Chapter 2

Mechanisms in Allergic Contact Dermatitis

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Introduction

During the past few decades, our understanding of why, where, and when allergic contact dermatitis (ACD) might develop has rapidly increased. Critical discoveries include the identification of T cells as mediators of cell-mediated immunity, their thymic origin and recirculation patterns, and the molecular basis of their specificity to just one or few allergens out of the thousands of allergens known. Progress has also resulted from the identification of genes that determine T-cell function, and the development of monoclonal antibodies that recognize their products. Moreover, the bio-industrial production of large amounts of these products, e.g. cytokines, and the breeding of mice with disruptions in distinct genes (knock-out mice) or provided with additional genes of interest (transgenic mice), have allowed in-depth analysis of skin-inflammatory processes, such as those taking place in ACD.

Although humoral antibody-mediated reactions can be a factor, ACD depends primarily on the activation of allergen-specific T cells [1, 2], and is regarded as a prototype of delayed hypersensitivity, as classified by Turk [3] and Gell and Coombs (type IV hypersensitivity) [4]. Evolutionarily, cell-mediated immunity has developed in vertebrates to facilitate eradication of microorganisms and toxins. Elicitation of ACD by usually non-toxic doses of small molecular-weight allergens indicates that the T-cell repertoire is often slightly broader than one might wish. Thus, ACD can be considered to reflect an untoward side effect of a well-functioning immune system.

Subtle differences can be noted in macroscopic appearance, time course, and histopathology of allergic contact reactions in various vertebrates, including rodents and man. Nevertheless, essentially all basic features are shared. Since both mouse and guinea-pig models, next to clinical studies, have greatly contributed to our present knowledge of ACD, both data sets provide the basis for this chapter.

In ACD, a distinction should be made between induction (sensitization) and effector (elicitation) phases [5] (Fig. 2.1). The induction phase includes the events following a first contact with the allergen and is complete when the individual is sensitized and capable of giving a positive ACD reaction. The effector phase begins upon elicitation (challenge) and results in clinical manifestation of ACD. The entire process of the induction phase requires at least 3 days to several weeks, whereas the effector phase reaction is fully developed within 1–2 days. Main episodes in the induction phase (steps 1–5) and effector phase (step 6) are:

1. *Binding of allergen to skin components.* The allergen penetrating the skin readily associates with all kinds of skin components, including major histocompatibility complex (MHC) proteins. These molecules, in humans encoded for by histocompatibility antigen (HLA) genes, are abundantly present on epidermal Langerhans cells (LC).
2. *Hapten-induced activation of allergen-presenting cells.* Allergen-carrying LC become activated and travel via the afferent lymphatics to the regional lymph nodes, where they settle as so-called interdigitating cells (IDC) in the paracortical T-cell areas.
3. *Recognition of allergen-modified LC by specific T cells.* In non-sensitized individuals the frequency of T cells with certain specificities is usually far below 1 per million. Within the paracortical areas, conditions are optimal for allergen-carrying IDC to encounter naïve T cells that specifically recognize the allergen–MHC molecule complexes. The dendritic morphology of these allergen-presenting cells

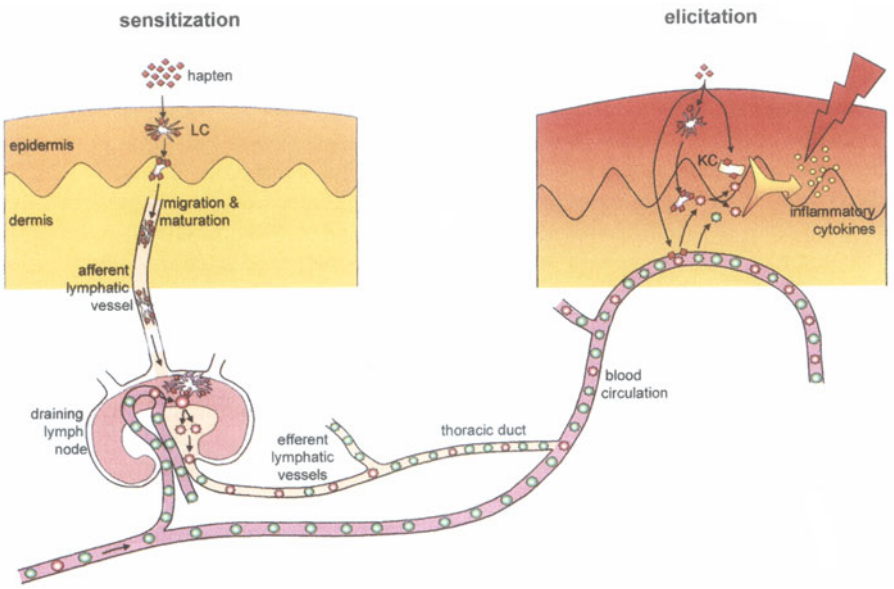


Fig. 2.1. Immunological events in allergic contact dermatitis (ACD). During the induction phase (*left*), skin contact with a hapten triggers migration of epidermal Langerhans cells (*LC*) via the afferent lymphatic vessels to the skin-draining lymph nodes. Haptenized LC home into the T cell-rich paracortical areas. Here, conditions are optimal for encountering naïve T cells that specifically recognize allergen–MHC molecule complexes. Hapten-specific T cells now expand abundantly and generate effector and memory cells, which are released via the efferent lymphatics into the circulation. With their newly acquired homing receptors, these cells can easily extravasate peripheral tissues. Renewed allergen contact sparks off the effector phase (*right*). Due to their lowered activation threshold, hapten-specific effector T cells are triggered by various haptenized cells, including *LC* and keratinocytes (*KC*), to produce proinflammatory cytokines and chemokines. Thereby, more inflammatory cells are recruited further amplifying local inflammatory mediator release. This leads to a gradually developing eczematous reaction, reaching a maximum within 18–48 h, after which reactivity successively declines

strongly facilitates multiple cell contacts, leading to binding and activation of allergen-specific T cells.

4. *Proliferation of specific T cells in draining lymph nodes.* Supported by interleukin (IL)-1, released by the allergen-presenting cells, activated T cells start producing several growth factors, including IL-2. A partly autocrine cascade follows since at the same time receptors for IL-2 are upregulated in these cells, resulting in vigorous blast formation and proliferation within a few days.
5. *Systemic propagation of the specific T-cell progeny.* The expanded progeny is subsequently released via the efferent lymphatics into the blood flow and begins to recirculate. Thus, the frequency of specific effector T cells in the blood may rise to as high as one in a thousand, whereas most of these cells display receptor molecules facilitating their migration into peripheral tissues. In the absence of further allergen contacts, their frequency gradually decreases in subsequent weeks or months, but does not return to the low levels found in naïve individuals.

6. *Effector phase.* By renewed allergen contact, the effector phase is initiated, which depends not only on the increased frequency of specific T cells, and their altered migratory capacities, but also on their low activation threshold. Thus, within the skin, allergen-presenting cells and specific T cells can meet, and lead to plentiful local cytokine and chemokine release. The release of these mediators, many of which have a pro-inflammatory action, causes the arrival of more T cells, thus further amplifying local mediator release. This leads to a gradually developing eczematous reaction that reaches its maximum after 18–48 h and then declines.

In the following sections, we will discuss these six main episodes of the ACD reaction in more detail. Furthermore, we will discuss local hyperreactivity, such as flare-up and retest reactivity, and hyporeactivity, i.e. upon desensitization or tolerance induction.

Binding of Contact Allergens to Skin Components

Chemical Nature of Contact Allergens. Most contact allergens are small, chemically reactive molecules with a molecular weight less than 400 Da. Since these molecules are too small to be antigenic themselves, contact sensitizers are generally referred to as haptens. Upon penetration through the epidermal horny layer, haptens readily conjugate to epidermal and dermal molecules. Sensitizing organic compounds may covalently bind to protein nucleophilic groups, such as thiol, amino, and hydroxyl groups, as is the case with poison oak/ivy allergens (reviewed in [6]). Metal ions, e.g. nickel cations, instead form stable metal–protein chelate complexes by co-ordination bonds [7, 8].

Hapten Presentation by LC. Sensitization is critically dependent on direct association of haptens with epidermal LC-bound MHC molecules, or peptides present in the groove of these molecules. Both MHC class I and class II molecules may be altered this way, and thus give rise to allergen-specific $CD8^+$ and $CD4^+$ T cells, respectively. Distinct differences between allergens can, however, arise from differences in chemical reactivity and lipophilicity (Fig. 2.2), since association with MHC molecules may also result from internalization of the haptens, followed by their intracellular processing as free hapten molecules or hapten-carrier complexes. Lipophilic haptens can directly penetrate into LC, conjugate with cytoplasmic proteins and be processed along the 'endogenous' processing route, thus favouring association with MHC class I molecules [9]. In contrast, hydrophilic allergens such as nickel ions may, after conjugation with skin proteins, be processed along the 'exogenous' route of antigen processing and thus favour the generation of altered MHC class II molecules. Thus, the chemical nature of the haptens can determine to what extent allergen-specific $CD8^+$ and/ or $CD4^+$ T cells will be activated [10–12].

Prohaptens. Whereas most allergens can form hapten–carrier complexes spontaneously, some act as prohaptens and may need activation, e.g. by light- or enzyme-induced metabolic conversion, or oxidation [13]. A prototype prohapten is *p*-phenylenediamine, which needs to be oxidized to a reactive metabolite, known as Bandrowski's base [14]. Tetrachlorosalicylanilide is a typical photoallergen, which undergoes photochemical dechlorination with UV irradiation, ultimately leading to photoadducts with skin proteins [15]. Reduced enzyme activity in certain individuals, related to genetic enzyme

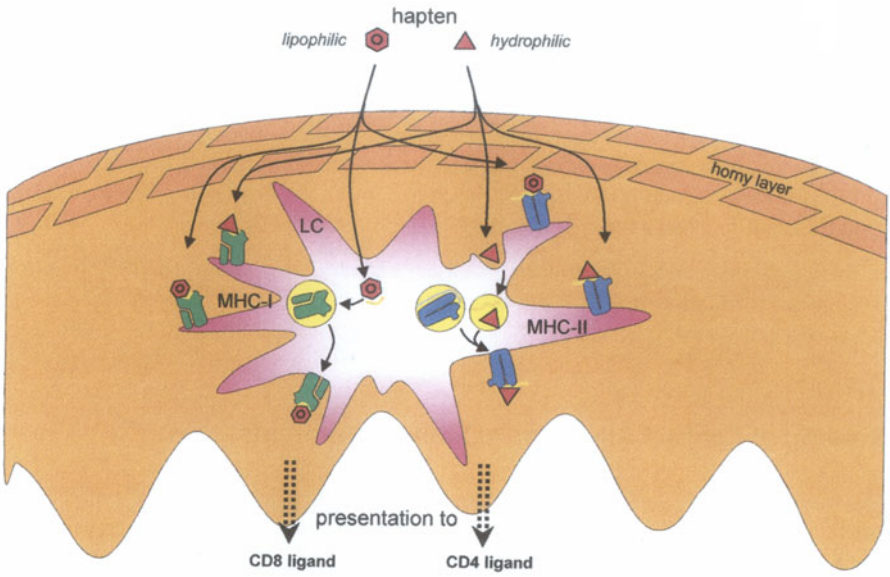


Fig. 2.2. Hapten presentation by epidermal Langerhans cells. Allergen penetrating into the epidermis readily associates with all kinds of skin components, including major histocompatibility complex (*MHC*) proteins, abundantly present on epidermal Langerhans cells (*LC*). Both MHC class I and class II molecules may be altered directly or via intracellular hapten processing and, subsequently, be recognized by allergen-specific $CD8^+$ and $CD4^+$ T cells

polymorphisms, explains the reduced risk of sensitization to prohaptens that need enzymatic activation [16]. Subsequent chapters of this book will present in extensive detail the numerous groups of molecules that have earned disrepute for causing ACD.

Conclusions. Allergenicity depends on several factors determined by the very physicochemical nature of the molecules themselves, i.e. their capacity to penetrate the horny layer, lipophilicity, and chemical reactivity. The sensitizing property of the majority of contact allergens could be predicted from these characteristics [17, 18]. Two other factors, however, further contribute to the allergenicity of chemicals, viz their pro-inflammatory activity and capacity to induce maturation of LC. These issues will be dealt with in more detail in the following sections.

Hapten-Induced Activation of Allergen-Presenting Cells

Physiology of Langerhans Cells. LC are 'professional' antigen-presenting dendritic cells (DC) in the skin [19]. They form a contiguous network within the epidermis and represent 2%–5% of the total epidermal cell population [20]. Their principal functions are internalization, processing, transport, and presentation of skin-encountered

antigens [20–22]. As such, LC play a pivotal role in the induction of cutaneous immune responses to infectious agents as well as to contact sensitizers [23–25]. LC originate from $CD34^+$ bone marrow progenitors, entering the epidermis via the blood stream [26]. Their continuous presence in the epidermis is also assured by local proliferation [21, 27, 28]. They reside as relatively immature DC, characterized by a high capacity to gather antigens by macropinocytosis, whereas their capacity to stimulate naïve T cells is still underdeveloped at this stage [22, 29]. Their prominent dendritic morphology and the presence of distinctive Birbeck granules were observed long ago [30–32]. In the last decade, their pivotal function in the induction of skin immune responses was explained by high expression of molecules mediating antigen-presentation (e.g. MHC class I and II, CD1), as well as of cellular adhesion and costimulatory molecules (e.g. CD54, CD80, CD86, and cutaneous lymphocyte antigen [CLA]) [33–35].

Hapten-Induced LC Activation. Upon topical exposure to contact sensitizers, or other appropriate stimuli (e.g. trauma, irradiation), up to 40% of the local LC become activated [36, 37], leave the epidermis, and migrate, via afferent lymphatic vessels, to the draining lymph nodes [23, 38] (Fig. 2.3). This process of LC migration results from several factors, including contact allergen-induced production of cytokines favouring LC survival [39–41] and loosening from surrounding keratinocytes [42–44]. Thus, within 15 min after exposure to a contact sensitizer, production of IL-1β mRNA and release of IL-1β protein from LC is induced [45, 46]. In turn, IL-1β stimulates release of tumour necrosis factor (TNF)-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) from keratinocytes [46, 47]. Together, these three cytokines facilitate migration of LC from the epidermis towards the lymph nodes [48]. IL-1β and TNF-α downregulate membrane-bound E-cadherin expression and thus cause disentanglement of LC from surrounding keratinocytes (Fig. 2.3) [44, 49, 50]. Simultaneously, adhesion molecules are increasingly expressed that promote LC migration by mediating interactions with the extracellular matrix and dermal cells, such as CD54, α_6 integrin, and CD44 variants [51–55]. Also, production of the epidermal basement membrane degrading enzyme metalloproteinase-9 is upregulated in activated LC [56]. Recently, it has been found that the transmembrane transporter molecule P-glycoprotein is essential for LC migration, which might relate to the putative role of P-glycoprotein in IL-1β release [57].

Next, LC migration is directed by hapten-induced alterations in chemokine receptor levels [58]. Upon maturation, LC downregulate expression of receptors for inflammatory chemokines (e.g. CCR1, 2, 5, and 6), whereas others (including CCR4, 7, and CXCR4) are upregulated (Fig. 2.3) (reviewed by [59] and [60–62]). Notably, CCR7 may guide maturing LC into the draining lymphatics and the lymph node paracortical areas, since one of its ligands (secondary lymphoid tissue chemokine, SLC) is produced by both lymphatic and high endothelial cells [63–65]. Notably, the same receptor-ligand interactions cause naïve T cells, which also express CCR7, to accumulate within the paracortical areas [66]. Final positioning of the LC within the paracortical T-cell areas may be due to another CCR7 ligand, EBI1-ligand chemokine (ELC), produced by resident mature DC [67]. Along with their migration and settling within the draining lymph nodes, haptenized LC further mature, as characterized by their increased expression of costimulatory and antigen-presentation molecules [68, 69]. In addition, they adopt a strongly veiled, interdigitating appearance, thus maximizing the chances of productive encounters with naïve T lymphocytes, recognizing altered self [47, 70, 71].

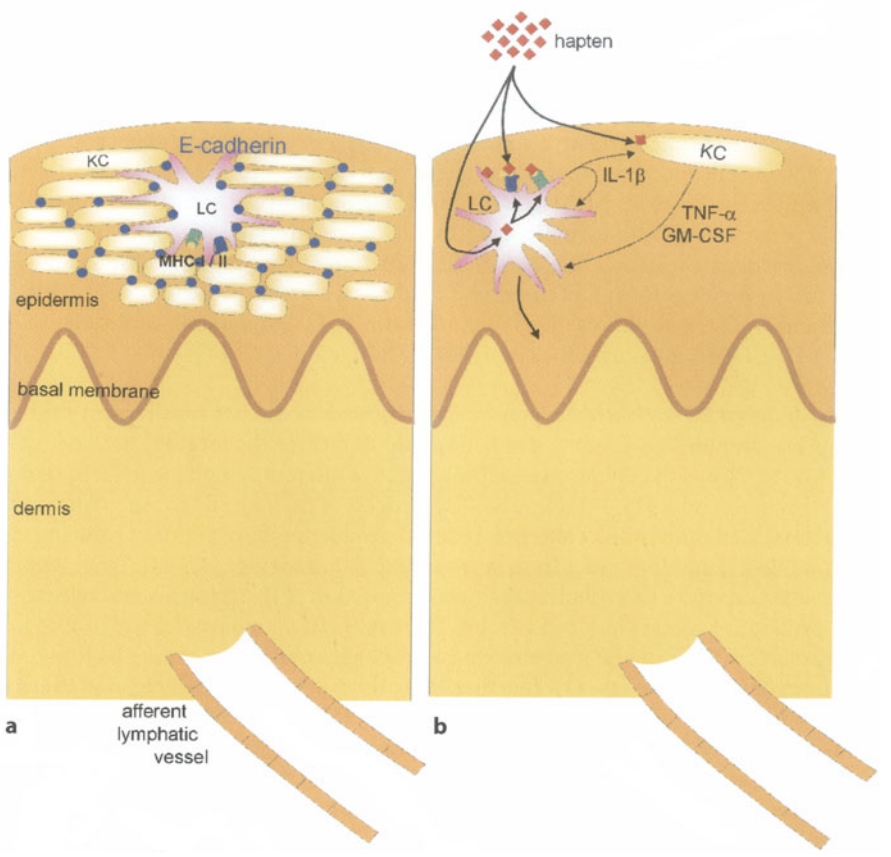


Fig. 2.3. Hapten-induced migration of Langerhans cells. **a** In a resting state, epidermal Langerhans cells (*LC*) reside in suprabasal cell layers, tightly bound to surrounding keratinocytes (*KC*), e.g. by E-cadherin. **b** Early after epidermal hapten exposure, LC produce IL-1β, which induces the release of TNF-α and GM-CSF from keratinocytes. Together, these three cytokines facilitate migration of LC from the epidermis towards the lymph nodes

Recognition of Allergen-Modified Langerhans Cells by Specific T Cells

Homing of Naïve T Cells Into Lymph Nodes. More than 90% of naïve lymphocytes present within the paracortical T-cell areas have entered the lymph nodes by high endothelial venules (HEV) [72]. These cells are characterized not only by CCR7 but also by the presence of a high molecular weight isoform of CD45 (CD45RA) [72, 73]. Entering the lymph nodes via HEV is established by the lymphocyte adhesion molecule L-selectin (CD62L), which allows rolling interaction along the vessel walls by binding to peripheral node addressins (PNAd), such as GlyCAM-1 or CD34 [74–76]. Next, firm adhesion is mediated by the interaction of CD11a/CD18 with endothelial CD54, resulting in subsequent endothelial transmigration. Extravasation and migration of naïve T cells to the

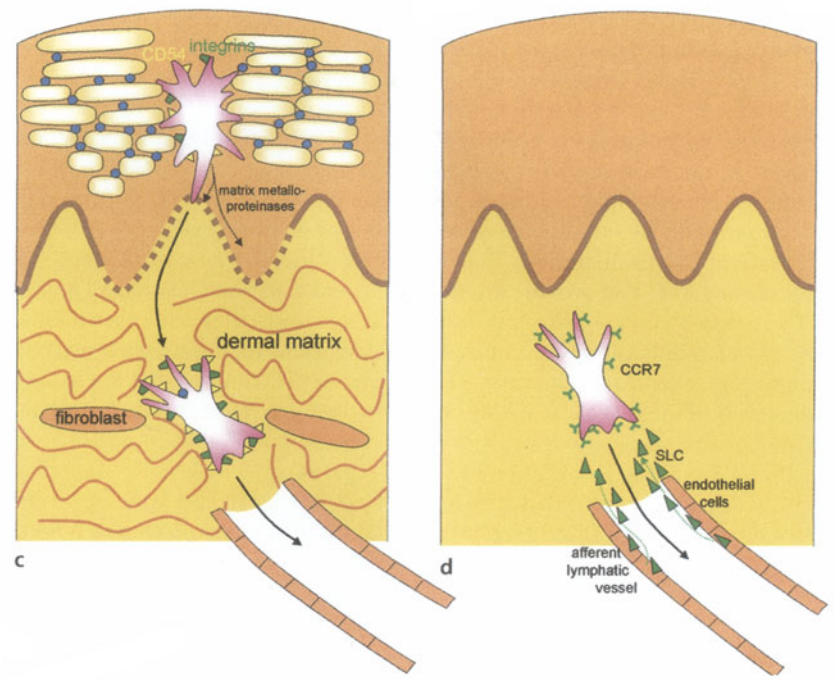


Fig. 2.3. **c** Emigration of LC starts with cytokine-induced disentanglement from surrounding keratinocytes (e.g. by downregulation of E-cadherin) and production of factors facilitating penetration of the basal membrane (e.g. matrix metalloproteinases) and interactions with extracellular matrix and dermal cells (e.g. integrins and integrin ligands). **d** Once in the dermis, LC migration is directed towards the draining afferent lymphatic vessels, guided by local production of chemokines (e.g. secondary lymphoid tissue chemokine, *SLC*) acting on newly expressed chemokine receptors, such as CCR7, on activated LC. Along their journey, haptenized LC further mature as characterized by their increased dendritic morphology and expression of costimulatory and antigen-presentation molecules

paracortical T-cell areas is supported by chemokines such as DC-CK-1, SLC, and ELC produced locally by HEV and by hapten-loaded and resident DC [65, 77–79]. In non-sensitized individuals, frequencies of contact-allergen specific T cells are very low, and estimates vary from 1 per 10^9 to maximally 1 per 10^6 [72, 80]. Nevertheless, the preferential homing of naïve T cells into the lymph node paracortical areas, and the large surface area of interdigitating cells, make allergen-specific T-cell activation likely with only few dendritic cells exposing adequate densities of haptenized-MHC molecules [81, 82].

Activation of Hapten-Specific T Cells. As outlined in "Binding of Contact Allergens to Skin Components", the chemical nature of the hapten determines its eventual cytoplasmic routing in antigen-presenting cells (APC), and thus whether presentation will be predominantly in context of MHC class I or II molecules (Fig. 2.2). T cells, expressing CD8 or CD4 molecules can recognize the hapten-MHC class I or II complex, which in turn stabilizes MHC membrane expression [83, 84]. Chances of productive interactions

with T cells are high since each MHC-allergen complex can trigger a high number of T-cell receptor (TCR) molecules ('serial triggering') [85]. Moreover, after contacting specific $CD4^+$ T cells, hapten-presenting DC may reach a stable super-activated state, allowing for efficient activation of subsequently encountered specific $CD8^+$ T cells [86]. The actual T-cell activation is executed by TCRζ-chain mediated signal transduction, followed by an intracellular cascade of biochemical events, including protein phosphorylation, inositol phospholipid hydrolysis, increase in cytosolic Ca^{2+} [87, 88], and activation of transcription factors, ultimately leading to gene activation (Fig. 2.4) [89].

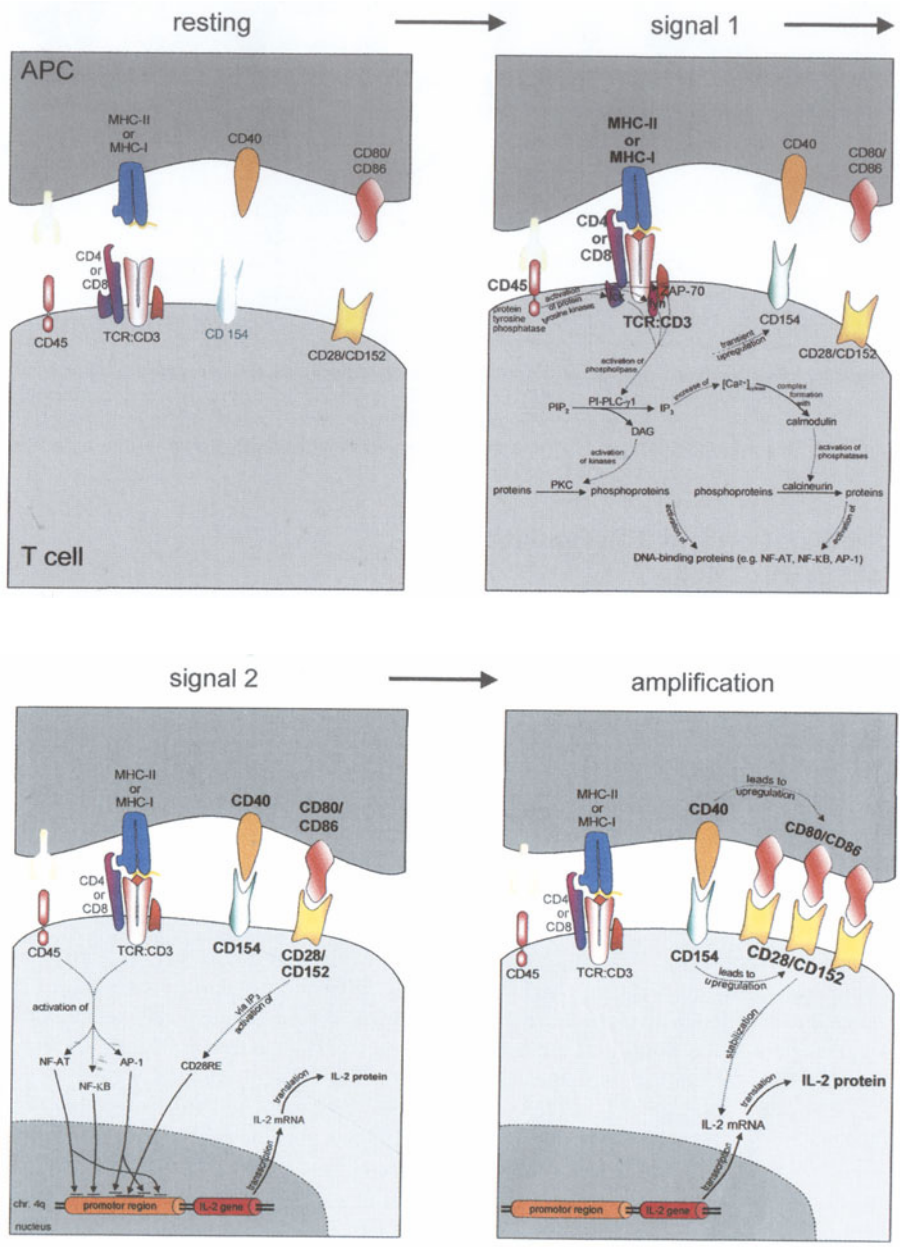
For activation and proliferation, TCR triggering ('signal 1') is insufficient, but hapten-presenting APC also provide the required co-stimulation ('signal 2'; Fig. 2.4) [90, 91]. The costimulatory signals may involve secreted molecules, such as cytokines (IL-1), or sets of cellular adhesion molecules (CAMs) and their counter-structures present on the outer cellular membranes of APC and T cells (summarized in Fig. 2.5). Expression levels of most of these CAMs vary with their activational status, and thus can provide positive stimulatory feedback-loops. For example, as mentioned above, after specific TCR binding and ligation of CD40L (CD154) on T cells with CD40 molecules, APC reach a super activated state, characterized by overexpression of several CAMs, including CD80 and CD86 (Fig. 2.4) [92, 93]. In turn, these molecules bind to and increase expression of CD28 on T cells. This interaction stabilizes CD154 expression, causing amplified CD154–CD40 signalling [93, 94].

The activational cascade is, as illustrated above, characterized by mutual activation of both hapten-presenting APC and hapten-reactive T cells. Whereas this activation protects the APC from apoptotic death and prolongs their life to increase the chance of activating their cognate T cells, only the latter capitalize on these interactions by giving rise to progeny. As discussed below, to promote T-cell growth, cellular adhesion stimuli need to be complimented by a broth of cytokines, many of which are released by the same APC. Together, elevated expression levels of (co-)stimulatory molecules on APC and local abundance of cytokines overcome the relatively high activation threshold of naïve T cells [95].

Conclusions. The intricate structure of lymph node paracortical areas, the differential expression of chemokines and their receptors, the characteristic membrane ruffling of IDC, and the predominant circulation of naïve T lymphocytes through these lymph

Fig. 2.4. Activation of hapten-specific T cells. T-cell receptor (TCR) triggering by hapten-major histocompatibility complex (*MHC*) complexes ('signal 1') is insufficient for T-cell activation. But 'professional' antigen-presenting cells (*APC*), such as Langerhans cells, can provide the required costimulation ('signal 2'), involving secreted molecules, such as cytokines, or sets of cellular adhesion molecules present on the outer cellular membranes of APC and T cells. T cells, stimulated in this way, activate nuclear responder elements (e.g. CD28RE). Together with nuclear transcription factors (*NF*), produced upon TCR triggering, these nuclear responder elements enable transcription of T-cell growth factors, e.g. IL-2. APC–T cell interaction gives rise to mutual activation ('amplification'): on APC, ligation of CD40 with CD154 molecules on T cells induces overexpression of several costimulatory molecules, including CD80 and CD86. In turn, these molecules bind to and increase expression of CD28 on T cells. This interaction stabilizes CD154 expression, causing amplified CD154–CD40 signalling, and preserves strong IL-2 production, finally resulting in abundant T-cell expansion

node areas provide optimal conditions for T cell-receptor binding, i.e. the first signal for induction of T-cell activation [96]. Intimate DC–T cell contacts are further strengthened by secondary signals, provided for by sets of cellular adhesion molecules, and growth-promoting cytokines (reviewed in [97, 98]).



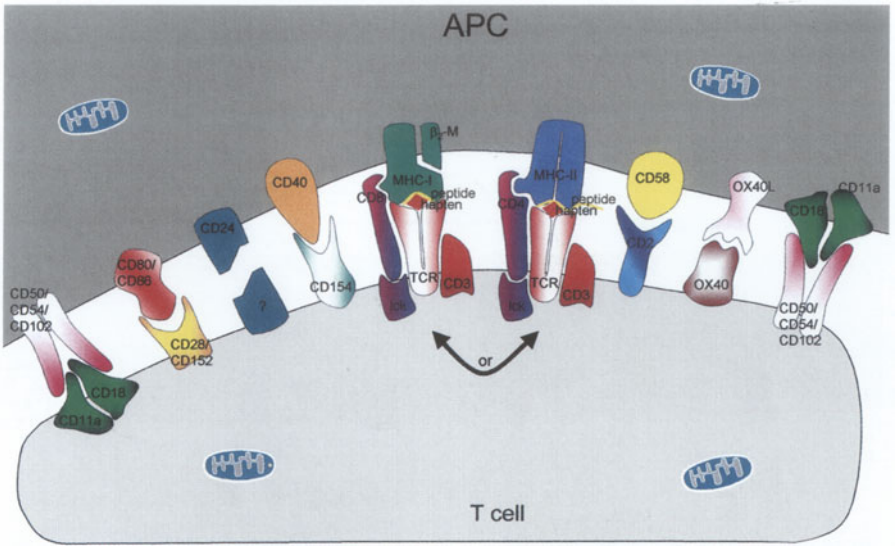


Fig. 2.5. Antigen-presenting cell and T-cell interaction molecules. On the outer cellular membranes of antigen-presenting cells (*APC*) and T cells, respectively, sets of interaction molecules are expressed. They include antigen presentation (like MHC class I and II) and recognition (such as T-cell receptor, TCR/CD8, and CD4 complexes, respectively) and various adhesion molecules

Proliferation and Differentiation of Specific T Cells

T-Cell Proliferation. When activated, naïve allergen-specific T cells start producing several cytokines, including IL-2, which is a highly potent T-cell growth factor [99–101]. Within 30 min after stimulation, IL-2 mRNA can already be detected [99, 102]. In particular, ligation of T cell-bound CD28 receptors augments and prolongs IL-2 production for several days [103]. Simultaneously, the IL-2 receptor α-chain gets upregulated, allowing for the assembly of up to approximately 10^4 high affinity IL-2 receptor molecules per T cell after 3–6 days [101]. This allows appropriately stimulated T cells to start proliferating abundantly. This process can be visible as an impressive, sometimes painful lymph node swelling.

T-Cell Differentiation. Whereas their allergen specificity remains strictly conserved along with their proliferation, the T-cell progeny differentiates within a few days into effector cells with distinct cytokine profiles [104, 105]. While naïve T cells release only small amounts of a limited number of cytokines, e.g. IL-2, activated T cells secrete a broad array of cytokines which, besides IL-2, include IL-4, IL-10, IFN-γ, and TNF-β ('type-0' cytokine profile) [106–108]. Within a few days, however, T-cell cytokine production can polarize towards one of the three major cytokine profiles, referred to as 'type 1' (characterized by a predominant release of IFN-γ and TNF-β), 'type 2' (IL-4 and/or IL-10), or 'type 3' [transforming growth factor (TGF)-β; Fig. 2.6] [109–111]. Evolutionarily, based on requirements for combating different exogenous microbial

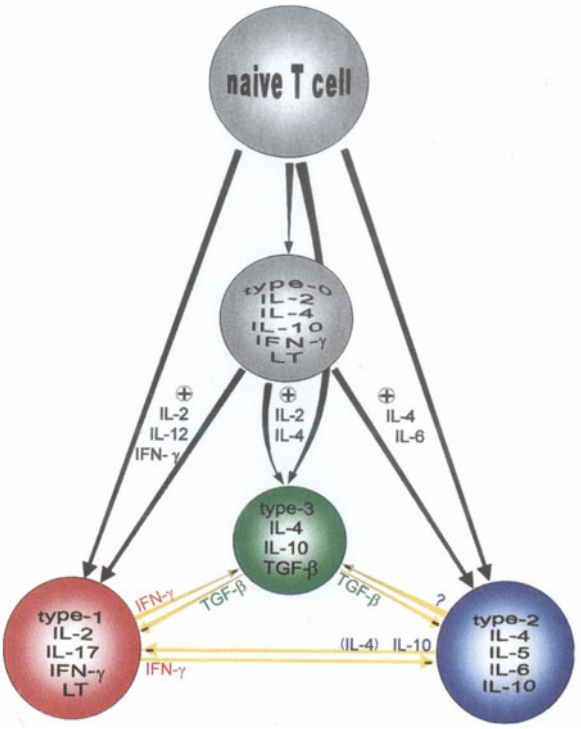


Fig. 2.6. Generation and cross-regulation of different types of T cells. Depending on the immunological microenvironment, activated naïve T cells, which only release low amounts of few cytokines (e.g. IL-2), can differentiate into type-0 cells, secreting a broad array of cytokines, or the more polarized T-cell types 1, 2, or 3, with their characteristic cytokine profiles. By secreting mutually inhibitory cytokines, the latter cell types can interactively regulate their activation and, thereby, control the type of immune response. *IL*, interleukin; *IFN*, interferon; *TGF*, transforming growth factor; *LT*, lymphotoxin

infections, these polarized cytokine profiles promote inflammation and cytotoxic effector cell functions (type 1), antibody production (type 2), or anti-inflammatory activities in conjunction with production of IgA (type 3) [112]. The latter excretory antibody excludes microbial entry, e.g. along mucosal surfaces [113]. As outlined above, both $CD4^+$ and $CD8^+$ allergen-specific T cells may become involved in contact sensitization, and it is now clear that both subsets can display these polarized cytokine profiles and, thereby, play distinct effector and regulatory roles in ACD [114–116].

Polarization of cytokine production depends on several factors, including (1) the site and cytokine environment of first allergenic contact, (2) the molecular nature and concentrations of the allergen, and (3) the neuroendocrine factors.

Cytokine Environment. In the skin-draining lymph nodes, allergen-activated LC and macrophages rapidly produce large amounts of IL-12, switching off IL-4 gene expression, thus promoting the differentiation of type-1 T cells [106, 117, 118]. Notably, this process

is reversible, and type-1 T cells retain high IL-4R expression throughout, leaving these sensitive for IL-4 as a growth factor [119]. On the other hand, functional IL-12R expression remains restricted to type-0 and type-1 cells [120]. Type-2 T cells, e.g. developing in mucosa-draining lymph nodes, lose the genes encoding the IL-12-R β2 chain and thus, type-2 differentiation is irreversible [120]. Early differentiation of type-1 T cells is co-promoted by IL-12 induced secondary cytokines, e.g. IFN-γ, released by non-specific 'bystander' lymphocytes, including NK cells, within the lymph nodes [121, 122]. Next, cell contact-mediated signals provided by APC during priming of naïve T cells constitute a critically important factor in skewing T-cell differentiation [123]: type-1 differentiation of T cells is strongly stimulated by CD154 triggering through CD40 on APC [124]. In contrast, ligation of CD134L (gp 34; on APC) by CD134 (OX40; on T cells) promotes the differentiation of type-2 T cells [125]. Also, CD86 expression on APC contributes to preferential differentiation of naïve T cells towards a type-2 cytokine profile [126–129].

After a few days type-1, but not type-2, T cells lose functional IFN-γR expression [130, 131] and thus become refractory to the growth inhibitory effects of IFN-γ [132]. Once established, the type-1-differentiated T cells produce IFN-γ and IL-18, thereby further suppressing development of type-2 T cells [133]. Thus, considering that contact allergens will mainly enter via the skin, type-1 pro-inflammatory T cells are thought to represent the primary effector cells in ACD. Nevertheless, in sensitized individuals, type-2 T cells also play a role, as shown by both IL-4 production and allergen-specific type-2 T cells in the blood and at ACD reaction sites (see "The Effector Phase of Allergic Contact Dermatitis") [134–136]. Their role may increase along with the longevity of sensitization, since several factors contribute to shifting type-1 to type-2 responses, including reversibility of the former and not of the latter T cells, as mentioned above [137].

After mucosal contacts with contact allergens, type-2 T cell responses are most prominent. In the mucosal (cytokine) environment, DC release only small quantities of IL-12, whereas IL-4 and IL-6 production by cells of the mast cell/basophil lineages, macrophages and NK(T) cells is relatively high [138–140], abundantly present within the mucosal layers. Moreover, these tissues, as compared to the skin, contain high frequencies of B cells, which, when presenting antigen, favour type-2 responses through the abundant release of IL-10 [141, 142]. IL-10 is known to inhibit type-1 differentiation, just as IFN-γ and IL-18 interfere with type-2 T-cell differentiation [105, 143, 144]. Along the mucosal surfaces, T cells may also develop exhibiting the third 'type-3' T cell-cytokine profile, characterized by TGF-β production (reviewed by [145]). Since these cells play critical regulatory roles in ACD, they will be described further in "Hyporeactivity: Tolerance and Desensitization".

Nature of the Allergen. A second factor in determining T-cell cytokine-production profiles, although still poorly understood, is the molecular character of the contact allergen itself, and the resulting extent of TCR triggering [105, 146, 147]. For both protein and peptide antigens, high doses of antigen might favour type-2 responses, whereas intermediate/low doses would induce type-1 T-cell responses [105, 148]. To what extent this translates to contact allergens is still unclear. Certainly, endogenous capacities of contact allergens to induce IL-12 by LC, vs IL-4 by mast cells, basophils, or NK(T) cells, will affect the outcome. In this respect, some contact allergens are notorious for inducing type-2 responses, even if their primary contact is by the skin route, e.g. trimellitic acid, which is also known as a respiratory sensitizer [149, 150].

Neuroendocrine Factors. Diverse neuroendocrine factors codetermine T-cell differentiation [151–153]. An important link has been established between nutritional deprivation and decreased T cell-mediated allergic contact reactions [154]. Apparently, adipocyte-derived leptin, a hormone released by adequately nourished and functioning fat cells, is required for type-1 T-cell differentiation. Administration of leptin to mice restored ACD reactivity in mice during starvation [154]. Also, androgen hormones and adrenal cortex-derived steroid hormones, e.g. dehydroepiandrosterone (DHEA), promote type-1 T-cell and ACD reactivity. DHEA, like testosterone, may favour differentiation of type-1 T cells by promoting IFN-γ and suppressing IL-4 release ([155, 156]; Giltay, personal communication). In contrast, the female sex hormone progesterone furthers the development of type-2 $CD4^+$ T cells and even induces, at least transient, IL-4 production and CD30 expression in established type-1 T cells [157]. Type-2 T-cell polarization is also facilitated by adrenocorticotrophic hormone (ACTH) and glucocorticosteroids [158], and by prostaglandin (PG)E_2 [159]. PGE_2, released from mononuclear phagocytes, augments intracellular cAMP levels, resulting in inhibition of pro-inflammatory cytokine, like IFN-γ and TNF-α, production [160–163] and thus can influence the development of effector T cells in ACD.

Conclusions. In healthy individuals, primary skin contacts with most contact allergens lead to differentiation and expansion of allergen-specific effector T cells displaying the type-1 cytokine profile. The same allergens, if encountered along mucosal surfaces, favour the development of type-2 and/or type-3 effector T cells. Factors skewing towards the latter profile are still unknown, despite their critical importance for understanding mucosal tolerance induction (see "Hyporeactivity: Tolerance and Desensitization"). For most, if not all allergens, prolonged allergenic contacts, also along the skin route, ultimately lead to a predominance of type-2 allergen-specific T cells which may take over the role of type-1 T cells in causing contact allergic hypersensitivity.

Systemic Propagation of the Specific T-Cell Progeny

T-Cell Recirculation. From the skin-draining lymphoid tissue, the progeny of primed T cells are released via the efferent lymphatic vessels and the thoracic duct into the blood where they circulate for several minutes, up to 1 h (Fig. 2.7) [164]. Like their naïve precursors, these effector/memory T cells can still enter lymphoid tissues upon adhering to HEV within the paracortical areas, because they continue to express L-selectin molecules (see "Recognition of Allergen-Modified Langerhans Cells by Specific T Cells") [165–167]. However, their lymph node entry via the afferent lymphatics increases as a consequence of their higher capacity to enter peripheral tissues [168]. The latter capacity relates to higher surface densities of adhesion molecules, such as VLA-4, facilitating migration through non-activated, flat endothelia, e.g. in the skin. Notably, vascular adhesion within peripheral tissues is strongly augmented when expression of vascular adhesion molecules, such as vascular cell adhesion molecule (VCAM), are upregulated, e.g. through cytokines released at inflammatory sites. Similarly, other ligand-counter structure pairs contribute to migration into peripheral tissues. Cutaneous lymphocyte-associated antigen and the P-selectin glycoprotein ligand (PSGL-1; CD162) are overexpressed on effector/memory T cells, and mediate binding to venules in the upper

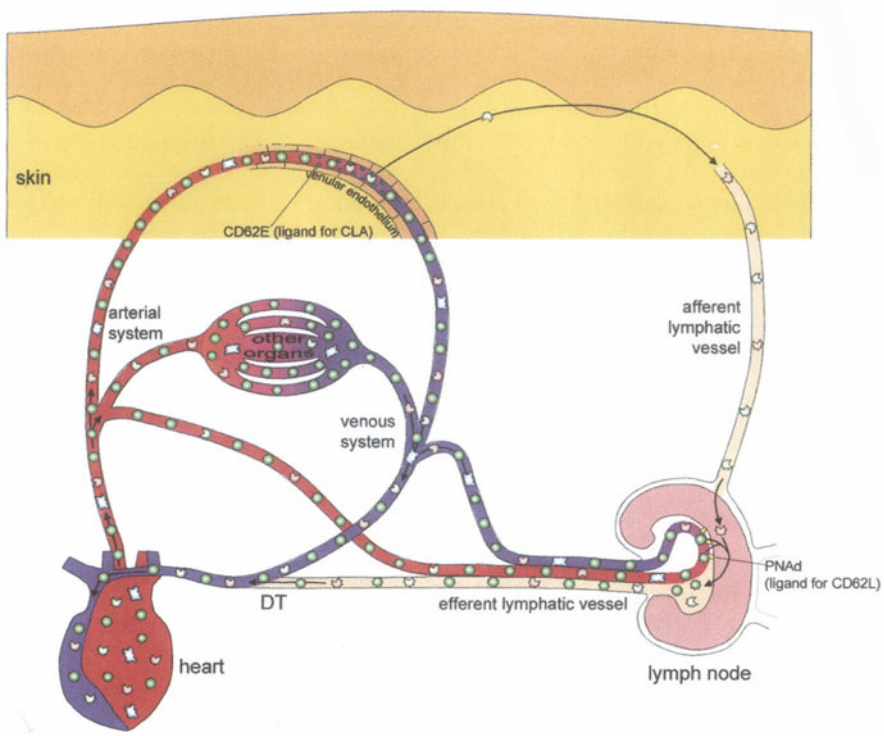


Fig. 2.7. Systemic propagation of hapten-specific T cells. From the skin-draining lymphoid tissue, the progeny of primed T cells is released via the efferent lymphatic vessels and the thoracic duct (*DT*) into the blood and becomes part of the circulation. Like their naïve precursors, these effector/memory T cells can still enter lymphoid tissues by binding to peripheral node addressins (*PNAd*). But increased expression of skin-homing molecules, e.g. cutaneous lymphocyte antigen (*CLA*), facilitates their migration in the skin. Via the afferent lymphatic vessels, cells re-enter draining nodes and the recirculating lymphocyte pool

dermis through the sugar-binding counter-structures CD62 E (E-selectin) and CD62P (P-selectin) [169–171]. Vascular expression of the latter molecules is also greatly increased by local inflammatory reactions [172–174]. Notably, expression of the lymphocyte-bound ligands is highest only for short periods after activation, thus endowing recently activated T cells with unique capacities to enter skin sites and exert effector functions. Upon repeated allergenic contacts, therefore, in particular within a few weeks after sensitization, recently activated effector T cells will give rise to allergic hypersensitivity reactions, as outlined below. However, within lymph nodes draining inflamed skin areas, they can also contribute to further expansion of the allergen-specific T-cell pool.

Different Homing Patterns. Effector/memory T cells show different recirculation patterns depending on their sites of original priming, e.g. within skin- or mucosa-draining lymphoid tissues [175, 176]. These differences are mediated by distinct vascular adhesion molecules and by the involvement of different chemokine-receptor pairs. First,

mucosal lymphoid tissue venules express yet another L-selectin binding molecule, the mucosal addressin MAdCAM-1. The latter molecule mediates preferential binding of lymphoid cells generated within the mucosal lymphoid tissues, showing overexpression of $\alpha_4\beta_7$, a MAdCAM-1 binding integrin. Thus, along the gut, Peyer's patches and lamina propria attract T lymphocyte progeny generated within other mucosal tissues, rather than contact allergen-specific cells derived from skin-draining lymph nodes. As outlined above, the latter are characterized by their high expression of CLA, facilitating preferential homing to the skin through its ligand CD62 E [177–179]. Second, T cells biased towards production of type-1 cytokines may show a higher propensity to enter skin sites, as compared to mucosal tissues. In mice, the early influx of type-1 T cells into delayed-type hypersensitivity (DTH) reactions was found to be more efficient than that of type-2 T cells, although both cell types expressed CLA. Here, CD162, highly expressed by type-1 T cells, was found to be important for this preferential homing [172, 180, 181]. Moreover, type-1 T cells express distinct chemokine receptors, notably CCR5 and CXCR3, contributing to skin entry [59, 182, 183]. In contrast, recirculation through mucosal tissues preferentially involves CCR3 and CCR4 [66, 184]. The latter chemokine receptors are not only overexpressed on type-2 cytokine-producing T cells, but also on basophils and eosinophils. Together, these cells contribute strongly to local immediate allergic hyperresponsiveness. Results obtained thus far favour the view that type-1 T cells enter skin sites most readily [180, 185]. Their primary function may be in the early control of antigenic pressure, e.g. through amplification of macrophage effector functions. However, subset recirculation patterns are not rigid, and, given the fact that type-1 cells can shift cytokine production towards a type-2 profile, allergic contact skin inflammatory lesions may rapidly be dominated by type-2 allergen-specific T cells (see "Proliferation and Differentiation of Specific T Cells").

Allergen-Specific T-Cell Recirculation: Options for In Vitro Testing. The dissemination and recirculation of primed, allergen-specific T cells through the body suggests that blood represents a most useful and accessible source for T cell-based in vitro assays for ACD. A major advantage of in vitro testing would be the non-interference with the patient's immune system, thus eliminating any potential risk of primary sensitization by in vivo skin testing. Although such tests have found several applications in fundamental research, e.g. on recognition of restriction elements, cross-reactivities and cytokine-profile analyses, their use for routine diagnostic purposes is limited. Even in highly sensitized individuals, frequencies of contact allergen-specific memory/effector cells may still be below 1 per 10^3 [116, 186]. Given the relatively small samples of blood obtainable by venepuncture (at only one or a few time points), numbers of specific T cells in any culture well used for subsequent in vitro testing would typically be below 100 cells/well. For comparison, in vivo skin test reactions recruit at least 1000 times more specific T cells from circulating lymphocytes passing by for the period of testing, i.e. at least 24 h [164, 187]. The sensitivities required, therefore, for direct in vitro read-out assays, e.g. allergen-induced proliferation or cytokine production, may often exceed the lowest detection limits. However, the observation that in vivo signal amplification may allow for the detection of a single memory/effector T cell [188, 189] suggests that it may be possible to solve sensitivity problems.

Appropriate allergen presentation, however, is a major hurdle for in vitro testing, with a broad range of requirements for different allergens with unique solubilities, tox-

icities, and reactivity profiles. Moreover, in the absence of LC, monocytes are the major source of APC, though their numbers in peripheral blood may vary substantially within and between donors. Of note, optimal APC function is particularly critical for recirculating resting/memory T cells to respond. In the absence of repeated allergenic contacts, most CD45RO memory cells may finally revert to the naïve CD45RA phenotype, with a higher threshold for triggering [190, 191]. Supplementing in vitro test cultures with an appropriate mix of cytokines may, however, compensate for this effect [186].

Conclusions. After antigenic activation the progeny of primed T cells, i.e. effector/memory cells, are released via the efferent lymphatics into the bloodstream. Like their naïve precursors, they can again leave the circulation into lymphoid organs anywhere in the body, thus rapidly ensuring systemic memory. They differ, however, from naïve T cells in many ways, including increased surface exposure of ligands facilitating entry into the peripheral tissues, such as the skin. On the vascular side, distinct exit patterns from the circulation are determined by tissue-dependent expression of vascular addressins and other adhesion molecules, and locally released chemoattractant molecules, i.e. chemokines. Once inside the tissues, these chemokines and stromal adhesion molecules determine the transit times before recirculating T cells eventually re-enter the blood stream. Thus, peripheral blood provides a good source for in vitro studies in ACD but, besides budgetary and logistical reasons, theoretical considerations argue against wide-scale applicability of in vitro assays for routine diagnostic purposes.

The Effector Phase of Allergic Contact Dermatitis

Elicitation of ACD. Once sensitized, individuals can develop ACD upon re-exposure to the contact allergen. Positive patch test reactions mimic this process of allergen-specific skin hyperreactivity. Thus, skin contacts induce an inflammatory reaction that, in general, is maximal within 2–3 days and, without further allergen supply, declines thereafter (Fig. 2.8). Looked at superficially, the mechanism of this type of skin hyperreactivity is straightforward: allergen elicitation or challenge leads to the (epi)dermal accumulation of contact allergen-specific memory/effector T lymphocytes which, upon encountering allergen-presenting cells, are reactivated to release pro-inflammatory cytokines. These, in turn, spark the inflammatory process, resulting in macroscopically detectable erythema and induration. As compared to immediate allergic reactions, developing within a few minutes after mast-cell degranulation, ACD reactions show a delayed time course, since both the migration of allergen-specific T cells from the dermal vessels and local cytokine production need several hours to become fully effective. Still, the picture of the rise and fall of ACD reactions is far from clear. Some persistent issues are discussed below, notably: (1) irritant properties of allergens, (2) role of early-phase reactivity, (3) T-cell patrol and specificity, (4) effector T-cell phenotypes, and (5) downregulatory processes.

Irritant Properties of Allergens. Within a few hours after allergenic skin contact, immunohistopathological changes can be observed, including vasodilatation, upregulation of endothelial adhesion molecules [192, 193], mast-cell degranulation [194, 195], keratinocyte cytokine and chemokine production [196], influx of leucocytes [197,

198], and LC migration towards the dermis [52, 199, 200]. These pro-inflammatory phenomena, which are also observed in non-sensitized individuals [201] and in T cell-deficient nude mice [202], strongly contribute to allergenicity [5]. Clearly most, if not all, of these effects can also be caused by irritants and, therefore, do not unambiguously discriminate between irritants and contact allergens [203–205]. Probably, true differences between these types of compounds depend on whether or not allergen-specific T cells become involved. Thus, only after specific T-cell triggering might distinctive features be observed, e.g. local release of certain chemokines, such as CXCL10 (IP-10) and CXCL11 (I-TAC/IP-9) [206]. The latter chemokines are produced by IFN-γ-activated keratinocytes and T lymphocytes [207].

Certainly, pro-inflammatory effects of contact allergens increase, in many ways, the chance of allergen-specific T cells meeting their targets. The first cells affected by skin contact, i.e. keratinocytes and LC, are thought to represent major sources of pivotal mediators such as IL-1β and TNF-α [45, 208]. First, as described in "Hapten-Induced Activation of Allergen-Presenting Cells", these cytokines cause hapten-bearing LC to mature and migrate towards the dermis [33, 47]. But, these cytokines also cause (over)expression of adhesion molecules on dermal postcapillary endothelial cells, and loosen intercellular junctions. Thereby, extravasation of leucocytes, including allergen-specific T cells, is strongly promoted [208–211]. Moreover, haptens can stimulate nitric oxide (NO) production of the inducible NO-synthase (iNOS) of LC and keratinocytes [212–214], which contributes to local oedema, vasodilatation, and cell extravasation [212, 214].

Histopathological analyses support the view that the major causative events take place in the papillary dermis, close to the site of entry of allergen-specific T cells, for instance at hair follicles, where haptens easily penetrate and blood capillaries are nearby [215]. Here, perivascular mononuclear cell infiltrates develop, giving the highest chance of encounters between allergen-presenting cells and specific T cells. Once triggered, extravasated T cells will readily enter the lower epidermal layers, in which haptenized keratinocytes produce lymphocyte-attracting chemokines, like CXCL10 (IP-10) [206]. Subsequently, since memory T cells can also be triggered by 'non-professional' APC, including KC, fibroblasts, and infiltrating mononuclear cells, ACD reactivity is amplified in the epidermis [95, 97, 201]. Together, these events result in the characteristic epidermal damage seen in ACD, such as spongiosis and hyperplasia. Notably, in ongoing ACD reactions, the production of chemokines attracting lymphocytes and monocytes/macrophages, in addition to the production of cytokines, adds to the non-specific recruitment and activation of leucocytes [59, 216, 217]. Thus, like the very early events in the effector phase reaction, the final response to a contact allergen is antigen-non-specific. It is therefore not surprising that allergic and irritant reactions are histologically alike.

Early Phase Reactivity. The role of an antibody-mediated early phase reaction in the development of ACD is still unclear in man, although Askenase and his colleagues have generated robust data to support this view in murine models [218–221]. Hapten-specific IgM, produced upon immunization by distant hapten-activated B-1 cells [222, 223], can bind antigen early after challenge [222, 224] and activate complement [225]. The resulting C5a causes the release of serotonin and TNF-α from local mast cells and platelets, leading to vascular dilatation and permeabilization, detectable as an early

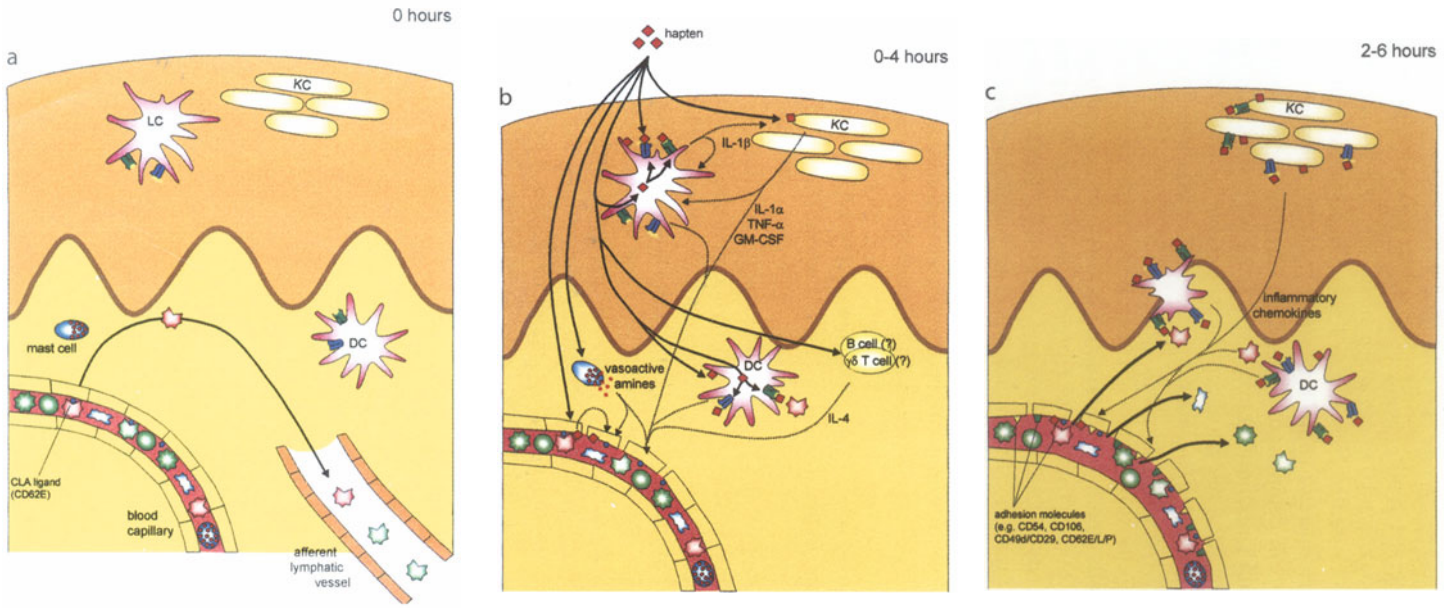


Fig. 2.8a–c. The effector phase of allergic contact dermatitis. **a** *0 h* In resting skin relatively few randomly patrolling, skin-homing CLA^+ T cells are present. **b** *0–4 h* Re-exposure of the contact allergen, binding to (epi)dermal molecules and cells, induces release of proinflammatory cytokines. **c** *2–6 h* Influenced by inflammatory mediators, activated epidermal Langerhans cells (*LC*) start migrating towards the basal membrane and endothelial cells express increased numbers of adhesion molecules. Endothelial cell-bound hapten causes preferential extravasation of hapten-specific T cells, which are further guided by inflammatory chemokines

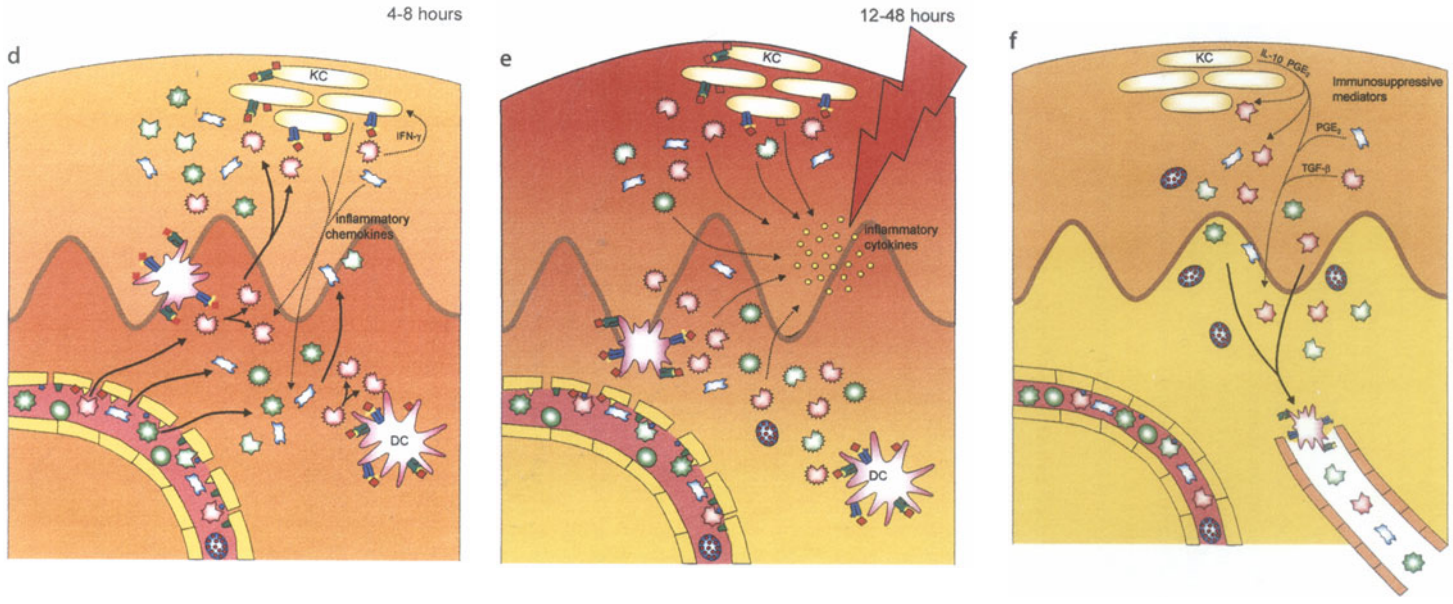


Fig. 2.8d–f. d *4–8 h* Hapten-activated T cells release increasing amounts of inflammatory mediators, amplifying further cellular infiltration. **e** *12–48 h* The inflammatory reaction reaching its maximum, characterized by (epi)dermal infiltrates, oedema, and spongiosis. **f** *48–120 h* Gradually, downregulatory mechanisms take over, leading to decreased inflammation and disappearance of the cellular infiltrate. Finally, primordial conditions are reconstituted except for a few residual hapten-specific T cells causing the local skin memory. *KC*, keratinocyte; *DC*, dendritic cell

ear swelling peaking at 2 h [221, 226, 227]. Furthermore, C5a and TNF-α induce the upregulation of adhesion molecules on local endothelial cells [228, 229], thereby contributing to the recruitment of T cells in hapten challenge sites [221, 229]. In addition, human T cells were recently found to express the C5a receptor and are chemoattracted to endothelium-bound C5a [230]. However, antibodies against most contact allergens, including nickel, are only occasionally detectable in man, arguing against humoral mechanisms playing more than a minor role in clinical ACD [231, 232]. In addition to an auxiliary role of humoral immunity, similar effects may be mediated by allergen-specific T cells with an unusual phenotype (CD3–CD4–CD8–Thy1$^+$), which recognize the hapten and, within 2 h of hapten application were found to elicit an early-phase response [220]. Also, γδ T cells might contribute in a non-antigen-specific, probably non-MHC-restricted manner, to (early) elicitation responses [233–236].

T-Cell Patrol and Specificity of T-Cell Infiltrates. Whereas early non-specific skin reactivity to contact allergens is pivotal for both sensitization and elicitation, full-scale development of ACD, of course, depends on allergen-specific T cells within the (epi)dermal infiltrates. In healthy skin there is a constant flow of memory T cells from the dermis towards the draining lymph nodes: about 200 T cells/h/cm^2 skin [55]. Since one single antigen-specific T cell can already trigger visible skin inflammation [189], randomly skin-patrolling memory/effector T cells might account for the initiation of the allergen-specific effector phase. However, since frequencies of hapten-specific T cells in sensitized individuals may still remain below 1 in 1000, this does not seem to be a realistic scenario. Thus, augmented random and/or specific T-cell infiltration accompanies the development of ACD. Apparently, local chemokine release is pivotal in this respect [237]. The question concerning the specificity of ACD T-cell infiltrates has so far received little attention. In a guinea-pig model, preferential entry of dinitrochlorobenzene (DNCB)-specific T cells was observed within 18 h after elicitation of skin tests with DNCB, as compared to non-related compounds [238]. Probably, extravasation of hapten-specific T cells benefits from T cell receptor-mediated interactions with endothelial MHC molecules, presenting hapten penetrated from the skin. Within minutes after epicutaneous application, hapten can indeed be found in dermal tissues and on endothelial cells [192, 239, 240]. Interestingly, whereas preferential entry may already contribute to extraordinarily high frequencies of allergen-specific T cells (within 48 h up to 10%) [135, 187], at later stages, when the ACD reaction fades away, the local frequency of allergen-specific T cells may increase even further, due to allergen-induced proliferation and rescue from apoptosis. Thus, at former skin reaction sites these cells can generate 'local skin memory' (see "Flare-up and Retest Reactivity").

Effector T-Cell Phenotypes. The debate on phenotypes of effector T cells in ACD is still ongoing, although recent studies have shed light on longstanding issues. This certainly holds true for expression of membrane molecules determining lymphocyte-migration patterns. Once released from reactive skin-draining lymph nodes to the blood, effector T cells express increased levels of molecules mediating adhesion to peripheral vascular endothelia, e.g. the cutaneous lymphocyte antigen CLA [241–243]. Notably, the same molecule is used by precursor LC to find their way to the skin [244]. To what extent other cellular adhesion molecules, associated with T-cell differentiation and maturation, in particular the low-molecular-weight CD45 isoforms, contribute to mi-

gration into skin-inflammatory foci is still unclear [245, 246]. Tissue-bound ligand molecules clearly involved in lymphocyte extravasation and extravascular migration in the skin are fibronectin and collagens [247–250].

Since cutaneous infiltrates show a clear preponderance of $CD4^+$ T cells, it is not surprising that these cells have most often been held responsible for mediating ACD. Nevertheless, as discussed in "Recognition of Allergen-Modified Langerhans Cells by Specific T Cells", infiltrates contain both allergen-specific $CD4^+$ and $CD8^+$ T cells [251, 252]. The latter might mediate skin inflammation through killing of hapten-bearing target cells. Indeed, it has become clear that both $CD4^+$ and $CD8^+$ T cells can act as effector cells in DTH and ACD reactions [253–256]. Thus, neither of these subsets can be regarded simply as regulatory or suppressor cells, although both of these subsets may, depending on the allergen models and read-out assays, play such roles [115, 257].

An essentially similar conclusion holds true for T-cell subsets (whether $CD4^+$ or $CD8^+$), releasing type-1 or type-2 cytokines, or both (type 0). Whereas type-1 cytokines, in particular IFN-γ, display well-established pro-inflammatory effects [132, 258], IL-4, a hallmark type-2 cytokine, can cause erythema and induration, when released in the skin [259, 260]. Indeed, blockage of IL-4 can interfere with ACD [260]. Furthermore, analyses of skin test biopsies demonstrate the presence of not only type-1 T cells, but also allergen-specific type-2 and type-0 T cells [116, 134, 135]. Entry of type-1 T cells into skin-inflammatory sites is facilitated by their expression of CCR1, 5, and CXCR3 receptors for IFN-γ-induced chemokines such as MIP-1α, MIP-1β, and IP-10 [59, 261, 262]. Type-2 T cells overexpress a partially different set of chemokine receptors, including, similar to eosinophils and basophils, CCR3, 4, and 8 [66, 263]. This would explain why local release of mediators commonly associated with immediate allergic reactions, such as eotaxins, preferentially involve type-2 T cells. Thus, a picture emerges in which ACD reactions can be caused both by allergen-specific type-1 or type-2 T cells [116, 264]. In retrospect, the downregulatory effects of IL-4 on ACD reactions observed earlier in some mouse models [265] might be ascribed to accelerated allergen-clearance rather than to blunt suppression. Still, both with time and repeated allergen-pressure, type-2 responsiveness may rapidly take over [266]. Allergen-specific T cells isolated from skin test sites of sensitized individuals, as compared to blood, showed a strong bias towards type-2 cytokine profiles [134]. Additional local IFN-γ release seems, however, indispensable, since for a broad panel of contact allergens, clinical ACD reactions were characterized by increased expression of mRNA encoding IFN-γ-inducible chemokines [206]. In addition, transgenic mice expressing IFN-γ in the epidermis showed strongly increased ACD reactivity [267].

Downregulatory Processes. Resolution of ACD reactions and risk factors for the development of chronicity are not yet fully understood. Of course, if the allergen source is limited, as with skin testing, local concentrations of allergen usually rapidly decrease, thus taking away the critical trigger of the ACD reaction cascade. Since even ACD reactions due to chronic exposure to allergen seldom result in permanent tissue destruction and scarification, immunoregulatory factors most likely contribute to prevention of excessive cytotoxicity and fatal destruction of the basal membrane. Both IL-1 and heparinase, secreted from activated keratinocytes and T cells, protect keratinocytes from TNF-α-induced apoptosis [268, 269]. Moreover, activated effector T cells can undergo activation-induced cell death (AICD) during the resolution phase

[270]. Notably, pro-inflammatory type-1 T cells, expressing high levels of Fas-ligand and low amounts of apoptosis-protecting FAP-1 protein, are more susceptible to AICD than type-2 cells [271]. This may partly explain the shift towards type-2 reactivity that is observed upon prolonged allergen exposure [266]. Moreover, during the late phase of ACD, keratinocytes, infiltrated macrophages and T cells start producing IL-10 [272–274], which has many anti-inflammatory activities, including suppression of antigen-presenting cell and macrophage functions [110, 275]. In addition, the release of factors, such as PGE_2 and TGF-β, derived from activated keratinocytes and infiltrated leucocytes, e.g. type-3 T cells, contribute to dampening of the immune response [276, 277]. Release of PGE_2, on the one hand, inhibits production of pro-inflammatory cytokines [163, 278] and, on the other hand, activates basophils [279]. These may constitute up to 5%–15% of infiltrating cells in late phase ACD reactions [280] and are also believed to contribute to downregulation of the inflammatory response [281, 282]. TGF-β silences activated T cells and inhibits further infiltration by downregulating the expression of adhesion molecules on both endothelial and skin cells [109]. Regulatory cells producing these suppressive mediators might even predominate in skin sites, frequently exposed to the same allergen, and known to show local (allergen-specific) hypo-responsiveness [283].

Conclusions. ACD reactions can certainly be mediated by classical effector cells, i.e. allergen-specific $CD4^+$ type-1 T cells which, upon triggering by allergen-presenting cells, produce IFN-γ to activate non-specific inflammatory cells like macrophages. However, $CD8^+$ T cells, and other cytokines, including IL-4, can also play major roles in ACD. The conspicuous difference with DTH reactions induced by intradermal administration of protein antigens, i.e. the epidermal infiltrate, can largely be attributed to hapten-induced chemokine release by keratinocytes.

Flare-up and Retest Reactivity

Flare-up Phenomena. Flare-up reactivity of former ACD and patch test reaction sites is sometimes observed [284–286]. From the basic mechanisms of ACD, it can be inferred that allergen-specific flare-up reactions depend either on local allergen or T-cell retention at these skin sites. Flare-up reactions due to locally persisting allergen can readily be observed in man, when from about 1 week after primary sensitization, sufficient effector T cells have entered the circulation to react with residual allergen at the sensitization site [287]. Pre-existing allergic reactivity and, thus, positive reactivity to formaldehyde apparently potentiated primary sensitization to penicillin, causing the other, previously negative, penicillin patch test sites to flare up from about 1 week after skin testing. Local allergen retention, however, is usually of short duration only. In experimental guinea-pig studies using DNCB, chromium and penicillin allergens for sensitization, and skin testing at different days before or after sensitization, we never observed allergen retention in the skin to mediate flare-up reactions for periods exceeding 2 weeks (Scheper et al., unpublished results).

Local Skin Memory. In contrast, allergen-specific T cells may persist for at least several months in the skin (Fig. 2.9) [288]. Thus, locally increased allergen-specific hy-

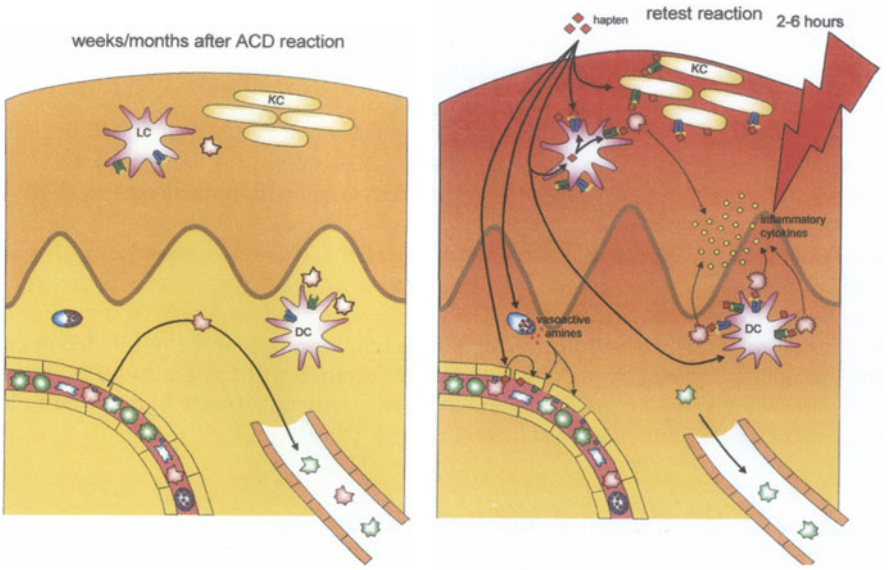


Fig. 2.9. Local skin memory. In former allergic contact dermatitis sites a few hapten-specific T cells can remain, mainly close to dermal dendritic cells (*DC*). Retest reaction: renewed hapten contact can induce a rapid onset of an erythematous reaction, sparked off by the residual hapten-specific T cells. *KC*, keratinocyte; *LC*, Langerhans cell

perreactivity, detectable through either accelerated 'retest' reactivity (after repeated allergenic contacts at the same skin site) or flare-up reactivity (after repeated allergen entry from the circulation, e.g. derived from food), may be observed for long periods of time at former skin reaction sites [289–292]. Typically, the erythematous reactions peak between 2 h and 6 h after contact with the allergen. Histological examination of such previous skin reaction sites shows that only a few T cells remain present during such periods. The remarkable flare-up reactivity at such sites can be understood by considering that just one specific effector T cell can be sufficient to generate macroscopic reactivity [189]. Moreover, a very high frequency of the residual T cells may be specific for the allergen, as discussed in "The Effector Phase of Allergic Contact Dermatitis". Notably, with higher allergen doses, in highly sensitized individuals, unrelated skin test sites may show flare-up reactions [288] and even generalized erythematous macular eruptions can be observed [293]. The latter reactivities are probably a corollary of the fact that recently activated T cells show strong expression of adhesion and homing molecules, e.g. CLA and chemokine receptors, such as CCR5, facilitating migration into peripheral tissues and thus allergen-specific T cell patrol in the skin [243, 262, 294]. Upon allergen entry from the circulation, these allergen-specific T cells could mediate generalized erythematous reactions.

Recently, we have explored the possibilities of exploiting the specific retest/'skin memory' phenomenon in both guinea-pig models and man, to differentiate between concomitant sensitization and cross-reactivity [295–298]. We hypothesized that, with preferential local retention of T cells reactive to the first allergen used for skin test-

ing, no accelerated retest reactivity would be observed with a second, non-cross-reactive allergen, even should the individual also be allergic to the latter allergen. However, if retests were made with a second allergen, cross-reactive with the same T cells, an accelerated erythematous reaction would again be observed. Indeed, this hypothesis was confirmed for several different combinations of contact allergens, in both guinea pigs and man. Thus, retesting guinea pigs previously sensitized to both methyl methacrylate (MMA) and DNCB, and skin tested with both allergens, showed accelerated retest reactivities with four different methacrylate congeners on the former MMA, but not DNCB, patch test sites [295, 296]. This retest model can also be readily applied in clinical practice to discriminate between cross-reactivity and concomitant sensitization. Matura et al. [297] confirmed positive cross-retest reactions for cloprednol and tixocortol pivalate, both belonging to group A, and budesonide, amcinonide, and triamcinolone, all belonging to group B corticosteroids [297]. In another recent study with this model, true cross-reactivity to Disperse Blue 106 and 124 was established by Seidenari et al. [299] (see also [300]).

Hyporeactivity: Tolerance and Desensitization

Of course, uncontrolled development and expression of T cell-mediated immune function would be detrimental to the host. During evolution, several mechanisms developed to curtail lymph node hyperplasia or to prevent excessive skin damage upon persisting antigen exposure.

Regulation of Immune Responses. First, allergen contacts, e.g. by oral or intravenous administration, may lead to large-scale presentation of allergen by cells other than skin DC (Fig. 2.10). In the absence of appropriate co-stimulatory signals (as described in "Recognition of Allergen-Modified Langerhans Cells") naïve T cells may be anergized, i.e. turned into an unresponsive state, eventually leading to their death by apoptosis (Fig. 2.11) [299–302]. With increasing density of MHC-antigen complexes on the surface of APC, multiple levels of T-cell tolerance might be induced, with the characteristic stages called ignorance, anergy, and deletion [303–306]. Unresponsiveness of T cells, induced by allergenic contacts at skin sites where LC/DC functions have been damaged, e.g. by UV irradiation, or are naturally absent, e.g. in the tail skin of mice, may be ascribed to T-cell anergy, frequently associated with TCR/CD4 or CD8 downregulation [307–309]. Whereas such anergy reflects 'passive' unresponsiveness, tolerance by 'active' suppression may also be induced under similar circumstances [306]. Actually, even regular epicutaneous allergenic contacts not only induce effector T cells but also lymphocytes regulating T-cell proliferation (afferently acting regulatory cells) or, with frequent skin contacts, causing decreased skin reactivity (regulatory cells of effector phase). Apparently, allergic contact hypersensitivity is the resultant of a delicate balance between effector and regulatory mechanisms [283].

Cellular Basis of Active Tolerance. Upon preferential stimulation of regulatory cells, e.g. by feeding non-primed, naïve individuals with contact allergens, strong and stable allergen-specific, active tolerance may develop [310–313]. The concept of active regu-

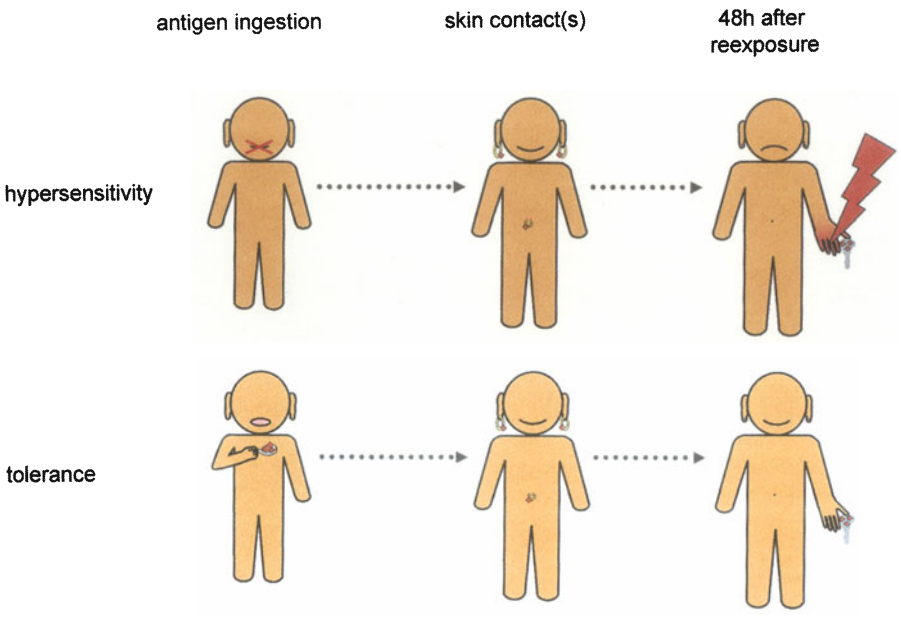


Fig. 2.10. Induction of oral tolerance. Hapten ingestion, prior to potential sensitizing skin contact(s), can induce hapten-specific tolerance

latory ('suppressor') cells controlling ACD is based on the fact that, in experimental animal models, such allergen-specific tolerance can be transferred by lymphoid cells from tolerant to naïve animals [236, 314]. Active suppression, as revealed by these adoptive cell transfers, is a critical event in regulating T-cell responses to contact sensitizers, and to all possible peptide/protein antigens, including bacterial, autoimmune, and graft rejection antigens [315–317].

Like effector T cells in ACD, regulatory cells are not a single subpopulation of cells. As outlined above, depending among other things on the nature of the allergen and route of exposure, ACD can be mediated by both $CD4^+$ and $CD8^+$ T cells, either or both releasing type-1 or type-2 cytokines. Probably, given a predominant effector phenotype for a particular allergen, each of the other phenotypes can act as regulatory cells [318]. Nevertheless, earlier data suggested that type-2 cytokine producing cells may be most prominent regulatory cells in ACD, since allergic contact hypersensitivity was found to be enhanced, and tolerance reversed, by appropriately timed treatment with cytostatic drugs, including cyclophosphamide [319–321], preferentially affecting type-2 T cells [322]. Interferons and IL-12, both impairing type-2 and -3 cells, were also shown to inhibit regulatory cells and to stimulate effector-cell functions in mouse models [323–325]. On the other hand, in particular after mucosal allergen contact stimulation, T cells predominantly producing TGF-β (type-3 cytokine profile) may act as regulatory cells [326, 327]. These T cells promote anti-inflammatory immunity, e.g. by switching antibody production to IgA, which mediates secretory immunity and thus contributes to antigen exclusion in the lumen, e.g. of the gas-

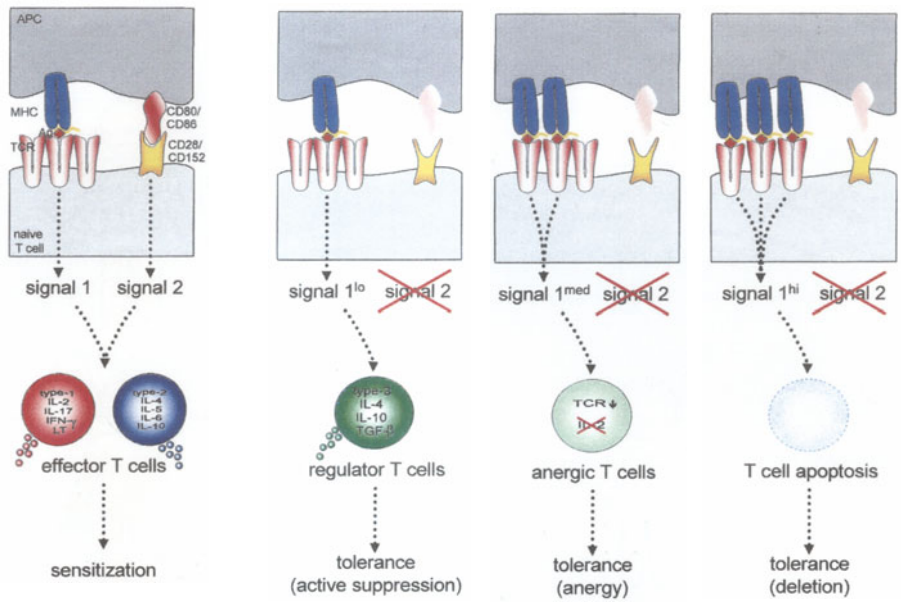


Fig. 2.11. The character of the APC–T cell interaction determines the immunological outcome. Sensitization: Naïve T cells, activated by antigen-presenting cells (*APC*) providing both hapten-specific ('signal 1') and appropriate costimulatory ('signal 2') signals, develop into effector T cells, characterized by type-0, -1, and -2 cytokine secretion profiles. Tolerance: In the absence of appropriate costimulatory signals, immunological tolerance may develop. With increasing density of MHC-hapten complexes on the surface of APC, activating 'signal 1' T-cell pathways, multiple levels of T-cell tolerance might be induced

tro-intestinal tract [328, 329]. Of note, TGF-β strongly suppresses development of both type-1 and -2 effector T cells, and can silence T cells in a semi-naïve state [109]. Whether these type-3 T cells, or their precursors, are more sensitive to cytostatic drugs is not known.

Regulatory Mechanisms of the Effector Phase. A critical feature of the regulatory principles involving mutual regulation of T-cell subpopulations by type-1 and -2 cytokines, and both of these in turn by TGF-β-producing T cells, is that their function is observed foremost in primary immune responses (Fig. 2.6). Regulation may also pertain to the actual ACD reactions, i.e. the effector phase. Several 'suppressive' pathways could lead to decreased allergic skin reactivity, including hapten removal by increased blood flow and metabolism by cells of the inflammatory infiltrate. Other regulatory mechanisms can also be involved, such as $CD8^+$ T cells, acting either as suppressor ($CD28^-CD11b^+$) or cytotoxic ($CD28^+CD11b^-$) T cells [330, 331], which may downregulate skin reactivity by focusing on allergen-presenting DC as their targets [331].

Redundancy of Tolerance Mechanisms. Besides these types of regulatory T cells, producing different cytokines, or exerting distinct cytotoxicities, other mechanisms may

also contribute to immune regulation and tolerance. Apparently, the risk of excessive immune reactivity should be very low. These mechanisms involve allergen-specific T cells shedding truncated T-cell receptors, acting as antagonists and blocking allergen presentation [332], and high-dose allergen-induced anergic T cells [306]. Possibly, the latter cells, by actively suppressing DC functions, can function as 'active' suppressor cells [306, 333]. Interestingly, DC, becoming suppressive by this mechanism [306] or by suppressive cytokines like IL-10 and PGE_2 [163, 334, 335], can, in turn, act themselves as suppressor cells by conferring antigen-specific anergy to subsequently encountered T cells [336–338]. Although, at present, consensus has been reached about a critical role of regulatory/suppressor cells in the development and expression of ACD, the relative contributions of each of the various mechanisms are still far from clear. Potential therapeutic applications of regulatory cells in various disorders, such as allergic contact dermatitis and autoimmune diseases, are currently under investigation.

Induction of Lasting Tolerance Only in Naïve Individuals. Both clinical and experimental findings indicate that full and persistent tolerance can only be induced prior to any sensitizing allergen contacts [311, 339, 340]. Upon primary allergenic contacts, naïve T cells differentiate to produce polarized cytokine profiles (Fig. 2.6). Once polarized, however, T-cell profiles are irreversible, due to loss of cytokine (receptor) genes, or at least very stable, due to the mutually suppressive activities of T-cell cytokines. An important corollary of the latter concept of active suppression is the bystander effect, in which the response to any antigen can be downregulated by immunosuppressive cytokines acting at a very local tolerogenic microenvironment [341]. The latter was observed for both protein antigens [342, 343] and methacrylate contact allergens [314]. The concept may also explain why even non-sensitizing doses of nickel applied to the skin prevented subsequent tolerance induction by feeding the metal allergen [344]. This may have contributed to incomplete tolerance induction in earlier clinical studies when feeding with poison ivy-/oak-derived allergens [345]. Apparently, the progeny of naïve allergen-specific cells, once 'on the stage', have been triggered to a 'subclinical' degree towards effector cells and become refractory to regulatory cell action. Indeed, to our knowledge, permanent reversal of existing ACD in healthy individuals has, as yet, never been achieved. Nevertheless, as described above, effector cells still seem susceptible, though transiently, to the downregulation of allergen reactivity, as was observed in desensitization procedures [344, 346].

Transient Desensitization in Primed Individuals. For dermatologists, methods by which patients might be desensitized for existing ACD would be a welcome addition to the currently prevailing symptomatic therapies, and investigators have made a wide variety of attempts to achieve this goal. Unfortunately, therapeutic protocols involving ingestion of poison ivy allergen, penicillin, or nickel sulphate were of only transient benefit to the patients [345–349]. Similarly in animal models, only a limited and transient degree of hyposensitization was obtained by Chase [350] when feeding DNCB-contact-sensitized guinea pigs with the allergen, whereas, for achieving persistent chromium-unresponsiveness in presensitized animals, Polak and Turk [351] needed a rigorous protocol involving up to lethal doses of the allergen. As outlined above,

mechanisms underlying specific desensitization in ACD probably depend on direct interference of allergen with effector T-cell function, by blocking or downregulating T-cell receptors, leading to anergy [352]. As the onset of desensitization is immediate, no suppressor mechanisms may initially be involved. Apparently in the absence of LC, MHC class II-positive keratinocytes can serve as APC and are very effective in rendering allergen-specific effector cells anergic [353]. Moreover, at later stages active suppression may come into play resulting from secondary inactivation of DC function by anergized T cells [306]. Nevertheless, major problems with in vivo desensitization procedures relate to the refractoriness of effector T cells to regulatory cell functions, and the rapid replacement of anergized effector cells by naïve T cells from relatively protected peripheral lymphoid tissues, which can be the source of a new generation of effector cells upon sensitizing allergen contacts. The same conclusions can be drawn from attempts to achieve local desensitization. It was found that local desensitization by repeatedly applying allergen at the same skin site did not result from local skin hardening or LC inactivation, as local reactivity to an unrelated allergen at the site was unimpaired [283]. Persistence of cellular infiltrates, in the absence of erythematous reactivity, at a desensitized skin site could reflect local anergy, but also locally active regulatory cells. Upon discontinuation of allergen exposure, however, local unresponsiveness was rapidly (within 1 week) lost. Collectively, this data illustrates the problems encountered in attempting to eradicate established effector-T-cell function, not only in ACD but also in autoimmune diseases [315].

Summary and Conclusions

Extensive research has led to a better understanding of the mechanisms of ACD. The basic immunology of ACD is now well-defined, including T-cell migratory patterns, recognition of distinct allergens, interactions with other inflammatory cells to generate inflammation, and cytokine profiles. But new complexities have emerged. For instance, in contrast to earlier belief, many of the currently known T-cell subpopulations can act either or both as effector and regulatory cells, depending on the nature of the allergen, the route of entry, frequency of exposure, and many other, still ill-defined factors. In particular, the poor understanding of regulatory mechanisms in ACD still hampers further therapeutic progress. So far, no methods of permanent desensitization have been devised.

Nevertheless, recently defined cellular interaction molecules and mediators provide promising targets for anti-inflammatory drugs, some of which have already entered clinical trials. Clearly, drugs found to be effective in preventing severe T-cell-mediated conditions, e.g. rejection of a vital organ graft, should be very safe before their use in ACD would seem appropriate. To date, prudence favours alternative measures to prevent ACD, be it through legal action to outlaw the use of certain materials or through avoiding personal contact with these materials. In the meantime, for difficult-to-avoid allergens, further studies on the potential value of tolerogenic treatments prior to possible sensitization seem warranted.

References

1. Boerrigter GH, Bril H, Scheper RJ (1988) Hapten-specific antibodies in allergic contact dermatitis in the guinea pig. Int Arch Allergy Appl Immunol 85:385–391
2. Bergstresser PR (1989) Sensitization and elicitation of inflammation in contact dermatitis. In: Norris DA (ed) Immune mechanisms in cutaneous disease. Dekker, New York, pp 219–246
3. Turk JL (1975) Delayed hypersensitivity, 2nd edn. North-Holland, Amsterdam
4. Gell PDH, Coombs RRA, Lachman R (1975) Clinical aspects of immunology, 3rd edn. Blackwell, London
5. Grabbe S, Schwarz (1998) Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 19:37–43
6. Roberts DW, Lepoittevin J-P (1998) in: Allergic Contact Dermatitis. Lepoittevin J-P, Basketter DA, Goossens A, Karlberg A-T (eds) Springer, Berlin Heidelberg New York, pp 81–111
7. Lecoeur S, Andre C, Beaune PH (1996) Tienilic acid-induced autoimmune hepatitis: anti-liver and -kidney microsomal type 2 autoantibodies recognize a three-site conformational epitope on cytochrome P4502C9. Mol Pharmacol 50:326–333
8. Eliasson E, Kenna JG (1996) Cytochrome P450 2E1 is a cell surface autoantigen in halothane hepatitis. Mol Pharmacol 50:573–582
9. (1999) Molecular mechanisms of $CD8^+$ T cell-mediated delayed hypersensitivity: implications for allergies, asthma, and autoimmunity. J Allergy Clin Immunol 103:192–199
10. Daly AK (1995) Molecular basis of polymorphic drug metabolism. J Mol Med 73:539–553
11. Liberato DJ, Byers VS, Ennick RG, Castagnoli N (1981) Region specific attack of nitrogen and sulfur nucleophiles on quinones from poison oak/ivy catechols (urushiols) and analogues as models for urushiol-protein conjugate formation. J Med Chem 24:28–33
12. Kalish RS, Wood JA, LaPorte A (1994) Processing of urushiol (poison ivy) hapten by both endogenous and exogenous pathways for presentation to T cells in vitro. J Clin Invest 93:2039–2047
13. Bourdi M, Tinel M, Beaune PH, Pessayre D (1994) Interactions of dihydralazine with cytochromes P4501 A: a possible explanation for the appearance of anti-cytochrome P4501A2 autoantibodies. Mol Pharmacol 45:1287–1295
14. Krasteva M, Nicolas JF, Chabeau G, Garrigue JL, Bour H, Thivolet J, Schmitt D (1993) Dissociation of allergenic and immunogenic functions in contact sensitivity to para-phenylenediamine. Int Arch Allergy Immunol 102:200–204
15. Epling GA, Wells JL, Ung Chan Yoon (1988) Photochemical transformations in salicylanilide photoallergy. Photochem Photobiol 47:167–171
16. Schnuch A, Westphal GA, Muller MM, Schulz TG, Geier J, Brasch J, Merk HF, Kawakubo Y, Richter G, Koch P, Fuchs T, Gutgesell T, Reich K, Gebhardt M, Becker D, Grabbe J, Szliska C, Aberer W, Hallier E (1998) Genotype and phenotype of N-acetyltransferase 2 (NAT2) polymorphism in patients with contact allergy. Contact Dermatitis 38:209–211
17. Lepoittevin JP (1999) Development of structure-activity relationships (SARs) in allergic contact dermatitis. Cell Biol Toxicol 15:47–55
18. Basketter DA (1998) Chemistry of contact allergens and irritants. Am J Cont Derm 9:119–124
19. Nestle FO, Burg G (1999) Dendritic cells: role in skin diseases and therapeutic applications. Clin Exp Dermatol 24:204–207
20. Teunissen MB (1992) Dynamic nature and function of epidermal Langerhans cells in vivo and in vitro: a review with emphasis on human Langerhans cells. Histochem J 24:697–716
21. Breathnach SM (1988) The Langerhans cell. Centenary review. Br J Dermatol 119:463–469
22. Aiba S, Katz SI (1990) Phenotypic and functional characteristics of in vivo-activated Langerhans cells. J Immunol 145:2791–2796
23. Braathen LR, Thorsby E (1980) Studies on human epidermal Langerhans cells. I. Allo-activating and antigen-presenting capacity. Scand J Immunol 11:401–408
24. Inaba K, Schuler G, Witmer MD, Valinsky J, Atassi B, Steinman RM (1986) Immunologic properties of purified epidermal Langerhans cells. Distinct requirements for stimulation of unprimed and sensitized T lymphocytes. J Exp Med 164:605–613
25. Kimber I, Cumberbatch M (1992) Dendritic cells and cutaneous immune responses to chemical allergens. Toxicol Appl Pharmacol 117:137–146

26. Dieu M-C, Vanbervliet B, Vicari A, Bridon J-M, Oldham E, Ait-Yahia S, Brière F, Zlotnik A, Lebecque S, Caux C (1998) Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J Exp Med 188:373–386
27. Stingl G, Katz SI, Clement L, Green I, Shevach EM (1978) Immunological functions of Ia-bearing epidermal Langerhans cells. J Immunol 121:2005–2013
28. Czernielewski SM, Demarchez M (1987) Further evidence for the self-reproducing capacity of Langerhans cells in human skin. J Invest Dermatol 88:17–20
29. Streilein JW, Grammer SF (1989) In vitro evidence that Langerhans cells can adopt two functionally distinct forms capable of antigen presentation to T lymphocytes. J Immunol 143: 3925–3933
30. Langerhans P (1868) Über die Nerven der menschlichen Haut. Virchows Arch Pathol Anat 44:325–337
31. Birbeck M (1961) An electron microscope study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. J Invest Dermatol 37:51–56
32. Braathen LR (1980) Studies on human epidermal Langerhans cells. III. Induction of T lymphocyte response to nickel sulphate in sensitized individuals. Br J Dermatol 103:517–526
33. Kimber I, Dearman RJ, Cumberbatch M, Huby RJ (1998) Langerhans cells and chemical allergy. Curr Opinion Immunol 10:614–619
34. Romani N, Schuler G (1992) The immunologic properties of epidermal Langerhans cells as a part of the dendritic cell system. Springer Semin Immunopathol 151:6535–6545
35. Park SH, Chiu YH, Jayawardena J, Roark J, Kavita U, Bendelac A (1998) Innate and adaptive functions of the CD1 pathway of antigen presentation. Semin Immunol 10:391–398
36. Weinlich G, Heine M, Stössel H, Zanella M, Stoitzner P, Ortner U, Smolle J, Koch F, Sepp NT, Schuler G, Romani N (1998) Entry into afferent lymphatics and maturation in situ of migrating murine cutaneous dendritic cells. J Invest Dermatol 110:441–448
37. Richters CD, Hoekstra MJ, van Baare J, Du Pont JS, Hoefsmit EC, Kamperdijk EW (1994) Isolation and characterization of migratory human skin dendritic cells. Clin Exp Immunol 98:330–336
38. Brand CU, Hunziker T, Limat A, Braathen LR (1993) Large increase of Langerhans cells in human skin lymph derived from irritant contact dermatitis. Br J Dermatol 128:184–188
39. Ozawa H, Nakagawa S, Tagami H, Aiba S (1996) Interleukin-1b and granulocyte-macrophage colony stimulating factor mediate Langerhans cell maturation differently. J Invest Dermatol 106:441–445
40. Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi YW (1997) TRANCE (Tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med 186:2075–2080
41. Aiba S, Tagami H (1999) Dendritic cell activation induced by various stimuli, eg exposure to microorganisms, their products, cytokines, and simple chemicals as well as adhesion to extracellular matrix. J Dermatol Sci 20:1–13
42. Inaba K, Schuler G, Steinman RM (1993) GM-CSF – a granulocyte/macrophage/dendritic cell stimulating factor. In: Van Furth R (ed) Hemopoietic growth factors and mononuclear phagocytes. Karger, Basel, pp 187–196
43. Jakob T, Udey MC (1998) Regulation of E-Cadherin mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells in vivo. J Immunol 160:4067–4073
44. Schwarzenberger K, Udey MC (1996) Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression in situ. J Invest Dermatol 106: 553–558
45. Enk AH, Katz SI (1992) Early molecular events in the induction phase of contact sensitivity. Proc Natl Acad Sci USA 89:1398–1402
46. Enk AH, Angeloni VL, Udey MC, Katz SI (1993) An essential role for Langerhans cell-derived IL-1b in the initiation of primary immune responses in skin. J Immunol 150:3698–3704
47. Steinman RM, Hoffman L, Pope M (1995) Maturation and migration of cutaneous dendritic cells. J Invest Dermatol 105:2S–7S

48. Wang BH, Amerio P, Sauder DN (1999) Role of cytokines in epidermal Langerhans cell migration. J Leuk Biol 66:33–39
49. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC (1993) Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. Nature 361:82–85
50. Jakob T, Udey MC (1998) Regulation of E-cadherin-mediated adhesion in Langerhans cell like dendritic cells by inflammatory mediators that mobilize Langerhans cells in vivo. J Immunol 160:4067–4073
51. Ma J, Wing J-H, Guo Y-J, Sy M-S, Bigby M (1994) In vivo treatment with anti-ICAM-1 and antiLFA-1 antibodies inhibits contact sensitization-induced migration of epidermal Langerhans cells to regional lymph nodes. Cell Immunol 158:389–399
52. Rambukhana A, Bos JD, Irik D, Menko WJ, Kapsenberg ML, Das PK (1995) In situ behaviour of human Langerhans cells in skin organ culture. Lab Invest 73:521–531
53. Price AA, Cumberbatch M, Kimber I (1997) a6 integrins are required for Langerhans cell migration from the epidermis. J Exp Med 186:1725–1735
54. Weiss JM, Sleeman J, Renkl AC, Dittmar H, Termeer CC, Taxis S, Howells N, Hofmann M, Kohler G, Schöpf E, Ponta H, Herrlich P, Simon JC (1997) An essential role for CD44 variant isoforms in epidermal Langerhans cell and blood dendritic cell function. J Cell Biol 137: 1137–1147
55. Brand CU, Hunger RE, Yawalkar N, Gerber HA, Schaffner T, Braathen LR (1999) Characterization of human skin-derived CD1a-positive lymph cells. Arch Dermatol Res 291:65–72
56. Kobayashi Y (1997) Langerhans cells produce type IV collagenase (MMP-9) following epicutaneous stimulation with haptens. Immunol 90:496–501
57. Randolph GJ, Beaulieu S, Pope M, Sugawara I, Hoffman L, Steinman RM, Müller WA (1998) A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. Proc Natl Acad Sci USA 95:6924–6929
58. Sozzani S, Allavena P, Vecchi A, Mantovani A (1999) The role of chemokines in the regulation of dendritic cell trafficking. J Leuk Biol 66:1–9
59. Sallusto F, Lanzavecchia A, Mackay CR (1998) Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. Immunol Today 19:568–574
60. Zlotnik A, Morales J, Hedrick JA (1999) Recent advances in chemokines and chemokine receptors. Crit Rev Immunol 19:1–47
61. Sozzani S, Luini W, Borsatti A, Polentarutti N, Zhou D, Piemonti L, D'amico G, Power CA, Wells TN, Gobbi M, Allavena P, Mantovani A (1997) Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. J Immunol 159: 1993–2000
62. Sallusto F, Palermo B, Lenig D, Miettinen M, Matikainen S, Julkunen I, Forster R, Burgstahler R, Lipp M, Lanzavecchia A (1999) Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol 29:1617–1625
63. Saeki H, Moore AM, Brown MJ, Hwan ST (1999) Secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 162:2472–2475
64. Kellermann S-A, Hudak S, Oldham ER, Liu Y-J, McEvoy LM (1999) The CC chemokine receptor-7 ligand 6kine and macrophage inflammatory protein-3b are potent chemoattractants for in vitro and in vivo-derived dendritic cells. J Immunol 162:3859–3864
65. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT (1998) A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naïve T lymphocytes. Proc Natl Acad Sci USA 95:258–263
66. Kim CH, Broxmeyer HE (1999) Chemokines: signal lamps for trafficking of T and B cells for development and effector function. J Leuk Biol 65:6–15
67. Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR, Qin S, Lanzavecchia A (1998) Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. Eur J Immunol 28:2760–2769
68. Cumberbatch M, Dearman RJ, Kimber I (1997) Interleukin 1-beta and the stimulation of Langerhans cell migration – comparisons with tumour necrosis factor alpha. Arch Dermatol Res 289:277–284

69. Heufler C, Koch F, Schuler G (1988) Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med 167:700–705
70. Furue M, Chang CH, Tamaki K (1996) Interleukin-1 but not tumor necrosis factor a synergistically upregulates the granulocyte-macrophage colony-stimulating factor-induced B7–1 expression of murine Langerhans cells. Br J Dermatol 135:194–198
71. Schuler G, Steinman RM (1985) Murine epidermal Langerhans cells mature into potent immune-stimulatory dendritic cells in vitro. J Exp Med 161:526–546
72. Haig DM, Hopkins J, Miller HRP (1999) Local immune responses in afferent and efferent lymph. Immunology 96:155–163
73. Altin JG, Sloan EK (1997) The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol 75:430–445
74. Hiraoka N, Petryniak B, Nakayama J, Tsuboi S, Suzuki M, Yeh JC, Izawa D, Tanaka T, Miyasaka M, Lowe JB, Fukuda M (1999) A novel, high endothelial venule-specific sulfotransferase expresses 6-sulfo sialyl Lewis(x), an L-selectin ligand displayed by CD34. Immunity 11:79–89
75. Von Andrian UH, Mrini C (1998) In situ analysis of lymphocyte migration to lymph nodes. Cell Adh Comm 6:85–96
76. Vestweber D, Blanks JE (1999) Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 79:181–213
77. Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, Foster J, Xu Y, Nooyen P, McClanahan T, Bacon KB, Figdor CG (1997) A dendritic-cell-derived C-C chemokine that preferentially attracts naïve T cells. Nature 387:713–717
78. Ngo VN, Tang LH, Cyster JG (1998) Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naïve T cells and activated B cells. J Exp Med 188:181–191
79. Nagira M, Imai T, Hieshima K, Kusuda J, Ridanpaa M, Takagi S, Nishimura M, Kakizaki M, Nomiyama H, Yoshie O (1997) Molecular cloning of a novel human CC chemokine secondary lymphoid-tissue chemokine that is a potent chemoattractant for lymphocytes and mapped to chromosome 9p13. J Biol Chem 272:19518–19524
80. Rustemeyer T, von Blomberg BME, de Ligter S, Frosch PJ, Scheper RJ (1999) Human T lymphocyte priming in vitro by dendritic cells. Clin Exp Immunol 117:209–216
81. Steinman RM, Pack M, Inaba K (1997) Dendritic cells in the T-cell areas of lymphoid organs. Immunol Rev 156:25–37
82. Ingulli E, Mondino A, Khoruts A, Jenkins MK (1997) In vivo detection of dendritic cell antigen presentation to CD4(+) T cells. J Exp Med 185:2133–2141
83. Griem P, Wulferink M, Sachs B, Gonzales JB, Gleichmann E (1998) Allergic and autoimmune reactions to xenobiotics: how do they arise? Immunol Today 19:133–141
84. Moulon C, Vollmer J, Weltzien H-U (1995) Characterization of processing requirements and metal crossreactivities in T cell clones from patients with allergic contact dermatitis to nickel. Eur J Immunol 25:3308–3315
85. Valitutti S, Müller S, Cella M, Padovan E, Lanzavecchia A (1995) Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature 375:148–151
86. Schoenberger SP, Toes REM, Vandervoort EIH, Offringa R, Melief CJM (1998) T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 393:480–483
87. Weiss A, Littman DR (1994) Signal transduction by lymphocyte antigen receptors. Cell 76:263–274
88. Cantrell D (1996) T cell receptor signal transduction pathways. Annu Rev Immunol 14:259–274
89. Kuo CT, Leiden JM (1999) Transcriptional regulation of T lymphocyte development and function. Annu Rev Immunol 17:149–187
90. Weintraub BC, Goodnow CC (1998) Immune responses – costimulatory receptors have their say. Curr Biol 8:R575–R577
91. Zuckerman LA, Pullen L, Miller J (1998) Functional consequences of costimulation by ICAM-1 on IL-2 gene expression and T cell activation. J Immunol 160:3259–3268
92. Ni K, Oneill HC (1997) The role of dendritic cells in T cell activation. Immunol Cell Biol 75:223–230

93. Grewal IS, Flavell RA (1998) CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 16:111–135
94. Vangool SW, Vandenberghe P, Deboer M, Ceuppens JL (1996) CD80, CD86 and CD40 provide accessory signals in a multiple-step T cell activation model. Immunol Rev 153:47–83
95. Viola A, Lanzavecchia A (1996) T cell activation determined by T cell receptor number and tunable thresholds. Science 273:104–106
96. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252
97. Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045
98. Cella M, Sallusto F, Lanzavecchia A (1997) Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 9:10–16
99. Gomez J, Gonzalez A, Martinez-A C, Rebollo A (1998) IL-2-induced cellular events. Crit Rev Immunol 18:185–220
100. Berridge MJ (1997) Lymphocyte activation in health and disease. Crit Rev Immunol 17:155–178
101. Theze J, Alzari PM, Bertoglio J (1996) Interleukin 2 and its receptors: recent advances and new immunological functions. Immunol Today 17:481–486
102. Lacour M, Arrighi J-F, Müller KM, Carlberg C, Saurat J-H, Hauser C (1994) cAMP up-regulates IL-4 and IL-5 production from activated $CD4^+$ T cells while decreasing IL-2 release and NF-AT induction. Int Immunol 6:1333–1343
103. Linsley PS, Ledbetter JA (1993) The role of the CD28 receptor during T cell responses to antigen. Annu Rev Immunol 11:191–212
104. Romagnani S (1997) The Th1/Th2 paradigm. Immunol Today 18:263–266
105. Constant SL, Bottomly K (1997) Induction of Th1 and Th2 $CD4^+$ T cell responses: the alternate approaches. Annu Rev Immunol 15:297–322
106. Nakamura T, Lee RK, Nam SY, Podack ER, Bottomly K, Flavell RA (1997) Roles of IL-4 and IFN-g in stabilizing the T helper cell type-1 and 2 phenotype. J Immunol 158:2648–2653
107. O'Garra A (1998) Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity 8:275–283
108. Thomas MJ, Kemeny DM (1998) Novel CD4 and CD8 T-cell subsets. Allergy 53:1122–1132
109. Sad S, Mosmann TR (1994) Single IL-2 secreting precursor CD4 T cell can develop into either Th1 or Th2 cytokine secretion phenotype. J Immunol 153:3514–3522
110. Morel PA, Oriss TB (1998) Crossregulation between Th1 and Th2 cells. Crit Rev Immunol 18:275–303
111. Salmon M, Kitas GD, Bacon PA (1989) Production of lymphokine mRNA by $CD45R^+$ and $CD45R^-$ helper T cells from human peripheral blood and by human $CD4^+$ T cell clones. J Immunol 143:907–912
112. Ehlers S, Smith KA (1991) Differentiation of T cell lymphokine gene expression: the in vitro acquisition of T cell memory. J Exp Med 173:25–36
113. Simecka JW (1998) Mucosal immunity of the gastrointestinal tract and oral tolerance. Adv Drug Deliv Rev 34:235–259
114. Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D, Revillard JP, Nicolas JF (1995) Major histocompatibility complex class I-restricted $CD8^+$ T cells and class II-restricted $CD4^+$ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. Eur J Immunol 25:3006–3010
115. Dilulio NA, Xu H, Fairchild RL (1996) Diversion of $CD4^+$ T cell development from regulatory T helper to effector T helper cells alters the contact hypersensitivity response. Eur J Immunol 26:2606–2612
116. Cavani A, Mei D, Guerra E, Corinti S, Giani M, Pirrotta L, Puddu P, Girolomoni G (1998) Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses. Evidence for the presence of effector $CD8^+$ and regulatory $CD4^+$ T cells. J Invest Dermatol 111:621–628
117. Kang KF, Kubin M, Cooper KD, Lessin SR, Trinchieri G, Rook AH (1996) IL-12 synthesis by human Langerhans cells. J Immunol 156:1402–1407

118. O'Garra A, Murphy K (1994) Role of cytokines in determining T-lymphocyte function. Curr Opin Immunol 6:458–466
119. Paul WE, Ohara J (1987) B-cell stimulatory factor-1/interleukin 4. Annu Rev Immunol 5:429–459
120. Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, Sinigaglia F (1997) Selective expression of an interleukin-12 receptor component by human T helper 1 cells. J Exp Med 185:825–831
121. Nakamura T, Kamogawa Y, Bottomly K, Flavell RA (1997) Polarization of IL-4- and IFN-gamma-producing CD4(+) T cells following activation of naïve CD4(+) T cells. J Immunol 158:1085–1094
122. Orange JS, Biron CA (1996) An absolute and restricted requirement for IL-12 in natural killer cell IFN-g production and antiviral defense. J Immunol 156:1138–1142
123. Mackey MF, Barth RJ, Noelle RJ (1998) The role of CD40/CD154 interactions in the priming, differentiation, and effector function of helper and cytotoxic T cell. J Leuk Biol 63: 418–428
124. Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G (1996) Ligation of CD40 on dendritic cells triggers production of high levels of interleukin 12 and enhances T cell stimulatory capacity. J Exp Med 184:747–752
125. Ohshima Y, Tanaka Y, Tozawa H, Takahashi Y, Maliszewski C, Delespesse C (1997) Expression and function of OX40 ligand on human dendritic cells. J Immunol 159:3838–3848
126. Kuchroo V, Prabhu Das M, Brown JA Ranger A, Zamvill MSS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH (1995) B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways. Application to autoimmune disease therapy. Cell 80:707–718
127. Ranger AM, Prabhu Das M, Kuchroo VK, Glimcher LH (1996) B7–2 (CD86) is essential for the development of IL-4 producing cells. Int Immunol 153:1549–1560
128. Schweitzer AN, Borriello F, Wong RCK, Abbas AK, Sharpe AH (1997) Role of costimulators in T cell differentiation – studies using antigen-presenting cells lacking expression of CD80 or CD86. J Immunol 158:2713–2722
129. Rulifson IC, Sperling AI, Fields PE, Fitch FW, Bluestone JA (1997) CD28 costimulation promotes the production of Th2 cytokines. J Immunol 158:658–665
130. Groux H, Sornasse T, Cottrez F, de Vries JE, Coffman RL, Roncarolo MG, Yssel H (1997) Induction of human T helper cell type-1 differentiation results in loss of IFN-g receptor b-chain expression. J Immunol 158:5627–5631
131. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, Rothman P (1995) Lack of interferon gamma receptor beta chain and the prevention of interferon signaling in Th1 cells. Science 269:245–247
132. Gajewski TF, Fitch FW (1988) Antiproliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. J Immunol 140:4245–4252
133. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S, Nakanishi K (1998) IL-12 up-regulates IL-18 receptor expression on T cells, TH1 cells, and B cells – synergism with IL-18 for IFN-gamma production. J Immunol 161:3400–3407
134. Werfel T, Hentschel M, Kapp A, Renz H (1997) Dichotomy of blood- and skin-derived IL-4-producing allergen-specific T cells and restricted V beta repertoire in nickel-mediated contact dermatitis. J Immunol 158:2500–2505
135. Probst P, Küntzlin D, Fleischer B (1995) T_H2-type infiltrating T cells in nickel-induced contact dermatitis. Cell Immunol 165:134–140
136. Zanni MP, Mauri-Hellweg D, Brander C, Wendland T, Schnyder B, Frei E, von Greyerz S, Bircher A, Pichler WJ (1997) Characterization of lidocaine-specific T cells. J Immunol 158:1139–1148
137. Perez VL, Lederer JA, Lichtman AH, Abbas AK (1995) Stability of Th1 and Th2 populations. Int Immunol 7:869–875
138. Rincon M, Anguita J, Nakamura T, Fikrig E, Flavell RA (1997) Interleukin (IL)-6 directs the differentiation of IL-4 producing $CD4^+$ T cells. J Exp Med 182:1591–1596

139. Yoshimoto T, Bendelac A, Watson C, Hu-Li J, Paul WE (1995) Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. Science 270:1845–1847
140. Hiroi T, Iwatani K, Iijima H, Kodama S, Yanagita M, Kiyono H (1998) Nasal immune system – distinctive Th0 and Th1/Th2 type environments in murine nasal-associated lymphoid tissues and nasal passage, respectively. Eur J Immunol 28:3346–3353
141. Banchereau J (1995) Converging and diverging properties of human interleukin-4 and interleukin-10. Behr Inst Mitteil 96:58–77
142. Itoh K, Hirohata S (1995) The role of IL-10 in human B cell activation, proliferation, and differentiation. J Immunol 154:4341–4350
143. Napolitano LM, Buzdon MM, Shi HJ, Bass BL (1997) Intestinal epithelial cell regulation cell regulation of macrophage and lymphocyte interleukin 10 expression. Arch Surg 132:1271–1276
144. Xu H, Banerjee A, Diulio NA, Fairchild RL (1996) T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (IL)-4/IL-10-producing (Th2) negative regulatory CD4+ T cells. J Exp Med 183:1001–1012
145. Letterio JL, Roberts AB (1998) Regulation of immune responses by TGF-beta. Annu Rev Immunol 16:137–161
146. Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A (1995) The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor phenotype development in a T cell receptor alpha/beta-transgenic model. J Exp Med 182:1579–1584
147. Constant SL, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K (1995) Extent of T cell receptor ligation can determine the functional differentiation of naïve CD4+ T cells. J Exp Med 5:1591–1596
148. Bretscher PA, Ogunremi O, Menon JN (1997) Distinct immunological states in murine cutaneous leishmaniasis by immunizing with different amounts of antigen: the generation of beneficial, potentially harmful, harmful and potentially extremely harmful states. Behring Inst Mitt 98:153–159
149. Dearman RJ, Basketter DA, Kimber I (1996) Characterization of chemical allergens as a function of divergent cytokine secretion profiles induced in mice. Toxicol Appl Pharmacol 138:308–316
150. Kanerva L, Hyry H, Jolanki R, Hytonen M, Estlander T (1997) Delayed and immediate allergy caused by methylhexahydrophthalic anhydride. Contact Dermatitis 36:34–38
151. Luger TA, Schwarz T (1995) The role of cytokines and neuroendocrine hormones in cutaneous immunity and inflammation. Allergy 50:292–302
152. Scholzen T, Armstrong CA, Bunnett NW, Luger TA, Olerud JE, Ansel JC (1998) Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. Exp Dermatol 7:81–96
153. Luger TA, Lotti T (1998) Neuropeptides: role in inflammatory skin diseases. J Eur Acad Derm Venereol 10:207–211
154. Lord GM, Matarese G, Howard LK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897–901
155. Tabata N, Tagami H, Terui T (1997) Dehydroepiandrosterone may be one of the regulators of cytokine production in atopic dermatitis. Arch Dermatol Res 289:410–414
156. Huber SA, Kupperman J, Newell MK (1999) Hormonal regulation of CD4(+) T-cell responses in coxsackievirus B3-induced myocarditis in mice. J Virol 73:4689–4695
157. Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annuziato F, Livi C, Romagnani S, Maggi E (1995) Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 155:128–133
158. Vieira PL, Kalinski P, Wierenga EA, Kapsenberg ML, Dejong EC (1998) Glucocorticoids inhibit bioactive IL-12P70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential. J Immunol 161:5245–5251

159. Calder PC, Bevan SJ, Newsholme EA (1992) The inhibition of T-lymphocyte proliferation by fatty acids is via an eicosanoid-independent mechanism. Immunology 75:108–115
160. Uotila P (1996) The role of cyclic AMP and oxygen intermediates in the inhibition of cellular immunity in cancer. Cancer Immunol Immunother 43:1–9
161. Demeure CE, Yang LP, Desjardins C, Raynauld P, Delespesse G (1997) Prostaglandin E-2 primes naïve T cells for the production of anti-inflammatory cytokines. Eur J Immunol 27:3526–3531
162. Abe N, Katamura K, Shintaku N, Fukui T, Kiyomasu T, Lio J, Ueno H, Tai G, Mayumi M, Furusho K (1997) Prostaglandin E2 and IL-4 provide naïve $CD4^+$ T cells with distinct inhibitory signals for the priming of IFN-gamma production. Cell Immunol 181:86–92
163. Kalinski P, Hilkens CMU, Snijders A, Snijdewint FGM, Kapsenberg ML (1997) IL-12 deficient dendritic cells, generated in the presence of prostaglandin E_2, promote type-2 cytokine production in maturing human naïve T helper cells. J Immunol 159:28–35
164. von Blomberg BME, Bruynzeel DP, Scheper RJ (1991) Advances in mechanisms of allergic contact dermatitis: in vitro and in vivo research. In: Marzulli FN, Maibach HI (eds) Dermatotoxicology, 4th edn. Hemisphere Publishing Corporation, New York, pp 255–362
165. Westerman J, Pabst R (1996) How organ-specific is the migration of 'naïve' and 'memory' T cells? Immunol Today 17:278–282.
166. Westerman J, Geismar U, Sponholz A, Bode U, Sparshott, Bell EB (1997) $CD4^+$ T cells of both the naïve and the memory phenotype enter rat lymph nodes and Peyer's patches via high endothelial venules: within the tissue their migratory behaviour differs. Eur J Immunol 27:3174–3181
167. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LWMM (1993) Control of lymphocyte recirculation in man: I. Differential regulation of the homing receptor L-selectin on T cells during the virgin to memory transition. J Immunol 150: 1105–1121.
168. Hall JG, Morris B (1965) The origin of cells in the efferent lymph from a single lymph node. J Exp Med 121:901–910
169. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC (1991) ELAM-1 is an adhesion molecule for skin homing T cells. Nature 349:796–799
170. Shimizu Y, Shaw S, Graber N, Gopal TV, Horgan KJ, Van Seventer GA, Newman W (1991) Activation- independent binding of human memory T cells to adhesion molecule ELAM-1. Nature 349:799–802
171. Mackay CR (1993) Homing of naïve, memory and effector lymphocytes. Curr Opin Immunol 5:423–427
172. Tietz W, Allemand Y, Borges E, Vonlaer D, Hallmann R, Vestweber D, Hamann A (1998) $CD4(^+)$ T cells migrate into inflamed skin only if they express ligands for E- and P-selectin. J Immunol 16:963–970
173. Rosen SD, Bertozzi CR (1994) The selectins and their ligands. Curr Opin Cell Biol 6:663–673
174. Bradley L, Watson SR (1996) Lymphocyte migration into tissue: the paradigm derived from CD4 subsets. Curr Op Immunol 8:312–320
175. Tanchot C, Rocha B (1998) The organization of mature T-cell pools. Immunol Today 19:575–579
176. Meeusen ENT, Premier RR, Brandon MR (1996) Tissue-specific migration of lymphocytes: a key role for Th1 and Th2 cells? Immunol Today 17:421–424.
177. Priest R, Bird MI, Malhotra R (1998) Characterization of E-selectin binding epitopes expressed by skin homing T cells. Immunology 94:523–528
178. Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, Terstappen LWMM (1993) Control of lymphocyte recirculation in man: II. Differential regulation of the cutaneous lymphocyte associated antigen, a tissue-selective homing receptor for skin homing T cells. J Immunol 150:1122–1136
179. Sunderkötter C, Steinbrink K, Henseleit U, Bosse R, Schwarz A, Vestweber D, Sorg C (1996) Activated T cells induce expression of E-selectin in vitro and in an antigen-dependent manner in vivo. Eur J Immunol 26:1571–1579

180. Austrup F, Vestweber D, Borges E, Lohning M, Brauer R, Herz U, Renz H, Hallmann R, Scheffold A, Radbruch A, Hamann A (1997) P- and E selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. Nature 385:81–83
181. Borges E, Tietz W, Steegmaier M, Moll T, Hallmann R, Hamann A, Vestweber D (1997) P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not on T helper 2 cells binds to P-selectin and supports migration into inflamed skin. J Exp Med 185:573–578
182. Tensen CP, Flier J, Rampersad SS, Sampat-Sardjoerpersad A, Scheper RJ, Boorsma DM, Willemze R (1999) Genomic organization, sequence and transcriptional regulation of the human CXCL 11 gene. Biochim Biophys Acta 1446:167–172
183. Sallusto F, Kremmer E, Palermo B, Hoy A, Ponath P, Qin SX, Forster R, Lipp M, Lanzavecchia A (1999) Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. Eur J Immunol 29:2037–2045
184. Baggiolini M (1998) Chemokines and leukocyte traffic. Nature 392:565–568.
185. Fuhlbrigge RC, Kieffer JD, Armerding D, Kupper TS (1997) Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. Nature 389:978–981
186. Rustemeyer T, von Blomberg BME, de Ligter S, Frosch PJ, Scheper RJ (1999) Human T lymphocyte priming in vitro by haptenated autologous dendritic cells. Clin Exp Immunol 117:209–216
187. Kapsenberg ML, Res P, Bos JD, Schootemijer A, Teunissen MB, van Schooten W (1987) Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. Eur J Immunol 17:861–865
188. Milon G, Marchal G, Seman M, Truffa-Bachi P (1981) A delayed-type hypersensitivity reaction initiated by a single T lymphocyte. Agents Actions 11:612–614
189. Marchal G, Seman M, Milon G, Truffa-Bachi P, Zilberfarb V (1982) Local adoptive transfer of skin delayed-type hypersensitivity initiated by a single T lymphocyte. J Immunol 129:954–958
190. Bell EB, Sparshott SM, Bunce C (1998) $CD4^+$ T-cell memory, CD45R subsets and the persistence of antigen – a unifying concept. Immunol Today 19:60–64
191. Bell EB, Sparshott SM, Ager A (1995) Migration pathways of CD4 T cell subsets in vivo: the CD45RC- subset enters the thymus via alpha 4 integrin- VCAM-1 interaction. Int Immunol 11:1861–1871
192. Goebeler M, Meinardus-Hager G, Roth J, Goerdt S, Sorg C (1993) Nickel chloride and cobalt chloride, two common contact sensitizers, directly induce expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule (ELAM-1) by endothelial cells. J Invest Dermatol 100:759–765
193. Goebeler M, Roth J, Brocker EB, Sorg C, Schulze-Osthoff K (1995) Activation of nuclear factor-kappa B and gene expression in human endothelial cells by the common haptens nickel and cobalt. J Immunol 155:2459–2467
194. Walsh LJ, Lavker RM, Murphy GF (1990) Determinants of immune cell trafficking in the skin. Lab Invest 63:592–600
195. Waldorf HA, Walsh LJ, Schechter NM, Murphy GF (1991) Early molecular events in evolving cutaneous delayed hypersensitivity in humans. Am J Pathol 138:477–486
196. Stoof TJ, Boorsma DM, Nickoloff (1994) Keratinocytes and immunological cytokines. In: Leigh I, Lane B, Watt F (eds) The keratinocyte handbook. Cambridge University Press, Cambridge, pp 365–399
197. Silberberg I, Baer RL, Rosenthal SA (1976) The role of Langerhans cells in allergic contact hypersensitivity: a review of findings in man and guinea pigs. J Invest Dermatol 66:210–217
198. Brasch J, Sterry W (1992) Expression of adhesion molecules in early allergic patch test reactions. Dermatology 185:12–17
199. Silberberg-Sinakin I, Thorbecke GJ, Baer RL, Rosenthal SA, Berezowsky V (1976) Antigen-bearing Langerhans cells in skin, dermal lymphatics, and in lymph nodes. Cell Immunol 25:137–151
200. Hill S, Edwards AJ, Kimber I, Knight SC (1990) Systemic migration of dendritic cells during contact sensitization. Immunology 71:277–281

201. Sterry W, Künne N, Weber-Matthiesen K, Brasch J, Mielke V (1991) Cell trafficking in positive and negative patch test reactions: demonstration of a stereotypic migration pathway. J Invest Dermatol 96:459–462
202. Herzog WR, Meade R, Pettinicchi A, Ptak W, Askenase PW (1989) Nude mice produce a T cell-derived antigen-binding factor that mediates the early component of delayed-type hypersensitivity. J Immunol 142:1803–1812
203. Willis CM, Young E, Brandon DR, Wilkinson JD (1986) Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. Br J Dermatol 115:305–316
204. Brasch J, Burgard J, Sterry W (1992) Common pathways in allergic and irritant contact dermatitis. J Invest Dermatol 98:166–170
205. Hoefakker S, Caubo M, van 't Herve EHM, Roggeveen MJ, Boersma WJA, van Joost Th, Notten WRF, Claassen E (1995) In vivo cytokine profiles in allergic and irritant contact dermatitis. Contact Dermatitis 33:258–266
206. Flier J, Boorsma DM, Bruynzeel DP, van Beek PJ, Stoof TJ, Scheper RJ, Willemze R, Tensen CP (1999) The CXCR3 activating chemokines IP-10, MIG and IP-9 are expressed in allergic but not in irritant patch test reactions. J Invest Dermatol 113:574-578
207. Tensen CP, Flier J, van der Raaij-Helmer EM, Sampat-Sardjoepersad S, van den Schors RC, Leurs R, Scheper RJ, Boorsma DM, Willemze R (1999) Human IP-9: a keratinocyte derived high affinity CXC-chemokine ligand for the IP-10/Mig receptor (CXCR3). J Invest Dermatol 112:716–722
208. Kondo S, Sauder DN (1995) Epidermal cytokines in allergic contact dermatitis. J Am Acad Dermatol 33:786–800
209. Wardorf HA, Walsh LJ, Schechter NM (1991) Early cellular events in evolving cutaneous delayed hypersensitivity in humans. Am J Pathol 138:477–486
210. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone MA Jr (1986) Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 136:1680–1687
211. Shimizu Y, Newman W, Gopal TV, Horgan KJ, Graber N, Beall LD, van Seventer GA, Shaw S (1991) Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. J Cell Biol 113:1203–1212
212. Ross R, Gilitzer C, Kleinz R, Schwing J, Kleinert H, Forstermann U, Reske-Kunz AB (1998) Involvement of NO in contact hypersensitivity. Int Immunol 10:61–69
213. Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V (1998) Nitric oxide and its implications in skin homeostasis and disease – a review. Arch Dermatol Res 290:643–651
214. Rowe A, Farrell AM, Bunker CB (1997) Constitutive endothelial and inducible nitric oxide synthase in inflammatory dermatoses. Br J Dermatol 136:18–23
215. Szepietowski JC, McKenzie RC, Keohane SG, Walker C, Aldridge RD, Hunter JA (1997) Leukaemia inhibitory factor: induction in the early phase of allergic contact dermatitis. Contact Dermatitis 36:21–25
216. Yu X, Barnhill RL, Graves DT (1994) Expression of monocyte chemoattractant protein-1 in delayed type hypersensitivity reactions in the skin. Lab Invest 71:226–235
217. Buchanan KL, Murphy JW (1997) Kinetics of cellular infiltration and cytokine production during the efferent phase of a delayed-type hypersensitivity reaction. Immunology 90:189–197
218. Ptak W, Askenase PW, Rosenstein RW, Gershon RK (1982) Transfer of an antigen-specific immediate hypersensitivity-like reaction with an antigen-binding factor produced by T cells. Proc Natl Acad Sci USA 79:1969 – 1973
219. Van Loveren H, Ratzlaff RE, Kato K, Meade R, Ferguson TA, Iverson GM, Janeway CA, Askenase PW (1986) Immune serum from mice contact-sensitized with picryl chloride contains an antigen-specific T cell factor that transfers immediate cutaneous reactivity. Eur J Immunol 16:1203–1208
220. Ptak W, Herzog WR, Askenase PW (1991) Delayed-type hypersensitivity initiation by early-acting cells that are antigen mismatched or MHC incompatible with late-acting, delayed-type hypersensitivity effector T cells. J Immunol 146:469–475

221. Tsuji RF, Geba GP, Wang Y, Kawamoto K, Matis LA, Askenase PW (1997) Required early complement activation in contact sensitivity with generation of local C5-dependent chemotactic activity, and late T cell interferon g: a possible initiating role of B cells. J Exp Med 186:1015–1026
222. Askenase PW, Kawikova I, Paliwal V, Akahira-Azuma M, Gerard C, Hugli T, Tsuji R (1999) A new paradigm of T cell allergy: requirement for the B-1 B cell subset. Int Arch All Appl Immunol 118:145–149
223. Hardy RR, Hayakawa K (1994) CD5+ B cells, a fetal B cell lineage. Adv Immunol 55:297–339
224. Askenase PW, Kawikova I, Paliwal V, Akahira-Azuma M, Gerard C, Hugli T, Tsuji R (1999) A new paradigm of T cell allergy: requirement for the B-1 cell subset. Int Arch Allergy Immunol 118:145-149
225. Feinstein A, Richardson N, Taussig MJ (1986) Immunoglobulin flexibility in complement activation. Immunol Today 7:169–173
226. Van Loweren H, Meade R, Askenase PW (1983) An early component of delayed type hypersensitivity mediated by T cells and mast cells. J Exp Med 157:1604–1617
227. Geba GP, Ptak W, Anderson GA, Ratzlaff RE, Levin J, Askenase PW (1996) Delayed-type hypersensitivity in mast cell deficient mice: dependence on platelets for expression on contact sensitivity. J Immunol 157:557–565
228. Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, Eddy SM, Ward PA (1994) C5a-induced expression of P-selectin in endothelial cells. J Clin Invest 94:1147–1155
229. Groves RW, Allen MH, Ross EL, Barker JN, MacDonald DM (1995) Tumor necrosis factor alpha is pro-inflammatory in normal human skin and modulates cutaneous adhesion molecule expression. Br J Dermatol 132:345–352
230. Nataf S, Davoust N, Ames RS, Barnum SR (1999) Human T cells express the C5a receptor and are chemoattracted to C5a. J Immunol 162:4018–4023
231. Wilkinson SM, Mattey DL, Beck MH (1994) IgG antibodies and early intradermal reactions to hydrocortisone in patients with cutaneous delayed-type hypersensitivity to hydrocortisone. Br J Dermatol 131:495–498
232. Shirakawa T, Kusaka Y, Morimoto K (1992) Specific IgE antibodies to nickel in workers with known reactivity to cobalt. Clin Exp Allergy 22:213–218
233. Ptak W, Askenase PW (1992) Gamma delta T cells assist alpha beta T cells in adoptive transfer of contact sensitivity. J Immunol 149:3503–3508
234. Salerno A, Dieli F (1998) Role of gamma delta T lymphocytes in immune response in humans and mice. Crit Rev Immunol 18:327–357
234. Szczepanik M, Lewis J, Geba GP, Ptak W, Askenase PW (1998) Positive regulatory gamma-delta T cells in contact sensitivity – augmented responses by in vivo treatment with anti-gamma-delta monoclonal antibody, or anti-V-gamma-5 or V-delta-4. Immunol Invest 27:1–15
236. Dieli F, Ptak W, Sireci G, Romano GC, Potestio M, Salerno A, Asherson GL (1998) Cross-talk between V-beta-8(+) and gamma-delta(+) T lymphocytes in contact sensitivity. Immunology 93:469–477
237. Tang HL, Cyster JG (1999) Chemokine up-regulation and activated T cell attraction by maturing dendritic cells. Science 284:819–822
238. Scheper RJ, van Dinther-Janssen AC, Polak L (1985) Specific accumulation of hapten-reactive T cells in contact sensitivity reaction sites. J Immunol 134:1333–1336
239. Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P (1987) Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. J Exp Med 166:1654–1667
240. Lappin MB, Kimber I, Norval M (1996) The role of dendritic cells in cutaneous immunity. Arch Dermatol Res 288:109–121
241. Santamaria-Babi LF, Pickel LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, Hauser C (1995) Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-homing receptor, the cutaneous lymphocyte antigen. J Exp Med 181:1935–1940

242. Teraki Y, Picker LJ (1997) Independent regulation of cutaneous lymphocyte-associated antigen expression and cytokine synthesis phenotype during human CD4+ memory T cell differentiation. J Immunol 159:6018–6029
243. Butcher EC, Picker LJ (1996) Lymphocyte homing and homeostasis. Science 272:60–66
244. Strunk D, Egger C, Leitner G, Hanau D, Stingl G (1997) A skin homing molecule defines the Langerhans cell progenitor in human peripheral blood. J Exp Med 185:1131–1136
245. Wroblewski M, Hamann A (1997) CD45-mediated signals can trigger shedding of lymphocyte L-selectin. Int Immunol 9:555–562
246. Burastero SE, Rossi GA, Crimi E (1998) Selective differences in the expression of the homing receptors of helper lymphocyte subsets. Clin Immunol Immunopathol 89:110–116
247. Wahbi A, Marcusson JA, Sundqvist KG (1996) Expression of adhesion molecules and their ligands in contact allergy. Exp Dermatol 5:12–19
248. Dailey MO (1998) Expression of T lymphocyte adhesion molecules: regulation during antigen-induced T cell activation and differentiation. Crit Rev Immunol 18:153–184
249. Oppenheimer-Marks N, Lipsky PE (1997) Migration of naïve and memory T cells. Immunol Today 18:456–457
250. Romanic AM, Graesser D, Baron JL, Visintin I, Janeway CA Jr, Madri JA (1997) T cell adhesion to endothelial cells and extracellular matrix is modulated upon transendothelial cell migration. Lab Invest 76:11–23
251. Zanni MP, von Greyerz S, Schnyder B, Brander KA, Frutig K, Hari Y, Valitutti S, Pichler WJ (1998) HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. J Clin Invest 102:1591–1598
252. Bos JD (1997) The skin as an organ of immunity. Clin Exp Immunol 107:3–5
253. Moulon C, Wild D, Dormoy A, Weltzien HU (1998) MHC-dependent and -independent activation of human nickel-specific CD8+ cytotoxic T cells from allergic donors. J Invest Dermatol 111:360–366
254. Pichler WJ, Schnyder B, Zanni MP, Hari Y, von Greyerz S (1998) Role of T cells in drug allergies. Allergy 53:225–232
255. Kehren J, Desvignes C, Krasteva M, Ducluzeau MT, Assossou O, Horand F, Hahne M, Kagi D, Kaiserlian D, Nicolas JF (1999) Cytotoxicity is mandatory for CD8+ T cell mediated contact hypersensitivity. J Exp Med 189:779–786
256. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ (1995) Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. J Immunol 155:462–472
257. Abe M, Kondo T, Xu H, Fairchild RL (1996) Interferon-gamma inducible protein (IP-10) expression is mediated by CD8+ T cells and is regulated by CD4+ T cells during the elicitation of contact hypersensitivity. J Invest Dermatol 107:360–366
258. Stark GR, Kerr IM, Williams BRG, Silverman RH, Schreiber RD (1998) How cells respond to interferons. Annu Rev Biochem 67:227–264
259. Rowe A, Bunker CB (1998) Interleukin-4 and the interleukin-4 receptor in allergic contact dermatitis. Contact Dermatitis 38:36–39
260. Asherson GL, Dieli F, Sireci G, Salerno A (1996) Role of IL-4 in delayed type hypersensitivity. Clin Exp Immunol 103:1–4
261. Yamada H, Matsukura M, Yudate T, Chihara J, Stingl G, Tezuka T (1997) Enhanced production of RANTES, an eosinophil chemoattractant factor, by cytokine-stimulated epidermal keratinocytes. Int Arch Aller Immunol 114:28–32
262. Moser B, Loetscher M, Piali L, Loetscher P (1998) Lymphocyte responses to chemokines. Int Rev Immunol 16:323–3244
263. Siveke JT, Hamann A (1998) T helper 1 and T helper 2 Cells respond differentially to chemokines. J Immunol 160:550–554
264. Szepietowski JC, McKenzie RC, Keohane SG, Aldridge RD, Hunter JAA (1997) Atopic and nonatopic individuals react to nickel challenge in a similar way – a study of the cytokine profile in nickel-induced contact dermatitis. Br J Dermatol 137:195–200
265. Asada H, Linton J, Katz SI (1997) Cytokine gene expression during the elicitation phase of contact sensitivity – regulation by endogenous IL-4. J Invest Dermatol 108:406–411

266. Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T (1995) Immediate-type hypersensitivity response followed by late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol 105:749–755
267. Carroll JM, Crompton T, Seery JP, Watt FM (1997) Transgenic mice expressing IFN-gamma in the epidermis have eczema, hair hypopigmentation, and hair loss. J Invest Dermatol 108:412–422
268. Lider O, Cahalon L, Gilat D, Hershkoviz R, Siegel D, Margalit R, Shoseyov O, Cohen IR (1995) A disaccharide that inhibits tumor necrosis factor alpha is formed from the extracellular matrix by the enzyme heparinase. Proc Natl Acad Sci USA 92:5037–5041
269. Kothny-Wilkes G, Kulms D, Poppelmann B, Luger TA, Kubin M, Schwarz T (1998) Interleukin-1 protects transformed keratinocytes from tumor necrosis factor-related apoptosis-inducing ligand. J Biol Chem 273:29247–2953
270. Orteu CH, Poulter LW, Rustin MHA, Sabin CA, Salmon M, Akbar AN (1998) The role of apoptosis in the resolution of T cell-mediated cutaneous inflammation. J Immunol 161:1619–1629
271. Zhang X, Brunner T, Carter L, Dutton RW, Rogers P, Bradley L, Sato T, Reed JC, Green D, Swain SL (1997) Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. J Exp Med 185:1837–1849
272. Enk AH, Katz SI (1992) Identification and induction of keratinocyte-derived IL-10. J Immunol 149:92–95
273. Schwarz A, Grabbe S, Riemann H, Aragane Y, Simon M, Manon S, Andrade S, Luger TA, Zlotnik A, Schwarz T (1994) In vivo effects of interleukin-10 on contact hypersensitivity and delayed-type hypersensitivity reactions. J Invest Dermatol 103:211–216
274. Berg DJ, Leach MW, Kuhn R, Rajewsky K, Muller W, Davidson NJ, Rennick D (1995) Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. J Exp Med 182:99–108
275. Lalani I, Bhol K, Ahmed AR (1997) Interleukin-10 biology, role in inflammation and autoimmunity. Ann Allergy Asthma Immunol 79:469–484
276. Epstein SP, Baer RL, Thorbecke GJ, Belsito DV (1991) Immunosuppressive effects of transforming growth factor beta: inhibition of the induction of Ia antigen on Langerhans cells by cytokines and of the contact hypersensitivity response. J Invest Dermatol 96:832–837
277. Lawrence JN, Dickson FM, Benford DJ (1997) Skin irritant-induced cytotoxicity and prostaglandin E-2 release in human skin keratinocyte cultures. Toxicol Vitro 11:627–631
278. Walker C, Kristensen F, Bettens F, deWeck AL (1983) Lymphokine regulation of activated (G1) lymphocytes. I. Prostaglandin E_2-induced inhibition of interleukin 2 production. J Immunol 130:1770–1773
279. Weston MC, Peachell PT (1998) Regulation of human mast cell and basophil function by cAMP. Gen Pharmacol 31:715–719
280. Dvorak HF, Mihm MC Jr, Dvorak AM (1976) Morphology of delayed-type hypersensitivity reactions in man. J Invest Dermatol 64:391–401
281. Marone G, Spadaro G, Patella V, Genovese A (1994) The clinical relevance of basophil releasability. J Aller Clin Immunol 94:1293–1303
282. Lundeberg L, Mutt V, Nordlind K (1999) Inhibitory effect of vasoactive intestinal peptide on the challenge phase of allergic contact dermatitis in humans. Acta Derm Venereol 79:178–182
283. Boerrigter GH, Scheper RJ (1987) Local and systemic desensitization induced by repeated epicutaneous hapten application. J Invest Dermatol 88:3–7
284. Andreassi M, Digioacchino M, Sabbioni E, Pietra R, Masci S, Amerio P, Bavazzano P, Boscolo P (1998) Serum and urine nickel in nickel-sensitized women – effects of oral challenge with the metal. Contact Dermatitis 38:5–8
285. Moller H, Larsson A, Björkner B, Bruze M, Hagstam A (1996) Flare-up at contact allergy sites in a gold-treated rheumatic patient. Acta Derm Venereol 76:55–58
286. Larsson A, Moller H, Björkner B, Bruze M (1997) Morphology of endogenous flare-up reactions in contact allergy to gold. Acta Derm Venereol 77:474 – 479
287. Skog E (1976) Spontaneous flare-up reactions induced by different amounts of 1,3-dinitro-4-chlorobenzene. Acta Derm Venereol 46:386–395

288. Scheper RJ, von Blomberg BME, Boerrigter GH, Bruynzeel D, van Dinther A, Vos A (1983) Induction of local memory in the skin. Role of local T cell retention. Clin Exp Immunol 51:141–148
289. Yarnashita N, Natsuaki M, Sagami S (1989) Flare-up reaction on murine contact hypersensitivity. I. Description of an experimental model; rechallenge system. Immunology 67:365–369
290. Christensen OB, Beckstead JH, Daniels TE, Maibach HI (1985) Pathogenesis of orally induced flare-up reactions at old patch sites in nickel allergy. Acta Derm Venereol 65:298–304
291. Hindsen M, Christensen OB (1992) Delayed hypersensitivity reactions following allergic and irritant inflammation. Acta Derm Venereol 72:220–221
292. Gawkrodger DJ, McVittie E, Hunter JA (1987) Immunophenotyping of the eczematous flare-up reaction in a nickel-sensitive subject. Dermatology 175:171–177
293. Polak L, Turk JL (1968) Studies on the effect of systemic administration of sensitizers in guinea-pigs with contact sensitivity to inorganic metal compounds. II. The flare-up of previous test sites of contact sensitivity and the development of a generalized rash. Clin Exp Immunol 3:253–262
294. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR (1998) The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 101:746–754
295. Rustemeyer T, von Blomberg BME, Scheper RJ (1997) Cross-reactivity studies in allergic contact dermatitis. EECDR meeting at Göteborg, Sweden, 30 Aug
296. Rustemeyer T, de Groot J, von Blomberg BME, Frosch PJ, Scheper RJ (in press) Cross-reactivity evaluation using the local skin memory based retest model
297. Matura M (1998) Contact allergy to locally applied corticosteroids. Thesis, Leuven Belgium
298. Seidenari S, Rustemeyer T, Scheper RJ (in press) The retest model for evaluation of cross-reactivity between Disperse blue 106 and Disperse 124 in humans
299. Kruisbeek AM, Amsen D (1996) Mechanisms underlying T-cell tolerance. Curr Opin Immunol 8:233–244
300. Tabi Z, McCombe PA, Pender MP (1995) Antigen-specific down-regulation of myelin basic protein-reactive T cells during spontaneous recovery from experimental autoimmune encephalomyelitis: further evidence of apoptotic deletion of autoreactive T cells in the central nervous system. Int Immunol 7:967–973
301. Rocha B, von Boehmer H (1991) Peripheral selection of the T cell repertoire. Science 251:1225–1228
302. Benson JM, Whitacre CC (1997) The role of clonal deletion and anergy in oral tolerance. Res Immunol 148:533–541
303. Ferber I, Schönrich G, Schenkel J, Mellor AL, Hämmerling GJ, Arnold B (1994) Levels of peripheral T cell tolerance induced by different doses of tolerogen. Science 263:674–676
304. Arnold B, Schönrich G, Hämmerling GJ (1993) Multiple levels of peripheral tolerance. Immunol Today 14:12–14
305. Morgan DJ, Kreuwel HTC, Sherman LA (1999) Antigen concentration and precursor frequency determine the rate of CD8(+) T cell tolerance to peripherally expressed antigens. J Immunol 163:723–727
306. Taams LS, van Eden W, Wauben MHM (1999) Dose-dependent induction of distinct anergic phenotypes: Multiple levels of T cell anergy. J Immunol 162:1974–1981
307. Semma M, Sagami S (1981) Induction of suppressor T cells to DNFB contact sensitivity by application of sensitizer through Langerhans cell-deficient skin. Arch Dermatol Res 271:361–364
308. Shreedhar V, Giese T, Sung VW, Ullrich SE (1998) A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. J Immunol 160:3783–3789
309. Niizeki H, Streilein JW (1997) Hapten-specific tolerance induced by acute, low-dose ultraviolet B radiation of skin is mediated via interleukin-10. J Invest Dermatol 109:25–30
310. Mowat A (1987) The regulation of immune responses to dietary protein antigens. Immunol Today 8:93–98

311. Van Hoogstraten IMW, Andersen JE, von Blomberg BME, Boden D, Bruynzeel DP, Burrows D, Camarasa JMG, Dooms-Goossens A, Lahti A, Menné T, Rycroft R, Todd D, Vreeburg KJJ, Wilkinson JD, Scheper RJ (1989) Preliminary results of a multicenter study on the incidence of nickel allergy in relationship to previous oral and cutaneous contacts. In: Frosch PJ, Dooms-Goossens A, Lachapelle JM, Rycroft RJG, Scheper RJ (eds) Current topics in contact dermatitis. Springer, Berlin Heidelberg New York, pp 178–184
312. MacDonald TT (1998) T cell immunity to oral allergens. Curr Opin Immunol 10:620–627
313. Weiner HL, Gonnella PA, Slavin A, Maron R (1997) Oral tolerance: cytokine milieu in the gut and modulation of tolerance by cytokines. Res Immunol 148:528–533
314. Rustemeyer T, de Groot J, von Blomberg BME, Frosch PJ, Scheper RJ (in press) Induction of cross-tolerance to methacrylate contact sensitizers
315. Miller SD, Sy M-S, Claman HN (1977) The induction of hapten-specific T cell tolerance using hapten-modified lymphoid membranes. II. Relative roles of suppressor T cells and clone inhibition in the tolerant state. Eur J Immunol 7:165–170
316. Polak L (1980) Immunological aspects of contact sensitivity. An experimental study. Monogr Allergy 15:4–60
317. Weiner HL (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol Today 18:335–343
318. Weigle WO, Romball CG (1997) $CD4^+$ T-cell subsets and cytokines involved in peripheral tolerance. Immunol Today 18:533–538
319. Zembala M, Ashershon GL (1973) Depression of T cell phenomenon of contact sensitivity by T cells from unresponsive mice. Nature 244:227–228
320. Boerrigter GH, Scheper RJ (1984) Local administration of the cytostatic drug 4-hydroperoxy-cyclophosphamde (4-HPCY) facilitates cell mediated immune reactions. Clin Exp Immunol 58:161–166
321. Boerrigter GH, de Groot J, Scheper RJ (1986) Intradermal administration of 4-hydoperoxy-cyclophosphamde during contact sensitization potentiates effector T cell responsiveness in draining lymph nodes. Immunopharmacology 1:13–20
322. Mokyr MB, Kalinichenko T, Gorelik L, Bluestone JA (1998) Realization of the therapeutic potential of CTLA-4 blockade in low-dose chemotherapy-treated tumor-bearing mice. Cancer Res 58:5301–5304
323. Knop J, Stremmer R, Neumann C, Dc Maeyer D, Macher E (1982) Interferon inhibits the suppressor T cell response of delayed-type hypersensitivity. Nature 296:775–776
324. Zhang ZY, Michael JG (1990) Orally inducible immune unresponsiveness is abrogated by IFN-gamma treatment. J Immunol 144:4163–4165
325. Claessen AME, von Blomberg BME, de Groot J, Wolvers DAE, Kraal G, Scheper RJ (1996) Reversal of mucosal tolerance by subcutaneous administration of interleukin-12 at the site of attempted sensitization. Immunology 88:363–367
326. Röcken M, Shevach EM (1996) Immune deviation – the third dimension of nondeletional T cell tolerance. Immunol Rev 149:175–194
327. Bridoux F, Badou A, Saoudi A, Bernard L, Druet E, Pasquier R, Druet P, Pelletier L (1997) Transforming growth factor beta (TGF-beta)-dependent inhibition of T helper cell 2 (Th2)-induced autoimmunity by self-major histocompatibility complex (MHC) class II-specific, regulatory CD4+ T cell lines. J Exp Med 185:1769–1775
328. Hafler DA, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Fukaura H (1997) Oral administration of myelin induces antigen-specific TGF-beta 1 secreting T cells in patients with multiple sclerosis. Ann NY Acad Sci 835:120–131
329. Fukaura H, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Hafler DA (1996) Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. J Clin Invest 98:70–77
330. Lonati A, Licenziati S, Marcelli M, Canaris D, Pasolini G, Caruso A, de Panfilis G (1998) Quantitative analysis "at the single cell level" of the novel $CD28^-CD11b^-$ subpopulation of $CD8^+$ T lymphocytes. ESDR meeting at Cologne
331. De Panfilis G (1998) $CD8^+$ cytolytic T lymphocytes and the skin. Exp Dermatol 7:121–131

332. Kuchroo VK, Byrne MC, Atsumi Y, Greenfeld E, Connol JH, Whitters MJ, O'Hara RM, Collins M, Dorf ME (1991) T cell receptor alpha chain plays a critical role in antigen-specific suppressor cell function. Proc Natl Acad Sci USA 88:8700–8704
333. Kumar V, Sercarz E (1998) Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. J Immunol 161:6585–6591
334. Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML (1998) Prostaglandin E_2 induces the final maturation of IL-12 deficient $CD1a^+CD83^+$ dendritic cells. J Immunol 161: 2804–2809
335. Steinbrink K, Wolf M, Jonuleit H, Knop J, Enk AH (1997) Induction of tolerance by IL-10-treated dendritic cells. J Immunol 159:4772–4780
336. Steinbrink K, Jonuleit H, Muller G, Schuler G, Knop J, Enk AH (1999) Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in $CD8(^+)$ T cells resulting in a failure to lyse tumor cells. Blood 93:1634–1642
337. Taams LS, Boot EPJ, van Eden W, Wauben MHM (2000) 'Anergic' T cells modulate the T-cell activating capacity of antigen-presenting cells J Autoimmun 14:335–341
338. Taams LS, van Rensen AJML, Poelen MC, van Els CACM, Besseling AC, Wagenaar JPA, van Eden W, Wauben MHM (1998) Anergic T cells actively suppress T cell responses via the antigen presenting cell. Eur J Immunol 28:2902–2912
339. Strobel S, Mowat AM (1998) Immune responses to dietary antigens: oral tolerance. Immunol Today 19:173–181
340. Strober W, Kelsall B, Marth T (1998) Oral tolerance. J Clin Immunol 18:1–30
341. von Herrath MG (1997) Bystander suppression induced by oral tolerance. Res Immunol 148:541–554
342. Inobe J, Slavin AJ, Komagata Y, Chen Y, Liu L, Weiner HL (1998) IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. Eur J Immunol 28:2780–2790
343. Fowler E, Weiner HL (1997) Oral tolerance: elucidation of mechanisms and application to treatment of autoimmune diseases. Biopolymers 43:323–335
344. Van Hoogstraten IMW, von Blomberg BME, Boden D, Kraal G, Scheper RJ (1994) Non-sensitizing epicutaneous skin tests prevent subsequent induction of immune tolerance. J Invest Dermatol 102:80–83
345. Epstein WL (1987) The poison ivy picker of Pennypack Park: the continuing saga of poison ivy. J Invest Dermatol 88:7–9
346. Morris DL (1998) Intradermal testing and sublingual in desensitization for nickel. Cutis 61:129–132
347. Wendel GD, Stark BJ, Jamison RB, Molina RD, Sullivan TJ (1985) Penicillin allergy and desensitization in serious infections during pregnancy. N Engl J Med 312:1229–1232
348. Panzani RC, Schiavino D, Nucera E, Pellegrino S, Fais G, Schinco G, Patriarca G (1995) Oral hyposensitization to nickel allergy: preliminary clinical results. Int Arch Allergy Immunol 107:251–254
349. Troost RJ, Kozel MM, van Helden-Meeuwsen CG, van Joost T, Mulder PG, Benner R, Prens EP (1995) Hyposensitization in nickel allergic contact dermatitis: clinical and immunologic monitoring. J Am Acad Dermatol 32:576–583
350. Chase MW (1946) Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med 61:257–259
351. Polak L, Turk SL (1968) Studies on the effect of systemic administration of sensitizers in guinea pigs with contact sensitivity to inorganic metal compounds. I. The induction of immunological unresponsiveness in already sensitized animals. Clin Exp Immunol 3:245–251
352. Polak L, Rinck C (1978) Mechanism of desensitization in DNCH-contact sensitive guinea pigs. J Invest Dermatol 70:98–104
353. Gaspari AA, Jenkins MK, Katz SI (1988) Class II MCH-bearing keratinocytes induce antigen-specific unresponsiveness in hapten-specific TH1 clones. J Immunol 141:2216–2220