

Early immune events in the induction of allergic contact dermatitis

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Abstract | The skin is a barrier site that is exposed to a wide variety of potential pathogens. As in other organs, pathogens that invade the skin are recognized by pattern-recognition receptors (PRRs). Recently, it has been recognized that PRRs are also engaged by chemical contact allergens and, in susceptible individuals, this elicits an inappropriate immune response that results in allergic contact dermatitis. In this Review, we focus on how contact allergens promote inflammation by activating the innate immune system. We also examine how innate immune cells in the skin, including mast cells and dendritic cells, cooperate with each other and with T cells and keratinocytes to initiate and drive early responses to contact allergens.

Delayed-type hypersensitivity (DTH). A T cell-mediated immune response marked by monocyte and/or macrophage infiltration and activation. DTH skin tests have classically been used for the diagnosis of infection with intracellular pathogens such as *Mycobacterium tuberculosis* and as a measure of the vigour of the cellular immune system. Classical DTH responses to intracellular pathogens are thought to depend on CD4⁺ T cells that produce T helper 1-type cytokines, such as interferon- γ .

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Allergic contact dermatitis is a common skin disease that is caused by type IV delayed-type hypersensitivity responses to antigens that come into contact with the skin¹ (BOX 1). Allergic contact dermatitis is a major cause of occupational skin disease, and it accounts for approximately 20% of all work-related health complaints. It results in an estimated 4 million lost work days and has an associated cost of almost US\$400 million per year in the United States alone^{2–4}. One of the clinically most important categories of contact allergens is small organic molecules that are chemically reactive (chemical sensitizers). They bind to self proteins to generate immunogenic neo-antigens through a process termed haptenization. Contact allergens are common in cosmetics, personal care products and jewellery, as well as in the workplace, regardless of whether it is in an industrial, health care or office setting. Examples of common allergens are inorganic chemicals, such as nickel, and organic chemicals, such as those found in fragrances and dyes.

There is a series of fundamental events that lead to immune recognition of the hapten–self complex (BOX 2). The first is percutaneous penetration through the stratum corneum barrier, which is the water-impermeable outer layer of the skin. Compounds must be less than 500 daltons for efficient penetration⁵. This step allows the chemical sensitizer to come into contact with and haptenize self proteins in the skin. Haptens or haptenated self proteins are recognized by innate immune mechanisms in the skin, and this leads to the induction of several pro-inflammatory mediators, including interleukin-1 β (IL-1 β). As a result,

skin-resident dendritic cells (DCs) become activated. These DCs — which may have been directly haptenated or could have acquired haptenated proteins from their surroundings — migrate to skin-draining lymph nodes, where they present peptides from haptenated proteins to activate memory and naive T cells. In the final step, hapten-induced inflammation recruits activated effector T cells back to the initial site of antigen encounter in the skin (BOX 3). The effector T cells release pro-inflammatory cytokines, such as interferon- γ (IFN γ), and promote the killing of haptenized cells, resulting in the development of the classic inflammatory rash that is seen in patients with allergic contact dermatitis^{5–8}.

In this Review, we first examine how the hapten–self complex and other contact allergens are recognized by the innate immune system. We then focus on each of the skin-resident immune cells that contribute to the allergen-induced inflammatory cascade and describe how they ultimately lead to the generation of the adaptive immune response that drives allergic contact dermatitis.

Hapten–self complex formation

The landmark article of Landsteiner and Jacobs published in 1936 on the sensitizing potential of 2,4-dinitrohalobenzene derivatives led to the hypothesis that the ability of a chemical to react covalently with a ‘carrier protein’ is a major determinant factor in its ability to act as a skin sensitizer^{9–11}. Indeed, the formation of the hapten–carrier complex, in which the xenobiotic chemical attaches itself to one or more host molecules, generates a neo-antigen that is eventually recognized by the

Hapten

A low-molecular-weight xenobiotic chemical that penetrates into the skin and chemically reacts with self proteins (either through covalent modification or the formation of a chelation complex). It is this hapten–self complex that is recognized by the immune system as a neo-antigen and is recognized by allergen-specific effector T cells in allergic contact dermatitis.

Box 1 | Allergic contact dermatitis versus contact hypersensitivity

Allergic contact dermatitis is a clinical term used to describe an inflammatory reaction in the skin. Allergic contact dermatitis is characterized by a wide range of symptoms, and patients are typically described as having an inflammatory reaction (dermatitis) with intensely pruritic erythema, oedema and even vesicles at sites where chemicals contact the skin. In humans, allergic contact dermatitis usually occurs in response to low-molecular-weight compounds after many repeated sub-threshold exposures that do not initially induce allergic signs or symptoms. This is termed the 'afferent' or 'sensitizing' phase of allergic contact dermatitis. The afferent phase may take weeks to months of repeated exposures to the sensitizing chemical. Gradually, the patient becomes allergic to the offending compound, and dermatitis occurs on further exposures to the allergen. This is the 'efferent' or 'elicitation' phase of allergic contact dermatitis. There are over 2,800 chemicals that have the potential to cause allergic contact dermatitis¹³. Patch testing is a standardized diagnostic procedure that is necessary to confirm allergic contact dermatitis. Potential allergens are applied onto the skin on a patient's back. After 2 days, the site of application of each potential allergen is evaluated. A positive patch test recapitulates the efferent phase of allergic contact dermatitis (that is, the allergen induces dermatitis), and this helps to identify the initial offending chemical. The treatment of choice for allergic contact dermatitis is strict avoidance of the offending chemical. Pharmacological measures, such as topical or systemic glucocorticosteroids and oral antihistamines, are also frequently used to control allergic contact dermatitis.

Contact hypersensitivity is an experimental model system for human allergic contact dermatitis. Typically, this model uses rodents, such as mice. Rather than using a series of subclinical exposures for induction (as occurs in humans), potent lipophilic compounds (such as dinitrofluorobenzene and other similar chemicals) are dissolved in an organic solvent and applied directly to the shaved abdominal skin of the experimental animal over a period of 1 or 2 days (this is the afferent phase of contact hypersensitivity). After 5 to 7 days, an elicitation response is induced by application of the same compound to the ear or footpad. The amount of swelling that occurs in the ear or footpad correlates with the intensity of the effector response generated. Contact hypersensitivity depends on the development of an early inflammatory response in the skin that is driven by innate mechanisms, but it ultimately requires antigen-specific CD8⁺ cytotoxic T lymphocytes and CD4⁺ helper T cells. Contact hypersensitivity is considered to be an example of a type IV hypersensitivity reaction (that is, a T cell-mediated allergic reaction). Although the circumstances of sensitization differ between humans with allergic contact dermatitis and mice with contact hypersensitivity, the contact hypersensitivity model is considered a reliable model for allergic contact dermatitis and has provided the framework for understanding the human disease.

immune system as 'altered self'. The amino acids with the most-reactive nucleophilic side chains are lysine, cysteine and histidine. However, their degree of ionization, and hence nucleophilicity, is dependent on the pH of the microenvironment, which is influenced by surrounding amino acids and by protein location within the epithelium¹².

The ability of a chemical species to react covalently with a carrier protein is related to electrophilic reactivity and hydrophobicity. Complete haptens are chemicals with skin sensitization potential that are electrophilic and can directly react with protein nucleophiles. Pro-haptens require activation by the host metabolism to convert them into reactive electrophilic intermediaries that can be conjugated to host proteins¹³ (FIG. 1). A large number of enzymes have been suggested to participate in the activation of pro-haptens, including the cytochrome P450 mixed-function oxidase system, alcohol dehydrogenases, aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases, hydrolytic enzymes, acyltransferases, glutathione S-transferases, uridine 5'-diphospho-glucuronosyltransferases and sulphotransferases^{14–16}.

Pre-haptens are chemicals that are not activated by host proteins but instead require chemical transformation by oxidative derivatization by ambient or air oxidation to form hydroperoxides. Common examples include urushiol (which is derived from the poison ivy plant), certain fragrance materials, and dyes used in hair colouring, such as *para*-phenylenediamine¹⁷. Finally, metal salts (such as nickel salts) interact with amino acids such as histidine to form non-covalent chelation complexes.

Allergen recognition

Innate recognition of haptens. It has long been recognized that the presence of foreign antigens alone is insufficient to generate immune responses: activation of the innate immune system is also required. In the case of vaccines, this is accomplished by combining antigens with adjuvants. In most cases, adjuvants contain microbial products (for example, mycobacteria in complete Freund's adjuvant) that engage families of receptors that have evolved to recognize specific microbial components termed pathogen-associated molecular patterns (PAMPs). Many of these receptors can also respond to endogenous ligands to sense cell damage or necrosis. Historically, research into the immunology of allergic contact dermatitis and contact hypersensitivity has overlooked hapten-mediated activation of the innate immune system to focus on the roles of cutaneous antigen-presenting cells (APCs), such as Langerhans cells and dermal DCs, and of conventional CD8⁺ and CD4⁺ T cells. The importance of hapten-mediated activation of innate immunity is highlighted

Box 2 | Essential steps for the induction of allergic contact dermatitis

- Percutaneous penetration of the hapten
- Haptenization of self proteins
- Epidermal and dermal inflammation
- Dendritic cell activation and migration from the skin to local lymph nodes

Box 3 | Development of the effector response

Allergic contact dermatitis can be considered as a cascade of sequential and parallel events that starts with sensing by the innate immune system of self damage that occurs during the haptization process. This generates an inflammatory response that induces cutaneous dendritic cells to mature, migrate and traffic haptenated self proteins to local lymph nodes for presentation to antigen-specific T cells. As a consequence, naive CD4⁺ and CD8⁺ T cells differentiate in the lymph node into effector T helper 1 (T_H1) cells, T_H17 cells and cytotoxic T lymphocytes (CTLs)¹¹⁴. These cells are then recruited back to the skin¹¹⁵, where CTLs induce the apoptosis of keratinocytes¹¹⁶. In addition to effector T cells, CD4⁺ regulatory T cells develop, and these cells inhibit allergic contact dermatitis and mediate tolerance in non-allergic individuals^{117,118}. Natural killer (NK) cells in mice have been shown to be capable of maintaining persistent, hapten-specific memory¹¹⁹. Although these studies have not been translated to humans, NK cells are present in significant numbers in cellular infiltrates in the skin of patients with allergic contact dermatitis^{120,121}. Invariant natural killer T (NKT) cells are thought to respond to endogenous glycolipids, and they secrete cytokines (such as interferon- γ) that help to amplify the early innate immune response¹²². Defective NKT cells or the application of antagonist lipids that inhibit NKT cell activation reduce contact hypersensitivity responses in mice^{123,124}.

by the clinical observation that the irritancy of chemicals (that is, the ability of the chemicals to cause grossly visible skin inflammation following primary exposure) correlates with their ability to act as contact sensitizers and to induce allergic contact dermatitis.

Haptens activate Toll-like receptors. Toll-like receptors (TLRs) are a family of receptors that recognize PAMPs expressed by bacteria, parasites, viruses and fungi. TLRs are evolutionarily conserved in vertebrates and recognize a wide variety of ligands, including lipids, lipoproteins, proteins and nucleic acids¹⁸. TLRs trigger specific biological responses. For instance, TLR4-mediated recognition of lipopolysaccharide (LPS), a cell wall component found in Gram-negative bacteria, induces the secretion of type I IFNs and other pro-inflammatory cytokines. By contrast, activation of a cell via other TLRs — such as TLR1–TLR2 and TLR2–TLR6 heterodimers or TLR5 homodimers — induces the secretion of pro-inflammatory cytokines but not IFN production. These differences are explained by differences in the downstream signalling pathways that are engaged by different TLRs. Most TLRs, with the exception of TLR3 and TLR4, signal exclusively via the adaptor MYD88, which activates the transcription factor nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPKs) to induce the expression of pro-inflammatory cytokines, such as IL-1 β ¹⁸. TLR3 signals via TIR-domain-containing adaptor protein inducing IFN β (TRIF), leading to activation of the transcription factors IFN-regulatory factor 3 (IRF3) and NF- κ B, and this results in the induction of type I IFNs and pro-inflammatory cytokines. TLR4 signals via both TRIF and MYD88, resulting in the activation of both signalling pathways. In addition to binding to PAMPs, TLRs recognize damage-associated molecular patterns (DAMPs), which are self molecules that can be released during necrotic cell death. DAMPs comprise a diverse set of proteins, nucleic acids and glycosaminoglycans; examples include heat shock proteins, high mobility group protein B1 (HMGB1), RNA, DNA, hyaluronan and heparan sulphate¹⁹.

Recently, a crucial role for TLR2 and TLR4 has been described in contact hypersensitivity, which is an experimental model of allergic contact dermatitis²⁰ (BOX 1). Although mice deficient in either TLR2 or TLR4 developed normal contact hypersensitivity responses, mice lacking both of these TLRs were not sensitized and failed to develop contact hypersensitivity in response to the haptens trinitrochlorobenzene (TNCB), oxazolone and fluorescein isothiocyanate (FITC). Interestingly, if the mice were unable to respond to IL-12, the absence of either TLR2 or TLR4 alone was sufficient to prevent the development of optimal contact hypersensitivity²⁰. These data suggest that there are two possible mechanisms of sensitization to contact allergens: an IL-12-dependent one, in which activation of TLR2 or TLR4 alone is sufficient for contact hypersensitivity to develop, and an IL-12-independent mechanism that requires the activation of both TLR2 and TLR4.

As TLR2 and TLR4 do not directly recognize haptens, a potential role for commensal skin bacteria was investigated by performing sensitization experiments in germ-free mice. These studies showed that contact hypersensitivity to TNCB develops normally in the absence of the bacterial flora²⁰. Thus, TLR2 and TLR4 appear to recognize endogenous host-derived ligands that are present in the skin, rather than components of the commensal flora. One candidate is low-molecular-weight breakdown products of high-molecular-weight hyaluronic acid²¹ (FIG. 1). Low-molecular-weight derivatives of hyaluronic acid activate DCs *in vitro* via TLR2 and TLR4 (REFS 22,23). In addition, reactive oxygen species that are produced in response to haptens can induce the breakdown of hyaluronic acid²⁴. Importantly, blockade of hyaluronic acid function in germ-free mice substantially reduces sensitization to haptens²⁰, which confirms a role for degraded hyaluronic acid as an endogenous activator of TLR2- and TLR4-mediated signalling in contact hypersensitivity responses. This observation has therapeutic implications, in that blockade of the formation of DAMPs may represent a novel approach to prevent allergic contact dermatitis in a naive host, or to lessen allergic contact dermatitis in an allergic host.

Haptens activate innate immunity via NOD-like receptors. A second system of PAMP recognition involves the formation of an intracellular complex of proteins termed the inflammasome²⁵ (FIG. 1). The inflammasome complex includes proteins of the NOD-like receptor (NLR) family that sense foreign PAMPs or endogenous ligands through an incompletely understood mechanism. Engagement of these receptors leads to signalling through the adaptor protein ASC to activate pro-caspase 1, which then cleaves pro-IL-1 β and pro-IL-18 into their active forms. As one outcome of TLR signalling is increased expression of the mRNA encoding IL-1 β , the TLR and inflammasome systems can work in a coordinated manner to induce the release of pro-inflammatory cytokines (TABLE 1).

The importance of the inflammasome system in responding to haptens is demonstrated by the reduced sensitization to TNCB and dinitrofluorobenzene (DNFB) that occurs in *Asc*^{-/-}, *Nlrp3*^{-/-} and

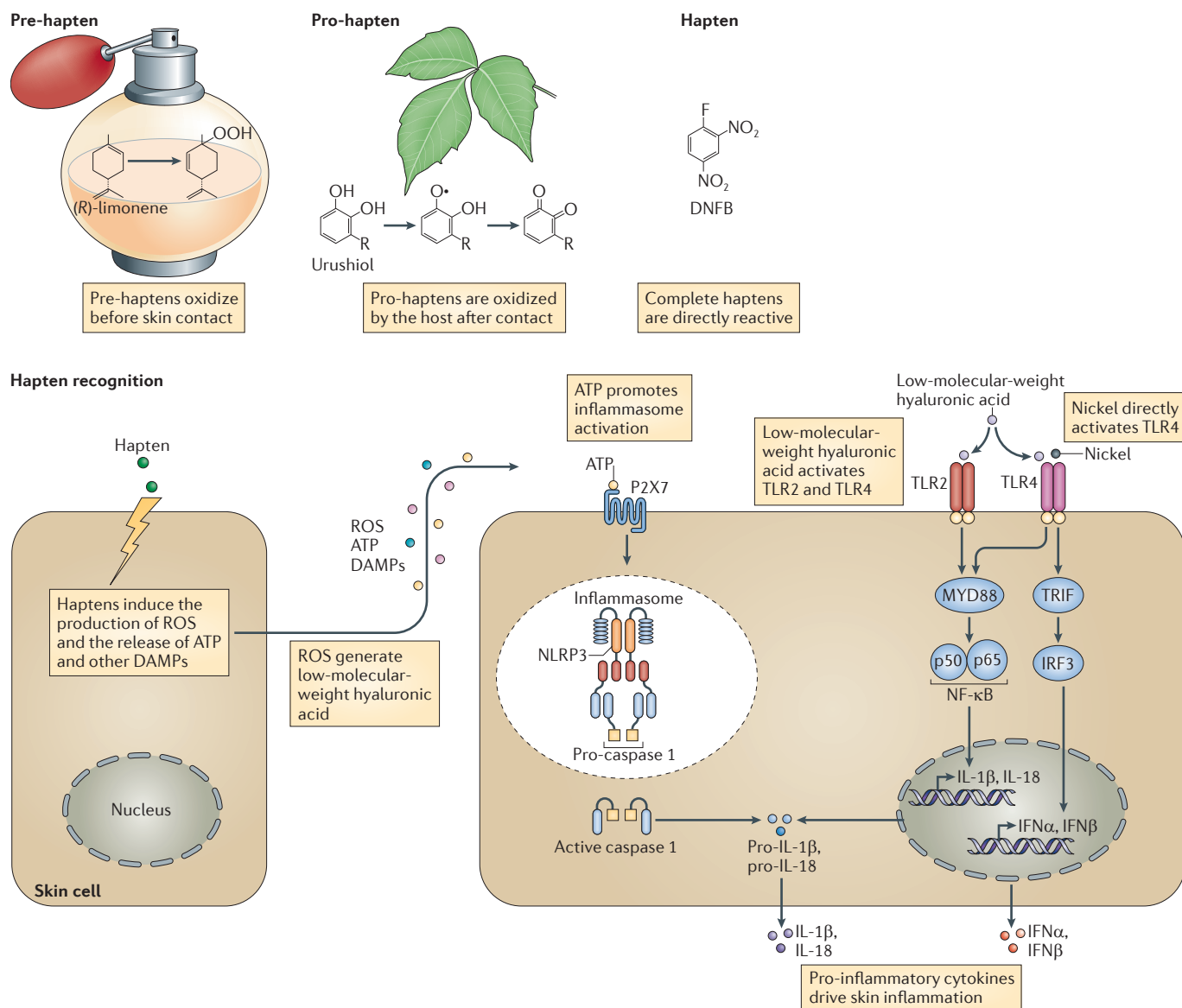


Figure 1 | Innate recognition of haptens. The earliest event in allergic contact dermatitis is the formation of hapten–self complexes. Complete haptens, such as dinitrofluorobenzene (DNFB), are directly reactive with protein nucleophiles and can form a complex without prior modification. Pre-haptens, such as the fragrance limonene, are activated by oxidation before skin contact. Pro-haptens, such as urushiol (a molecule found in poison ivy), are activated enzymatically once on the skin. Through a poorly described process, haptens induce the production of reactive oxygen species (ROS), which lead to the release of ATP and possibly other damage-associated molecular patterns (DAMPs), as well as to the generation of low-molecular-weight hyaluronic acid. Low-molecular-weight hyaluronic acid is sensed by neighbouring cells via Toll-like receptor 2 (TLR2) and TLR4, resulting in increased expression of inactive pro-interleukin-1 β (pro-IL-1 β) and pro-IL-18. ATP is sensed through the purinergic receptor P2X7 and activates the inflammasome, resulting in caspase 1 activity and the generation of active IL-1 β and IL-18. Nickel has a unique adjuvant property and can directly bind to histidine residues in the extracellular domain of TLR4, triggering the activation of this receptor. IRF3, IFN-regulatory factor 3; NF- κ B, nuclear factor- κ B; NLRP3, NOD-, LRR- and pyrin domain-containing 3; TRIF, TIR-domain-containing adaptor protein inducing IFN β .

Casp1^{−/−} mice^{26–28}. At least in the case of TNCB and oxazolone, inflammasome activation also depends on the ATP receptor P2X7 (REF. 29). This indicates that inflammasome activation occurs indirectly, as a result of cell damage and the release of ATP. Interestingly, dinitrothiocyanobenzene (DNTB) is a weak sensitizer that can induce tolerance to the structurally related hapten DNFB. DNTB does not induce inflammasome-mediated

secretion of active IL-1 β and IL-18 (REF. 30). However, artificial inflammasome induction with sodium dodecyl sulphate (SDS) or co-administration of IL-1 β converts DNTB into a sensitizing agent. In addition, administration of low, sub-threshold doses of many haptens, including DNFB, can induce a long-lasting tolerance³¹. Thus, inflammasome activation may be a feature common to all sensitizing haptens.

Table 1 | A comparison of common skin contact allergens

Allergen	Allergic response	Mechanism(s) of recognition	Comment
Nickel	Allergic contact dermatitis in humans	Engages two histidine residues in human TLR4; does not bind to mouse TLR4 (REF. 32)	Most common contact allergen; barrier disruption increases the rate of sensitization ³⁶
House dust mite allergens (Der p 2, Der f 2)	Allergic contact dermatitis in humans	These allergens are homologues of MD2, an LPS-binding protein that forms a complex with TLR4 (REF. 34)	Important skin and respiratory antigens for patients with atopic dermatitis
Dinitrofluorobenzene (DNFB), trinitrochlorobenzene (TNClB)	Contact hypersensitivity in mice	Induce hyaluronic acid degradation products that activate TLR2 and TLR4, and induce ATP release that leads to inflammasome activation ^{20,29}	Commonly used experimental haptens
Fluorescein isothiocyanate (FITC)	Contact hypersensitivity in mice	Unknown	The commonly used vehicle for FITC (DBP) induces TSLP to promote a T _H 2-type response ⁵⁰
Dinitrothiocyanobenzene (DNTB)	Contact hypersensitivity in mice	Weak hapten that does not activate the inflammasome ³⁰	Can induce tolerance to DNFB, a structurally similar hapten ³⁰
Dimethylbenzanthracene (DMBA)	Contact hypersensitivity in mice	Unknown mode of recognition; in the absence of TLR4, contact hypersensitivity is unaffected but the T _H 1/T _H 17 cell balance is altered ¹²⁵	Carcinogen and contact allergen

DBP, dibutyl phthalate; LPS, lipopolysaccharide; T_H, T helper; TLR, Toll-like receptor; TSLP, thymic stromal lymphopoietin.

Other skin allergens engage pattern-recognition receptors.

Although the above mechanisms appear to be broadly applicable to allergic contact dermatitis, there are also very specific examples of how pattern-recognition receptors (PRRs) are activated by certain human skin allergens. Nickel is the most common cause of allergic contact dermatitis and is known to activate NF- κ B and MAPK signalling. Recently, human TLR4 was shown to be the crucial receptor for nickel-induced pro-inflammatory gene expression³². Nickel allergies in humans appear to be an accident of nature. Binding of nickel, but not of the natural ligand LPS, to human TLR4 requires the presence of two non-conserved histidine residues (H456 and H458) in human TLR4. These histidines are absent from mouse TLR4, which explains the mystery of why mice fail to develop contact hypersensitivity to nickel³².

House dust mites can exacerbate eczema in patients with atopic dermatitis, and are also a major source of aeroallergens for patients with allergic asthma. These allergens activate innate immune responses through a unique and important mechanism³³. Concentrated in mite faecal pellets, the major group 2 allergens (Der p 2 and Der f 2) are highly allergenic; among defined dust mite antigens they have the highest rates of skin test positivity in patients with atopic dermatitis and can also result in exacerbations of eczema. Sequence similarity places these allergens in the recently recognized MD2-related lipid-recognition domain family of proteins³⁴. Der p 2 can also function in a similar manner to the TLR4 accessory molecule MD2, as it facilitates signalling through direct interactions with the TLR4 complex and can reconstitute LPS-driven TLR4 signalling in the absence of MD2. This property provides it with natural adjuvant properties that are highly relevant for its potency as a common skin and respiratory tract allergen.

Cellular responses to cutaneous allergens

In the preceding section, we described the molecular mechanisms that underlie the recognition of haptens. In this section, we examine how the different cell types

in the skin contribute to the generation of adaptive, hapten-specific immune responses. The skin can be broadly divided into two distinct regions: the epidermis and the dermis (FIG. 2). The epidermis is derived from ectoderm and is an epithelial layer composed primarily of keratinocytes, which provide the integrity of the skin. Langerhans cells and dendritic epidermal T cells (DETCs) reside within the epidermis. The dermis is a relatively cell-sparse tissue derived from mesoderm that forms a stromal layer immediately below the epidermis. It is populated by fibroblasts that secrete components of the complex extracellular matrix. In the steady state, a variety of immune cells can be found in the dermis, including T cells, mast cells and dermal DCs.

Keratinocytes. Keratinocytes are a crucial cell type for the development of allergic contact dermatitis owing to their numerical abundance in the epidermis and their role in the formation of the anatomical barrier function of the skin. Although haptens can penetrate through intact skin, the importance of an intact skin barrier in limiting sensitivity to allergic contact dermatitis has long been hypothesized, based on the observation that patients with certain disease states that impair barrier function (for example, leg ulcers and peri-anal dermatitis) have an increased risk of sensitization to topically applied medications and their vehicle components. Recently, direct evidence has been obtained through the discovery that many patients with atopic dermatitis (an inherited form of allergic chronic dermatitis) or allergic contact dermatitis to nickel harbour a defective form of the filaggrin gene^{35,36}. Filaggrin helps to aggregate the cytoskeletal proteins that form the cornified cell envelope. In its absence, the barrier is defective, as determined by increased loss of water through the epidermis. The importance of filaggrin has been confirmed in mice into which the mutations observed in patients with atopic dermatitis were genetically introduced³⁷. Mice

Dendritic epidermal T cells (DETCs). $\gamma\delta$ T cells that are localized purely in the epidermis. They are present in rodents and cattle but not in humans. In mice, essentially all DETCs express precisely the same T cell receptor, forming a prototype lymphocyte repertoire of limited diversity.

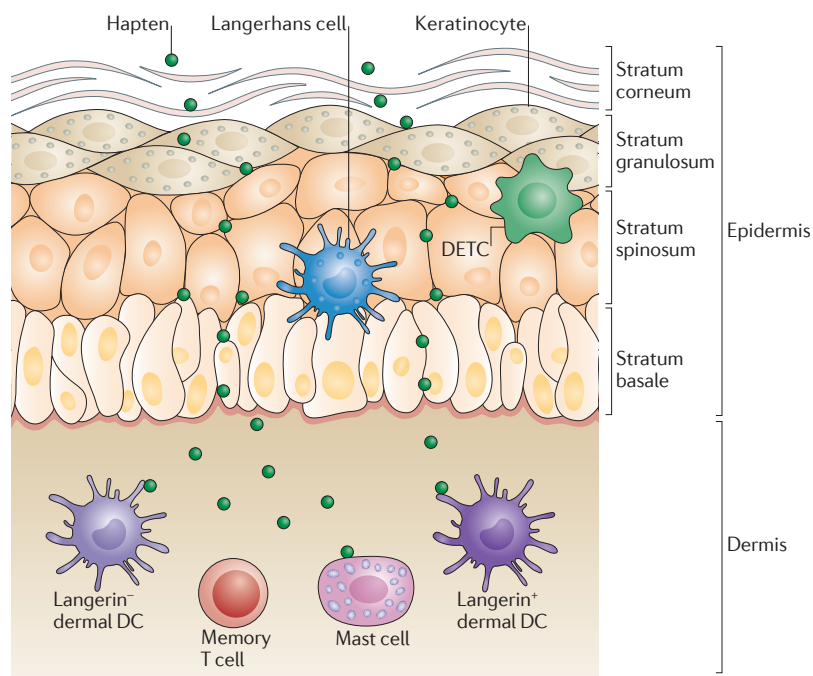


Figure 2 | Anatomy of the skin. The skin can be divided into two broad anatomical units: the epidermis and the dermis. The epidermis is a stratified epithelial layer that is composed primarily of keratinocytes that produce the water-impermeable stratum corneum. The dermis is a cell-poor stromal layer below the epidermis. Several immune cell types reside within the skin. A scheme for mouse skin is shown, although the cell types in human skin are similar. Langerhans cells are the only dendritic cell (DC) population resident in the epidermis. In the dermis, there are at least two subsets of DCs, which can be distinguished based on the expression of langerin. The dermis also contains mast cells and memory T cells. Dendritic epidermal T cells (DETCs) that have invariant $\gamma\delta$ T cell receptors (TCRs) are present in mouse but not human epidermis. Haptens, shown as green dots, penetrate into the dermis and are available to all the cell types shown.

with a defective filaggrin gene develop exaggerated responses to percutaneous immunization with proteins and haptens, and this results in a reduced threshold for allergic dermatitis^{37–39}.

In addition to physically blocking skin penetration, keratinocytes also recognize haptens via the mechanisms discussed above to generate the innate responses that are required for contact hypersensitivity (FIG. 3a). Keratinocytes express most TLRs, except TLR7 and TLR8, and they respond to TLR4-triggering haptens, such as DNFB and nickel⁴⁰. Inflammasome-mediated secretion of IL-1 β and IL-18 occurs in response to certain haptens. In addition to producing IL-1 β and IL-18, keratinocytes secrete many other pro-inflammatory cytokines and chemokines (reviewed in REFS 41,42). Of particular importance is tumour necrosis factor (TNF), which, along with IL-1 β and IL-18, is required for hapten-induced DC maturation and migration from the skin to the local lymph nodes^{43–45}. However, TNF may be partially redundant, as *Tnf*^{-/-} mice have only somewhat reduced contact hypersensitivity responses⁴⁶.

It has been recently recognized that, in addition to inducing DC migration, keratinocytes can modulate the T cell response through the 'conditioning' of skin-resident DCs. Perhaps the best example of this is the conditioning effects of keratinocyte-derived thymic

stromal lymphopoietin (TSLP) on DCs⁴⁷. TSLP stimulates DCs to induce the differentiation of naive T cells into T helper 2 (T_H2) cells, which promote allergic responses^{48,49}. The expression of TSLP is induced by dibutyl phthalate (DBP), a vehicle often used in conjunction with FITC to generate a T_H2-type contact hypersensitivity response. The importance of TSLP has been confirmed by the observation that mice deficient in the TSLP receptor develop reduced contact hypersensitivity responses to FITC-DBP⁵⁰. TSLP is also expressed by keratinocytes isolated from patients with atopic dermatitis and may be a key contributor to this disease⁴⁸. A second conditioning factor, receptor activator of NF- κ B ligand (RANKL), is expressed by keratinocytes in response to inflammation. Overexpression of RANKL by keratinocytes increases the number of regulatory T (T_{Reg}) cells, probably through effects on Langerhans cells that express RANK⁵¹. Interestingly, ultraviolet (UV) light and vitamin D3, which both increase the expression of RANKL by keratinocytes, suppress contact hypersensitivity responses in mice and allergic contact dermatitis in humans^{52–54}.

In addition to promoting T_{Reg} cell responses through RANKL expression, keratinocytes can directly participate in the suppression of the contact hypersensitivity response. For example, the antimicrobial peptide cathelicidin is secreted by keratinocytes and inhibits hyaluronan-induced cytokine release⁵⁵. Cathelicidin-deficient mice develop exaggerated contact hypersensitivity to DNFB. Furthermore, activated keratinocytes express high levels of MHC class I and MHC class II molecules but have only low levels of expression of the co-stimulatory molecule CD80 (also known as B7.1). Transgenic overexpression of CD80 on keratinocytes leads to exaggerated contact hypersensitivity responses, which suggests that an absence of CD80 on keratinocytes could inhibit or anergize effector T cells^{56,57}. Keratinocytes are also a source of IL-10, an immunosuppressive cytokine that limits the extent of contact hypersensitivity⁵⁸.

Dendritic cells. The key event in the initiation of allergic contact dermatitis is the priming of hapten-specific naive CD4⁺ and CD8⁺ T cells, which then become activated, proliferate and differentiate into effector T cell subsets. This occurs in the regional lymph nodes. Although haptens can drain to the lymph node via lymphatic flow, this does not lead to a productive T cell response^{59,60}. Instead, antigen presentation in the lymph node by migratory DCs is absolutely essential for the generation of responses to peripheral antigens^{59–62}.

In the skin at steady state, there are several populations of DCs. Langerhans cells are the only DC subtype in the epidermis. Langerhans cell precursors first populate the epidermis on embryonic day 18 and proliferate *in situ* to form a radio-resistant, self-renewing population that is unique among DCs^{63,64}. Like all skin-resident DCs, Langerhans cells efficiently acquire antigens in the periphery and then migrate to regional lymph nodes, where they present antigens to naive and memory T cells⁶⁵. Haptens also penetrate into the dermis and are acquired by dermal DCs⁶⁶. Langerin⁺

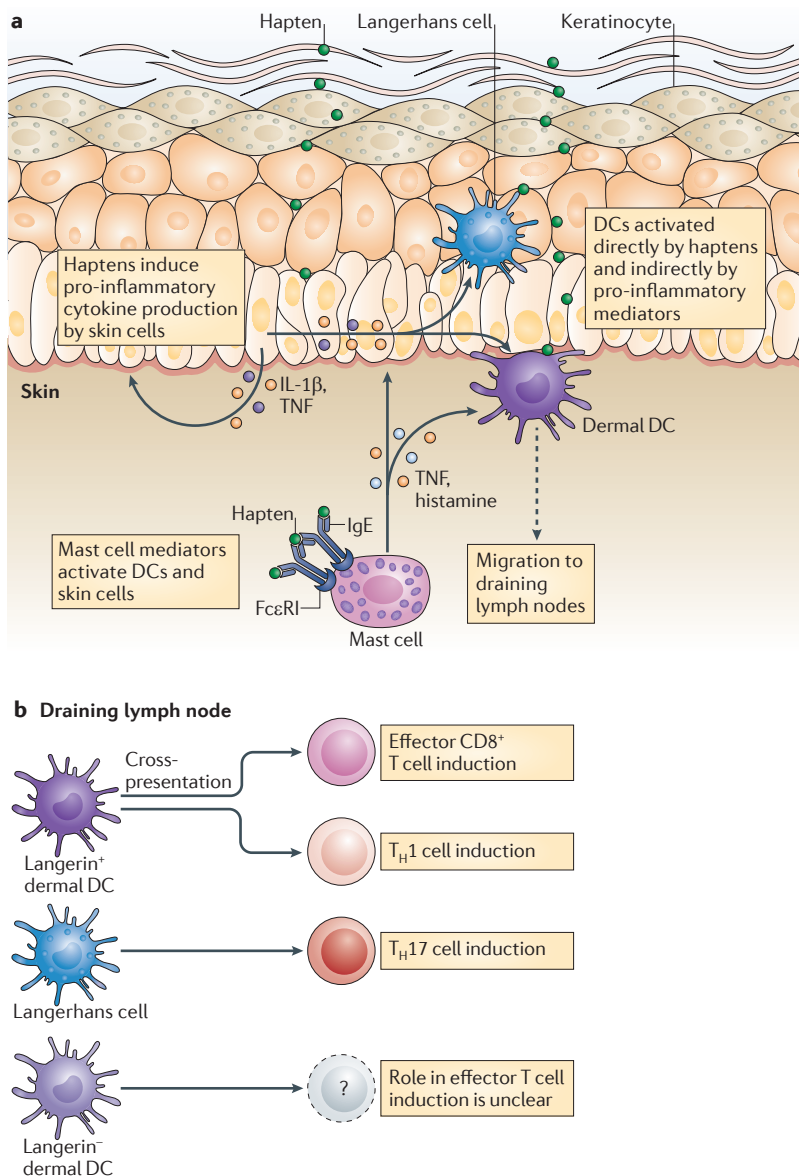


Figure 3 | Cellular responses to cutaneous antigens. a Although the precise sequence of events that occurs shortly after an encounter with an antigen has not been rigorously established, it is clear that most cells in the skin participate in the innate recognition of haptens and other cutaneous antigens. Keratinocytes secrete a large number of factors, including pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumour necrosis factor (TNF). Similarly, mast cells in the dermis also secrete many factors, including TNF and histamine. These pro-inflammatory mediators activate neighbouring cells, thereby amplifying the immune response. Dendritic cells (DCs) also have the intrinsic ability to sense haptens, and they then secrete pro-inflammatory cytokines and probably participate in escalating the inflammatory cascade. A combination of direct sensing of the hapten and the inflammatory milieu causes the activation of Langerhans cells in the epidermis and DCs in the dermis, resulting in their migration to the skin-draining lymph nodes. **b** In the lymph node, migratory DCs present antigens acquired in the skin to naive and memory CD4⁺ and CD8⁺ T cells. As the relative importance of skin-resident DC subsets during the establishment of hapten-specific adaptive immune responses remains controversial, this model is based on data from skin infections. Langerin⁺ dermal DCs are the only skin-derived DC subset specialized for the cross-presentation of antigens to CD8⁺ T cells. These DCs also present antigens to naive CD4⁺ T cells and promote the differentiation of these cells into T helper 1 (T_H1)-type effector cells. Presentation of antigens by Langerhans cells to naive CD4⁺ T cells promotes the differentiation of these cells into T_H17-type effector cells. Fc ϵ RI, high-affinity receptor for IgE.

dermal DCs (also referred to as CD103⁺ dermal DCs) are a distinct DC subset that arises from bone marrow precursors in an FMS-like tyrosine kinase 3 (FLT3)-dependent manner^{67–71}. Although they represent only a small percentage (~3%) of the total DC population in the dermis, langerin⁺ dermal DCs are in a high state of flux and are continually replaced by new recruits from the blood^{68,72}. Examination of subset ontogeny has clearly identified that Langerhans cells and langerin⁺ dermal DCs are distinct DC subsets^{63,71,73,74}. The second major dermal population, langerin⁻ dermal DCs (also referred to as classic dermal DCs), make up the bulk of dermal DCs (~80%) and are heterogeneous; there are at least two subsets, which can be distinguished based on the expression of CD11b⁷². In addition, in response to inflammation, GR1⁺ monocytes are recruited to the skin, where they develop into DCs^{75,76}. The functional relevance of this important DC subset to contact hypersensitivity is unclear.

Studies examining the specific contributions of steady-state DC subsets to the hapten-specific immune response have been made possible by the existence of several mouse strains that lack specific DC subsets (TABLE 2). HuLangerin-DTA mice express the active subunit of diphtheria toxin (DTA) specifically in Langerhans cells (under the control of the human langerin promoter), and this leads to a constitutive absence of epidermal Langerhans cells⁷⁷. HuLangerin-DTR mice express the primate diphtheria toxin receptor (DTR) in Langerhans cells, and this enables inducible and specific ablation of Langerhans cells after injection of diphtheria toxin⁷⁸. These mice develop enhanced contact hypersensitivity responses to various contact allergens, but not to irritants such as benzalkonium chloride. Experiments in which Langerhans cells were depleted at different time points showed that Langerhans cells have suppressive functions during the priming phase but not during the effector phase of the contact hypersensitivity response^{77–79}. Contact hypersensitivity responses are also exaggerated in huLangerin-Cre MHC-II^{fllox} mice and huLangerin-Cre *Il10*^{fllox} mice (which have a Langerhans cell-specific deficiency of MHC class II molecules and IL-10, respectively). This suggests that Langerhans cells suppress contact hypersensitivity responses in an IL-10-dependent manner and require cognate interaction with CD4⁺ T cells^{79,80}. In addition to inhibiting contact hypersensitivity responses, Langerhans cells have suppressive functions in other immune models; for example, they have been shown to promote tolerance to minor-mismatched skin grafts, to suppress the clearance of *Leishmania major* infection and to mediate UV-induced immunosuppression^{81–84}.

As discussed above, langerin is expressed in Langerhans cells and in a subset of dermal DCs. In mice that express DTA or DTR under the control of the human langerin promoter, dermal DCs are not deleted. This is presumably due to differences between the human and mouse langerin promoters. By contrast, in muLangerin-DTR mice (generated using the endogenous mouse promoter), diphtheria toxin injection ablates Langerhans cells and langerin⁺ dermal DCs, as

Table 2 | Effects of genetic ablation of DC subsets on the development of contact hypersensitivity

Mouse strain	Effects on dermal DC subsets				Effect on intensity of contact hyper-sensitivity response	Refs
	Epidermal Langerhans cells	Langerin ⁺ CD103 ⁺ dermal DCs	Langerin ⁻ dermal DCs	CD8 ⁺ DCs		
Constitutive systems						
HuLangerin-DTA	Absent	Normal	Normal	Normal	Increased	77
HuLangerin-Cre x <i>Il10^{fllox}</i> or huLangerin-Cre x MHC-II ^{fllox}	Functionally defective	Normal	Normal	Normal	Increased	79
<i>Batf3^{-/-}</i>	Normal	Absent	Normal	Absent	Normal	73
Inducible systems						
MuLangerin-DTR (diphtheria toxin administered on day -1)	Absent	Absent	Normal	~25% of normal population present	Decreased	85,86, 91
MuLangerin-DTR (diphtheria toxin administered on day -7 or day -13)	Absent	~20–50% of normal population present	Normal	Normal	Normal	67,91
HuLangerin-DTR (diphtheria toxin administered on day -1)	Absent	Normal	Normal	Normal	Increased	78

Batf3, basic leucine zipper transcriptional factor ATF-like 3; DC, dendritic cell; DTA, diphtheria toxin fragment A; DTR, diphtheria toxin receptor.

well as CD103⁺ DCs from many other peripheral tissues^{85,86}. CD8⁺ DCs that reside in lymph nodes and the spleen are also depleted with reduced efficiency. Contact hypersensitivity is greatly diminished in these mice if diphtheria toxin is administered before priming, but not before elicitation^{85–87}. Langerhans cells repopulate the skin after diphtheria toxin-mediated ablation much more slowly than langerin⁺ dermal DCs⁶⁷. Interestingly, contact hypersensitivity responses develop normally in muLangerin-DTR mice if priming is delayed until 7–13 days after diphtheria toxin administration, a time point at which Langerhans cells are absent but langerin⁺ dermal DCs have partially returned. This suggests that langerin⁺ dermal DCs, rather than Langerhans cells, promote the development of contact hypersensitivity^{67,88}. This is supported by the observation that contact hypersensitivity responses can be adoptively transferred by dermal DCs, but not Langerhans cells, that are isolated from sensitized mice⁸⁹. However, by using the delayed contact hypersensitivity technique or muLangerin-DTR bone marrow chimaeras to selectively ablate Langerhans cells or langerin⁺ dermal DCs, other groups have found that langerin⁺ dermal DCs and Langerhans cells are functionally redundant^{90,91}. Thus, distinguishing the functions of Langerhans cells and langerin⁺ dermal DCs using muLangerin-DTR mice is complicated by the complex experimental manipulations that are required to deplete individual DC subsets. However, in *Batf3*^{-/-} mice, which have a constitutive absence of langerin⁺ dermal DCs⁷³, contact hypersensitivity responses develop normally, indicating that, at least in the setting of a constitutive absence of langerin⁺ dermal DCs, these cells are not required for contact hypersensitivity.

A major obstacle to untangling the functions of skin-resident DCs during contact hypersensitivity is the high degree of experimental variability that is intrinsic to the contact hypersensitivity assay. In addition, it is difficult to efficiently examine hapten-specific

responses. In the setting of skin infections, however, the functional distinction between skin DC subsets is clearer. Langerin⁺ dermal DCs isolated from skin-draining lymph nodes during recurrent herpes simplex virus infection efficiently cross-prime antigen-specific CD8⁺ T cells *in vitro*⁹². By contrast, other skin-resident DCs and lymph node-resident DCs are much less efficient. Similarly, skin infection with *Candida albicans* induces efficient activation of CD8⁺ T cells *in vivo* in langerin-DTA mice, which lack Langerhans cells, whereas CD8⁺ T cell activation is defective in infected *Batf3*^{-/-} mice, which lack langerin⁺ dermal DCs⁹³. Moreover, langerin⁺ dermal DCs are required for T_H1 cell differentiation in response to *C. albicans* infection, whereas Langerhans cells are required for the development of T_H17 cell responses. Thus, langerin⁺ dermal DCs and Langerhans cells are clearly functionally distinct in the setting of skin infection. This may also be true for contact hypersensitivity, but these experiments will require the development of new tools to analyse hapten-specific responses.

Mast cells. Mast cells are haematopoietically derived cells that reside long-term in barrier tissue sites, such as the skin and gut. Mast cells express the high-affinity receptor for IgE (FcεRI) and can be activated via antigen-mediated crosslinking of surface IgE (FIG. 3a). In addition, mast cells express TLRs and respond to many microbial products⁹⁴. In response to IgE-mediated activation, mast cells induce an immediate ‘wheal and flare’ response (within minutes) by releasing preformed granules that contain a wide variety of immunologically active compounds, such as histamine, proteases, proteoglycans and TNF. Mast cells also secrete various pro-inflammatory ‘late’ mediators, such as IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-13, TNF, CC-chemokine ligand 2 (CCL2; also known as MCP1), CCL3 (also known as MIP1α) and CCL4 (also known as MIP1β).

Although the participation of mast cells in the development of immune responses against pathogens has been well described (recently reviewed in REF. 95), the importance of mast cells during contact hypersensitivity is quite controversial. Early studies were contradictory, with some authors reporting that mast cells are redundant for contact hypersensitivity and others suggesting an essential role for mast cells in contact hypersensitivity responses^{96,97}. More recently, mast cell activation by hapten-specific IgM and complement has been shown to participate in the elicitation phase of contact hypersensitivity⁹⁸. In addition, mast cell activation has been shown to induce the migration of skin-resident DCs and to enhance the efficacy of immunizations^{99,100}. The availability of mice that lack mast cells owing to mutations in the *Kit* gene (that is, B6-*Kit*^{W-sh/W-sh} or WBB6F1-*Kit*^{W/W-v} strains) has greatly facilitated the evaluation of mast cell function *in vivo*¹⁰¹. These mast cell-deficient mice can be reconstituted with *in vitro*-generated mast cells derived from mice deficient in a particular cytokine, thereby functionally generating mice with a mast cell-specific cytokine deletion. Contact hypersensitivity responses to FITC are defective in B6-*Kit*^{W-sh/W-sh} mice. Reconstitution of these mice with mast cells derived from wild-type mice, but not from TNF-deficient mice, restores contact hypersensitivity¹⁰². Similarly, contact hypersensitivity to oxazalone is defective in WBB6F1-*Kit*^{W/W-v} mice, as well as in both IgE-deficient mice and IgE receptor-deficient mice¹⁰³. Thus, mast cells, mast cell activation via FcεRI and mast cell-derived TNF are required for the induction of contact hypersensitivity. Intriguingly, contact hypersensitivity to urushiol in B6-*Kit*^{W-sh/W-sh} mice develops normally but fails to appropriately resolve, resulting in increased ear swelling compared with that of wild-type mice several days after challenge. Reconstitution with wild-type mast cells, but not *Il10*^{-/-} mast cells, rescues the phenotype¹⁰⁴. These experiments suggest that, at least in response to urushiol, mast cells also suppress the late phase of the contact hypersensitivity response by producing IL-10.

An important caveat to experiments using mice with *Kit* mutations is that these mice also have other defects. WBB6F1-*Kit*^{W/W-v} mice develop macrocytic anaemia, have reduced numbers of neutrophils, lack melanocytes and are sterile¹⁰⁵. Although B6-*Kit*^{W-sh/W-sh} mice have a milder phenotype, subtle effects outside the mast cell compartment cannot be excluded. Recently, transgenic mice have been engineered that have mast cell-specific expression of Cre recombinase (*Mcpt5*-Cre mice)¹⁰⁶. Breeding *Mcpt5*-Cre mice with inducible-DTR mice (which ubiquitously express a *loxP*-stop-DTR construct) creates a mouse strain in which mast cells can be ablated by injection of diphtheria toxin¹⁰⁷. Mast cell ablation before sensitization or elicitation impairs contact hypersensitivity responses to FITC and oxazalone. In addition, *Mcpt5*-Cre *Il10*^{fllox} mice do not develop exaggerated contact hypersensitivity. Thus, mast cells are required for contact hypersensitivity, but mast cell-derived IL-10 does not appear to be necessary for the resolution of the contact hypersensitivity response. Interestingly, similarly to earlier data using urushiol, late contact

hypersensitivity responses to haptens are increased in B6-*Kit*^{W-sk/W-sh} mice, but this result is not due to the absence of mast cells but rather a result of a previously unrecognized neutrophil defect in B6-*Kit*^{W-sk/W-sh} mice.

γδ T cells. DETCs that express identical γδ (Vγ5–Vδ1) T cell receptors (TCRs) populate the epidermis of virtually all strains of inbred mice but are not found in other tissues. Their location in the epidermis brings them into contact with haptens during the sensitization stage of contact hypersensitivity. It is unknown whether they directly sense the presence of haptens, but they do respond to stress-induced molecules expressed by keratinocytes; for example, DETCs recognize retinoic acid early-inducible protein 1 (RAE1) via NKG2D¹⁰⁸. Several studies have suggested that these cells participate in contact hypersensitivity by modulating the response of αβ T cells¹⁰⁹. However, *Tcrd*^{-/-} mice, which lack all γδ T cells, develop normal contact hypersensitivity on a C57BL/6 genetic background. Interestingly, *Tcrd*^{-/-} mice on either the FVB or non-obese diabetic (NOD) genetic backgrounds develop spontaneous dermatitis and enhanced contact hypersensitivity responses that can be rescued by the adoptive transfer of Vγ5⁺Vδ1⁺ T cells, but not by transfer of other γδ T cells that do not express a Vγ5–Vδ1 TCR¹¹⁰. Owing to a point mutation in a gene involved in the intrathymic positive selection of Vγ5⁺Vδ1⁺ cells before their migration to the skin, the epidermis of the substrain of FVB mice produced by Taconic Farms (FVB Tac mice) lacks Vγ5⁺Vδ1⁺ DETCs and is instead populated by Vγ5⁺Vδ1⁻ DETCs. DETCs in FVB Tac mice are functionally defective, and these mice exhibit spontaneous dermatitis and develop exaggerated contact hypersensitivity responses that are similar to those seen in *Tcrd*^{-/-} mice¹¹¹. Thus, Vγ5⁺Vδ1⁺ DETCs appear to suppress contact hypersensitivity through still poorly understood mechanisms. γδ T cells are also found in human skin and may be the functional equivalent of the rodent invariant epidermal γδ T cells¹¹².

Conclusion

Understanding how contact allergens promote allergic contact dermatitis is important, not only for preventing this disease, but also to enable us to develop a better understanding of other inflammatory skin diseases. Keratinocytes, mast cells and skin-resident DCs all contribute to the recognition of contact allergens. The precise role of each cell type and the sequence of events are still being determined. For the small number of contact allergens studied so far, it appears that they are sensed by TLRs and the inflammasome. As these recognition systems evolved to sense microbial pathogens, the inflammation induced by contact allergens appears to be an accident of nature. This raises the possibility that the activation of these pathways is a trait shared by all compounds with sensitizing potential. This has important implications, as determining whether specific compounds are sensitizers is required for ingredients that come into contact with human skin (for example, skin care products). Whether all 2,800 of the currently known sensitizing chemicals also trigger inflammation via TLR and NLR pathways remains to be determined¹¹³.

NKG2D

(Natural killer group 2, member D). A lectin-type activating receptor that is encoded by the NK complex and is expressed at the surface of NK cells, NKT cells, γδ T cells and some cytolytic CD8⁺ αβ T cells. The ligands for NKG2D are MHC class I polypeptide-related sequence A (MICA) and MICB in humans, and retinoic acid early-inducible protein 1 (RAE1) and H60 in mice. Such ligands are generally expressed at the surface of infected, stressed or transformed cells.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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