPx activity as detected by a specific substrate spectrophotometric assay. In contrast to a maximal 4.5-fold induction of specific MMP-1 mRNA levels in mock-transfected cells at 24h after UVA-irradiation (30kJ/m^2), no MMP-1 induction occurred at any studied time point after UVA treatment of PHGPx overexpressing fibroblasts. As interleukin-6 (IL-6) was earlier shown to mediate the UVA-induction of MMP-1, we studied whether PHGPx overexpression might interfere with the NFκB-mediated IL-6 induction and downstream signalling. Using transient transfection of IL-6 promoter constructs containing NFκB sites revealed a 50-fold induction of the reporter gene luciferase in control cells and a significantly lower induction in PHGPx overexpressing fibroblasts following UVA-irradiation. This was confirmed by the reduced constitutive and UVA-enhanced release of IL-6 in PHGPx overexpressing fibroblasts compared to control cells. The reduced induction of IL-6 in PHGPx overexpressing cells following incubation with different PCOOH concentrations as well as the suppression of UVA-induced IL-6 release in the presence of Trolox, a chain breaker of PCOOH-initiated lipid peroxidation, suggest that UV-induced PCOOH and subsequent lipid peroxides initiate the NFκB-mediated induction of IL-6 which - as earlier proven by IL-6 antisense oligonucleotides and neutralizing antibodies - mediates the induction of MMP-1. Our finding is particularly relevant in the light of already available small molecule mimetics of PHGPx.

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UVA-1 mediated cytokine release in vivo,- a time course and dose dependence analysis

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The UVA-1 spectrum is used for treating various conditions. Especially for some cases of atopic eczema and sclerosing disorders it seems to be more effective than UVB. We therefore became interested in the question how UVA-1 modulates the inflammatory cytokine network within the skin and whether it exerts different effects on inflammatory cytokine levels in vivo than UVB. Thus a study involving 35 healthy human non-smokers of skin type 2 and 3 was set up. Suction blisters were raised at several timepoints 3-48h after UVA-1 irradiation of previously unexposed buttock skin to provide a time course analysis of the changes in levels of Il-1α, Il-1β, TNF-α, and Il-6 measured by ELISA compared to unirradiated neighbouring skin. The UVA-1 doses administered were orientated on the 24h pigmentation threshold values (MTD) obtained for each volunteer individually before they were enrolled into the study. Three sets of experiments were performed, one time course analysis each after a single dose of 2MTD, respectively 0.5MTD and one after 3x0.5 MTD. The last set mimicked repetitive exposure such as it occurs during phototherapy. We found a significant increase in the DNA-damage dependent cytokine IL-6 in all three sets of experiments in the time window of 3-10 hours after the UVA-1 exposure. This increase was neither correlated to skin type, nor the physical actual dose of UVA-1 administered. Strikingly, all the other cytokine levels did not rise at all at any timepoint after the UVA-1 exposures. This is in contrast to published data with the same method, i.e. suction blister raising, after MED based solar simulated radiation (SSR) including the UVB and UVA-2 spectra. Our finding that UVA-1 does not raise Il-1α, Il-1β or TNF-α levels, which were all found to be highly elevated after SSR, prompts the conclusion that the release of these inflammatory cytokines in vivo is a wavelength dependent effect involving UVB and possibly also UVA-2. The fact of a spectrum dependent modification of the cytokine milieu within the skin should prompt attempts to correlate it with the therapeutical efficacy for a given skin disease. In this respect IL-6 might also serve as a suitable biomarker for UVA-1 irradiation in vivo.

P155

Human skin explants are useful to assess different effects of UV irradiation in vitro

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Complex human in vitro methods to test the different effects of UV irradiation are rare. We used human skin explants to investigate the effect of different doses of solar simulated UV irradiation on the sunburn cells formation, the migratory capacity of dendritic cells (DC), the expression of the co-stimulatory B7.1 and B7.2 molecules on migrated DC, the reduction of CD1 positive cells in the skin, the production of TNF-alpha and interleukin 10.
Skin samples of the thigh were obtained from 12 different individuals (6 females, 6 males; skin type II: 3, skin type III: 9; mean age: 65.9) undergoing plastic surgery. The skin was divided into 4 parts, which were irradiated with a 1000 W Xenon solar simulator (Oriel Corp) with 0 0.5, 1, and 2 minimal erythema dose equivalents (MEDE). After irradiation at least three 6-mm skin biopsies were taken from each part and floated on RMPI medium for 48h. The emigrating cells and the skin explants were histologically and immunohistochemically analyzed and the soluble mediators were measured by ELISA.
The number of emigrated DC ($p<0.006$), the expression of the B7.2 molecule on the emigrated DC ($p<0.0001$), and the number of CD1-positive cells in the epidermis ($p<0.002$) decreased in an UV dose dependent manner. A significant UV dose dependent increase was observed for the formation of sunburn cells ($p<0.0001$) and the production of TNF-alpha ($p<0.003$). No correlation with the UV doses was found for the expression of the B7.1 molecule on the emigrated cells and the production of interleukin 10.
Skin explants can be used to measure the effects of UV -irradiation on the formation of sunburn cells, the capacity of DC to emigrate, the expression of co-stimulatory molecules, and the UV induced TNF-alpha production.

P156

UVB-induced keratinocyte apoptosis: redundant signalling upstream and downstream of the mitochondria

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Death receptor-mediated signals critically require the activation of caspases for apoptosis induction. In contrast, the critical signals required during UVB-induced apoptosis are less well defined. We therefore analyzed apoptotic signal transduction pathways activated after UVB irradiation. Interestingly, while a pancaspaseinhibitor (zVAD-fmk) fully protected clonogenic survival following TRAIL treatment, UVB induced loss of clonogenic capacity cannot be prevented by zVAD-fmk. However, detectable cleavage of caspases 3, 7 and 8 as well as in vitro DEVDase activity is seen within few hours post UVB, and are inhibited to undetectable levels by zVAD-fmk. In order to elucidate the role of two important caspases (9 and 3) for UVB-induced apoptosis, we examined caspase 9-deficient HeLa and caspase 3-deficient MCF7 cells. When compared to control cells, disruption of the mitochondrial transmembrane potential (MTP) following UVB was delayed in both cell types, indicating a critical role of these caspases for early MTP loss. In line, UVB induced decrease of MTP is delayed, but not prevented by zVAD-fmk in keratinocytes. Interestingly, Caspase 9-, but not caspase 3-deficiency increased clonogenic survival. Moreover, the release of cytochrome C from mitochondria, not blocked by zVAD-fmk, was detectable as early as 1 hour postirradiation, suggesting a caspase-independent initiation phase of apoptotic signals upstream of mitochondria in response to UVB. Our data suggest that caspase 9, rather than caspase 3 is critically involved in the execution phase of UVB-induced apoptosis and clonogenic survival. In contrast, a functional autoamplification loop involving caspase 3 accelerates UVB-induced cell death, but does not affect clonogenic survival. We conclude that currently available caspase inhibitors are ineffective to block critical steps of UVB induced apoptosis. The invariant release of cytochrome C from mitochondria in the presence of zVAD-fmk indicate that redundant signalling pathways are involved in UVB induced cell death. Thus, for preventing major skin damage following UVB due to excessive apoptosis, multiple signalling pathways might have to be inactivated for therapeutic benefit.

P157

***Ha-ras* and *p53* mutation spectra of PUVA keratoses from psoriasis patients**

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Psoriasis patients exposed to high cumulative doses of Psoralen and UVA (PUVA) photochemotherapy have an increased skin cancer risk, particularly for squamous cell carcinoma. Moreover, those patients frequently have so called PUVA keratoses (PK), i.e. hyperkeratotic lesions with mild cellular atypia. However, no conclusive evidence yet has been provided whether PK are premalignant skin tumors or only indicators of an increased skin cancer risk. Furthermore, no definite information is present on the exact causes and molecular mechanisms of the formation of PK. Thus, we have performed a molecular mutation analysis on *Ha-ras* and *p53* in PK. Those genes are most often mutated in human cancers of any origin and, thus, their mutation analysis may allow to disclose potential causative factors of PK and to determine the relation of formation of PK to that of skin cancers. We analyzed 25 paraffin-embedded PK from 10 psoriasis patients treated with PUVA (122 to 1144 exposures; 511 to 5204 J/cm2 total UVA dose). DNA sequencing of exon 1 to 4 of *Ha-ras* and exon 4 to 9 of p53 revealed that PK carried mutations at both *Ha-ras* and *p53* in 28% (7/25), at *Ha-ras* only in 16% (4/25), at p53 only in 28% (7/25), and at none of the genes in 28% (7/25) of the lesions. Thirteen of a total of 18 (72%) *Ha-ras* mutations and 22 of a total of 39 (57%) p53 mutations were at dipyrimidine sites and of UV fingerprint type (C>T and CC>TT transitions). Of the *Ha-ras* and *p53* mutations 2 (11%) and 13 (33%) were at potential psoralen binding sites (5 TpA, 5 TpG, or 5 TpT DNA sequences) and thus possibly of PUVA origin, 3 (17%) and 2 (5%) were of ambiguous origin (UV and/or PUVA), and 0 (0%) and 2 (5%) were of other type, respectively. Based on these findings, we conclude that (1) *Ha-ras* and *p53* are often mutated in PK and may cooperate in their formation, (2) most *Ha-ras* and *p53* mutations were of UV type and thus environmental and/or therapeutic UV(B) exposure seems to be the major causing factor, and (3) PUVA itself seems to play only a minor direct role in causing these mutations. In the formation of PK other genes than Ha-ras and p53 may be the major direct targets of PUVA and/or other PUVA effects, such as tumor promotion and/or immunosuppression, may play a role.

P158

The effect of the antioxidants Vitamin C, αTocopherol and Melatonin in UVA-irradiated skh-1-hairless mouse skin

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UV-irradiation is known to be an important factor for generating reactive oxygen species in the skin which finally leads to cancer and premature skin aging. To pre´vent this UV-induced photodamage it is necessary to investigate antioxidative substances in the skin. The skh-1-hairless mouse is a well established model to investigate skin changes caused by ultraviolet irradiation. The skin changes seen in chronically irradiated hairless mice are similarly to that in human skin. Vitamin C and E are known to be substances with high antioxidative potential. Melatonin as an important hormone for the regulation of biorhythm acts as an radical scavenger in the leucocyte model and suppresses erythema in UV-irradiated human skin. To investigate the UVA-light induced skin damage we compared the effect of topically applied creans containing melatonin, vitaminC or E on the back for 15 minutes and than irradiated with UVA for 10 weeks.The dose per each exposure was 120J/cm^2. The total cumulative dose was approximately 3000J/cm^2. skin biopsies were stained with HE and immunostained using immunofluorescence technique.The epidermal thickness increased and dermal thickness decreased significantly in the antioxidant-treated groups compared with untreated and vehicle-treated groups. Furthermore it was remarkable that the epidermis in the melatonin treated group was significantly thicker compared to the vitamin C and E treated groups, whereas the dermal thickness was similar between the three groups. This epidermal effect may be due to a cream caused irritation. The decreased dermal thickness in antioxidant treated groups compared to the vehicle treated group probably shows a protective effect against UVA-irradiation. Changes in matrixcomponents were also detectable and show an influence of antioxidants in the process of ultraviolet induced photodamage. topically applied antioxidants prevent UVA-caused deep skin damage in the skh-hairless-1 mouse and probably also display an effect in human skin

P159

ADENOVIRAL VECTORS AND HUMAN SKIN RECONSTRUCTS TO INVESTIGATE THE CONTRIBUTION OF INDIVIDUAL GENES FOR MELANOMA DEVELOPMENT AND PROGRESSION

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Based on clinical and histopathological features a model of melanoma development and progression has been proposed by Clark et al. This model suggests that melanoma develops and progresses in a sequence of steps: melanocyte-nevus-dysplastic nevus-radial growth phase melanoma-vertical growth phase melanoma-metastatic melanoma. In order to investigate the relative contribution of individual genes for melanoma development and progression we used adenoviral vectors as gene delivery tools and developed an in vitro model to study the biological effects in a physiological context. This in vitro model consists of artificial skin rebuilt from isolated cutaneous cell populations with incorporated melanocytic cells from different stages of progression. We examined the role of basic fibroblast growth factor and the adhesion molecules Mel-CAM and ß3 integrin in melanoma development and progression. Basic fibroblast growth factor induced development of early radial growth phase melanoma whereas Mel-CAM and ß3 integrin promoted progression from radial to vertical growth phase melanoma. Molecular engineering of the tumor cells and of each normal skin component will allow a better dissection of each step of melanoma development and progression holding the promise of design of effective treatment for metastatic melanoma.

P160

Soluble HLA-DR is a potent predictive serum marker in melanoma patients.

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HLA antigens are structures of major importance for the interaction between tumor cells and the host immune system. In the present study we investigated the clinical impact of soluble HLA-DR (sHLA-DR) in comparison to S100-β in serum from 183 melanoma patients compared to 86 healthy controls using immunosorbent assays. sHLA-DR levels were reduced ($p < 0.0005$) in melanoma patients (0.70 ± 0.08 µg/ml) compared to healthy controls (1.38 ± 0.16 µg/ml). Reduced sHLA-DR was associated with advanced disease stages and tumor load. Low serum sHLA-DR was correlated with poor overall ($p=0.021$) and progression-free ($p < 0.0005$) survival. In contrast to S100-β, low sHLA-DR serum concentrations were strongly associated ($p=0.0001$) with poor progression-free survival in $n = 60$ early-stage (I/II) melanoma patients. To find one possible cause for the decrease in serum sHLA-DR concentration, we investigated the expression of MHC class-I and -II molecules, of the costimulatory molecules CD80/B7-1 and CD86/B7-2 and of the marker of proliferation CD71 on CD14+ peripheral blood monocytes (PBM) from 144 melanoma patients and 43 healthy controls by flow cytometric analysis. We found decreased expression of HLA-DR ($p < 0.0005$), HLA-DP ($p < 0.0005$), HLA-DQ ($p = 0.006$) and CD86/B7-2 ($p = 0.001$) on PBM from melanoma patients compared to healthy controls, associated to advanced stages of tumor disease and burden, whereas no significant differences could be detected in HLA class-I and CD80/B7-1 expression. In contrast, expression of CD71 was increased on PBM from melanoma patients compared to healthy controls ($p = 0.024$). In conclusion, our results suggest sHLA-DR as a potent prognostic serum marker in melanoma patients, particularly useful for the identification of early-stage melanoma patients at high risk of disease progression. Reduced cell surface HLA-DR expression on PBM could be one possible origin of reduced sHLA-DR serum levels in melanoma patients.

P161

Upregulation of the transcriptional repressor Snail leads to loss of E-cadherin expression in melanoma cells in vitro and in vivo

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Malignant transformation of melanocytes frequently coincides with loss of E-Cadherin expression. We could show that loss of E-Cadherin in melanoma cell lines involves upregulation of the transcriptional repressor Snail. In comparison to primary human melanocytes, where Snail expression was not detected by RT-PCR, significant expression was found in all eight melanoma cell lines. In parallel, western blot and RT-PCR analysis revealed strong reduction of E-Cadherin expression in the melanoma cells. Consistently, transient transfection of a Snail expression plasmid into human primary melanocytes led to significant downregulation of E-Cadherin, whereas transient and stable transfection of an antisense snail construct induced reexpression of E-Cadherin in melanoma cell lines. Promoter analysis of an E-Cadherin promoter fragment further proved that the downregulation of E-Cadherin was Snail dependent as a promoter with mutated snail binding site had an increased activity in melanoma cells. In vivo, strong Snail expression and consequently loss of E-Cadherin expression was found in most of the melanoma specimens analysed. In summary, we conclude that activation of Snail expression plays an important role in downregulation of E-Cadherin and tumorigenesis of malignant melanomas in vivo.

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Expression of the inducible nitric oxide synthase does not correlate with apoptosis in human melanocytic cells

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Nitric oxide (NO) is a gaseous messenger involved in the regulation of physiological processes in various cell types including skin cells. Three different NO synthases (nNOS, eNOS, iNOS) have been identified. Only the expression of the inducible NO synthase (iNOS) requires signaling by cytokines and/or lipopolysacharides (LPS). Elevated levels of iNOS protein and formation of high levels of NO were suggested to cause apoptosis in murine K1735 melanoma cells. As such an effect could be also employed against human melanoma, we studied expression of iNOS, NO production and apoptosis in human melanoma cells and in normal human melanocytes.
In Northern blots, iNOS mRNA was detected in 4 of 15 melanoma cell lines cultured without iNOS inducing agents. Induction of iNOS mRNA by TNF-α, INF-γ and LPS was found in normal melanocytes and in the mammary carcinoma cell line MCF-7 used as a control, but not in melanoma cells. Western blot analysis using two different iNOS-specific antibodies identified a 150 kDa protein expressed in MCF-7 cells, in normal human melanocytes and in melanoma cells. This protein was inducible in cultures of normal melanocytes, whereas in melanoma cell lines, iNOS was found weakly expressed before and after induction. Elevated levels of iNOS protein in normal human melanocytes, however, did not result in higher NO levels as determined by quantification of the stable NO products nitrite in the culture fluid and nitrotyrosine in cellular protein. The apoptotic rates of melanoma cells and of several normal melanocyte cultures were significantly increased by TNF-α, IFN-γ and LPS, but apoptosis was not prevented by the specific NOS inhibitor L-NMMA. Also, addition of the NOS cofactor tetrahydrobiopterin did not enhance induction of apoptosis.
Our data indicate, that human melanoma cells, unlike to normal melanocytes, show weak and constitutive iNOS expression, but they are resistant to iNOS induction. This regulation defect may be important for melanocytic transformation and malignancy. Treatment of melanoma cells and melanocytes with iNOS inducing agents resulted in increased apoptosis. However, experiments with a specific NOS inhibitor and measurement of stable NO products after induction suggested that iNOS was not involved in TNF-α, IFN-γ and LPS-dependent apoptosis.

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The combination of melanoma-specific peptides and genetic transfection of DC leads to a significantly enhanced induction of melanoma-specific CTL: Implications for vaccination strategies.

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Genetic immunization through ex vivo transduction of dendritic cells (DC) is suggested to enhance antitumor immunity by activating a broad range of peptide-specific $CD8^+$ T cells. To analyze whether in vitro priming of T cells with gp100-transduced DC enhanced the induction of CTL in comparison to peptide-pulsed DC, $CD8^+$ T cells were repetitively stimulated with gp100 peptide-pulsed or gp100-transfected DC. T cells activated with Ad-transduced DC (Ad-DC) showed an early and strong induction of cytokine synthesis even after primary stimulation. Reactivity against several gp100-epitopes suggested simultaneous presentation of multiple peptide-epitopes in an immunogenic form. However, gp100-specific T cell responses induced by Ad-DC decreased upon several restimulations, while, in parallel, Ad-specific T cell responses were elicited. In contrast, peptide-pulsed DC were able to induce long-lasting, melanoma-antigen-specific CTL. Combination of both strategies - priming with gp100-transduced DC and expansion with peptide-pulsed DC - led to increased frequencies of gp100-specific T cells with high affinity and in addition limited the Ad-specific T cell responses. Thus, anti-adenoviral T cell responses may provide a "helper" effect in the induction-phase of CTL by inducing the release of high amounts of IL-2/IFN-γ and by activation of a broad range of T cells. In addition, priming with Ad-DC might help to identify the epitope-specificity of $CD8^+$T cells in individual melanoma patients. A prime/boost vaccination strategy with the initial induction of immune responses by Ad-DC and a boost with peptide-loaded DC for expansion of CTL as shown already in vitro should be evaluated based on these data in vivo.

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Tyrosinase mRNA fragments in serum and plasma of melanoma patients: detection and evidence for their origin from apoptotic cells

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Increased levels of RNA fragments have been found in the blood of patients with different forms of cancer. Amplifiable fragments of tyrosinase mRNA in blood plasma fractions from melanoma patients were detected recently. The purpose of the present study was to reproduce these data and to expand these observations, characterizing mRNA expression of three melanoma-specific tumor-markers in serum and plasma samples from patients with disseminated disease (stage IV). Thus we analysed the presence of specific mRNA for tyrosinase, gp100, and MART-1 by RT-PCR amplification of specific cDNA from serum and plasma of 10 melanoma patients. Tyrosinase mRNA could be detected in serum/plasma of 6 of 10 melanoma patients whereas gp100 and MART-1 specific transcripts were not detectable in any of the samples tested. The presence and integrity of amplifiable RNA was shown in all serum and plasma samples of patients and controls by RT-PCR-specific amplification of porphobilinogendesaminase (PBDG) mRNA. To investigate the possibility that plasma tyrosinase mRNA originates from apoptotic melanoma cells, we performed in vitro studies with apoptotic bodies isolated from a melanoma cell line (Bu-Hom). Induction of apoptotic cell death and presence of apoptotic bodies was confirmed by different methods: Light microscopy evaluation of morphologic changes characterizing apoptotic cell death and Annexin-V/PI- staining of cell-free supernatants containing apoptotic bodies. The presence of RNA in apoptotic bodies was shown by staining with the dye pyronin Y. Incubation of fresh serum-samples with free mRNA isolated from the melanoma cell line Bu-Hom or cell-free supernatants containing apoptotic bodies from Bu-Hom cells, following mRNA extraction and subsequent RT-PCR analysis, demonstrates amplifiable tyrosinase mRNA in serum samples mixed with apoptotic bodies, but not in serum samples spiked with free mRNA. Furthermore performing time course analyses, amplifiable tyrosinase mRNA associated with apoptotic bodies was found after 30 min, but not after an incubation period of 60 min. In conclusion the present study demonstrates that circulating tyrosinase mRNA is detectable in serum and plasma of melanoma patients and that apoptotic tumor cells are a source for plasma RNA that is protected from serum RNases within apoptotic bodies.

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Reconstitution of E-Cadherin in G361 Human Melanoma Cells Triggers Apoptosis

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Cell-cell adhesion is considered important in the development and maintenance of organ tissue. Cadherins mediate calcium-dependent homophilic cell-cell interaction by a so-called zipper mechanism. In various tumors including melanomas cadherin expression is downregulated which is thought to be a key event in cell invasion.
In the present study E-cadherin levels of different cell lines were compared by western blotting. Protein extracts derived from the human keratinocyte line HaCaT gave strong E-cadherin signals whereas G361 human melanoma cells show only very weak signals. Extracts from a melanoma line derived from a freshly excised metastasis showed no E-cadherin staining. In order to investigate the role of E-cadherin in melanoma we stably transfected an inducible full-length E-cadherin construct (kind gift from Keith Johnson; Toledo, USA) into G361 human melanoma cells. After selection of vector carrying cells by using an eukariotic antibiotic the vector promoter became induced by dexamethasone. Already 8h after induction cells featured visible signs of cell apotosis including blebbing and ruffling of cell membranes, partly cells became detached from the substrate. The levels of cytosolic cytochrom c - an early marker of apoptosis - were markedly elevated in E-cadherin expressing G361 cells compared to mock transfectants. These results show that reconstitution of E-cadherin expression in G361 human melanoma cells is able to control cell fate. The mechanism by which E-cadherin triggers apotosis is yet not clear, but a βcatenin dependent mechanism seems to be likely.

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Tumor-Stroma-Interactions in malignant melanoma - characterisation of a novel cDNA

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In previous investigations we could demonstrate the activation of fibroblasts by tumour cells from malignant melanoma in vitro. The fibroblasts become activated by culture supernatants of melanoma cells, express elevated levels of MMPs and support the tumour cells to invade collagenous matrices in vitro. Beside the upregulation of known genes, soluble mediators of melanoma cells are able to induce a so far unknown cDNA in fibroblasts detected by PCR-Select (Clontech) technology.
Using RACE-PCR we have isolated the complete coding sequence giving a hypothetical protein of about 40 kDa. The cDNA is only weakly expressed in healthy skin and in biopsies of non-malignant nevi detected by in situ hybridisation (ISH).
The cDNA is strongly expressed in most of the melanomas (n=22) investigated. However, there are different results on the expressing cells in situ: We have found melanomas with preferential expression by cells located in HMB45-positive regions suggesting that in contrast to in vitro data the melanoma cells themselves express this gene. In contrast, we have got also data on melanomas where the cDNA is expressed in regions staining for mAb AS02 (fibroblasts and activated endothelial cells) suggesting an activation of stromal cells known from in vitro experiments. The evaluation of clinical and ISH characteristics should give some answers whether differentiation of the tumour plays a role in the expression of this cDNA.
Using in vitro expression studies we investigated the nature of the inducing agent (cytokine or chemokine?) in the supernatants of melanoma cells that induce the cDNA in fibroblasts in vitro. Fibroblast cultures have been exposed to various cytokines and chemokines in serum free medium and the gene expression has been analysed by RealTime RT-PCR. TGFβ1 and IL-1β obviously induce the expression of this mRNA in dermal fibroblasts in vitro. Investigations with blocking antibodies should show the significance of our results.

P167

Downregulation of TAP-associated glycoprotein (tapasin) is associated with progression of human primary melanoma

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Tumor cells with alterations of the MHC class I peptide-loading complex are unable to load peptide antigens onto MHC class I molecules. Those alterations result in destabilization of MHC class I expression on the tumor cell surface and thus play a critical role in escape from immunological recognition by the acquired cellular immune response. MHC class Ia downregulation has been repeatedly described on melanoma cells, and the comparative analysis of the expression of components of the class I peptide-loading complex may help to get further insight in the mechanisms employed by tumor cells to evade immunosurveillance. By forming physical links between class I and TAP molecules a component of the class I peptide-loading complex, tapasin, plays an important role in the assembly of MHC class I molecules with peptides in the endoplasmic reticulum. In the present study we compared 87 primary human melanoma lesions representing different invasion levels and 19 metastatic melanoma lesions for the expression of tapasin in tumor cells by immunohistochemistry with specific rabbit polyclonal anti-human Ab (StressGen). By scoring immunostaining patterns as homogeneously positive (>75% melanoma cells stained in the entire lesion), heterogeneously positive (between 25 and 75%), and negative (<25%) we found significant downregulation of tapasin in primary melanomas with an invasion level > 0.75 mm (p=0.009, chi-square test) as well as in metastatic melanoma lesions (p=0.02) as compared to primary melanomas with an invasion level ?0.75 mm. Our data suggest that downregulation of tapasin may be associated with failure of the acquired cellular immune response to control progression and metastatic spread of melanoma cells in vivo and that analysis of tapasin expression may be useful to select patients to be treated with T cell-based immunotherapy.

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Antibodies in the serum of patients with melanoma-associated retinopahty (MAR) recognize antigens expressed from melanoma cell lines

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Melanoma-associated retinopathy (MAR)is a paraneoplastic syndrome in patients with metastatic melanoma. The pathomechanism is supposed to result from antibody production against not yet identified melanoma antigens; these antibodies cross-react with epitopes of retinal cells. If a critical number of retinal cells is destroyed the ophthalmological symptoms appear. Recently, clinical studies proved that the majority of patients in advanced stages of disease suffer from subclinical MAR symptoms. Furthermore, immunofluorescent examinations, using a rabbit anti-retina serum and autologous serum from melanoma patients could show that these serum probes were able to react with retinal tissue from healthy donors as well as with autologous tumor tissue. In this study we screened serum from 20 melanoma patients in the various stages of disease and a polyclonal rabbit anti-retina serum on several melanoma cell lines and on fibroblasts using flow cytometry. As negative control normal rabbit serum was used. All serum probes were evaluated in a dilution of 1:1000, detection was performed using a FITC-conjugated goat anti-rabbit or goat anti-human antibody. The autologous serum probes from the 20 patients enrolled in this study as well as the rabbit anti-retina serum reacted with 4 of 5 tested melanoma cell lines. Flow cytometry on fibroblasts was always negative. We could show that the serum of melanoma patients contains antibodies against not yet identified melanoma antigens which seem to be expressed from most melanoma cell lines. Further investigations are required to identify and characterize the antigen(s) able to induce the production of antibodies cross-reative with retinal tissue.

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Differential downregulation of MHC class I-chaperones calnexin and calreticulin during metastatic spread of human malignant melanoma

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Characterization of the molecular basis of tumor recognition by T cells has shown that major histocompatibility complex (MHC) class I molecules play a crucial role presenting antigenic peptide epitopes to cytolytic lymphocytes. MHC class Ia downregulation has been repeatedly described on melanoma cells and is thought to be involved in the failure of the immune system to control tumor progression. Shortly after their synthesis, heavy chains of the MHC class I molecules bind to the ER-residing chaperone calnexin. Calnexin stabilizes free heavy chains, protects them from degradation, and allows assembly of heavy chains with β2-m. Binding of class I heavy chains to β2-m results in the exchange of chaperone calnexin for the chaperone calreticulin that allows incorporation of class I molecules into the so-called class I peptide-loading complex. Alterations in the expression of these chaperones may have important implications for MHC class I assembly and peptide loading and presentation on the tumor cell surface and thus may play a critical role in escape from immunological recognition by the acquired cellular immune response. In the present study we compared 106 and 111 melanoma lesions representing different stages of tumor progression for the expression of calnexin and calreticulin, respectively, in tumor cells by immunohistochemistry with mouse monoclonal anti-human calnexin Ab (Chemicon Inc.) and rabbit polyclonal anti-human calreticulin Ab (StressGen). By scoring immunostaining patterns as homogeneously positive (>75% melanoma cells stained in the entire lesion), heterogeneously positive (between 25 and 75%), and negative (<25%) we found significant downregulation of calnexin in metastatic as compared to non-metastatic primary melanoma lesions ($p=3.7 \times 10^{-8}$, chi-square test). In contrast, chaperone calreticulin showed stable expression in melanoma cells of primary as well as of metastatic lesions. Our data suggest that downregulation of calnexin but not calreticulin molecules may be associated with failure of the acquired cellular immune response to control metastatic spread of melanoma cells in vivo and that expression analysis of calnexin may may be useful to select patients to be treated with T cell-based immunotherapy.

P170

Malignant melanoma is associated with an activation of antioxidant enzymes and increased lipid peroxidation

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Solar UV radiation is known to induce the formation of reactive oxygen species (ROS) in skin. ROS participate in a number of pathophysiological processes including DNA damage and lipid peroxidation (LPO) and are believed to be a key factor for tumor progression. LPO-products, such as malondialedyde (MDA) or 4-hydroxynenal (4-HNE) can tie spontaneously to DNA, RNA or proteins and thus lead to mutagenesis. Malignant melanoma cells are known to exhibit aberrant regulation of redox sensitive pathways. We hypothesized that in human melanoma the natural redox balance is disturbed and results in accumulation of LPO-products. Biopsies of patients with superficial spreading melanoma (n=18) were compared to age-matched benign melanocytic naevi (n=18) and young healthy controls (n=10). Expression of the antioxidant enzymes copper-zinc superoxide dismutase (CuZnSOD), manganese SOD (MnSOD) and catalase (CAT) was analyzed by immunohistochemical techniques. To detect lipid peroxidation products, protein-bound-malondialdehyde (MDA) was immunohistochemically visualized. In all cases, stained sections were analyzed using densitometric image analysis (Analysis 3.0, Soft Imaging System, Muenster, Germany). Statistical analysis was performed by ANOVA. In human melanoma biopsies, a significant overexpression of all antioxidant enzymes was found when compared to surrounding non-tumor tissue as well as to benign melanocytic naevi, and young controls. Intriguingly, the lipid peroxidation marker MDA was significantly increased in melanoma tissue. MDA was found not only in typical melanoma cells, but also occured in surrounding keratinocytes.

In conclusion, enzymatic antioxidants in human malignant melanoma are activated. The lipid peroxidation product MDA accumulates within the melanoma area indicating severe oxidative damage. Thus, oxidative stress is very likely to play an important role in the development and progression of human malignant melanoma.

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Malignant melanoma cells induce endothelial cells to form tubulo-capillary structures by ephrinB2-ephB4 signal transduction

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Cell surface located Eph-receptor tyrosin kinases and their ligands, the ephrins, are known to be multitalented effectors of morphogenesis during the embryonal development with particular impact on the development of the brain and the vasculature. Recently, it was suggested that ephrinB2 may play a collaborative role in neo-angiogenesis as well, together with VEGF and angiopoetins. Since we had shown in the past that overexpression of ephrinB2 mRNA is a characteristic finding in cultured melanoma cells with increased metastatic potential and advanced primary as well as metastatic MMs (Vogt et al., Clin Cancer Res 4: 791-797, 1998), we addressed a possible involvement of ephrinB2 in tubular-formation mechanisms in vitro. Equal numbers of HUVEC cells were cocultivated with A375SM human MM cells on a layer of matrigel under serum starved conditions. The formation of tubolo-capillary structures (microvascular density cm-2) was quantitated by time-lap photography and interactive image analysis tools. We could show that the addition of A375 cells led to a significant increase of tubulo-capillary structures formed by HUVECs within 24 hours after MM cells were added, similar to VEGF positiv control (about 3-fold). Since ephB4 was previously demonstrated to be the leading receptor on HUVEC cells for possible ephrin ligands in the surrounding microenvironment, the assumed signal transduction via ephrinB2 (MM cell)-ephB4 (HUVEC) was specifically blocked by adding recombinant soluble monomers of ephrinB2 to the media in increasing amounts up to 1 µg/ml. Toxic effects on the cells were excluded by viability assays. The effects were a significant reduction of the microvascular density down to the level of the HUVEC-alone control. These observation suggests a model in which the MM-cells present an ephrin-ligand on their surface to induce endothelial cells to form primitive vasculogenic structures. Since this kind of blocking of this specific interaction could become a novel approach to antiangiogentic therapy in MM, we currently test this concept in the mouse-dorsal skin fold chamber angiogenesis model.

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Differential regulation of apoptosis by Bcl-X proteins in human melanoma cell lines

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Bcl-X, a member of the Bcl-2 gene family is expressed in two known splice variants, Bcl-X_S (proapoptotic) and Bcl-X_L (antiapoptotic) which regulate cell death in eukaryotic cells. Bcl-X proteins can form homo- and heterodimers with other Bcl-2-related proteins, their active form being localized in the outer mitochondrial membrane. By heterodimerization, Bcl-X_S was shown to block the antiapoptotic potential of Bcl-2 and Bcl-X_L. This has been shown in several cell types to result in increased sensitivity to proapoptotic stimuli as to chemotherapeutics. Expression analyses of Bcl-2-related proteins in melanoma cell lines and normal melanocytes by Northern and Western blotting revealed high levels of Bcl-X_L and Bcl-2 protein in melanoma cell lines, whereas Bcl-X_S was not or only weakly expressed. In order to investigate the role of Bcl-X_S and Bcl-X_L for apoptotic processes in human melanoma cell lines, we subcloned full-length cDNA fragments of both mRNAs by RT-PCR. Identity of the clones was confirmed by sequence analysis. For overexpression, both cDNAs were further subcloned in sense and in antisense-orientation downstream of a tetracyclin-regulatable promoter and were transfected into established Tet-On (SK-Mel-13, Bro) and Tet-Off (Mel-2a) melanoma cell lines. Overexpression of Bcl-X_L and of Bcl-X_S after transient transfection was verified on the mRNA level by Northern and on the protein level by Western analysis; apoptosis was quantified by the cell death detection ELISA (Roche). Induction of Bcl-X_S by doxycyclin led to significantly increased apoptosis as compared to non-induced cultures (1.2 to 1.7-fold), whereas induction of Bcl-X_L resulted in reduced apoptoptotic rates. Several cell clones were established which were stably transfected with Bcl-X_L. In these cell clones, sensitivity to the proapoptotic signal Fas (CD95, Apo-1) ligand was strongly reduced after induction. These findings suggest that exogenous Bcl-X_S may provide a novel tool for initiating cell death in human melanoma cells. An even stronger effect may be obtained by combination with chemotherapeutics. On the other hand, Bcl-X_L turned out as equipotent to Bcl-2 in prohibiting induced apoptosis in melanoma cells as shown for Fas ligand.

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Constitutive activation of extracellular signal-regulated kinases 1 and 2 in human melanoma cells in vitro and in situ

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The extracellular signal-regulated kinases 1 and 2 (ERK1 and 2) belong to the best characterized mammalian mitogen-activated protein kinase (MAPK) modules and are crucially involved in complex biological responses such as proliferation and differentiation. For example, sustained activation of ERK1/2 by a mutated upstream kinase results in transformation of 3T3 fibroblasts. Since melanocyte peptide growth factors induce rapid but transient ERK1/2 activation in normal human melanocytes (NHM) we studied the expression/activation of ERK1/2 in 8 human melanoma cell lines as well as in 68 melanomas in situ. In contrast to NHM, all melanoma cell lines displayed constitutive ERK1/2 tyrosine phosphorylation, but only ERK2 was dually phosphorylated on Thr183 and Tyr185, the critical phosphorylation sites required for full enzymatic activity of ERK2. ERK2 phosphorylation correlated with in vitro kinase activity as shown by phosphorylation of myelin basic protein. ERK2 tyrosine phosphorylation of melanoma cells in situ correlated with tumor invasion/thickness as shown by immunohistology. Incubation of melanoma cells with 5?-methyl-thioadenosine, an inhibitor of basic fibroblast growth factor (bFGF) receptor tyrosine kinase, strongly suppressed autonomous ERK1/2 phosphorylation in all melanoma cell lines and markedly inhibited in vitro proliferation. These findings suggest that constitutive activation of ERK1/2 in melanoma cells originates mainly from constitutively activated bFGF receptor but do not exclude deviations of dual-specific phosphatases, i. e., MKP1 and MKP3, which are capable of dephosphorylating ERK1/2. Our data also indicate that pharmacological intervention of the MAPK pathway leading to ERK1/2 deactivation may represent a novel therapeutic strategy for melanoma.

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Suppressor of cytokine signaling 1 (SOCS1) is expressed in human melanoma and in situ expression correlates with tumor progression

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Cytokine resistance is an important feature of melanoma progression and has been reported for interleukin-6 (IL-6) and interferon (IFN). We recently demonstrated that IL-6 resistance of melanoma cells is linked to impaired activation of the signal transducer and activator of transcription 3 (STAT3), a key player in IL-6 signal transduction. Since it was reported that suppressor of cytokine signaling 1 (SOCS1) can suppress IL-6- and IFN-induced responses by interfering with activation of STAT members, we examined the expression of SOCS1 in a panel of human melanoma cell lines derived from different tumor stages and with differential IL-6 sensitivity. RT-PCR revealed SOCS1-specific transcripts in all cell lines as well as in normal human melanocytes grown in absence or presence of TPA. Quantitative real-time RT-PCR did not demonstrate any correlation between IL-6 sensitivity, tumor progression and the level of SOCS1 expression. However, SOCS1 protein expression was confined to melanoma cell lines and was absent in normal melanocytes. SOCS1 protein was expressed in melanoma cells within the cytoplasm as shown by immunofluorescence. In situ SOCS1 expression in melanomas correlated with tumor progression and was uniformly positive in metastases but absent in normal skin. Our data for the first time show that melanoma cells express SOCS1. By acting as a negative regulator of cytokine signaling SOCS1 may mitigate biological responses of melanoma cells to therapeutically administered IFN or endogenously secreted pro-inflammatory cytokines.

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Targeting lymphotoxin-alpha to the tumor microenviroment elicts an infiltration of tumor-specific CD8+ T cells evidenced by*in situ* tetramer staining

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We previously demonstrated that antibody-mediated targeting of lymphotoxin-alpha (LT) to the tumor microenvironment mounts an effective cellular response against murine melanoma. Moreover, melanoma differentiation antigen specific T cells were detectable within the peripheral blood and spleen subsequent to targeted LT therapy. However, the relevance of anti-tumor T cell responses in the peripheral blood to predict clinical effect of these has been challenged since these cells seems to have only a limited capacity to infiltrate the tumors. Thus, it seems more appropriate to examine specific immune responses to solid tumors *in situ*. To this end, we used TRP-$2_{180\text{-}188}$ peptide/H-$2K^b$ multimers to stain frozen tissue sections. The specificity and the sensitivity of this technique was validated using a TRP-2-reactive T-cell line injected s.c.. In tumor samples obtained subsequent to targeted-LT therapy we detected up to 5% TRP-$2_{180\text{-}188}$ reactive cells among infiltrating CD8+ T cells. In contrast, for control animals treated with a soluble form of LT this percentage was below 0.5%. Moreover, the total number of infiltrating CD8+ T cells was substantial lower. This observation was confirmed by IFN-gamma ELISPOT assays detecting specific reactivity against TRP-$2_{180\text{-}188}$ peptides among freshly isolated CD8+ T cells from such tumors. However, the background reactivity against unpulsed target cells in these assays was rather high which is likely due to contaminating T cell epitopes derived from residual tumor cells in the T-cell preparation.
In summary, we have obtained direct *in situ* evidence that targeted-LT therapy is able to induce infiltration of tumor-specific T cells. Future studies will address whether these data will show a better correlation to the clinical course than that obtained from peripheral blood.

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Transcriptome analysis of melanoma metastases and their daughter passages - implications for vaccination protocols

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Most vaccine protocols for the treatment of malignant melanoma are based on cultured cells, which were prepared from tumor tissue. The cells are grown for different numbers of passages and then used for the stimulation of patients´ T-cells or dendritic cells which are reinjected to the patient. So far, no systematic analysis has been published comparing the transcriptome of the tumor tissue with the cultured cells of different numbers of passages. Gene expression profiling of melanoma metastases and their daughter cell lines of different numbers of passages was performed. Using Incyte® life grid arrays we could detect about 3.200 genes from 8.400 genes spotted on the array. The differences in gene expression correlate with passage numbers. The reproducible comparison of a melanoma metastasis with the daughter cell line after 5 passages revealed 2 % differentially regulated genes; after 27 and 34 passages already 10 % and 34 % of the detected genes showed a different expression level in the cell lines compared to the tumor, respectively. In all cell lines a subset of 23 genes was differentially regulated (21 Incyte ESTs, KIA A0863 protein, interleukin-6). For interleukin-6 it was shown to support the growth of melanoma cells and protects against apoptosis.Our data suggest, that in vitro cultured cells from tumors do respond strongly on the culture conditions still after a number of passages. The induction of interleukin-6 may indicate an autokrine growth stimulation within the cell culture. If cultured cells are taken for stimulation of immune cells it has to be considered, that the dramatic changes in the transcriptome are accompanied by changes in the proteome (e.g. the pattern of surface molecules and peptides presented to the immune cells). The differences observed in this study may explain the lack of therapeutic response of this therapies in some patients.

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Skin mast cells suppress the development and growth of epidermal tumors

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Our recently reported findings showing increased susceptibility to skin carcinogenesis in genetically CD117 (c-kit)-deficient $Kit^{W}/Kit^{W\text{-}v}$-mice, which lack skin mast cells (MC) and melanocytes, suggest that these cell populations provide protection from tumor development. Here, we have subjected MC knock-in mice generated by adoptive transfer of MC to the skin of $Kit^{W}/Kit^{W\text{-}v}$-mice to classical two-stage chemical carcinogenesis and have assessed the development and growth of epidermal tumors. $Kit^{W}/Kit^{W\text{-}v}$-mice that had been repaired of their cutaneous MC-deficiency by reconstitution with bone marrow-derived cultured MC ($Kit^{W}/Kit^{W\text{-}v}$+MC mice) proceeded to develop tumors significantly slower than MC-deficient $Kit^{W}/Kit^{W\text{-}v}$-mice when treated topically with a carcinogen (DMBA, once) followed by a promotor (TPA, 2x/week for 17 weeks). After 13 weeks of treatment only 50 ± 16.4% of $Kit^{W}/Kit^{W\text{-}v}$+MC mice, but 82.4 ± 6.6% of $Kit^{W}/Kit^{W\text{-}v}$-mice showed 1 or more papilloma ($p<0.005$). Interestingly, after 13 weeks of treatment $Kit^{W}/Kit^{W\text{-}v}$+MC mice also exhibited reduced numbers of papillomas per treatment site (1.8 ± 0.8 papillomas/mouse) as compared to $Kit^{W}/Kit^{W\text{-}v}$-mice (4.5 ± 0.7 papillomas/mouse, $p<0.02$). Susceptibility to skin carcinogenesis in $Kit^{W}/Kit^{W\text{-}v}$+MC mice was comparable to that seen in normal *Kit+/+ mice* (week 12: 1 or more papilloma in 51.7 ± 6.5% of *Kit+/+*mice, 2.3 ± 0.4 papillomas per mouse), indicating that activation of MC is a critical host anti-tumor defense mechanism. Most notably, MC appear to also regulate tumor growth. After 10 weeks of treatment the average tumor volume per papilloma was markedly increased in $Kit^{W}/Kit^{W\text{-}v}$-mice as compared to Kit+/+ mice and $Kit^{W}/Kit^{W\text{-}v}$+MC mice: 3.3 ± 1.2 vs. 0.9 ± 0.1 and 0.5 ± 0.1 mm^3/papilloma, respectively. These data support a dual role for MC in murine epithelial carcinogenesis: MC confer resistance to skin tumor development and suppress tumor growth.

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Higher Viral Load in HPV Associated Benign as Well as Non-Melanoma Skin Cancer of Immunosuppressed Versus Non-Immunosuppressed Patients

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Objective: A causal role for HPV and anogenital cancer, especially cervical carcinoma was shown fulfilling the WHO criteria for viral carcinogenesis in 1995. The relationship between HPV and non-melanoma skin cancer (NMSC) is still unclear as well as the true HPV prevalence rate of this disease mainly due to the low virus load and the high number of different HPV types. The risk of NMSC and the HPV prevalence rates in organ-transplanted recipients (immunosuppressed) are higher compared to the normal population (non-immunosuppressed). Therefore, we have estimated the amount of HPV DNA in cutaneous verruca vulgares, anal condyloma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC). **Patients and Methods**: Fresh frozen biopsies of HPV positive patients, 10 SCC, 3 BCC, 4 cutaneous warts, and 3 anal condyloma were examined using PCR based HPV detection methods. SCC biopsies were taken of 6 organ-transplanted recipients and 4 immunocompetent patients. The sensitivity of the PCR method using MY- or CP-primer was 100-10,000 viral genome copies dependent on the HPV type. The quality of all specimens was controlled by beta-globin specific PCR and only beta-globin positive cases were included in this study. The sensitivity of the beta-globin PCR was 500-1,000 genomes analysing serial dilutions of blood cells.**Results**: The relation of viral genomes to cellular genomes of acuminate warts and verruca vulgares was 4.86 (range 1-10) and 0.007 of SCC and BCC (range 0.01-0.001). The virus load of non-melanoma skin cancer (BCC, and SCC) was lower compared to benign lesions. The ratio of immunosuppressed (n=6) versus non-immunosuppressed SCC patients (n=4) was 0.01 vs 0.006. The viral load in tumors of immunosuppressed patients was higher than in non-immunosuppressed patients.**Conclusions**: HPV seems to be a risk factor for SCC especially in organ-transplanted recipients indicated by the increased virus load. Further studies investigating the mechanism of skin tumourgenesis are warranted.

P179

Increased Human Papillomavirus (HPV) Prevalence Rate in Normal Skin and Non-Melanoma Skin (Pre)cancer Using a Combination of Four Different PCR Methods

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Objective: HPV is the major risk factor of several benign (anogenital and cutaneous warts) and (pre)malignant anogenital lesions. In addition, HPV 16 and 18 are carcinogenic to humans (WHO, 1995). The role of HPV in skin carcinogenesis is still unclear, mainly due to specimen quality and the lack of HPV detection methods with a high sensitivity. Therefore, we have analysed 61 cutaneous specimens of 48 patients with normal skin and various cutaneous tumours using four different PCR methods. **Patients and Methods**: Fresh frozen biopsies (n=61) of 48 patients with 7 normal skin (7), 8 seborrhoic keratoses (SK) (11), 4 Bowens disease (BD) (6), 9 actinic keratoses (AK) (15), 15 squamous cell carcinoma (SCC) (21) and 5 basal cell carcinoma (BCC) (5) were examined. HPV status was assessed using consensus primers for genital (MY09/11) and cutaneous EV-associated HPV types (CP-primer). In addition, we have used non-consensus primers containing either HPV 5 (MY-5) or HPV 8 (MY-8) homologous sequences of the MY binding region. The quality of each specimen was controlled by beta-globin specific PCR and only beta-globin positive cases were included in this study. **Results**: By using only consensus primers HPV DNA was detected in 14/48 (29%) of patients and 21/61 (34%) of specimens. Positive HPV results with consensus and non-consensus primers were obtained in 23/48 (48%) of patients and 31/61 (51%) of specimens. HPV DNA was only detected in 9 of the patients (19%) and 10 of all specimens analysed (16%) using a combination of consensus and non-consensus primers. In these patients HPV 5, HPV 8, and EV-associated HPV types were present. HPV was detected in 1/7 normal skin (14%), in 4/8 SK (50%), in 1/4 BD (25%), in 6/9 AK (67%), in 11/15 SCC (73%), and none of 5 BCC. **Conclusions**: The broad spectrum of different HPVs in skin lesions cannot be detected by only employing consensus primers for genital and cutaneous virus types. This should be considered by epidemiological studies analysing the risk of HPV and skin cancer. HPV seems to be involved in skin carcinogenesis indicated by the prevalence rate increasing with severity of skin disease.

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Plasticity in tumor migration strategies: conversion of proteolytic mesenchymal migration to non-proteolytic ameboid crawling

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Cancer progression involves a cascade of adhesive and proteolytic cell-matrix interactions. In a 3D collagen matrix model, highly collagenolytic HT1080 fibrosarcoma cells expressing matrix metalloproteinases, serine proteases and cathepsins develop a spindle-shaped, mesenchymal migration type resulting in: (1) adhesive binding of leading filopodia to collagen fibers, (2) focal coclustering of beta 1 integrins and MMPs at fiber binding sites, (3) contractile force generation, fiber traction and bundling, (4) migration-associated degradation of collagen fibers and tube-like matrix defect generation accompanied by (5) the shedding of cell surface determinants into newly generated matrix defects. After blocking total endoproteolytic activity, however, this constitutive mesenchymal migration converted towards spherical, non-proteolytic morphodynamics reminiscent of ameboid crawling, while total migration efficiency remained unaffected. Induced ameboid movements resulted from striking morphological adaptation and squeezing through the fiber network. While blocked MMPs were excluded from fiber-binding sites, ameboid morphodynamics were independent of structural matrix breakdown. This "evolutionary recall" from proteolytic mesenchymal towards protease-independent ameboid tumor cell motility highlights unexpected plasticity in invasive cell migration strategies and represents an efficient escape mechanism upon protease inhibitor treatment.

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Collective cell invasion from primary melanoma explants: plasticity of cell-cell-interaction, b1 integrin function, and migration strategies

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Collective cell movement in epithelial and mesenchymal cancer represents an important invasion and migration strategy that is able to generate both, active and passive translocation of heterogeneous sets of cells. In primary melanoma explants cultured in three-dimensional (3D) collagen lattices, invasive migration of multicellular clusters was dependent on the function of ß1 integrins, as shown by preferential ß1 integrin expression and clustering in a subset of promigratory cells at the leading edge ("guiding cells") and the abrogation of multicellular migration by blocking anti-ß1 integrin antibody. Concomitantly, loss of ß1 integrin function induced complex morphological and functional changes, including loss of front-rear asymmetry followed by formation of two or several opposing leading edges. Ultimately, cluster disruption resulted from the detachment and dissemination of individual cells that utilized ß1 integrin-independent „ameboid" crawling. The conversion from collective ß1 integrin-dependent to individual ß1 integrin-independent ameboid migration shows considerable plasticity and adaptation in tumor invasion and migration strategies. While multicellular migration may allow the dissemination of neoplastic cells of different clonality and function within one functional unit, the conversion to ameboid single cell crawling implies the availability of a more basic, less tightly regulated migration program. Therefore, future therapeutic targeting of the invasion cascade will require to cope with cellular and molecular plasticity in tumor cell migration.

P182

Chemokine receptor expression on neoplastic and non-neoplastic T cells in mycosis fungoides

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Mycosis fungoides (MF) is a cutaneous lymphoma in which clonally expanded T lymphocytes home to the skin with an initial tropism towards the epidermis. With progression to the tumor stage, the dermal infiltrate becomes more diffuse and the epidermotropism may no longer be present. In addition, MF lesions are typically infiltrated by “reactive” non-neoplastic T lymphocytes.

We were interested to define the contribution of chemokine receptors (CCR) to the characteristic infiltration pattern of neoplastic and reactive T lymphocytes in MF. To this end,
lesional skin biopsies from 6 patch/plaque and 6 tumor stages of MF were analyzed by immunohistology using mAb specific for the 13 CCR (CCR1-CCR7, CXCR1-CXCR5, CX3CR1). Neoplastic and reactive T cells were differentiated by cytomorphology and their characteristic localization in the tissue. In selected patients, T cells isolated directly from lesions were analyzed in parallel for expression of activation markers and chemokine receptors using flow cytometry.

CCR1-3, CXCR2 and CX3CR1 were not observed on neoplastic or reactive T cells, and expression of CCR5, CCR6 and CXCR1 was variable. In contrast, CCR4, CXCR3 and CXCR4 were markedly expressed on both, neoplastic and reactive T cells, whereas CCR7 was mainly present on neoplastic lymphocytes. Interestingly, in the majority of tumor-stage MF specimens a loss of chemokine receptors, in particular CXCR3, was observed on neoplastic T cells. The immunohistology data were in good concordance with the results obtained by flow cytometry on the same specimens.

Taken together, we have identified several chemokine receptors which appear to be closely associated with MF. In addition, our data suggest that the loss of chemokine receptors contributes to the loss of the epidermotropism observed in the tumor stage of MF, in which neoplastic T cells spread into lymph nodes and other organs.

P183

Identification of sample-specific sequences in mammalian cDNA andgenomic DNA by the novel ligation mediated subtraction (Limes)

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Primary cutaneous T- and B-cell lymphoma exhibit a considerablevariation in clinical presentation, histology, immunophenotype as wellas prognosis and have a largely unknown etiology. Accordingly, we triedto isolate tumor-associated mutations from a cutaneous lymphoma cellline (My-La) by applying the representational difference analysis (RDA).RDA and other subtraction techniques allow to eliminate ubiquitoussequences existing in both, the sample of interest (tester) and thesubtraction partner (driver). As a result, tissue-specific (ortester-specific) sequences are enriched. After application of RDA,however, repetitive sequences and artificial fusion products ofotherwise independent PCR fragments (PCR hybrids) were predominatelyisolated that severely interfered with the isolation of tumor-relevantfragments. Accordingly, we developed a considerably more robust andefficient approach, which is termed ligation mediated subtraction(Limes). These improvements are consequences of the usage of a highlyspecific ligase mediating the ligation of oligonucleotides to the endsof perfectly matched tester/tester hybrids. In first applications ofLimes, genomic sequences and/or transcripts of genes involved in theregulation of transcription - such as the transforming growth factor bstimulated clone 22 related gene (TSC-22R) -, cell death and cytokineproduction (caspase-1), or antigen presentation (HLA class II sequences)were found to be absent in My-La. Moreover, Limes allowed to identifysequences that were tester-specifically absent at genomic andtranscriptional level. Due to these results, we are currently able toinvestigate a panel of lymphoma-specifically expressed sequences withunknown function and expect, that Limes may substitute/supplement othertechniques such as RDA or DNA microarray techniques in a variety ofdifferent research fields.

P184

Induction of apoptosis via a CD95/Fas-mimicking pathway in epithelial tumor cells is a novel anticarcinogenic principle exerted by imiquimod and resiquimod, immune response modifiers of the imidazoquinoline family

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are increasingly common tumors in fair-skinned populations. Topical imiquimod, an immune response modifier, was efficacious in the treatment of BCC or solar keratoses. It is thought that imiquimod exerts its effects through proinflammatory cytokines, thus stimulating a tumor-directed T cell response. However, the exact molecular basis of the antineoplastic effects has not been completely unraveled yet. In particular, direct antitumoral activity has not been shown.We demonstrate that imiquimod and, to a lesser extent, resiquimod increased apoptosis between 100% and 750% in five different SCC cell lines as well as HaCaT cells at doses that are relevant *in vivo*. As detected by DNA-fragmentation, induction of apoptosis in normal keratinocytes was considerably weaker (increase of 30-40% as compared to vehicle-treated controls), thus implying a relative tumor selectivity. Apoptosis of tumor cells induced by imiquimod or resiquimod resembled that seen with antibody-mediated signaling through CD95/Fas and included activation of caspase 3 as detected by Western-blot analysis. In addition, the pro-apoptotic activity of imiquimod and resiquimod could be inhibited completely by specific tetrapeptide antagonists to caspases 3, 8 and 9. In addition, stable overexpression of murine *bcl*-2 in HaCaT cells rendered these cells unresponsive to the pro-apoptotic activity of imiquimod and resiquimod, respectively, as compared to mock-transfectants. These results provide strong evidence that imiquimod and resiquimod induce apoptosis in keratinocyte-derived tumor cells by mimicking the pathway activated by CD95/Fas or other death receptors. Thus, our findings strongly suggest direct antitumoral activity of imidazoquinoline family members through preferential induction of apoptosis in cutaneous epithelial tumor cells. This activity may bypass certain mechanisms developed by malignant tumors to resist death signals transduced via the CD95/Fas or related signaling pathways, thus contributing to the specific antineoplastic effects of imiquimod seen *in vivo*.

P185

Androgen reduction by 17β-hydroxysteroid dehydrogenase but not 5α-reductase type 1 activity may indicate the tumorogenic potential of skin gland cells and other tissues

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Steroid 5α-reductase plays a major role in androgen activation because it catalyses the conversion of testosterone into 5α-dihydrotestosterone, the most potent androgen. Two isoforms have been characterised. Type 2 is mainly expressed in the prostate while the tissue distribution of type 1 is still under investigation. Moreover, hormone-related cancers share a unique mechanism of development: Endo- and exogenous hormones drive cell proliferation, and thus the opportunity for the accumulation of somatic mutations. The genes involved in progression of hormone-related cancers are still unknown. Since skin glands and breast tissue show similarities concerning their differentiation, we studied the expression of 5α-reductase isoforms in normal skin cells, in established cell lines such as the human sebaceous gland cell line SZ95, the spontaneously immortalized human keratinocyte line HaCaT and the breast cancer cell lines BT-20, T47-D, MDA-MB 435S and MCF-7. By rtPCR, northern blot and western blot studies as well as immunocytochemistry we found the expression of 5α-reductase type 1 in the cytoplasm of all skin cells as well as breast cancer cell lines, while type 2 could not be detected in any cell type. Furthermore, the expression pattern was correlated with the 5α-reductase activity by evaluation of testosterone metabolism by HPTLC in primary sebocytes, SZ95 sebocytes, HaCaT keratinocytes and MDA-MB 435S BT-20, T47-D and MCF-7 breast cancer cell lines. The total amount of 5α-reductase products was 12.9% in MCF-7, 7.0% in HaCaT, 6.7% in primary sebocytes, 6.5% in MDA-MB 435S, 4.7% in T47-D, 4.3% in BT-20 and 2% in SZ95, showing no correlation between testosterone oxidation and tumorogenic potential. In contrast, androstanedione, a 17β-hydroxysteroid dehydrogenase reduction product of testosterone, was the main molecule identified in benign cells, namely 13.5% in primary sebocytes, 15.0% SZ95, 7.0% in HaCaT, while in the breast cancer cells the total recovered radioactivity was below 0.5% indicating high levels of 5α-dihydrotestosterone. These data suggest a major role of active androgens in the tumorogenesis and should be subject of further investigations.

P186

Differential expression of MMP-9, MMP-2 and MT1-MMP correlates with melanoma progression in a syngenic EGFP transgenic mouse model

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Recently, we have demonstrated that expression of MT1-MMP and activation of MMP-2 in tumor cells is correlated with melanoma progression both in xenograft models and in human melanocytic lesions. However, not only tumor cells but also tumor surrounding and/or infiltrating stromal as well as immune cells express MMPs and TIMPs. Until now the complex interaction between tumor cells and tumor surrounding stromal cells is not fully understood. Therefore, we established a syngenic model, consisting of three murine melanoma cell lines which display different metastatic behaviour in vivo to scrutinize the expression of MMP-9, MMP-2, MT1-MMP, TIMP-1 and TIMP-2. Enhanced Green Fluorescent Protein (EGFP)-transgenic mice allows to discriminate tumor from host cells.To this end, MMP-9, MMP-2, MT1-MMP, TIMP-1 and TIMP-2 protein was expressed by all cell lines. Moreover, Western blot analysis and zymography revealed that expression and/or activation of MMP-9 and MMP-2 as well as expression of their specific inhibitors TIMP-1 and TIMP-2 were increased in the highly metastatic cell line B16.G3.12.BM2 compared to low and intermediate metastatic cell lines. Cell surface expression of MT1-MMP as determined by flow cytometry was similar on all cell lines. Characterization of subcutaneous tumors in EGFP mice revealed a high expression of MMPs and TIMPs not only in tumor cells but also in tumor surrounding/infiltrating stromal cells, which was pronounced at the tumor invasion front. Thus, the expression of MMP-9, MMP-2 and MT1-MMP both by tumor itself as well as by non-neoplastic stromal cells stipulated the metastatic behavior. Future studies are addressing the signals transmitted by the tumor cells to modulate these environmental changes.

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Reduction of expression of tuberin, the tuberous-sclerosis-complex-gene-2 product in tuberous sclerosis complex associated and sporadic skin tumors

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Background: Patients affected with tuberous sclerosis complex (TSC) are prone to the development of multiple benign tumors of the skin and other organs. Tuberin, the protein product of the tuberous-sclerosis-complex-2 tumor suppressor gene (TSC2) has been shown to inhibit cell proliferation. In TSC associated kidney tumors and sporadic brain tumors the loss/reduction of tuberin has been shown.
Methods: Specimens of 9 squamous cell carcinomas (SCC) and 5 basal cell carcinomas (BCC) from patients without TSC and 6 biopsies of connective tissue nevi (CTN) of patients with TSC were obtained. Specimens were analyzed by immunoblotting for the expression of tuberin.
Results: Absent or reduced levels of tuberin were detected in the dermal parts of 3 of 6 shagreen patches, 2 of 5 BCC, and 4 of 9 SCC.
Conclusions: In tumors/hamartomas of patients with TSC the complete loss of TSC2 and tuberin is a mechanism which could be shown for CTN, thereby excluding the possibility of haploinsufficiency of TSC2. In a substantial number of cutaneous BCC and SCC the loss or downregulation of tuberin seems to be epigenetic, as alterations of TSC2 are not known in these tumors. The absence or reduction of tuberin might contribute to their proliferation.

P188

Differential expression of Calpain isozymes 1 and 2 (CAPN1 and 2) and calpastatin in cutaneous malignancies.

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Two major isozymes of the ubiquitious intracellular cytoplasmic non-lysosomal cysteine endopeptidase calpain (CAPN) are known. Many substrates of the different calpain isoenzymes known today, such as the transcription factors c-Fos and c-Jun, the tumor suppressor protein p53, protein kinase C, pp60src, and the adhesion molecule integrin have been implicated in the pathogenesis of cutaneous malignancies including malignant melanoma (MM) as well as squamous (SCC) and basal (BCC) cell carcinomas, suggesting an important role of the calpain isoenzymes in malignant diseases. We have analyzed for the first time expression of CAPN1 and CAPN2 in MM, BCC and SCC of human skin. Immunoreactivity (streptavidin-peroxidase technique) for CAPN1, CAPN2 and calpastatin was detected in MM, metastases of MM, acquired melanocytic nevi, BCC and SCC. Interestingly, CAPN1 staining was markedly reduced in BCC as compared to normal human skin (NS) or SCC, while in contrast CAPN1 mRNA levels (real time PCR, LightCycler) were markedly elevated in BCC (median 4,4; p=0,001) and SCC (median 11,22; p=0,01) as compared to NS (median 0,0084). No differences were found analyzing mRNA expression of CAPN2 (northern analysis) as well as immunoreactivity of CAPN2 and the endogenous CAPN-inhibitor calpastatin in NS, BCC and SCC. Our findings indicate that calpain isozymes may be involved in the tumorigenesis and growth regulation of MM, SCC and BCC. Pharmacological modulation of calpain activity may be a target for the treatment of MM and SCC.

P189

Distinct periodic acid-Schiff (PAS)-positive patterns correlate with tumor thickness in melanoma

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PAS-positive patterns have been described to represent vascular channels in melanomas. An association between distinct PAS-positive patterns (e.g. networks) and outcomes of patients with melanomas was suggested previously. However, an association between these patterns and tumor thickness has not been analyzed so far. Therefore we retrospectively analyzed PAS-positive patterns in cutaneous melanomas with known thickness and outcome. 120 patients with melanoma (> 1mm) were followed up for 5 years. In 50 of the patients, the melanomas recurred, in 70 they did not. We were able to match 15 pairs of recurring versus non-recurring tumors of identical thickness. The PAS-positive patterns were categorized into straight or parallel patterns, arcs, loops, networks, and reticular ropes. We assigned the 120 melanomas to three groups of tumor thickness (1 - 2 mm, 2 - 3 mm, > 3mm). Straight or parallel PAS-positive pattern were preferentially detected in thin melanomas whereas loops, networks, and reticular ropes were detected in thick melanomas. No substantial correlation between any distinct PAS-positive pattern and outcome was found in the analyzed matched pairs of melanomas.We conclude that distinct PAS-positive patterns correlate with tumor thickness but do not represent a prognostic parameter independent of tumor thickness. PAS-positive patterns have been described as vascular channels with a PAS-positive basal membrane but without endothelial cells, and are believed to contribute to the supply of the tumor cells. Possibly, an acceleration in tumor growth with increasing tumor thickness causes a change from rather organized parallel and straight structures of the vascular channels to more disorganized structures such as networks, arcs, and loops. This could explain the correlation between distinct PAS-positive patterns and tumor thickness.

P190

DCoH/PCD, dimerization cofactor of hepatocyte nuclear factor 1/pterin-4α-carbinolamine dehydratase is overexpressed in primary human melanoma and squamous cell cancer

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DCoH/PCD, dimerization cofactor of hepatocyte nuclear factor 1/pterin-4α-carbinolamine dehydratase has a bifunctional role: A: transcription factor, B: enzymatic function in the biosynthesis of melanin. Dysfunction of DCoH/PCD is associated with the human disorders hyperphenylalaninemia and vitiligo.We studied the expression of the DCoH/PCD in different human skin cancers and normal organs of different individuals by immunostainig of tissue arrays.Tissue arrays are microscopic glass sildes with multiple tissue samples suitable for immunohistochemistry.Using melanoma tissue arrays, we identified 17 of 50 melanoma specimens of various stages being positve for DCoH/PCD. In contrast, all tested normal skin specimens were negative, human squamous skin cancers were stained for DCoH/PCD in relation to the degree of differentiation (8 of 19 highly differentiated; 2of 8 moderately differentiated; 0 of 8 poorly differentiated specimens, and 4 of 6 metastatic squamous cell cancer). In addition to positive DCoH/PCD staining in liver that has already been shown, normal human organs such as kidney, esophagus, stomach, prostate and cervix were DCoH/PCD positive. In conclusion we show that benign nevi (n=20) were only rarely stained for DCoH/PCD, while melanoma and squamous cell cancer were frequently DCoH/PCD positive, rendering DCoH/PCD a potentially useful tumor marker for cancer of the skin.

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Functional analyzis of Jararhagin, a collagen mimetic substrate on MMP expression and cell morphology: Characterization of functional domains for integrin receptor activation

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Cell matrix interactions play an important role in a variety of physiological and pathological processes like wound healing, cell invasion and metastasis.
Previously we have shown, that binding of the cell adhesion molecule integrin a2ß1 to its ligand type I collagen initiates several signalling events including the regulation of matrix metalloprotease (MMP) synthesis and activation, in particular of MMP-1 and MMP-14. Due to the function to degrade matrix components these enzymes are crucial for tissue remodeling.
Synthesis of MMP-1 and MMP-14 was found to be induced upon treatment of fibroblasts in monolayer culture with Jararhagin which was comparable to that obtained in fibrillar collagen lattices. This snake venom metalloprotease containing a disintegrin and a metalloprotease domain binds to a2ß1 integrin and induces intracellular signalling events similar to collagen I. In addition treatment with Jararhagin leads to a change of cell morphology to a spindlelike, "motile" phenotype and reduced collagen gel contraction indicating an inhibition of collagen/integrin interaction.
To get more insights in the mechanisms underlying the Jararhagin/integrin interaction we further chararcterized integrin binding of different domains of Jararhagin. We used the disintegrin domain as well as peptides corresponding to different regions of the metalloprotease and disintegrin domains of the snake venom.
Adhesion assays showed that the "RKKH" peptide and the disintegrin domain are bound by fibroblasts and in addition inhibit collagen/integrin interaction. The disintegrin domain only was able to induce MMP-1 synthesis and MMP-14 mediated pro MMP-2 activation and to reduce collagen gel contraction, while peptides failed to show an effect.
These data show that the disintegrin domains is responsible for a2ß1 activation and induction of MMP synthesis.
In contrast to the full length Jararhagin protein, however, treatment of fibroblasts with the disintegrin domain did not alter cell morphology, suggesting that the metalloprotease and disintegrin domains exert different effects on cellular functions.

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ANTAGONIST SIGNALING: A RAFT-INDEPENDENT MECHANISM FOR RECEPTOR DESENSITIZATION

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Chemokine receptors belong to the family G protein coupled receptors. Agonistic triggering results in migration, secretion, elevation of intracellular calcium, and activation of extracellular signal-regulated kinase (ERK)-2. Recently, we characterized Eotaxin as antagonist of the chemokine receptor CCR2. In contrast to the agonist MCP-1, Eotaxin does not induce chemotaxis, calcium mobilization, enzyme release, or receptor internalization via CCR2 but inhibits the MCP-1-mediated responses. Here we report that in CCR2 transfected cells Eotaxin induces ERK-2 phosphorylation comparable to MCP-1. Activation of receptor-coupled G proteins is accomplished by the GDP/GTP exchange. In isolated membranes Eotaxin did not stimulate GTP-binding whereas MCP-1 induced GDP/GTP exchange indicating distinct pathways of ERK-2 activation. Therefore we tested different inhibitors the ERK activation pathway. MCP-1- and Eotaxin-induced ERK phosphorylation was inhibited by pertussis toxin which attenuates Gi proteins and by PD98059 which blocks MEK the upstream kinase of ERK. Conversely, PP2, an inhibitor of Src kinases, only abolished the MCP-1-stimulated but not Eotaxin-dependent ERK activation. Src kinases are known to associate with lipid microdomains in the membrane, called rafts. We investigated if the two ligands have different dependence on raft formation for ERK activation. Cholesterol depletion by cyclodextrin (Cdx) disables cells to form rafts. In our system, Cdx abolished ERK-2 activation only by MCP-1 but not by Eotaxin. To further assess the role of Eotaxin signaling we investigated the effect of ERK-2 on CCR2 desensitization. We have reported that Eotaxin desensitizes CCR2 for MCP-1-dependent calcium mobilization. We show here that this effect was reversed if the cells were pretreated with PD98059. These data support a new concept of receptor antagonism: the antagonist Eotaxin induces a signaling cascade via CCR2 and ERK that causes desensitization of CCR2, thereby enhancing its antagonistic effect. Raft formation in moving cells is important for induction of cell polarity, a prerequisite for site directed migration. We show that a non-polarizing stimulus (Eotaxin) does not require raft formation for signaling whereas the CCR2 agonist MCP-1 needs lipid rafts for signal transduction.

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Expression of PAF-R in human embryonal cells, HaCaT cells and psoriasis

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Platelet activating factor (PAF) is a lipid mediator of inflammation. Moreover, its receptor (PAF-R) plays a role in bone marrow hematopoiesis and in murine kidney-, bladder- and colon-tumors. Recently PAF-R has been shown in keratinocytes, rarely and in the basal layer of human epidermis, the relevance of this findings being unknown. Therefore, we studied for the pregnancy week (PW) PAF-R is turned on in embryonal cells, for its role in the inflammatory disease psoriasis and for its expression in HaCaT cells.
Indirect immunoperoxidase technique was applied to primary human keratinocytes and fibroblasts from embryonal epidermis (informed consent of parents after abortions, n=3 per PW), HaCaT cells, normal skin and psoriatic lesions and to controls with the primary polyclonal goat antibody against human PAF-R (1:100, Fa. Santa Cruz), the secondary antibody donkey anti goat (1:50, Fa. Santa Cruz) and the chromogen DAB. Additionally Western Blot analysis was performed with all these cells and specimens.
Embryonal keratinocytes of the 20. PW were negative for PAF-R, but in the 21. PW positively labeled in the cytoplasm with perinuclear dots in 4,2 ± 0,05 %. In normal human epidermis from adults PAF-R was found in the basal cell layer, but not in all cells and in a plaque like staining pattern in the cytoplasm. In contrast, in psoriasis cytoplasmic PAF-R positive keratinocytes were found in the spinous cell layer in a band like staining pattern. In HaCaT cells, Western blot analysis revealed a positive band for PAF-R at 68 kDa. Embryonal fibroblasts were negative for PAF-R in the 20. and 21. PW, in normal human dermis and in psoriasis.
PAF-R seems to play a role in the differentiation of embryonal keratinocytes after the 21. PW, still present in a few cells in the basal layer also in human skin of adults. In psoriasis PAF-R in the spinous cell layer may mark differentiation. In the tumor cell line HaCaT PAF-R might stand for its role in epithelial tumors.

P194

Microarray analysis of human dermal papilla and connective tissue sheath cells reveals multiple factors with potential for hair follicle growth promotion and cycling control.

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Previous research has indicated dermal papilla cells are derived from the connective tissue sheath and studies have clearly demonstrated the ability of dermal papilla cells to induce hair follicle development and differentiation. Advances in technology now make it possible to use gene array analysis to investigate dermal papilla and connective tissue sheath cell gene expression profiles.
We compared the gene-expression profiles of hair follicle derived cultured dermal papilla cells versus connective tissue sheath cells, obtained from individual humans with informed consent using high-density oligonucleotide arrays. This approach resulted in the identification of 15 known, differentially expressed genes in dermal papilla derived cells and 26 in connective tissue sheath derived cells, encoding products potentially involved in morphogenic signaling, transcriptional regulation, extra-cellular matrix modeling, cell adhesion and cell migration. Of greatest interest, significant expression was noted for MST1, HGFAC, MMP1, MMP3, TMSB10, IGFBP2, ANXA6, EXTL1, and PTTG1IP. Additional ESTs were also defined.
Our preliminary data suggests several gene products with potential to modify hair follicle activity. More extensive micro-array use may yield more candidates worthy of further analysis. Gene expression profiling using gene arrays is a comprehensive approach to evaluating hair follicle compartments. In the future, by profiling hair follicles at different stages during embryological development and cycling it may be possible to identify the key regulatory products involved. Gene array profiling of normal versus disease affected hair follicle compartments may also reveal regulatory factors behind the disease and identify candidate genes for targeting with new therapeutic regimes.

P195

MYOPATHY IN DERMATOMYOSITIS IS ASSOCIATED WITH EXPRESSION OF CALCIUM-BINDING PROTEINS MRP8 AND MRP14

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Dermatomyositis is an idiopathic inflammatory myopathy with characteristic cutaneous manifestations. The mechanisms of muscular and cutaneous damage involve a mononuclear infiltrate, but are not well defined. Monocytes exhibit various phenotypes with different functional properties. One proinflammatory subtype of macrophages is characterized by expression of MRP8 and MRP14, two calcium binding S100-proteins. They comprise about 5% of the cytosolic protein in monocytes, but their functions are still not fully revealed.
In the present study we investigated the expression and local distribution of MRP8 and MRP14 immunohistochemically in muscle biopsies of dermatomyositis (n=12), polymyositis (n=12) and inclusion body myositis (n=9) as well as their effects on muscle cells in vitro (in vitro assays on differentiation, proliferation and apoptosis).
In endomyosial mononuclear infiltrates monocytes and macrophages (CD68+) express MRP8 and MRP14 at a very low level. In contrast, MRP8 and MRP14 high expressing monocytes (CD68+) are the most abundant cell type in necrotic myofibers of all three disorders (double-labeling). Thus there was a clear association of expression of MRP8 and MRP14 by infiltrating macrophages with necrosis of myofibers. As MRP8 and MRP14 have recently been shown to be secreted by activated macrophages we investigated if they have direct extracellular effects on myocytic cells. We found a direct inhibitory effect of MRP8/MRP14 on proliferation and differentiation of C2C12 myoblasts as well as a time and dose dependent induction of apoptosis via activation of caspase 3 after treatment with purified MRP8/MRP14 complex.
This is the first description of myopathic effects of MRP8 and 14, two proteins abundant in mononcytic cytoplasm. Our results indicate that activated macrophages exhibit a destructive effect on myofibers in the process of inflammatory myopathies via secretion of MRP8/MRP14.

P196

Differential Requirements for Calcium Mobilization in p38 MAP Kinase and ERK1&2 Activity in Human Basophils Between Immunological and Non-Immunological Stimuli.

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Calcium mobilization has long been appreciated to play a crucial role in mast cell and basophil activation. However, little is known concerning which key signalling pathways are affected by the ion, particularly following cell activation by immunological stimuli. We therefore compared the effects of calcium-containing versus calcium-free conditions on the IgE-dependent and -independent phosphorylation of p38 MAPK and ERK1&2 in purified human basophils from allergic donors. Our data show that both kinases, which are vital for controlling histamine, LTC-4 and cytokine secretion, require intracellular calcium as well as influx of the ion into the cells. In the absence of extracellular calcium the early kinetics of p38 MAPK and ERK1&2 activity (caused by anti-IgE, fMLP or ionophore) remained unaffected but the magnitude of phosphorylation was sharply reduced and waned more rapidly than for calcium-containing conditions. Similar effects were also observed using thapsigargin, which specifically releases intracellular calcium into the cytosol. This demonstrates that the early activation of both p38 MAPK and ERK1&2 depends on intracellular calcium mobilization whilst the magnitude and longevity of phosphorylation requires calcium entry into the cell. The sensitivity of the above kinases, which are well known to control mediator output from basophils, to calcium highlights the need to develop pharmacological strategies to block its actions on basophil signalling.

P197

Minocycline does not alter collagen type I metabolism of dermal fibroblasts in culture

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Antibiotic drugs such as Penicillamine, Penicillin G and Minocycline are used in the treatment of Systemic Sclerosis (SSc). However, there is little knowledge on the mechanism of anti-fibrotic action of these drugs. In general, excluding cell proliferation data, the influence of antibiotics on the metabolism of eukaryotic cells is poorly investigated.
Penicillin G that may improve skin status of SSc-patients significantly did not influence collagen metabolism in vitro during previous investigations . Minocycline is another drug that may improve the clinical phenotype of SSc .
We studied the effects of Minocycline by analysing the influence of various concentrations of Minocycline on cell proliferation, synthesis and degradation of collagen by human dermal fibroblast from healthy donors and SSc-patients (collagen high producer).
More detailed, collagen metabolism of cultured dermal fibroblasts was studied by Northern Hybridisation for mRNA of collagen I, proline-4-hydroxylase, lysyl-hydroxylase, matrix metalloproteinase I and determination of collagen content in culture supernatants.
Minocycline did not alter the expression of the investigated mRNA, independently on the dosage and the incubation times used. The amount of collagen I protein was not influenced.

In conclusion, there is no evidence of a direct anti-fibrotic effect of Minocycline on dermal fibroblasts. Therefore, other mechanisms might be responsible for its effect in the treatment of SSc-patients. In the case of Minocycline, the anti-inflammatory action might be relevant for the positive effects known from SSc therapy.

P198

The Thy1 / Thy-1-ligand-interaction is involved in binding of inflammatory cells and melanoma cells to activated Thy-1-positive microvascular endothelial cells

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Cell adhesion plays a central role in metastasis of tumour cells as well as in inflammation. Specific adhesion molecules direct and regulate the invasion of these cells in certain tissues.
In recent studies, the human Thy-1 molecule was characterised as an inducible activation associated molecule on human dermal microvascular endothelial cells (HDMEC). Thy-1 expression has never been detected on resting endothelial cells in situ and in vitro. So far the physiological function of Thy-1 and the Thy-1-ligand were unknown. Now, we could detect a Thy-1-ligand on polymorphnuclear cells (PNC) and monocytes. Our experiments proved the involvement of the Thy-1 / Thy-1-ligand interaction in binding of PNC and monocytes to activated Thy-1-positive HDMEC. The strong connection of inflammatory events and Thy-1 expression suggests a specific role during binding of monocytes and PNC to activated endothelium establishing an event-specific interaction with PNC and monocytes from peripheral blood vessels during inflammatory responses or cell activation processes.
Furthermore, we could demonstrate a strong expression of Thy-1 on endothelial cells in tissue section of melanoma. In vitro, an induction of Thy-1 expression on HDMEC was seen after stimulation with melanoma cell derived soluble mediators. The Thy-1-ligand was found on different melanoma cell lines in vitro and on melanoma cells in situ. In cell adhesion assays the involvement of the Thy-1 / Thy-1-ligand interaction in binding of melanoma cells to activated Thy-1-positive HDMEC was shown.
Taken together, we could demonstrate for the first time a physiological role of Thy-1 /Thy-1-ligand as an inducible activation associated cell adhesion molecule in the human system. Furthermore, the investigation of Thy-1 and Thy-1 ligand expression might allow a more detailed insight in the direction and regulation of the invasion of cells in inflammatory tissues as well as in metastasis of certain tumour cells.

P199

Transactivation of EGF-receptors and induction of c-fos expression by angiotensin II in human dermal fibroblasts

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Angiotensin receptors are expressed by diverse human cutaneous cells including dermal fibroblasts. This study was designed to identify angiotensin receptor coupled second messengers in this cell type. Signal transduction mechanisms known to be coupled to angiotensin receptors on fibroblasts of other origin comprise the transactivation of EGF-receptors and, subsequently, the induction of c-fos expression.
Primary dermal fibroblasts, isolated from human foreskin, were incubated for 1 to 5 min (for determination of EGF-receptor transactivation) or 15 to 60 min (for estimation of c-fos expression), respectively, with angiotensin II (10-7 to 10-11 M). Total RNA was isolated from treated or untreated (control) cells and forwarded to semiquantitative RT-PCR analysis of c-fos transcripts. The protein fractions of treated and untreated cells were immunoprecipitated using an antibody directed against the phosphorylated, activated EGF-receptor and subsequently analysed by Western Blotting.
Angiotensin II time-dependently elicited a transactivation of EGF-receptors with a maximal effect as early as 2 min after stimulation. It induced furthermore time- and dose-dependently the expression of c-fos expression, peaking after 30 min at a concentration of 10-7 M angiotensin II.
From these results we conclude, that human dermal fibroblasts express functional angiotensin receptors. Since physiological functions of angiotensin II in non-dermal fibroblasts via a transactivation of EGF-receptors or an induction of c-fos expression have been described to include the stimulation of fibronectin- and TGF-beta-synthesis, suggesting a role in fibrotic processes, this possibility may also pertain to dermal fibroblasts and is currently under investigation.

P200

Psoriasis like skin disease after specific ablation of I kappa B kinase 2 from the epidermis of mice

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Despite convincing evidence for a role of T lymphocytes in the pathogenesis of psoriasis the mechanisms responsible for the initiation of this disease are still controversial. The T cell camp of skin researchers, on one side, attempts to describe psoriasis as an autoimmune disease that is the consequence of dysregulated T cell function. The other side believes in a role of epidermal keratinocytes that, probably due to a genetic defect, respond abnormally to environmental challenges and thus initiate the disease process. Using conditional gene targeting we ablated a single gene, I kappa B kinase 2, specifically in epidermal keratinocytes of mice. After exposure to the extra maternal environment, these mice develop a severe skin phenotype with striking similarities, both macroscopically and histologically, to human psoriasis. Typical patterns of keratin expression, immune cell invasion and production of soluble mediators of inflammation further confirm the resemblence of this phenotype to psoriatic skin. Our results demonstrate that elimination of a single gene in epidermal keratinocytes only is sufficient to trigger psoriasis like skin disease.

P201

Expression of prolactin mRNA and Protein in human skin and its role in catagen control of human hair follicles

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The pituitary hormone prolactin (PRL) exerts a wide variety of growth-regulatory effects in mammals. We have recently demonstrated that prolactin is expressed in a hair cycle- dependent manner in mice However, it has been published that prolactin RNA is not expressed in human skin(Slominski et al, 2000). Here, we provide the first study of prolactin and prolactin receptor expression in human hair follicles (HF) by immunohistology and RT-PCR, and have analyzed the influence of prolactin on hair growth in human hair follicles.Human HF from scalp hair transplants were isolated and cultured for up to eight days in Williams E medium and prolactin (20ng, 200ng or 400ng/ml) was added every other day. Hair shaft elongation was measured at regular intervals. For RT-PCR RNA was extracted from full-thickness human scalp skin. Prolactin immunoreactivity (IR) was detected in human anagen VI HF in the inner root sheath (IRS), matrix cells of the hair bulb, and outer root sheath (ORS). The dermal papilla (DP) fibroblasts were negative. Prolactin receptor IR showed a very similar pattern, as it was detected in the ORS, proximal IRS, and in most of the matrix cells. In catagen HF, prolactin and PRL receptor immunoreacvtivity appeared to be upregulated. ORS and matrix cells showed IR, while in the DP and IRS no prolactin expression could be detected. Treatment of human HF with 400ng/ml of prolactin resulted in significant inhibition of hair shaft elongation and induction of a catagen-like HF transformation. Compared to vehicle controls Ki-67/TUNEL double-staining showed a significantly reduced number of proliferating and increased number of apoptotic cells in the hair bulb of PRL-treated HF. Prolactin transcripts could be detected in human skin by RT-PCR. In this study we show contrary to a previous report- human skin is both a source and target of PRL, with the HF as a likely candidat for substantial Prl-gene and protein expression, supporting the hypothesis that PRL acts as an autocrine hair growth modulator, which inhibits proliferation and induces apoptosis in HF keratinocytes.

P202

RESISTANCE TO TRAIL INDUCED APOPTOSIS IN PRIMARY KERATINOCYTES: A ROLE FOR EFFECTOR CASPASE INHIBITION

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has been shown to exert potent cytotoxic activity against transformed keratinocytes, whereas primary keratinocytes are relatively resistant against TRAIL. In this report, we examined intracellular mechanisms of resistance of primary keratinocytes to TRAIL. The presumed antiapoptotic transcription factor NF-KB was rapidly activated by TRAIL in primary keratinocytes. Moreover, upon inhibition of TRAIL-induced NF-KB activation with the proteasomal inhibitor MG115, primary keratinocytes were dramatically sensitized to TRAIL-induced apoptosis. In contrast, transient transfection of keratinocytes with mutant forms of IKB kinase 2 did not modulate TRAIL sensitivity in keratinocytes. At the molecular level, sensitization to TRAIL-induced apoptosis by MG115 was found to be downstream of the native TRAIL death inducing signalling complex (DISC) and its components FADD, caspase 8, caspase 10 and cFLIP, which was analyzed for the first time in human keratinocytes. In line with these findings, rapid partial cleavage of caspase 3 to a 20 kDa fragment was detected. However, full caspase 3 maturation to enzymatically active p17 and p15 fragments and the release of cytochrome c and smac/DIABLO from mitochondria required the inhibition of the proteasome. Taken together, our data suggest that modulation of proteasomal function sensitizes keratinocytes to TRAIL-induced apoptosis at or upstream of mitochondria, but downstream of caspase 8 or 10 activation at the DISC, leading to cytochrome c and smac/DIABLO release from the mitochondrium and concomitant full caspase 3 maturation. Taken together, our data suggest an important role of effector caspase maturation in the regulation of death receptor sensitivity, particularly in primary keratinocytes. This mechanism of resistance to TRAIL-mediated apoptosis may well be important in many different cellular systems and might lead to unwanted side effects on primary cells when proteasomal inhibitors are used for cancer therapy.

P203

Expression of a vascular endothelial growth factor (VEGF) antagonist, sFlt-1, in chronic non-healing wounds

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During cutaneous wound healing, the development of granulation tissue requires the formation of new capillaries, and the absence of this tissue is a typical feature of chronic wounds. Angiogenesis is tightly regulated by factors that act by either stimulating or inhibiting the growth of new vessels. We hypothesize that the balance of positive and negative regulators of neoangiogenesis is disturbed in the chronic wound environment, and thereby may influence the progression of vessels growth in chronic wounds. To test this hypothesis we evaluated the effect of chronic wound fluid on endothelial cell proliferation in vitro.
The proliferation of human umbilical vein endothelial (HUVE) cells was significantly decreased in the presence of wound fluid collected from chronic wounds. In contrast, wound fluid collected from acute wounds stimulated endothelial cell proliferation. An explanation for this apparent decrease of HUVE cell proliferation in the presence of chronic wound fluid could be the result of degradation of angiogenic factors by proteases found in the chronic wound environment, or the expression of negative endothelial growth regulators in the chronic wound environment.
In the present study we evaluated the expression of sFlt-1, a soluble form of the VEGF receptor Flt-1, in wound fluid from acute and chronic wounds. sFlt-1 is able to inhibit VEGF induced endothelial cell proliferation and is so far the only naturally occurring antagonist of VEGF. Semiquantitative RT-PCR analysis indicates that the expression of Flt-1 and sFlt-1 is increased in chronic wounds in comparison to normal skin. Further, an ELISA specific for sFlt-1 and western-blot analysis could demonstrate the presence of sFlt-1 in chronic and acute wound fluid. In several chronic wound fluids tested, sFlt-1 levels are significantly increased over levels in acute wound fluids. Soluble Flt-1 may be important in regulating the actions of VEGF and angiogenesis during wound healing and its increased expression in chronic wounds may contribute to an impaired wound healing response.

P204

Keratinocyte transplants cultivated in autologues serum

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The etiology of chronic non-healing wounds is still unknown. A variety of different underlying diseases is discussed including chronic venous insufficiency, arterial occlusive diseases, vasculitis and diabetes mellitus. The medical costs of ambulant and hospitalize wound management estimates milliards and demand the development of innovative and efficient treatment regimes. New strategies for treating chronic wounds include complex woundcouverages, growth factors and cellular wound dressings. Transplants of keratinocytes and/or fibroblasts are used which are placed on the wound surface with different carriermaterials; allogenic or autologues cells are used. Allogen transplants carry the risk of transmitting infectious diseases such as viruses/prions and transplants are rejected. In contrast, autologues transplants are permanent on the wound surface and there is no risk of transmitting diseases. To further reduce the risk of transmitting diseases Smola et al. showed that the autologues culture system can be extended by replacing fetal calf serum by autologues patient serum (Lancet 1999). In our study chronic wounds of different etiology (chronic venous insufficiency, traumatic injury and postoperative wounds) were treated with autologues keratinocyte transplants which were cultivated in autologues patient serum (4% in DMEM). Keratinocytes were isolated from 4x6 mm punch biopsies, co-cultered with mouse 3T3 fibroblasts in autologues serum. Within three weeks keratinocytes could be expanded up to 250 cm2. Cells were transplanted until passage three. In all cases there was a clear reduction of wound size following 4-6 autologues keratinocyte transplantations applied on a weekly schedule. Currently we perform studies assessing the influence of autologues keratinocyte transplants on the micromillieu of chronic wounds.

P205

Detection of mu-opioid receptors on human SZ95 sebocytes and modulation of proliferation by beta-endorphin via a calcium-dependent mechanism

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Many lines of evidence indicate that the sebaceous gland is a target organ for stress hormones. We recently detected specific receptors for alpha-MSH, namely melanocortin-1 receptors, in human sebaceous glands in situ as well as in the immortalized sebaceous gland cell line SZ95. We demonstrate here by RT-PCR that SZ95 sebocytes express specific transcripts for the mu-opioid receptor (MOR). Expression of the MOR on the protein level was verified in total cell lysates from SZ95 sebocytes. Western immunoblotting revealed a single band of approximately 38 kDa in SZ95 sebocytes as well as in SK-N-MC cells, a neuroblastoma cell line used as a positive control. Lysates from PC12 cells, which previously were reported not to express the MOR on the RNA level, did not contain any immunoreactive protein as demonstrated by Western immunoblotting. As shown by immunofluorescence, MOR immunoreactivity was detected on the surface of SZ95 sebocytes and was distributed in a punctate staining pattern. Stimulation of SZ95 sebocytes with beta-endorphin (beta-ED), a natural ligand for the MOR, resulted in dose-dependent rise of intracellular cAMP. To further assess the biological relevance of these findings, we evaluated the effect of beta-ED on the proliferation of SZ95 sebocytes alone and in combination with epidermal growth factor (EGF) in serum-free medium. When co-incubated with EGF, beta-ED inhibited the proliferation of SZ95 sebocytes in a dose-dependent fashion. This effect was calcium-dependent since in calcium-deficient medium beta-ED failed to have any effect. Taken together, our data show that sebocytes express the MOR; their biological activity can be regulated by beta-ED in a calcium-dependent mechanism.

P206

Gene Regulation in Fibroblasts under Tensile Load

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Modulation of gene expression in dermal fibroblasts due to changes in the mechanical force balance plays a key role in skin function in the context of wound healing, scar formation, and fibrosis. However, the mechanisms underlying these mechanically regulated phenomena are still poorly understood.
To analyze the influence of self-generated tensile forces on human dermal fibroblasts, cells were cultured within three-dimensional collagen lattices, either under low (relaxed lattice) or under high tension (stressed lattice). Gene expression was assessed at two time-points: before the onset of gel contraction (2 hours) and at macroscopic completion of contraction (20 hours). Employing cDNA microarray technology, subtractive and Northern hybridization analysis we could demonstrate differential regulation of many different genes and substantial modulation of cellular morphology at 20 hours. Tensile forces induced a myofibroblastic phenotype, transcription of extracellular matrix constituents, fibrogenic mediators, cytoskeletal components and protease inhibitors, while repressing transcription of proteases and inflammatory mediators. We also identified various mechano-inducible transcripts with yet unknown function. One of these genes has previously been isolated from mechanically stressed tissues and shows a significant upregulation during early scar formation.
Further, SAGE-technology (Serial Analysis of Gene Expression) was used to compare gene expression profiles of fibroblasts in the presence/absence of mechanical stimulation at the beginning of gel contraction (2 hours), and a number of differentially regulated genes including potentially relevant transcription factors was identified. At this point, it is unclear whether a master regulatory gene has been detected which in turn regulates a cluster of genes in a coordinate fashion.

P207

Evidence for expression of melanocortin receptors and components of the enzymatic machinery required for posttranslational processing of pro-opiomelanocortin in human dermal papilla cells

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There is accumulating evidence that dermal papilla cells (DPC) are involved in regulating hair follicle activity by producing growth factors and cytokines. In mice DPCs are also known to produce agouti-signaling protein, a paracrine regulator capable of inhibiting melanogenesis. We show here that human dermal papilla cells in culture express components of the melanocortin system, i. e., melanocortin receptors (MC-R), prohormone convertases (PC) and the prohoromone pro-opiomelanocortin (POMC) which is a common precursor for the neuropeptides alpha-melanocyte-stimulating hormone, adrenocorticotropin (ACTH) and beta-endorphin. By means of RT-PCR with specific primers pairs against all MC-Rs, we detected specific transcripts for both MC-1R and MC-4R on the RNA level while MC-2R, MC-3R and MC-5R transcripts were absent. PC2 and its essential cofactor 7B2, a maturation factor of this enzyme, were also found to be expressed on the RNA level whereas transcripts for PC1 could neither be detected in unstimulated DPCs nor in DPCs treated with several pro-inflammatory stimuli. Interestingly, despite absent PC1 expression, DPCs expressed POMC on the RNA and protein level as shown by Western immunoblotting using an anti-ACTH antibody that cross-reacts with the 31 kDa protein of POMC. These results suggest that human DPCs are target cells for the pleiotropic actions of melanocortins and, possibly, by releasing melanocortins may modulate biological responses of other cell types, i. e. melanocytes and keratinocytes, within the hair follicle.

P208

Epi-Flow - a physiological cell culture system ?

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Although there is a broad knowledge about morphology and biochemistry of melanocytes, data characterizing the cross-talk between melanocytes and keratinocytes are limited. This is due to the different culture conditions necessary for the growth of both cell types in vitro. Melanocytes in culture require phorbolester (TPA), which is toxic for keratinocytes, and cholera toxin which enhances the mitogenic effect of TPA. These substances have a growth promoting effect on cells simulating transforming conditions leading to severe modifications of gene expression in comparison with cells under *in vivo* conditions.
To establish a more physiological *in vitro*system for investigating the cross-talk of melanocytes and keratinocytes we established the Epi-Flow cell culture system. This system allows maintenance of cells in the absence of growth promotors because the Epi-Flow simulates more physiological conditions by the supply of a continuous flow of medium and a permanent gas exchange. In addition, two or more cell types can be cocultured with different media. This is enabled by using membranes to which cells can be attatched on both sides, without exchange of the different culture media on both sides of the membrane. The membranes have a pore-size which just allows pseudopodia to grow towards the other side, enabling the direct contact between melanocytes and keratinocytes. In another setting possible effects of melanocytes on keratinocyte conditioned medium including parakrine factors without direct cell-cell contact can be monitored.
To prove the concept of the Epi-Flow to be a more physiological cell culture system, we analysed the gene expression patterns by cDNA array hybridisation in melanocytes grown in normal cell culture and in the Epi-Flow system in the presence of medium with and without PMA. Most importantly, genes involved in proliferation and apoptosis were downregulated (e.g. MAPKK kinase, epithelial membrane protein 3, p300, caspases 2, 10 and 8) in the Epi-Flow system. In addition genes involved in the maintenance of cells in the G0/G1 phase of the cell cycle (e.g. MARCKS and galectin) were found to be induced. In conclusion, our data comparing the transcriptome of melanocytes in Epi-Flow system with conventional culture support the hypothesis, that the Epi-Flow system provides more physiological conditions. Therefore the system may be also of high relevance for therapeutic studies.

P209

Decreased expression of inhibitor SMAD 6 and 7 and TGF-β mRNA in **fibroblast cultures from keloid scarring**

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Keloids are benign skin tumors occurring during wound healing in genetically predisposed patients. They are characterized by an abnormal deposition of extracellular matrix components, in particular collagen. There is evidence that transforming growth factor-beta (TGF-β) is involved in keloid formation. SMAD proteins play a crucial role in TGF-β signalling and in terminating the TGF-β signal by a negativ feedback loop through SMAD 6 and 7. Until today it is unknown how TGF-β signalling might be involved in the pathogenesis of keloids. Therefore we investigated the expression of SMAD mRNA in keloids and in normal scars as well as the expression of TGF-β 1, 2, and 3 and their receptors. Fibroblast obtained from biopsy specimen from keloids, normal scars or normal skin were cultured under standard conditions. After 2-4 passages cells were stimulated with TGF-β or left without stimulation. Total mRNA was extracted and the expression of SMAD 2, 3, 4, 6 and 7 mRNA analysed by realtime PCR using the Lightcycler technique. In addition the expression of TGF-β 1, 2 and 3 and the expression of TGF-β receptors I and II (TβI and II) was analyzed in cultured fibroblasts as well. Our data demonstrate a decreased mRNA expression of the inhibitory SMAD 6 and 7 in keloid fibroblasts as compared to normal scar ($p<0.01$) and normal skin fibroblasts ($p<0.05$). Furthermore, significantly higher TGF-β 2 mRNA expression was found in keloid fibroblasts as compared to normal scar fibroblasts ($p<0.05$). In contrast, significantly lower TGF-β 3 mRNA expression was found in keloid fibroblasts in comparison to normal scar-derived fibroblasts ($p<0.01$). No significant difference of SMAD2, SMAD3, SMAD4, Tβ I and Tβ II mRNA expression was found between keloid-, normal scar- and normal skin-derived fibroblasts. These results strongly support the important role of TGF-β 2 in keloid development and of TGF-β 3 in keloid prevention. Furthermore, our data suggest that the high expression of the inhibitory SMADs (SMAD 6 and 7) mRNA in normal scars may inhibit further activity of the receptor to TGF-β responses and terminate TGF-β signalling in order to prevent abnormal fibroblast proliferation and collagen deposition. The decreased expression of the inhibitory SMAD 6 and 7 could explain why TGF-β signaling is not terminated in keloids by an negative feedback loop.

P210

The epidermal cholinergic system

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Acetylcholine (Ach)and its nicotinic (nAch-R) and muscarinic (mAch-R)receptors are produced extraneuronally in the tegumental cells covering the inner and outer surfaces of the body. Ach and nicotine have been suggested to be involved in epidermal diffrentiation, keratinocyte proliferation and locomotion. However, the exact role of the epidermal cholinergic system remains unclear, so far. We could confirm the presence of mRNA of alpha 3, 5, 7 9, beta 2 and 4 nAch-R and the m1-m5 mAch-R in human skin by PCR. In order to study the role of the cholinergic system in the skin under physiologic and pathologic circumstances, we have produced and characterized polyclonal guinea pig antisera against human nicotinic and muscarinic acetylcholine receptors. Using immunohistochemistry, we were able to localize the alpha 3, 5, beta 2 and 4 n-Ach-R subunits, which form the heteropentameric ion channels of the alpha 3* type, to the basal layer of the epidermis and to the basal layer of the anagen hair follicle. The alpha7 and 9 subunits, which form homooligomeric receptors, were predominantly expressed in the suprabasal layers. The G-protein coupled muscarinic m3 isoform was localized predominantly to the epidermal basal layer. Like in other extraneuronal systems, the m4 and m5 isoforms of the mAch-R were broadly distributed, while the m1 isoform was restricted to the suprabasal cells of the upper spinous and granular layers. We therefore conclude, that proliferating basal cells of the epidermis express two types of Ach-R: the heterooligomers of the nAch-R alpha 3* type and the mAch-R m3, weakly also m4 and m5. Upon differentiation keratinocytes of the spinous and granular layer downregulate the alpha 3* and the m3 receptors and upregulate alpha 7, alpha 9 and m1. Further studies will be needed to functionally understand the importance of the epidermal cholinergic system.

P211

Transient control of androgen receptor expression in human keratinocytes using antisense oligonucleotides

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Having recognized the key effects of biologically active androgens on skin, their local synthesis in sebocytes and degradation in keratinocytes have gained special interest. The effects of androgens is mediated by their binding to nuclear androgen receptor (AR) molecules available in skin cells. An association of a possible local overproduction of active androgens and skin disorders is obvious in acne and androgenetic alopecia in males. Androgen activity can be inhibited by systemic administration of compounds, which have strong AR affinity and antagonize AR binding of agonists. In this study we applied a new technology to realize the same purpose: we tested the activity of antisense oligonucleotides against the AR in human keratinocytes. Antisense molecules are highly specific because they bind to mRNA targets at multiple points of interaction at a single receptor site. In a previous successful antisense study we could diminish the protein expression of AR in SZ95 sebocytes with antisense oligonucleotides. To transfer the antisense oligonucleotides into human keratinocytes a liposome-mediated transfection system with Poly-L-ornithine (12 microg/ml) over 4 hrs was evaluated. The transfection efficiency was assessed using FITC-labeled $(ACTG)_5$ random oligonucleotides, which were localized in cytoplasm structures of the keratinocytes. AR expression on the protein level was investigated by Western blotting. The transient transfection of foreskin keratinocytes with thioate antisense molecules showed a reduction of the AR expression about 25% compared to native keratinocytes after 14 h recovery time. After 24 h, the AR levels returned to the levels of non-transfected keratinocytes. The AR knock down in epidermal keratinocytes of two women (30 and 60 years old) was stronger in older, more differentiated keratinocytes. This procedure provides a specific tool for studies of the sensitivity of epidermal cells of different individuals against biologically active androgens.

P212

Expression of hormone receptors in human skin cells

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Hormones play a significant role in skin homeostasis, whereas recent reports indicate that human skin is an independent peripheral endocrine organ. Hoever, little is known about the expression of several hormone receptors in the different skin cells. The aim of this study was to investigate the expression and regulation of growth hormone receptor (GHR), insulin-like growth factor I receptor (IGF-IR), androgen receptor (AR), estrogen receptor alpha (ER-alpha) and estrogen receptor beta (ER-beta) in human sebocytes, keratinocytes and fibroblasts at the mRNA level by reverse transcription polymerase chain reaction (RT-PCR). The cells were cultured under defined conditions. The sizes of the RT-PCR obtained cDNA transcripts were 201 bp for GHR, 265 bp for IGF-IR, 603 bp for IR, 998 bp for AR, 483 bp for ER-alpha and 283 bp for ER-beta. AR expression was detected in all skin cells. ER-alpha was expressed in all skin cells, while ER-beta in sebocytes only. GHR was expressed in all skin cells, while IGF-IR was strongly expressed in keratinocytes and sebocytes. The mRNA levels of all receptors were upregulated by 17beta-estradiol, whereas the mRNA levels of GHR and IGF-IR were also upregulated by insulin and Ca^{2+}. These results indicate gene expression for GHR, IGF-IR, AR, ER-alpha and ER-beta in human skin cells providing a basis for further studies on endocrine regulation in the skin.

P213

Agonists of Proteinase-activated Receptor-2 Stimulate Activation of Nuclear Transcription Factor kappa B and Upregulation of ICAM-1 in Human Keratinocytes.

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It is well known that serine proteases are generated in the skin and are involved in several biological processes such as growth, differentiation and inflammation. Proteinase-activated receptor-2 (PAR-2) belongs to a new G protein-coupled receptor subfamily activated by various serine proteases. PAR-2 has been demonstrated to play a role during cutaneous inflammation in vivo. PAR-2 is expressed by human keratinocytes (KTC) and regulates inflammatory responses, proliferation and differentiation in these cells. However, the underlying mechanisms of PAR-2 activation in KTC are still incomplete. Northern blot analysis revealed PAR2-RNA in primary KTC and endothelial cells. Moreover, increased RNA levels were detected in lesional skin of patients with atopic dermatitis. Ca-mobilization studies demonstrated that PAR-2 is functional in human KTC induced by PAR2 agonist tryptase. Electro mobility shift assays and morphological transduction studies revealed PAR2-induced activation and translocation of NFkB with a maximum after 1h. Use of a NFkB inhibitor prevented upregulation of the cell adhesion molecule ICAM-1 in KTC. In conclusion, PAR-2 induces NFkB activation and upregulation of cell adhesion molecules such as ICAM-1. Thus, PAR-2 may play an important regulatory role on human KTC function under physiological and pathophysiological conditions.

P214

Dimethylfumarate inhibits TNF-induced CD62E expression in a NF-κB-dependent manner

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Fumaric acid esters (FAE) are thought to improve psoriasis by altering leukocyte, keratinocyte and/or endothelial functions. To determine specificity, kinetics and molecular mechanisms of different FAE in their ability to inhibit endothelial cell activation, we analyzed CD62E and CD54 expression in endothelial cells in vivo and in vitro. In lesional skin of psoriatic patients, oral FAE treatment resulted in a marked reduction of CD62E but not of CD54 expression on dermal microvessels. Using human umbilical vein endothelial cells (HUVEC), dimethylfumarate (DMF) almost completely inhibited TNF-induced CD62E, but not CD54 expression at concentrations ≤ 70 µM, mimicking the situation in vivo. A 60 min DMF preincubation was sufficient to block TNF-induced CD62E expression for up to 24 h. In contrast, equimolar concentrations of methylhydrogen fumarate (MHF), the hydrolysis product of DMF, did not suppress TNF-induced CD62E expression. Likewise, all FAE other than DMF were ineffective. Using CD62E, NF-κB or AP-1-responsive promoter constructs, DMF inhibited TNF-induced activation of the CD62E and the NF-κB but not of the AP-1 promoter construct. In summary, at a dose range ≤ 70 µM, DMF appeared to be a specific inhibitor of CD62E expression in a NF-κB-dependent manner.

P215

Dimethylfumarate inhibits tumor necrosis factor-induced nuclear entry of NF-κB/p65 in **hum**an **e**ndo**the**lial c**e**ll**s**

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Dimethylfumarate (DMF), a diester of fumaric acid, is known to inhibit TNF-induced activation of endothelial cells in vivo and in vitro. To characterize the molecular basis of DMF action, we analyzed TNF- and/or VEGF-induced tissue factor expression in human endothelial cells in culture. We show that DMF inhibited TNF-induced tissue factor mRNA transcription and protein expression, as well as TNF-induced DNA binding of NF-κB in endothelial cells. In contrast, DMF has no effect on VEGF-induced tissue factor protein and mRNA expression as well as DNA binding of EGR-1 and constitutive SP1. Employing immunoblots of nuclear extracts and FACS analysis of nuclei isolated from green fluorescent protein-NF-κB/p65-transfected endothelial cells we show that DMF inhibited the TNF-induced nuclear entry of p65. This is not due to inhibition of TNF-signaling down to IκB, because DMF does not alter TNF-induced IκBα phosphorylation and IκBα degradation. Also the constitutive shuttling of inactive p65/IκB complexes into and out of the nucleus is not altered by DMF. We conclude that DMF inhibits NF-κB-induced gene transcription at the level of the nuclear entry of p65, after its release from IκB.

P216

Retinoic acid upregulation of TGF-β in t**he** d**e**r**m**al **p**a**p**illa o**f hum**an ana**g**e**n hair follicles likely causes premature catagen induction**

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Retinoic acid and TGF-β have recently been shown to induce catagen in human anagen hair follicles (HF) in vitro. TGF-βisoforms are up-regulated in several cell types after retinoic acid treatment suggesting mediator activities of TGF-β for retinoic acid. Since systemic retinoid treatment is often associated with telogen effluvium, we have investigated the influence of all-trans retinoic acid (RA) on the growth of human hair follicles in culture and expression of TGF-β by histomorphometry, immunohistochemistry and light-cycler RT-PCR. Human anagen hair follicles (HF) were dissected, and organ-cultured for 2, 4 or 6 days with retinoic acid (10-6 -10-11 M), and hair shaft elongation was measured. For light-cycler PCR hair follicles after two days of RA treatment were divided into hair bulb (incl. the DP) and the distal part of the HF. Histomorphometry showed that approx. 80% of the RA-treated hair follicles at day 6 had entered catagen, compared to 30% of control HFs. Hair shaft length decreased already significantly after two days in the RA-treated group, compared to control. This corresponded to a relative upregulation of apoptotic cells and a downregulation of proliferating cells in the RA-treated follicles. TGF-β immunoreactivity (ir) was detected in the outer root sheath (ORS) of untreated anagen VI scalp hair follicles. The lower portion of the hair bulb and the dermal papilla (DP) were negative for TGF-β. After 4 days of RA treatment, TGF-β ir was significantly up-regulated in anagen hair follicles in the DP and the connective tissue sheath. In control HFs TGF-β RNA levels were very low in the hair bulb (incl. DP), while there were significantly upregulated TGF-β transcripts in the RA-treated HFs. Taken together these data support the hypothesis that TGF-β acts as a key-mediator of RA by inducing catagen.

P217a

Tight-junction (re)-organisation in human epidermis and keratinocytes

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Tight-junctions (TJ) are well known structures in simple epithelia and endothelia connecting cells and controlling tissue functions. They separate the molecular composition of the basolateral and the apical site of the cells ("fence-function") and form and maintain barriers between different compartments of the body. The existence of TJ in stratified epithelia has been shown by us and others only lately. To enlarge our knowledge of these TJ we have studied various TJ-proteins during differentiation and stratification and during wound-healing in the epidermis and in cultured keratinocytes by using biochemistry, immunocytochemistry, cell culture and the generation of new antibodies recognizing various TJ-proteins. We demonstrate that TJ-proteins occur very early during the stratification of skin-equivalents and during the regneration of the epidermis in wound-healing. Furthermore we show the different localisation of various TJ-proteins, especially claudins, in the epidermis and discuss the existence of different TJ-systems with a variated permeabilty for molecules.

P217b

Tight Junctions: Do they play a role in the barrier-function of the skin?

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Tight Junctions (TJ) fulfil important barrier functions in simple epithelia and endothelia, thereby supporting e.g. the generation of the blood-brain barrier. As we and others have shown lately, TJ exist also in stratified epithelia, e.g. the human epidermis which is known to play an important role in the barrier function of the skin, protecting the body from the surrounding environment and from the loss of water and solutes. Therefore we investigated the fate of TJ-proteins in diseases accompanied with an impaired barrier function of the skin, e.g. psoriasis, lichen ruber and eczema by using immunocytochemistry and the generation of new antibodies. We demonstrate the down-regulation of TJ-proteins in affected skin, especially in areas with throughbreaking granulocytes. Moreover we show that the restriction of the TJ-proteins occludin and ZO-1 to the stratum granulosum and the transition layer is abolished. Regarding these results we discuss the possible role of TJ in the barrier-function of the skin.

P218

ABSENCE OF THE TRANSCRIPTION FACTOR FOXN1 IN NUDE MICE CAUSES A SEVERE DYSPLASIA OF CLAWS

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BACKGROUND: Nude mice and their human and rat equivalent lack the evolutionarily conserved transcription factor FOXN1 that i) is exclusively expressed in epthelial cells, ii) is known to be a key regulator of hair keratin genes, and iii) is presumed to be involved in early keratinocyte differentiation. Homozygous animals lack a thymus and any macroscopically visible fur, due to bending of the improperly keratinized hair shaft within the hair canal. Nude mice have extensively been used as a biomedical tool but surprisingly little is known about the morphological alterations in the skin and its appendages. This particularly applys to the claws of nude mice that have not been studied thoroughly so far. QUESTIONS ADRESSED: We aimed to investigate the morphological alterations in the claws of nude mice, in order to further dissect the functions of FOXN1 in keratinocyte differentiation. MATERIAL AND METHODS: The claws of homozygous and heterozygous NMRI nude-mice of different age and sex were investigated by histology, immunohistology and transmission electron microscopy. RESULTS: Claws of homozygous nude mice are much shorter than claws of heterozygous littermates and are abnormally bent, but have the same diameter. Claws of homozygous mice have blund tips and exhibit a severe parakeratosis of the dorsal plate which normally builds the sharp tip of the claw. This parakeratosis is based on a dyslasia of the claw matrix which shows impaired keratinization of keratinozytes. CONCLUSIONS: The lack of the transcription factor FOXN1 in nude mice leads to dysplasia of the claw matrix and impairs keratinocyte-differentiation. Claws in nude mice might provide an intriguing novel model for dissecting the full spectrum of FOXN1 functions, including and beyond the regulation of keratin gene expression.

P219

Ameboid morphodynamics and contact guidance: T cell crawling through fibrillar collagen is independent of proteolytic matrix remodeling

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Leukocyte trafficking through interstitial matrix is a cycling multi-step process that couples shape change to adhesion events. In T cells, the penetration of basement membrane is thought to involve limited proteolysis provided by MMP-2 and MMP-9. However, is remains unclear whether migration within interstitial tissues includes proteolysis and matrix remodeling. We here show, that highly migratory human peripheral preactivated T blasts as well as T lymphoma (SupT1) cells express mRNA of several proteases, including MMP-9, MT1-MMP, MT3-MMP cathepsin B, K, L, and uPA and uPAR as well as ADAM-9, -10, -12, -15, -17 and TIMP-1 and -2 (RT-PCR). Upon long-term migration in 3D collagen matrices, however, no breakdown of collagen fibers was detected by a sensitive quantitative FITC-release assay as well as confocal reflection microscopy, suggesting that the collagen fiber network remained intact. Consistent with a non-proteolytic process, T cell migration efficiency was not impaired by MMP inhibitor BB-2516 (marimastat) or a highly efficient cocktail of proteinase inhibitors simultaneously targeting MMPs, serine proteases, cathepsins, and cysteine proteases. The mechanism of non-proteolytic T cell migration followed the hallmarks of ameboid crawling, as established for ameba of the lower eucaryote *Dictyostelium discoidum*. These features included simple polarized shape, dynamic pseudopod protrusion and retraction, flexible oszillatory morphodynamics, adaptation to preformed matrix structures and squeezing through matrix gaps, resulting in a rapid low-adhesive crawling style, as shown by high-resolution time-lapse confocal microscopy. We conclude that ameboid T cell migration and biophysical contact guidance along the preformed matrix pattern support an efficient non-proteolytic migration strategy generated by a plastic and highly adaptive actin cytoskeleton. These characteristics set T cells apart from proteolytic migration in other cell types such as fibroblasts and tumor cells.

P220

HAIR CYCLE-DEPENDENT EXPRESSION OF VEGF-SPLICE VARIANTS IN MURINE SKIN SUGGESTS KEY FUNCTIONS IN THE REGULATION OF PERIFOLLICULAR MICROVASCULATURE REMODELING

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BACKGROUND: Angiogenesis and regression of the perifollicular microvasculature occur temporally and spatially restricted throughout the hair growth cycle. This suggests that hair follicles meet their changing demand in a blood supply by influencing the perifollicular vasculature. VEGF, comprising various isoforms with different biological activities, is a key regulatory factor of blood vessel homeostasis and has also been demonstrated in the hair follicle epithelium. QUESTIONS ADRESSED: We investigated qualitatively and quantitatively which VEGF isoforms are expressed in murine skin throughout the hair growth cycle, and how they correlate to the expression of the two VEGF tyrosine kinase receptors. MATERIALS AND METHODS: Immunohistochemistry, semiquantitative RT-PCR and real-time (TaqMan) PCR were performed. RESULTS: VEGF protein is predominantly expressed in follicular outer root sheath keratinocytes during early anagen development. Three splice variants of the primary VEGF gene transcript, encoding for the isoforms VEGF164, VEGF144 and VEGF120, are differentially expressed throughout the hair growth cycle and are temporally associated with angiogenesis and regression of the perifollicular vasculature. CONCLUSIONS: Telogen skin appears to be associated with vascular quiescence, achieved by high expression of VEGF164. Anagen-associated angiogenesis is obviously mediated by VEGF120, predominantly deriving from keratinocytes of the outer root sheath, whereas catagen-associated vascular regression seems to be mediated by downregulation of VEGF gene transcription in follicular keratinocytes. Thus, the hair follicle appears to actively influence the perifollicular vasculature by modulating VEGF gene transcription in follicular keratinocytes. The murine hair cycle offers an intriguing model for dissecting the molecular controls of cutaneous vascular homeostasis.

P221

Drebrin and drebrosomes: components regulating actin dynamics in cell protrusions and lamellipodia

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Drebrins, actin-binding 70-kDa proteins containing a proline-rich, profilin-binding motif, were originally identified in - and thought to be specific for - the nervous system. More recently, the splice variant drebrin E2 has been also detected in a wide range of non-neuronal tissues and cultured cells[1,2]. In locomotory cells of various origins we have localized drebrin to lamellipodia and filopodia and, in some cells, to cytoplasmic granular structures clustered nearby[3]. In vitro wounding experiments show drebrin recruitment to lamellipodia of fibroblasts upon onset of migration. Biochemical analyses have revealed a series of drebrin particles: a 4S monomeric form and oligomers of 8S, 13S, ~20S and ~32S. The 8S particles are, most likely, drebrin trimers, whereas the larger complexes, *drebrosomes*, also contain actin. We conclude that drebrin particles are located in strategic positions for the regulation of actin dynamics, thereby contributing to cell motility, formations of protrusions and plasticity. Recruitment of drebrin-containing material to cell protrusions might be of pathophysiological significance, e. g. during wound healing.

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P222

Basal and cytokine-regulated expression of PDGF and its corresponding receptors in the human hair follicle

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Platelet-derived growth factor (PDGF) is a potent mitogen for dermal fibroblasts and other cell types and is able to stimulate proliferation and chemotaxis. The mitogenic role of PDGF is mediated by two different PDGF receptors which dimerize upon binding of their ligands and exert their further biological functions.As PDGF is important for hair follicle formation we investigated the expression of PDGF receptors in dermal papilla cells (DPC) and follicular keratinocytes (FK). In addition we examined the regulation of PDGF factors by different cytokines (Il-1β, TNFα, TGFβ1, Il-4, IFNγ).Expression of PDGF-A mRNA is detected in both, FK and DPC whereas PDGF-B mRNA is exclusively detected in FK. In addition, the expression of PDGF-A mRNA is down-regulated in DPC and FK upon Il-1β and IFNγ treatment. A significant down-regulation of PDGF-B mRNA in FK occurs after treatment with Il-1β.DPC strongly express both types of PDGF receptors (PDGFRs) on the mRNA level whereas PDGF receptor expression in FK was only weakly detectable. In addition, we investigated the expression of PDGFRs in DPC and FK by Western Blot analysis. Surprisingly the detection of PDGFR α in DPC failed but both types of PDGF receptors were confirmed in FK. Moreover, the corresponding bands were shown to be phosphorylated upon stimulation with PDGF ligands using anti-phosphotyrosine antibodies confirming the identity of PDGFRs in DPC and FK.Our findings indicate that PDGF ligands and their corresponding receptors are expressed in cultured DPC and FK of the human hair follicle. The regulation of PDGF expression levels by different cytokines might be important in regulating proliferation processes of the human hair follicle.

P223

Sphingosine kinase activity is functionally expressed in the human immortalized cell line HaCaT

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Normally, cell growth and differentiation are well balanced processes but may be disturbed in pathological reactions of the skin. Thus, atrophy or hyperkeratosis may be considered as directly related to proliferation and programmed cell death, i.e. apoptosis.
Sphingosine-1-phosphate (SPP) is a highly bioactive product of sphingolipid metabolism which is formed in a wide variety of mammalian cells in response to various stimuli including cytokines, growth factors (PDGF), serum and others. Activation of sphingosine kinase (SphK) by these stimuli leads to the phosphorylation of sphingosine yielding SPP. SPP was shown to exert both intracellular and extracellular function. Intracellular SPP was confirmed to influence signaling pathways leading to cellular proliferation and inhibition of apoptosis in various cell types. In contrast, SPP exerts extracellular functions as the ligand for members of the EDG-receptor family.
As the intracellular balance of SPP and ceramides, which are known inducers of apoptosis in HaCaT, may determine the fate of the cell, we investigated the role of SPP in HaCaT keratinocytes.
In the present study, we demonstrate for the first time the expression of sphingosine kinase activity in the keratinocyte cell line HaCaT. Sphingosine kinase experiments using cytosols of HaCaT keratinocytes showed a time and concentration-dependent formation of SPP. Examinating the substrate specifity of SphK, we could show that 30 µM *N*,*N*-dimethylsphingosine or 20 µM DL-*threo*-dihydrosphingosine decreased the SphK activity by 50 %, indicating that both compounds are potent inhibitors of SphK.
Our data clearly show that sphingosine kinase is functionally expressed in human keratinocytes and may therefore be involved in cellular processes concerning proliferation and differentiation. Furthermore, SphK inhibitors may be an interesting new group of drugs targeting epidermal homoeostasis.

P224

Constitutive and androgen induced NO production in dermal papilla cells of the human hair follicle

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Nitric oxide (NO) has been identified as an important mediator in various physiological and pathophysiological processes of the skin, such as regulation of blood flow, melanogenesis, wound healing, and hyperproliferative skin diseases. However, little is known about the role of NO in cell populations forming the human hair follicle. By measuring nitrate and nitrite levels in culture supernatants we demonstrate that dermal papilla cells (DPC) derived from human hair follicles spontaneously produce NO. This biomolecule is apparently formed by the endothelial isoform of nitric oxide synthase (NOS), which could be detected by reverse transcription polymerase chain reaction (RT-PCR) and immunological protein analysis. Remarkably, basal NO level was markedly enhanced by stimulating DPC with 5α-dihydrotestosterone (DHT) but not by testosterone. Addition of N-(3-(aminomethyl)benzyl-acetamidine (1400W), a highly selective inhibitor of human inducible NOS, restrained the elevation of NO level induced by DHT. Analysis of androgen-stimulated cells on RNA as well as on protein level, respectively, confirmed the expression of inducible NOS. Thus, we suggest that endothelial NOS as a constitutive enzyme accounts for a physiological role of NO in hair follicle cells, whereas, upregulation of NO production by androgens via stimulating inducible NOS expression may indicate an involvement of NO in the pathogenesis of androgen-dependent hair loss, such as androgenetic alopecia. These findings indicate that NO could be part of an important signaling pathway in the human hair follicle.

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Influence of Melatonin on HaCaT cell proliferation

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Introduction: The epiphysial hormone melatonin is characterized by a lot of pharmacological effects in biological systems. Due to the evaluation of the cytotoxic ability after application of different substances the DNA-synthesis rate was determined by Brd - insertion. The decrease of the DNA synthesis rate could be shown after treatment of keratinocytes with melatonin in high concentrations (1 nM). However, low concentrations of melatonin (10 µM -1 nM) caused an increase of BrdU - insertion after 24h. It was found already the inhibitory as well as the stimulating effects of melatonin depending on its concentration regarding to growth of hair . The aim of the present study was the finding of optimal melatonin doses for increasing of HaCaT cell proliferation.
Material and Methods: Human HaCaT ?keratinocytes were cultured in Dulbecco´s modified Eagle´s medium and were supplemented with 1% antibiotic- antimycotic solution and 10% fetal bovine serum. The culture was performed 5-7 days in a 200 mL cell culture bottle at 37°C and in 5% CO_2 - atmosphere.The cells were resolved by trypsin-EDTA , transfered into a 96-well- micro titer plate (20 - 40000 cells /cm^2) and incubated 48 h. After the HaCaT- cells were incubated with melatonin in different concentrations for 24 h, 40 h, 48 h or 72 h. The proliferation of HaCaT-cells was investigated by means of the following methods: ^{3}[H]-thymidine incorperation, fluorometric assay with Hoechst dye 33342 and ATP-bioluminescence assay.
Results: The investigations of HaCaT-keratinocyte proliferation influenced by melatonin could confirme the positive effects of the hormon melatonin in general. The effects depend on the concentration, the time of incubation and the used methods of testing. The activity of melatonin shows an inverse dependence on the concentration; the activity is higher at lower concentrations. Melatonin in concentrations between 5 µM and 10 µM (24 h incubation) can generate a significant proliferation of HaCaT cells.
Conclusions: In this study the influence of melatonin on the proliferation of HaCaT cells was measured by different analytical methods. The ATP bioluminescence assay is the recommended method because of its high sensitivity.

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PUVA-induced S-phase arrest involves activation of the checkpoint kinase Chk1

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Induction of interstrand crosslinks (ICLs) in chromosomal DNA is considered a major reason for the anti-proliferative effect of psoralen plus ultraviolet A (PUVA). It is unclear as to whether PUVA induced cell cycle arrest is caused by ICLs mechanically stalling replication forks or by triggering cell cycle checkpoints. Cell cycle checkpoints serve to maintain genomic stability by halting cell cycle progression to prevent replication of damaged DNA templates or segregation of broken chromosomes. In response to DNA damage, human Chk1 kinase is activated by phosphorylation at Ser345. Activated Chk1 phosphorylates Cdc25 dual-specificity protein phosphatases, which promote cell cycle transitions by dephosphorylating cyclin-dependent kinases. Phosphorylation of Cdc25 phosphatases inhibits their function or targets them for degradation. We show that HaCaT keratinocytes treated with low-dose PUVA arrest with a near-4n DNA content. The higher the dose of PUVA the earlier cells arrest during replication, consistent with an increase in the ratio of ICLs to origins of replication and, subsequently, the number of stalled replication forks. In addition, PUVA induced rapid phosphorylation of Chk1 at Ser345 and a concomitant decrease in Cdc25A levels, consistent with the activation of an S-phase DNA damage checkpoint. Future studies will demonstrate as to which degree both mechanisms contribute to PUVA induced cell cycle arrest.

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Zonula occludens protein 1 (ZO-1): Induction in Malignant Melanoma and Downregulation in Adjacent Epidermis

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The barrier function of the skin is held upright mainly by corneocytes and lipids. However it is still under discussion, whether cell-cell-junctions, e.g. Tight Junctions, are involved as well. In simple epithelia and endothelia Tight Junctions are known to be responsible for the formation and maintainance of the tissue barrier between compositionally distinct compartments, e.g. the blood-brain-barrier. They are composed of various transmembrane (Claudin 1-20, Occludin, junctional adhesion molecule (JAM)) and plaque proteins (Zonula occludens proteins 1-3 (ZO-1-3), Symplekin).
In this work we focus on the occurrence of Occludin, Claudin 1 and ZO-1 in malignant melanoma compared to normal interfollicular epidermis. Investigations were performed by immunofluorescence microscopy using previously described specific antibodies.
As previously shown by us and others in normal interfollicular epidermis Occludin and ZO-1 are expressed in the Stratum granulosum and the transition layer, while Claudin 1 occurs in all layers.
Now we show that in contrast there is no expression of ZO-1 in epidermis adjacent to malignant melanoma. There is a strong expression of ZO-1 in malignant melanoma, in contrast to melanocytes, which are known to be negative for ZO-1. Whereas Occludin and Claudin 1 are absent from malignant melanoma cells. They occur in normal interfollicular epidermis adjacent to malignant melanoma, but Occludin is present in all layers and is no more restricted to the Stratum granulosum and the transition layer.
These results clearly show a downregulation of ZO-1 in normal interfollicular epidermis adjacent to a malignant melanoma and an induction of ZO-1 in melanoma cells.
We discuss a central role of ZO-1 in the genesis of malignant melanoma. Ongoing investigations will elucidate the function of ZO-1 and other Tight Junction proteins in malignant melanoma.

P228

Caspase-14 is Regulated by Ap-1 Transcription Factors during Keratinocyte Terminal Differentiation

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Expression of caspase-14, a member of the caspase family of proapoptotic proteases, was linked to keratinocyte terminal differentiation in the past. In order to understand the regulation of this highly specific expression, we set out to identify factors controlling keratinocyte specific caspase-14 gene-expression as well as examining factors involved in the differentiation process.
In this study we have explored the molecular mechanisms underlying the regulation of caspase-14 transcription in primary keratinocytes. Deletion mapping of the 5´-region of caspase-14 revealed a minimal promoter sequence of ~270bp relative to the start site of transcription. This minimal promoter element contains two potential AP-1 sites (AP-1.2, AP-1.3). Further transfection experiments and electrophoretic mobility shift assays encompassing these transcription factor binding sites and their mutated counterparts showed that only AP1.3 displayed functional activity in keratinocytes. PMA, a potent inducer of AP-1 increased caspase-14 promoter driven luciferase activity more than two-fold in postconfluently growing keratinocytes. We could also demonstrate that caspase-14 promoter driven luciferase activity decreased progressively as the sequence length increased, suggesting the presence of an upstream suppresser activity. Postconfluent transfected keratinocytes exhibited a higher luciferase activity than preconfluent growing cells, demonstrating that the promoter is triggered more potently in postconfluent cells than in non differentiated keratinocytes, which highly corresponds to the caspase-14 expression pattern in human epidermis. Our data demonstrate that the specific expression of caspase-14 in the uppermost layers of the skin is regulated to some extent by members of AP-1 transcription factor complex.

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Expression Patterns of Connexins in Basal Cell Carcinoma, Squamous Cell Carcinoma, Malignant Melanoma and Adjacent Epidermis

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Communication between cells is performed by exchange of small molecules through gap junctions. Gap junctions connect adjacent cells and form communicating channels. They consist of two connexons (one of each cell), each formed by six transmembrane proteins called connexins (Cx). Seven of the known fourteen different connexins are expressed in human skin. Gap junctions formed by different connexins are selective for different small molecules (e.g. second messengers). Thereby they control the specificity of cell-cell-communication.
This work concerns the expression of Cx30, Cx26 and Cx43 in various skin tumours compared to normal epidermis. Investigations were performed by immunofluorescence microscopy using previously described specific antibodies.
Both Cx30 and Cx26 are only weakly, if at all, expressed in normal interfollicular epidermis while Cx43 is present in all layers.
Now we show that in basal cell carcinoma and squamous cell carcinoma both Cx30 and Cx26 are strongly expressed. Cx30 is absent from malignant melanoma cells. Even more striking is the expression of Cx30 in unaffected interfollicular epidermis neighbouring squamous cell carcinoma and malignant melanoma. In contrast there is no expression of Cx30 in normal epidermis adjacent to basal cell carcinoma. Cx43 is expressed in both investigated carcinomas at a lower degree than in normal epidermis. There is no expression of Cx43 in malignant melanoma cells.
In basal cell carcinoma and squamous cell carcinoma these results clearly show an induction of Cx30 and Cx26 and in contrast a downregulation of Cx43. Interestingly there is an induction of Cx30 in unaffected epidermis adjacent to squamous cell carcinoma and malignant melanoma. This reminds on the induction of Cx30 in other hyperproliferative skin diseases (e.g. psoriasis).
We discuss a central role of connexins in hyperproliferation of keratinocytes. Ongoing investigations will elucidate the communication in hyperproliferative epithelia and the function of Cx30 in their gap junctions.

P230

ALL-TRANS-RETINOIC-ACID SENSITIZES NORMAL HUMAN EPIDERMAL KERATINOCYTES TO APOPTOSIS

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Recent work in our laboratory has shown that treatment of keratinocytes (KC) with all-trans-retinoic-acid (ATRA) leads to an increase of the protein level of pro-caspase-3. In addition we observed an increase of cells with apoptotic morphology in in vitro reconstructed epidermis. Immunoblotting of lysates of KC in monolayer culture exposed to 10-6 M ATRA revealed that besides caspase 3, the proforms of caspases -6, -7, and -9 were induced. Despite this upregulation cells did not show apoptotic morphology, and when applying methods such as histone-ELISA, and DNA-laddering, only minor levels of cell-death could be detected. To test whether overexpression of pro-caspase 3 can sensitize KCs to apoptotic stimuli without leading to spontaneous apoptosis, we exposed ATRA-treated keratinocytes to UV irradiation or staurosporine (250 ng/ml). At doses of 20 mJ/cm^2 UVB it was not possible to cause detectable cell-death when exposing non-ATRA-treated, confluent, differentiated keratinocytes, while irradiation of ATRA-treated, confluent keratinocytes led to massive apoptosis (> 70%). Furthermore, when KC pretreated with ATRA were exposed to 250 ng/ml staurosporine, the majority of the cells detached, and a DNA-ladder was detected, both signs of apoptosis. Cells that were not pretreated with ATRA, did not detach from the culture plate, and instead of a DNA-ladder only a smear, suggestive of necrosis could be observed.Our results show that pretreatment of human KC with ATRA sensitizes these cells to apoptotic stimuli, probably via an upregulation of pro-apoptotic pro-caspases. Of special interest is the observation, that ATRA, that inhibits the expression of the differentiation-associated caspase-14, upregulates pro-apoptotic caspases. Therefore, we conclude that ATRA has the ability to block terminal differentiation, and instead direct keratinocytes to the apoptotic pathway.

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Caspase-14 is Regulated by Ap-1 Transcription Factors during Keratinocyte Terminal Differentiation

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Expression of caspase-14, a member of the caspase family of proapoptotic proteases, was linked to keratinocyte terminal differentiation in the past. In order to understand the regulation of this highly specific expression, we set out to identify factors controlling keratinocyte specific caspase-14 gene-expression as well as examining factors involved in the differentiation process.
In this study we have explored the molecular mechanisms underlying the regulation of caspase-14 transcription in primary keratinocytes. Deletion mapping of the 5´-region of caspase-14 revealed a minimal promoter sequence of ~270bp relative to the start site of transcription. This minimal promoter element contains two potential AP-1 sites (AP-1.2, AP-1.3). Further transfection experiments and electrophoretic mobility shift assays encompassing these transcription factor binding sites and their mutated counterparts showed that only AP1.3 displayed functional activity in keratinocytes. PMA, a potent inducer of AP-1 increased caspase-14 promoter driven luciferase activity more than two-fold in postconfluently growing keratinocytes. We could also demonstrate that caspase-14 promoter driven luciferase activity decreased progressively as the sequence length increased, suggesting the presence of an upstream suppresser activity. Postconfluent transfected keratinocytes exhibited a higher luciferase activity than preconfluent growing cells, demonstrating that the promoter is triggered more potently in postconfluent cells than in non differentiated keratinocytes, which highly corresponds to the caspase-14 expression pattern in human epidermis. Our data demonstrate that the specific expression of caspase-14 in the uppermost layers of the skin is regulated to some extent by members of AP-1 transcription factor complex.

P232

Class II-mediated cell death in dendritic cells requires cellular interactions

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Class II ligation is known to induce a rapid form of cell death in all types of mature dendritic cell populations tested from either human or mouse. Our data has shown the killing to be independent of caspase activation. However, intracellular signalling mechanisms for class II-mediated death remain unknown. Class II ligation on DC induces a rapid homotypic adhesion. We now show that this homotypic adhesion is essential for class II-mediated cell death.
By limiting dilution analysis we observed that both, loss of mitochondrial membrane potential and binding of annexin depend on cellular interactions between class II treated DC. Furthermore, class II mAb-treated DC failed to die when incorporated into collagen gels (where cell: cell contact is minimal). However, when class II-ligated wild type DC were mixed with class $II^{-/-}$ DC, only the former died. Thus, the mechanism of class II killing requires molecules associated with the class II surface complex or the class II molecules themselves. Blocking mAb against most known adhesion molecules, a wide range of lectins or sugars failed to inhibit DC: DC clustering and cell death. Enzyme inhibitors revealed no obvious role for various proteases, such as serine and cysteine proteases including calpeptin, and matrix metalloproteinases or the proteasome complex. Furthermore, inhibitors of intracellular transport of molecules such as monensin or brefeldin A or inhibitors of lysosomal acidification did not show any effect on class II mediated apoptosis. Only inhibitors of cytoskeletal motion, such as cytochalasin D or iodoacetic acid profoundly inhibited class II mediated cell death.
Since mature DC are not susceptible to TRAIL or Fas mediated cell death, class II mediated apoptosis may be one of the few mechanisms functioning for the control of DC populations in vivo. Further studies have to elucidate the currently unknown intracellular signalling pathways activated by MHC class II ligation.

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An analysis of dendritic cell populations in obf-1 deficient mice

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The expanding family of dendritic cells (DC) encompasses progenitor cells of both myeloid and lymphoid origin. We have recently suggested a role for the octamer motif binding transcription factor OCT-2 in DC biology, since OCT-2 is downregulated during differentiation of both human and murine DC. OBF-1 (BOB-1, OCA-B) is a cofactor for a subgroup of OCT transcription factors including OCT-2, which is constitutively and inducibly expressed in B and T cells.
Since oct-$2^{-/-}$ mice die at birth we utilised obf-$1^{-/-}$ mice to further elucidate a role of octamer factors in the generation of DC. First, we generated DC in vitro from bone marrow cultures and found yield and maturation phenomena comparable to wild type. Next we looked in vivo at DC within peripheral tissues. Analysis of ear skin from obf-$1^{-/-}$ mice revealed normal numbers and distribution of dermal DC and epidermal LC. We then analyzed DC of secondary lymphoid organs, which in obf-$1^{-/-}$ mice lack germinal center formation. By FACS analysis the recently described subpopulations of spleen CD4+ and CD4-/CD8a- DC, discussed to be of myeloid origin, as well as spleen lymphoid CD8a+ could be detected at normal 2:1:1 ratios and were of normal phenotype. In addition, both migratory and resident DC within peripheral lymph nodes of OBF-1 knockout mice did not differ from wild type mice.
Taken together, despite lack of germinal center follicles obf-$1^{-/-}$ knockout mice show normal number, distribution and phenotype of immature and mature DC. Thus, our data do not imply a role for OBF-1 in the generation or maturation of murine dendritic cells, neither of myeloid nor of lymphoid origin.

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A model for for the initiation of non-apoptotic neurodegeneration - induction of Alzheimer´s disease-specific epitopes by oncogenic transformation of primary neurons

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Cell cycle markers have been detected in brain tissue from Alzheimer's disease (AD) and other neurodegenerative diseases, suggesting that either dysregulated expression of cell cycle-related genes or the inappropriate entry into the cell division cycle may be involved in the onset and development of neurodegeneration. In addition, increased ploidy has been observed in AD neurons. This has led us to the idea that some degenerative disorders might arise from a mechanism similar to oncogenesis. To create a model system to directly determine whether oncogenic signaling and cell cycle re-entry initiates neuronal degeneration similar to that observed in AD, primary postmitotic rat cortical neurons (E18) were infected with a combination of adenovirus vectors encoding the two oncogenes, c-myc and a mutationally active form of ras (H-ras61L). Myc/ras-infected but not control (green fluorescent protein)-infected neurons stain positive for the expression of two AD diagnostic markers, the TG-3 mitotic phospho-epitope and the Alz-50 conformational epitope of tau. Clear co-localization with the neuronal marker MAP2 was observed. There was a positive correlation between the DNA content and the TG-3 immunoreactivity in myc/ras-infected cells. In addition, infected neurons express the mitotic cyclin B1, show evidence of DNA replication and an increase in mitochondrial content. We propose that early elements of AD neuropathology may arise due to inappropriate re-entry into the cell cycle. The results suggest that age-dependant loss of cell cycle control may play a role in the initiation of neurodegenerative diseases. These experiments represent the first in vitro model for the initiation of non-apoptotic neurodegeneration that mimics several features of AD.

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Expression and Function of Histamine Receptors in Human Monocyte-Derived Dendritic Cells

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Histamine is a well-known mediator involved in urticaria and anaphylaxis. In order to analyse the function of histamine on other immunological reactions we performed studies with monocyte-derived dendritic cells (DC). Thereby we have shown that immature and mature DC express the mRNA for H_1, H_2 and H_3 histamine receptors. Histamine induced intracellular Ca^{2+} transients, actin polymerization and chemotaxis in immature human DC, through activation of H_1 and H_3 receptors. Maturation of DC resulted in the loss of histamine-induced chemotaxis, actin polymerization and Ca^{2+} responses, while histamine now enhanced intracellular cAMP levels, resulting in an inhibition of interleukin (IL)-12 and increase of IL-10 production, through stimulation of H_2 and H_3 receptors. As a consequence, histamine impaired the ability of mature DC to generate allogeneic Th1 response favoring Th2 lymphocyte-dominated immunity. Our study suggests that the function of histamine in allergic reaction might not be limited to urticaria and anaphylaxis. It might have a broader function in allergic responses by regulating DC functions and favoring Th2 lymphocyte-dominated immunity.

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