

Review Article

Dendritic cells: biology of the skin

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Allergic contact dermatitis results from a T-cell-mediated, delayed-type hypersensitivity immune response induced by allergens. Skin dendritic cells (DCs) play a central role in the initiation of allergic skin responses. Following encounter with an allergen, DCs become activated and undergo maturation and differentiate into immunostimulatory DCs and are able to present antigens effectively to T cells. The frequency of allergic skin disorders has increased in the past decades. Therefore, the identification of potential sensitizing chemicals is important for skin safety. Traditionally, predictive testing for allergenicity has been conducted in animal models. For regulatory reasons, animal use for sensitization testing of compounds for cosmetic purposes is shortly to be prohibited in Europe. Therefore, new non-animal-based test methods need to be developed. Several DC-based assays have been described to discriminate allergens from irritants. Unfortunately, current *in vitro* methods are not sufficiently resilient to identify allergens and therefore need refinement. Here, we review the immunobiology of skin DCs (Langerhans' cells and dermal dendritic cells) and their role in allergic and irritant contact dermatitis and then explore the possible use of DC-based models for discriminating between allergens and irritants.

Key words: allergen; allergic contact dermatitis; animal alternative; dendritic cell; dermal dendritic cell; irritant; Langerhans' cell; predictive testing for allergenicity; skin immunology. © Blackwell Munksgaard, 2008.

Accepted for publication 29 June 2008

Dendritic Cells

Dendritic cells (DCs) represent a heterogeneous cell population residing in most peripheral tissues, particularly at sites of interphase with the environment, e.g. skin and mucosa. They represent 1–3% of the total cell numbers of these tissues (1). DC patrol through the blood, peripheral tissues, lymph, and secondary lymphoid organs (Fig. 1). In the periphery, immature DC (iDC) can capture and process antigens. Thereafter, they migrate towards T-cell-rich areas of the secondary lymphoid organs through the afferent lymphatics. During this migration, DC lose their capacity to internalize further antigens and acquire the capacity to present antigens to naïve T cells, a process referred to as maturation of DC. Antigens are presented in such a way that antigen-specific naïve T cells become activated and start to proliferate (T-cell priming). Moreover, when DC initiate a T-cell-mediated adaptive immune response, they also play an important role in polarization of T-cell reactivity towards type-1 and/or type-2 responses.

Cutaneous DCs

DCs in the epidermis and dermis participate in the recognition of invading pathogens. These cells are dedicated antigen-presenting cells (APCs) and play a key role in sensing danger and initiating both innate and adaptive responses. The two main populations of DC present in normal human skin are epidermal Langerhans' cells (LC) and dermal (or interstitial) dendritic cells (DDC) (Table 1).

Langerhans' cells

Paul Langerhans', as a medical student, identified DC residing in the epidermis. These LC represent approximately 3% of epidermal cells and form a constituent part of the skin immune system. They are found regularly spaced throughout the epidermis (2). LC are typically characterized by the expression of CD1a and a unique cytoplasmic organelle named the Birbeck granule (BG). BGs constitute a subdomain of the endosomal recycling compartment, perhaps being involved in

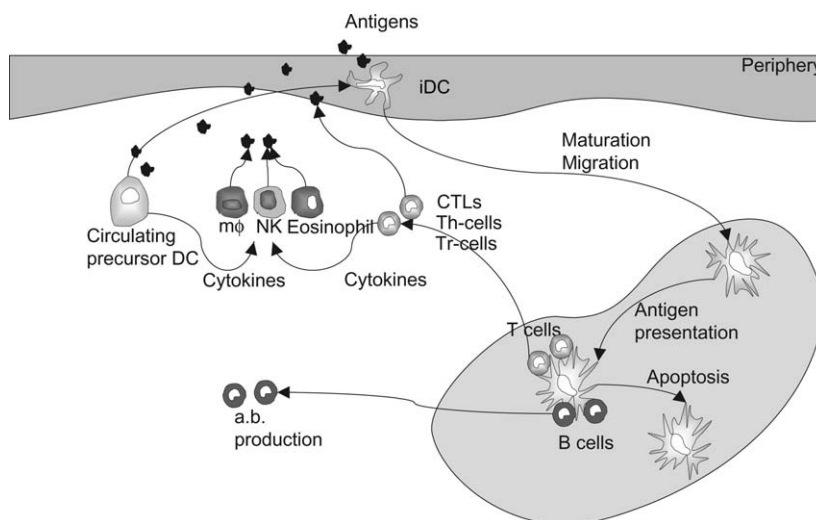


Fig. 1. The life cycle of DC. Circulating precursor DCs enter tissue as iDC. Upon uptake of antigens (e.g. bacteria and viruses), DCs induce secretion of cytokines and chemokines, which in turn can attract and activate eosinophils, macrophages, and NK cells at the site of antigen entry. After antigen capture, iDC migrate to secondary lymphoid organs. They present peptide–major histocompatibility complexes, which allow selection of antigen-specific lymphocytes. These lymphocytes help DC in terminal maturation, which allows T-cell expansion and differentiation. Activated antigen-specific T cells migrate to the injured tissue. Th cells secrete cytokines, which permit activation of macrophages, eosinophils, and NK cells. CTLs eventually lyse these cells. In parallel, B cells become activated after contact with T cells and DC. They migrate into specialized areas where they mature into plasma cells, which produce antibodies that neutralize the initial antigen. Finally, after interaction with lymphocytes, DCs die by apoptosis. CTLs, cytotoxic T cells; DC, dendritic cells; iDC, immature DC; NK, natural killer; Th, T-helper; Tr₁, T regulatory-1 cells.

antigen loading processing. The C-type lectin Langerin (Lag, CD207) is responsible for BG formation and thus a key marker of the LC lineage (3).

LC originate from bone-marrow-derived progenitors, as indicated by their CD45 expression (4). However, the process of LC differentiation and their migration into the epidermis is not clearly understood. It has been postulated that bone-marrow-derived myeloid cutaneous lymphocyte-associated antigen (CLA)-expressing LC precursors travel through peripheral blood through the dermis into the epidermis (5). Other studies suggested that skin-resident LC precursors exist in the dermis under normal non-inflammatory conditions. These LC precursors co-express Langerin and CD14, and their final migration into the supra-basal layer of the epidermis is most probably controlled by keratinocyte-derived CXCL14 (6, 7). Additionally, it is suggested that a combination of transforming growth factor (TGF)- β_1 and CCL20 is required for the attraction of human LC precursors through the dermal–epidermal barrier (8).

The final differentiation of LC precursors depends on the cytokine environment of the epidermis (7). The cutaneous cytokines granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-15, and TGF- β_1 contribute to the establishment of immature LC in the epidermis. During inflammation, however, circulat-

ing precursors might have an important role in replenishing the local pool of LC (5).

In vitro, LC can be cultured from cord blood-derived or bone-marrow-derived cells (9, 10). These CD34⁺ haematopoietic progenitors acquire LC features upon differentiation with a combination of cytokines, e.g. TGF- β_1 , GM-CSF, and tumour necrosis factor- α (TNF- α). In addition, several blood-derived cell types can be differentiated into LC-like cells as depicted in Table 2. Undoubtedly, the presence of TGF- β_1 in the cytokine milieu is pivotal in the differentiation to a LC phenotype. In the absence of TGF- β_1 , *in vitro* cultured LC lack the development of BGs, whereas the other DC characteristics, e.g. CD1a and langerin expression, can still be presented.

Dermal dendritic cells

The dermis consists of a large variety of cell types, including fibroblasts, macrophages, mast cells, T cells, and DDC. These latter APCs are located at the level of the capillaries and in the higher part of the reticular human dermis. Two major subsets of DDC are present in the dermis: CD1a^{high}/CD4⁺/CD14[−]/CD16[−]/CD206^{high}/CD207[−]/CD209⁺ and CD1a^{low}/CD4⁺/CD14⁺/CD16[−]/CD206^{low}/CD207[−]/CD209⁺ cells (11). It has been suggested that the latter DC subset shows weaker allogeneic stimulatory capacities compared with the CD1a^{high}

Table 1. Characteristics of LC and dermal dendritic cells

Marker	LC	DDC	Remark	Reference
Adhesion molecule				
CD11a	+/-	+	Upregulated upon migration	(12)
CD11b	-	+	Upregulated upon migration	(157, 158)
CD11c	+	+	Upregulated upon migration	(158, 159)
CD54	+/-	+	Upregulated upon migration	(12, 159)
CD58	+/-	+	Upregulated upon migration	(12, 159)
CD324 (E-cadherin)	+	-		(160, 161)
CLA	+	+/-		(162)
Antigen presentation				
MHC-class I	+	+		(163)
MHC-class II	+	+		(163)
CD1a	+	+/-	Defines two subsets	(164)
CD1b	+	+		(158, 165)
CD1c	+/-	+		(158, 165)
CD1d	-	+		(166)
CD45	+	+		(167)
Birbeck granule	+	-		(168)
Chemokine receptor				
CCR2	-	+	Downregulated upon migration	(169)
CCR6	+	+	Downregulated upon migration	(169)
CCR7	-	-	Upregulated upon migration	(169)
CXCR1/2	-	-	Upregulated upon migration	(6, 158)
CXCR4	-	ND	Upregulated upon migration	(59)
Cytokine receptor				
IFN-gamma R	+	+		(6, 158)
IL-1R1	+	+		(6, 158)
IL-1R2	+	+		(6, 158)
IL-2R α	-	+		(6, 12, 158)
IL-2R β	-	-		(6, 158)
IL-2R γ	ND	+		(6, 158)
IL-3R	ND	+		(6, 158)
IL-4R	-	+		(6, 158)
IL-5R	ND	+/-		(6, 158)
IL-6R	+/-	+		(6, 158)
IL-7R	-	+		(6, 158)
G-CSFR	-	-		(6, 158)
GM-CSFR α	+	+		(6, 158)
GM-CSFR β	+/-	+/-		(6, 158)
M-CSFR	-	-		(6, 158)
TNFR 55000 MW	-	-		(6, 158)
TNFR 75000 MW	+	+		(6, 158)
Fc receptor				
CD16 (Fc γ RIII)	-	-		(12)
CD32 (Fc γ RII)	+	+		(12, 158)
CD64 (Fc γ RI)	-	+		(12)
Other				
CD14	-	+/-	Defines two subsets	(12)
CD36	-	+/-		(12)
CD205 (DEC205)	+/-	+	Upregulated upon maturation	(170)
CD206 (MMR)	-	+		(171)
CD207 (Langerin)	+	-	Downregulated upon maturation	(172)
CD208 (DC-LAMP)	-	-	Upregulated upon maturation	(170)
CD209 (DC-SIGN)	-	+		(11, 170)
Factor XIII	-	+		(173)

-, negative; +/-, only a percentage positive; +, positive; CLA, cutaneous lymphocyte-associated antigen; G-CSFR, granulocyte colony-stimulating factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GM-CSFR, GM-CSF receptor; IFN, interferon; IL, interleukin; IL-2R, interleukin-2 receptor; LC, Langerhans' cells; MHC, major histocompatibility complex; MMR, mannose receptor; ND, not described; TNF- α , tumour necrosis factor- α ; TNFR, TNF- α receptor.

subset (12). Some investigators claim that the CD1a^{low} subset can be defined as macrophages rather than DC (13). Expression of CD36, CD209, and the coagulation factor XIII (FXIIIa) has been suggested to be specific markers for both populations of DDC. However, macrophages also

express these markers (11, 14). Therefore, macrophages and DDC may be hard to distinguish.

In vitro, DDC can be generated from CD34⁺-haematopoietic progenitor cells or CD14⁺ circulating monocytes with cytokine cocktails (15, 16). All combinations of IL-3, TNF- α , IL-13, or IL-4

Table 2. Culture methods for human peripheral blood-derived Langerhans' cell

Characteristics progenitor cells	Cytokine cocktail	Reference
CD34 ⁺ CLA ⁺ cells	GM-CSF, TNF- α	(10)
CD1a ⁺ CD11c ⁺ CD14 ⁺ cells	GM-CSF, TGF- β_1 , IL-4	(174)
CD14 ⁺ cells	GM-CSF, TGF- β_1 , IL-4	(175)
CD14 ⁺ cells	GM-CSF, TGF- β_1 , IL-13	(48)
CD14 ⁺ cells	GM-CSF, TGF- β_1 , IL-15	(176)

CLA, cutaneous lymphocyte-associated antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; TGF, transforming growth factor; TNF- α , tumour necrosis factor- α .

together with GM-CSF stimulate the development of DDC (15–18). As is to be expected, the presence of TGF- β_1 in the cytokine milieu appears to inhibit DDC development and promote the differentiation of DC precursors to LC.

DC Migration

Migration of DC from the skin is one of the first steps in the initiation phase of an immune response (Fig. 2). Skin contact with antigens is known to stimulate various epidermal cytokines, e.g. IL-6, TNF- α , GM-CSF, and CXCL2/CXCL3 (macrophage inflammatory protein-2, MIP-2) (19), among which IL-1 β and TNF- α are essential in DC migration (20, 21).

Disentanglement of skin DC

Cytokine signalling results in altered expression of adhesion molecules, which facilitates DC migra-

tion out of the epidermis. The mobilization of DC is associated with loss of CD324 (E-cadherin), caused by TNF- α , IL-1 β , or IL-1 α production (21, 22). In brief, it is proposed that LC-derived IL-1 β performs two functions. First, DC-derived IL-1 β activates the DC in an autocrine loop through the IL-1 receptor 1 (IL-1RI). Second, IL-1 β also stimulates epidermal keratinocytes (KCs) to secrete TNF- α , which acts in a paracrine manner to facilitate DC migration through TNF-RII (23). If the availability of either of these cytokines is compromised, then allergen-LC migration and DC mobilization in draining lymph nodes and the development of contact sensitization are inhibited partially (24, 25).

Simultaneously, production of epidermal basement membrane degrading enzymes, such as matrix metalloproteinases (MMPs), is upregulated in activated LC (26). The MMP-2, MMP-3, and MMP-9 have been described to facilitate in the passage of LC across the basement membrane. Subsequently, MMPs are also essential for migration of LC and DDC through the dermal tissue matrix, by cleavage of type IV collagen (27). The activity of MMP-2 and MMP-9 is under the control of naturally occurring proteinase inhibitors such as tissue inhibitor metalloproteinase (TIMP)-1 and TIMP-2, respectively. Several studies have demonstrated that inhibitors of MMPs prevented the emigration of LC from the epidermis and DC accumulation to the afferent lymph node in the response to the epicutaneous application to sensitizers, such as nickel, oxazolone (OXA), and dinitrochlorobenzene (DNCB) (27–29). These findings suggest that the development

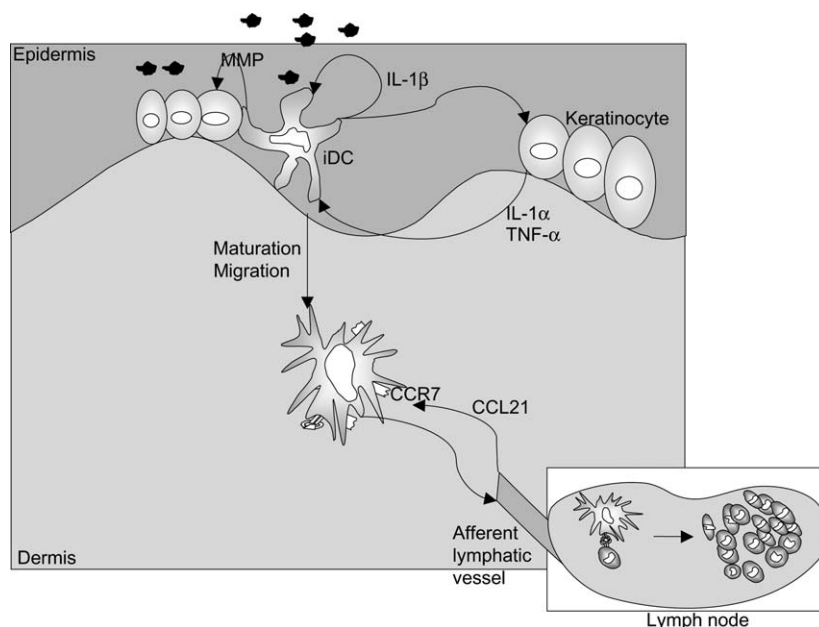


Fig. 2. Dendritic cell migration. Skin contact with a haptens triggers migration of dendritic cells through the afferent lymphatic vessels to the skin-draining lymph nodes. Production of cytokines – particularly TNF- α , IL-1 α , and IL-1 β – facilitates migration of Langerhans' cells (LC) out of the epidermis. At later stage, CCR7–CCL21 interaction plays a pivotal role in the migration of LC into the T-cell areas of lymph nodes. DC, dendritic cell; IL, interleukin; MMP, matrix metalloproteinases; TNF- α , tumour necrosis factor- α .

of contact hypersensitivity could be prevented by inhibition of the MMP activity. However, the development of contact hypersensitivity was not impaired in MMP-9-deficient mice, although LC migration was dramatically inhibited in these gene-deficient mice (27). Apparently, the few DDC and LC that still migrate in the absence of MMP-9 successfully induce contact hypersensitivity. In addition, contact hypersensitivity might be induced by the free diffusion of the contact allergen into the afferent lymph nodes where it can be presented by local DCs (30).

Meanwhile, differential expression of adhesion molecules is induced to mediate interactions with the extracellular matrix, epidermal, and dermal cells. Among the most important events is the reduced expression of CD324, which allows LC to dissociate from surrounding KCs (31). Additional roles are thought for CD54 (intracellular adhesion molecule, ICAM-1) and lymphocyte function-associated antigen-1 because blockage of these adhesion molecules inhibited migration of epidermal DC to regional lymph nodes. The upregulated expression of $\alpha 6$ -integrin and $\beta 1$ -integrin comprises very-late antigen 6 that confers on LC laminin-binding activity and thereby the ability to interact with basement membrane. Another adhesion molecule, junctional adhesion molecule 1 (JAM-1), is expressed by DC and therefore affects DC migration (32, 33). Absence of JAM-1 facilitates DC trafficking to lymph nodes (34). Combined, these events result in disentanglement of LC from surrounding KCs, thus allowing LC migration.

Chemokines and chemokine receptors

LC migration is also associated with the expression of chemokine receptors on the surface of the cells. iDC express chemokine receptors, and these can guide them to inflammatory sites where antigen sampling occurs. Upon antigen sampling, DCs rapidly differentiate into a mature phenotype. This maturation process is associated with a switch in chemokine receptor profile. Skin-homing chemokine receptors are downregulated (CCR1, CCR2, CCR5, and CCR6), whereas receptors involved in homing to the local lymph node are upregulated (CCR4, CXCR4, and CCR7) (35). Importantly, the mobilization of DC from the periphery to the afferent lymph nodes is regulated by the 'gate-keeper' chemokine receptor CCR7 (9, 36). Both lymphatic and high endothelial venules produce CCL21, a major CCR7 ligand (37, 38). Residing DC in the paracortical areas of the lymph nodes produce CCL19, a second ligand of CCR7. Next to antigen-bearing

LC also naïve T cells express CCR7. This orchestrates the encounter of DC and naïve T cells in the paracortical lymph node areas.

The lipid mediators, cysteinyl leukotrienes (cysLTs) and prostaglandin E_2 (PGE₂), all produced at sites of inflammation, are also required for LC migration (39, 40). These lipid mediators promote, by upregulation of CCR7, optimal DC chemotaxis to the chemokine CCL19 but not to CCL21. In addition, the cysLT transporter multidrug resistance protein-1 and lipid transporter *p*-glycoprotein (multi-drug resistance, MDR-1) are also expressed by DC and are necessary for their entry into afferent lymphatics (41, 42).

Negative regulation of DC migration

In mice, regardless of the nature of the stimulus (e.g. skin contact to allergens, pathogens, or trauma), only up to 30% of epidermal LC leave the tissue (43, 44). Possibly, these residential LC have the function to guard the skin for subsequent exposures. LC retention might be caused by the rapid onset of the production of negative regulators of LC migration such as epidermal-derived IL-4, IL-10, and TGF- β_1 . The former cytokine inhibits the migration of human LC through downregulation of TNF-RII expression (44). The two latter cytokines induce the expression of CCR6, also facilitating the retention of LC in the epidermis (8). During cutaneous inflammation, IL-10 provides homeostatic counterbalance for IL-1 β and TNF- α (19). TGF- β_1 inhibits DC migration through inhibition of CCR7 expression and upregulation of CD324 expression (45).

Differences in LC and DDC migration

In mice, LC and DDC show different migratory capacities upon epicutaneous exposure to antigen sensitizers. Mouse studies showed that upon exposure of the skin to the fluorescent dye tetramethyl rhodamine iso-thiocyanate, DDC migration clearly preceded LC migration (46). This might be due to the fact that LC take longer to reach afferent lymph nodes because they first need to detach from neighbouring KCs. Interestingly, within the T-cell zone, DDC are located in the outer paracortex just beneath the B-cell follicles, whereas LC are located in the inner T-cell-rich paracortex (46). These findings suggest that DDC are involved in B-cell priming, whereas LC are involved in T-cell activation. The differential migration capacity and lymph node destination between LC and DDC might result from a distinct chemokine or lipid mediator receptor expression on the LC and DDC (47). Recently,

it was suggested that CCR7 expression plays an important role in this controversy. *In vitro* generated DDC acquire a low CCR7 expression, whereas LC acquire a high level of CCR7 expression (48). This finding might explain the migration of LC, and not DDC, to T-cell zones within the lymph node.

DC Maturation

DCs located in peripheral tissues are continuously monitoring their environment (49). Tissue injury, microbial, and other changes of homeostasis provide danger signals, which activate DC and trigger their transition from immature antigen-capturing cells to mature antigen-presenting DC.

Maturation process

Once in contact with antigens, iDC use a broad range of pathways to facilitate antigen uptake. Many of these pathways, e.g. through $\alpha_v\beta_5$ -integrins or CD36, may also be used for uptake of self antigens (50). Following antigen uptake, maturation of DC is associated with several co-ordinated events: loss of endocytic/phagocytic receptors, upregulation of co-stimulatory molecules (e.g. CD80 and CD86), change in morphology, and upregulation of class II major histocompatibility complex (MHC) molecules (51). Functionally matured DCs (mDC) accumulate in T-cell-rich areas of lymphoid tissues. Within the paracortical areas, conditions are optimal for antigen-bearing mDC to encounter naïve T cells that specifically recognize the antigen–MHC molecule complexes. The dendritic morphology of these antigen-presenting cells strongly facilitates multiple cell contacts, leading to binding and activation of antigen-specific T cells. Antigen-specific T cells now expand abundantly and generate effector and memory T cells, which are released by the efferent lymphatics into the circulation. In conclusion, a variety of ‘danger’ signals induce DC maturation, ultimately resulting in generation of antigen-specific T cells, which in turn facilitates immunity.

Intracellular pathways

DC maturation can be initiated *in vitro* by inflammatory stimuli, such as proinflammatory cytokines (e.g. TNF- α and IL-1 β), lipopolysaccharide (LPS), CD40 ligation, and contact allergens (52, 53). These maturation stimuli induce phosphorylation of mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPK. These three

MAPK signalling pathways have distinct roles in the maturation process. Stress stimuli and inflammatory cytokines can strongly activate the JNK and p38 MAPK pathways, whereas the ERK pathway is strongly activated by growth factors through receptors for tyrosine kinases (54). In the maturation process of DC, the expression of surface molecules such as CD40, CD80, CD83, CD86, and MHCII and production of inflammatory cytokines is upregulated. Upregulation of these membrane molecules can be prevented by specific kinase inhibitors, such as p38 MAPK inhibitor (SB203580) (55).

Stimulators of DC maturation

In vivo, DC may encounter many different pathogens, for example prokaryote-derived lipoproteins, flagellin, cytosine-phosphate-guanosine, and LPS (56). iDC express a large array of receptors that can specifically recognize specific motives of pathogens, pathogen-associated molecular patterns (PAMPs). These PAMPs are ligands for Toll-like receptors (TLRs) on APCs. Basically, bacterial components can be recognized by DC through TLR1, TLR2, TLR4, TLR5, and TLR6 and viral RNA through TLR3 and TLR7 (57, 58). In addition, iDC express C-type lectins, like CD209, recognizing carbohydrate structures on pathogens (59).

Contact sensitizers may activate the innate immune system in a similar way as PAMPs of bacteria and viruses. Like PAMPs, contact sensitizers can induce DC activation by signalling cascades, upregulation of co-stimulatory molecules, and cytokine production (60, 61). p38 MAPK is considered to be a critical player in the initiation of contact hypersensitivity. *In vitro* exposure of DC to the allergens DNCB and nickel leads to activation and translocation of p38 MAPK and ultimately to the upregulation of CD80, CD83, and CD86 (55, 62, 63). However, it is unclear whether TLRs are involved in allergen-induced DC activation.

In contrast to inducing immune responses against invading pathogens, skin DCs can also play a role in induction of tolerance. By expression of inducible costimulatory molecule ligand (ICOS-L; B7-H2) or the immunoregulatory enzyme indoleamine 2,3-dioxygenase, LCs are capable of maintaining a state of tolerance (64). In the absence of the appropriate costimulatory signals, naïve T cells may be anergized, frequently associated with T-cell receptor (TCR)/CD4 or CD8 downregulation (65). T cells may turn into an unresponsive state and might also eventually die through apoptosis.

DC Polarization

Once DCs enter the regional lymph node, T cells that possess TCRs complementary to the MHC molecule–antigen complex on the surface of the LC can become activated. The site of interaction between the APC and the naïve T cell is called the ‘immunological synapse’, and sufficient stimulation by a set of three distinct groups of signals is pivotal for T-cell activation (66). Signal 1 is provided by interaction between the peptide-bearing MHC molecule on the DC and the TCR on the naïve T cell (‘antigen-specific’ signal) (67). Signal 2 consists of contact-dependent interactions, of which the costimulatory molecules CD80 or CD86 expressed on DC interact with CD28 expressed on T cells – this is the most potent signal (‘costimulatory’ signal) (68). In addition, interactions of CD54 and CD102 (ICAM-2) and CD252 (OX40 ligand) with their T-cell counterparts can contribute to signal 2 (69). The combination of signal 1 and 2 results in antigen-specific activation and proliferation of naïve T cells and the development of effector and/or memory T cells. Additional factors (signal 3) are needed for polarization of T cells towards type-1 or type-2 helper T cells (for CD4⁺ T cells) and type-1 or type-2 cytotoxic T cells (for CD8⁺ T cells) (70).

Type-1 T cells produce high levels of interferon (IFN)- γ , TNF- α , and TNF- β . These cytokines are important in the induction of cell-mediated immunity against intracellular pathogens. Type-2 T cells produce high levels of IL-4, IL-5, and IL-13. These cytokines are important in production of antibodies by B cells. Next to type-1 and type-2 T cells, a less polarized T-cell type (type-0) can develop that secretes a broad range of cytokines including IL-2, IFN- γ , IL-4, and IL-10. It has

been suggested that during the first antigen contact, the majority of emerging effector/memory T cells belong to this group of less polarized cells. Upon repeated antigen stimulation, they can be further polarized into the more pronounced type-1 or type-2 cytokine secreting cell populations (71).

Polarization of DC

DCs play an important role in the polarization of T cells (Fig. 3). Type-1 DC-derived cytokines strongly stimulate IFN- γ production in naïve T cells and thereby promote type-1 T-cell responses (72). Among the type-1 T-cell-polarizing cytokines of DC, IL-12 is the most important member of the IL-12 cytokine family, which includes IL-12, IL-18, IL-23, and IL-27 (73, 74). iDC are imprinted to secrete IL-12 upon stimulation by various pathogen types (e.g. bacteria, viruses, and protozoa). After migration to the draining lymph nodes, these pathogen-exposed DC will produce even higher levels of IL-12 upon CD40–CD40L interactions (75). Mature DCs express the membrane molecule CD40 that responds to their counter structure CD40L on naïve T cells to stimulate type-1 T-cell proliferation. In addition to an IL-12-conditioned cytokine environment, also intracellular interactions between CD54 and CD11a can promote the induction of type-1 T-cell responses (76).

Type-2 DC-derived cytokines potently stimulate IL-4, IL-5, and IL-13 production in naïve T cells and thereby promote generation of type-2 T cells (72). Whereas type-1 T-cell-inducing factors are well studied, little is known about mediators promoting type-2 T-cell responses. It has been

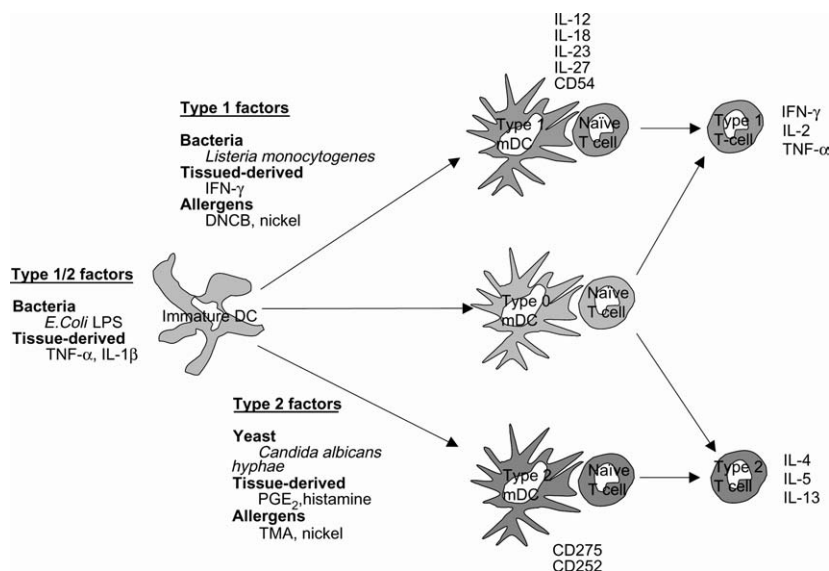


Fig. 3. DC polarization. Schematic representation of factors that influence DC polarization and ultimately polarization of naïve T cells as proposed by De Jong et al. (76). DC, dendritic cell; DNCB, dinitrochlorobenzene; *E. coli*, *Escherichia coli*; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; mDC, mature DC; PGE₂, prostaglandin E₂; TMA, trimellitic anhydride; TNF- α , tumour necrosis factor- α .

suggested that type-2 T-cell polarization is only induced in the absence of type-1 DC cytokines (77). DC or T-cell-derived IL-10 is able to downregulate DC-derived IL-12 production, facilitating type-2 responses (78). As to specific type-2-polarizing surface interactions, presence of the costimulatory molecules CD275 (ICOS-L), CD252 (OX40L), and Notch-ligand Jagged on DC has been implicated in this regard (79, 80).

Type-0 DC/regulatory iDC

DCs cannot only induce immune activation but also control the level of activation by regulatory loops. Thus, next to type-1 and type-2 polarization, DCs also play a role in the induction of peripheral tolerance. Two subsets of T_{regs} are described: naturally occurring regulatory T cells (T_{regs}) and adaptive T_{regs} (81). In contrast to natural T_{regs} , adaptive T_{regs} originate from uncommitted peripheral naïve or central memory T cells. By the selective induction of adaptive T_{regs} (82), 'regulatory iDC' can induce peripheral tolerance after repetitive stimulation of T cells in the absence of sufficient costimulatory signals (83). *In vitro*, 'regulatory iDC' can be induced after culturing iDC with the anti-inflammatory cytokines TGF- β_1 , IL-10, or anti-inflammatory drugs like corticosteroids (82, 83). These 'regulatory iDC' express several factors, e.g. CD274 (PD-L1) and CD273 (PD-L2) and can secrete high levels of IL-10, as a negative signal for T-cell responses (84, 85).

Inducers of DC polarization

Different compounds have been described as typical inducers of type-1 or type-2 immune responses in DC. Certain viruses, (myco)bacteria, and protozoa will induce a type-1 immune response, whereas helminths can induce a type-2 response (76). For example, the synthetic double-stranded RNA, polyriboinosinic polyribocytidylic acid (poly I:C), is known for its type-1 polarization. Direct exposure of DC with poly I:C results in type-1 DC, which subsequently trigger type-1 T-cell responses (79).

As to skin sensitization, it has been reported that different allergens can also provoke qualitatively distinct immune responses (86). Topical exposure of contact allergens (e.g. DNCB) in rodents will induce a prototypical type-1 response in allergen-activated lymph node cells, with high levels of type-1 and low levels of type-2 cytokines. In contrast, skin contact with respiratory allergens (e.g. trimellitic anhydride) results in the opposite. However, to what extent this translates to the human situation is largely unclear.

Role of microenvironment on DC polarization

The plasticity of DC to act as type-0, type-1, or type-2 polarizing antigen-presenting cells is influenced by many factors, e.g. by epithelial cells (route of entry of the antigen), antigen dose, and molecular nature of the antigen. By receiving danger signals from antigen-affected cells and tissues, DC can also indirectly obtain information about the molecular identity of the antigen. Considering the fact that DCs reside in close proximity of epithelial cells, such as skin KCs or airway epithelial cells, these cells most likely influence and enhance the local polarization of type-1 or type-2 APCs.

As mentioned above, direct exposure of DC with the synthetic double-stranded RNA poly I:C results in type-1 DC, which subsequently trigger type-1 T-cell responses (79). Indirectly, poly I:C can also modulate DC function through interactions with KCs, the epithelial cells of the skin (87). In that study, keratinocyte-derived type-1 IFNs and IL-18 were identified as soluble factors driving the type-1 phenotype of DC and T cells. On the other hand, exposure of KCs to a well-known non-polarizing cytokine cocktail, IL-1 β and TNF- α , did not result in type-1 IFNs and IL-18 production and subsequently induced both type-1 and type-2 immunity. Apparently, keratinocyte-derived factors can modulate DC function.

In contrast to type-1-skewing cytokines, thymic stromal lymphopoietin (TSLP) and PGE₂ are important tissue-derived inducers of type-2 immunity (88, 89). Under homeostatic conditions, TSLP is produced in epithelia of mucosal tissues and in the thymus (90, 91). TSLP can also be found in atopic dermatitis skin or in bronchial epithelium and submucosa in allergic asthma (92). Recently, it has been proposed that normal levels of TSLP in mucosa are important for the maintenance of tolerance-inducing type-2 T cells, whereas high levels of TSLP result in the development of proinflammatory type-2 T cells as found in allergic asthma (93, 94).

As to skin exposure to contact allergens, keratinocyte-derived IL-18 is involved in the cutaneous type-1 immune response (88). Exposure of human KCs to the contact sensitizer DNCB results in the secretion of IL-18, which subsequently trigger type-1 T-cell responses. In contrast, after mucosal contact with contact allergens, type-2 T-cell responses are most prominent. In the mucosal cytokine environment, DCs release only small quantities of IL-12, whereas IL-4 and IL-6 production of, e.g., macrophages and natural killer cells is relatively high, favouring type-2 responses (76).

As described earlier, the skin contains a network of CD1a⁺ DCs, which are responsible for defence

against pathogens. Compared with the skin, the situation of the gut appears to be more complex. Mucosal DCs allow on one hand the continuous uptake of nutrients and fluids through the epithelial layers, whereas on the other hand, mucosal DCs should prevent entry of harmful pathogens. In response to commensal bacterial signals, murine DCs produce mainly anti-inflammatory cytokines like IL-6, IL-10, and TGF- β , prime T cells towards a type-2, regulatory T-cells response, and induce B cell to produce IgA (95). In contrast, pathogenic bacterial signals induce production of inflammatory cytokines and chemokines, prime type 1 T cells, and induce IgG and IgA production by B cells (96).

The Role of DCs in Allergic and Irritant Contact Dermatitis

Allergic contact dermatitis (ACD) is a result of a T-cell-mediated inflammatory reaction occurring in sensitized individuals upon allergen challenge (Fig. 4). In the sensitization phase, allergens that have penetrated into the skin are internalized by skin DC, which subsequently migrate to the draining lymph nodes and present the hapten–peptide–MHC complexes to naïve allergen-specific T cells. This process results in clonal expansion of specific T cells, which subsequently can be recruited, from the circulation into the skin. Re-exposure to the relevant allergen can ini-

tiate the elicitation phase and thereby the clinical manifestation of the disease. As a consequence of the local release of cytokines and chemokines, circulating allergen-specific T cells and APCs meet at the site of allergen challenge. At this stage, non-dedicated APCs such as KCs also present the allergen to T cells. This results in the release of proinflammatory cytokines and chemokines of epidermal cells. These mediators attract more T cells, mainly allergen-non-specific, and other inflammatory cells, which further amplify the local mediator release. Finally, the skin site clinically develops an eczematous reaction. After several days to weeks, allergen removal and down-regulatory factors (e.g. IL-10, PGE₂, and TGF- β) are locally released, which results in clinically healed skin.

ACD: Sensitization Phase

Sensitization is critically dependent on association of allergens with epidermal LC-bound MHC molecules or peptides present in the groove of these molecules.

Formation of hapten–protein complexes

Allergens are typically chemically reactive, low-molecular-weight compounds that are unable themselves to stimulate directly the immune response (97). Some allergens are reactive, but

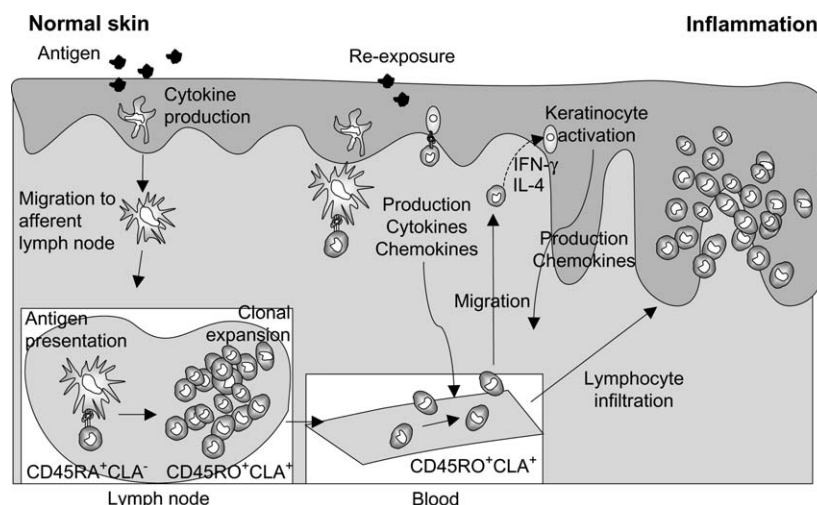


Fig. 4. Allergic contact dermatitis. Epidermal Langerhans' cells reside in normal skin in a functional resting state, characterized by high antigen-processing capacity. Topical allergen application triggers migration of LC through the afferent lymphatics towards regional skin-draining lymph nodes. In the T-cell-rich paracortical area of the lymph node, LC encounter naïve T cells and activate these through interaction between major histocompatibility complex–peptide complex and T-cell receptor. Hapten-specific T cells expand and generate effector or memory function and will go into the circulation. Upon re-exposure to the allergen, epidermal cells release proinflammatory cytokines and chemokines, which will recruit hapten-specific T cells. Infiltrating T cells release inflammatory cytokines, interferon- γ , and IL-4, which stimulate keratinocytes to produce chemokines, amplifying cellular infiltration. These infiltrating cells migrate into dermal and epidermal compartments. Ultimately, inflammation characterized by, e.g., oedema and spongiosis reaches its maximum at 24–48 hr after allergen exposure. CLA, cutaneous lymphocyte-associated antigen; IL, interleukin; IFN, interferon; LC, Langerhans' cells.

the vast majority of allergens interact with soluble or cell-associated skin proteins to form a covalent binding and to become immunogenic. Contact allergens that have to be converted before becoming reactive are referred to as prohaptens. This binding is dependent on their chemical reactivity. Many chemicals have electrophilic properties and are able to react with various nucleophiles within the skin. The human skin contains a very large array of biological structures, e.g. hydroxyl, amino, and thiol functionalities on proteins, containing electron-rich groups. As electrophiles, contact allergens are capable of reacting with these electron-rich groups to form a covalent and therefore irreversible bond. For example, the hapten trinitrophenyl binds covalently to the ϵ -amino group of the side chain of lysine residues (98). For sesquiterpene lactones, binding to thiol groups is needed to become a hapten (99). It has been suggested that typically 30–40% of sensitizing potential of allergens is associated with their electrophilicity (100). However, covalent binding is not necessary for all sensitizers. Metals react with skin proteins and form co-ordinate bonds. For nickel, different modes of co-ordinative binding to MHC and/or MHC-bound peptides and the TCR have been shown to be relevant in the activation of Ni-specific T cells (101). Recently, a large number of heat-shock proteins were described as Ni-interacting proteins (102). Other chemicals may directly connect MHC and TCR without binding (103). Ultimately, each approach results in the activation of chemical-reactive T cells that can cause ACD.

The hapten-carrier protein complexes form the complete antigen and are processed by LC and DDC (104). Both MHC class I and II may be altered in this way, and thereby both CD8⁺ and CD4⁺ T cells, respectively, can be involved in ACD. Lipophilic haptens, e.g. DNCB, can directly penetrate into the LC, conjugate with cytoplasmic proteins, and be processed along the endogenous processing route, which results in MHC class I presentation. In contrast, hydrophilic allergens such as nickel ions may rather be presented through the exogenous processing route and thus favour the generation of MHC class II molecules (105).

Hapten-induced activation and migration of DCs

Cutaneous DC continuously migrate out of the skin at a low rate, which is dramatically increased after allergen exposure. The emigration of skin DC is one of the first events in ACD and is regulated by various factors. Within 15 min after allergen painting, LC start to produce and express

IL-1 β (19). As discussed before, IL-1 β stimulates the production of the cytokines TNF- α and GM-CSF and differential expression of adhesion molecules and MMPs, which facilitates the migration of LC. Following encounter with an allergen, LC become activated and undergo maturation and differentiate from antigen capture and processing cells into potent immunostimulatory DC, able to present antigen effectively to effector T cells. Among the changes reported in LC as a result of exposure to the chemical allergens are internalization of surface MHC class II molecules through endocytosis, induction of tyrosine phosphorylation, the modulation of the costimulatory molecules CD80 and CD86 and increased expression of IL-1 β (19, 106). During this maturation, the shape of mDC changes from a round to a more ruffling appearance. This maximizes the chance to encounter with naïve T cells in the local lymph nodes and results in optimal antigen presentation.

ACD: Elicitation Phase

Allergen re-exposure at former challenged site

The effector phase of ACD is initiated upon re-exposure to a contact allergen. Allergen re-exposure results in production of proinflammatory cytokines by epidermal cells. After 4–8 hr, hapten-specific T cells are recruited by inflammatory cytokines and chemokines. Infiltrating T cells release inflammatory cytokines, such as IFN- γ and IL-4, which stimulate KCs to produce chemokines (CXCL9, CXCL10, and CXCL11). This in turn results in the attraction of CXCR3⁺ T cells (107). In humans, approximately 70% of the infiltrating CD4⁺ and CD8⁺ T cells express the receptor CXCR3 (104). Most of these co-express the cutaneous lymphocyte antigen CLA (108, 109). Subsequently infiltrating cells, including monocytes, DC and non-antigen-specific T cells migrate into dermal and epidermal compartments of the skin. These activated cells are an additional source of chemokines and cytokines, which further amplify the inflammatory response. CCL5 and CCL22 appear after 12 hr concomitantly with the infiltration of mononuclear cells into the dermis and epidermis (110). The expression of CXCL9, CXCL10, CXCL11, CCL17, CCL18, and CCL21 expression begins at 12 hr and peaks at 72 hr, paralleling the strong infiltration of lymphocytes (111). Ultimately, inflammation reaches its maximum, characterized by (epi)dermal infiltrates, oedema and spongiosis. In conclusion, the variety of chemokines expressed during ACD determines a robust and rapid recruitment of leukocytes into the skin.

The inflammatory infiltrate is composed mainly of CD4⁺ and CD8⁺ T cells, monocytes, and DC, with an early and transient presence of neutrophils. Murine contact hypersensitivity mainly correlates with the activity of hapten-specific CD8⁺ T cells, exerting their effector function through cytotoxic activity as well as the release of cytokines. Whereas allergen-specific CD8⁺ T cells may contribute to local release of cytokines, allergen-specific CD4⁺ T cells are major sources of proinflammatory cytokines in human ACD. CD4⁺ T cells play a more complex role, with both type-1 and type-2 subsets contributing to disease expression, and regulatory T cells primarily involved in the downregulation of the clinical manifestation of the disease (112).

Downregulation of inflammation

Gradually, the inflammatory reaction decreases and inflammation disappears. This down-regulatory process is mediated by secretion of anti-inflammatory mediators, such as IL-10, TGF- β , and PGE₂ and cell–cell contact-dependent mechanisms.

In these processes, adaptive and naturally occurring regulatory T cells play a central role (113). First, the adaptive allergen-reactive T regulatory-1 (Tr₁) T cells block, in an IL-10-dependent manner, the maturation of DC and thereby inhibit IL-12 release, thus impairing their capacity to activate type-1 effector T cells (114). During the late phase of ACD, CCL1, ligand for CCR8, is produced by KCs and activated T cells (115). Subsequently, CCR8⁺-bearing Tr₁ cells are attracted to the site of allergen contact. Second, another group of regulatory T cells prevent T-cell responses in a cell–cell contact-dependent manner. These naturally occurring CD4⁺CD25⁺ regulatory T cells are anergic and represent approximately 10% of CD4⁺ T cells in peripheral blood (116). Suppression of both CD4⁺ and CD8⁺ T-cells reactivity may occur through the regulatory markers cytotoxic T-lymphocyte antigen-4 and glucocorticoid-induced tumour necrosis factor receptor (117).

Next to T cells, KCs and macrophages are also capable of producing IL-10 (118). In addition, KCs also release the immunosuppressive cytokines TGF- β and PGE₂, thus contributing to the downregulation of ACD (119, 120). TGF- β silences activated T cells and inhibits further infiltration by downregulating the expression of adhesion molecules of both endothelial cells and skin cells (70). PGE₂ inhibits the production of proinflammatory cytokines, thus contributing to dampening of the skin immune system.

In resting skin, few skin-homing T cells are present. The so-called local skin memory at former contact eczematous sites can be explained by the persistent production of CCL27 by local KCs. It has been shown that the interaction of CCL27 with its receptor CCR10 is critical in the retention of CD4⁺ T cells but not CD8⁺ T cells at previously challenged ACD sites (121). The persistence of CD4⁺CCR10⁺ T cells seems to be crucial for local skin memory as *in vitro* results show that these cells, and not CD8⁺ T cells from allergic patients, show proliferative responses and produce cytokines in response to allergen (121).

Immune tolerance

One of the mechanism by which specific suppression can be mediated is by induction of immune tolerance. Oral ingestion of antigen might modify systemic immune responses by inducing a state of hyporesponsiveness or oral tolerance. For example, individuals having had oral contacts with nickel releasing dental braces prior to ear piercing showed a reduced frequency of nickel hypersensitivity, whereas hypersensitivity to other potential allergens were unaffected (122). Individuals who had dental braces after ear piercing did not show a decreased frequency but a slight increase of nickel ACD. These data show that oral administration of antigen prior to potential sensitization can induce systemic oral tolerance. Characterization of cytokine profiles produced by T cells from these tolerized individuals demonstrated an increased TGF- β and IL-10 production (123). These T cells act as suppressor cells by secreting anti-inflammatory cytokines.

Differences Between Allergic and Irritant Contact Dermatitis

The immunological mechanisms underlying allergic and irritant contact dermatitis reactions are different. However, both phenomena result in vasodilatation, upregulation of endothelial adhesion molecules (124), mast cell degranulation (125), keratinocyte cytokine and chemokine production (126), influx of leukocytes (127, 128), and LC migration into the dermis (129, 130). The similarity of these events can be explained by the irritant properties of allergens, which strongly contribute to their allergenicity (131). Differences between allergen and irritant exposure have been studied in murine models. MHC class II, IL-1 α , IL-1 β , CXCL10, and CXCL2/CXCL3 (MIP-2) were found to be only upregulated after allergen painting (19). In humans, expression of CXCL9, CXCL10, and CXCL11 has been reported to be

specifically upregulated in ACD and not in irritant contact dermatitis (ICD) (132). Of course, true differences between an allergic and an irritant reaction depend on whether specific T cells become involved. Only those chemicals with sufficient affinity for MHC molecules and for which matching TCRs exist can ultimately cause ACD. As DC maturation is required for proper T-cell activation, one can speculate that allergens and not irritants are able to sufficiently activate maturation of skin DC. Indeed, *in vitro* studies showed that potential contact sensitizers can be distinguished from irritants by their different ability to initiate LC/DDC migration and maturation (60, 61, 133).

Predictive Assays for Skin Sensitizing Testing

The frequency of allergic skin disorders has increased in the past decades. Therefore, the identification of potential sensitizing chemicals is important for skin safety. Traditionally, predictive testing for allergenicity has been conducted in guinea-pigs (134). In recent years, the murine local lymph node assay has become the preferred test method for assessing skin sensitization potential (135). For regulatory reasons, animal use for sensitization testing of compounds for cosmetic purposes is shortly to be prohibited in Europe. Therefore, new non-animal-based test methods need to be developed.

The following events/properties in skin sensitization are crucial in skin sensitization and might, therefore, be explored by various *in silico*, *in vitro*, and *ex vivo* models.

The ability of the chemical to penetrate through the stratum corneum

In order to induce skin sensitization, the physico-chemical properties of a compound must enable it to penetrate into the epidermis. Low molecular size, net charge, and octanol–water partition coefficient (logP) contribute to the epidermal bioavailability. LogP represents the relative affinities of the allergen in an octanol–water mixture and is a descriptor of hydrophobicity (136). Overall, small, non-polar, and moderately lipophilic substances penetrate the stratum corneum best, whereas highly polar, water-soluble compounds with high molecular weight penetrate poorly. Different *in silico* models have been developed that aim to predict skin penetration and sensitization potential of chemicals by structure-activity relationships (137, 138). Unfortunately, these methods are not suitable for identification of all allergens. For example, metal allergens, such as Ni^{2+} , are not identified as sensitizing compounds.

The potential of the chemical to form stable conjugates to create an immunogenic complex

For immunogenicity, the hapten must form a stable association with a macromolecule (e.g. protein). Either skin sensitizing chemicals are naturally protein reactive or can be metabolically transformed within the skin into a protein-reactive species, the so-called prohaptens (139). Sensitizing organic compounds bind covalently to protein nucleophilic groups, such as thiol, amino, and hydroxyl groups, as is the case with poison oak/ivy allergen (140). In contrast, metal ions, e.g. nickel cations, form stable metal–protein chelate complexes by co-ordination bonds (141).

The availability of skin-derived cytokines that facilitate chemical-induced LC migration

Migration of LC is stimulated by e.g. $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-18 (142). These cytokines are important for LC migration and the development of skin sensitization in mice.

The ability of a chemical to induce maturation of DCs

In this paragraph, we will discuss human *in vitro* models for allergen screening based on phenotypical changes in DC.

The ability of a chemical to provoke T-cell response by DC

The activation and clonal expansion of allergen-specific memory and effector T cells are pivotal in the acquisition of ACD. Sensitization is dependent on direct association of haptens with MHC molecules or peptides in the groove of these molecules of epidermal LC. For a number of chemical allergens, e.g. 2,4,6-trinitrophenyl or sesquiterpene lactones, it has been shown that they generate hapten-modified peptides that are presented and recognized by CD8^+ or CD4^+ T cells on MHC class I and class II molecules, respectively. For metal chemicals, co-ordinative binding to MHC molecules and/or MHC-bound peptides and the TCR have been shown to be relevant in the activation of allergen-specific T cells (101). Other chemicals seem to directly connect MHC and TCR without binding (103). Ultimately, each approach results in the activation of chemical-reactive T cells, which can cause ACD. Only those chemicals with sufficient affinity for MHC molecules and for which matching TCRs exist can ultimately cause an adapted allergic reaction.

DCs play a crucial role in the initiation phase of contact hypersensitivity reactions in the skin.

Given the importance of skin DCs in the initiation of skin sensitization, various models (*ex vivo* and *in vitro*) have been proposed for screening of potential sensitizing chemicals and will be discussed below.

Ex Vivo: Human Skin Explant Cultures

Models have been developed for predictive testing of the sensitizing capacity of allergens in human skin explant cultures. The group of Das and colleagues (143) used short-term human skin organ cultures to examine the time-course effects of either allergens or irritants on human LC. The advantage of this approach is the involvement of the additional danger signals provided by skin residential cells, e.g. KCs and fibroblasts, which can enhance the allergenic effects on LC. In unchallenged skin, LC preserved their characteristics and distribution within the epidermis of these cultures. Epicutaneous applications of the contact allergens, e.g. DNCB, OXA, nickel sulfate (NiSO_4), or diphenycprone, showed reduction of LC in the epidermis, whereas remaining LC were localized along the epidermal–dermal junction after 24 hr. Furthermore, the assay showed that decrease of LC in the epidermis was dependent on the concentration of the contact allergens and the sensitizing capacities of the test compounds (133). In contrast, the irritants, e.g. sodium dodecyl sulfate (SDS), croton oil, nonanoic acid, or benzalkonium chloride (BC), did not induce these changes. Although the LC migration assay using human skin explants seems effective in identifying allergens, broader introduction of this assay might be hampered by its time-consuming and costly features as well as its dependence of a ready supply of freshly excised skin from surgery.

In Vitro: Peripheral CD14⁺ Blood- or CD34⁺-Progenitor-Derived DCs

Because Sallusto and Lanzavecchia (15) introduced a method to generate large numbers of DCs *in vitro* from peripheral blood-derived monocytes (moDC), numerous studies have been conducted on the effects of allergens on DCs. Aiba et al. (52) was the first to show that allergens can induce phenotypic alterations of immature moDC. They have shown that the allergens nickel and DNCB can significantly upregulate the surface expression of the markers CD54, CD86, HLA-DR, and IL-1 β production, while the irritants SDS, BC, and zinc chloride (ZnCl_2) could not augment these markers. IL-1 β gene expression in moDC is upregulated within 30 min after allergen exposure (144). However, allergen-induced

upregulation of IL-1 β messenger RNA (mRNA) seems to be donor dependent. Exposure to dinitrofluorobenzene (DNFB) resulted in upregulation of IL-1 β mRNA (twofold to threefold) in cells derived from only three of eight donors. MoDC from donors that had increased IL-1 β mRNA to DNFB also induced gene expression of IL-1 β in response to the other allergens, e.g. paraphenylenediamine (PPDA) and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) (145). Likewise, moDC from donors, which failed to respond to DNFB, also did not respond to treatment with either PPDA or MCI/MI. Responder and non-responder donors are stable over time, thus these observations are not due to inter-experimental differences. We observed that DCs from atopic individuals seem to be more susceptible to allergen-induced DC polarization than DC from healthy individuals (Toebak MJ, Moed H, Gibbs S, Bruynzeel DP, Scheper RJ, Rustemeyer T, in preparation). Likewise, it might be possible that DC derived from atopic individuals in the study of Pichowski reflect the responder donors.

Collectively, several investigators reported alterations in expression of CD40, CD54, CD83, CD86, HLA-DR (52), CXCR4, CCR5 (126), CCR7 (146) or production of CXCL8 (61), intracellular IL-1 β (147), or mRNA expression of CCL2, CCL3, and CCL4 (148) after allergen exposure in either monocyte or CD34⁺-derived DC. Recently, gene expression profiles of allergen or irritant-exposed DC were investigated in two different studies (60, 149). First, nickel induced significant changes of 283 genes in CD34⁺-derived DC (60). The following interesting genes were affected: cytokine(receptor)s (e.g. IL-1 β , IL-6, IL-1RA, IL-7R, CXCL1, and CXCR4), membrane markers (CD1c, CD83, and CD86), adhesion molecules (E-cadherin and MMPs), and signalling pathways (MAPK, NF- κ B, and signal transducer and activator of transcription). Unfortunately, in these studies, only nickel is used as an allergen and no irritants were included. Gildea et al. (149) reported that a group of 11 genes are strongly associated with allergen-induced DC activation. However, some of these genes (e.g. ABCA6, CCL23, and Signaling Lymphocytic Activation Molecule) were also affected by one of the four irritants. Strikingly, only one gene, HML2, was able to discriminate the 11 allergens from the four irritants.

In Vitro: DC Lines

During the past decade, human cell lines have been explored as potential surrogates for DC for screening potential sensitizers. The great advantage of using cell lines is the avoidance of donor

variability as discussed above. Cytokine treatment of the human acute myeloid leukaemia cell line KG-1 has been shown to induce a DC-like phenotype and morphology (150, 151). Exposure to chemical allergens had little or no effect on the expression of HLA-DR, CD54, CD80, and CD86 in these cells (152). The THP-1 cell line, derived from human monocytic leukaemia cells, has been examined for its potential to replace moDC in the development of an *in vitro* predictive test for contact sensitizers (152, 153). Exposure to a number of skin sensitizers has been shown to enhance surface expression of CD86 and CD54. In addition, THP-1 cells demonstrated an increased internalization of MHC class II molecules in response to allergen exposure (146). Most recently, the human myeloid leukaemia-derived MUTZ-3 cell line has been proposed as a DC line (154, 155). Azam et al. (156) tested MUTZ-3 against a wide panel of sensitizers and irritants on the cell surface marker expression of HLA-DR, CD40, CD54, CD80, B7-H1, B7-H2, and B7-DC. In general, some of the DC cell lines, such as MUTZ-3 and THP-1, show promising tools for sensitization testing. However, it should be noted that the parameters of activation examined to date have shown relatively less expression changes in DC cell lines compared with blood-derived DC. Identification of novel markers or development of new culture methods might enhance the sensitivity of the cell lines.

Conclusions

At present, surface expression of CD86 (52, 156), CXCL8 production (61), and gene expression of HML2 (149) seem to be the most reliable markers for screening potential sensitizers in both blood-derived DCs and DC lines. In the future, the most robust method to identify the sensitizing capacity of all groups of allergens might benefit from a broad panel of markers, which are restricted to allergen-induced DC maturation. Additionally, it may be possible to exploit DC responses *in vitro* not only for identification of allergens but also for predicting whether type-1 or type-2 immune responses will be induced. Notwithstanding, a more detailed understanding of the ways in which allergens interact with DC will result in robust tools for identification of allergens.

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