



Immunological mechanisms in allergic contact dermatitis

Stefan F. Martin

Purpose of review

Allergic contact dermatitis is a skin disease resulting from an adverse reaction of the immune system to low-molecular-weight organic chemicals or metal ions. This review summarizes recent findings that highlight new details of the complex orchestration of the cellular and molecular immune response to contact allergens.

Recent findings

Progress has been made in the characterization of the roles of natural killer T (NKT) cells, natural killer cells, mast cells and neutrophils, as well as in the elucidation of signaling pathways triggered by contact allergens. Global technologies begin to reveal gene signatures for contact allergen identification and improved diagnostics.

Summary

Recent progress in contact allergy research has deepened our understanding of the molecular and cellular pathomechanisms, and opens new avenues towards improved diagnostics and treatments, as well as prevention and risk assessment strategies.

Keywords

contact dermatitis, dendritic cell, innate immunity, skin, T cell

INTRODUCTION

Low-molecular-weight organic chemicals and metal ions cause allergic contact dermatitis (ACD), which affects 5–10% of the general population and is one of the most important occupation-related skin diseases. Chemicals that cause ACD must be protein-reactive. By covalent protein modification in the case of organic chemicals or by complex formation with proteins in the case of metal ions, they form T-cell epitopes. The molecular details of T-cell recognition have been elucidated for some contact allergens, and T-cell-based in-vitro assays for the identification of contact allergens are being developed [1,2]. Protein reactivity is also required for the activation of the innate immune response, which is an essential element of ACD [3,4]. Progress is being made in the understanding of the role of different immune cell types and their interplay, as well as in the identification of signaling pathways and their mechanisms of activation by contact allergens. Here, I review a selection of the recent findings that deepen our understanding of the orchestration of the cellular and molecular immune response to contact allergens.

NEW ALLERGENS AND 'HYPOALLERGENIC' CHEMICALS

The most recent example for newly emerging allergens in consumer products is the preservative methylisothiazolinone (MI) [5,6]. MI is used alone or in combination with methylchloroisothiazolinone (MCI) in many products such as cosmetics and paints. The recent 'epidemic' of ACD caused by MI clearly shows the limitations of the in-vivo and in-vitro assays for the identification of contact allergens, of the final product testing, as well as the importance of considering exposure conditions and concentrations used in consumer products and in patch testing. Interesting approaches to reduce

Allergy Research Group, Department of Dermatology, Medical Center, University of Freiburg, Freiburg, Germany

Correspondence to Professor Dr Stefan F. Martin, PhD, Allergy Research Group, Department of Dermatology, Medical Center – University of Freiburg, Hauptstrasse 7, D-79104 Freiburg, Germany. Tel: +49 761 270 67380; fax: +49 761 270 66550; e-mail: stefan.martin@uniklinik-freiburg.de

Curr Opin Allergy Clin Immunol 2015, 15:000–000

DOI:10.1097/ACI.0000000000000142

KEY POINTS

- DETC and NKT cells are important effector/regulatory cells in allergic contact dermatitis.
- Mast cells and neutrophils are essential for the sensitization and elicitation phase of allergic contact dermatitis.
- Contact allergens trigger DAP12/Syk/CARD9 signaling.
- Human Toll-like receptor (TLR)4, Keap1 and TRPA1 are functional targets of contact allergens.
- Genomic profiling of cell lines and skin uncovers chemical and disease-specific gene signatures of contact allergens.

the risk of skin sensitization are the chemical modification of known contact allergens to generate less allergenic molecules. In the case of p-phenylenediamine (PPD), which is a contact allergen contained, for example, in hair dyes, addition of a 2-methoxymethyl side chain reduced its sensitizing potency [7[■]]. A recent study [8[■]] showed that a significantly lower number of PPD-allergic individuals reacted to methyl-PPD as compared with their PPD reactivity. A similar approach resulted in the reduction of the sensitization capacity of epoxy resin monomers [9[■]]. These strategies reveal the importance of the mechanistic understanding of the chemistry and biological effects of contact allergens which aids in the development of in-vitro assays for the identification of contact allergens, novel diagnostic techniques and therapies for contact dermatitis.

DENDRITIC EPIDERMAL T CELLS IN CONTACT HYPERSENSITIVITY

Mouse epidermis harbors a $\gamma\delta$ T cell receptor (TCR)-expressing T-cell population called dendritic epidermal T cells (DETCs) [10]. Recently, they were identified as producers of interleukin (IL)-17, a pro-inflammatory cytokine that is involved in attracting neutrophils [11[■]]. The activation of DETCs by contact allergens seems to involve IL-1 β produced by keratinocytes and up-regulation of an as yet undefined ligand for the $\gamma\delta$ TCR [12]. As shown previously [13], this study also confirmed that blocking IL-1 β by the IL-1 receptor antagonist Anakinra inhibited contact hypersensitivity (CHS) underlining the central role of this cytokine. Thus, both dermal $\gamma\delta$ T cells [14] and epidermal DETCs are a source of IL-17 in skin inflammation. In humans, dermal $\gamma\delta$ T cells with pro-inflammatory activities in psoriasis have been identified [15].

NATURAL KILLER AND NKT CELLS IN CONTACT HYPERSENSITIVITY

Natural killer (NK) cells are found in murine and human ACD skin. They are pro-inflammatory cells in human ACD due to their rapid interferon (IFN)- γ production, which amplifies the T-cell response [16]. A subset of liver-derived CD49a+DX5– NK cells was now shown to mediate CHS to oxazolone in mice [17[■]]. Transfer of this subset isolated from the liver of oxazolone-sensitized T/B-cell-deficient recombination activation gene (RAG) knockout mice allowed induction of CHS in recipient mice following oxazolone application to the ear skin. Another study demonstrated a role for DX5+ liver NK cells in mediating antigen-specific CHS in severe combined immunodeficiency mice and in RAG knockout mice. This response was dependent on IL-12, IFN- γ and IL-1 α receptor expressed by the transferred CHS-inducing cells [18]. These studies [19,20] underline the memory-like properties of NK cells and demonstrate that they can induce an antigen-specific CHS-like skin disease as previously shown. The origin of the NK cells contributing to T-cell-mediated CHS is not clear, but it is likely that liver-derived NK cells also play a role here. However, in human ACD, no antigen-specificity of the NK cells was detected [16].

NKT cells recognize lipid antigens on major histocompatibility complex I-like CD1d molecules. They can be effector cells as well as immunoregulatory cells [21,22]. In mice, CD1d-restricted invariant NKT (iNKT) cells expressing an invariant $\alpha\beta$ TCR play a role in CHS [23]. They were recruited to the skin in 2,4-dinitrofluorobenzene (DNFB)-induced CHS in C57BL/6 mice, required activation by CD11c+CD1d+ dendritic cells and CD8+ T-cell-derived IFN- γ , and suppressed CHS by their expression of IL-4 and IL-13. Mice lacking iNKT cells had exacerbated CHS responses. This study identified iNKT cells as important regulatory cells which, independently of Foxp3+ regulatory T cells, but in concert with them, limit CHS. In another study [24[■]], it was shown that NKT cells can also be effector cells in the CHS response to DNFB in Balb/c mice. Mice lacking NKT cells had significantly reduced CHS responses due to impaired dendritic cell maturation, lymph node migration and decreased T-cell proliferation and cytokine production. In conclusion, these data demonstrate strain-dependent roles of NKT cells and highlight the important communication between the liver and the skin in CHS. Part of this communication may be the rapid accumulation of stimulatory lipids in the liver that rapidly and efficiently activate CD1d-restricted NKT cells. It was speculated that these lipids may be derived from the skin in CHS [25].

MAST CELLS AND NEUTROPHILS IN CONTACT HYPERSENSITIVITY

Mast cells are found in large numbers in the dermis [26,27]. They are important for the recruitment of neutrophils in the elicitation phase of CHS [28]. In mast cell-deficient mice and mice with inducible mast cell ablation, the early ear swelling response, which occurs a few hours after ear challenge of DNFB-sensitized mice, was completely absent and CHS was significantly reduced. This response was dependent on mast cell-derived histamine [29]. We have now shown that neutrophils are already required in the sensitization phase of CHS [30]. In their absence, due to genetic deficiency or antibody depletion, dendritic cell migration to and T-cell priming in the skin draining lymph nodes were significantly impaired. Also, here the recruitment of neutrophils to the skin was mast cell-dependent. In the elicitation phase, neutrophil deficiency abrogated the recruitment of adoptively transferred effector T cells. These studies indicate rapid mast cell activation in the skin by contact allergens and a subsequent mast cell-dependent neutrophil recruitment that is required for the lymph node migration of skin dendritic cells in the sensitization phase as well as for the recruitment of effector T cells in the elicitation phase.

TH9 CELLS IN ALLERGIC CONTACT DERMATITIS

One of the most recent T-cell subsets identified in ACD is the T helper (Th)9 cell. These T cells are skin-homing or skin-resident cells and produce mostly IL-9 [31]. Th9 cells were found in lesional skin in psoriasis. In that study [32], they had pro-inflammatory activity since blockade of IL-9 reduced IFN- γ , IL-13 and IL-17 production by cutaneous lymphocyte antigen+ Th cells *in vitro*. Another study [33^{***}] identified IL-9 and PU.1+CD4+ T cells, consistent with Th9 cells, in patch test biopsies from patients with ACD to different allergens. Peripheral blood T cells from patients produced IL-9 in response to nickel. Blocking IL-9 reduced IL-4 and increased IFN- γ levels *in vitro*. IL-9-deficient mice showed increased CHS to DNFB and elevated levels of IFN- γ in ear skin. Interestingly, IL-9-deficient mice had reduced irritant contact dermatitis (ICD) in response to croton oil [33^{***}]. This study identified Th9 cells as new players in ACD and demonstrated a regulatory role due to the action of IL-9 on the Th1/Th2 cytokine expression with a direct regulation of IFN- γ expression. Thus, IL-9 can have pro-inflammatory or regulatory activities. This may depend on the context in which this cytokine is produced. Future work will have to solve the current controversies.

T-CELL ACTIVATION IN THE SKIN

The elicitation of CHS depends on the activation of contact allergen-specific effector/memory T cells in the skin. In CHS to DNFB inducible clusters of dermal dendritic cells are formed in the perivascular regions of the skin. These clusters support antigen-specific proliferation and IFN- γ production of CD8+ T cells in an leukocyte function associated molecule-1/intercellular adhesion molecule-1-dependent manner [34^{***}]. In that study, CD4+ T cells showed only a low proliferation and Langerhans cells were dispensable for CD8+ T-cell activation. The formation of dendritic cell clusters was dependent on mast cells and on IL-1 α which may derive from keratinocytes. IL-1 α -induced CXCL2 in IL-1R-expressing M2 macrophages was required for dendritic cell cluster formation. The clusters were maintained by antigen-specific T cells which accumulated to proliferate and produce IFN- γ *in situ*.

Chronic ACD is suspected to promote skin cancer development as reported for an invasive squamous cell carcinoma associated with chronic ACD to an orthopedic implant [35^{***}]. In a mouse model of chronic ACD and application of a tumor promoter it was shown that epidermal hyperplasia and inflammation, as well as the development of tumors, were dependent on GATA3+ Th2 type T cells. The levels of IL-4 and IL-6, and the number of M2 macrophages, mast cells and blood vessels were markedly increased. These observations clearly show that chronic ACD creates an inflammatory milieu that can promote tumorigenesis.

T-CELL TOLERANCE

Since ACD is mediated by T cells, immunotherapy approaches that induce T-cell tolerance are studied. A recent study has shown that tolerization of mice by injection of isogenic erythrocytes modified with the contact allergens 2,4,6-trinitrochlorobenzene (TNCB) or oxazolone-induced CD8+ suppressor T cells which release exosome-like nanovesicles. These may act on the T cells or the antigen-presenting cells [36^{***}]. The nanovesicles contained microRNA-150 (miR-150), and they were coated by contact allergen-specific antibodies or light chains which may derive from B-1 B cells. Systemic application of nanovesicles suppressed CHS even when injected in the elicitation phase. Nanovesicles from immunoglobulin-deficient joining heavy or microRNA (miR)-150-deficient mice treated with contact allergen-modified erythrocytes were not suppressive. However, their activity was restored by adding back contact allergen-specific antibodies or miR150, respectively. A role for Foxp3+ regulatory T cells (Treg) was ruled out by showing functional

tolerance induction in depletion of regulatory T cells mice which lack Treg by depletion with diphtheria toxin.

INNATE IMMUNE RESPONSES TO CONTACT ALLERGENS

Contact allergens such as TNCB fail to fully activate dendritic cells *in vitro* due to a lack of tissue-derived signals such as TLR2/4-activating fragments of hyaluronic acid [3,37]. The important role of hyaluronic acid fragments as contact allergen-induced damage associated molecular patterns (DAMPs) in CHS was now underlined by a study [38[■]] in mice overexpressing human hyaluronidase 1 in keratinocytes. Constitutive overexpression resulted in inhibition of CHS due to the depletion of epidermal and dermal dendritic cells, most likely a consequence of ongoing inflammation due to overproduction of hyaluronic acid fragments. Inducible overexpression at the time of sensitization of mice with the contact allergen DNFB resulted in exacerbated CHS due to increased production of pro-inflammatory hyaluronic acid fragments. All effects were abrogated in mice lacking TLR4. These results are in line with our previous studies in which we observed enhanced CHS after injection of low doses and abrogation of CHS after injection of high doses of active hyaluronidase into the skin of C57BL/6 mice [37]. The activation of IL-18 production in the human keratinocyte cell line NCTC2544 by contact allergens *in vitro* was recently shown to depend on hyaluronic acid breakdown [39]. Contact allergens up-regulated hyaluronidases and metabolism of hyaluronic acid. Blocking this breakdown prevented IL-18 production as did antioxidants. These data illustrate the contact allergen-dependent reactive oxygen species (ROS) and hyaluronidase-mediated hyaluronic acid breakdown, and the immune stimulatory role of the hyaluronic acid fragments in skin cells and CHS [37,38[■]].

The nuclear high mobility group protein B1 (HMGB1) is another DAMP that can act via TLR4. Galbiati *et al.* [40] have now shown that HMGB1 is released from NCTC2544 keratinocytes upon stimulation with different contact sensitizers and participates in the triggering of IL-18 production. These findings underline the important role of endogenous danger signals and DAMPs for activation of innate immune responses by contact allergens.

ROS regulate many aspects of the immune response to contact allergens. A recent study [41[■]] demonstrated a role for ROS in the opening of pannexin 1 hemichannels involved in the release of ATP from HaCaT keratinocytes. Extracellular ATP contributes to the activation of the NOD-like

receptor protein 3 (NLRP3) inflammasome via the purinergic receptor P2X7R in CHS [13]. Interestingly, ROS production, ATP release and cell death as measured by lactate dehydrogenase release and propidium iodide positive cells triggered by the organic contact allergens DNCB, diphenylcyclopropenone (DPCP) and 4-nitrobenzylbromide (4-NBB) were sensitive to N-acetylcysteine (NAC) antioxidant treatment. NiCl₂ and the irritants sodium dodecylsulfate (SDS) and lactic acid induced cell death which was not prevented by NAC. These results indicate that thiol reactivity of nonmetal contact allergens is crucial for the ROS-mediated ATP release involving the opening of pannexin 1 hemichannels. Treatment of mice with a pannexin1 inhibitor before sensitization reduced the CHS response to DNCB.

Nickel and cobalt ions can directly trigger TLR4 dimerization and nuclear factor kappa B (NF- κ B) activation in ACD [42]. Now it has been shown that nickel can also activate the NLRP3 inflammasome resulting in the production of mature IL-1 β [43[■]]. Also, in this study [44], an important role for ROS was demonstrated. The TLR4 of mice lacks the metal-binding sites. Therefore, induction of CHS to nickel in mice requires the injection of nickel and an adjuvant such as lipopolysaccharide (LPS). A TLR4-independent, but MyD88 and IL-1 β -dependent, CHS model for nickel was recently reported [45[■]]. Here, nickel in petrolatum was repeatedly applied epicutaneously to the skin of mice. This resulted in CHS which was absent in MyD88-deficient mice or mice treated with the IL-1 receptor antagonist Anakinra. Mice lacking TLR4 still mounted CHS with this protocol. Nickel stayed in the epidermis for more than 20 h in contrast to a more rapid clearance and distribution in epidermis and dermis upon skin injection. This study implies that prolonged exposure to nickel overcomes the need for TLR4 stimulation. Alternative inflammatory stimuli may be given as a consequence of nickel-induced tissue damage and stress via DAMPs and other TLRs triggering MyD88-dependent signaling and inflammasome activation [46].

A direct *in-vitro* activation of dendritic cells by 2,4,6-trinitrobenzene sulfonic acid (TNBS) has now been shown by Yasukawa *et al.* [47[■]]. They identified a DAP12/Syk/Card9-dependent signaling cascade that was triggered by contact allergens and resulted in NF- κ B activation. Furthermore, activation of the NLRP3 inflammasome and production of the essential IL-1 β were dependent on Syk-mediated, but CARD9-independent, control of ROS production. CARD9-deficient mice and mice with a dendritic cell-specific deficiency of DAP12, Syk or CARD9 were resistant to CHS induced by

TNCB. Moreover, dendritic cells lacking CARD9 failed to sensitize wild-type mice for CHS. It will be of interest to identify whether an upstream receptor is directly activated by TNBS modification to transmit a signal via the DAP12 adaptor protein.

Mizukami *et al.* [48] demonstrated a role for the mitogen activated protein (MAP) kinase Apoptosis signal-regulating kinase 1 (ASK1) in CHS. ASK1 mediates the activation of p38 MAP kinase. Mice lacking ASK1 had strongly impaired CHS responses to DNFB or fluorescein isothiocyanate, and lymph node cells from sensitized ASK1-deficient mice failed to transfer CHS to recipient wild-type mice, whereas sensitized wild-type cells induced CHS in the ASK1-deficient recipient. T-cell proliferation *in vitro* was normal as was IFN- γ production. However, IL-17 production was absent in CD4⁺ T cells after sensitization. Pharmacological ASK1 inhibition prior to elicitation of CHS strongly attenuated the response. It remains to be shown which T-cell subset is functionally impaired in CHS and whether the reduction of IL-17 is responsible for the phenotype. The results of ASK1 inhibition are in line with studies in the CHS model, demonstrating prevention of sensitization to TNCB by blocking p38MAPK [37]. This pathway is involved in the up-regulation of hyaluronidase activity which is an important element of the innate inflammatory response in CHS.

PROTEIN TARGETS FOR FUNCTIONAL MODIFICATION BY CONTACT ALLERGENS

Up to now, only three target proteins for functional contact allergen modification have been identified. Human TLR4, the receptor for LPS from the cell walls of gram-negative bacteria, directly binds nickel and cobalt ions. The complex formation of these metal ions with histidine residues induces TLR4 dimerization and activation of the signaling cascade, leading to the activation of NF- κ B [42]. Palladium was now shown to also act via TLR4 [49].

The cytosolic sensor for oxidative and electrophilic stress, Keap1, is modified by organic chemical allergens that bind covalently to cysteine residues. That triggers the antioxidant phase 2 response via activation of the transcription factor Nrf2. Failure of the Keap1/Nrf2 pathway in Nrf2-deficient mice results in facilitated sensitization and increased CHS to lower doses of contact allergens and in induction of CHS to weak allergens that normally fail to induce CHS [50]. These data clearly underline the importance of oxidative and electrophilic stress in determining thresholds for inflammatory responses.

The transient receptor potential ankyrin (TRPA1) channel, a cation channel of the plasma

membrane of sensory neurons, and also of keratinocytes and fibroblasts, plays a role in itching and pain. TRPA1 triggering also has pro-inflammatory effects. DNFB and cinnamal can activate TRPA1 [51,52[¶]]. Mice lacking TRPA1 or treated with pharmacological TRPA1 inhibitors had reduced CHS responses to oxazolone [53]. Several pro-inflammatory cytokines, peptide mediators and pruritogens were reduced in the skin of these mice. Levels of endogenous TRPA1 antagonists such as 4-hydroxy-2-nonenal (HNE) were elevated. HNE is a product of membrane lipid oxidation by ROS, which are important mediators in CHS.

GLOBAL TECHNOLOGIES: TOWARDS ALLERGEN AND DISEASE-SPECIFIC GENE SIGNATURES

Global gene expression analysis using array technology is a promising strategy for the identification of gene signatures that characterize contact allergens and irritants, as well as subtypes of eczema such as ACD or atopic eczema. Stimulation of human MUTZ-3 dendritic cell progenitor cells with different contact allergens identified a predictive gene signature for the identification of contact allergens [54,55]. Grouping of contact allergens according to their reaction mechanisms identified 33 signaling pathways involved in sensitization. A correlation between sensitizing potential and reactivity groups and the number of signaling pathways engaged was revealed that may allow potency assessment [56[¶]].

Gene expression analysis of human skin performed with biopsies from patch tests of patients with ACD to nickel, fragrance or rubber revealed an allergen-specific regulation of gene expression with an overlap of 149 genes common to all three allergen groups [57[¶]]. Whereas nickel induced a Th1-dominated gene profile, fragrance and rubber induced a Th2-dominated polarization. Th9-related genes were similarly regulated by all three allergen groups. In another study [58[¶]], in patients with both psoriasis and nonatopic or atopic eczema, eczema subtypes and psoriasis could be differentiated by a two-gene classifier using C-C motif ligand 27 and nitric oxide species. Expression profiling of positive nickel patch test biopsies from a subgroup of these patients with nickel sensitization revealed characteristic changes in the expression of genes related to skin barrier (down-regulation of late differentiation markers of the late cornified envelope (LCE)2 and LCE3 families, up-regulation of extracellular matrix proteins hyaluronic acid synthetase 3 and epithelial stromal interaction 1) and acute inflammation (up-regulation of IL-1 β , AIM2 inflammasome, the Th1-associated chemokines

CXCL8, 9, 10, 11) in comparison to noninvolved skin.

CONCLUSION

The recent progress in our understanding of the pathogenesis of contact dermatitis has provided important new insights into the orchestration of the cellular and molecular immune response to chemical allergens. Along with genomic and proteomic profiling studies, important pathogenesis pathways will become amenable for therapeutic targeting and for use in in-vitro assay development for contact allergen identification. Disease and contact allergen/irritant specific gene signatures will provide invaluable information for the development of modern diagnostics and of targeted, mechanism-based therapies.

Acknowledgements

I am grateful to Dr Philipp Esser for carefully reading the manuscript.

Financial support and sponsorship

None.

Conflicts of interest

The author declares no conflict of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Richter A, Schmucker SS, Esser PR, *et al.* Human T cell priming assay (hTCPA) for the identification of contact allergens based on naive T cells and DC-IFN-gamma and TNF-alpha readout. *Toxicol In Vitro* 2013; 27:1180–1185.
 2. Vocanson M, Achachi A, Mutez V, *et al.* Human T cell priming assay: depletion of peripheral blood lymphocytes in CD25(+) cells improves the in vitro detection of weak allergen-specific T cells. *Exs* 2014; 104:89–100.
 3. Martin SF, Esser PR, Weber FC, *et al.* Mechanisms of chemical-induced innate immunity in allergic contact dermatitis. *Allergy* 2011; 66:1152–1163.
 4. Martin SF. New concepts in cutaneous allergy. *Contact Dermatitis* 2015; 72:2–10.
 5. Lundov MD, Krongaard T, Menne TL, Johansen JD. Methylisothiazolinone contact allergy: a review. *Br J Dermatol* 2011; 165:1178–1182.
 6. Mahler V, Geier J, Schnuch A. Current trends in patch testing: new data from the German Contact Dermatitis Research Group (DKG) and the Information Network of Departments of Dermatology (IVDK). *J Dtsch Dermatol Ges* 2014; 12:583–592.
 7. Goebel C, Troutman J, Hennen J, *et al.* Introduction of a methoxymethyl side chain into p-phenylenediamine attenuates its sensitizing potency and reduces the risk of allergy induction. *Toxicol Appl Pharmacol* 2014; 274:480–487.
- This study demonstrates the reduction of allergenicity by chemical modification of PPD.
8. Blomeke B, Pot LM, Coenraads PJ, *et al.* Cross-elicitation responses to 2-methoxymethyl-p-phenylenediamine under hair dye use conditions in p-phenylenediamine-allergic individuals. *Br J Dermatol* 2014. [Epub ahead of print]
- This study evaluates human data for the sensitizing capacity of chemically modified PPD.
9. O'Boyle NM, Niklasson IB, Tehrani-Bagha AR, *et al.* Epoxy resin monomers with reduced skin sensitizing potency. *Chem Res Toxicol* 2014; 27:1002–1010.
- The authors show how chemical modification of epoxy resin monomers can reduce allergenicity.

10. Macleod AS, Havran WL. Functions of skin-resident gammadelta T cells. *Cell Mol Life Sci* 2011; 68:2399–2408.
 11. Nielsen MM, Lovato P, MacLeod AS, *et al.* IL-1beta-dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Immunol* 2014; 192:2975–2983.
- This study identifies DETC as an important source of IL-17 in contact hypersensitivity.
12. Komori HK, Witherden DA, Kelly R, *et al.* Cutting edge: dendritic epidermal gammadelta T cell ligands are rapidly and locally expressed by keratinocytes following cutaneous wounding. *J Immunol* 2012; 188:2972–2976.
 13. Weber FC, Esser PR, Muller T, *et al.* Lack of the purinergic receptor P2X(7) results in resistance to contact hypersensitivity. *J Exp Med* 2010; 207:2609–2619.
 14. Cai Y, Shen X, Ding C, *et al.* Pivotal role of dermal IL-17-producing gamma-delta T cells in skin inflammation. *Immunity* 2011; 35:596–610.
 15. Laggner U, Di Meglio P, Perera GK, *et al.* Identification of a novel pro-inflammatory human skin-homing Vgamma9Vdelta2 T cell subset with a potential role in psoriasis. *J Immunol* 2011; 187:2783–2793.
 16. Carbone T, Nasorri F, Pennino D, *et al.* CD56highCD16-CD62L- NK cells accumulate in allergic contact dermatitis and contribute to the expression of allergic responses. *J Immunol* 2010; 184:1102–1110.
 17. Peng H, Jiang X, Chen Y, *et al.* Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. *J Clin Invest* 2013; 123:1444–1456.
- This study underlines the function of NK cells as effector cells with adaptive properties in CHS.
18. Majewska-Szczepanik M, Paust S, von Andrian UH, *et al.* Natural killer cell-mediated contact sensitivity develops rapidly and depends on interferon-alpha, interferon-gamma and interleukin-12. *Immunology* 2013; 140:98–110.
 19. Paust S, Gill HS, Wang BZ, *et al.* Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol* 2010; 11:1127–1135.
 20. Rouzaire P, Luci C, Blasco E, *et al.* Natural killer cells and T cells induce different types of skin reactions during recall responses to haptens. *Eur J Immunol* 2011; 42:80–88.
 21. Balato A, Unutmaz D, Gaspari AA. Natural killer T cells: an unconventional T-cell subset with diverse effector and regulatory functions. *J Invest Dermatol* 2009; 129:1628–1642.
 22. McKee SJ, Mattarollo SR, Leggatt GR. Immunosuppressive roles of natural killer T (NKT) cells in the skin. *J Leukoc Biol* 2014; 96:49–54.
 23. Goubier A, Vocanson M, Macari C, *et al.* Invariant NKT cells suppress CD8(+) T-cell-mediated allergic contact dermatitis independently of regulatory CD4(+) T cells. *J Invest Dermatol* 2013; 133:980–987.
 24. Shimizuhiro C, Otsuka A, Honda T, *et al.* Natural killer T cells are essential for the development of contact hypersensitivity in BALB/c mice. *J Invest Dermatol* 2014; 134:2709–2718.
- The authors demonstrate a strain-specific difference of the role of NKT cells in CHS.
25. Dey N, Szczepanik M, Lau K, *et al.* Stimulatory lipids accumulate in the mouse liver within 30 min of contact sensitization to facilitate the activation of naive iNKT cells in a CD1d-dependent fashion. *Scand J Immunol* 2011; 74:52–61.
 26. Tong PL, Roediger B, Kolesnikoff N, *et al.* The skin immune atlas: three-dimensional analysis of cutaneous leukocyte subsets by multiphoton microscopy. *J Invest Dermatol* 2015; 135:84–93.
 27. Wang XN, McGovern N, Gunawan M, *et al.* A three-dimensional atlas of human dermal leukocytes, lymphatics, and blood vessels. *J Invest Dermatol* 2014; 134:965–974.
 28. Biedermann T, Kneilling M, Mailhammer R, *et al.* Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. *J Exp Med* 2000; 192:1441–1452.
 29. Dudeck A, Dudeck J, Scholten J, *et al.* Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. *Immunity* 2011; 34:973–984.
 30. Weber FC, Nemeth T, Csepregi J, *et al.* Neutrophils are required for both the sensitization and elicitation phase of contact hypersensitivity. *J Exp Med* 2015. [Epub ahead of print]
 31. Eyerich S, Zielinski CE. Defining Th-cell subsets in a classical and tissue-specific manner: examples from the skin. *Eur J Immunol* 2014; 44:3475–3483.
 32. Schlappbach C, Gehad A, Yang C, *et al.* Human TH9 cells are skin-tropic and have autocrine and paracrine proinflammatory capacity. *Sci Transl Med* 2014; 6:219a8.
 33. Liu J, Harberts E, Tammara A, *et al.* IL-9 regulates allergen-specific Th1 responses in allergic contact dermatitis. *J Invest Dermatol* 2014; 134:1903–1911.
- This study establishes a role for Th9 cells as regulators in human allergic contact dermatitis and in the mouse CHS model.
34. Natsuaki Y, Egawa G, Nakamizo S, *et al.* Perivascular leukocyte clusters are essential for efficient activation of effector T cells in the skin. *Nat Immunol* 2014; 15:1064–1069.
- The role of in-situ formation of DC/T-cell clusters in the skin and its mechanistic basis is shown here.

35. Demehri S, Cunningham TJ, Hurst EA, *et al.* Chronic allergic contact dermatitis promotes skin cancer. *J Clin Invest* 2014; 124:5037–5041.
 This study analyzes the impact of chronic inflammation due to ACD on skin cancer.
36. Bryniarski K, Ptak W, Jayakumar A, *et al.* Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. *J Allergy Clin Immunol* 2013; 132:170–181.
 Here, a role for immune regulation of T cells by microRNAs from exosomes is uncovered.
37. Esser PR, Wolfe U, Durr C, *et al.* Contact sensitizers induce skin inflammation via ROS production and hyaluronic acid degradation. *PLoS One* 2012; 7:e41340.
38. Muto J, Morioka Y, Yamasaki K, *et al.* Hyaluronan digestion controls DC migration from the skin. *J Clin Invest* 2014; 124:1309–1319.
 This study elegantly demonstrates the role of the endogenous danger signal function of hyaluronic acid in the inflammatory response to contact allergens.
39. Nikitovic D, Berdiaki A, Galbiati V, *et al.* Hyaluronan regulates chemical allergen-induced IL-18 production in human keratinocytes. *Toxicol Lett* 2014; 232:89–97.
40. Galbiati V, Papale A, Galli CL, *et al.* Role of ROS and HMGB1 in contact allergen-induced IL-18 production in human keratinocytes. *Eur J Immunol* 2014; 44:3475–3483.
41. Onami K, Kimura Y, Ito Y, *et al.* Nonmetal haptens induce ATP release from keratinocytes through opening of pannexin hemichannels by reactive oxygen species. *J Invest Dermatol* 2014; 134:1951–1960.
 This study shows the link between ROS production, pannexin hemichannels and ATP release in the danger signaling triggered by contact allergens.
42. Raghavan B, Martin SF, Esser PR, *et al.* Metal allergens nickel and cobalt facilitate TLR4 homodimerization independently of MD2. *EMBO Rep* 2012; 13:1109–1115.
43. Li X, Zhong F. Nickel induces interleukin-1 beta secretion via the NLRP3-ASC-caspase-1 pathway. *Inflammation* 2014; 37:457–466.
 This study identifies the NLRP3 inflammasome as an important element in the innate immune response to nickel.
44. Schmidt M, Raghavan B, Muller V, *et al.* Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. *Nat Immunol* 2010; 11:814–819.
45. Vennegaard MT, Dyring-Andersen B, Skov L, *et al.* Epicutaneous exposure to nickel induces nickel allergy in mice via a MyD88-dependent and interleukin-1-dependent pathway. *Contact Dermatitis* 2014; 71:224–232.
 This study provides a protocol for TLR4 independent CHS induction in mice.
46. Martin SF. Adaptation in the innate immune system and heterologous innate immunity. *Cell Mol Life Sci* 2014; 71:4115–4130.
47. Yasukawa S, Miyazaki Y, Yoshii C, *et al.* An ITAM-Syk-CARD9 signalling axis triggers contact hypersensitivity by stimulating IL-1 production in dendritic cells. *Nat Commun* 2014; 5:3755.
 This study identifies a signaling cascade that is triggered by contact allergens *in vitro* and activates dendritic cells.
48. Mizukami J, Sato T, Camps M, *et al.* ASK1 promotes the contact hypersensitivity response through IL-17 production. *Sci Rep* 2014; 4:4714.
49. Rachmawati D, Bontkes HJ, Verstege MI, *et al.* Transition metal sensing by Toll-like receptor-4: next to nickel, cobalt and palladium are potent human dendritic cell stimulators. *Contact Dermatitis* 2013; 68:331–338.
50. El Ali Z, Gerbeix C, Hemon P, *et al.* Allergic skin inflammation induced by chemical sensitizers is controlled by the transcription factor Nrf2. *Toxicol Sci* 2013; 134:39–48.
51. Silva CR, Oliveira SM, Rossato MF, *et al.* The involvement of TRPA1 channel activation in the inflammatory response evoked by topical application of cinnamaldehyde to mice. *Life Sci* 2011; 88:1077–1087.
52. Saarnilehto M, Chapman H, Savinko T, *et al.* Contact sensitizer 2,4-dinitrochlorobenzene is a highly potent human TRPA1 agonist. *Allergy* 2014; 69:1424–1427.
 Together with other studies, this study underlines the agonist properties of contact allergens for TRPA1.
53. Liu B, Escalera J, Balakrishna S, *et al.* TRPA1 controls inflammation and pruritogen responses in allergic contact dermatitis. *FASEB J* 2013; 27:3549–3563.
54. Johansson H, Lindstedt M, Albrekt AS, Borrebaeck CA. A genomic biomarker signature can predict skin sensitizers using a cell-based *in vitro* alternative to animal tests. *BMC Genomics* 2011; 12:399.
55. Johansson H, Albrekt AS, Borrebaeck CA, Lindstedt M. The GARD assay for assessment of chemical skin sensitizers. *Toxicol In Vitro* 2013; 27:1163–1169.
56. Albrekt AS, Johansson H, Borje A, *et al.* Skin sensitizers differentially regulate signaling pathways in MUTZ-3 cells in relation to their individual potency. *BMC Pharmacol Toxicol* 2014; 15:5.
 This genomic profiling study suggests that the broadness of the spectrum of signaling pathways depends on the potency of a contact allergen.
57. Dhirga N, Shemer A, Correa da Rosa J, *et al.* Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. *J Allergy Clin Immunol* 2014; 134:362–372.
 This study identifies gene expression signatures for different contact allergens.
58. Quaranta M, Knapp B, Garzorz N, *et al.* Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. *Sci Transl Med* 2014; 6:244ra90.
 The authors provide intra-individual differences in the gene expression profiles for psoriasis and eczema.