DOI:10.1111/j.1600-0625.2012.01471.x
www.blackwellpublishing.com/EXD

Contact dermatitis: from pathomechanisms to immunotoxicology

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Abstract: Contact allergens are small reactive chemicals. They cause allergic contact dermatitis (ACD) by activating the innate and adaptive immune system. Contact allergens are very peculiar because of their built-in autoadjuvanticity that allows them to trigger sterile inflammation following skin penetration. The innate inflammatory response involves the triggering of pattern recognition receptors either by direct chemical interaction with such receptors or by induction of endogenous activators. I discuss here the recent findings regarding prevalence and predisposition, the identification of innate immune and stress response mechanisms relevant for sensitization and the orchestration of the innate and adaptive immune response to contact allergens. Despite still significant gaps of knowledge, recent advances in our understanding of the immunopathogenesis of ACD can now be used for the development of causative treatment strategies and of *in vitro* alternatives to animal testing for the identification of contact allergens in immunotoxicology.

Abbreviations: ACD, allergic contact dermatitis; AhR, arylhydrocarbon receptor; BMDC, bone marrow-derived dendritic cell; CHS, contact hypersensitivity; CYP, cytochrome P450; DAMPs, damage-associated molecular patterns; DC, dendritic cell; DNCB, 2,4-dinitrochlorobenzene; ECM, extracellular matrix; HA, hyaluronic acid; LLNA, local lymph node assay; NLR, NOD-like receptor; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognition receptor; PTM, post-translational modification; TCR, T-cell receptor; TLR, Toll-like receptor; TNCB, 2,4,6-trinitrochlorobenzene; Treg, regulatory T cell.

Key words: contact allergen – inflammation – innate immunity – local lymph node assay – skin

Accepted for publication 14 February 2012

Contact dermatitis – prevalence and predisposition

Irritant and allergic contact dermatitis (ACD) may pose a serious problem to human health. These inflammatory eczematous skin diseases are mostly caused by chemicals that exert toxic effects without inducing a T-cell response (irritants) or by small reactive chemicals that modify proteins and induce innate and adaptive immune responses (contact allergens). ACD is eventually mediated by contact allergen-specific T cells.

The prevalence of ACD is high: 15–20% of the general population suffers from ACD to at least one chemical, most commonly nickel, fragrances and preservatives (1,2). Of utmost importance is occupational contact dermatitis. It is the most frequent work-related skin disease and is associated with significant economic costs (3,4). Identified risk factors are sex, with higher frequency of ACD in women, age, with frequent onset at young age, exposure in the workplace, use of consumer products and genetic predisposition. However, solid evidence for an increased risk associated with specific MHC haplotypes is missing. This is different from some drug hypersensitivity reactions (5). Nevertheless, genetic predisposition is evident and involves polymorphisms in genes that regulate xenobiotic metabolism and biotransformation as well as cellular stress responses including redox balance (e.g. *N*-acetyltransferase, glutathione-*S*-transferase), inflammation including innate and adaptive immunity (e.g. IL-1α, IL-1β, IL-6, IL-10, TNF-α) and skin barrier function (e.g. filaggrin) (Table 1) (6,7). Most likely, polymorphisms in innate pattern recognition receptors (PRR) such as Toll-like receptors (TLR) and NOD-like receptors (NLR) or in cytokine receptors such as IL-12R also contribute to genetic predisposition. In this context, the skin microbiome may also play an important role (8,9).

The current data on predisposing genetic polymorphisms with relevance for sensitization identify in part the same pathways as identified in the mouse contact hypersensitivity (CHS) model (10), thereby providing evidence for their relevance in the human disease, and by genomic and proteomic studies on contact allergen-treated human cells (Table 1) (11–17) (Sens-it-iv Newsletter 44, http://www.sens-it-iv.eu).

Xenobiotic chemicals and xenoinflammation – learning from pathogens

Xenobiotic chemicals including drugs, respiratory and contact allergens can cause and contribute to adverse reactions including autoimmune diseases or allergies (18). Many of these chemicals are very peculiar as they represent exogenous triggers for sterile inflammation. As in other cases of sterile inflammation such as trauma, PRR for anti-microbial defense are activated as shown, for example, for contact allergens, even in germ-free mice, suggesting a role of endogenous activators such as damage-associated molecular patterns (DAMPs) (19). As chemical-induced inflammatory responses are initially mechanistically different from auto-inflammation and from microbe-induced inflammation, but result in the activation of similar signalling pathways I have proposed the term *xenoinflammation* (20). One form of xenoinflammation is the contact allergen-induced skin inflammation.

We knew for a long time that contact allergens induce skin inflammation involving the production of proinflammatory cytokines. We also knew that this is coupled to their chemical reactivity, but it was unclear how this reactivity was translated into the activation of innate immune and stress responses. Therefore, we started to analyse the role of well-known innate inflammatory pathways triggered by pathogens assuming that they may play a role in ACD. With this strategy, we were able to demonstrate a physiological role for TLR, $P2X_7$-dependent NLRP3 inflammasome activation and for reactive oxygen species (ROS) in ACD and in

Table 1. Signalling pathways and cellular responses relevant to allergic contact dermatitis (ACD). Listed are signalling pathways and cellular responses triggered by contact allergens as identified by genomic studies in human MUTZ-3 progenitor cells (13) and by proteomic studies in human keratinocytes (Sens-it-iv Newsletter 44, http://www.sens-it-iv.eu) (16,17) and pathways identified based on human polymorphisms associated with susceptibility to ACD (6,7)

Genomics (MUTZ-3)	Proteomics (keratinocytes)	Human polymorphisms
Oxidative/cellular stress response (e.g. Keap1/Nrf2 pathway)	Oxidative/cellular stress response (e.g. Keap1/Nrf2 pathway)	Oxidative/cellular stress response
Xenobiotic metabolism	Metal toxicity	Xenobiotic metabolism
Protein ubiquitination	Metabolic response	Inflammation (innate/adaptive immunity)
LPS/IL-1 mediated RXR inhibition	Cytoskeletal reorganization	Skin barrier function
AhR signaling		
Protein kinase A signaling		

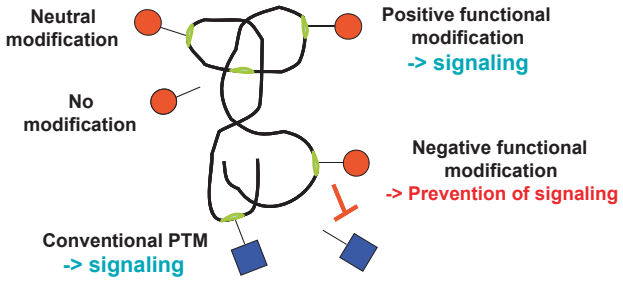

Figure 1. Consequences of protein modification by chemicals. The chemical modification of proteins by contact allergens (red) can remain neutral, can induce signalling processes or interfere with conventional post-translational modification (blue), for example, with phosphorylation. This may inhibit signalling. Only permissive target sites within the protein can be chemically modified. Other putative target sites remain unmodified because of their steric inaccessibility or biochemical features.

the CHS model (21–23) (P. R. Esser, U. Wölfle, C. Dürr, F. D. von Loewenisch, C. M. Schempp, M. A. Freudenberg, T. Jakob, S. F. Martin, manuscript submitted for publication.).

Chemicals as modifiers of biomolecules

The sequencing of the human genome has disclosed an evolutionary dilemma. The high biological complexity of the species *Homo sapiens* is based on roughly only 23 000 protein-coding genes (24,25) and about 10^{14} cells, with the number of genes not so much different from the small nematode worm *Caenorhabditis elegans* with about 19 000 genes and 959 cells in the adult hermaphrodite and 1031 cells in the male worm (26). To achieve this high-complexity combinatorial diversity is used with proteins assembled in different combinations to build, for example, different signalling pathways. In addition, chemical modification of proteins [post-translational modification (PTM)] and other biomolecules is used to create diversity. Changes in protein localization, protein–protein interactions and alteration of protein functions are achieved by PTM. Other levels of diversity are generated by epigenetic modifications, including DNA methylation and post-translational histone modification by methylation and acetylation, and by the use of non-coding small interfering (si)RNAs and micro(mi)RNAs as sequence-specific post-transcriptional regulators of gene expression (27,28).

Examples for PTM that alter proteins are methylation, acetylation, ubiquitination, sumoylation, myristoylation, gylcosylation and phosphorylation. It is conceivable to assume that many drugs and chemical allergens have their business exactly here by performing the reversible or irreversible modification of proteins and other biomolecules, thus mimicking or interfering with conventional PTM (Fig. 1). Thus, binding of contact allergens, mostly electrophilic chemicals or metal ions, to proteins will result in changes of protein function, localization, protein–protein interactions and most likely also in conformational changes up to the level of interference with proper protein folding with increasing extent of chemical modification. This may result in the activation of the unfolded protein response and endoplasmic reticulum stress (29,30).

Contact allergens are peculiar in their dual function as 'half-antigenic' compounds (haptens) that generate antigenic T-cell epitopes and as adjuvants for innate immune system activation

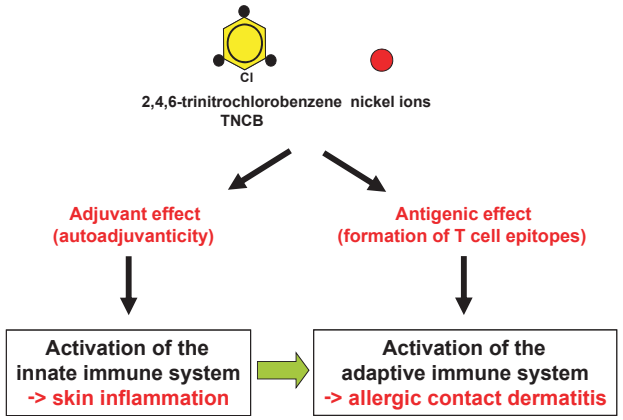

Figure 2. Dual effects of contact allergens. Contact allergens are very peculiar, because as sterile chemicals, they can simultaneously activate the innate and adaptive immune system, even in germ-free mice. Both, their 'built-in' autoadjuvanticity, which induces sterile inflammation, and the formation of antigenic T-cell epitopes, which leads to a contact allergen-specific T-cell response, depend on their chemical reactivity.

with a built-in *autoadjuvanticity* (Fig. 2) (31). This term is used to describe, for example, the adjuvant effect of protein allergens such as Derp2 from house dust mite, that mimics MD-2, a structural component of the TLR4 receptor complex, and which can thereby contribute to – and amplify – TLR4 signalling (32). Both the antigenicity and the autoadjuvanticity of contact allergens are based on chemical reactivity, that is, their capacity to modify proteins and other biomolecules by covalent binding in the case of organic chemicals or by complex formation in the case of inorganic molecules such as metal ions like nickel and cobalt. Autoadjuvanticity of contact allergens can result from direct activating effects on innate immune signalling pathways or from indirect effects that involve the formation or release of endogenous danger signals including DAMPs as activators of innate immunity (10). Examples for direct effects are the interaction of nickel ions with conserved histidine residues in the human TLR4 and the activation of the Keap1/Nrf2-dependent antioxidant response by interaction of organic molecules such as TNCB and DNCB with cysteine residues in Keap1. Indirect activation of TLR2 and TLR4 by TNCB, oxazolone and most likely also other contact allergens involves the

degradation of the extracellular matrix (ECM) component hyaluronic acid (HA) (21) (P. R. Esser, U. Wölfle, C. Dürr, F. D. von Loewenisch, C. M. Schempp, M. A. Freudenberg, T. Jakob, S. F. Martin, manuscript submitted for publication.). Fragments of HA can activate TLR2 and TLR4 (33–35). However, up to now, direct binding of pure, synthetic HA fragments to these TLR has not been demonstrated, yet. Similarly, NLRP3 inflammasome activation by these chemicals involves the activation of the purinergic receptor $P2X_7$ by the release of the endogenous danger signal ATP from stressed or damaged skin cells into the extracellular space (23).

Orchestration of the innate cellular and molecular immune response to contact allergens

Removing cells from their tissue context may completely alter their function, as many cellular processes and functions are not cell autonomous, but strongly depend on – and are regulated by – the tissue context (36,37). Therefore, we have to go back from the reductionist approaches to the complexity of the tissue and the organism as aimed for by systems biology. Contact dermatitis is a good example to describe the interplay of cells and signalling pathways in an inflammatory disease of the skin. A highly suitable term to describe this interplay is *orchestration*. Notably, in our experimental approaches, we always identify one cell type or one molecule or pathway that is crucial for skin sensitization to contact allergens as revealed by successful prevention of sensitization and, in some cases, also elicitation upon genetic knockout or pharmacologic inhibition of a single one of the cellular and molecular players involved. Because similar results, that is, abrogation of CHS, can be observed for different cell types, molecules and signalling pathways (10), these findings clearly illustrate the essential functional interaction of non-redundant, complementary cellular and molecular mechanisms.

We must unravel the qualitative and quantitative contribution of the different cellular and molecular players and their spatiotemporal interplay. There is intensive cross-talk between the different cellular signalling pathways and cell types, as well as between organ compartments such as epidermis and dermis or whole organs like skin and lymph nodes, which altogether makes the symphony sound perfect. Using this comparison, removing either the violins or the trombones from the orchestra is equally devastating. Therefore, we have to understand the orchestration of these immune responses in the respective tissue context.

Orchestration of the innate cellular immune response in contact dermatitis

The skin consists of resident tissue stromal and hematopoietic cells and cells that are constantly recirculating for immune surveillance purposes and of cells that are recruited during an immune response. During the rapidly triggered innate immune response to contact allergens, among others, keratinocytes, mast cells and dendritic cells (DCs) are activated, and NK cells and neutrophils are recruited. Mast cells increase vascular permeability, thereby facilitating the recruitment of innate inflammatory cells. It has also been shown that mast cells contribute to the recruitment of leukocytes via TNFα and support skin DC maturation, migration and polarization of T-cell responses to IL-17 and IFN-γ production (38–41). Constitutive absence or conditional depletion of mast cells in mice abrogates CHS (41). Likewise, monoclonal antibody-mediated depletion of Gr-1+ cells prevents elicitation of CHS. A role for Gr-1+ neutrophils in the recruitment of T cells to the skin was suggested (42), but also a role for Gr-1+CCR6+ monocytes that are recruited from the blood and are efficient in the cross-priming of CD8+ T cells (43). These data nicely show that depletion of either mast cells or Gr1+ cells is sufficient to abrogate the CHS response and that careful orchestration of the presence of both cell types during the different stages of inflammation plays an important role in the innate immune response to contact sensitizers.

Moreover, liver NKT cells are required especially in the early phase of the elicitation of CHS. By their production of IL-4, they activate B1 B cells to produce IgM which plays a role in T-cell recruitment (44). It was now shown that stimulatory lipids accumulate in the liver following sensitization to trigger CD1d-dependent NKT cell activation (45). Here a participation of B1 B cells and $\gamma\delta$ T cells was claimed. IL-33, a member of the IL-1 family that is produced by stromal cells and mast cells in the skin, may play a role in the activation of these B1 B cells in CHS (46). It also activates mast cells and plays a role in neutrophil recruitment (47), thereby providing a further link between the different immune players. Interestingly, a proinflammatory role of IL-17-producing $\gamma\delta$ T cells in skin inflammation was now shown in mice and humans (48–50).

A further cell type recently shown to be crucially involved in CHS is NK cells. Liver NK cells induce a contact allergen-specific CHS-like response in an artificial T/B-cell free situation in RAG-deficient mice or in CD3ε-deficient mice (51–53). However, the inflammatory ear swelling response differs from T-cell-induced *bona fide* CHS by the absence of an inflammatory cellular infiltrate, lack of the upregulation of characteristic markers of inflammation and cytotoxicity in the skin and of a boosting effect upon repeated contact allergen challenge (53). The antigen specificity of the NK cell response may be because of haptenation of a ligand on antigen-presenting cells, which is recognized by a corresponding receptor on the NK cell. One may speculate that direct hapten modification of self-MHC molecules or their presentation of hapten-modified peptides is perceived by NK cells as 'missing self' disabling recognition by inhibitory receptors and enabling recognition of altered, allo-like self-MHC by activating receptors. The induction of hapten-specific conformational changes may underlie these processes. In normal CHS, one also finds NK cells in the inflamed skin, but their contribution to the disease is so far unclear (51). NK cells are also detected in human skin in ACD to nickel. It was shown that they are activated by Th1 and Th17 cells and amplify the inflammatory response. It remains to be shown whether an upregulation of activating NK receptor ligands because of chemical-induced cellular stress is involved in NK cell activation in ACD. Evidence for antigen specificity of the human NK cell response is missing up to now (54).

Because of the slower kinetics of the priming and polarization of naive T cells by DCs emigrated from the skin, the T cells arrive later than innate immune cells in the skin and complete the hypersensitivity reaction by triggering the elicitation phase. In mice, an experimental elicitation by a contact allergen challenge does not seem to be absolutely required, because a primary CHS occurs with a single application of strong contact allergens (55–57). These data indicate that thanks to their autoadjuvanticity, contact allergens induce an innate inflammatory response that triggers all components required for the priming, polarization and

skin homing of effector T cells, at least in the case of strong contact allergens.

An interesting concept is now developing that links innate and adaptive cellular responses in the skin. The initial innate cellular response results in the early recruitment of contact allergen-specific T cells, which in mice are CD8+ IFN-γ producing, cytotoxic T cells (Tc1). This may also be the case in human ACD in analogy to early initiator CD8+ T-cell recruitment in atopic skin disease (58). This antigen-specific T cell–dependent initiation phase is then followed by an amplification phase, which is in part contact allergen non-specific and involves NK cells (54) and maybe also skin NKT cells, that may recognize self-lipids (59), as well as inflammatory skin $\gamma\delta$ T cells (48).

Orchestration of innate molecular immune and stress responses

The identification of the innate immune and cellular stress response pathways triggered by contact allergens has seen significant progress during the last years. These pathways are of great interest for therapeutic intervention and for the development of *in vitro* alternatives to animal testing for the identification of contact allergens in immunotoxicology (60,61). Most studies focus on DCs and keratinocytes. Studies in the mouse CHS model have revealed that contact allergens activate pathways triggered by pathogens (21–23,62–65), (P. R. Esser, U. Wölfle, C. Dürr, F. D. von Loewenisch, C. M. Schempp, M. A. Freudenberg, T. Jakob, S. F. Martin, manuscript submitted for publication.). While nickel directly interacts with the human TLR4, organic chemical allergens such as TNCB and oxazolone promote the degradation of HA to proinflammatory fragments that can be endogenous activators of TLR2 and TLR4. These findings also demonstrate that degradation and release of ECM components including HA and biglycan signals to the innate immune system and thereby contributes to inflammation (66). Moreover, contact allergens trigger the release of ATP from skin cells, an endogenous danger signal that activates the NLRP3 inflammasome via $P2X_7$ to generate mature IL-1β and IL-18. Interestingly, we found a central role for the functionality of TLR2, TLR4, IL-12Rβ2, ASC, NLRP3 and $P2X_7$ in DCs. Bone marrow-derived dendritic cells (BMDCs) deficient in any combination of two of the three receptors TLR2, TLR4, and IL-12Rβ2 or a single one of the others lost their ability to induce sensitization when modified with the contact allergen TNBS and injected intracutaneously into wild-type mice. In addition, in all the corresponding knockout mouse strains, CHS cannot be induced by direct topical sensitization of the mice. However, wild-type BMDCs successfully sensitized the corresponding knockout mice (21,23,62,63). These findings underscore the necessity to induce TLR and inflammasome triggering in DCs for successful sensitization to contact allergens and once again illustrate the complementary interaction of the different pathways and their cooperation in the induction of skin inflammation. Such interactions are also observed in infections (67,68).

In addition to TLR and inflammasome activation, oxidative stress responses are induced by contact allergens that lead to ROS production and activation of the antioxidant phase 2 response. This involves Keap1-mediated Nrf2 activation and upregulation of antioxidant response genes (69,70).

Recent evidence suggests that there is a role for the arylhydrocarbon receptor (AhR) in CHS. On the one hand, mice lacking AhR have decreased CHS responses that may be because of a defect in the maturation of Langerhans cells (71). On the other hand, some contact allergens such as the prohaptens eugenol and isoeugenol seem to activate AhR directly and thereby suppress cell cycle progression as shown in the keratinocyte cell line HaCaT (72,73). The AhR signalling pathway was also prominent in contact allergen-stimulated human MUTZ-3 cells in genomic studies (13). The regulation of cell cycle progression may be one physiological function of AhR. Interestingly, some of the xenobiotic metabolizing enzymes of the cytochrome P450 (CYP) family and some ABC transporters including multidrug resistance proteins are regulated by AhR (74,75). This may be of relevance for the metabolic conversion of chemically non-reactive prohaptens by CYPs to haptens in the skin (76–78) and their export from skin cells via multidrug resistance proteins (79,80). Moreover, AhR contributes to the development of Th17 cells and has an important role in the production of IL-17 and IL-22 (81,82). Th17 cells are also involved in the pathology of CHS (10). Moreover, AhR plays a role in $\gamma\delta$ T cells that are a source for IL-17 (83) and is important for the homeostasis of skin $\gamma\delta$ T cells (84) that are involved in skin inflammation (48). It is tempting to speculate on a role of contact allergens in the polarization of T-cell responses via the AhR pathway and in the regulation of their own metabolization and cellular transport in skin cells.

Genomic and proteomic profiling studies of keratinocytes and DCs unravel new pathways and their interactomes as activated by contact allergens (15–17). Much work lies ahead of us with respect to the validation of the identified candidates genes, proteins and pathways with respect to their relevance in the sensitization process of ACD.

Gaps of knowledge and their practical consequences for immunotoxicology

The problems that arise from our still limited knowledge of the immune pathology of ACD are not only the lack of causative treatment strategies but also the difficulty to develop reliable *in vitro* alternatives to replace the gold standard animal test in immunoxicology, the local lymph node assay (LLNA) for the identification and potency assessment of contact allergens (60). The 7th Amendment to the Cosmetics Directive is in effect since March 2009 with exceptions until 2013 and prohibits animal testing for the contact sensitizing potential of cosmetic ingredients (85). In addition, the testing of 40–60 000 already marketed chemicals in animal-based tests for their sensitizing potential because of the EU regulation REACh would require enormous animal numbers. Product and consumer safety necessitates immunotoxicological risk assessment, and the challenge is to develop *in vitro* assays that cover the most important steps of the sensitization process (60,61). At this point, it is highly likely that only a combination of assays will be able to cover these different steps and are needed to develop an optimal integrated testing strategy (86). The current gaps of knowledge and the reductionism of *in vitro* assays that eliminates many factors that play a role in *in vivo* sensitization pose a significant problem for accurate risk assessment and the actual predictivity of *in vitro* assays. Eventually, only *in vivo* exposure will tell whether a chemical induces sensitization (87). In the future, we have to translate our knowledge from the identification of molecular pathways and interactomes into an understanding of the impact of the complex interactions of cells

Table 2. *In vitro* assays for the assessment of the sensitizing potential of chemicals. Listed are current *in vitro* assays for the identification of contact allergens. None of these assays is formally validated, yet

Sensitization step	*In vitro* assay	Cell type/Model	End point	References
Skin protein modification	Direct peptide reactivity assay (DPRA)	–	Peptide reactivity	89–91
Keratinocyte activation	NCTC2544 assay	NCTC2544	IL-18 production	94,95
Keratinocyte antioxidant response	Keratinosens assay	HaCaT	Keap-1/Nrf2 activation	96,97
DC activation	MUSST	U937	Induction of CD86, IL-8	92
	hCLAT	THP-1	Induction of CD54, CD86	92
	PBMDC assay	CD14+ monocyte derived DC	Induction of CD86	93
	Vitosens®	CD34+ progenitor derived DC	Gene signature	98,99
	GARD	MUTZ-3 progenitor	Gene signature	13,15
DC migration	DC migration assay	MUTZ-LC	CCR5/CXCR4 dependent migration	100
Chemical skin toxicity, tissue stress	EE potency	Epidermal equivalent	Toxicity, induction of IL-1α	101
T cell priming	Human T cell priming assay (hTCPA)	CD14+ monocyte derived DC + autolog. naive human T cells	T cell cytokine production/proliferation	102–104

within the tissue context and the interactions of different tissues via soluble mediators and migratory cells in the whole organism.

Current assays under development or in prevalidation address different steps of the sensitization process (Table 2) (60,61,88). Chemical reactivity of haptens and prohaptens is assessed in the direct peptide reactivity assay (DPRA) (89–91), DC activation in the MUSST, hCLAT and PBMDC assays (92,93), keratinocyte IL-18 production and Keap1/Nrf2 activation in the NCTC2544 and KeratinoSens assays, respectively (94–97). Genomic profiling is performed in the VITOSENS (98,99) and the GARD assay (13,15). DC migration is tested using a chemokine-based assay and MUTZ-3-derived Langerhans-like cells (MUTZ-LC) (100). One of the major shortcomings of all current assays is their inability to assess allergen potency. This is a distinctive feature of the LLNA. The DPRA assay and a tiered approach that combines the NCTC and the epidermal equivalent (EE) potency assay (101) aim for this goal. It remains to be seen whether these assays are suited to provide such information. The most specific assays to identify putative contact allergens are human T-cell priming assays (hTCPA) (102–104). Here, chemicals are tested for their ability to prime naive human T cells. The hTCPA is now also tested for its ability to assess potency. If there is a correlation between allergen potency and the frequencies of primed contact allergen-specific T cells and regulatory T cells (Treg) and their T-cell receptor (TCR) repertoire diversity, this assay may be useful in this respect (105). It remains to be determined whether allergen potency really correlates with the size of the T-cell pool or whether the extent of immune regulation of the T-cell pool is more important. In this respect, it is interesting that CHS responses that are comparable in magnitude to those induced by strong contact allergens can be induced in mice upon repeated sensitization to weak contact allergens and to drugs upon depletion of CD4+ cells (106–108) and in the hTCPA upon depletion of CD25+ and CD56+ immunoregulatory T cells (103). This may indicate the existence of comparably sized T-cell pools and more efficient suppression of these cells by immunoregulatory CD4+ cells like Treg and NKT cells in the case of weak contact allergens. This may, in part, be correlated to the potential of contact allergens of different potencies to induce skin inflammation that impacts the balance between effector and Treg cells (109). However, determination of T-cell frequencies and TCR repertoires has yet to be performed to clarify this issue. Tightly linked to the balance between effector and Treg cells is the strength of the innate inflammatory response. It seems obvious that allergenic potency strongly correlates with the ability to induce skin inflammation, which is a prerequisite to break homeostatic tolerance and induce adaptive immunity. Therefore, establishment of *in vitro* assays for the qualitative and quantitative assessment of the innate inflammatory response to chemical allergens should yield potency information.

A problem arises from the fact that immunotoxicological testing is performed with single compounds and not with mixtures and formulations or the final product that often contains a combination of weak contact allergens that as single compounds represent a low risk to cause sensitization. Furthermore, irritants can often be encountered in the final product as well or in the workplace. They may facilitate sensitization to contact allergens or amplify ACD. From a mechanistic point of view, one can envisage additive or synergistic effects of the combination of several weak contact allergens that cooperatively cause sufficient inflammation to overcome a critical threshold and hence allow for sensitization

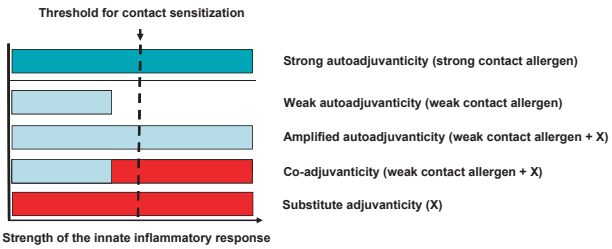

Figure 3. Hypothetical possibilities for triggering of the innate inflammatory response in ACD. Strong contact allergens (dark green) exhibit sufficiently strong autoadjuvanticity to overcome the sensitization threshold, while weak contact allergens may exhibit insufficient autoadjuvanticity (light green). In the latter case, amplified autoadjuvanticity may be achieved (dose–response effect), for example, by irritants such as detergents that may increase skin penetration of the contact allergen or by other contact allergens, infection or other factors (light green bar). Alternatively, additive or synergistic effects may overcome the threshold because of co-adjuvanticity provided by irritants, other contact allergens, microbial pathogen-associated molecular patterns (PAMPs) because of coincidental infection, by damage-associated molecular patterns (DAMPs) and other factors that trigger innate inflammatory signalling (light green/red bar). In some cases, contact allergen autoadjuvanticity may be inefficient or missing, for example, because of genetic polymorphisms that prevent innate signalling or delete interaction sites for the contact allergen in a critical target protein. In that case, sufficient substitute adjuvanticity may be provided by other contact allergens, irritants, PAMPs, DAMPs and other factors (red bar). X = irritants, contact allergens, PAMPs, DAMPs, other factors (nanoparticles etc.).

to one or several of the compounds (Fig. 3). Such cooperative effects have been described in the mouse CHS model (110), and there is evidence for the clinical relevance of such effects (111–113). In the case of irritants, one can envisage effects such as the facilitation of skin penetration, for example, by detergents. This will increase the local contact allergen concentration and thereby amplify the insufficient magnitude of the autoadjuvant effect over the threshold. Irritants may also induce skin inflammation by triggering pathways critical for sensitization and elicitation (Fig. 3). Some irritants cannot trigger inflammatory responses that may help to overcome the threshold for sensitization to a weak contact allergen as shown, for example, for croton oil and suboptimal doses of TNCB (110), while others are able to do that as shown for sodium lauryl sulphate and DNTB (64). We must realize that we lack sufficient knowledge on the action of irritants. Because of the existence of genomic and proteomic profiling data for contact allergens and, notably, unpublished data for irritants that are used as controls in these studies, it is now possible to identify crucial pathways for skin sensitization to contact allergens, which can also be triggered by irritants and may, therefore, lead to the amplification of ACD. It is to be expected that one will find a certain overlap of the genomic and proteomic profiles of some irritants with contact allergen-specific signatures, but one should also find pathways that are not triggered by irritants and others that are exclusively triggered by them.

The important lesson to be learnt from animal testing is that it has several limitations. Examples are false-positive and false-negative results for some chemicals in the LLNA or the species-specific differences for the interaction of nickel with TLR4. Moreover, systematic testing of the sensitization potential of mixtures and formulations, which may allow additive and synergistic effects of different compounds, and of the final and marketed product is lacking. In addition, there are still significant gaps of knowledge in our understanding of the sensitization process. These evident problems should therefore warrant better postmarketing and epidemiologic surveillance.

Genomics and proteomics in basic research and immunotoxicology

Modern large-scale profiling studies are very powerful in the identification of contact allergen-specific genomic and proteomic signatures which may help to develop high throughput assays. However, it may become evident with the increasing number of substances used in such studies that there is a strong variation in the profiles with different chemicals that may not allow to identify a single common contact allergen signature. This may be because of the wide variety of physicochemical properties of the reactive compounds that can cause ACD. One way to resolve such issues may be the grouping of chemicals according to the mechanistic domains, that is, reaction mechanism for adduct formation with proteins (114,115). This will be a very interesting approach and may reveal the existence of class-specific chemical profiles based on mechanistic domains. In the optimal situation, one would find common denominators between several or all classes of chemicals that can be used for the identification of contact allergens. Similar studies should be carried out with respiratory allergens and drugs that can cause T cell–mediated hypersensitivities that may have many commonalities with contact allergens regarding innate immune and stress responses as well as for irritants.

One of the future tasks of proteomics is the identification of the *hapten proteome*, that is, the array of extracellular and cellular proteins that interact with contact allergens (16). Such studies have been initiated for the identification of nickel-interacting proteins in human B cells and DNP-modified proteins in human THP-1 monocytes and RAW264.7 macrophages (116–118). The aim of these studies must be the identification of the target proteins whose modification by chemical allergens results in the activation of the pathways that are relevant for sensitization and elicitation of ACD. Moreover, the identification of the functionally relevant target sites within these proteins must be identified. These studies are essential to clarify the molecular basis for the autoadjuvanticity of chemical allergens.

The ugly, the bad and the good

Besides the ugly adverse effects of contact allergens leading to chronic eczematous disease or less severe but recurrent ACD, these chemicals have a good side and can be used for topical immunotherapy of skin diseases (119). One example is alopecia areata. In this skin disease that causes hair loss on the head, contact allergens such as DNCB, diphenylcyclopropenone (DPCP) or squaric acid are used to stimulate hair growth (120). Another example is the use of contact allergens for chemoimmunotherapy of cutaneous melanoma (121,122). In that case, the activation of acute inflammation by contact allergens may help to break tolerance mechanisms of the tumor. Moreover, hapten modification of self-antigens may prime tumor-specific effector T cells that might also recognize the unmodified self-antigens. This self-reactivity of T cells may explain the occasional vitiligo-like depigmentations that are most likely caused by the killing of normal melanocytes presenting such self-antigens on their MHC molecules.

A further interesting, still speculative aspect is the potential use of contact allergens to better understand and to modulate protein function. It is likely that some of the PTM introduced by contact allergens may differ from conventional PTM because of the molecular structure of the chemical and its target sites and specific amino acids within the protein. We may, therefore, learn that known protein functions can be further modulated by contact allergens with regard to the quality and quantity of the resulting response. A useful comparison may be the variation in disease phenotypes in the case of mutations of the same protein in different protein domains as seen, for example, in IPEX patients with different mutations in FOXP3 (123–125). Studies in this field of chemical biology seem interesting and may help to develop drugs that mimic or prevent contact allergen-dependent modulation of protein function.

All roads lead to Rome – implications of innate immune and stress responses to contact allergens

The realization that contact allergens and infectious agents can trigger the same innate immune and stress pathways has important implications. Infections may be trigger factors for contact sensitization and elicitation by either substituting for ineffective or missing autoadjuvanticity or amplifying weak autoadjuvanticity of contact allergens helping to surpass a critical threshold for the activation of the innate immune system (Fig. 3). Evidence for these hypotheses comes from our experiments rendering BMDCs, which because of their double deficiency for IL-12Rβ2 and TLR4 are incapable of sensitizing mice for TNCB-induced CHS, competent for sensitization by *in vitro* pretreatment with CpG oligonucleotides, ligands for

TLR9 (21). This also shows that not necessarily the same PRR responsible for contact allergen-mediated signalling must be triggered to substitute for ineffective autoadjuvanticity. Other studies made use of the resistance of mice to nickel-induced CHS because of the lack of nickel-binding sites in mouse TLR4. The ineffective autoadjuvanticity was substituted by co-injection of nickel with LPS (126) or microbial compounds (127,128). In this context, it is interesting to consider microbial adjuvants that can induce immune deviation and immunoregulation or tolerance and, therefore, may help in the design of treatment strategies as suggested by a recent study (129).

The fact that infections induce TLR signalling and can also lead to inflammasome activation and ROS production makes the co-adjuvant/substitute adjuvant concept of microbial trigger factors for ACD very attractive. Thus, coincidental infections can provide exogenous adjuvants (PAMPs) or trigger the production and/or release of endogenous danger signals such as DAMPs and may, thus, substitute for ineffective or missing autoadjuvanticity and amplify insufficient autoadjuvanticity of contact allergens. Such effects may not be restricted to bacterial or viral pathogen-associated molecular patterns (PAMPs) and other proinflammatory microbe-induced factors but may also include other non-microbial agents like silica or asbestos crystals as inflammasome activators (130), nanoparticles (131,132) and many other compounds that can trigger the relevant innate inflammatory pathways directly or via the induction of endogenous activators such as DAMPs (Fig. 3). It is possible that our increased exposure to such exogenous *co-adjuvants* in our environment and in consumer products explains in part the increase in the prevalence of ACD over the last decades.

Conclusion

The emerging theme from basic research in chemical allergy is the triggering of innate immune and stress responses by contact allergens that are used for antimicrobial/antiviral defense (10,133). This does not only apply to chemical allergens but is also observed for some protein allergens (31,134) and relies on intrinsic autoadjuvanticity of the allergens. In addition, biogenic cofactors from the allergen carriers, for example, pollen-associated NADPH oxidases or lipid mediators (135) play a role. These exciting news teach us new lessons on what makes an allergen an allergen.

Similar mechanisms may be operated by chemical respiratory allergens and drugs such as β-lactam antibiotics that cause T cell–mediated adverse reactions (5,108,136). The specific effects of these chemicals with respect to the type of immune responses may be because of their intrinsic properties but most likely also due to the respective tissue microenvironment. The different sets of tissue stromal cells in the skin, the lung, the gut, the liver and other organs and the respective immune cells will certainly impact the outcome of chemical exposure (36,37). This may explain in part why topically applied chemical respiratory allergens are positive in the LLNA, but fail to induce ACD. Another, chemical intrinsic factor is the induction of different cytokine profiles by contact and respiratory allergens (60,137–141). Interestingly, sensitization for respiratory hypersensitivity may occur via the skin.

The identification of the defining molecular features of specific tissue microenvironments, especially on epithelial cells which also imprint tissue-specific features of immune cells, the so-called epimmunome, is a major challenge and a very exciting task for future research (142).

With respect to the treatment of ACD, the revelation of the complementary action of innate immune and stress pathways resulting in cooperative induction of skin inflammation opens new avenues to causative anti-inflammatory treatment strategies. As shown in the CHS model, blocking a single one of the pathways is sufficient to prevent sensitization and in some cases also elicitation. Future work has to asses the relevance of these pathways in the elicitation phase and in chronic ACD. Anti-inflammatory strategies that block more than a single pathway combined with strategies that get rid of contact allergen-specific effector and memory T-cell responses, for example, by specific immunotherapy to re-establish tolerance can be envisaged for the future.

Acknowledgements

I am very grateful to Dr Philipp R. Esser for fruitful discussions and careful and critical reading of the manuscript. The work reviewed here was supported in part by a grant of the European Commission as part of the project 'Novel Testing Strategies for In Vitro Assessment of Allergens (Sens-it-iv)', LSHB-CT-2005-018681.

Conflict of interests

The author declares no conflict of interest.

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