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Early events in the induction of allergic contact dermatitis

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Preface

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. Recently, it has been appreciated that pattern-recognition receptors are also engaged by chemical contact allergens. This elicits an inappropriate immune response in susceptible individuals resulting in allergic contact dermatitis. In this Review, we will focus on how contact allergens promote inflammation. We will also examine how immune cells in the skin, including mast cells, T cells and dendritic cells, cooperate with each other and with keratinocytes to drive early responses to contact allergens.

Introduction

Allergic contact dermatitis is a common skin disease that is caused by type IV delayed type hypersensitivity responses to antigens that come in contact with the skin (Box 1)[1]. Allergic contact dermatitis is a major cause of occupational skin disease, and 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 $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 with self proteins to generate immunogenic neo-antigens through a process termed haptenization. Contact allergens are common in cosmetics, personal care products, jewelry and in the workplace whether it is in an industrial, health care, or office setting. Examples of common allergens are inorganic chemicals such a nickel and organic chemicals such as those found in fragrances and dyes.

There are 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 skin. Compounds must be less than 500 Daltons for efficient penetration[5]. This step allows the chemical sensitizer to come into contact 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 elaboration of a number of pro-inflammatory mediators, including interleukin-1β (IL-1β). 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 naïve 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

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T cells release pro-inflammatory cytokines, such as IFNγ, and promote the killing of haptenized cells resulting in the development of the classic inflammatory rash that is seen in patients with allergic contact dermatits[5-8].

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

Hapten–self complex formation

The landmark article of Landsteiner and Jacobs published in 1935 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 host molecule(s) generates a neo-antigen that is eventually recognized by the immune system as ‘altered self’. The most reactive amino acid nucleophilic side-chains are those that are found on lysine, cysteine and histidine residues. However, their degree of ionization, and hence nucleophilicity is dependent on the pH of the microenvironment, which is influenced by surrounding amino acids as well as by protein location within the epithelium[12].

The ability of the chemical species to react covalently with the carrier protein is related to electrophilic reactivity and hydrophobicity. Complete haptens are chemicals with skin sensitization potential that are directly reactive (electrophilic) with protein nucleophiles. Pro-haptens, which are also hydrophobic molecules, require activation by the host metabolism to make them into a reactive electrophile intermediary that can conjugate with host proteins (Figure 1)[13]. A large number of enzymes have been suggested to participate in activation of pro-haptens, including the cytochrome P450 (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 sulfotransferases[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 coloring, such as para-phenylene diamine[17]. Finally, metal salts such as nickel 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 antigen 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 antigen with adjuvants. In most cases, adjuvants contain microbial products (for example, mycobacteria in complete Freund’s adjuvant) that engage families of receptors which have evolved to recognize specific components of microbial organisms, that is, 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 acute contact dermatitis and contact hypersensitivity has overlooked hapten-mediated activation of the innate

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immune system to focus on the role of cutaneous antigen-presenting cells (APCs), such as Langerhans cells and dermal DC, and conventional $CD8^+$ and $CD4^+$ T cells. The importance of hapten-mediated activation of innate immunity is highlighted by the clinical observation that the irritancy of chemicals (that is, the ability of these chemicals to cause grossly visible skin inflammation upon primary exposure) correlates with their ability to act as contact sensitizers and to induce acute 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 acids[18]. 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 interferons (IFNs) and other pro-inflammatory cytokines. In contrast, activation of a cell via other TLRs, such as heterodimers of TLR1–TLR2 and TLR2–TLR6 or TLR5 homodimers**,** induces the secretion of pro-inflammatory cytokines but not IFN production. These differences are explained by differences in the downstream signaling pathways engaged by different TLRs. Most TLRs, with the exception of TLR3 and TLR4, signal exclusively via the adapter MyD88, which activates the transcription factor nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) to induce the expression of pro-inflammatory cytokines, such as IL-1β[18]. TLR3 signals via TIR-domain-containing adaptor protein inducing IFNβ (TRIF) leading to activation of the transcription factors interferon-regulatory factor 3 (IRF3) and NF-κB and results in induction of type I IFN and inflammatory cytokines. TLR4 signals via both TRIF and MyD88 resulting in activation of both signalling pathways. In addition to PAMPs, TLRs also recognize damage associated molecular patterns (DAMPs), which are self molecules that can be released during necrotic cell death. PAMPs comprise a diverse set of proteins, nucleic acids, and glycosaminoglycans; examples include heat shock proteins, HMGB1, RNA, DNA, hyaluronan, and heparin sulfate[19].

Recently, a crucial role for TLR2 and TLR4 has been described in contact hypersensitivity, an experimental model of allergic contact dermatitis (Box 1)[20]. 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 hypersensitivity[20]. 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 a IL-12-independent mechanism that requires activation of both TLR2 and TLR4.

Since TLR2 and TLR4 do not directly recognize haptens, a role for commensal skin bacteria was evaluated by performing sensitization experiments in germ-free mice. These studies showed that contact hypersensitivity to TNCB develops normally in the absence of the bacterial flora[20]. 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 acid **(Figure 1)**[21]. Low molecular weight-hyaluronic acid derivatives activate DCs *in vitro* via TLR2 and TLR4[22, 23]. In addition, reactive oxygen species that are produced in response to haptens can induce breakdown of hyaluronic acid[24]. Importantly, blockade of hyaluronic acid function in germ-free mice significantly reduces sensitization to haptens[20], which

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confirms a role for degraded hyaluronic acid as an endogenous activator of TLR2 and TLR4-mediated signaling 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 naïve host, or lessen allergic contact dermatitis in an allergic host.

Haptens activate innate immunity via NLR receptors

A second system of PAMP recognition involves the formation of an intracellular complex of proteins termed the inflammasome **(Figure 1)**[25]. The inflammasome complex includes proteins of the nucleotide-binding domain, leucine-rich repeat-containing (NLR) gene family that sense foreign PAMPs or endogenous ligands through an incompletely understood mechanism. Engagement of these receptors leads to signaling through the adaptor protein ASC (apoptosis associated speck-like protein containing a caspase recruitment domain) to activate pro-caspase 1, which cleaves pro-IL-1β and pro-IL-18 into active forms. Since one outcome of TLR signaling is increased expression of mRNA for IL-1β, the TLR and inflammasome systems can work coordinately to induce the release of pro-inflammatory cytokines (Table 1).

The importance of this system in responding to haptens is demonstrated by the reduced sensitization to TNCB and dinitrofluorobenzene (DNFB) that occurs in $ASC^{-/-}$, $NALP3^{-/-}$, and $caspase1^{-/-}$ mice[26-28]. At least in the case of TNCB and oxazalone, inflammasome activation also depends on the ATP receptor $P2X_{7}$[29]. This indicates that inflammasome activation occurs indirectly as a result of cell damage and 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-1β and IL-18[30]. However, artificial inflammasome induction with sodium dodecyl sulfate (SDS) or co-administration of IL-1β converts DNTB into a sensitizing agent. In addition, administration of low, sub-threshold doses of many haptens including DNFB can induce a long-lasting tolerance[31]. Thus, inflammasome activation may be a feature common to all sensitizing haptens.

Other skin allergens engage PRRs

Although the above mechanisms appear to be broadly applicable to allergic contact dermatitis, there are very specific examples of how PRRs are activated by certain human skin allergens. Nickel is the most common cause of allergic contact dermatitis and is known to activate NF-kB and MAPK signaling. Recently, human TLR4 was shown to be the crucial receptor for nickel-induced pro-inflammatory gene expression[32]. 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 in human TLR4, H456 and H458. These histidines are absent from mouse TLR4, which explains the mystery of why mice fail to develop contact hypersensitivity to nickel[32].

House dust mites can exacerbate eczema in patients with atopic dermatitis, and are also a major source of aeroallergens for patients with allergic asthma and activate innate immunity through a unique and important mechanism[33]. 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 homology places these allergens in the recently recognized MD2-related lipid-recognition domain family of proteins[34]. Der p 2 also has functional homology, facilitating signaling through direct interactions with the TLR4 complex, and reconstituting LPS-driven TLR4 signaling in the absence of MD-2. This

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property provides it with natural adjuvant properties that are highly relevant for its potency as a common skin and respiratory track allergen.

Cellular response to cutaneous allergen

In the preceding section, we have examined the molecular mechanisms underlying recognition of haptens. In this section we will examine how the different cells 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 dermis **(Figure 2)**. The epidermis is derived from ectoderm and is an epithelial layer composed primarily of keratinocytes that provide for 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 due to their numerical abundance in the epidermis and through their role in the formation of the anatomic 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 harbor a defective form of the *filaggrin* gene[35, 36]. Filaggrin helps aggregate 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 atopic dermatitis patients were genetically introduced[37]. Mice with defective *filaggrin*develop exaggerated responses to percutaneous immunization with proteins and haptens that results in a reduced threshold for allergic dermatitis[37-39].

In addition to physically blocking penetration, keratinocytes also recognize haptens via the mechanisms discussed above to generate the innate responses that are required for contact hypersensitivity **(Figure 3a)**. Keratinocytes express most TLRs, except TLR7 and TLR8, and this allows them to respond to TLR4-triggering haptens, such as DNFB and nickel[40]. Inflammasome-mediated secretion of IL-1β and IL-18 occurs in response to certain haptens. In addition to IL-1β and IL-18, keratinocytes secrete a large number of other pro-inflammatory cytokines and chemokines (reviewed in [41, 42]). Of particular importance is TNFα which, along with IL-1β and IL-18, is required for hapten-induced DC maturation and migration from the skin to the local lymph node[43-45]. TNFα may be partially redundant, though, as TNFα$^{-/-}$ mice have only somewhat reduced CHS[46].

It has been recently appreciated that in addition to inducing DC migration, keratinocytes can modulate the T cell response through ‘conditioning’ of skin-resident DCs. Perhaps the best example of this is the conditioning effects of keratinocyte-derived thymic stromal lymphopoietin (TSLP) on DCs[47]. TSLP activates DCs to induce naive T cell differentiation into T helper 2 (T_H2) cells, which promote allergic responses[48, 49]. The expression of TSLP is induced by dibutylphthalate (DBP), a vehicle often used in conjunction with FITC to generate a T_H2-type contact hypersensitivity response. The importance of TSLP has been

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confirmed with the observation that mice deficient in the TSLP receptor develop reduced contact hypersensitivity responses to FITC–DBP[50]. TSLP is also expressed by keratinocytes isolated from patients with atopic dermatitis and may be a key contributor to the disease[48]. A second conditioning factor, the receptor activator of –NF-κB ligand (RANKL), is expressed by keratinocytes in response to inflammation. Overexpression of RANKL by keratinocytes increases the number of regulatory T (T_{Reg}) cells, most likely through effects on Langerhans cells that express RANK[51]. Interestingly, UV-light and vitamin D3, which both increase 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 also participate in suppressing the response. For example, the anti-microbial peptide, cathelicidin, is secreted by keratinocytes and inhibits hyaluronan-induced cytokine release[55]. Cathelicidin-deficient mice develop exaggerated contact hypersensitivity to DNFB. Activated keratinocytes express high levels of MHC class I and MHC class II but have only low levels of expression of the costimulatory molecule CD80 (also known as B7.1). Transgenic overexpression of CD80 on keratinocytes leads to exaggerated contact hypersensitivity, which suggests that the 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 hypersensitivity[58].

Dendritic cells

The key event in allergic contact dermatitis is the priming of hapten-specific naïve $CD4^+$ and $CD8^+$ T cells, which then become activated, proliferate, and differentiate into effector T cell subsets. This occurs in the regional lymph node. 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 antigen[59-6162].

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 dendritic cells (DCs)[63, 64]. Like all skin-resident DCs, Langerhans cells efficiently acquire antigen in the periphery and migrate to regional lymph nodes where they present antigen to naïve and memory T cells[65]. Hapten also penetrates into the dermis and is acquired by dermal DCs[66]. $Langerin^+$ dermal DCs (also referred to as $CD103^+$ dermal DCs) are a distinct DC subset and arise from bone marrow precursors in a FMS-related tyrosine kinase 3 (FLT3)-dependent manner[67-71]. Although they represent only a small percentage of the total DC population in the dermis (~3%), $Langerin^+$ 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, with at least two subsets that can be distinguished based on the expression of CD11b[72]. In response to inflammation, GR1+ monocytes are recruited to the skin where they develop into DC[75, 76]. The functional importance to CHS of this important DC subset is unclear.

Studies examining the specific contribution 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 and this leads to a constitutive absence of epidermal Langerhans cells [77]. HuLangerin-DTR mice express the primate

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diphtheria toxin receptor (DTR) in Langerhans cells and this enables inducible and specific ablation of Langerhans cells after injection of diphtheria toxin[78]. These mice develop enhanced contact hypersensitivity responses to various contact allergens, but not 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^{flox} and huLangerin-Cre IL-10^{flox} mice suggesting that Langerhans cells suppress contact hypersensitivity responses in an IL-10-dependent manner and require cognate interaction with CD4$^+$ T cells **(Figure 3b)**[79, 80]. In addition to inhibiting contact hypersensitivity responses, Langerhans cells have suppressive functions in other immune models, and have been shown to promote tolerance to minor-mismatched skin grafts, suppress the clearance of Leishmania infection and mediate UV-induced immunosuppression [81-84].

As discussed above, Langerin is expressed in Langerhans cells and a subset of dermal DC. In mice that express DTA or DTR under control of the human *Langerin* promoter, dermal DCs are not deleted. This is presumably due to difference between the human and murine *langerin* promoters. In contrast, in muLangerin-DTR mice, diphtheria toxin injection ablates Langerhans cells, Langerin$^+$ dermal DCs and also CD103$^+$ DCs from many other peripheral tissues[85, 86]. CD8$^+$ DCs that reside in lymph nodes and spleen are also eliminated with reduced efficiency. Contact hypersensitivity is greatly diminished in these mice if diphtheria toxin is administered prior to priming, but not prior to elicitation[85-87]. Langerhans cells repopulate the skin after diphtheria toxin-mediated ablation much more slowly than Langerin$^+$ dermal DCs[67]. 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 when 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 with dermal DCs, but not with Langerhans cells, isolated from sensitized mice[89]. However, by using the delayed contact hypersensitivity technique or muLangerin-DTR bone marrow chimeras 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 function of Langerhans cells from 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 DCs[73], 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]. In 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, but CD8$^+$ T cell activation is defective in infected Batf3−/− mice, which lack Langerin$^+$ dermal DCs[93]. 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

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functionally distinct in the setting of skin infection. This may also be true for CHS but its demonstration requires the development of new tools to analyze hapten-specific responses.

Mast Cells

Mast cells are hematopoetically derived cells that reside long-term in barrier tissue sites, such as skin and gut. Mast cells express the high affinity receptor for IgE, FcεRI, and can be activated via antigen-mediated cross-linking of surface IgE (**Figure 3a**). In addition, mast cells express TLRs and respond to many microbial products[94]. In response to IgE-mediated activation, mast cells induce an immediate wheal and flare response (within minutes) by releasing pre-formed granules that contain a wide variety of immunologically active compounds, such as histamine, proteases, proteoglycans and TNF. Mast cells also secrete a number of pro-inflammatory 'late' mediators, such as IL-3, IL-4, IL-5, IL6, IL-8, IL-9, IL-11, IL-13, TNF, CCL3 (also known as MIP-1α), CCL4 (also known as MIP-1β) and CCL2 (also known as MCP1).

Though the participation of mast cells in the development of immune responses against pathogens has been well described (recently reviewed [95]), the importance of mast cells during contact hypersensitivity is quite controversial. Early work found that mast cells were both redundant and required for contact hypersensitivity [96, 97]. More recently, mast cell activation by hapten-specific IgM and complement has been shown to participate in the elicitation phase of contact hypersensitivity[98]. In addition, mast cell activation has been shown to induce migration of skin-resident DCs and to enhance the efficacy of immunizations[99, 100]. The availability of mice that lack mast cells due to mutations in the *kit* gene (that is, B6Kit$^{W\text{-}sh/W\text{-}sh}$ or WBB6F1-Kit$^{W/Wv}$ strains) has greatly facilitated evaluation of mast cell function *in vivo*[101]. These mast cell-deficient mice can be reconstituted with *in vitro*-derived 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 B6Kit$^{W\text{-}sk/W\text{-}sh}$ mice. Reconstitution of these mice with mast cells derived from wild-type mice, but not from TNF-deficient mice, restores contact hypersensitivity[102]. Similarly, contact hypersensitivity to oxazalone is defective in WBB6F1-Kit$^{W/Wv}$ mice as well as in both IgE$^{-/-}$ and IgE receptor-deficient mice[103]. Thus, mast cells, mast cell activation via FcεRI, and mast cell-derived TNF are required for induction of contact hypersensitivity. Intriguingly, contact hypersensitivity to urushiol in B6Kit$^{W\text{-}sh/W\text{-}sh}$ develops normally but fails to appropriately resolve resulting in increased ear swelling compared with wild-type mice several days after challenge. Reconstitution with wild-type, but not IL-10$^{-/-}$ mast cells, rescues the phenotype[104]. 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 *kit* mutant mice is that these mice also have other defects. WBB6F1-Kit$^{W/Wv}$ mice develop macrocystic anemia, have reduced numbers of neutrophils, lack melanocytes and are sterile [105]. Though B6Kit$^{W\text{-}sh/W\text{-}sh}$ 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)[106]. Breeding Mcpt5-Cre mice with inducible-DTR mice, which ubiquitously express a loxP-stop-diphtheria toxin receptor construct, creates a mouse strain in which mast cells can be ablated by injection of diphtheria toxin [107]. Mast cell ablation prior to sensitization or elicitation impairs contact hypersensitivity responses to FITC and oxazalone. In addition, Mcp5-Cre IL-10^{flox} 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, similar to earlier data using urushiol, late contact

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hypersensitivity responses to hapten are increased in B6Kit$^{W\text{-}sk/W\text{-}sh}$ mice but this result is not due to the absence of mast cells, but rather from a previously unrecognized neutrophil defect in B6Kit$^{W\text{-}sk/W\text{-}sh}$ mice.

γδ T cells

Dendritic epidermal T cells (DETC) that express the identical γδ TCR (Vγ5/Vδ 1) 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 hapten during the sensitization stage of contact hypersensitivity. It is unknown whether they directly sense the presence of hapten, but they do respond to stress-induced molecules expressed by keratinocytes; for example, DETCs recognize RAE1 via NKG2D[108]. Several studies have suggested that these cells participate in contact hypersensitivity by modulating the response of αβ T cells[109]. However, TCR δ $^{-/-}$ mice, which lack all γδ T cells, develop normal contact hypersensitivity on a C57BL/6 genetic background. Interestingly, TCRδ $^{-/-}$ mice on either the FVB or NOD genetic backgrounds develop spontaneous dermatitis and enhanced contact hypersensitivity responses that can be rescued by the adoptive transfer of Vγ5/Vδ 1^{+} T cells, but not by transfer of other γδ T cellsthat do not express Vγ5/Vδ 1[110]. Due to a point mutation in a gene involved in the intrathymic positive selection of Vγ5/Vδ 1^{+} cells prior to their migration to the skin, the epidermis of the substrain of FVB mice produced by Taconic Farms (FVBTac mice) lacks Vγ5/Vδ 1^{+} DETCs and is instead populated by Vγ5/Vδ 1^{-} DETCs. DETCs in FVBTac mice are functionally defective and these mice exhibit spontaneous dermatitis and develop exaggerated contact hypersensitivity responses that are similar to those seen in TCR δ −/− mice[111]. Thus, Vγ5/Vδ 1^{+} 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 cells[112].

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 is still being determined. For the small number of contact allergens studied so far, it appears that they are sensed by TLRs and the inflammasome. Since 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 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 in contact with human skin (for example, skin care products). Whether all 2800 of the currently known sensitizing chemicals also trigger inflammation via TLR and NRR pathways remains to be determined[113].

Biographies

Gaspari Biography

Dr. Gaspari is currently the Shapiro Professor and Chairman of the Department of Dermatology at the University of Maryland Baltimore. He attended medical school at Jefferson Medical College, followed by a Dermatology residency at Emory Univeristy. Then, he performed his post-doctoral studies at the Dermatology Branch of the NCI in Bethesda, Maryland. His laboratory studies keratinocyte-lymphocyte interactions and how

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this regulates skin immunity. His laboratory also studies toll-like receptor signaling in the skin and how this influences responses to environmental challenges.

Kaplan Biography

Dr. Kaplan is currently the Zelickson Professor and Assistant Professor of Dermatology at the University of Minnesota, Minneapolis, MN. He completed his MD/PhD training at Washington University in St. Louis followed by training and fellowship at the Yale School of Medicine, New Haven, CT. His laboratory currently studies skin dendritic cells and how they contribute to the generation and regulation of cutaneous adaptive responses.

Igyarto Biography

Dr. Igyarto is currently a Resarch Associate in the department of Dermatology at the University of Minnesota, Minneapolis, MN. He completed his PhD training at Semmelweis University in Budapest. His work currently focuses on examining the mechanisms that determine why different skin DC subsets to promote distinct T-helper phenotypes.

Glossary

hapten	A molecule that can bind antibody but cannot by itself elicit an immune response. Antibodies that are specific for a hapten can be generated when the hapten is chemically linked to a protein carrier that can elicit a T-cell response.
dendritic epidermal T cells	(DETCs). γδ-TCR$^+$ cells localized purely in the epidermis that are present in rodents and cattle, but not in humans. In mice, essentially all DETCs express precisely the same TCR, forming a prototype lymphocyte repertoire of limited diversity.
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, γδT cells and some cytolytic CD8+ αβT cells. The ligands for NKG2D are MHC-class-I-polypeptide-related sequence A (MICA) and MICB in humans, and retinoic acid early transcript 1 (RAE1) and H60 in mice. Such ligands are generally expressed at the surface of infected, stressed or transformed cells.

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20. Martin SF, et al. Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. J Exp Med. 2008; 205:2151–62. [PubMed: 18725520] Comment: This is the first article to suggest that innate immune receptors, in the form of TLR, play an important role in CHS

29. Weber FC, et al. Lack of the purinergic receptor P2X(7) results in resistance to contact hypersensitivity. J Exp Med. 2010; 207:2609–19. [PubMed: 21059855] Comment: This study demonstrated that self-molecules (ATP) released as a result of cellular damage by haptens activates the inflammasome.

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107. Dudeck A, et al. Mast Cells Are Key Promoters of Contact Allergy that Mediate the Adjuvant Effects of Haptens. Immunity. 2011; 34:973–84. [PubMed: 21703544] Comment: This paper demonstrated that the adjuvant effects of haptens requires mast cells and histamine. Mast cell are also required for hapten-induced DC migration to regional lymph nodes and for CHS

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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, edema 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 and 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 2800 chemicals that have the potential to cause allergic contact dermatitis [113]. Patch testing is a standardized, diagnostic procedure that is necessary to confirm allergic contact dermatitis. Potential allergens are applied onto a patient's back skin. 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 allergen avoidance of the offending chemical. Pharmacologic measures, such as topical and 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 utilizes rodents, such as mice. Rather than a series of sub-clinical exposures 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 five to seven 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 ultimately requires antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) and $CD4^+$ helper T cells. Contact hypersensitivity is considered an example of a type IV hypersensitivity reaction (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 this disease.

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Box 2

Essential steps for induction of ACD

Percutaneous penetration of hapten

Haptenization of self proteins

Epidermal and dermal-derived inflammation

Dendritic cell activation and migration from skin to local lymph node

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Box 3

Development of the effector response

Allergic contact dermatitis can be considered a cascade of sequential and parallel events that starts with the innate immune system sensing self-damage that occurs during the haptenization process. This generates an inflammatory response that mobilizes cutaneous DCs to mature, migrate and traffic haptenated self proteins to local lymph nodes for presentation to antigen-specific T cells. As a consequence, naïve $CD4^+$ and $CD8^+$ T cells differentiate in the lymph node into effector T 114 H1, T_H17 and CTL cells . These cells are then recruited back to the skin[115] where CTL mediate apoptosis of keratonicytes[116]. In addition to effectors, regulatory CD4+ Treg develop that inhibit ACD and mediate tolerance in nonallergic individuals[117, 118]. NK cells in mice have been shown capable of maintaining persistent, hapten-specific memory[119]. Although these studies have not been translated to humans, NK cells are present in significant numbers in cellular infiltrates of patients with allergic contact dermatitis[120, 121]. Invariant NKT cells are thought to respond to endogenous glycolipids and secrete cytokines such as IFNγ that help amplify the early innate response[122]. Defective NKT cells or application of antagonist lipids that inhibit NKT cell activation results in reduced contact hypersensitivity in mice[123, 124].

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Summary Bullet points

1. 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. The formation of the hapten–carrier complex generates a neo-antigen that is eventually recognized by the immune system as ‘altered self’.
2. Haptens induce production of endogenous ligands for TLR and NLR pattern receptors that activate an innate immune response and is required for adaptive immune responses to hapten–carrier complex.
3. Keratinocytes and mast cells produce inflammatory cytokines in response to hapten exposure that mobilizes skin-resident DC and recruits effector lymphocytes into the skin which are responsible for immune-mediated inflammatory response associated with ACD.
4. Skin-resident dendritic cells are required for the development of an anti-hapten T cell response.
5. The Langerhans cells subset of skin dendritic cells is not required for the generation of the anti-hapten response but the precise contribution of each dendritic cell subset is unclear.

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Table 1

Comparison on common skin contact allergens.

Allergen	Allergic response	Mechanism(s) of Recognition	Comment
Nickel	Acute contact dermatitis in humans	Engages critical histidine residues in human TLR4. Does not bind mouse TLR4[32]	Most common contact allergen. Barrier disruption increases rate of sensitization[36]
House Dust mite (Der p2, Der f2)	Acute contact dermatitis in humans	Homologues of MD-2, LPS-binding protein that complexes with TLR4[34]	Important skin and respiratory antigen for patients with atopic dermatitis
Dinitrofluorobenzene (DNFB), trinitrochlorobenzene (TNCB).	Contact hypersensitivity in mice	Induce hyaluronic acid degradation products that activate TLR2 and TLR4 and induction of ATP release with inflammasome activation[20, 29]	Commonly used experimental haptens
Fluorescein isothiocyanate (FITC)	Contact hypersensitivity in mice	Unknown	Commonly used vehicle (DBP) induces TSLP that promotes Th2 [50]
Dinitrothiocyanobenzene (DNTB)	Contact hypersensitivity in mice	Weak hapten that does not activate inflammasome[30]	Can induce tolerance to DNFB, a structurally similar hapten[30]
Dimethylbenzanthracene (DMBA)	Contact hypersensitivity in mice	Unknown mode of recognition. In absence of TLR4, contact hypersensitivity is unaffected but the Th1/Th17 cell balance is altered[125]	Carcinogen and contact allergen.

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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 hypersensitivity response
	Epidermal Langerhans cells	Dermal Langerin$^+$ DCs	Dermal Langerin- DCs	CD8$^+$ DCs	
Constitutive systems					
huLangerin-DTA[77]	absent	normal	normal	normal	⇧
huLangerin-Cre[79]x IL-10^{flox} or huLangerin-Cre x I-Ab^{flox}	Functionally defective	normal	normal	normal	⇧
Batf3$^{-/-}$[73]	normal	absent	normal	absent	normal
Inducible systems					
muLangerin-DTR (diphtheria toxin administered day +1)[85], [86, 91]	absent	absent	normal	~25% of DCs present	⇩
muLangerin-DTR (diphtheria toxin administered day ++7/13)[67, 91]	absent	~20-50% of DCs present	normal	normal	normal
huLangerin-DTR (diphtheria toxin administered day +1)[78]	absent	normal	normal	normal	⇧