

New concepts in cutaneous allergy

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Summary

Allergic contact dermatitis affects a worrying proportion of the general population. The mechanisms underlying this chemical-triggered delayed-type hypersensitivity are still not fully understood. In recent years, basic research has shown that the immune system reacts to contact allergens by activation of signalling pathways that are usually used to fight infections. Ongoing work is aimed at the elucidation of the path that leads from the chemistry of contact allergens to the inflammatory skin disease. The cellular players and their complex interactions are being characterized. Proteins are being identified whose chemical modification by contact allergens results in the activation of signalling pathways involved in pathogenesis. Pathway identification is supported by genomic and proteomic techniques. All of these efforts will yield a cellular and molecular understanding of the orchestration of the innate and adaptive immune response to contact allergens. This knowledge will help in the identification of gene and protein signatures for improved diagnostics, the identification of novel drug targets for targeted treatments, as well the development of *in vitro* assays for contact allergen identification.

Key words: chemical; contact dermatitis; dendritic cell; inflammation; innate immunity; skin; T cell.

The chemical world comprises an innumerable diversity of natural and synthetic chemicals. Among these, ~4000 chemicals can cause T cell-mediated allergic contact dermatitis in humans. Contact allergens are reactive organic chemicals or metal ions that modify proteins and most likely other biomolecules by covalent binding and complex formation, respectively. The most frequent contact allergies are caused by nickel, fragrance substances, and preservatives. Up to 15–20% of the general population is sensitized to at least one contact allergen, and the prevalence of allergic contact dermatitis is ~7% in

the German population (1, 2). Irritant contact dermatitis is caused by chemicals that have toxic effects resulting in skin inflammation and eczema, but do not involve T cells. Contact dermatitis, as one of the most frequent occupation-related diseases, is a severe health problem (1). Chronic contact dermatitis remains a major challenge for clinicians. Symptomatic treatment with corticosteroid drugs is currently used, but new and mechanism-based, targeted treatments should be developed on the basis of our increasing mechanistic understanding of the pathology. Chemical allergens penetrate into the skin and activate skin cells such as immune cells and structural cells. Most importantly, dendritic cells (DCs), such as epidermal Langerhans cells and different subsets of dermal DCs, become activated. These DCs then upregulate costimulatory molecules, migrate to the skin-draining lymph nodes, and present contact allergens in the context of major histocompatibility complex molecules to naïve T cells (3). Owing to a contact allergen-dependent polarization process, DCs secrete a combination of cytokines that polarize the T cell response towards Tc1/Th1 and

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Tc17/Th17 effector/memory T cells. The inflammatory milieu is needed to overcome constitutive immune regulation. It removes the brakes on the immune system, and enables a contact allergen-specific T cell response. The priming of naïve T cells seems to be paralleled by the induction and expansion of contact allergen-specific regulatory T cells (Tregs) (4) that fail to prevent allergic contact dermatitis in the case of sufficiently strong allergens, but may prevent allergic contact dermatitis being caused by weak contact allergens. In the elicitation phase of allergic contact dermatitis, these Tregs help to downregulate the immune response. In their absence, the immune response is stronger and prolonged. The ratio between effector/memory T cells and Tregs seems to be determined by the potency of the respective contact allergen, and the strength of the innate inflammatory response could be the major determinant influencing this ratio and the decision between immunity and tolerance (5). Allergic potency may be mainly defined by the strength of the innate inflammatory response induced by a contact allergen, but this remains to be studied further.

The elicitation phase of allergic contact dermatitis is initiated by repeated contact with the sensitizing contact allergen. The innate inflammatory response triggers the release of cytokines and T cell-attracting chemokines. Endothelial cells are activated, and the contact allergen-specific T cells infiltrate the inflamed skin to exert their effector function. The clinical picture of allergic contact dermatitis is the result. The eczematous skin reaction resolves within a few days, in part because of the action of regulatory T cells. Apart from cells that are newly recruited to the skin and, as shown for Tregs, can recirculate to the draining lymph nodes, skin-resident T cells may be important in allergic contact dermatitis (6, 7).

***In vitro* assays for contact allergen identification**

The induction of contact allergen-specific T cells concludes the clinically inconspicuous sensitization phase. This phase of allergic contact dermatitis is an important topic of immunotoxicology with respect to contact allergen identification. Replacement of the murine local lymph node assay (Organization for Economic Co-operation and Development Guideline 429) is a pressing issue, and animal testing is already prohibited for the cosmetics industry. Several *in vitro* assays that cover different aspects of the complex sensitization phase, ranging from peptide reactivity to T cell priming, have been developed, and some of them are almost fully validated. A combination of selected assays in an integrated testing strategy may allow for complete replacement of animal testing (8, 9).

A major remaining problem up to now has been the lack of *in vitro* assays that predict allergenic potency, but this problem may be solved in the future if the parameters that determine it are identified and translated into *in vitro* assays. The chemical reactivity and the innate inflammatory response are plausible candidates.

The chemistry of contact dermatitis

Allergic contact dermatitis is an eczematous inflammatory skin disease. The activation of the innate immune system by metal ions or organic chemicals eventually breaks immunological tolerance and results in the activation of the adaptive immune response, that is, the generation of a contact allergen-specific effector and memory T cell response. For this purpose, the chemicals must be reactive to proteins, that is, form complexes like nickel and cobalt ions or bind covalently to proteins. The consequence of this chemical reactivity is twofold: it results in the activation of the innate immune system and in the formation of T cell epitopes (10).

The direct or indirect activation of the innate immune system by contact allergens (Fig. 1) is essential for the activation of the adaptive immune response. It is caused by the so-called irritant effect of contact allergens. Interestingly, it is unknown how much mechanistic overlap there is between irritants and contact allergens with respect to their irritant effect, that is, the ability to cause skin inflammation. Future studies including genomic profiling will hopefully clarify these aspects and help us to understand the mostly unknown mechanisms of action of irritants. Here, a classification of irritants according to physicochemical properties may be interesting with respect to their ability to trigger signalling pathways. Attempts such as this have been made for contact allergens by grouping them according to mechanistic domains and analysing the co-segregation of this classification with the allergenicity and allergenic potency (11–14). These approaches may be extremely useful in elucidating the link between the chemistry and biology of irritants and contact allergens.

Chemical protein modification by contact allergens is essential for their ability to induce skin inflammation. Modifications may mimic or interfere with classic post-translational protein modifications, and thereby alter the function and localization of proteins, and protein–protein interactions (15). There is a large gap in our knowledge regarding the identity of the functionally relevant target proteins that are modified by contact allergens and the relevant sites of their modification. One study using the human monocyte cell line THP-1 has shown modification of a limited number of proteins and

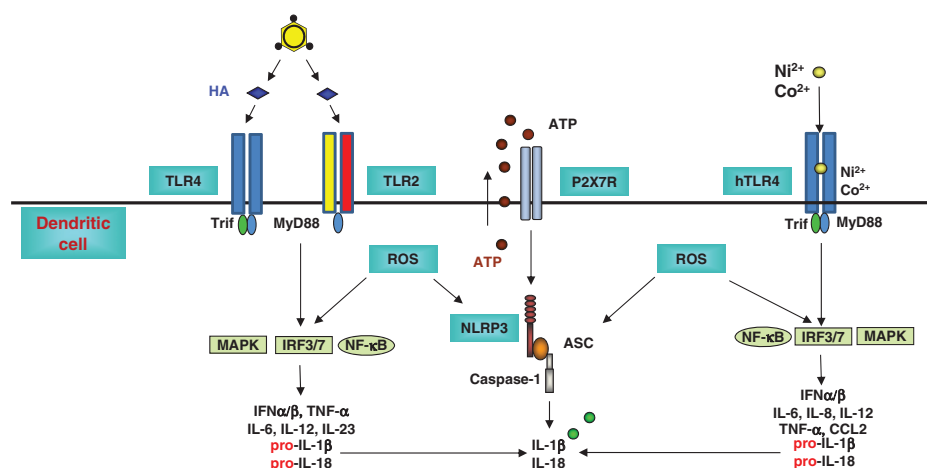


Fig. 1. Direct and indirect triggering of innate signalling pathways by contact allergens. Nickel and cobalt ions bind to and dimerize human Toll-like receptor (TLR) 4 (hTLR4) and induce signalling. Contact allergens such as 2,4,6-trinitrochlorobenzene and oxazolone induce the generation of hyaluronic acid (HA) fragments that activate TLR2 and TLR4. The TLRs signal via the adaptor proteins MyD88 and Trif to activate nuclear factor- κ B (NF- κ B), mitogen-activated protein kinases (MAPKs) and interferon (IFN) response factors (IRFs) 3 and 7. Pro-inflammatory cytokines and chemokines are produced. The contact allergens induce reactive oxygen species (ROS) production and ATP release. ROS promote TLR signalling and NLRP3 inflammasome activation, together with ATP, which activates the potassium channel P2X7R. Pro-interleukin (IL)-1 β and pro-IL-18 are processed by caspase-1. TNF, tumour necrosis factor.

the lack of correlation of protein abundance and modification by the contact allergen 2,4-dinitrochlorobenzene (16). Thus, there is selectivity in the modification of proteins by contact allergens.

Up to now, three proteins have been identified whose modification is relevant for allergic contact dermatitis. These proteins are Keap1, transient receptor potential ankyrin 1 (TRPA1), and human Toll-like receptor (TLR) 4. Keap1 is a cytosolic sensor protein that detects oxidative and electrophilic stress by oxidation or chemical modification of cysteines. This results in the activation and nuclear translocation of the transcription factor Nrf2, which is otherwise ubiquitinated and shuttled to the proteasome by Keap1 for degradation. Nrf2 activates genes with antioxidant response elements in their promoters. This activates the antioxidant phase II response (17, 18). Mice lacking Nrf2 have a much lower threshold for sensitization by contact allergens and increased contact hypersensitivity (CHS), most likely because of the pro-inflammatory role of oxidative and electrophilic stress (19, 20). In these mice, even weak contact allergens such as fragrances induce CHS (20). The Keap1–Nrf2 pathway seems to be commonly triggered by contact allergens, and is the basis for the KeratinoSensTM assay for the *in vitro* identification of contact allergens (21). TRPA1 is a calcium influx channel of nociceptive neurons, and is involved in pain and itching, but also in inflammation (22). It is also expressed on keratinocytes and endothelial cells. Its activation, like

that of Keap1 or the transient receptor potential vanilloid 1 (TRPV1) channel that detects reactive chemical compounds from onions, mustard, garlic, and cinnamon, depends on chemical modification of cysteines (17, 23–25). Interestingly, both CHS caused by oxazolone or urushiol and cinnamal-induced oedema are reduced in TRPA1-deficient mice (26, 27). Human TLR4 is the receptor for endotoxin/lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria. Nickel and cobalt ions can directly bind to conserved histidines in human TLR4, thereby inducing its dimerization and inflammatory signalling via nuclear factor- κ B (NF- κ B) (28, 29). Palladium also acts via human TLR4 (30). It will be interesting to identify more such proteins and the associated signalling pathways. Contact allergen-triggered signalling pathways and contact allergen-driven regulation of gene expression are being identified by genomic and proteomic profiling studies using human cell lines (31–33) or skin biopsies from patch tests (34, 35). Such studies will allow us to find susceptibility genes, identify molecular pathways that are relevant for pathogenesis, identify drug targets, and establish gene and protein signatures that can be used for the *in vitro* identification of contact allergens and the diagnosis of allergic contact dermatitis and its differentiation from other forms of eczema. Irritants must also be subjected to such studies. An interesting aspect here, as mentioned before, will be the correlation of mechanistic domains and mechanisms of action of contact allergens and irritants.

Orchestration of the cellular innate immune response to contact allergens

During the innate immune response to contact allergens, the composition of the array of skin cells, the 'cellome', changes dramatically (10). Among the early events in the initiation of CHS is the activation of mast cells (36). By an as yet unknown mechanism, contact allergens activate mast cells to secrete histamine, which acts on endothelial cells and allows for the infiltration of neutrophils. Depletion of mast cells before sensitization or the use of mast cell-deficient mice significantly reduces CHS. Neutrophils are also important innate effector cells in CHS. Their depletion before elicitation impairs CHS by impairing the recruitment of CD8⁺ T cells (37). We have shown that neutrophils are already required in the sensitization phase of CHS (Weber F. C. et al. in revision) (38). Their recruitment is promoted by mast cells, and their absence or depletion during sensitization prevents CHS. DC migration, T cell priming and effector T cell recruitment require neutrophils. Neutrophils also infiltrate human skin in allergic contact dermatitis, but their number seems to be low and their functional role is unclear, indicating species-specific differences (39). The interdependence of mast cells and neutrophils and the similar reduction in CHS in their absence nicely illustrates the orchestration of the response: different cell types contribute qualitatively and quantitatively to the immune response, and there is crosstalk between these effector cells. If one cell type is absent, a critical biological threshold may no longer be reached (e.g. strength of the inflammatory response) or a unique but essential effector function may be missing. Future work must identify the contributions of the different cell types, especially unique contributions, and their spatio-temporal interplay.

Orchestration of the molecular innate immune response to contact allergens

The innate immune system detects infection via recognition of so-called pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs), such as bacterial cell wall components or microbial nucleic acids, by pattern recognition receptors (PRRs) located in cellular membranes, the cytosol, or the extracellular space. PRRs belong to receptor families such as the TLRs, NOD-like receptors, RIG-I-like receptors, or C-type lectin receptors. Besides recognition of PAMPs/MAMPs, these PRRs can also recognize endogenous damage-associated molecular patterns (DAMPs) as danger signals that are formed and released following tissue stress and cellular damage, for example in autoimmune or auto-inflammatory diseases and

sterile inflammation (40). Evidence is accumulating that DAMPs and PRRs play a central role in allergic contact dermatitis. As shown for the innate cellular immune response, the molecular innate immune response to contact allergens is well orchestrated, owing to the crosstalk between signalling pathways. The irritant effect of contact allergens has, in part, been elucidated. As in anti-infectious responses, TLRs, reactive oxygen species (ROS), the NLRP3 inflammasome and Syk-mediated signalling processes are essential for allergic contact dermatitis (41, 42). However, the activation of these pathways can occur in the absence of microbial triggers that serve as exogenous adjuvants. This highlights the auto-adjuvantivity of many/most contact allergens that can be direct as shown for nickel, cobalt and palladium, or indirect owing to endogenous triggers such as hyaluronic acid for innate signalling pathways. Apart from the direct activation of human TLR4 by nickel, cobalt and palladium ions, indirect triggering of PRRs and the generation of endogenous danger signals for innate immune system activation have been shown (Fig. 1). Contact allergen application to the skin of mice triggers CHS. The innate inflammatory response involves the rapid generation of ROS. ROS contribute to the oxidative breakdown of the extracellular matrix component hyaluronic acid (HA) and to the p38 mitogen-activated protein kinase (MAPK)-mediated upregulation of hyaluronidase activity and complete degradation of HA to pro-inflammatory fragments that are important for DC activation and migration from the skin to the draining lymph nodes (43). ROS also promote TLR-mediated and NLRP3 inflammasome-mediated inflammation. HA fragments trigger TLR2 and TLR4 activation and the subsequent production of pro-inflammatory cytokines and chemokines and of pro-interleukin (IL)-1 β and pro-IL-18. Moreover, the release of ATP is triggered and sensed by the potassium channel P2X7R, and thereby contributes to the activation of the NLRP3 inflammasome and the generation of mature and secreted IL-1 β and IL-18 (Fig. 1). These mechanisms must be functional in DCs in order to enable sensitization (44–46). In addition, contact allergens can trigger DAP12-mediated activation of the kinase Syk and Card9/Bcl10-mediated NF- κ B activation, as well as Syk-dependent and ROS-dependent but Card9/Bcl10-independent activation of the NLRP3 inflammasome (42). Interference with these innate immune mechanisms by topical antioxidants, hyaluronidase or p38 MAPK inhibitors, the P2X7R antagonist KN-62 or the IL-1 receptor antagonist anakinra can prevent sensitization (45, 46), and, in some cases, elicitation of CHS as shown for antioxidants (46) or

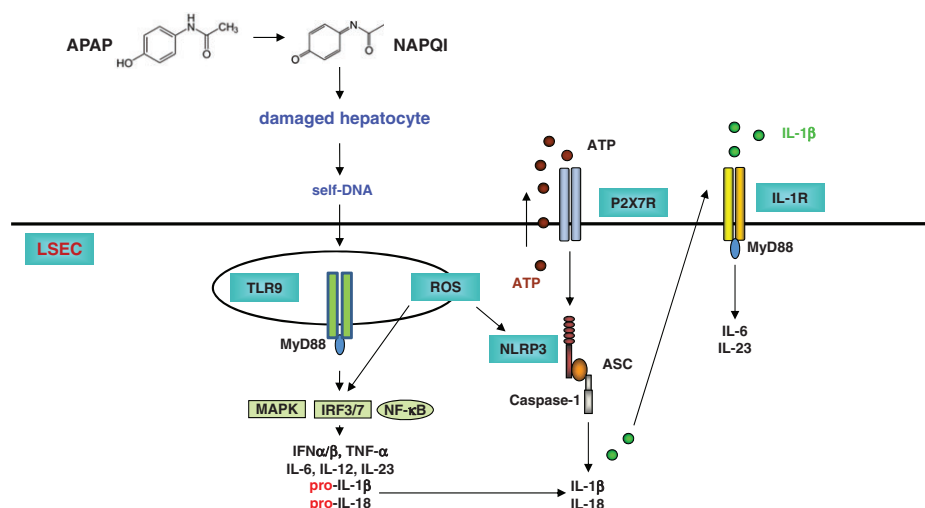


Fig. 2. Indirect triggering of innate signalling pathways by acetaminophen (APAP). APAP is metabolized in the liver to the toxic metabolite *N*-acetyl-*p*-benzo-quinoneimine (NAPQI) which damages liver cells. Self-DNA is released and triggers Toll-like receptor (TLR)9 on liver sinusoidal epithelial cells (LSECs). Reactive oxygen species (ROS) and ATP promote TLR signalling and NLRP3 inflammasome activation via P2X7R. Caspase-1 then produces mature interleukin (IL)-1 β , which acts via the IL-1 receptor IL-1R of target cells. ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; IFN, interferon; IRF, interferon response factor; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor- κ B.

the IL-1 receptor antagonist anakinra (our unpublished data).

Some chemically reactive drugs or reactive drug metabolites can induce immune-mediated adverse drug reactions that are mechanistically similar to contact dermatitis. A strikingly similar innate immune response is found for the drug acetaminophen (APAP, paracetamol), which can cause drug-induced liver injury (Fig. 2). Its toxic and chemically reactive metabolite *N*-acetyl-*p*-benzo-quinoneimine (NAPQI) damages liver cells, which then release self-DNA and other DAMPs (47). Self-DNA can trigger TLR9 on liver sinusoidal epithelial cells and lead to the production of pro-inflammatory cytokines, including pro-IL-1 β and pro-IL-18. ATP is released, P2X7R is triggered, and the NLRP3 inflammasome is activated (48, 49). Moreover, ROS play a role in this response as well (50). In another model of APAP-induced liver inflammation, a role for TLR2 and TLR4 triggering by histones released from damaged hepatocytes was shown (51). Like some contact allergens, NAPQI triggers TRPA1 (52). Owing to these similarities, some adverse drug reactions may be amenable to targeted therapies similar to those suggested for contact dermatitis.

Heterologous innate immune stimulation in allergic contact dermatitis

The central role of the innate immune response in allergic contact dermatitis has important consequences. A

feature of this response is its broad specificity at the level of PRRs. For example, different microbial or endogenous agonists can trigger the same TLR or activate the NLRP3 inflammasome. Moreover, different PRRs activate the same signalling pathways (Fig. 3). This is in striking contrast to the exquisite specificity of the T cell or B cell response. As a consequence, with respect to allergic contact dermatitis or T cell-mediated adverse drug reactions, it is not obligatory for the contact allergen or the drug that induces the specific T cell response to also trigger the innate immune response. Heterologous innate stimuli may amplify, substitute for or perhaps even antagonize the autologous innate stimulus (Fig. 4) (53). This is best illustrated for the TLR system, but also holds for other PRRs. The different TLRs, except for TLR3, signal via the adaptor protein MyD88 to NF- κ B and MAPK. Contact allergens such as nickel and cobalt ions may trigger human TLR4 and induce a contact allergen-specific T cell response. Alternatively, and as shown in mice, which do not have nickel-binding or cobalt-binding sites in their TLR4, a heterologous innate TLR4 stimulus can also be given by LPS, a bacterial PAMP, instead (54). In the case of IL-12 receptor β 2/TLR4-deficient mice, which, like TLR2/TLR4-deficient mice, are resistant to CHS, the missing innate signal can be substituted for by CpG-oligodeoxynucleotides, which trigger TLR9 and mimic a bacterial infection (44). These examples show that heterologous innate immune stimulation can

overcome resistance to contact allergy. Most likely, heterologous innate immune stimuli can also be given by infectious agents via PAMPs and by endogenous factors such as DAMPs. Moreover, the combination of autologous and heterologous innate stimuli may result in amplification of insufficient stimuli over a critical threshold, thereby enabling sensitization. This may be a problem encountered, for example, with cosmetics and household products, which contain a combination of different contact allergens and irritants (55, 56). These augmentation effects need to be studied more systematically in the future, as they may account for increased sensitization rates and allergic contact dermatitis prevalence, because of problematic combinations of chemical ingredients in consumer products and in chemicals used in the workplace (57–59). Understanding this feature of the innate immune system and its consequences may allow interference with innate immune stimulation by the use of antagonistic heterologous innate immune stimuli.

Conclusion and future perspectives

Much progress has been made in our understanding of the pathomechanisms of allergic contact dermatitis. The immune response to contact allergens resembles an anti-infectious immune response resulting from the involvement of PRRs that normally play a role in defence against viruses and bacteria. In allergic contact dermatitis, these PRRs are triggered directly by contact allergens or indirectly via the generation and release of DAMPs. Whereas the resulting T cell response is highly specific for the inducing contact allergen, the innate immune response can be triggered or amplified by heterologous innate immune stimuli given by infectious agents, DAMPs, or other chemicals such as contact allergens or irritants. This has many implications with regard to the interactions of different chemicals and exogenous trigger factors for sensitization and elicitation of allergic contact dermatitis. Many different cell types, including mast cells and neutrophils, interact to orchestrate the cellular innate immune response, and the mechanisms of their activation by contact allergens, their crosstalk and their contribution to the pathogenesis are being explored. Regulation of gene and protein expression by contact allergens is being analysed in primary cells, cell lines, skin models, and skin samples. Epigenetic regulation, including DNA methylome and microRNA analyses, is being studied (60, 61). *In situ* protein modification by contact allergens has been established, and may help to identify target proteins whose modification is of functional relevance for innate immune and stress responses, and for the formation of T cell epitopes (62). Modern

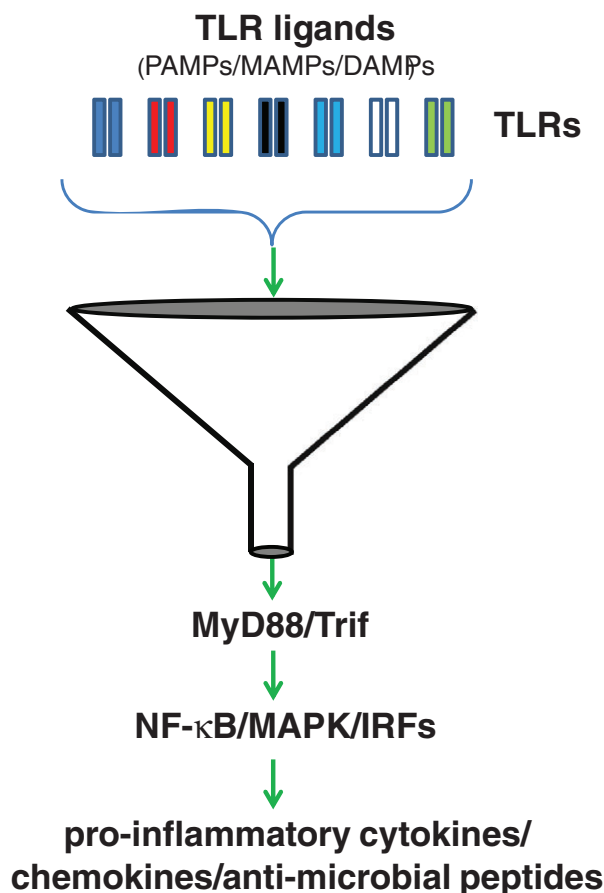


Fig. 3. Activation of the same signalling pathways by different Toll-like receptors (TLRs). As though through a funnel, the signals triggered by recognition of pathogen-associated molecular patterns (PAMPs)/microbe-associated molecular patterns (MAMPs)/damage-associated molecular patterns (DAMPs) by different TLRs are focused on MyD88 and Trif for activation of nuclear factor- κ B (NF- κ B), mitogen-activated protein kinases (MAPKs), and interferon response factors (IRFs). Pro-inflammatory cytokines, chemokines and antimicrobial peptides are produced.

proteomic techniques will allow for the identification of contact allergen interactions with proteins (63).

In the future, global technologies such as genomic and proteomic profiling of contact allergen-regulated gene and protein expression will identify additional relevant signalling pathways that can be explored to refine our molecular understanding of allergic contact dermatitis, and help in the development of drugs for targeted treatments and *in vitro* assays for contact allergen identification. Thorough analysis of the chemistry of contact allergens and also of irritants will hopefully enable correlation of the chemistry with the biological responses elicited by these chemicals, and allow for the synthesis of modified chemicals with reduced sensitization potential

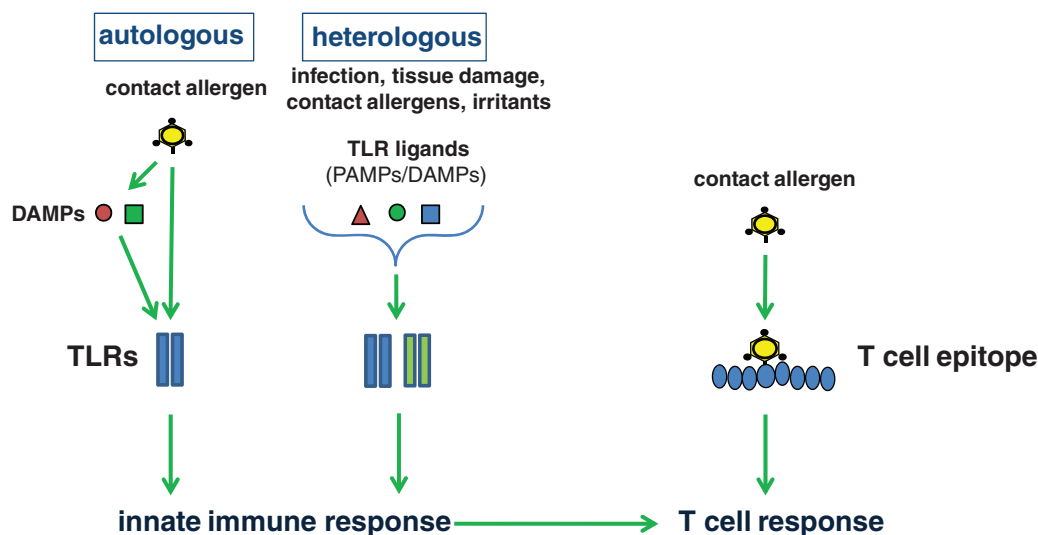


Fig. 4. Heterologous innate immune stimulation in allergic contact dermatitis. Contact allergens can form T cell epitopes and induce a specific T cell response. At the same time, they provide autologous innate immune stimulation directly or indirectly via damage-associated molecular patterns (DAMPs), as exemplified for Toll-like receptors (TLRs). The innate immune response is a prerequisite for the T cell response. Heterologous innate immune stimulation can be provided by infectious agents [pathogen-associated molecular patterns (PAMPs)], tissue damage (DAMPs), or other contact allergens or irritants. The same or other TLRs can be triggered by the heterologous innate stimuli.

(64, 65). As a consequence, chemical class-specific activities such as specific gene expression profiles can be used to compile characteristic gene signatures for the prevention and improved diagnosis of allergic contact dermatitis. Understanding the mechanisms of action of contact allergens and irritants is crucial for prevention, diagnosis, treatment, hazard identification, and risk assessment. Much work lies ahead of us, given the large number of chemicals causing irritant contact dermatitis and allergic contact dermatitis and the small number of experimental contact allergens used in the mouse CHS model. All murine and human data have to be constantly cross-validated and epidemiological, clinical

and (immuno)toxicological data have to be considered to constantly determine and re-evaluate safety thresholds for prevention and reduction of the incidence of allergic contact dermatitis (66–68). The new and exciting concepts in allergic contact dermatitis that evolve from basic research and human studies will eventually result in improved prevention, diagnosis, and treatment strategies.

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