

REVIEW ARTICLE

Immunological mechanisms of contact hypersensitivity in mice

ANNE DEEN CHRISTENSEN^{1,2} and CLAUS HAASE¹

¹Experimental Immunology, Department of Immunopharmacology, Novo Nordisk A/S, Måløv; and
²Department of Veterinary Disease Biology, Section of Biomedicine, Faculty of Life Sciences, University
of Copenhagen, Frederiksberg, Denmark

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Contact hypersensitivity (CHS) is an animal model in which the immunological mechanisms of allergic contact dermatitis (ACD) in humans can be studied but is also widely used in the study of many basic immunological mechanisms. In CHS, a pre-sensitized animal is re-exposed to an antigen, thereby eliciting an immunological reaction at the site of antigen exposure. CHS consists of two phases: sensitization and elicitation phase. In the sensitization phase, the first contact of the skin with a hapten leads to binding of the hapten to an endogenous protein in the skin where they form hapten-carrier complexes which are immunogenic. The hapten-carrier complex is taken up by Langerhans cells (LCs) and dermal dendritic cells (dDCs) which migrate from the epidermis to the draining lymph node. Here, they present the haptenated peptides to naive T cells which are subsequently activated. The newly activated T cells proliferate and migrate out of the lymph node and into circulation. In the elicitation phase, re-exposure of the skin to the hapten activates the specific T cells in the dermis and triggers the inflammatory process responsible for the cutaneous lesions. Originally CHS was regarded as being solely driven by T cells but recently other cell types such as B1 cells, natural killer (NK) T cells and NK cells have shown to mediate important functions during the response as well. Here, we have described the molecular and cellular pathways in the development of CHS and have focused on recent advances and novel knowledge in the understanding of the immunoregulatory mechanisms involved in CHS.

Key words: Contact hypersensitivity; haptens; antigen-presenting cell; T cell; CD8 T cell; CD4 T cells.

Anne Deen Christensen, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.
e-mail: adc@novonordisk.com

Contact hypersensitivity (CHS) is traditionally considered as an animal model in which the immunological mechanisms of allergic contact dermatitis (ACD) in humans can be studied. Additionally, CHS is also one of the most intensively studied *in vivo* models of immune activation and the regulation hereof. It is therefore an

animal model where many immunological mechanisms are involved and the model is used in a much wider sense than for the investigation of ACD. It provides the option of studying many aspects of the immune system and basic immunological mechanisms *in vivo*, which is relevant for a whole range of inflammatory diseases and not only for ACD. In the following, a sequential and chronological overview of the CHS reaction in mice will be described from

start to end, including the different cell types and effector molecules playing a role in the different phases. This will hopefully provide the reader with a comprehensive overview of the entire reaction together with a general impression of the basic immune mechanisms involved.

Classically, acute CHS is divided into two distinct phases. The first phase is called the *sensitization phase* and is terminated after the priming of hapten-specific T cells. As described below, haptens are low molecular weight chemicals, which are non-immunogenic by themselves but upon binding to proteins – which in the skin usually implies epidermal proteins – form strongly immunogenic hapten-carrier complexes. In the CHS model, the sensitization phase is initiated by painting of a sensitizing chemical on the abdominal skin of a mouse (1). Subsequently, the hapten-carrier complex is taken up by Langerhans Cells (LCs) and dermal dendritic cells (dDCs) and is expressed as haptenated peptides in the antigen-binding groove of MHC classes I and II molecules on the cell surface (2). When the skin is exposed to the hapten, LCs start to migrate from the epidermis to the draining lymph node (dLN) where the hapten/protein/MHC complex is presented to naive T cells. The newly activated T cells proliferate and migrate out of the dLN to the bloodstream where they re-circulate between the lymphoid organs and the skin. The second phase is initiated after re-exposure of the skin to the hapten and is known as the *elicitation phase*. In the CHS model in mice, this phase involves application of the sensitizing chemical to a previously unexposed site (such as the ear); however, in some protocols the same skin area is used for both sensitization and elicitation. Subsequently, the hapten-specific recall response can be measured during subsequent days as an increased ear thickness due to inflammation, vasodilation and edema (1). The elicitation response can be divided in two phases; the early phase (approximately the first 2 h after challenge) and the late phase (approximately 24 h after challenge) (3). The early phase is thought to involve activation of complement C5/C5a (4–7), activation of skin mast cells (3, 8) and local platelets (9) and their release of vasoactive and inflammatory mediators such as serotonin (3, 9) and tumor necrosis factor α (TNF α) (10). During this phase,

neutrophils but also antigen-specific T cells are recruited into the challenged skin. The late phase is characterized by infiltration of effector T cells and it is shown that when one or more of the pathways that are important during the early phase are blocked, infiltration of effector T cells during the late phase is inhibited (11, 12). The effector T cells produce type 1 cytokines such as interferon γ (IFN γ) which subsequently mediates further cellular infiltration together with the characteristic edema (13). The ear-swelling response peaks after 24–48 h and dampening of the response is mediated by recruitment of immunoregulatory cells (14). It is generally believed that the response is driven mainly by CD8 T cells and down-regulated by CD4 T cells (13–20) but that a minor subset of CD4 T cells (Th1) can act as effector cells as well (15, 21, 22). Furthermore, a role for B-1 cells (4–6, 8, 23–26), natural killer (NK) cells (27, 28) and natural killer T (NKT) cells (8, 25, 29–32) have been demonstrated in the CHS response. The major events involved in the CHS response are illustrated in Fig. 1, which represents a simplified version of the complex immunological process. The cellular components, the interactions between cells, including cytokines and chemokines and other mechanisms will be discussed in details in the next section. The cytokines, chemokines, receptors, adhesion molecules and other immunological pathways involved in the CHS response are listed in Tables 1–5, respectively.

Haptens

As described above, the initiating event in CHS is the first contact of the skin with a hapten. Haptens are a class of low molecular weight (<1000 Da) compounds that include a limited number of sensitizers used in animal models of CHS, such as dinitrofluorobenzene (DNFB), oxazolone, fluorescein isothiocyanate (FITC) as well as trinitrochlorobenzene (TNCB) and thousands of haptens responsible for human ACD. Nearly all haptens are electrophilic molecules, which can bind covalently with nucleophilic residues of cutaneous proteins and because of their small size and lipophilic nature, they are able to penetrate the skin. Even though haptens are

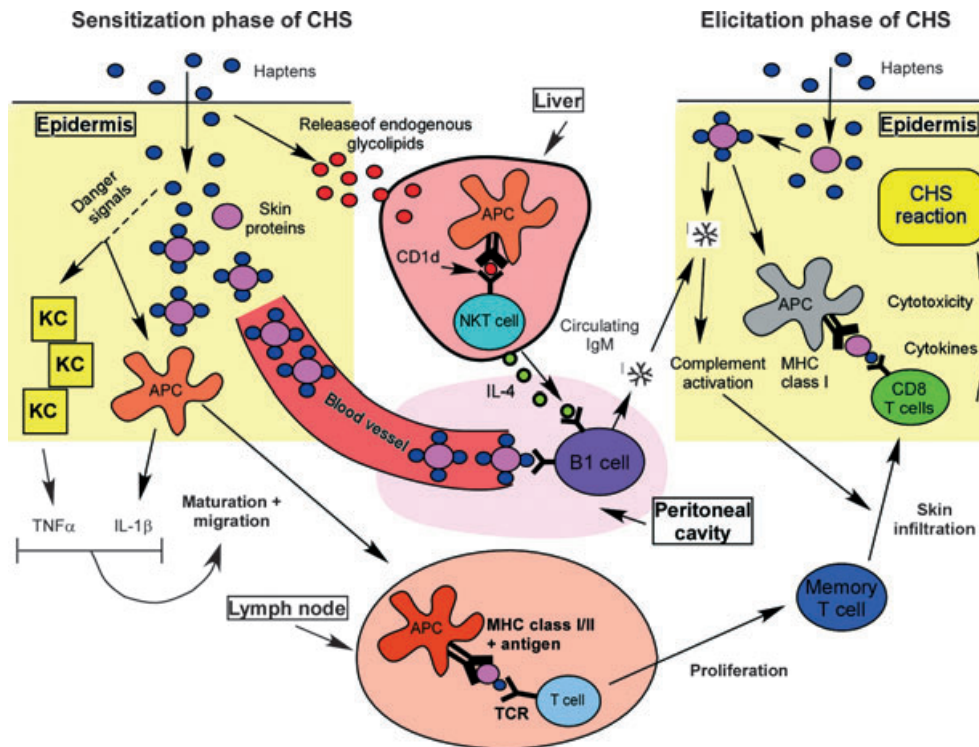


Fig. 1. Illustration of the sensitization and elicitation phase of contact hypersensitivity (CHS). In the first step of the sensitization phase, haptens applied to the skin interact with epidermal proteins to form hapten-protein complexes. These are taken up by antigen-presenting cells (APCs; LCs and dDCs) and presented on MHC class I or II. Furthermore, the hapten application induces a danger signal that activates KCs after which they release cytokines such as $TNF\alpha$ and $IL-1\beta$. Together with APC-derived $IL-1\beta$, they promote APC maturation and migration to the dLN. In the dLN, APCs activate antigen-specific T cells, which divide and ultimately become memory T cells. The cutaneous application of hapten further leads to the release of endogenous glycolipids, which are presented by CD1d-bearing APCs in the liver to NKT cells leading to release of $IL-4$. Simultaneously, hapten-protein complexes are released from the sensitization site into circulation and transferred to the peritoneal cavity. In the presence of $IL-4$ and hapten-protein complexes, B-1 cells in the peritoneal cavity are activated and migrate to the spleen and lymph nodes where they release circulating IgM. In the elicitation phase, IgM interacts with the hapten-protein complex and induces complement-activation, which leads to the release of inflammatory and chemotactic mediators from mast cells and endothelial cells. Finally, antigen-specific CD8 T cells migrate to the site of elicitation and interact with local APCs resulting in release of inflammatory cytokines as $IFN\gamma$ and cell-mediated cytotoxicity. APCs, antigen-presenting cells; NKT cells, natural killer T cells; KCs, keratinocytes; TCR, T-cell receptor.

very diverse, they share an important common feature: they are not immunogenic by themselves due to their small size. However, by binding to proteins they form a hapten-carrier complex which is immunogenic (33, 34). In addition to this, haptens must also possess pro-inflammatory properties, which imply that they are capable of activating the innate immune response in the skin and thereby deliver activation signal which mediate the recruitment, migration and maturation of LCs and dDCs (35–38).

INITIATION PHASE

The role of antigen-presenting cells

The initiating (or sensitization) phase is initiated upon the first contact between the skin and a hapten. Subsequently, the hapten interacts with epidermal proteins to form hapten-protein complexes which are taken up by antigen-presenting cells (APCs). The current hypothesis is that LCs are the main APCs in CHS but that other APCs can participate in the antigen presentation as

Table 1. Cytokines involved in the contact hypersensitivity (CHS) response in mice. For discussion, see text

Cytokine	Principal function in CHS	
IL-1 β ¹	Important for LC migration	IL-1 β KO – reduced response (127, 166) Anti-IL-1 β mAb – reduced response (54) Administration of IL-1 receptor antagonist (IL-1Ra) – reduced response (48, 167) IL-1 receptor KO – reduced response (48) Anti-IL-2 – increased response (150) IL-2 KO – unchanged response (92)
IL-2	T-cell proliferation	
IL-4	Stimulates activated B- and T-cell proliferation and the differentiation of CD4 ⁺ T cells into Th2 cells	<i>Anti-inflammatory:</i> Anti-IL-4 in TNCB-treated mouse – increased response (161) Injection of IL-4 in TNCB-treated mouse – reduced response (168) <i>Proinflammatory:</i> Anti-IL-4 in TNCB-treated mouse – reduced response (165) IL-4 KO treated with TNCB (162), DNFB (163) and DNFB (164) – reduced response <i>No effect:</i> IL-4 KO treated with oxazolone – unchanged response (162;167)
IL-6 ¹	T-cell polarization	IL-6 KO – reduced response (92)
IL-10	Anti-inflammatory cytokine B-cell proliferation	IL-10 injection prior to hapten challenge – reduced response (157, 169) IL-10 KO – increased response (158) and enhanced migration of LC (170) Anti-IL-10 – increased response (157)
IL-12 ¹	Drives polarization of TH1 cells	IL-12 p40 KO – unchanged response (81) IL-12 receptor β 2 KO – unchanged response (35) Anti-IL-12 mAb – reduced response (90, 91)
IL-15	Lymphocyte homeostasis NK cell survival	Overexpression of IL-15 by KCs – increased response (171) IL-15 receptor α KO – reduced response (172)
IL-17	Proinflammatory cytokine, regulate infiltration of leukocyte into inflammatory sites	IL-17A KO – reduced response (101) Anti-IL-17 mAb – reduced response (102) IL-17 receptor KO – reduced response (18)
IL-18	Important for LC migration	IL-18 KO – reduced response (58)
IL-20	Belonging to IL-10 family	IL-20 receptor 2 KO – increased response (173)
IL-21	Implicated in Th17 differentiation Regulatory function on APCs	DC incubation with IL-21 blocks their ability to induce CHS after transfer (174) IL-21 receptor KO – reduced response (175)
IL-33	Induces helper T cells, mast cells, eosinophils and basophils to produce type 2 cytokines	IL-33 receptor (ST2) KO – reduced response (176) IL-33 injection – increased response (176)
IFN γ	Effector molecule during the elicitation phase Potentiates inflammatory response of APCs	IFN γ receptor KO – reduced cell infiltration (143) and reduced response (18)
TNF α ¹	Proinflammatory cytokine, important for LC migration	TNF α receptor p75 KO – reduced response (177) TNF α receptor p55 KO – reduced response (178) Anti-TNF α mAb – reduced response (54, 55) TNF α KO – reduced response (127)

LC, Langerhans cell; KO, knock-out; TNCB, trinitrochlorobenzene; DNFB, dinitrofluorobenzene; KCs, keratinocytes; IFN γ , interferon γ ; APC, antigen-presenting cell.

¹marks molecular targets for marketed anti-inflammatory drugs.

Table 2. Chemokines and their receptors involved in the contact hypersensitivity (CHS) response in mice. For discussion, see text

Chemokine	Receptor	Principal function in CHS	Effect in the CHS model
CCL3 (MIP-1 α) CCL4 (MIP-1 β) CCL5 (RANTES)	CCR1 and CCR5	Retention of LCs in the skin under normal conditions	Not directly investigated in CHS CCR1 and CCR5 are downregulated on DCs when stimulated with TNF α and IL-1 β (59)
CCL19 (MIP-3 β , ELC)	CCR7	Expressed by DCs in the paracortex Guides LCs to the T-cell area of the dLN	Increased gene-expression of CCL19 and CCL21 in the tissue after challenge (123)
CCL21 (SLC)		Expressed by high-endothelial-venules in the LN. Directs the LCs to the LN	CCR7 KO – abolished response (66) Anti-CCL21 mAb – reduced response (85)
CCL17/CCL22	CCR4	Direct CCR4 ⁺ T cells to the site of inflammation	Simultaneous administration of anti-CCL17-, anti-CCL22- and anti-CCL27 mAb treatment – reduced response to oxazolone (129) Expression of CCL17 and CCL22 shown in the tissue after oxazolone challenge (129, 130)
CXCL10 (IP-10)	CXCR3	Directs CXCR3 ⁺ T cells to the site of inflammation	Anti-CXCL10 mAb – reduced response to TNCB (127) CXCR3 and CXCL10 expression is shown in the tissue after DNFB challenge (122)
CCL27	CCR10	Homing receptor of memory T cells to the skin	CCL27 KO – unchanged response (179)
CXCL1 (Gro α)	CXCR2 (neutrophils)	Recruitment of neutrophils	CXCR2 KO – reduced response (111)

KO, knock-out; TNCB, trinitrochlorobenzene.

well. Nevertheless, the following section reflects the difficulty in precisely defining the role of different subsets of APCs and describes how different conclusions based on different experimental set-ups have been drawn.

The skin hosts a network of specialized APCs which are all related to classical dendritic cells (DCs). They detect exogenous substances that have penetrated the epithelial barrier and bring this information to the dLN. One of the major DC subsets in the skin are the LCs, which can capture haptens that have penetrated the skin barrier very efficiently due to their multiple dendritic processes and organization in an epidermal cellular network. Furthermore, LCs are active phagocytes and are phenotypically

characterized by the presence of the intracellular organelles known as Birbeck granules. These granules are thought to be an endosomal recycling compartment and their development within LCs are dependent on Langerin (CD207), which is an endocytic receptor expressed on the surface of LCs (39, 40).

The role of LCs in the development of CHS has recently been studied by the use of different approaches. Firstly, naive mice could be sensitized by injection of either epidermal cells or purified LCs sensitized *in vitro*, but injection of total epidermal cells depleted of LCs was not able to induce sensitization (41). Secondly, the role of LCs has been studied using knock-in (KI) mice in which diphtheria toxin receptor

Table 3. Receptors and their ligands involved in the contact hypersensitivity response in mice. For discussion, see text

Receptor	Ligand	Principal function in CHS	Effect in the CHS model
CD25	IL-2	Alpha-chain of the IL-2 receptor	Anti-CD25 (PC61) – increased response (150, 156) Suppressed response upon transfer with CD4 ⁺ CD25 ⁺ Tregs (149, 154)
CD40	CD154 on T cells	Migration of LC Co-stimulation during activation of CD8 T cells	CD154 KO – reduced response (78) CD154 KO – unchanged response (82) Anti-CD154 mAb – reduced response (80) Agonist anti-CD40 mAb – increased response (79)
CD69	–	Lymphocyte activation marker	CD69 KO – reduced response (180)
CD80 (B7-1) ¹ CD86 (B7-2) ¹	CD28 or CTLA-4 on T cells	Co-stimulation during T-cell activation when binding to CD28, down-regulation of response when binding to CTLA-4	Anti-CD80 mAb – unchanged response (68, 75) Anti-CD86 mAb – reduced response (68, 75) CD28 KO – abolished response (76) CTLA4-Ig treatment – abolished response (94) Anti-CTLA-4 mAb – increased response (152)
E-cadherin	E-cadherin	Retainment of LCs within the epidermis	TNF α and IL-1 β have shown to downregulate E-cadherin on DCs <i>in vitro</i> (60)
OX40L	OX40 (CD134) expressed by activated T cells	Co-stimulation during T-cell activation	OX40L KO – reduced response (181)
P2X7	ATP	Activates LCs	P2X7 KO – abolished response (48)
TLR 2 and 4	TLR 2: variety of microbial components TLR4: LPS	Activates LCs	TLR2 KO – reduced response (35, 182) TLR4 KO – unchanged response (35) TLR2/TLR4 KO – abolished response (35)

¹marks molecular targets for marketed anti-inflammatory drugs.

LC, Langerhans cell; KO, knock-out; TLR, toll-like receptors; LPS, lipopolysaccharide.

(DTR) is inserted into the Langerin locus thereby allowing Langerin⁺ cells to be ablated by administration of diphtheria toxin (DT). Interestingly, conflicting results were derived from experiments using this specific LC-depleted animal; one group obtained a reduced CHS response (42, 43), whereas another group gained an unmodified response (44). In addition, contradictory results were obtained from experiments using bacterial artificial chromosome (BAC)-transgenic mice in which the DT

subunit A (DTA) is cloned into the human Langerin locus, resulting in mice devoid of LCs. Here, absence of LCs resulted in an increased response, which suggests that LCs might also serve a regulatory function (40). These data were confirmed by Igyarto et al. who also showed that BAC-transgenic mice lacking LCs developed exaggerated CHS (45). Furthermore, a study has suggested that in the transgenic BAC mouse only the epidermal Langerin⁺ cells are depleted and not Langerin⁺ cells in the

Table 4. Adhesion molecules involved in the contact hypersensitivity (CHS) response in mice. For discussion, see text

Adhesion molecule	Ligand	Principal function in CHS	Effect in the CHS model
ICAM-1 (CD54)	LFA-1 (CD11a:CD18) on T cells	LC migration and T-cell activation	Anti-ICAM-1 mAb - reduced response (183) ICAM-1 KO – reduced response (184, 185)
$\alpha 6$ -integrin	Laminin in the basement membrane	LC migration by regulating access across the epidermal basement membrane	Anti- $\alpha 6$ -integrin mAb – reduced migration of LC after oxazolone application (62)
CD44	Hyaluronic acid, collagen, others	LC migration	CD44 KO mice – unchanged response (186) Anti-CD44 mAb – unchanged response (187)
LFA-3 (CD58) ICAM-2 (CD102)	LFA-2 (CD2) on T cells LFA-1 (CD11a:CD18) on T cells	Facilitate T cell-LC interaction	Not directly investigated in the CHS response
DC-SIGN (CD209) P-selectin (CD62P) E-selectin (CD62E)	ICAM-3 (CD50) on T cells PSGL-1 on lymphocytes CD43 and PSGL-1 on T cells	Mediates rolling interaction of lymphocytes on the endothelia	Upregulated during T-cell-LC interaction (72) P- and E-selectin KO – reduced response (11) Simultaneous administration of anti-P-selectin and anti-E-selectin mAb – reduced response (187, 188) P-selectin KO – reduced response (189) CD43 and PSGL-1 double KO – reduced response (104)
L-selectin (CD62L)	CD34 on endothelial cells	Homing receptor to lymphoid tissue	L-selectin KO – reduced response (185)
VLA-4 ¹ ($\alpha 4$: $\beta 1$ integrin) $\beta 7$ integrin	VCAM-1 on activated endothel VCAM-1 and MadCAM-1 on activated endothel	Leukocyte extravasation Leukocyte extravasation – mainly described in gut-associated lymphoid tissue	Anti-VLA-4 mAb – reduced response (187) $\beta 7$ integrin KO – reduced response (190)

LC, Langerhans cell; LFA, leukocyte function-associated antigen; ICAM-1, intercellular adhesion molecule-1; PSGL, P-selectin glycoprotein ligand; VCAM, vascular cell adhesion molecule; KO, knock-out.

¹marks molecular targets for marketed anti-inflammatory drugs.

dermis and dLN (39). The conflicting results most likely rely on the fact that Langerin is not a marker exclusively expressed on LCs but also on various subtypes of dermal DCs (39), making the interpretation of the results difficult.

Dermal DCs (dDCs) comprise another subset of DCs in the skin that participates in antigen uptake (39). The dDCs are phenotypically distinct from the LCs but share the ability to stimulate T cells (46) and it has been suggested that in the absence of epidermal LCs, dDCs can mediate sensitization in the CHS response (39,

42). Classically, it was believed that Langerin was exclusively expressed on LCs but recently a Langerin⁺ subtype of dDCs has been described. This study showed that when both LCs and Langerin⁺ dDCs are depleted using DTR KI mice and subsequently administration of DT, the CHS response is abrogated. In contrast, in the transgenic BAC, DTA mice that lack only epidermal LCs but have normal numbers of dDCs, the CHS response is unaffected. This suggests that Langerin⁺ dDCs can mediate sensitization in the absence of LCs (39). The following

Table 5. Other pathways involved in the contact hypersensitivity (CHS) response in mice. For discussion, see text

Other	Ligand	Principal function in CHS	Effect in the CHS model
MMP3, -9 and -19		Extra-cellular, matrix-degrading enzymes	MMP3 KO – reduced response (191) MMP9 KO – increased response (191) MMP19 KO – abrogated response (192)
C5a	C5aR	Anaphylatoxin; causing the release of histamine from mast cells Leukocyte chemoattractant especially for neutrophils	Anti-C5a – reduced response (5) C5aR KO – reduced response (6) C5 KO – reduced response (7)
Prostaglandin E2	EP1-4 expressed on LC	Activates LCs	EP4 KO – reduced response (49)

LC, Langerhans cell; KO, knock-out.

discussion will describe LCs as the main APCs involved in CHS even though dDCs probably contribute to antigen-presentation as well (39;42). In addition to LCs and dDCs, keratinocytes (KCs), mast cells and infiltrating macrophages may function as APCs as well (47).

Interactions between langerhans cells and keratinocytes

An important aspect of the sensitization phase of CHS is the cross-talk between LCs and KCs. The interaction between LCs and KCs is important in the first hours following primary application of the hapten where LCs migrate and mature into professional APCs. The interaction is the result of the danger signals caused by the pro-inflammatory properties of the hapten and these signals affect both KCs and LCs. The danger signals are thought to comprise fragments of hyaluronic acid (HA) from the extracellular matrix (35;48), extracellular ATP (48), prostaglandin E2 (49) or a cellular redox imbalance in LCs (50). HA is a ligand for the toll-like receptors (TLR) and it has been demonstrated that activation of TLR-4 on the APC by HA leads to stimulation of intracellular signal transduction pathways including NF- κ B or MAP kinases which in the end leads to activation of the APC (48). The importance of TLRs is further demonstrated by the observation that TLR2/TLR4 double-deficient mice are resistant to CHS (35). Additionally, it is shown that haptens mediate upregulation of CD80 and CD86 and

expression of TNF α and IL-1 β (52, 53) and in this activation of the APC a cellular redox imbalance, induced by the hapten, is shown to play a role (50). Beside TLRs and redox imbalance, a role for purinergic receptor P2X7 has been suggested. P2X7 is an ATP receptor, expressed on many cell types and important in the release of IL-1 β . It is shown that lack of P2X7 leads to resistance to CHS and that application of the hapten TNCB leads to ATP release in the skin (51). This study confirms the importance of extracellular ATP in the CHS response and its role as a danger signal. The role of danger signals and how haptens activate the innate immune response are under intense investigation and most likely more mediators and pathways will be described in the coming years.

Ultimately, danger signals from the hapten lead to production of TNF α and IL-1 β from KCs and LCs, respectively (54–56). IL-1 β performs two functions: it acts on local KCs to produce more TNF α and it works in an autocrine fashion on LCs to deliver one of two signals necessary for their migration (57). The other signal is delivered by TNF α and both IL-1 β and TNF α appear to be required for the migration of LCs to the dLN during sensitization (54). In addition, IL-18 has been suggested to play a role in LC migration upstream of the cytokines IL-1 β and TNF α (58). Furthermore, IL-1 β and TNF α possess a central role as they inhibit expression of certain chemokine receptors such as CCR1 and CCR5 on the LC cell membrane.

Ligands for CCR1 and CCR5 are inflammatory chemokines as CCL3 (MIP-1 α), CCL4 (MIP-1 β) and CCL5 (RANTES) that normally ensure retention of LCs in the skin (59). Activation of LCs is also associated with up-regulation of the chemokine receptor CCR7 (59) and loss of E-cadherin on the LC cell membrane (60). E-cadherin on the LC binds to E-cadherin expressed on the KCs and in that way represents the 'glue' that under normal circumstances retains LCs within the epidermis (60). Instead, expression of adhesive molecules such as intercellular adhesion molecule-1 (ICAM-1/CD54) (61), $\alpha 6$ integrin (62) and CD44 (63) are upregulated which permit interaction between LCs and the extracellular matrix, thereby allowing migration. Collectively, the induced changes in expression of adhesion molecules give the LCs the ability to free themselves from the epidermis and to interact with the cells and the extracellular matrix proteins necessary for moving from the skin to the dLN. To initiate the migration and reach the lymphatic system, the LCs also need to cross the dermo-epidermal junction and the dermal tissue matrix. In response to IL-1 β and TNF α , LCs secrete different enzymes, such as matrix metallo-proteinase (MMP) 3 and 9 that cleave the macromolecules at the dermo-epidermal junction (64). When the LC has

crossed the dermis and reached the lymphatic vessels, migration is mediated by the expression of CCR7 which directs the LCs to the CC chemokine ligand 21 (CCL21/SLC) – expressed in high-endothelial-venules (HEV) of the lymph nodes (65). The importance of CCR7 is illustrated by the observation that CCR7 knock-out (KO) mice fail to develop a CHS response (66). In the lymph node, another CCR7-ligand – CCL19 (MIP-3 β) – is expressed in the paracortex and further guides LCs to the final position within the T cell area of the dLN (67). CCL21/CCL19-CCR7 interactions are therefore critically important for the sensitization phase. The changes are illustrated in Fig. 2 (see also Tables 1 and 2).

During migration, LCs undergo a classical maturation-process which transforms them into professional APCs. The maturation is closely linked to migration and the same factors influence both processes. Before maturation and migration, LCs are highly endocytic and therefore very efficient at antigen capture but – at the same time – retain most of their MHC molecules intracellularly. Signals from TNF α and IL-1 β change immature LCs to professional APCs by inducing elevated membrane levels of MHC class II and I, decreasing the expression of Fc-receptors and blocking the intracellular

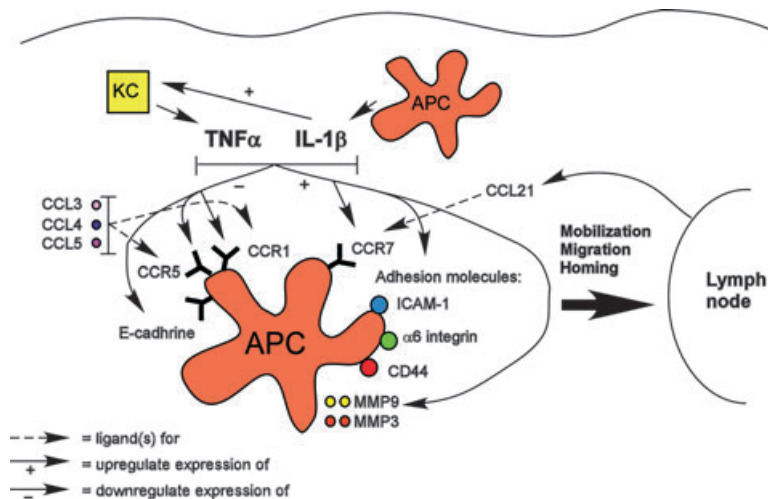


Fig. 2. The coordinated roles of cytokines and chemokines in Langerhans cells migration. TNF α and IL-1 β induce the changes in LC necessary for their migration from the skin and direct their movement to the dLN. Such changes include down-regulation of the chemokine receptors CCR5 and CCR1, loss of E-cadherin, upregulation of the chemokine receptor CCR7, increased expression of various adhesion molecules such as ICAM-1, $\alpha 6$ integrin and CD44 and secretion of MMP 3 + 9. APC, antigen-presenting cell; KC, keratinocyte; MMP3 + 9, matrix metallo-proteinase 3 + 9.

machinery which controls macropinocytosis and phagocytosis (64). In addition, maturation is accompanied by enhanced expression of co-stimulatory molecules such as CD80 (B7-1) and CD86 (B7-2) and adhesion molecules such as ICAM-1, which are necessary for optimal activation of naive T cells in the lymph node (68, 69). The expression of these markers is specific for hapten-exposed (or activated) LCs, whereas immature LCs have low levels of CD80, CD86 and ICAM-1 (69).

T-cell activation

After migration and entrance into the dLN, LCs activate hapten-specific naive T cells. The importance of T cells to the elicitation phase is illustrated by the fact that adoptive transfer of CD8 T cells from sensitized mice into naive recipient can transfer sensitivity to a given hapten (18) and that depletion of both CD4- and CD8 T cells with mAb simultaneously nearly abolishes the CHS response (15). The following section addresses the different steps and interactions that T cells go through during the CHS response, and the contribution from the different subtypes of T cells and their effector function will be discussed as well.

Circulating CD4 and CD8 T cells enter the paracortical area of the dLN through the post-capillary HEV. As naive T cells travel through the cortical area of the lymph node they interact with a high number of APCs. The first contact between LCs and naive T cells is dependent on adhesion molecules and is not due the engagement of the T-cell receptor (TCR) and consequently, this allows the TCRs to 'scan' the LCs for their specific antigen (70). Mature LCs express a high level of adhesive molecules such as leukocyte function-associated antigen 3 (LFA-3 or CD58), ICAM-1 (CD54), ICAM-2 (CD102) and the lectin DC-SIGN (CD209), which facilitate T cell-LC interactions. These molecules interact with their respective partners on T cells; LFA-2 (CD2), LFA-1 (CD11a:CD18) and ICAM-3 (CD50), respectively. The binding of ICAM-3 to DC-SIGN is unique to the interaction between naive T cells and DC and could be part of the explanation why DCs are the only APC capable of stimulating naive T cells (71, 72). In general, if the T cell recognizes its specific antigen, a more stable contact is established

which consists of a contact zone – or immunological synapse – formed by extension of actin-based membrane protrusions from the T cell toward the LC (73).

As for most immune responses, activation of T cells is dependent on three signals and the first signal is provided by the interaction of the TCR and the MHC/peptide complex (2). In this context, the nature of the haptens can explain the different routes of processing and presentation by APC. First, haptens can bind to extracellular cell-surface proteins that subsequently are internalized and processed into peptides in the endosomal/lysosomal compartment, followed by presentation on MHC class II molecules and activation of CD4 T cells. Alternatively, haptened extracellular surface proteins may be cross-presented to CD8 T cells. Secondly, as most haptens are lipid-soluble molecules, they may also enter the cell via passive diffusion where they can bind to intracellular proteins inside the cell. After processing via the endogenous route, they will bind to MHC class I molecules and be presented to CD8 T cells. Alternatively, the haptens can bind directly, without processing, to a peptide in the groove of either MHC class I and II and thus contribute to recognition by CD8 and CD4 T cells, respectively (74).

The second signal is provided by the up-regulation of co-stimulatory molecules on the APC – e.g. CD80 and CD86, which are ligands of the CD28 molecule constitutively expressed on T cells (75). This pathway is mandatory for the CHS response as illustrated by the fact that mice deficient for CD28 show an almost completely abolished CHS response (76). Furthermore, CD86 is crucial for development of effector CD4 and CD8 T cells (77) and blockade of CD86 leads to a prominently diminished response (75). In addition, this shows that in CHS, CD86 seems to play a more important role than CD80, which may be related to the observation that LCs express higher levels of CD86 than CD80 (75, 77). Another pair of important co-stimulatory molecules is CD40/CD40-ligand (CD154). CD40 is expressed on APCs and it appears to mediate two functions in the CHS response. First, CD40 participates in the signals which initiate maturation of the antigen-bearing LCs and their migration from the skin to the dLN (78, 79). Secondly, it is

known that the interaction between CD40 (expressed by APCs) and CD154 (expressed on CD4 T helper cells) induces higher expression of CD80/CD86 on the APC, which subsequently allows the APC to activate naive CD8 T cells. Different conclusions have been drawn on the role of CD40 and CD4 T helper cells in CHS. Some studies have suggested that CD40–CD154 interaction is crucial for the priming of effector T cells during CHS (78, 80), while others have suggested that CD8 T-cell priming occurs independently of CD40–CD154 interaction, but that the development of effector CD4 Th1 cells still requires CD40–CD154 interaction (81, 82). However, as will be discussed later, effector CD8 T cells in CHS may indeed develop without help from CD4 T cells if these are depleted by monoclonal antibodies (mAbs) (15, 83) – an observation that strengthens the statement that CD8 T cells may develop without CD40–CD154 interaction. Generally, it is believed that CD8 T cells can be primed without the help from CD4 T cells if (i) the immunogen generates a danger signal resulting in high levels of CD86 and CD80 on the APC, (ii) the affinity of TCR for the MHC-complex is high or (iii) the frequency of CD8 T cell precursors is high – e.g. as in some virus infections. Haptens have two properties that explain why they can be immunogenic in the absence of CD4 help. First, they do indeed provide a danger signal as mentioned previously (35) and second, they activate a high number of CD8 T cell precursors (in one study about 0.1–0.2% of the CD8⁺ T cells in the dLN were reported to be hapten-specific Tc1 cells) (84). Why different conclusions have been drawn on the role of CD40–CD154 in CHS is probably explained by the experimental setup – mainly the issue whether KO mice or mAb is used and what hapten is applied. In this context, Tang et al. (1997) observed a reduced CHS response when treating with anti-CD154 mAb (80) and later Gorbachev et al. (2001) found that anti-CD154 mAb-mediated inhibition of CHS is not due to direct blockade of CD40–CD154 interactions during CD8 T-cell priming but is due to an active process requiring CD4 T cells (82). Moodycliffe et al. (2000) obtains a decreased response using CD154 KO mouse when using a FITC-induced CHS model (78). In contrast, Gorbachev et al. (2001) gains an unchanged CHS response in CD154 KO mice

when using DNFB, thus concluding that T-cell priming in CHS is independent of CD40–CD154 (82). The difference might be explained by the different haptens used in the two particular studies; FITC have been observed to be a weaker sensitizer than DNFB (85) and probably needs more co-stimulation in priming CD8 T cells – thus sensitization to FITC might be dependent on CD40–CD154 interaction while DNFB is independent of this interaction. Nevertheless, the role of CD40–CD154 needs more investigation and emphasizes that the CHS response induced by different haptens are not similar in all aspects and cannot be regarded as a uniform process. Table 3 provides an overview of the studies investigating the co-stimulatory molecules in the CHS model.

The third signal is pivotal in driving T-cell differentiation into the different subsets of effector T cells. Differentiation of primed CD4 precursors is primarily dependent on the type of cytokines secreted during APC-T-cell interaction. In the presence of IL-12 and IFN γ , CD4 T cells develop into Th1 cells, which produce IL-2 and IFN γ ; in the presence of IL-4, CD4 T cells develop into Th2 cells producing IL-4, IL-5 and IL-13 (86). Recent studies have revealed that CD8 T cells can synthesize a pattern of cytokines similar to CD4 T cells and can also be divided into Tc1 (producing IL-2, IFN γ and TNF α) and Tc2 (producing IL-4, IL-5 and IL-10). The differentiation of CD8 Tc1 and Tc2 cells is dependent on the same cytokines as differentiation of CD4 Th1 and Th2 cells (87). Therefore, ‘type 1’ and ‘type 2’ cytokine patterns are subsequently used for both CD8- and CD4 T cells. Information related to production of polarizing cytokines by LCs and dDCs in response to contact allergens are limited. One of the few studies using cultured DCs shows that the contact allergens DNCB (dinitrochlorobenzene) induces production of the type 1 driving cytokine IL-12 (88). Subsequently, the role of IL-12 has been more extensively studied and revealed that treatment with IL-12 at the time of sensitization increases the magnitude and the duration of the CHS response and augments the development of IFN γ -producing CD4- and CD8 T cells (81, 89). However, the role of IL-12 in CHS is controversial, as another study showed that the response is independent of endogenous IL-12 as neither treatment with

anti-IL-12 mAb nor IL-12/23-deficient p40 KO mice revealed a reduced CHS response (81). In contrast, two different studies showed that anti-IL-12 treatment prevented induction of contact hypersensitivity (90, 91). The reason for this discrepancy is currently unknown but could be due to differences in the protocols used. As another example, the cytokine IL-6 is produced by LCs in the dLN and is involved in T-cell proliferation following sensitization – IL-6 is important in development of CHS since IL-6 KO mice exhibit a diminished CHS response (92, 93). Whether LCs polarize a type 1 or type 2 T cell response probably depends on the nature of the hapten together with other factors such as if the response is acute or chronic. FITC is known to induce type 2 cytokines (94, 95), whereas oxazolone (96), TNCB (97) and DNFB (98) are reported to induce a type 1 cytokine response. In contrast, oxazolone is also shown to induce a mixed response that is not dominated by either type 1 or type 2 T cells (99), so it seems that oxazolone induces a response that is not clearly defined as type 1 or type 2. Nevertheless, the generally accepted hypothesis states that polarization of type 1 T cells is for most cases crucial to the development of specific effector T-cell population and optimal CHS reaction (13).

Besides type 1 and 2 T cells, the role of Th17 cells has also been studied in CHS. In mice, differentiation to Th17 cells from naive T cells is dependent on TGF β , IL-6 and IL-21, whereas IL-23 stabilizes the generation of Th17 cells. In contrast, TGF β is not essential for the differentiation in humans (100). IL-17 is a proinflammatory cytokine and one of its primary effects is to regulate the infiltration of leukocytes into the inflammatory tissue but also to regulate the production of a panel of cytokines and chemokines (18). IL-17A-deficient mice demonstrated a marked suppression of the CHS response and decreased infiltration of inflammatory cells but the cellular source of IL-17 was not revealed (101). Later studies have further suggested a role for IL-17-producing CD8 T cells in CHS by showing that treatment with anti-IL-17 mAbs results in reduced edema and diminished infiltration of T cells, monocytes/macrophages and neutrophils during the elicitation of CHS. This study also indicates that IL-17-producing CD8 T cells are distinct from IFN γ -producing CD8 Tc1 cells (102). Likewise, it is demonstrated that

IL-17 signals are primarily required for the infiltration of leukocytes, whereas IFN γ signals are more important for the regulation of ROS activities during inflammation but also that both signals are required for a optimum response (18). Finally, a recent study indicates that IL-17- and IFN γ -producing CD8 T cells are recruited into the challenged skin very rapidly after hapten challenge and thereby play a central role in initiating the CHS response (103). Together, these studies suggest that IL-17 plays a role in the early inflammatory mechanism of CHS by mediating recruitment of inflammatory cells inclusive neutrophils, macrophages and T cells.

After activation, T cells migrate out of dLN through the efferent lymphatic vessels and into circulation. Two subsets of memory T cells are generated – effector memory T cells (T_{em}) lack the chemokine receptor CCR7 and can rapidly enter the inflamed tissue and mediate a quick response. In contrast, central memory T cells (T_{cm}) express CCR7 by which they maintain the ability to re-circulate from the blood to the LN, and are therefore capable of quick expansion upon re-exposure to antigen. Migration of T cells out of the dLN is further associated with expression of new surface molecules that allows migration from the circulation to the tissue such as CD43 and P-selectin glycoprotein ligand 1 (PSGL-1) (104–106), which will be discussed further below. The migration of the activated antigen-specific T cells out of dLN marks the end of the sensitization phase. The cytokines, receptors and adhesion-molecules important during the sensitization phase and which are investigated in the CHS model are listed in Tables 1, 3 and 4, respectively.

ELICITATION PHASE

The elicitation phase is initiated upon subsequent exposure of the hapten used for sensitization. The elicitation response can be divided in two phases; early phase in the first 2 h after challenge and late phase 24 h after challenge (3).

Early elicitation phase

The early phase was first described in details by van Loveren et al. (1984) who showed that the

ear-swelling response was bi-phasic with the first peak after 2 h, declined completely at 4 h and rose again to the second peak after 24 h. They further showed that the early phase was mediated by T cells and mast cells (3). Later, the early phase was also shown to involve complement C5/C5a (4–7), activation of skin mast cells (3, 8) and local platelets (9) including their release of vasoactive and inflammatory mediators such as serotonin (3, 9) and $\text{TNF}\alpha$ (10). In this process, C5a is thought to bind both to C5a receptors on the endothelia, but also on local mast cells and platelets (107) which cause mast cells to release vasoactive $\text{TNF}\alpha$ and serotonin, and the platelets to release serotonin (6). These vasoactive mediators stimulate the post-capillary venules to express ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1, CD106) on the luminal surface, which direct effector T cells to the dermis and the epidermis (see Fig. 3) (108). In this context, several hypotheses exist regarding the role of the various cell types in the

tissue during the early phase. First, it is reported that CD4 T cells are recruited into the challenged site during the first 2 h after the challenge. The recruitment is dependent on endothelial expression of E- and P-selectin (11) and their interaction with CD43 and PSGL-1 on the CD4 T cell, respectively (104–106). The early recruitment of CD4 T cells is reported to be essential – at least in the oxazolone-induced model – for the later adhesion and recruitment of leukocytes 4–24 h after challenge (11). Secondly, a role for neutrophils has been suggested, since challenge with a hapten induces CXC chemokine ligand 1 (CXCL1, $\text{Gro}\alpha$) production, which mediates a subsequent neutrophil infiltration. In turn, neutrophils recruit antigen-primed T cells into the challenged site, most likely through the release of chemoattractant factors such as CXCL9 (MIG) and CXCL10 (IP-10) (103, 109, 110). In mice, only one CXCL1-binding receptor (CXCR2) has been identified, and infiltration of neutrophils and

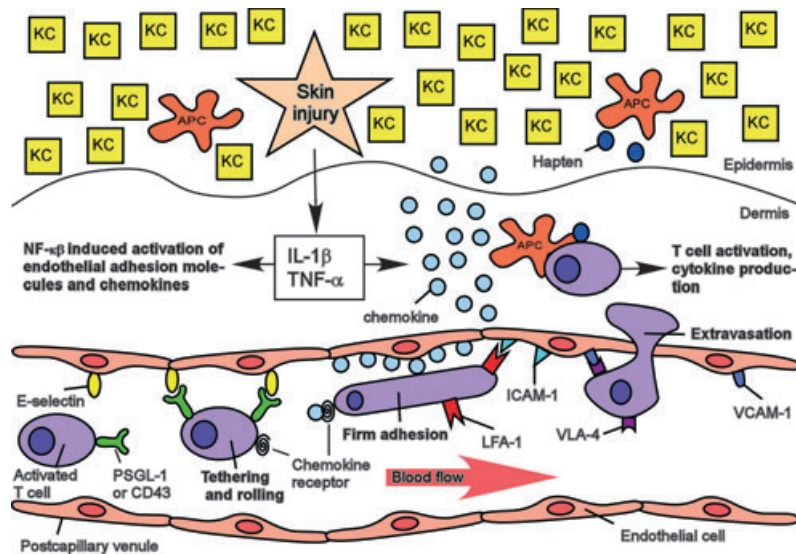


Fig. 3. Extravasation of recently activated T cells into the inflamed skin. The irritating effect from the hapten causes skin injury and stimulates KCs and LCs to produce $\text{TNF}\alpha$ and $\text{IL-1}\beta$. The $\text{NF-}\kappa\text{B}$ pathway in the endothelial cells is activated by these cytokines and this leads to the transcription of adhesion molecules E-selectin, ICAM-1 and VCAM-1. E-selectin expressed on the luminal surface of the vessel binds to CD43 and PSGL-1 expressed on T cells and this interaction slows the movement of the T cell in circulation and this is the first step in the extravasation. KCs on the abluminal surface of the vessel produce chemokines, which T cells are exposed to when they roll slowly on the endothelial surface. These chemokines yield the signal to the second step of extravasation, which is the strong interaction between VLA-4 and LFA-1 receptors on T cells and ICAM-1 and VCAM-1, respectively on endothelial cells. In this way, the movement of the T cells is stopped and the cells migrate into the dermis and further to the epidermis. $\text{NF-}\kappa\text{B}$, nuclear factor- κB ; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1; PSGL-1, p-selectin glycoprotein ligand 1; VLA-4, very late antigen 4; $\alpha_4\beta_1$ integrin; LFA-1, leukocyte function-associated antigen-1.

the CHS response are dramatically inhibited in CXCR2-deficient mice (111). Thirdly, Kish et al. (2009) suggested that during the early phase, IL-17- and IFN γ -producing CD8 T cells induce the production of CXCL1 and CXCL2 (Groß, MIP-2) – the functional analogue of human IL-8 – in the challenged skin, which subsequently recruit neutrophils. The neutrophils then direct more CD8 T cells into the skin to elicit the response (103). Finally, it is thought that resident mast cells can control neutrophil recruitment in the early phase after hapten challenge via release of TNF α and CXCL2 (112). Together, these studies show that the recruitment of primed T cells into the challenge site is dependent on complex interactions between components of the innate immune system such as neutrophils, mast cells and complement but also describe a role for CD4 and/or CD8 T cells in the early phase.

Originally, CHS was believed to be mediated almost entirely by T cells. However, recent studies have provided a significant role for B cells and especially the subset of B-1 cells has been studied in detail. The hypothesis that B cells and antibodies could play a role in CHS was further substantiated after the involvement of complement was described. It was shown that T-cell recruitment after challenge requires early activation of complement and generation of the C5a complement fragment (4–7), which can be detected already within 1 h after challenge (6). This hypothesis was further confirmed by the fact that depletion of C5a with antibodies (5), use of C5a-receptor KO mice (6) and the use of C5-deficient mice (7) all demonstrated a decreased ear-swelling response after 2 and 24 h. The discovery that C5a played a role in the elicitation of CHS suggested a role for B cells and/or antibodies in the process, e.g. via complement-fixing antibodies (26). It was later confirmed that B-1 cells produce IgM antibodies immediately after sensitization. These antibodies form immune-complexes with the challenge-haptenized antigen and hence activate complement. The complement cascade then generates C5a, which initiates recruitment of effector T cells and this finally mediates the classical 24 h response (23). Later, B-1 cells were shown to derive from the peritoneal cavity (24) and it is thought that within the first hour of sensitization, B-1 cells in the peritoneal cavity are

activated and subsequently migrate to the spleen and lymph nodes where they produce hapten-specific IgM antibodies which are detectable in circulation within one day after sensitization (4–8, 23–26). Although B-1 cells are described as T cell independent, NKT cells have been shown to play a role in the activation of these cells. In this context, the ability of NKT cells to recognize highly conserved endogenous and microbial glycolipids, presented on CD1d molecules by APCs, is of interest (25, 32). It is known that NKT cells play an important role in CHS as mice deficient in CD1d or the TCR variable region associated with NKT cells (J α 18), have been found to develop diminished CHS response (25, 32). The role of NKT cells in the activation of B-1 cells is suggested to involve a response to endogenous lipids from the skin that are released during sensitization. These lipids bind to CD1d on APCs in the liver, which induce hepatic NKT cells to release IL-4. Simultaneously, hapten-protein complexes are released from the sensitization site and together with IL-4, this activates B-1 cells in the peritoneum (8, 25, 29, 30, 32). Moreover, it is suggested that TLR4 expression and activation of hepatic NKT cells are involved in the production of IL-4 and the subsequent activation of B-1 cells (31).

Beside NKT cells, a novel role for NK cells has recently been described in the CHS model. NK cells are traditionally regarded as cells of the innate immune system but recently several studies have shown that at least some subsets show characteristics of a memory phenotype. This new role of NK cells as part of the adaptive immune system was suggested by two studies showing that mice deficient in T cells and B cells develop antigen-specific immunological memory to haptens. This memory response was further shown to be driven by a subset of primed hepatic NK cells (28, 113). These findings suggest a new role for NK cells and will be more thoroughly investigated in the future both in the CHS model but presumably also in other types of inflammatory response.

The role of the classical (B-2) B cells has not been as extensively studied as B-1 B cells and many studies do not differentiate between B-1 and B-2 cells. One study using B- cell deficient mice (μ MT-mice KO mice) showed that B cells are not absolutely required for developing CHS; however, a lack of B cells resulted in a decreased

CHS response (114). Apparently, B cells or their products may be a response-amplifier. This is further demonstrated by finding that in the dLN of the sensitized mice, B cells are the dominating cell type and during the elicitation phase nearly twice as many B cells are recruited to the dLN as compared to the sensitization phase (115). Because B-1 cells are described to migrate to the spleen and draining lymph nodes (inguinal and axillary) immediately after sensitization (estimated to 4500 IgM producing cells/spleen and 500 IgM producing cells/lymph node on day 4 after sensitization) (24) and because B-1 cells do not elicit memory responses, B-2 cells could be the main subtype found in the draining lymph node (auricular) after elicitation. In support of this, a few studies have shown that antibodies other than IgM also play a role in CHS. DNCB was shown to induce the formation of antigen-specific antibodies mainly IgG1, which were further reported to play an indispensable role in the pathogenesis of DNCB-induced CHS (116). Also, sensitization to oxazolone leads to enhanced serum levels of antigen-binding IgM, IgG and IgE (99, 117, 118) i.e. antibodies which may help to opsonize the hapten-modified protein. Finally, a regulatory role of B cells in CHS has been suggested based on the fact that CD19 KO mice exhibit a prolonged and increased ear-swelling response (119) and that a regulatory IL-10 producing B-cell subset (called B10) has recently been described (120). The absence of these cells could perhaps explain the enhanced ear swelling observed in the CD19 KO mice. In conclusion, the role of antibodies and classical B-2 cells is not well described and probably also depends on the hapten used. However, B-cell deficient mice develop a reduced CHS reaction compared to wild-type (23, 114) so B cells do play a role in CHS although they may not be critically required to elicit a response.

Late elicitation phase

After the early phase, recruitment of effector cells drives the response into the late phase and the peak of the inflammatory response 24–48 h after challenge. The recruitment of effector cells is controlled by a coordinated and sequential release of chemokines by KCs after their exposure to TNF- α and IL-1 β . The most studied chemokines in CHS are CXCL9 (MIG),

CXCL10 (IP-10) and CXCL11 (I-TAC), which are the three known ligands for the CXCR3-receptor (121–123). They contribute to accumulation of CXCR3-bearing T cells in the inflamed skin. Interestingly, T cells bearing CXCR3 are known to belong mainly to type 1 T cells, including CD4 Th1 cells (124) and cytotoxic CD8 T cells (125). It is further believed that the CXCR3⁺ CD8 T cells are attracted to the challenge sites by CXCL10, where they secrete IFN γ which in turn stimulates local KCs to produce more CXCL10 and thereby completes a positive feedback loop (122, 126). Accordingly, CHS is suppressed by treatment with anti-CXCL10 mAb; clearly illustrating a critical role for CXCL10 (127). Another chemokine receptor that has been studied in CHS is CCR4 and its ligands, CCL17 (TARC) and CCL22 (MDC). CCR4 is shown to be primarily expressed on CD4 Th2 cells in both humans and mice (128, 129) and in DNFB-induced CHS, no infiltration of CCR4⁺ T cells was demonstrated. Instead, only CXCR3-expressing- and IFN γ -producing T cells were demonstrated in both the lymph node and the hapten-challenged skin (122). In contrast, oxazolone-induced CHS shows an elevated expression of CCR4 ligands, CCL17 and CCL22 in the inflamed skin and in the draining lymph node (129, 130). Likewise, blockade of CCL17 and CCL22 and CCR10 ligand (CCL27) with neutralizing antibodies simultaneously led to a suppression of the CHS response in the oxazolone model (129). This indicates that in DNFB-induced CHS, CXCR3 might be the main chemokine-receptor expressed by T cells, whereas in the oxazolone-induced model, the most important chemokine-receptor involved seems to be CCR4. Furthermore, this illustrates an interesting difference between the two models, in that tissue-infiltrating cells in the DNFB model express chemokine-receptors which are consistent with type 1 T cells whereas the T cells in the oxazolone model express chemokine-receptors consistent with type 2 T cells (see also Table 2).

The different steps of extravasation of recently activated T cells into the inflamed skin are depicted in Fig. 3. The recruitment of T cells in the elicitation phase is initiated by activation of the innate immune response in the skin, which causes the release of TNF α and IL-1 β from KCs and LCs as mentioned previously

(131). Subsequently, these cytokines activate the NF- κ B pathway in the endothelial cells which further induces induction of adhesion molecules E-selectin, P-selectin, ICAM-1 and VCAM-1. To extravasate into the skin, T cells must slow their movement in the circulation and this is achieved by interaction between CD43 and PSGL-1 (104–106) on T cells and their ligands E-selectin and P-selectin on the luminal surface of the cutaneous post-capillary venules, a process called ‘tethering’ (132). When the T cells roll slowly on the endothelial surface, they are exposed to chemokines produced by KCs on the abluminal surface of the vessel. These chemokines give the signal to the second step of extravasation, which is the firm interaction between $\alpha_4\beta_1$ integrin (very late antigen 4, VLA-4) and LFA-1 receptors on T cells and VCAM-1 and ICAM-1, respectively on endothelial cells. This arrests T-cell motion and the cells migrate into the dermis and further to the epidermis where the LCs are located ready for the second antigen presentation (132). Table 4 gives a summary of the specific adhesions molecules which have been investigated in the CHS model.

It was previously believed that during the elicitation phase, haptens are taken up by LCs and presented to recently activated T cells in the dermis and epidermis. Although LCs are certainly capable of functioning as APCs, there is evidence indicating that they are not required during the elicitation phase. Grabbe et al. (1995) suggested that resident epidermal LCs are not the relevant APCs in the effector phase and that they may even provide inhibitory signals during elicitation (133). Instead, KCs and other skin resident cells as macrophages are thought to function as APCs during the elicitation phase (47, 134). Despite this controversy, there is general agreement that the second antigen presentation takes place in the skin and not in the dLN, consistent with the altered migration pattern of the recently activated T cells as described above.

After the second antigen presentation and activation of the hapten-specific T cells they exert their effector function and mediate the inflammatory response that characterizes CHS. Whether the effector T cells belong mainly to the subtype of CD4⁺ or CD8⁺ T cells has been both under intensive investigation and discussion. Classic DTH responses are known to be mediated by CD4 T cells and it was traditionally

believed to be similar in the CHS response (135). Nevertheless, this conclusion has been challenged by a number of experiments, suggesting that CD8 T cells work as effector cells, whereas CD4 T cells mainly function to down-regulate the response (13–20) but that a minor subset of CD4 T cells (Th1) can act as effector cells as well (15, 21, 22). This has been illustrated using *in vivo* depletion of CD4 and CD8 T cells with mAbs (13–15). Additionally, studies using gene-targeted mice have confirmed these results as MHC class I KO mice (16) or CD8 KO mice (136) are unable to develop CHS to haptens. The regulatory function of CD4 T cells is demonstrated by the fact that mice depleted of CD4 T cells – either by KO of the MHC-II-gene (16, 20) or by mAb treatment (13, 15) – develop an enhanced and sustained CHS reaction.

Although recent studies suggest that CD8 T cells are the major effector cells in CHS, it cannot be excluded that CD4 T cells in some circumstances are able to contribute as effector cells as well. A few studies directly suggest CD4 T cells as the effector cells in CHS (22, 135, 137–139). These studies were based both on CD4 KO mice that develop a reduced CHS response (22), but also on the observation that CHS can be transferred by CD4 T cells from sensitized mice into naive mice (139), and that CD4 molecules are crucial for the induction of the cytokines in the skin required for optimal development of CHS (138). In a study using CD4- and CD8 KO mice, Wang et al. (2000) suggested that both CD8 Tc1 cells and CD4 Th1 cells contribute significantly to the development of the CHS response (136). The different results might be explained by important functional differences between CD4 KO- and MHC class-II KO mice. In this context, it is observed that CD4 KO mice exhibit a significantly decreased response to DNFB (22) and oxazolone (136) while MHC class II KO mice reveal an increased CHS response (16, 20). Interestingly, a later study has found that in CD4 KO mice, a CD4-negative regulatory cell population develops, which suppresses the cytotoxic activity of hapten-specific CD8 T cells in CHS (140). Thus, although MHC class II KO mice are deficient in CD4 T cells, CD4 KO mice can select MHC-II-restricted T cells in the thymus, despite the lack of the CD4 co-receptor (141). This fact may explain some of the differences observed

between CD4- and MHC-II KO studies, and suggests that the most reliable results are obtained from CD4-depletion studies or MHC-II KO studies and not from CD4 KO studies. The hypothesis that CD4 T cells can work as effector cells *in the absence* of CD8 T cells has been suggested by a few studies. First, Gocinski et al. (1990) suggested that the CD4 population in CHS consists of two sub-populations – effector cells and suppressor/regulatory cells, where effector cells express a higher density of CD4 antigen than the suppressor/regulatory cells. By using sub-optimal doses of depleting mAb only the effector population was depleted [i.e. regulatory T cells (Tregs) are still present] and the CHS response was diminished instead of being enhanced as previously observed when high doses of depleting mAb were used (15). Furthermore, Martin et al. (2000) illustrate in a study with trinitrophenyl (TNP) that under certain circumstances, CD4 T cells can act as effector cells but that the response is clearly dominated by CD8 T cells (21). A possible explanation could be that during sensitization with TNP, CD8 T cells trigger apoptosis of CD4 T cells and consequently ensure that CD8 T cells are the dominant effector cells (142). In summary, despite the different results, it is believed that CD8 T cells are the major effector cells and that CD4 cells are split between a minor fraction of effector CD4 Th1 cells and a major fraction of suppressor/regulatory CD4 T cells. When CD8 T cells are absent, CD4 Th1 cells are able to mediate a diminished but significant response.

The main function of CD8 T cells is cytotoxicity, which is mandatory for the development of CHS. The cytolytic activity is dependent on perforin and the Fas/Fas ligand pathway (19). Hence, an association between recruitment of CD8 T cells into the challenged skin and induction of apoptosis in KCs has been described (14). Beside cytotoxicity, the production of IFN γ is also an important effector mechanism (18, 83, 102) and IFN γ -producing CD8 T cells can transfer the response from sensitized mice into naive animals (83). Furthermore, the importance of IFN γ is demonstrated by the observation that CD4-depleted mice treated with IFN γ -neutralizing antibodies (136) as well as IFN γ -receptor KO (143) mice exhibit reduced inflammation (see also Table 1). IFN γ appears to have an effect on the production of cytokines,

chemokines and ROS. ROS, as iNOS and MPO, are produced by macrophages and granulocytes and it is shown that IFN γ stimulates the infiltration of these cells into the inflamed skin (18). Subsequently, during the elicitation phase, iNOS seems to direct T cells, macrophages and neutrophils to the site of inflammation and to cause vasodilation, which further enhances migration of effector cells (144, 145).

Resolution of the elicitation phase

After 24–48 h, the ear-swelling response peaks and dampening of the response are mediated by recruitment of down-regulatory cells (14). Tregs are a subtype of T cells with anti-inflammatory properties and are important players in the development and maintenance of immune tolerance. The group of CD4⁺ Tregs has been the most widely studied and in the following section two subsets – CD4⁺ CD25⁺ and IL-10 producing Tregs (Tr1 cells) (146) – will be discussed together with the role of CD4 Th2 cells.

CD4⁺ CD25⁺ T cells express high levels of CD25 and are dependent of the transcription factor FoxP3. They represent 5–10% of the peripheral CD4 T cells in normal adult mice or humans and are characterized by a low sensitivity to stimulation via the antigen receptor (TCR) and inability to produce IL-2 (147). The first evidence for a regulatory function of a subset CD4 T cells was suggested by the studies showing that CD4 depletion with mAb (15, 148) or the use of MHC-II KO (16, 20) leads to an increased CHS response. Subsequently, the role of CD4⁺ CD25⁺ T cells in CHS has been extensively investigated. Adoptive transfer of freshly isolated or *in vitro* expanded CD4⁺ CD25⁺ T cells has been demonstrated to reduce the ear-swelling response in CHS significantly (149). Furthermore, treatment of wild-type mice with anti-CD25 mAb during hapten sensitization increases the magnitude and duration of the CHS response and also augments the numbers of hapten-specific IFN γ -producing CD8 T cells in the dLN (150). The CD4⁺ CD25⁺ T cells can exert their regulatory function in two different phases of the CHS response: during the expansion and differentiation of CD8 effector T cells in the dLN or during the inflammatory process generated in the skin. The immunosuppressive mechanisms by which CD4⁺ CD25⁺

T cells function are still not fully understood, but several pathways have been described. In the dLN, several mechanisms have been suggested: First, it is shown that a gap junction can be formed between the CD4⁺ CD25⁺ T cell and the APC, which may transfer cAMP or cAMP-inducing second messengers to the APC. This intercellular communication leads to downregulation of T-cell co-stimulatory molecules on the surface of the APC, abrogating the priming, activation and proliferation of CD8 T cells and thereby blocking sensitization (151). Secondly, the development and expansion of antigen-specific CD8 T cells are shown to be restricted by CD4⁺ CD25⁺ T cells through a FasL-dependent mechanism (17). Thirdly, CTLA-4 is also an important suppressive mechanism in the dLN, as CTLA-4 exposed on CD4⁺ CD25⁺ T cells can interact with CD80 and CD86 expressed by APCs; hence inhibits IL-2 secretion important for effector T-cell proliferation (94). In the CHS model treatment with CTLA4-Ig results in a long-lasting nonresponsiveness (94) and anti-CTLA-4 enhances the response significantly (152) which underline the importance of this inhibitory pathway in the CHS response. In the dLN, it is further suggested that IL-2 is required to maintain the presence and activity of CD4⁺ CD25⁺ T cells that restrict the development of effector CD8 T cells and further the magnitude and duration of the CHS response (150). Additionally, it is shown that CCR7-expression on CD4⁺ CD25⁺ T cells is required for their migration into the LNs and contributes to their suppressive function (153). In the skin, it is shown that the suppressive function of CD4⁺ CD25⁺ T cells is due to a decreased migration of effector T cells into the challenged tissue and that IL-10 produced by the CD4⁺ CD25⁺ T cells is involved in this effect (154). Furthermore, a role for adenosine in this control of recruitment has been suggested since adenosine down-regulates endothelial cell expression of E- and P-selectin needed for cell recruitment (155). The trafficking of Tregs has been studied during the CHS response and revealed that Tregs migrate from the skin upon sensitization into the dLN where they exert a stronger immunosuppressive function than the LN-resident Tregs. Moreover, these migrating Tregs re-circulate into the skin after challenge to terminate the inflammatory response (156). This

suggests that it might be same population of Tregs that mediates a suppressive function both during T cell priming in the dLN and through the inflammatory reaction in the challenged ear.

Another subtype of Tregs is the IL-10 producing Tregs, which are characterized by a high production of IL-10. Tr1 arises in the periphery when naive CD4 T cells are activated by tolerogenic APCs in the presence of IL-10 (146). IL-10 is known to suppress CHS since administration of IL-10 prior to hapten challenge strongly decreases the effector phase of CHS (157). Accordingly, IL-10 KO mice (158) and mice treated with IL-10 neutralizing antibodies (157) show an enhanced CHS response. Additionally, it is shown that IL-10 in the inflamed ear peaks at 10–14 h after challenge and returns to the background by 24 h (157, 159), which probably indicates that IL-10 participates in limiting the response during the first 24 h (see also Table 1). Moreover, it should be mentioned that other cell-types than T cells such as KCs (159) and mast cells (160) also are capable of producing IL-10 during CHS.

The suppressive contribution of CD4 Th2 cells remains ill described, but it seems that they also play a minor role by production of IL-10. The CD4 Th2 cells also produce IL-4 and IL-5 but the role of IL-4 in CHS has been controversial. First, it has been demonstrated that IL-4 negatively regulates CHS to TNCB (161). By contrast, IL-4 has also been ascribed a proinflammatory role since IL-4 KO mice exhibit a reduced CHS response to TNCB (162), DNCB (163) and DNFB (164) and blockade of IL-4 with an antibody in a TNCB model also reduced the response (165). On the other hand, the response to oxazolone was unchanged in IL-4 KO mice (158, 163). The heterogeneity of these data may reflect that IL-4 is able to express both proinflammatory and suppressive capacities dependent on the specific hapten applied (see also Table 1).

CONCLUSION AND FUTURE PERSPECTIVES

The inflammatory response in contact hypersensitivity involves many different pathways and mechanisms of the immune system and comprises a wealth of different cell types, cytokines,

chemokines and receptors (see Tables 1–5). Additionally, it involves both local and systemic immune responses and covers both the innate and the adaptive immune system. This makes the CHS model a suitable *in vivo* model for many mechanistic studies. Interestingly, many novel biologic-based immunomodulatory drugs (such as the TNF α inhibitors, IL-6 antagonism, IL-12 inhibition or CTLA4-Ig administration) have shown significant efficacy in the CHS model as well. This suggests that the model may also be useful as a mechanistic model in pre-clinical *in vivo* testing of new anti-inflammatory drug candidates in a broader sense. Still, there are still many undiscovered and unknown aspects of the CHS response that need more thorough investigation in the future. For example, the question how haptens activate the innate immune system and mediate danger signals leading to activation of the APCs. In this context, new receptors, pathways and mediators will certainly be discovered in the future. Additionally, the second antigen presentation event requires further investigation. It will be important to study the localization as well as the cell types involved in the second antigen presentation. Also the exact molecular events and the cellular subsets responsible for the regulation of the inflammatory response is an area where the understanding has expanded dramatically and the future most likely will provide even more knowledge and insight. Finally, the emerging roles of B cells, NK cells and NKT cells in CHS have made the picture much more complex than previously regarded and more cell types (including KCs, mast cells and platelets) will expand the list of critical participants in the CHS response in the future.

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