V01

Identification and analysis of the transcriptional regulation of a new hypoxia-inducible angiogenesis factor in malignant melanoma

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Tissue hypoxia is a critical regulatory factor for tumor progression and metastasis in a variety of tumors. In the present report we used the oligonucleotide microarray technique to analyse the gene expression pattern of hypoxic melanoma cells in order to gain a more complete understanding of the hypoxic tumor cell response. More than 1300 genes were found to be hypoxia-inducible in five malignant melanoma cell lines differing in their metastatic behavior. Making use of biostatistical cluster analysis a group of new hypoxia-inducible genes showing tumor stage-dependent expression were identified. Among these a new angiogenesis factor for malignant melanoma was identified. Further analyses of the underlying transcriptional mechanisms by in vitro luciferase assays and electrophoretic mobility shift assays revealed that AP-1 and hypoxia-inducible factor (HIF)-1α are critically involved in the hypoxic gene regulation of this factor in malignant melanoma cells. In immunoprecipitation studies it could be shown that HIF-1α directly interacts with AP-1. Further analyses of the immunoprecipitated proteins by use of mass spectrometry identified transcriptional co-activators and molecular chaperons active under hypoxia. In conclusion, we were able to identify a new angiogenesis factor for malignant melanoma and characterize the molecular mechanisms of its transcriptional regulation. The presented findings might provide targets for future therapeutic approaches.

V02

IκB-α controls perinatal myeolopoiesis via Jagged1

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The regulation of myelopoiesis in the perinatal period is scarcely understood. Here we show that the inhibitor of the transcription factor NF-κB, IκB-α, and Jagged1 are of crucial importance for this process. IκB-α-deficient mice developed an extensive granulopoiesis immediately after birth. This was paralled by an enhanced expression of Notch1 and Hes1 in neutrophils and the Notch ligand Jagged1 in the liver of five day old mice IκB-α-deficient mice. NF-κB was found to be constitutively active in the liver and in neutrophils. In contrast, in mice that selectively lacked IκB-α expression in neutrophils using conditional mutagenesis (IκB-$\alpha^{flox/flox}$ LysMCre) or in irradiated hosts that received fetal liver-derived IκB-$\alpha^{-/-}$ stem cells, granulopoiesis was unobtrusive. Analysis of embryonic day 12 (E12) livers demonstrated a strong expression of Jagged1 in IκB-α-deficient and wildtype mice. Irradiated $IgH^{-/-}$ mice that received fetal liver-derived IκB-$\alpha^{-/-}$ stem cells did not show any Jagged1 expression in the liver. Whereas the expression of Jagged1 declined in wildtype mice, it persisted in IκB-α-deficient mice. These data demonstrate that (1) Jagged1 is a key regulator for perinatal myelopoiesis, (2) myelopoiesis is strictly controlled by stromal expression of Jagged1 and (3) Jagged1 expression is controlled by IκB-α. Hence, IκB-α is an important regulator of Jagged1 in perinatal myelopoiesis, resulting in hypergranulopiesis, if downregulation of NF-κB is inhibited.

V03

Mast Cells control Angiogenesis in Arthritis

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K/BxN-arthritis is commonly used as a murine rheumatoid arthritis (RA) model because of its various similarities to human RA. In the K/BxN mouse model of RA, transfered serum with auto-antibodys specific for the ubiquitously expressed antigen, glucose-6-phosphate isomerase (GPI) bind directly to pre-existing etracellular GPI in normal healthy mouse joints and rapidly induce joint-specific inflammation, independent from B- and T cells. Previous studies confirmed that mast cell deficiency protects from K/BxN-arthritis. Mast cells as well as the complement network and Fc receptors are essentiall in the pathogenesis of K/BxN-arthritis. Since proliferation of new blood vessels from pre-existing capillaries play a key role in RA and since we found enhanced blood vessel formation in arthritic joints from wild-type but not from mast cell deficient W/Wv mice we examined the vascular cell integrin αvβ3 which is selectively induced during angiogenesis. We found enhanced RGD-peptide uptake in arthritic joints from wild-type but not from W/Wv mice using radiolabeld RGD-peptide which selectively binds to activated αvβ3 integrin detected in vivo by positron emission tomography (PET), most important αvβ3 expression and blood vessel formation was restored by mast cell engraftment, postulating an essential role of mast cells in arthritic angiogenesis. To further analyse we examined the effect of established mast cell degranulation inhibitors on K/BxN-arthritis. Strongly reduced ankle swelling, joint inflammation,αvβ3 expression and angiogenesis was found in Cromolyn, Salbutamol and Tranilast treated mice. Thus, mast cell degranulation inhibitors have important effects in inflammatory responses and a significant therapeutic action in K/BxN-arthritis and may led to new strategies for the treatment of human psoriasis arthritis and RA.

V04

Inhibition of Melanoma Inhibitory Activity (MIA) expression in melanoma cells leads to molecular and phenotypic changes

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Acquisition of an invasive and metastatic phenotype is a key event in tumor progression. Recent evidence reveals that the secreted protein MIA (melanoma inhibitory activity) is highly expressed in malignant melanomas and associated with tumor progression in vivo. We therefore further investigated the role of MIA in the metastatic process by inhibiting MIA expression of the human melanoma cell line HMB2 via stable antisense MIA cDNA transfection and analysing the cell clones. MIA-deficient cell clones showed changes in cell morphology. Further, in monolayer culture and spheroid system enhanced cell-cell-contacts were formed. Real-time RT-PCR showed a downregulation of N-Cadherin expression and an induction of E-Cadherin expression in the MIA-deficient cell clones. Further, a re-induction of pigment synthesis in comparison to the amelanotic parental cell line HMB2 was observed. Molecular analysis revealed a re-expression of TRP-1 in the MIA-deficient cell clone responsible for melanin synthesis. By the use of cancer cDNA arrays a marked downregulation of MT1-MMP, tPA, integrin ß3 and fibronectin mRNA was observed, which was confirmed by real-time RT-PCR. These gene are known to be associated with melanoma metastasis and progression. In summary, expression of MIA by malignant melanoma may regulate expression of other melanoma-associated genes and could therefore be an early event in melanoma development.

V05

Mutations in the epidermal lipoxygenase gene *ALOXE3* cause autosomal recessive congenital ichthyosis (ARCI) in a family mapped to chromosome 17p13.1

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of hereditary keratinization disorders characterized by a more or less intensive scaling of the whole integument often associated with erythema. Up to the present, five loci for ARCI have been mapped to human chromosoms 2q33-q35, 14q11.2, 17p13.1, 19p13.1-p13.2, and 19p12-q12. We have identified the locus on 17p13.1 by homozygosity mapping in four affected siblings from a German family with common ancestors ten generations earlier. Recently, mutations in two new genes on 17p13.1, *ALOX12B* and *ALOXE3*, were identified in ARCI patients. Here we have analysed the German family mapping to 17p13.1 for mutations. In *ALOXE3* we found a single nucleotide exchange, C1889T, which substitutes a leucine for a highly conserved proline at position 620. The siblings were homozygous for the mutation, in accordance with homozygosity-by-descent in the family. The gene *ALOXE3* codes for the epidermal lipoxygenase-3 (e-LOX-3), a member of the epidermis-type subclass of mammalian lipoxygenases. The enzymes share the common structure of lipoxygenases with a PLAT/LH2 and a lipoxygenase domain. The enzyme specificity of the epidermis-type lipoxygenases, however, has not yet been clarified. The mutation in the family described here affects the lipoxygenase domain of e-LOX-3. Further investigations are being performed in order to elucidate the functional consequences of the mutation. Detailed analysis of lipoxygenase mutations will give insight into the molecular basis of the clinicial and genetic heterogeneity in ARCI.

V06

CRIC3, a chemokine receptor inactivator as a novel approach in the treatment of allergic and inflammatory diseases

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The chemokine receptor CCR3 is expressed on different inflammatory cells, such as eosinophils, basophils, mast cells and Th2 cells, and is responsible for the invasion of these cells to the site of inflammation, particularly in allergic and autoimmune diseases. Whereas most anti-inflammatory strategies act on their target cells after migration to the site of inflammation, we propose a concept on how to prevent the cellular recruitment by inactivation of the chemokine receptor by means of a potent agonist, before leukocytes leave the blood vessels. We generated different truncated and chemically modified peptides derived from the chemokine CCL14 (HCC-1). We tested these derivatives on human eosinophils (release of reactive oxygen species, chemotaxis, internalization of CCR3, calcium mobilization) and in OVA-sensitized mice. Replacement of the ultimate N-terminal amino acid of CCL14[9-74] by nonanoic acid (NNY) converts it into a potent agonist, termed **C**D26-**r**esistant **i**nactivator of **C**CR**3** (CRIC3) for human eosinophils exhibiting an activity profile identical to CCL11 (eotaxin), the most potent chemokine agonist of CCR3 known with respect to the release of reactive oxygen species, intracellular calcium mobilization, chemotaxis, and CCR3-internalization. This modification results in a resistance to degradation by dipeptidyl peptidase IV (CD26/DPP IV) due to the substitution of the Gly-Pro motif by the NNY-Pro motif. Interestingly, intravenous administration of CRIC3 in ovalbumin-sensitized mice, prior to allergen aerosol challenge, resulted in a significant reduction of eosinophils in airways and lung tissue. CRIC3 can be therefore used to induce internalization and inactivation of CCR3 on circulating cells. Thus, the use of CRIC3 demonstrates a novel approach in the therapy of CCR3-mediated diseases.

V07

POLLEN DERIVED FACTORS IMPAIR THE CAPACITY OF DENDRITIC CELLS TO INITIATE TH1 RESPONSES

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The primary site of exposure to pollen grains is the epithelium of the upper respiratory tract, which is densely populated by immature dendritic cells that act as sentinels of the immune system. We recently demonstrated that pollen grains not only function as allergen carriers but are also a rich source of bioactive lipid mediators. Aqueous extracts from phleum pratense and betula alba pollen contained predominantly monohydroxylated derivatives of linoleic and linolenic acid. GC-MS analysis of betulla alba aqueous pollen extracts (APE) demonstrated the presence of phytoprostanes E1, F1, B1/2 in the nM range. Since DC critically influence the outcome of the ensuing T cell polarization, we investigate the biological activity of APE on immature DC. Human pB monocytes-derived DC generated under the aegis of GM-CSF and IL-4 displayed morphology and phenotype of immature DC (CD1a+, MHC class IImed, mannose receptor+, CD14neg., CD83neg., CD86neg.). Exposure to phleum pratense or betula alba APE induced DC maturation as documented by upregulation of MHC class II, CD83, CD86 and down regulation of mannose receptor expression. APE-induced DC maturation was accompanied by increased allostimulatory activity. In comparison to LPS, APE induced DCs to release significantly less IL-12 p40 and p70. APE inhibited dose-dependently the LPS-induced IL-12 p40 mRNA levels and IL-12 p70 release, while no effect on LPS induced IL-6 release was observed. Quantitative mRNA analysis demonstrated that IL12-production was regulated primarily at the level of IL12p40 mRNA expression while IL12p35 mRNA was not regulated. Consistent with that, APE-activated DCs induced significant less IFN-g production in allogeneic naïve CD45RA+, CD4+ T cells. These results suggest that pollen derived factors act as regulatory signal to dampen IL-12 production by DCs and modulate DC function in a fashion that may result in reduced Th1 polarization.
C.T.-H. and T.J. contributed equally.

V08a

Activation of the MAP kinase p38 is mandatory for the induction of antigen-specific anergy and suppressor activity of $CD4^+$ T cells

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Previous results demonstrated that human IL-10-treated dendritic cells (DC) induce anergic $CD4^+$ T cells with antigen-specific regulatory function. This study analysed altered signal transduction pathways in these anergic T cells compared to optimally stimulated T cells. During primary culture and after restimulation, lysates of anergic (cocultured with IL-10-DC) and control T cells (stimulated with mature DC) were prepared. Subsequently, immunoprecipitation, SDS gel electrophoresis, western blot analysis and *in-vitro*-kinase-assays were performed. We observed a reduced activity of the MAP kinases JNK1/2 and ERK1/2 in anergic T cells as compared to control T cells. More importantly, a markedly enhanced and sustained activity of the MAP kinase p38 during primary culture and after restimulation was found. Additional analysis of the kinase MAPKAP2/3, the downstream substrate of p38, revealed a significantly increased activity of MAPKAP2/3, suggesting an altered critical pathway of signal transduction in anergic T cells. Inhibition of the MAP kinase p38 using the specific inhibitor SD203580 completely impaired the induction of anergic $CD4^+$ T cells as demonstrated by restimulation experiments and IL-2 production. Notably, compared to control T cells these T cells lost their antigen-specific suppressor function. Our data show an important role of the MAP kinase p38 for the induction of antigen-specific anergy and regulatory function in $CD4^+$ T cells induced by IL-10-treated DC.

V08b

Suppressor activity of anergic T cells is associated with IL-2- and CTLA-4-dependent G_1 arrest of the cell cycle regulated by $p27^{Kip1}$

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We have previously shown that human IL-10-treated dendritic cells induce anergic $CD4^{+}$ T cells with antigen-specific regulatory function. These suppressor T cells are characterized by an inhibited proliferation, a reduced production of IL-2 and enhanced expression of CTLA-4. In this study we investigated the mechanisms underlying the anergic state and regulatory function of these T cells. Cell cycle analysis by DNA staining and western blot experiments revealed an arrest of anergic $CD4^{+}$ T suppressor cells in the G_1 phase. Accumulation of the G_1-specific cyclin D3/D2-cdk4 complexes and high levels of the IL-2 dependent cdk inhibitor $p27^{Kip1}$ were observed resulting in an inhibited activation of Rb and an arrest of cell cycle progression in the G_1 phase. Addition of IL-2, but not blocking of the highly expressed CTLA-4 molecule restored the proliferation of the anergic $CD4^{+}$ suppressor T cells in restimulation experiments. In contrast, both treatments (addition of IL-2 or inhibition of the CTLA-4 pathway) induced a downregulation of $p27^{Kip1}$ and a complete inhibition of the antigen-specific regulatory function as demonstrated by high proliferation and enhanced IFN-γ production of cocultured T cells. Our data show that the regulatory function of anergic $CD4^{+}$ suppressor T cells is associated with an arrest in the G_1 phase of the cell cycle mediated by increased levels of the IL-2- and CTLA-4-dependent cdk inhibitor $p27^{Kip1}$.

V09

In vivo induction of CD4+/CD25+ regulatory T cells by molecular targeting of antigens to immature dendritic cells by αDEC-205 antibodies.

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Dendritic cells (DC) express a distinct receptor for adsorptive endocytosis, DEC-205. Coupling of ovalbumin (OVA) to anti-DEC-205 mAb (αDEC) induced proliferation of OVA specific T cells in vivo. However, this expansion is shortlived, as OVA specific T cells isolated 8 days after injection of αDEC-OVA conjugates failed to proliferate upon restimulation with OVA pulsed DC. These effects were abolished when a DC-maturation stimulus such as a αCD40 antibody was simultaneously injected with αDEC-OVA. Analysis of the anergic T cells revealed sustained expression of CD25 and CTLA-4 and after coculture with conventional CD4+ T cells in MLR assays, suppression of proliferation and IL-2 production was evident. Further in vivo studies revealed that coupling of αDEC with OVA or the contact allergen TNCB resulted in an antigen-specific suppression of CD4+ T cell mediated footpad swelling and CD8+ T cell mediated contact hypersensitivity reactions. We conclude that targeting of antigens to immature DC via αDEC mAb, leads to induction of regulatory T cells in vivo. These studies provide the basis for a novel strategy to induce regulatory T cells for therapeutic purposes in different autoimmune diseases in vivo.

V10

Why does ultraviolet A radiation (UVAR) induce gene expression in human keratinocytes (NHK) in a biphasic pattern?

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Solar UVAR exerts deleterious effects on human skin. It is therefore important to study the underlying molecular mechanisms. Analysis of UVAR-induced gene expression in NHK has revealed that UVAR induces gene transcription through activation of transcription factor AP-2. Interestingly, AP-2 activation and mRNA expression of UVAR-inducibel genes follow a biphasic pattern. This biphasic pattern is highly characteristic for UVAR, because it is not observed after UVB irradiation or cytokine stimulation, and includes an early peak after 1-2 hours and a second, more sustained maximum 16 to 48 hr after exposure. The molecular basis for this biphasic activation pattern is unknown. By employing ICAM-1 as a model gene we have recently shown that the early activation is due to the generation of second messenger ceramide which were formed within minutes after irradiation through a non-enzmyatic mechanism. We now report that ceramide formation follows a biphasic pattern. Analysis of lipid extracts from UVA-irradiated NHK by sequential HPTLC revealed a first ceramide peak between 30 min to 2 hours (17-fold increase) and a second increase between 16 and 48 hours (4-fold increase). This second ceramide peak, in contrast to the 1st peak, was enzymatic in nature: (i) it was associated with significant upregulation of serine-palmitoyl transferase expression, an enzyme that is critically involved in the de novo synthesis of ceramide, and (ii) it could be completely prevented by preincubation of NHK with an inhibitor of serine-palmitoyltransferase (myriocin). This inhibition was specific because identical concentrations of this inhibitor did not affect the 1st ceramide peak in UVA-irradiated cells and because it did not inhibit IL-1-induced ceramide formation in unirradiated cells, which is known to be due to hydrolysis of sphingomyelin by neutral or acid sphingomyelinase. Inhibition of the 2nd ceramide peak was of functional relevance because myriocin treatment of NHK prevented the 2nd increase in ICAM-1 mRNA expression, whereas the 1st peak remained unaltered. Equivalent data were obtained, when human skin equivalents rather then NHK were employed. Thus, ceramide signaling constitutes the molecular basis for the biphasic gene expression pattern that is induced by UVAR.

V11

Distinct protein complexes induce expression of the chemokine CCL22 in dendritic cells and B cells

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Despite of the central role of dendritic cells (DC) for the induction of novel immune responses, little is known about gene regulation in DC. We recently characterized Dendritic cell and B cell-derived chemokine (DC/B-CK) / CCL22, the murine homologue of human macrophage-derived chemokine (MDC). Murine CCL22 attracts activated T cells and is of critical importance in various disease models.

The tightly controled differential expression of CCL22 and its high expression levels in mature DC and activated B cells prompted us to investigate the regulatory elements of the CCL22 gene. The gene was isolated, sequenced completely and two transcriptional start sites were identified by 5'-RACE. Reporter gene assays with expression constructs encompassing various fragments of the 5'-flanking promoter region indicate that a stretch of 250 bp proximal to the translation initiation site contains all major regulatory elements to mediate a strong expression in mature DC and activated B cells but not in other cell types tested including immature DC and fibroblasts. Using primary cells and cell lines to analyze different stages of DC maturation and B cell activation we identified three regions within these 250 bp which bind proteins. A heterodimer of the NFκB subunits p50 and p65 binds to the most distal box 1 and interacts with proteins bound by box 3. SP1 binds to box 3 in B cells but not in DC indicating that distinct complexes are formed.

V12

Cultured peri-bulbar dermal sheath and dermal papilla cells induce new hair follicles from mouse epidermis and modify the growth and cycling properties of hair follicles present through natural embryogenesis

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Mesenchyme derived dermal papilla (DP) cells control development, differentiation and cycling of hair follicles. These cells are highly differentiated and non-proliferative in vivo. Significant progress has been made in defining progenitor cells of the epidermal hair follicle component but the progenitor cell source for the DP is unknown. Transgenic GFP-expressing mouse, wildtype, and non-transgenic mouse vibirssa follicle cells were cultured and implanted to CBySmn.CB17-*Prkdc*$^{scid/J}$ mouse ears. DP-derived cells and cells from the peribulbar dermal sheath "cup" (DSC) induced new hair follicles while non-bulbar dermal sheath (DS) cells did not. Confocal microscopy revealed that GFP-expressing DSC cells induced hair growth through the formation of a new DP, DSC and the lower hair follicle DS, whereas DP cells only capable of forming a DP. In addition to inducing entirely new hair follicles, DP and DSC cells also integrated with hair follicles already present through natural embryogenesis. Biochemically, DSC cells where characterized *in vivo* and *in vitro* by low alkaline phosphatase activity in contrast to high alkaline phosphatase expression in differentiated DP cells. Thus, transplanted autogeneic and allogeneic cells derived from adult vibrissa follicles were capable of inducing new hair follicles from mouse epidermal cells and modified the growth and cycling properties of hair follicles present through natural embryogenesis. We suggest that the functional capacity of cultured DSC cells to form DP and DS and induce hair follicle development is an indicator of their pluripotency. In principle, it may be possible to utilize cultured hair follicle mesenchyme cells as a treatment for androgenetic alopecia and other forms of hair loss.

V13

Evidence for a role of pyrimidine dimers in UVA mutagenesis

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Several pieces of evidence point to longwave ultraviolet light (UVA) being a particular risk factor for melanoma. While it is well established that UVA is a mutagen and skin carcinogen, its mechanisms of mutation formation are not well understood. Since UVA, in contrast to UVB, is hardly able to generate pyrimidine dimers, UVA-induced oxidative DNA damage has been suggested to mediate UVA-mutagenesis. However, a detailed analysis of UVA- and UVB-induced somatic mutations in human cells has not been described. In order to investigate mutation formation by UVA and UVB, we used the HPRT-mutagenesis assay with primary human fibroblasts. This assay measures the frequency of spontaneous or induced hprt-mutations by selection for 6-thioguanine-resistance. In 3 to 5 independent experiments, we found a mutation frequency of 54 ± 17 hprt-mutants/10^6 cells with 20 J/cm^2 UVA1 and of 20 ± 9 hprt-mutants with 20 mJ/cm^2 UVB. To further characterize UVA- and UVB-induced mutations, we performed nested RT-PCR and sequence analysis of the hprt-gene of UVA- and UVB-induced hprt-mutants. All mutations were confirmed by sequencing the hprt cDNA in two directions. In a total of 24 sequenced mutants, more than 90 % carried single base substitution mutations, the remaining deletions. 50 % of UVB-induced mutations were C→T transitions. This is consistent with a predominant role of pyrimidine dimers in UVB-mutagenesis, as these mutations are considered "signature mutations" for pyrimidine dimers. A "hotspot" with four independent C→T transitions was observed at bp 659, which is located within a pyrimidine run of CCTTTT, a likely target for pyrimidine dimer formation. Surprisingly, the same frequency and the same hotspot of C→T transitions was observed also with UVA, pointing to a mutagenic role of pyrimidine dimers also with UVA. 20 % of UVA and of UVB-induced mutations were G→T or T→G transversions. Since these are considered "signature mutations" for oxidative guanine base damage, this result points to a similar role of oxidative DNA damage with both UVA and UVB. Beside these similarities between the UVA- and UVB-induced mutation spectra, there were also significant differences. Eg., a "hotspot" A→G transition mutation at bp 170 was only seen with UVB, but not UVA. Our data suggest similar mechanisms of mutation formation, including a prominent role of pyrimidine dimers, with UVA and UVB.

V14

CHILD syndrome: Molecular analysis of 25 patients

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CHILD syndrome (congenital hemidysplasia with ichthyosiform nevus and limb defects) is an X-linked dominant male lethal trait characterized by an inflammatory nevus (CHILD nevus) distributed in a unique lateralization pattern with strict midline demarcation. Skeletal defects such as hypoplasia or aplasia of limbs are found to be ipsilateral to the nevus. In addition, ipsilateral visceral or neurological defects may be associated. Recently, we were able to demonstrate that mutations in the gene NSDHL at Xq28, encoding a 3-beta-hydroxysteroid dehydrogenase, cause CHILD syndrome. Sterol analyses in the homologous murine Nsdhl mutants bare patches and striated had previously revealed a role of this enzyme in the late cholesterol synthetic pathway. Here we present the results of clinical data and mutational analyses in 25 patients. 11 of these were observed in 4 families, the remaining 14 cases were sporadic. Mutations in NSDHL were identified by SSCA and genomic sequence analysis in peripheral blood leukocytes and cultured skin fibroblasts. In addition, analysis of the X-inactivation status at the HUMARA locus was performed in two patients by use of a methylation-based assay. Heterozygous point mutations in NSDHL were demonstrated in all patients including various missense and nonsensense mutations as well as one splice site mutation. A mutational hotspot was found in exon 4, corresponding to a murine hotspot described earlier. Thus we were able to confirm the diagnosis in patients with the full-blown phenotype and also in patients with minimal or atypical involvement. The broad clinical spectrum of CHILD syndrome can so far not be attributed to a genotype-phenotype correlation; most probably the degree of involvement is a result of the random effect of lyonization. Skewed X-inactivation could be excluded in peripheral blood leukocytes and in skin fibroblasts from affected skin. However, skewing was observed in cells derived from normal skin of the unaffected body side. This finding allows the hypothetic conclusion that selection processes against the mutant allele occur on the unaffected body side in early embryogenesis, a mechanism that might be disturbed on the opposite side of the body resulting in hemidysplasia.

V15

Enhanced RANK-RANKL signalling in transgenic mice leads to increased numbers of regulatory T cells

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RANK-RANKL interaction plays a role in the regulation of T cell/DC communication, DC survival as well as lymph node formation. RANK is expressed on DC, whereas RANKL is found on activated T cells. Accordingly, epidermal Langerhans cells (LC) express RANK and RANKL transcripts are detectable in skin upon inflammation, ultraviolet irradiation as well as during viral infection. To investigate the role of RANK-RANKL signaling in cutaneous immunity, transgenic (tg) mice were generated that overexpress RANKL under control of the skin-specific Keratin-14 (K14) promoter. K14-RANKL-tg mice develop normally and have a uniform transgene expression in basal keratinocytes yet RANKL is not detectable in the serum. The number of epidermal LC was normal in tg mice. Blockade of RANK-RANKL interactions has been described to inhibit the development of CD4+CD25+ regulatory T cells (Treg). Since in K14-RANKL-tg mice increased signaling via this pathway is induced we hypothesized that these tg mice harbour enhanced numbers of Treg. Indeed, a 2-3-fold increase in numbers of CD4+CD25+ Treg cells is found in K14-RANKL-tg mice. These Treg cells are characterized by expression of GITR, CTLA-4, and CD45RBlow. In vitro, the Treg cells from tg mice were anergic, suppressive and produce IL-10. The anergic state can be overcome by stimulation with CD3, CD28 and IL?2. To investigate if these Treg cells from tg mice were functional in vivo contact hypersensitivity (CHS) was induced using DNFB as a hapten. K14?RANKL-tg mice show significantly reduced CHS responses. This suppression was transferrable since injection of isolated Treg cells from DNFB-sensitized K14-RANKL-tg mice into littermates previously immunized with DNFB inhibited the elicitation of CHS responses. This suppression was antigen-specific because transfer into oxazolone-immunized recipients failed to inhibit the elicitation of CHS. Treg cells play an integral role in controlling autoimmunity. Therefore, we used K14-CD40L-tg mice, which develop a severe systemic autoimmune disease and crossed them to K14-RANKL-tg mice. The double transgenics did not develop autoimmune disease implying that the RANK-RANKL induced Treg suppressed the K14-CD40L-induced autoimmune phenotype. Together, these data provide first evidence that stimulation of RANK-RANKL signaling by overexpression of RANKL induced Treg cells in vivo.

V16

Selective inactivation of the interleukin-10 gene in T cells results in enhanced contact hypersensitivity reactions

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Interleukin-10 (IL-10) is an immunomodulatory cytokine which mediates potent suppression of T helper 1 (TH1) responses. IL-10 is secreted by a variety of different cell types including macrophages which are considered the main source of IL-10, but also keratinocytes and T cells. In order to elucidate the function of T cell derived IL-10 we have generated mice with selective deficiency for IL-10 only in T cells by means of the Cre lox-recombination system. Unexpectedly, the T cell specific IL-10 mutants spontaneously developed inflammatory bowel disease and show enhanced immunopathology upon microbial infection. In addition, the mutants mount enhanced contact hypersensitivity reactions as compared to control animals. This phenotype is very similar to that of conventional IL-10 k.o. mice which lack IL-10 in all cell types and is probably a result of unbalanced TH1 responses. These results show that T cell derived IL-10 serves important regulatory functions which cannot be substituted for by IL-10 from other cell types. Future experiments will aim at defining T cell subsets carrying the IL-10 dependent regulatory function. Interestingly, responses of the skin to nonspecific irritation which are drastically enhanced in IL-10 k.o. mice were not different between T cell specific IL-10 mutants and controls. Cell type specific deletion of the IL-10 gene in macrophages or keratinocytes will help to determine whether IL-10 secreted by these cell types regulates cutaneous irritant responses.

V17

Vaccination with TAT-LACK fusion protein-transduced dendritic cells (DC) protects against murine cutaneous leishmaniasis

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Cutaneous leishmaniasis is a major world health problem of increasing importance. At present, a vaccine does not exist. The development of protective immunity depends on IFNγ producing $CD4^+$ Th1- (and $CD8^+$ Tc1-) cells. DC, the most potent of all APC, are able to prime CD4 as well as CD8 cells. Fusion proteins containing the 11 amino acid protein transduction domain (PTD) of HIV-1-TAT translocate antigens into the cytosol of DC facilitating MHC-class-I-dependent antigen presentation. We have reported recently, that TAT-transduced DC induce $CD8^+$ cytotoxic T-cells and elicit antitumor immunity [JI 168:2393, 2002]. Based on this result, we generated fusion proteins comprised of TAT-PTD and the *Leishmania*-specific antigen LACK and utilized TAT-LACK as a component of a DC vaccine against murine leishmaniasis. Bone marrow-derived DC (BMDC) were transduced *in vitro* with TAT-LACK fusion protein or appropriate controls and injected intradermally on day -7 into *Leishmania*-suceptible BALB/c mice. On day 0, we initiated infections using standard high dose inocula ($2x10^5$) as well as physiologically more relevant low dose inocula ($1x10^3$ infectious stage promastigotes/ear). In all experiments, we observed that administration of TAT-LACK transduced DC was superior to injection of DC loaded with LACK alone in mediating protection against progressive disease. In high dose infections, the lesion volume after 7 wks was 182±40 in TAT-LACK-, 350±30 in LACK-, and 272±40 in PBS-treated groups (mm^3, n=4 mice/group). Seven wks after infection with $1x10^3$ parasites, the lesion volumes were as follows: TAT-LACK 55±8, LACK 110±8, and PBS 106±15 (mm^3, n=4 mice/group). These data suggest that priming of $CD8^+$ T-cells, in addition to $CD4^+$ T-cells, is beneficial for the efficacy of a vaccine against leishmaniasis in highly susceptible BALB/c mice. These results also demonstrate that TAT-fusion proteins serve as promising tools for the induction of strong protective immunity in murine leishmaniasis. Since we have now established a basis for the development of an effective vaccine against progressive disease, future studies will exploit the potential of TAT-LACK pulsed DC as treatment for, as well as therapeutic vaccine against, leishmaniasis.

V18

Fibroblast growth factor-2 induces Lef/Tcf-dependent transcription in human endothelial cells

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Lef/Tcf proteins belong to a family of architectural transcription factors, which control developmental processes and play an important role in oncogenesis. Classical activators of Lef/Tcf-dependent transcription comprise the Wnt family of proteins, which translocate b-catenin into the nucleus and allow the formation of transactivation-competent Lef/Tcf/b-catenin complexes. Here we show that in human endothelial cells fibroblast growth factor-2 (FGF-2) reduces GSK-3 activity and augments nuclear levels of b-catenin. FGF-2 induced Lef/Tcf-dependent transcription of a cyclin D1-luciferase construct. Gel shift assays revealed binding of Tcf-4 as the only Lef/Tcf family member and of b-catenin to the Lef/Tcf site in the cyclin D1 promoter. Cotransfection with a dominant-negative Tcf-4 construct inhibited the FGF-2-induced cyclin D1 promoter activity. Overexpression of an uninhibitable GSK-3b mutant resulted in partial inhibition of FGF-2-mediated cyclin D1 induction. The importance for cyclin D1 in FGF-2-induced angiogenesis in vivo is shown in cyclin D1-/- mice, where FGF-2-induced new vessel formation was significantly reduced compared with FGF-2-induced angiogenesis in cyclin D1+/+ mice. In conclusion, FGF-2 is a novel modulator of Lef/Tcf/b-catenin signaling in endothelial cells, suggesting that angiogenic properties of FGF-2 are at least in part mediated by Lef/Tcf/b-catenin activation.

V19

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 (DC). In general, this interaction takes place in secondary lymphoid organs to which both naive T cells as well as mature DC preferentially home. However, this physiological scenario differs substantially from therapeutic DC-based vaccinations used to treat human cancer. In fact, only a small fraction of intradermally injected DC migrate to the draining lymph node and the majority of cells remains 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 expressing blood vessels represent possible entry channels for such 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 infiltrating T cells specifically recognize peptide epitopes presented by the injected DC. Thus, the fraction of DC not migrating to secondary lymphatic tissue after therapeutic inoculation might be actively involved in the induction and modulation of a specific immune response.

V20

Low zone tolerance to contact allergens: the role of immunosuppressive cytokines for the regulatory function of $CD8^+$ suppressor T cells

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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, IL-10, and TGF-β. Here, we studied whether these cytokines are involved in the regulatory properties of the $CD8^+$ effector T cells of the LZT using IL-10-/-, IL-4-/-, dnTGRII mice (expressing the dominant-negative TGF-β-RII under the control of the CD2 promotor), and normal wild type (WT) mice. LZT was assessed by determining the decrease of ear swelling in tolerized vs vehicle treated sensitized animals, as well as proliferation of LNC and cytokine secretion after hapten-specific restimulation.Performing the tolerance protocol resulted in robust LZT response in IL-4-/- and dnTGRII-mice (*in vivo* model of TGF-β insensitive T cells) as measured by ear swelling, but failed to induce LZT in IL-10-/- mice. These data were confirmed by hapten-specific restimulation experiments: lymph node cells (LNC) from tolerized IL-10-/- mice vigorously proliferated in vitro, while LNC from tolerized WT, IL-4-/- and dnTGRII mice displayed strongly reduced proliferation. Analysis of Th1/Tc2 cytokines produced by LNC obtained from tolerized IL-4-/-, IL-10-/- and dnTGRII mice showed no significant differences compared to WT but we observed increased TNF-α production in tolerized IL-4-/- and dnTGRII mice. To analyse the role of IL-10 in the develpoment of LZT, IL-10-/- mice were reconstituted with IL-10 during the induction and/or the effector phase of LZT. The experiments revealed that IL-10 is mandatory for the induction of LZT, whereas, like IL-4 and TGF-β, IL-10 is not contributed to the regulatory functions of the $CD8^+$ effector T cells in LZT.

V21

***P. aeruginosa* produces a specific inhibitor of human β-Defensin-2 induction in primary keratinocytes**

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The gram-negative bacterium *Pseudomonas aeruginosa* (PA) is an ubiquitous human pathogen which causes a variety of epithelial infections. Our recent studies have shown that antimicrobial peptides and proinflammatory cytokines are induced in keratinocytes by supernatants of biofilm-forming PA. As antimicrobial peptides (like the defensin hBD-2) are highly effective antimicrobial agents, we speculate that pathogens like PA might possess escape strategies to abolish these host defense mechanisms. Therefore it was the aim of this study to investigate whether bacteria produce inhibitors of antimicrobial peptide synthesis.We investigated different strains of PA grown for 6 hours to 14 days and evaluated their ability to induce the production of the antimicrobial peptide hBD-2 and the proinflammatory cytokine IL-8 in primary human keratinocytes. The mRNA-expression in keratinocytes was measured by using Realtime-PCR (LightCycler). We found that maximum induction of hBD-2 and IL-8 was achieved by stimulation with supernatants from bacteria grown for 24 hours. Strikingly, hBD-2 induction was found to be decreased when bacteria where grown for extended time periods (up to 14 days), whereas induction of IL-8 remained to be unaffected. Furthermore, when an hBD-2 inducing supernatant and an inhibiting supernatant were mixed a diminished hBD-2 induction was observed. Additionally, size exclusion HPLC of hBD-2 inhibiting supernatants revealed fractions containing inducing activity as well as others with inhibitory activity. A molecular characterization of these fractions is currently in progress. We conclude from these findings that highly pathogenic PA seem to be capable of releasing factors which abolish innate epithelial host defense. These factors specifically inhibit production of hBD-2 in keratinocytes as this effect was not observed for induction of IL-8, TNFα and IL-1β and therefore may represent a novel innate immune escape mechanism of PA.

V22

Protection from UVB-induced apoptosis - a novel activity of the neuropeptide alpha-melanocyte-stimulating hormone

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UVB-induced apoptosis is a tightly regulated and complex biological response which eliminates DNA-damaged cells. Thus, disruption of this process may lead to cellular transformation. We demonstrate that the neuropeptide alpha-melanocyte-stimulating hormone (alpha-MSH) suppresses UVB-induced apoptosis in cultured normal human melanocytes as shown by reduced amounts of oligo- and mononucleosomes and reduced annexin V staining. This effect was not related to enhanced melanogenesis as keratinocytes but not melanoma cells were likewise protected by alpha-MSH from UVB-induced apoptosis. Colony assays revealed that alpha-MSH or its superpotent analogue NDP-MSH did not delay UVB-induced cell death but indeed promoted long-term survival of melanocytes. To elucidate the mechanism of the anti-apoptotic activity of alpha-MSH, we performed DNA content and cell cycle analysis. alpha-MSH treatment of UVB-irradiated melanocytes did not alter the percentage of cells in any phase of the cell cycle as compared to cells treated with UVB alone ruling out that the suppression of UVB-induced apoptosis by alpha-MSH is due to G1 or G2 phase delay. alpha-MSH also did not reduce the expression of Fas (APO-1/CD95) and Fas ligand. In contrast, alpha-MSH treatment of UVB-irradiated melanocytes resulted in a marked reduction in the formation of pyrimidine dimers as shown by South Western dot blot. Our results highlight a novel biological activity of alpha-MSH in pigment cell biology. Moreover, these findings may explain the biological consequences of the recently reported loss of function mutations of the melanocortin-1 receptor in patients with red hair and light skin and melanoma, respectively.

V23

Environment and Genotype: how a water-sensitive transglutaminase-1 mutation explains the dynamic phenotype of the self-healing collodion baby

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Spontaneous healing with no or only very mild ichthyosis distinguishes the "self-healing collodion baby" (SHCB) from other congenital ichthyoses. We describe two SHCB siblings with compound heterozygosity for the transglutaminase 1 (TGase1) mutations G278R and D490G. Histochemical assays at the newborn stage revealed very low TGase1 activity that increased as the children grew older. Although paralleled by the typical clinical improvement, the TGase1 activity always remained markedly subnormal. Since immunohistochemical studies suggested no significant lack of the enzyme the reduced activity had to be due to functional losses of the mutant proteins. Indeed, molecular modeling allowed us to predict a loss of function in G278R and with D490G a chelation of water molecules that locks the mutated enzyme in an inactive *trans* conformation depending on the environmental factor of hydrostatic pressure. To confirm this on a functional level recombinant wildtype and mutant human TGase1 were expressed in a baculovirus system and specific activities of recombinant proteins were compared. G278R showed a complete loss of function, in contrast, D490G displayed more than 80% of wild type activity. Elevated hydrostatic pressure (115 mm Hg), however, reduced D490G activity to 30%. Our data can explain the dynamic SHCB phenotype on the basis of functional heterozygosity: *in utero*, at an average hydrostatic water pressure of about 100 mm Hg, the D490G mutant enzyme is maximally hydrated and therefore largely constrained in the inactive *trans* configuration. Therefore, together with the G278R mutation, the compound heterozygous siblings have very low total TGase 1 activity, resulting in a collodion membrane phenotype at birth. Soon after, the skin becomes less hydrated and the D490G mutant enzyme reverts to the partially active *cis* configuration. This regained TGase 1 activity amounting to upwards of 40% of total normal may be sufficient to maintain a minimal threshold that allows for better epidermal function and improved clinical phenotype.

V24

Dendritic cells genetically modified to secrete a T cell receptor mimic peptide suppress T cell activation in allergy and autoimmune disease models.

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A T cell receptor mimic peptide (TCRpep) consists of a 8-amino acid peptide, homologous to the transmembrane region of the T cell receptor (TCR) a chain. This peptide blocks T cell proliferation by preventing the assembly of an intact TCR. When Dendritic cells (DC) were transfected with the DNS sequence encoding for the peptide, these DCs secreted the TCRpep, causing a reduced antigen-specific T cell proliferation in an OVA specific, transgenic mouse model. To test the effects in vivo, we injected mice with TCRpep transduced DC that had been pulsed with the hapten TNBS. After 6 days mice were challenged with TNCB and ear swelling reaction was determined 24h later. Here ear swelling was markedly reduced in mice injected with TCRpep transduced DCs as compared to controls. Next we tested the effect of TCRpep tranfected DC in an experimental autoimune encephalitis (EAE) model, a murine model for multiple sklerosis. For this, TG4 transgenic mice were injected with TCRpep transduced or control DC respectively that had been pulsed with the EAE antigen, spinal cord homogenate. After 3 successive injections, the EAE was induced by s.c. injection of the antigen emulsified in CFA. Mice in control groups showed significant symptoms of EAE, 1 week after induction and 100% died within 3 weeks. In contrast, mice that received TCRpep secreting DC had significant less severe EAE symptoms and 70% of the animals survived. This effect was antigen-specific since transfected DC that did not express the respective EAE antigen failed to convey protection. Thus these data show, that DC expressing the TCRpep are able to prevent T cell activation in vivo and might be a useful tool to induce antigen specific immune suppression.

V25

Psoriasiform dermatitis in IκB-α-deficient mice is dependent on TNF, LT-α and LT-β and independent of T cells

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IκB-α-deficient mice develop skin changes typical for a psoriasiform dermatitis with acanthosis, parakeratosis and subcorneal microabscesses. The transcription factor NF-κB was found to be constitutively active in keratinocytes isolated from IκB-α-deficient mice. CD31 and CD54 expression was induced in the skin of IκB-α-deficient mice. Psoriasiform dermatitis is discussed to be T-cell dependent. However, in the mouse model presented here, T-cells are not present in the dermis. Transplantation of fetal liver-derived IκB-$\alpha^{-/-}$ stem cells into irradiated hosts did not result in a psoriasiform dermatitis, demonstrating its independency of IκB-α-deficiency in T-cells. Cross-breeding of IκB-α-deficient mice with TNFIII mice, deficient for TNF, LT-α and LT-β, abolished completely the skin phenotype found in IκB-α-deficient mice. CD31 and CD54 expression in the skin was also downregulated. The data presented here clearly show that in contrast to TNF, LT-α and LT-β, T-cells are dispensable for the induction of psoriasiform skin changes in mice.

V26

A Role for p50 in the Molecular Mechanisms of Interleukin-10 to inhibit NF-κB activity

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Nuclear factor kappa B (NF-κB) is a pivotal transcription factor for the development of inflammation. Upon activation, NF-κB is released from its inhibitory protein IκBα in the cytoplasm enabling its translocation to the nucleus where it regulates the expression of a wide array of pro-inflammatory genes such as MIP-2α and IL-6. Although classical NF-κB, a heterodimer composed of the p50 and p65 subunits, has been well-studied, little is known about gene regulation by other hetero- and homodimeric forms of NF-κB. While p65 possesses a transactivation domain, p50 does not. Indeed, p50/p50 homodimers have been shown to inhibit transcriptional activity. We have recently shown that Interleukin-10 exerts its anti-inflammatory activity in part through the inhibition of NF-κB by blocking IκB kinase activity and by inhibiting NF-κB already found in the nucleus. Since the inhibition of nuclear NF-κB could not be explained by an increase of nuclear IκB, we sought to further investigate the mechanisms underlying this observation. Western blot and gel shift analysis demonstrated that treatment of monocytic U937 cells and human peripheral blood mononuclear cells (PBMCs) with IL-10 selectively induced nuclear translocation and DNA-binding of p50/p50 homodimers. TNF-α treatment led to a complete degradation of IκBα and strong translocation of p65 and p50, whereas pre-treatment with IL-10 followed by TNF-α stabilized IκBα and blocked p65 translocation but did not alter the strong translocation of p50. Macrophages of p105/p50-deficient mice exhibited a significantly decreased constitutive production of MIP-2α and IL-6 in comparison to wildtype (wt) controls. Importantly, IL-10 inhibited high constitutive levels of these cytokines in wt macrophages but not in p105/p50 deficient cells. Furthermore, TNF-α-stimulated MIP-2α and IL-6 secretion was about 2-fold higher in p105/p50 -/- cells vs. wt cells. In contrast, IL-10 significantly inhibited TNF-α-induced cytokine production in wt and p105/p50 -/- macrophages. Our findings suggest that the selective induction of nuclear translocation and DNA-binding of the repressive p50/p50 homodimer is an important anti-inflammatory mechanism utilized by IL-10 to repress inflammatory gene transcription.

V27

TRAIL-INDUCED GENE INDUCTION IN HUMAN KERATINOCYTES: DIFFERENTIAL CONTRIBUTION OF TRAIL RECEPTOR 1 AND 2

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) exerts potent cytotoxic activity against transformed keratinocytes whereas primary keratinocytes are resistant to TRAIL-induced apoptosis. However, the specific role of the individual TRAIL receptors 1 and 2, both expressed on the surface of keratinocytes, has not been analysed in detail. Here we have examined TRAIL receptor-specific induction of apoptosis as well as non-apoptotic responses (e.g. NF-κB activation) in human keratinocytes. As recently reported, TRAIL induces caspase-independent activation of NF-κB in keratinocytes. Interestingly, within a panel of NF-κB target genes TRAIL specifically induced IL-8 and, to a lesser extent, IL-1 receptor antagonist whereas mRNA levels of many other potential NF-κB target genes were unaffected. In contrast to findings in other cell types, retroviral infection of keratinocytes with IκBα (IκBα-TD) mutant or kinase dead IKK2 (IKK2-KD) dominant-negative mutants, potent inhibitors of NF-κB, did not modulate sensitivity to TRAIL-induced apoptosis but fully inhibited TRAIL-induced IL-8 secretion suggesting that TRAIL-induced NF-κB activation is critically required for the induction of IL-8. Surprisingly selective inhibition of TRAIL signalling mediated by TRAIL receptor 1 and TRAIL receptor 2 using receptor-specific blocking antibodies demonstrated that in human keratinocytes mainly TRAIL receptor 1 rather than TRAIL receptor 2 leads to induction of apoptosis, caspase and NF-κB activation as well as IL-8 release. In conclusion, we show that similar to TNFα, TRAIL is able to modulate different pro-inflammatory and chemotactic cellular responses via activation of NF-κB. Our data suggest that beside its potent pro-apoptotic role, TRAIL induces pro-inflammatory responses which are mainly mediated by TRAIL receptor 1 in human keratinocytes. Therefore, similar to TNFα TRAIL might be implicated in the pathogenesis of dermatoses involving neutrophilic infiltrates like psoriasis.

V28

Modification of the human allergic immune response by allergen-DNA-transfected dendritic cells in vitro

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Atopic/allergic diseases are characterized by Th2 dominated immune responses resulting in IgE production. DNA-based immunotherapies have been shown to shift the immune response towards Th1 in animal models.
The aim of the study was to analyze wether DC transfected with allergen-DNA are able to stimulate human autologous CD4+ and/or CD8+ T cells from atopic individuals to produce Th1 cytokines instead of Th2 cytokines.
For this purpose, human mature DC from atopic donors were transfected with an adenovirus encoding the allergen Phl p 1. Autologous CD4+ and CD8+ T cells were stimulated with these transfected DC and proliferation and cytokine production were measured.
Using an adenoviral vector a transfection rate of 92% could be achieved. The proliferative response of CD4+ T cells stimulated with autologous transfected DC was dose dependent and almost as high as that of T cells stimulated with mature allergen pulsed DC. The proliferation of CD8+ T cells stimulated with transfected DC however, was higher than that of cells stimulated with allergen pulsed DC. The cytokine pattern showed a shift towards a Th1 immune response compared with T cells stimulated with allergen-pulsed DC.
Human DC can be transfected with allergen-DNA very efficiently using an adenoviral vector yielding DC with high T cell stimulatory capacities directing the atopic/allergic immune response from TH2-dominance towards TH1.

V29

Analysis of syndecan and glypican proteoglycans in human papillomavirus (HPV) infectivity pathways

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It has been shown recently that heparan sulfate proteoglycans (HSPG) are required for attachment of human papillomaviruses (HPV) capsids to the cell surface and for infection with HPV pseudovirions. We have reported last year that syndecan-1, the major HSPG of human keratinocytes, is the primary attachment protein for HPV16 virus-like particles (VLP) to its natural host cell. However, it is unclear if the core protein is important for capsid to cell binding. In addition, the possible importance for infectivity with native virions has not been examined. We therefore analyzed the role of HSPG in capsid binding and in infectivity pathways of bovine papillomavirus (BPV) and HPV11 virions isolated from bovine or human genital warts. K562 cells stably transfected with syndecan-1, syndecan-4, glypican-1 or empty vector control cells (G. David, Leuven) were analyzed for expression of the transgene and the total amount of HSPG on the cell surface by FACS, and for their ability to bind purified HPV16 VLP. Binding was stronger to syndecan-1 K562 cells as compared to the remaining transfectants and was correlated with the total amount of HSPG cell surface expression. In addition, using the appearance of spliced viral mRNA as readout (RT-PCR) we determined in in vitro infectivity assays that all stable transfectants had become permissive for infection with native virions. HPV11 infectivity was specifically neutralized by preincubation of virions with a neutralizing monoclonal antibody. These results indicated that similar to other viruses, the amount of HSPG cell surface expression determines the capacity for papillomavirus binding and infectivity. Since syndecan-1 is specifically induced in basal cells of injured epithelia these results are consistent with a model implicating syndecan-1 as the primary cellular receptor in natural papillomavirus infection.

V30

Heparin reduces melanoma cell rolling in ear postcapillary venules through inhibition of P-selectin

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Blood vessels constitute the routes by which cancer cells spread from the primary tumor to distant sites. Interaction of cancer cells with vascular endothelium influences the localization of circulating tumor cells to form metastasis. Previous preclinical studies and clinical observations suggested a therapeutic benefit of heparin treatment in cancer metastasis. Our studies employing the murine B16 melanoma lung metastasis model revealed that heparin treatment prior to i.v. inoculation of melanoma cells inhibits lung colony formation by more than 80%, whereas continued heparin treatment initiated after inoculation only marginally decreased lung metastasis formation. These data suggested that heparin interferes with extravasation rather than proliferation or metastatic growth. As interaction of P-selectin with its ligands represents a critical step in cell extravasation from the bloodstream, we hypothesized that heparin may reduce melanoma metastasis by inhibition of P-selectin binding to its ligands. The contribution of P-selectin in melanoma rolling was substantiated by intravital microscopy of the murine ear, studying interactions of melanoma cells with vascular endothelium: Compared to wild-type mice, melanoma rolling was indeed reduced by 80% in P-selectin deficient (P-sel -/-) mice. To test whether heparin inhibits melanoma rolling in a P-selectin-dependent fashion, we investigated the effect of heparin on melanoma rolling in both wild-type and P-sel -/- mice: Whereas in P-sel -/- mice rolling remained unaffected by heparin, in wild-type mice rolling of three melanoma cell lines (NW Mel1539, NW Mel624, B16F10) was reduced in a dose-dependent manner after heparin injection (inhibition by 70 ± 10%), supporting the assumption that P-selectin is a potential target of heparin action in melanoma metastasis formation.

V31

Decreased clearance of apoptotic cells and enhanced cross-presentation in milk fat globule-E8 (MFG-E8) deficient mice

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Milk fat globule-associated protein MFG-E8 is produced in large amounts in lactating and involuting breast and by thioglycollate-elicited macrophages, and MFG-E8 mRNA is abundant in immature dendritic cells. Known to bind av integrins via its RGD-containing amino terminus and phosphatidyl serine via its carboxy terminus, the predicted involvement of MFG-E8 in ingestion of apoptotic cells by macrophages in vitro has recently been confirmed. We generated MFG-E8-deficient (MFG-E8-/-) mice to assess the role of MFG-E8 in clearance of apoptotic cells and in cross-presentation in vivo. MFG-E8-/- mice were indistinguishable from normal littermates and were fertile. Although no gross morphologic or routine histological abnormalities were detected, the number of TUNEL-positive thymocytes in resting and dexamethasone-stressed thymi of adult MFG-E8-/- mice exceeded that found in controls. In vitro studies suggested that resident, but not thioglycollate-elicited, peritoneal macrophages from MFG-E8-/- mice ingested apoptotic thymocytes less efficiently than control macrophages. In an assay if cross-presentation, immunization of female 129Sv/B6 MFG-E8-/- mice with beta-2 microglobulin-deficient male C57BL/6 splenocytes resulted in more vigorous immune responses than immunization of wild-type littermate control mice. We conclude that MFG-E8 plays a role in clearance of apoptotic cells by resident macrophages and that MFG-E8 is not essential for cross presentation.

V32

IL-12 instructs in vivo skin homing of human Th2 cells independently of Th cell differentiation

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Th2 cells expressing the E-Selectin ligand cutaneous leukocyte antigen (CLA) dominate early atopic dermatitis (AD) lesions, can be isolated from AD skin, and home to human skin in vivo. In contrast, studies with in vitro polarized Th cells showed that Th2 cells do not express E-Selectin ligands and cannot home to the skin. To investigate this apparent paradox we selected from peripheral blood fully differentiated CLA^- Th2 cells, which expressed the skin and Th2 cell associated chemokine receptor CCR4. CLA^-, $CCR4^+$ Th2 cells were either cultered in the prescence of IL-12 and anti-IL-4 mAbs or IL-4 and anti-IL-12 mAbs. In contrast to resting Th2 cells, we found that activated Th2 cells expressed the IL-12 receptor β2 chain, especially when activated in the presence of IL-12. Fucosyltransferase (FucT) VII is necessary for the synthesis of E-Selectin ligands like CLA. Consequently, CLA^- Th2 cells were negative for FucT-VII, but the presence of IL-12 induced FucT-VII mRNA expression within hours resulting in subsequent surface expression of CLA. Expanded former CLA^- Th2 cells from the different culture conditions were analyzed for in vitro rolling and in vivo migration capacities. Rolling on E-Selectin was exclusively seen in IL-12 treated Th2 cells and only IL-12 treated Th2 cells migrated to human skin grafts on SCID mice in response to i.c. injection of CCR4 ligands. This in vivo migration of human Th2 cells could be completely blocked by anti-E-Selectin mAbs. Interestingly, in contrast to the IFN-γ inducing effect of IL-12 on naïve Th cells, the cytokine phenotype of these IL-12 treated fully differentiated Th2 cells was not changed. Moreover, transfecting Th2 cells with the Th1 transcription factor T-bet mediated strong IFN-γ production but CLA expression remained unchanged indicating that these events can be independent. Our findings demonstrate that IL-12, independently of its role in Th cell differentiation, instructs CLA on Th2 cells. This explains differences between in vitro and in vivo polarized Th2 cells in regard to the skin homing potential. Furthermore, IL-12 mediated CLA upregulation in Th2 cells may also be a cause for exacerbations of AD during infections.

V33

Antigen specific interactions of T cells with B cells or DC differ fundamentally in terms of dynamics, interaction plane and outcome

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T cell activation requires the physical interaction with APC. Based on the analysis of T-B conjugates current models generally propose long and stable T-APC contacts as indispensable prerequisite for successful signaling. We have recently described, that DC engage T cells only transiently and in a very dynamic way, when the activation process is performed in tissue like 3-D collagen matrices. While these differences have been attributed to the experimental setup employed here, we suspected, that the used APC might be a major factor. Therefore, we directly compared T cell activation within 3-D collagen matrices by B cells and DC.
Highly purified naive TCR-transgenic DO11.10 T cells were mixed with either freshly purified B cells or bone marrow derived DC in the presence or absence of the relevant antigen (chicken OVA, aa323-339). Cell-cell interactions were recorded by videomicroscopy and T cell activation was monitored by [3H]Thymidine incorporation, FACS and Ca2+-signaling.
As expected, DC were by far more effective than B cells in antigen specific T cell activation. Furthermore, T cells were activated much faster by DC and contacts to DC of 4-6 h in total were sufficient to achieve full activation, whereas with B cells, T cells needed contact times of up to 60 h. These functional differences were reflected by strikingly different biophysics of T-APC contacts. B cells indeed formed long-lasting, unipolar contacts with T cells being stably attached to the leading edge of single motile T cells. These synapse-like structures were strictly dependent on the presence of high amounts of antigen. DC, in contrast, engaged single or multiple T cells in a very dynamic manner involving the entire T cell body, which did not require the presence of cognate antigen.
These data show fundamental differences between DC and B cells in the way they interact with T cells and suggest, that stable contacts to B cells are much less effective for T cell activation than dynamic interactions with DC.

V34

Fcα-Dependent Granulocyte Activation Is Required for Blister Induction by IgA1 Autoantibodies to the Cleaved Ectodomain of Type XVII Collagen/BP180

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IgA, the second most prevalent serum immunoglobulin, plays an important role in immune exclusion at mucosal surfaces. In addition, by binding to its receptor on myeloid cells, IgA is able to mediate a plethora of effector functions. While IgA is important for the immune defense, IgA autoantibodies are associated with different autoimmune conditions, including blistering disorders, connective tissue diseases, and haemolytic anemia. However, a direct pathogenic role of IgA autoantibodies has not yet been demonstrated. Linear IgA disease (LAD), a severe subepidermal autoimmune blistering disease, is associated with IgA autoantibodies to type XVII collagen. The 120 kDa ectodomain of the 180 kDa full-length type XVII collagen may be cleaved from the cell surface. The aim of the present study was to investigate the blister-inducing potential of IgA autoantibodies in LAD. Interestingly, serum from patients with LAD (n=10), but not from normal controls, induced subepidermal splits in cryosections of human skin when co-incubated with leukocytes from healthy volunteers. To characterize the mechanisms of this dermal-epidermal separation, we purified IgA1 from LAD patients' sera by Jacalin affinity chromatography. Eluted IgA1 fractions retained the blister inducing capacity, while serum depleted of IgA1 was not pathogenic. Recruitment of granulocytes at the dermal-epidermal junction, but not of peripheral blood mononuclear cells, was a prerequisite for split induction. These granulocytes were activated as revealed by their capacity to reduce nitro blue tetrazolium to formazan. $F(ab')_2$ fragments of patients' IgA, lacking the effector Fc portion, lost their split-inducing capacity. Importantly, preadsorption of IgA autoantibodies against the cell-derived cleaved ectodomain, but not against a recombinant form of full-length type XVII collagen, abolished their blister-inducing potential. In summary, for the first time, we demonstrate the pathogenicity of autoantibodies of the IgA isotype. In addition, our findings suggest that epitopes, generated by cleavage of the type XVII collagen ectodomain, are important for binding of blister-inducing IgA autoantibodies to this target antigen.

V35

The Thy-1 molecule on activated human dermal microvascular endothelial cells specifically interacts with CD11b (aM-integrin) on leukocytes - impacts on inflammatory processes.

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Our previous work demonstrated that human Thy-1 is a newly identified activation associated cell adhesion molecule on human dermal microvascular endothelial cells (HDMEC). Thy-1 is involved in the adhesion of monocytes and polymorphnuclear cells (PNC) to activated HDMEC. In this study we identified the corresponding ligand of the Thy-1 molecule on these cells.
First, we generated a monoclonal antibody (mAb AW2) against PNC and monocytes blocking the binding of Thy-1 protein to its ligand. This antibody shows the same binding pattern as an anti-CD11b antibody. Blocking experiments revealed that the mAb AW2 inhibits the binding of an anti-CD11b antibody to monocytes. Second, purified and labeled Thy-1 protein binding to its ligand on monocytes and PNC has a similar binding profile as the anti-CD11b-antibody and mAb AW2 in flow cytometry. Additionally, purified Thy-1 protein was able to reduce binding of anti-CD11b antibody as well as mAb AW2 suggesting that Thy-1 binds to CD11b (am-chain of integrins) on PNC and monocytes.
Cell adhesion studies showed that mAb AW2 as well as anti-CD11b-antibodies were able to block adhesion of PNC and monocytes to Thy-1 transfected COS-cells emphasizing an essential role of Thy-1 / CD11b interaction for cell adhesion.
The CD11b is a well characterized cell adhesion molecule on monocytes and PNC. Until now it is thought that ICAM-1 is the main cellular ligand of CD11b. Our data first time provide evidence that human Thy-1 on activated HDMEC is a second cellular ligand of CD11b mediating the binding of monocytes and PNC to activated endothelium, possibly regulating invasion of leukocytes into inflamed tissues.

V36

Rapid induction of melanoma in human skin by exposure to growth factors and ultraviolet-b radiation

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Exposure to ultraviolet (UV) radiation likely plays a key role in melanomagenesis as well as yet unknown factors. We demonstrate that in normal human skin an increased expression of a combination of three growth factors - basic fibroblast growth factor (bFGF), stem cell factor (SCF) and endothelin-3 (ET-3) - together with exposure to UVB can transform melanocytes into melanoma within four weeks. Increased cutaneous expression of the growth factors was achieved by intradermal injection of adenoviral vectors (Ad5) containing the respective genes into human skin grafted to severe combined immunodeficiency disease (SCID) mice. While each growth factor alone stimulated pigmentation and melanocyte proliferation, only a combined expression of bFGF, ET-3 and SCF led to melanocytic clusters in the epidermo-dermal junction zone and to migration of single melanocytes into the upper layers of the epidermis within 2-4 weeks. When the weekly induced expression of bFGF, ET-3 and SCF was combined with 50 mJ/cm2 UVB irradiation three times weekly, severe pigmented lesions developed that were found to be in situ melanomas or invasive melanomas by histopathologic criteria. Of 79 human skin grafts that received the combination treatment, melanoma was found in 34 %. The lesions were positive for S100, HMB45, Melan-A and NKIC3. Melanocytic cells isolated and cultured from these lesions formed colonies in soft agar indicating anchorage-independent growth. Flow cytometry analysis for melanoma antigens revealed expression of beta3 integrin, MEL-CAM, melanotransferrin, chondroitinsulfate proteoglycan and acetyl-GD3. In summary, this is the first report of human cancer induction in which malignant transformation of normal human cells in vivo is achieved by a combination of natural environmental factors, while each factor alone has no such carcinogenic potential.

V37

Processing and possible mechanism of action of the antibiotic peptide Dermcidin in human sweat

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Recently we identified in human sweat the novel antimicrobial peptide DCD-1 derived from the Dermcidin (DCD) gene (Schittek et al., Nature Immunol. 2, 1133-1137 (2001)). We showed that DCD-1 has antimicrobial activity against a variety of pathogenic microorganisms in a dose-dependent manner. Furthermore, the activity of DCD-1 is maintained over a broad pH range and in high salt concentrations resembling the conditions in human sweat. To analyze whether there exist individual differences in the presence and the amount of the DCD-1 peptide in human sweat we applied surface-enhanced laser desorption/ionisation (SELDI) technology for the detection of DCD-1 directly from microlitre amounts of human sweat. Profiling of human sweat from healthy donors as well as from patients with atopic dermatitis revealed that in addition to DCD-1 other DCD-derived peptide species were present in significant quantities. Four of five identified peptides were DCD-1 related while the fifth corresponded to a portion of the DCD protein outside the DCD-1 core. This provides clues as to how the novel protein is processed to its active form. Furthermore, in order to reveal the mechanism of bacterial cell killing we performed ultrastructural analyses of *S. aureus* treated with DCD-1. Using electronmicroscopy analysis we could not detect perforation of the bacterial membrane. This indicates that DCD – in contrast to other antimicrobial peptides like the defensins - does not kill the bacteria by pore formation of the bacterial membrane and indicates an alternative killing mechanism of DCD. To analyze the mechanism in more detail we localized the peptide in DCD-treated *S. aureus* by immunelectronmicroscopy. The analyses indicated that DCD is mainly localized at the bacterial membrane. This suggests that DCD binds to receptors on the bacterial membrane and induces a signalling cascade leading to cell death.

V38

Absence of beta2 integrins on dendritic cells does not impair antigen presentation, but their expression on t lymphocytes is necessary for t cell activation

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Beta2 integrins are important for transendothelial migration of leukocytes as well as for T cell activation during antigen presentation. To determine the relevance of beta2 integrins for the DC-T cell interaction during antigen presentation, we investigated bone marrow-derived DC (bmDC) and T cells from CD18-deficient (-/-) mice in vitro. Increasing with age, CD18-/- mice, which lack all functional beta2 integrins, spontaneously generate profoundly increased numbers of activated cells (CD44high, CD62Llow) in peripheral blood, lymph nodes and spleen in vivo. Thus, the absence of beta2 integrins results in T cell activation, rather than immunodeficiency in vivo, despite profoundly impaired proliferation of CD18-/- T cells after allogeneic stimulation with DC in vitro. Furthermore, cytokine production (IFNγ and IL-2) is also significantly impaired in T cells of CD18 -/- mice. In contrast, the T cell stimulatory capacity of bmDC from CD18 -/- mice is not altered as determined by mixed lymphocyte reactions (MLR). Except for the absence of CD18, bmDC from wild type and CD18 -/- mice are phenotypically similar as measured by flow cytometry. DC-T cell contact, as determined by time-lapse videomicroscopy of collagen gel-embedded leukocytes, is independent of beta2 integrin expression by DC, but strongly dependent on LFA-1 expression on T cells.
Taken together, expression of the beta2 integrins on T cells, but not on DC, is essential for antigen presentation, and subsequent T cell activation.

V39

Identification and characterisation of peptide epitopes derived from murine MAGE

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Immunogenic peptide epitopes from tumor-associated antigens serve as targets for naturally occurring or therapeutic induced anti-tumor immune responses. Despite the fact that numerous clinical trials use such peptide for vaccination against cancer in humans, several prevailing questions can only be addressed in animal tumor models. However, while in the case of human melanoma abundant peptide epitopes derived from either melanocytic differentiation antigens like MART-1, gp 100, tyrosinase and tyrosinase related protein-1/2 or cancer-testis antigens like MAGE, BAGE, GAGE are known, for murine melanoma the available selection is restricted to differentiation antigens, i.e. gp100 and TRP2.
To overcome this limitation, we applied have searched the mouse MAGE family for Kb-restricted peptide epitopes applying reverse immunology and have identified 2 peptides derived from murine MAGE 1 and MAGE 5. *In vitro* binding assays revealed that these peptides bind equally well to Kb as the known strong binding peptide derived from the Sendai virus nucleoprotein. The *in vivo* immunogenicity of these peptides was examined by induction of tumor specific cytotoxic T lymphocytes in mice which were vaccinated twice with MAGE-derived peptide loaded dendritic cells. Spleen cells obtained from these vaccinated mice were used in cytotoxicity assay after one round of *in vitro* stimulation. Indeed, mice vaccinated with MAGE-peptide loaded DC showed specific lysis of melanoma cells while syngeneic fibroblasts were not killed. Moreover, after intravenous challenge with a lethal dose of syngeneic melanoma cells, lungs of mice vaccinated with MAGE-peptide loaded DC showed hardly any metastases, whereas control mice receiving dendritic cells loaded with an irrelevant peptide developed high numbers of lung metastases.
In summary, we describe herein two Kb-restricted peptide epitopes derived from murine MAGE proteins which can be used to induce anti-tumor immunity *in vivo*. These peptides are likely to serve as valuable tools to improve vaccination strategies *in vivo*.

V40

CCR10 Expression by Malignant Melanoma Cells: Implications for Tumor Growth and Metastasis

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Malignant Melanoma shows aggressive primary tumor growth and metastasizes into local draining lymph nodes, lung, liver, brain and bone marrow. In contrast to other malignancies, malignant melanoma also shows a high frequency of skin metastases. Recently, we showed that chemokine receptors such as CXCR4 are involved in the development of organ-specific metastasis to lymph nodes and lung. Furthermore, we identified the novel skin-associated chemokine CCL27(CTACK, ALP, ESkine) and its receptor CCR10(GPR-2) which specifically mediate the recruitment of lymphocytes into the skin. In the present study, we show that malignant melanoma cell lines as well as primary malignant melanoma tumors express high levels of CCR10 mRNA. Notably, skin metastases of malignant melanoma show significantly increased CCR10 mRNA expression compared to primary tumors suggesting the selection for CCR10-high expressing malignant clones. Immunohistochemical analyses confirmed CCR10 expression by MelanA-positive melanoma cells. *In vitro*, CCR10 signaling mediated migration and invasion of malignant melanoma cells and significantly induced their proliferation. *In vivo*, neutralization of mCCL27 resulted in delayed primary tumor growth of human melanoma cells in a SCID mouse model. Taken together, our findings suggest that CCR10 expression by malignant melanoma cells plays a role in primary tumor growth and the development of skin metastases.

V41

Cockayne Syndrome A and B proteins are present in mitochondria and involved in the repair of oxidative mtDNA damage

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Cockayne Syndrome (CS), which is characterised by neurodegeneration, photosensitivity and premature aging, is caused by mutations in the CSA and CSB gene. In addition to its role in nuclear excision repair, recent evidence suggests that the CSB protein participates in the repair of oxidative DNA damage in the nucleus. Mitochondria (mt) are the cells locus with the highest turnover of reactive oxygen species (ROS) and it is known that ROS-induced mutations of mtDNA play a role in (i) the normal aging process and (ii) neurodegenerative mt-syndromes with clinical features similar to CS. In the present study we have assessed whether the CSA and CSB protein are localized in the mt and whether they may play a role in protecting the mt genome against oxidative stress. Western blot analysis of protein extracts from mitochondria, which had been purified from normal human fibroblasts and were free of nuclear contaminants, revealed that the CSA and CSB proteins were detectable in the mt. In marked contrast, CSA and CSB were undetectable in mt-extracts of CSA and CSB patients. We have previously demonstrated that mt DNA mutations can be induced in human fibroblasts by repetitive exposure to UVA radiation as a source for oxidative stress. In order to assess the potential relevance of CSA and CSB proteins for protection of the mt genome we next compared the generation of mt DNA mutations in UVA-irradiated fibroblasts from normal individuals and CS patients. UVA irradiation induced mtDNA mutations after 36 repetitive exposures in normal controls, whereas only 12 irradiations were required in CSA and CSB cells. Moreover, stable transfection of the CSB protein into CSB-deficient cells not only restored presence of the CSB protein in their mt, but also normalized their susceptibility towards UVAR-induced mtDNA-mutagenesis. These results indicate a role of the CSA and CSB protein in the repair of oxidative damage in mtDNA and provide the first evidence for their role in the process of aging.

V42

NOD2 mediates beta-defensin expression in human keratinocytes

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Recent studies have been demonstrated that human skin-derived keratinocytes are capable of releasing antimicrobial proteins which might protect human skin from infections.
Two antimicrobial proteins, human beta-defensin-2 and -3 (hBD-2 and hBD-3) have been isolated from human skin. Expression of these peptide antibiotics in keratinocytes is induced by proinflammatory cytokines and bacteria. However, the signal transduction pathway leading to expression of hBD-2 and hBD-3 in keratinocytes is not known. Recently a novel nucleotide-binding site leucine-rich repeat protein termed NOD2 has been discovered. NOD2 shows high similarity to a class of plants disease resistance proteins which have been implicated in the recognition and mediation of a wide spectrum of pathogen components. It has been shown that NOD2 is primarily expressed in monocytes and that NOD2 activates NF-kappa B in response to bacterial products.
We addressed the question whether NOD2 might be involved in the epithelial innate immunity of human skin. We could show for the first time that NOD2 mRNA is expressed in skin-derived primary keratinocytes. NOD2 mRNA in keratinocytes was slightly increased upon stimulation with the proinflammatory cytokines tumor necrosis factor-alpha, interleukin-1-beta and interferon-gamma. Furthermore we observed that bacterial challenge of keratinocytes resulted in increased NOD2 gene expression indicating that NOD2 is involved in mediating a defense response in keratinocytes towards bacteria. To verify this hypothesis we cloned the NOD2 coding sequence in an expression plasmid which we used to transfect keratinocytes. Interestingly NOD2 overexpression in keratinocytes resulted in an upregulation of hBD-2 and hBD-3 gene expression. This was proven by activation of co-transfected gene reporter plasmids containing the promoter region of hBD-2 and hBD-3. These studies indicate that NOD2 may play an important role in the innate immune response of human keratinocytes. Further studies are in progress to elucidate the NOD2-mediated signal transduction pathways leading to increased gene expression of hBD-2 and hBD-3.

V43

Molecular topography of a dynamic immunological synapse

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Productive interactions of T cells with antigen-presenting cells result from stable and long-lived cell-to-cell binding, that leads to the accumulation of TCR, adhesion receptors, and signaling molecules at the contact zone, termed „immunological synapse“. Besides adhesive and long-lived contacts, more dynamic short-lived and serial encounters between T cells and dendritic cells (DC) can result in T cell activation and proliferation. Using a dynamic contact model in 3D collagen matrices and reconstruction by confocal microscopy, we here examine the morphology of the contact plane and the related distribution of TCR and LFA-1, as well as associated signaling events, i. e. the distribution of phosphotyrosine residues, lipid rafts, and calcium influx. Upon migratory scanning across the DC surface, T cells established a mobile, elongated, and asymmetric contact plane which includes the leading T cell edge, cell body, and the posterior uropod. While TCR remained evenly distributed on the T cell surface lacking accumulation at the contact plane, a prominent yet diffuse redistribution of LFA-1 towards the junction zone was obtained. The productivity of these contacts was confirmed by strong phophotyrosine staining along the junction, either in a bipolar dot-like pattern with maximum intensity at leading edge and uropod, or evenly distributed along the entire contact plane. Calcium influx in crawling T cells was initiated by initial contact acquisition via leading edge and persisted until detachment and complete uropod retraction were achieved. The importance of the uropod in TCR triggering and signalling was further supported by accumulation of cholesterol-rich microdomains (lipid rafts) in the uropod upon detachment. Together, the cellular mobility coupled to an asymmetric molecular topography of the junction zone represent a „dynamic immunological synapse“ in T cells scanning across the DC surface, implicating both, leading T cell edge and trailing uropod in the generation and maintenance of sustained TCR triggering and signaling.