Chapter 20
Contact Dermatitis

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Abstract Allergic contact dermatitis (ACD) is one of the most common skin diseases, prevailing in 15–20 % of the general population all over the world. It is classified as one type of delayed-type hypersensitivity response, and murine contact hypersensitivity (CHS) is a frequently used and suitable animal model of ACD. During the last decade, new subsets of immune cells, such as regulatory T cells (Tregs) and $CD4^+$ T helper 17 (Th17) cells have been identified both in mouse and human, and the important roles of those cell subsets in ACD have been implicated from the studies of CHS. In addition, the discovery of Langerin-positive dermal dendritic cells (DCs) questioned the relevance of epidermal Langerhans cells as key antigen-presenting cells (APCs) in cutaneous immune responses. We summarize the recent reports of CHS and integrate recent advances into the classic view of CHS, and discuss the updated mechanisms of its development.

20.1 Introduction

Allergic contact dermatitis (ACD), such as metal allergy or plant allergy, is one of the most common skin diseases [1]. ACD is a kind of delayed-type hypersensitivity response, an essential immune response that eliminates the pathogens from the host. The antigens in ACD are usually not pathogens and not harmful to our body. However, once the antigen enters into skin and is recognized as harmful (sensitization phase), our host-defense system tries to eliminate them and provokes inflammation in skin, which is called the elicitation phase. In the sensitization phase, antigen-specific effector T cells are induced in the draining lymph nodes (LNs). In elicitation phase, the effector T cells infiltrate to the skin area where the antigens

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enter, and are activated by their specific antigen, and provoke inflammation in ACD.

The contact hypersensitivity model (CHS) is a classic but easy and appropriate animal model for ACD [2]. By using CHS as a model of ACD, a number of studies have been performed and our knowledge of the immunological mechanisms of ACD has significantly expanded. In the past decade, several kinds of new types of immune cells have been discovered, such as regulatory T cells (Tregs) or langerin positive dermal dendritic cells (DCs). The roles of such new types of cells in ACD have been investigated using CHS, and some of the key dogma in ACD has been changing. In this section, we integrate the recent advancement of immunological mechanisms of both the sensitization and elicitation phases of CHS into the classic view, and discuss updated mechanisms on its development.

20.2   General Protocols of CHS

As this section discusses the mechanisms of ACD mainly based on mouse studies (CHS), we first briefly explain the experimental systems of CHS. For the induction of CHS, dinitrofluorobenzen (DNFB), oxazolone, Trinitrochlorobenzene (TNCB), and fluorescein isothiocyanate (FITC) are generally used as haptens [2]. Although there exist some variations in the protocol of CHS, the basic procedure is as follows. On day 0 (the first day of experiment), mice are painted with hapten (ex. 0.5 % DNFB) on their shaved abdominal skin or ear skin. This is the day of sensitization. Five or 7 days later, the same hapten of less concentration (ex. 0.3 % DNFB) is applied on the ear skin (elicitation), and ear thickness change is evaluated as a parameter of skin inflammation, which peaks at 24–48 h later. Histologically, severe edema and inflammatory cell infiltration are detected in dermis 24–48 h after elicitation, which correspond well with the ear thickness change.

As described above, the experimental system of CHS is quite simple, but well reflects many characteristic features of ACD.

20.3   Mechanisms of Sensitization Phase

20.3.1   *Haptens and Keratinocytes*

Most of the chemicals that induce ACD are small compounds called haptens, which typically have a molecular mass of less than 500. Haptens need to have chemical interactions with proteins to elicit adaptive immune responses [3]. Upon hapten application, keratinocytes are activated and produce various chemical mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and prostaglandin (PG) E2, which promote the migration and maturation of skin DCs

[4–6]. Keratinocytes are activated by haptens through innate immune systems, such as Toll-like receptors (TLRs) and cytosolic NOD-like receptors (NLRs). Among the NLR family, NACHT-, LRR-, and pyrin (NALP) 3 control the production of pro-inflammatory cytokines through activation of caspase-1. Without NALP3 or its adaptor protein ASC, the production of IL-1β and IL-18 from keratinocytes was inhibited, which resulted in impaired DC migration and T cell priming, and led to impaired CHS [7–10]. Innate immune activation is also important for dendritic cell activations.

20.3.2 Roles of Cutaneous DC Subsets in Sensitization

There are at least three subsets of DCs in mouse skin: Langerhans cells in epidermis, langerin$^+$ dermal DCs, and langerin–dermal DCs in dermis. After haptens enter into skin, they are captured by cutaneous DCs, which become mature and migrate to draining lymph nodes (dLNs), and present the antigen to naïve T cells to induce effector T cells. For the activation of DCs in the sensitization phase, both TLR and NOD pathways are required [11]. For example, among TLRs, TLR2 and TLR4 play critical roles for DCs maturation in CHS induced with trinitrochlorobenzene (TNCB), oxazolone (Ox), and fluorescein isothiocyanate (FITC) [12]. Among cutaneous DCs, LCs have long been considered to be central cells for antigen presentation in the sensitization phase of CHS. However, novel depletion systems of LCs (langerin–diphtheria toxin receptor [DTR] knockin mice) have revealed that langerin$^+$ dermal DCs, but not LCs, may play a crucial role in sensitization, because depletion of langerin$^+$ dermal DCs reduced CHS response whereas depletion of LCs did not [13–16]. Furthermore, it has also been reported that LCs play a regulatory role, rather than a stimulatory role, during sensitization in CHS by producing IL-10 [17, 18] or inducing ICOS$^+$Foxp3$^+$Tregs in dLNs [19]. We and others have, however, recently reported that these two populations work in a compensatory manner to initiate sensitization in CHS [20, 21]. Consistently, Batf3-deficient mice that lack langerin$^+$ dDCs exhibited a normal CHS phenotype, suggesting the compensation of its function by other DCs [22].

Thus, although the function of LCs in sensitization remains controversial, langerin$^+$ dDCs probably exert stimulatory effects during sensitization. As for the function of langerin$^-$ dDCs in sensitization, it has not yet been investigated intensively. However, they also appear to have stimulatory functions in sensitization, because ablation of both LCs and langerin$^+$ dDCs prior to sensitization impairs CHS response but is unable to abrogate it completely [13, 20].

20.3.3 Metal Allergy and Innate Immune Activation

Nickel (Ni^{2+}) is one of the most frequent causes of ACD, although it rarely occurs in mice. Earlier studies have reported that coadministration of adjuvant, such as complete Freund's adjuvant or lipopolysaccharide, efficiently induced Ni^{2+} allergy in mice, suggesting the important role of TLR4 signaling in efficient sensitization with Ni^{2+}. Very recently, Schmidt et al. reported that Ni^{2+} directly activates human TLR4 but not mouse TLR4, and that the transgenic expression of human TLR4 in TLR4-deficient mice resulted in efficient sensitization and elicitation to Ni^{2+} [23]. These reports indicate the crucial roles of TLR4 in Ni^{2+} allergy in both mice and humans.

Together, these hapten-induced irritant effects through innate immune systems are essential to the activation of cutaneous APCs and thereby determine the allergenic potential of a hapten during the sensitization phase.

20.3.4 Role of Mast Cells in Sensitization Phase

As the other authors describe this topic in the chapter, "Mast Cells and Basophils in Cutaneous Immune Response," in this book, we just briefly explain this here. The role of mast cells in CHS has been controversial, because conflicting results have been reported in the CHS model using mice lacking mast cells constitutively [24–27]. However, a novel mast cell ablation system was recently established in two independent groups [28, 29]. In these mice, mast cells can be conditionally depleted through the administration of DT. Both groups reported that mice depleted of mast cells exhibited reduced CHS. It has also been revealed that mast cells stimulated DCs via intercellular adhesion molecule (ICAM)-1 or lymphocyte function-associated antigen (LFA)-1 interaction and by membrane-bound TNF- α on mast cells, and stimulated migration/maturation and subsequent T cell priming in the sensitization phase [28].

Overall, it would be very likely that mast cells play stimulatory roles both in the sensitization and elicitation phases in CHS.

20.4 Mechanisms of Elicitation Phase

20.4.1 Antigen Nonspecific Inflammation: Keratinocytes, Mast Cells, and Neutrophil Activation

The mechanism of inflammatory cell infiltration in the elicitation phase has been explained using two different inflammatory signals: antigen nonspecific and antigen specific [2, 30, 31]. Keratinocytes, neutrophils, and mast cells are the main players

to create the antigen nonspecific inflammation at elicitation. First, haptens stimulate keratinocytes to produce pro-inflammatory cytokines such as IL-1β and TNF-α in an NLR-dependent manner [7, 10]. Those cytokines then activate vascular endothelial cells to express adhesion molecules such as ICAM-1 and P/E-selectins, which guide T cells in the blood to transmigrate to tissues. Haptens also increase vascular permeability through mast cell-derived histamin [29], which help neutrophils infiltrate the skin. In addition, haptens activate mast cells and keratinocytes to produce neutrophil-recruiting chemokines, such as CXCL1 and CXCL2, which further contribute to the neutrophil recruitment [25]. The initial neutrophil recruitment is proposed to be essential for subsequent T cell infiltration, because the depletion of neutrophils reduces the CD8^{+} T-cell infiltration and leads to impaired CHS [32, 33]. The aforementioned mechanism creates the first round of antigen nonspecific inflammation, which is an important step for subsequent antigen specific inflammation (Fig. 20.1a).

20.4.2 Antigen Specific Inflammation: T-Cell Activation, CD4^{+} T (Th1/17) Cells, and CD8^{+} T (Tc1/Tc17) Cells

Following the antigen nonspecific inflammation, T-cell–mediated antigen-specific inflammation is initiated. When T cells infiltrate the skin, they are activated by cutaneous APCs, and produce cytokines such as IFN-γ and IL-17. In fact, stable interaction between skin DCs and T cells was observed in live imaging analysis [34] (Fig. 20.1b), and inhibition of CD86 expression by siRNA resulted in reduced inflammation [35], suggesting that effector T cells are activated locally by hapten-
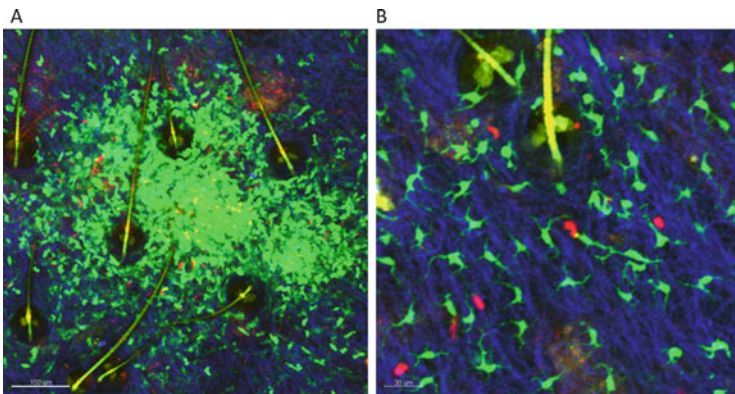
Fig. 20.1 Representative image of neutrophil infiltration and T cells–DCs interaction in skin during elicitation phase. (**a**) Accumulation of neutrophils in dermis 24 h after elicitation (*Green*: neutrophils in Lysm-eGFP mice. *Red*: transferred CD4/CD8 T cells). (**b**) The interaction between T cells and dendritic cells in skin 24 h after elicitation (*Green*: Langerin positive cells in Langerin-GFP mice. *Red*: transferred CD4/CD8 T cells)

carrying APCs. Intriguingly, it has been reported that depletion of skin DCs in hapten-sensitized mice enhanced the effector phase of CHS [36], suggesting the existence of some DC subsets that play a regulatory function in the elicitation phase.

Cytokines produced by activated T cells then stimulate skin resident cells, which lead to further recruitment of T cells and amplify the inflammation. Each T-cell subset (i.e., Th1/Tc1, Th2, Th17/Tc17) activates the skin resident cells differently and forms their specific type of inflammation.

CHS was first considered to be a CD4^{+} T cell-mediated response as a representative of delayed-type hypersensitivity, but it is now recognized that both CD4^{+} and CD8^{+} T cells are important in the elicitation of CHS. CD8^{+} T cells mainly have pro-inflammatory effector functions, whereas CD4^{+} T cells have both pro- and anti-inflammatory functions that are dependent on their cytokine production pattern or subset.

CD4^{+} T helper (Th) cells and CD8^{+} T cytotoxic (Tc) cells can be subdivided into at least three subsets that are relevant for cutaneous immune responses: Th1/Tc1, Th2/Tc2, and Th17/Tc17 cells, respectively. Th1/Tc1 cells are characterized by the secretion of interferon (IFN)-γ; Th2/Tc2 cells by IL-4, IL-5, and IL-13; and Th17/Tc17 cells by IL-17A and IL-22 production. Although there exists some controversy regarding roles of each cytokine in CHS, the general trend is that IFN-γ from Tc1 is the major effector cytokine that provokes inflammation [37], and IFN-γ from Th1 and IL-17 from Th17/Tc17 also contribute for the full development of CHS [38–40]. It remains unknown whether Th2 cells contribute to the development of CHS, but several reports suggest that Th2 cells may also play important roles for the development of CHS in certain situations, depending on mouse strain or haptens [41].

IFN-γ or IL-17 produced by activated T cells induces various chemokine production from keratinocytes. Keratinocytes are an important source of chemokines in skin, and produce multiple chemokines, such as CXCL1, CXCL2, CXCL9, CXCL10, CCL8, CCL17, and CCL27. CXCL10 is a ligand for CXCR3, which are strongly expressed on Th1 cells and regulates their infiltration into skin [42, 43]. The CCL27-CCR10 and CCL17/22-CCR4 axes are another important mechanism for T-cell recruitment to skin [44, 45]. Neutrophil-recruiting chemokines also play important roles, because a blockade of CXCL1 or a deficiency of its receptor (CXCR2) leads to reduced CHS [32, 46].

20.5   Regulation of Inflammation: Regulatory T Cell (Treg)s

Evidence has accumulated regarding the regulatory mechanisms of Tregs in CHS [47]. Transfer of Tregs before elicitation suppresses the ear-swelling response [48], and depletion of endogenous Tregs before sensitization or elicitation enhances CHS response [49–51], indicating that Tregs play essential roles not only for the resolution of inflammation but also the initiation of T-cell priming. Studies of nickel

**Table 20.1** An overview of recently published papers about Tregs and CHS

	Major findings	References
Sensitization	Attenuated sensitization by Treg induced by orally administered antigen in an oral tolerance model	[54]
	Treg attenuate sensitization by modifying DC function through gap junction formation	[55]
	Treg acquire an activated phenotype by means of ATP in draining LNs	[56]
	Enhanced ear swelling response resulting from the depletion of endogenous Treg	[49]
	Enhanced ear swelling response resulting from the depletion of endogenous Treg	[51]
Elicitation	Reduced ear swelling response resulting from the inhibition of the leukocyte influx through IL-10 from Treg	[48]
	Reduced ear swelling response resulting from the inhibition of the leukocyte influx through adenosine from Treg via CD39/CD73 (inhibition of E- and P-selectin expression in endothelial cells)	[53]
	Treg acquire activated phenotype by means of ATP in blood	[56]
	Enhanced and prolonged ear swelling response resulting from depletion of endogenous Treg	[50]
	Prolonged ear swelling response resulting from depletion of endogenous Treg	[51]

allergy illustrated the existence of antigen-specific Tregs in healthy control individuals [52], suggesting the important roles of Tregs for the tolerance to allergens. As for the Tregs suppression mechanisms in CHS, several mechanisms such as IL-10 or CD39/73 dependent pathways are proposed [48, 53]. Table 20.1 summarizes the recent reports of Tregs function in CHS. Moreover, it has been revealed that Tregs display their inhibitory function by recirculating from skin to the draining [50]. As skin is an organ full of Tregs, such inhibitory mechanisms may also work in other skin diseases.

20.6 Conclusion

Taken together, the broad view of CHS development during the sensitization and elicitation phases is summarized in Fig. 20.2 and Table 20.2.

The CHS model has provided us with valuable lessons on the mechanisms of ACD as discussed above. However, there still remains a compelling need to reveal whether such findings in CHS are relevant to human ACD. In addition, recent reports suggest that innate immune cells, such as innate lymphoid cells, macrophages, natural killer T cells, or gamma-delta T cells contribute to the development

Flow of CHS development
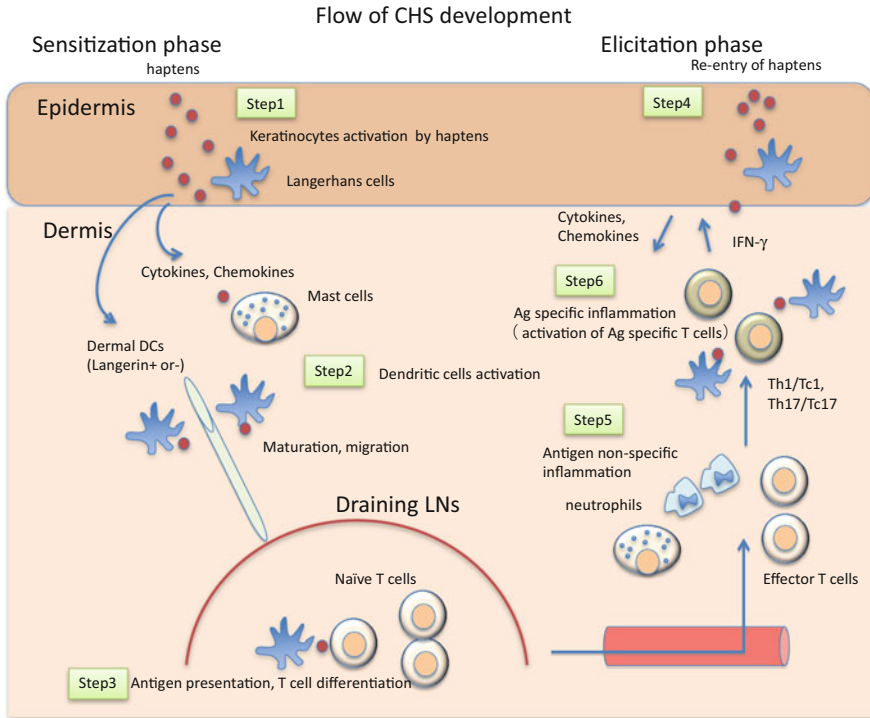
Fig. 20.2 A schematic view of the development of CHS. *Step 1*: Haptens activate keratinocytes (KCs) and mast cells directly or indirectly through innate immune systems. The activated KCs and mast cells produce various chemical mediators, which activate cutaneous DCs. *Step 2*: The activated DCs capture antigens and start maturation and migrate to the dLNs via afferent lymphatics. *Step 3*: Migrated DCs present antigen to naïve T cells in dLNs. Antigen-specific clones differentiate and proliferate into effector T cells. Tregs affect DCs function and play a suppressive role in effector T cell generation. *Step 4*: Upon re-exposure to haptens, KCs and mast cells are activated and produce various chemical mediators, which activate endothelial cells and cause inflammatory cell infiltration, including antigen-specific T cells. *Step 5*: Infiltrated antigen-specific effector T cells are activated and produce pro-inflammatory cytokines and chemokines, which activate KCs and cause further inflammatory cell infiltration. *Step 6*: In addition to effector T cells, Tregs infiltrate inflammatory sites and exert a suppressive function. Some infiltrated Tregs return to dLNs and may contribute to the resolution of inflammation

of many more diseases than previously thought. Investigation of the roles of such cells in CHS would also be important for the understanding of ACD, which may lead to an innovative therapy for allergic skin inflammation.

Table 20.2 Summary of the functions of immune cells in CHS

	Cell subsets	Sensitization phase	Elicitation phase
	Keratinocytes	Initiation of DC migration and maturation via production of TNF-α, IL-1β, IL-18, PGE$_2$	Recruitment of inflammatory cells through chemokine (CXCX1, CXCL2, CCL17, CCL27, CXCL9, CXCL10) production
	Mast cells	Initiation of DC migration and maturation via TNF-α	Endothelial activation and promotion of inflammatory cell infiltration via TNF-α, histamine, CXCL1
	Neutrophils		Production of pro-inflammatory cytokines (TNF-α, etc), which contributes to Ag-nonspecific inflammation and subsequent Ag-specific inflammation
Cutaneous dendritic cells	Langerhans cells	Ag presentation in dLNs to promote or suppress T cell differentiation and proliferation. Peak of migration: around 72–96 h after haptens application	Possible Ag presentation in dLNs and skin
	Langerin$^+$dDCs (approximately 10 % of dDCs)	Ag presentation in dLNs to promote effector T cell differentiation and proliferation. Peak of migration: around 24–48 h after haptens application	
	Langerin$^-$dDCs (approximately 90 % of dDCs)	Ag presentation in dLNs to promote effector T cell differentiation and proliferation	
T cells	Th1/Tc1		IFN-γ production: stimulate KCs to produce chemokines, and amplify the inflammation
	Th2		IL-4 production: stimulate/regulate the inflammation
	Th17/Tc17	Promotion of effector T cell generation by IL-17	IL-17 production: stimulate KCs to produce chemokines and amplify the inflammation
	Treg	Suppression of T cells differentiation and proliferation by inhibiting the function of DCs	Suppression of endothelial cell activation and inhibition of T cell infiltration via IL-10 and/or adenosine degradation through CD39/73

Ag; Antigen

References

1. Peiser M, Tralau T, Heidler J, Api AM, Arts JH, Basketter DA, English J, Diepgen TL, Fuhlbrigge RC, Gaspari AA, Johansen JD, Karlberg AT, Kimber I, Lepoittevin JP, Liebsch M, Maibach HI, Martin SF, Merk HF, Platzek T, Rustemeyer T, Schnuch A, Vandebriel RJ, White IR, Luch A (2012) Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. Current knowledge assembled at an international workshop at BfR, Germany. Cell Mol Life Sci 69(5):763–781. doi:10.1007/s00018-011-0846-8

2. Honda T, Egawa G, Grabbe S, Kabashima K (2013) Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. J Invest Dermatol 133(2):303–315. doi:10.1038/jid.2012.284

3. Lepoittevin JP, Karlberg AT (1994) Interactions of allergenic hydroperoxides with proteins: a radical mechanism? Chem Res Toxicol 7(2):130–133

4. Cumberbatch M, Dearman RJ, Kimber I (1997) Interleukin 1 beta and the stimulation of Langerhans cell migration: comparisons with tumour necrosis factor alpha. Arch Dermatol Res 289(5):277–284

5. Cumberbatch M, Kimber I (1995) Tumour necrosis factor-alpha is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. Immunology 84(1):31–35

6. Kabashima K, Sakata D, Nagamachi M, Miyachi Y, Inaba K, Narumiya S (2003) Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. Nat Med 9 (6):744–749. doi:10.1038/nm872 nm872 [pii]

7. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, Bertin J, Coyle AJ, Galan JE, Askenase PW, Flavell RA (2006) Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity 24 (3):317–327. doi:S1074-7613(06)00140-3 [pii] 10.1016/j.immuni.2006.02.004

8. Antonopoulos C, Cumberbatch M, Dearman RJ, Daniel RJ, Kimber I, Groves RW (2001) Functional caspase-1 is required for Langerhans cell migration and optimal contact sensitization in mice. J Immunol 166(6):3672–3677

9. Antonopoulos C, Cumberbatch M, Mee JB, Dearman RJ, Wei XQ, Liew FY, Kimber I, Groves RW (2008) IL-18 is a key proximal mediator of contact hypersensitivity and allergen-induced Langerhans cell migration in murine epidermis. J Leukoc Biol 83 (2):361–367. doi:jlb.0604352 [pii] 10.1189/jlb.0604352

10. Watanabe H, Gaide O, Petrilli V, Martinon F, Contassot E, Roques S, Kummer JA, Tschopp J, French LE (2007) Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. The Journal of investigative dermatology 127 (8):1956–1963. doi:5700819 [pii] 10.1038/sj.jid.5700819

11. Martin SF, Esser PR, Weber FC, Jakob T, Freudenberg MA, Schmidt M, Goebeler M (2011) Mechanisms of chemical-induced innate immunity in allergic contact dermatitis. Allergy 66 (9):1152–1163. doi:10.1111/j.1398-9995.2011.02652.x

12. Martin SF, Dudda JC, Bachtanian E, Lembo A, Liller S, Durr C, Heimesaat MM, Bereswill S, Fejer G, Vassileva R, Jakob T, Freudenberg N, Termeer CC, Johner C, Galanos C, Freudenberg MA (2008) Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. J Exp Med 205 (9):2151–2162. doi:jem.20070509 [pii] 10.1084/jem.20070509

13. Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, Hogquist KA (2007) Identification of a novel population of Langerin+ dendritic cells. J Exp Med 204 (13):3147–3156. doi:jem.20071966 [pii] 10.1084/jem.20071966

14. Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N, Tripp CH, Douillard P, Leserman L, Kaiserlian D, Saeland S, Davoust J, Malissen B (2005) Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 22 (5):643–654. doi:S1074-7613 (05)00131-7 [pii] 10.1016/j.immuni.2005.04.004

15. Wang L, Bursch LS, Kissenpfennig A, Malissen B, Jameson SC, Hogquist KA (2008) Langerin expressing cells promote skin immune responses under defined conditions. J Immunol 180 (7):4722–4727. doi:180/7/4722 [pii]

16. Poulin LF, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B (2007) The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. J Exp Med 204 (13):3119–3131. doi:jem.20071724 [pii] 10.1084/jem.20071724

17. Kaplan DH, Jenison MC, Saeland S, Shlomchik WD, Shlomchik MJ (2005) Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. Immunity 23 (6):611–620. doi:S1074-7613(05)00349-3 [pii] 10.1016/j.immuni.2005.10.008

18. Igyarto BZ, Jenison MC, Dudda JC, Roers A, Muller W, Koni PA, Campbell DJ, Shlomchik MJ, Kaplan DH (2009) Langerhans cells suppress contact hypersensitivity responses via cognate CD4 interaction and langerhans cell-derived IL-10. J Immunol 183 (8):5085–5093. doi:183/8/5085 [pii] 10.4049/jimmunol.0901884

19. Gomez de Aguero M, Vocanson M, Hacini-Rachinel F, Taillardet M, Sparwasser T, Kissenpfennig A, Malissen B, Kaiserlian D, Dubois B (2012) Langerhans cells protect from allergic contact dermatitis in mice by tolerizing CD8(+) T cells and activating Foxp3(+) regulatory T cells. J Clin Invest 122(5):1700–1711. doi:10.1172/JCI59725

20. Honda T, Nakajima S, Egawa G, Ogasawara K, Malissen B, Miyachi Y, Kabashima K (2010) Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity. J Allergy Clin Immunol 125 (5):1154–1156 e1152. doi:10.1016/j.jaci.2009.12.005

21. Noordegraaf M, Flacher V, Stoitzner P, Clausen BE (2010) Functional redundancy of Langerhans cells and Langerin+ dermal dendritic cells in contact hypersensitivity. J Invest Dermatol 130(12):2752–2759. doi:10.1038/jid.2010.223

22. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, Moon C, Albring JC, Ise W, Michael DG, Bhattacharya D, Stappenbeck TS, Holtzman MJ, Sung SS, Murphy TL, Hildner K, Murphy KM (2010) Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J Exp Med 207 (4):823–836. doi:10.1084/jem.20091627

23. Schmidt M, Raghavan B, Muller V, Vogl T, Fejer G, Tchaptchet S, Keck S, Kalis C, Nielsen PJ, Galanos C, Roth J, Skerra A, Martin SF, Freudenberg MA, Goebeler M (2010) Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. Nat Immunol 11 (9):814–819. doi:10.1038/ni.1919

24. Askenase PW, Van Loveren H, Kraeuter-Kops S, Ron Y, Meade R, Theoharides TC, Nordlund JJ, Scovern H, Gerhson MD, Ptak W (1983) Defective elicitation of delayed-type hypersensitivity in W/Wv and SI/SId mast cell-deficient mice. J Immunol 131(6):2687–2694

25. Biedermann T, Kneilling M, Mailhammer R, Maier K, Sander CA, Kollias G, Kunkel SL, Hultner L, Rocken M (2000) Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. J Exp Med 192(10):1441–1452

26. Galli SJ, Hammel I (1984) Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. Science 226(4675):710–713

27. Mekori YA, Galli SJ (1985) Undiminished immunologic tolerance to contact sensitivity in mast cell-deficient W/Wv and Sl/Sld mice. J Immunol 135(2):879–885

28. Otsuka A, Kubo M, Honda T, Egawa G, Nakajima S, Tanizaki H, Kim B, Matsuoka S, Watanabe T, Nakae S, Miyachi Y, Kabashima K (2011) Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity. PLoS One 6(9):e25538. doi:10.1371/journal.pone.0025538

29. Dudeck A, Dudeck J, Scholten J, Petzold A, Surianarayanan S, Kohler A, Peschke K, Vohringer D, Waskow C, Krieg T, Muller W, Waisman A, Hartmann K, Gunzer M, Roers A (2011) Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. Immunity 34(6):973–984. doi:10.1016/j.immuni.2011.03.028

30. Grabbe S, Schwarz T (1998) Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 19 (1):37–44. doi:S0167569997011869 [pii]

31. Grabbe S, Steinert M, Mahnke K, Schwartz A, Luger TA, Schwarz T (1996) Dissection of antigenic and irritative effects of epicutaneously applied haptens in mice. Evidence that not the antigenic component but nonspecific proinflammatory effects of haptens determine the concentration-dependent elicitation of allergic contact dermatitis. J Clin Invest 98 (5):1158–1164. doi:10.1172/JCI118899

32. Dilulio NA, Engeman T, Armstrong D, Tannenbaum C, Hamilton TA, Fairchild RL (1999) Groalpha-mediated recruitment of neutrophils is required for elicitation of contact hypersensitivity. Eur J Immunol 29 (11):3485–3495. doi:10.1002/(SICI)1521-4141(199911)29:11<3485::AID-IMMU3485>3.0.CO;2-B [pii]

33. Engeman T, Gorbachev AV, Kish DD, Fairchild RL (2004) The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. J Leukoc Biol 76 (5):941–949. doi:10.1189/jlb.0304193 jlb.0304193 [pii]

34. Egawa G, Honda T, Tanizaki H, Doi H, Miyachi Y, Kabashima K (2011) In vivo imaging of T-cell motility in the elicitation phase of contact hypersensitivity using two-photon microscopy. J Invest Dermatol 131(4):977–979. doi:10.1038/jid.2010.386

35. Ritprajak P, Hashiguchi M, Azuma M (2008) Topical application of cream-emulsified CD86 siRNA ameliorates allergic skin disease by targeting cutaneous dendritic cells. Mol Ther 16 (7):1323–1330. doi:mt200891 [pii] 10.1038/mt.2008.91

36. Grabbe S, Steinbrink K, Steinert M, Luger TA, Schwarz T (1995) Removal of the majority of epidermal Langerhans cells by topical or systemic steroid application enhances the effector phase of murine contact hypersensitivity. J Immunol 155(9):4207–4217

37. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H (2009) IL-17 and IFN-gamma mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses. J Immunol 183 (2):1463–1470. doi:jimmunol.0804108 [pii] 10.4049/jimmunol.0804108

38. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H (2006) CD8+ IL-17-producing T cells are important in effector functions for the elicitation of contact hypersensitivity responses. J Immunol 177 (10):6852–6858. doi:177/10/6852 [pii]

39. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 17 (3):375–387. doi:S1074761302003916 [pii]

40. Mori T, Kabashima K, Yoshiki R, Sugita K, Shiraishi N, Onoue A, Kuroda E, Kobayashi M, Yamashita U, Tokura Y (2008) Cutaneous hypersensitivities to hapten are controlled by IFN-gamma-upregulated keratinocyte Th1 chemokines and IFN-gamma-downregulated langerhans cell Th2 chemokines. The Journal of investigative dermatology 128 (7):1719–1727. doi:jid20085 [pii] 10.1038/jid.2008.5

41. Yokozeki H, Ghoreishi M, Takagawa S, Takayama K, Satoh T, Katayama I, Takeda K, Akira S, Nishioka K (2000) Signal transducer and activator of transcription 6 is essential in the induction of contact hypersensitivity. J Exp Med 191(6):995–1004

42. Nakae S, Komiyama Y, Narumi S, Sudo K, Horai R, Tagawa Y, Sekikawa K, Matsushima K, Asano M, Iwakura Y (2003) IL-1-induced tumor necrosis factor-alpha elicits inflammatory cell infiltration in the skin by inducing IFN-gamma-inducible protein 10 in the elicitation phase of the contact hypersensitivity response. Int Immunol 15(2):251–260

43. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD (2002) IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol 168(7):3195–3204

44. Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bunemann E, Lehto M, Wolff H, Yen D, Marxhausen H, To W, Sedgwick J, Ruzicka T, Lehmann P, Zlotnik A (2002) CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med 8 (2):157–165. doi:10.1038/nm0202-157 nm0202-157 [pii]

45. Reiss Y, Proudfoot AE, Power CA, Campbell JJ, Butcher EC (2001) CC chemokine receptor (CCR)4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lympho-cyte trafficking to inflamed skin. J Exp Med 194(10):1541–1547
46. Cattani F, Gallese A, Mosca M, Buanne P, Biordi L, Francavilla S, Coletti G, Pellegrini L, Melillo G, Bertini R (2006) The role of CXCR2 activity in the contact hypersensitivity response in mice. Eur Cytokine Netw 17(1):42–48
47. Honda T, Miyachi Y, Kabashima K (2011) Regulatory T cells in cutaneous immune responses. J Dermatol Sci 63(2):75–82. doi:10.1016/j.jdermsci.2011.06.004
48. Ring S, Schafer SC, Mahnke K, Lehr HA, Enk AH (2006) CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. Eur J Immunol 36(11):2981–2992. doi:10.1002/eji.200636207
49. Honda T, Otsuka A, Tanizaki H, Minegaki Y, Nagao K, Waldmann H, Tomura M, Hori S, Miyachi Y, Kabashima K (2011) Enhanced murine contact hypersensitivity by depletion of endogenous regulatory T cells in the sensitization phase. J Dermatol Sci 61(2):144–147. doi:10.1016/j.jdermsci.2010.11.001
50. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, Waldmann H, Hori S, Cyster JG, Watanabe T, Miyachi Y, Kanagawa O, Kabashima K (2010) Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. J Clin Invest 120(3):883–893. doi:10.1172/JCI40926
51. Lehtimaki S, Savinko T, Lahl K, Sparwasser T, Wolff H, Lauerma A, Alenius H, Fyhrquist N (2012) The temporal and spatial dynamics of Foxp3+ Treg cell-mediated suppression during contact hypersensitivity responses in a murine model. J Invest Dermatol 132(12):2744–2751. doi:10.1038/jid.2012.212
52. Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G (2003) Human CD25 + regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. J Immunol 171(11):5760–5768
53. Ring S, Oliver SJ, Cronstein BN, Enk AH, Mahnke K (2009) CD4+CD25+ regulatory T cells suppress contact hypersensitivity reactions through a CD39, adenosine-dependent mechanism. J Allergy Clin Immunol 123 (6):1287–1296 e1282. doi:S0091-6749(09)00491-6 [pii] 10.1016/j.jaci.2009.03.022
54. Dubois B, Chapat L, Goubier A, Papiernik M, Nicolas JF, Kaiserlian D (2003) Innate CD4 +CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. Blood 102(9):3295–3301. doi:10.1182/blood-2003-03-0727
55. Ring S, Karakhanova S, Johnson T, Enk AH, Mahnke K (2010) Gap junctions between regulatory T cells and dendritic cells prevent sensitization of CD8(+) T cells. J Allergy Clin Immunol 125 (1):237–246 e231–e237. doi:10.1016/j.jaci.2009.10.025
56. Ring S, Enk AH, Mahnke K (2010) ATP activates regulatory T Cells in vivo during contact hypersensitivity reactions. J Immunol 184(7):3408–3416. doi:10.4049/jimmunol.0901751