Effector and regulatory T cells in allergic contact dermatitis

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Allergic contact dermatitis is a prototypic T-cell-mediated disease that has a socio-economic impact in industrialized countries. Here, Andrea Cavani and colleagues highlight recent developments in the T-cell-based effector and regulatory mechanisms of this common skin disorder.

Allergic contact dermatitis (ACD) results from a T-cell response to harmless, low-molecular-weight chemicals (haptens) applied to the skin. In the sensitization phase, haptens penetrating the skin are collected by resident dendritic cells that migrate to the regional lymph nodes to activate and clonally expand specific T-cell precursors[1]. Re-exposure to the relevant hapten initiates the efferent phase and clinical expression of ACD, characterized by the rapid recruitment and activation of specific T cells at the sites of hapten challenge. In spite of the longlasting persistence of the hapten in the skin, the reaction is self-limited, suggesting that regulatory mechanisms are actively involved in the termination of ACD.

T-cell recruitment in ACD

The recruitment of T cells in the skin is regulated by the expression of proper homing receptors, such as the cutaneous lymphocyte-associated antigen (CLA), which mediates rolling of T cells over activated endothelial cells expressing E-selectin[1]. More recently, chemokine receptors have been proposed as important regulators of the tissue targeting of T cells. In line with this concept, it has been shown that skin-seeking CLA^+ T cells co-express CC chemokine receptor 4 (CCR4), the ligand for thymus and activation-regulated chemokine [TARC; CC chemokine ligand 17 (CCL17)] and macrophage-derived chemokine (MDC; CCL22). CCR4 triggered by TARC exposed on the endothelial cell surface during inflammatory skin disorders is thought to augment integrin-dependent firm adhesion of T cells to endothelial intercellular adhesion molecule 1 (ICAM-1)[2]. T-cell migration into peripheral tissues mostly depends on their chemokine receptor profile. Owing to the high expression of CCR5 and CXCR3, T helper 1 (Th1) cells preferentially migrate to the respective ligands, macrophage inflammatory protein 1β (MIP-1β; CCL4) and interferon γ (IFN-γ)-inducible protein 10 [IP-10; CXC chemokine ligand 10 (CXCL10)]. By contrast, T helper 2 (Th2) cells are mostly attracted by eotaxin (CCL11), TARC and MDC, and I-309 (CCL1), because of the high levels of CCR3, CCR4 and CCR8, respectively[3].

Epidermal keratinocytes have been extensively investigated as a source of inflammatory mediators for the initiation and amplification of skin immune responses, and T-cell-derived lymphokines are among the most potent activators of keratinocytes. Treatment with IFN-γ or IFN-γ plus tumor necrosis factor α (TNF-α) induces keratinocytes to express ICAM-1 and mature MHC class II molecules, and to release a vast array of growth factors, chemokines and cytokines such as interleukin 1 (IL-1), TNF-α and granulocyte–macrophage colony-stimulating factor (GM-CSF)[4,5]. IL-17, a cytokine produced by skin-infiltrating Th1 and Th2, but not $CD8^+$ T cells, reinforces many of the effects induced by IFN-γ (Ref. 4). Interestingly, the Th2 cytokine IL-4 acts synergistically with IFN-γ to enhance keratinocyte ICAM-1 expression and release of the CXCR3 agonistic chemokines, IP-10, monokine induced by IFN-γ (Mig; CXCL9) and IFN-inducible T-cell α-chemoattractant (I-TAC;

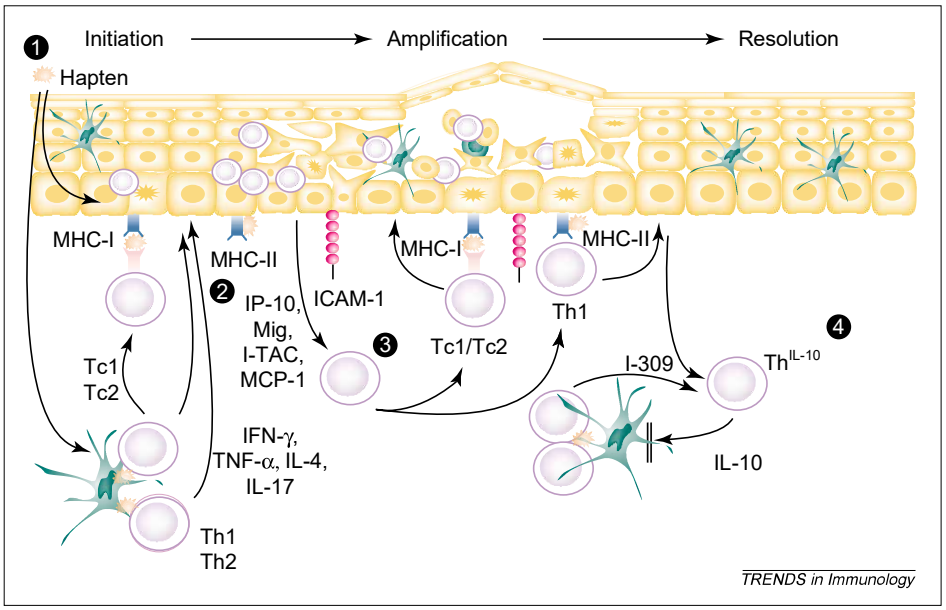

Fig. 1. Initiation, amplification and resolution of the elicitation phase of allergic contact dermatitis (ACD). In sensitized individuals, hapten-specific memory T cells recirculate in the skin environment at both epidermal ($CD8^+ > CD4^+$) and dermal level. (1) Haptens penetrating the skin are picked up by dendritic cells that induce activation and expansion of both $CD8^+$ and $CD4^+$-specific T cells. (2) $CD8^+$ T cells with cytotoxic potential can directly target resting keratinocytes and, together with $CD4^+$ T cells (both Th1 and Th2), release lymphokines that promote keratinocyte synthesis of proinflammatory cytokines (e.g. IL-1, TNF-α and GM-CSF) and chemokines (e.g. IP-10, Mig, I-TAC, MCP-1 and RANTES), and *de novo* expression of adhesion (e.g. ICAM-1) and MHC class II molecules. (3) This results in the massive recruitment of T cells, especially type 1, and their retention in the epidermis. MHC class II expression allows Th1, but not Th2 cells to exert cytotoxic activity against keratinocytes. In addition, upregulation of MHC class I induced by IFN-γ allows increased $CD8^+$-mediated cytotoxicity. Consequently, the skin shows the typical eczematous changes, including infiltration of mononuclear cells in both dermis and epidermis, intercellular edema between keratinocytes (spongiosis) and keratinocyte damage. (4) Resolution of ACD probably involves $CD4^+$ T cells secreting high levels of IL-10 ($Th^{IL\text{-}10}$), which impairs the functions of dendritic cells, but can also directly affect T cells. $Th^{IL\text{-}10}$ cells are attracted more selectively by the chemokine I-309, produced by activated T cells, but also by keratinocytes. Abbreviations: GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon γ; IL-4, interleukin 4; IP-10, IFN-γ-inducible protein 10; I-TAC, IFN-inducible T-cell α-chemoattractant; MCP-1, monocyte chemotactic protein 1; MHC, major histocompatibility complex; Mig, monokine induced by IFN-γ; Tc1, T cytotoxic 1; Th1, T helper 1; TNF-α, tumor necrosis factor α.

CXCL11), and thus can augment the recruitment and retention of Th1 cells in lesional skin[5].

Effector T cells in ACD

T cells involved in ACD are extremely heterogeneous in their cytokine profile and function, with $CD4^+$ and $CD8^+$ T cells playing distinct roles in the expression of the disease. In particular, $CD8^+$ T cells seem to provide the most relevant effector mechanisms of tissue damage[6]. Mice depleted of $CD8^+$ T cells or deficient in MHC class I molecules show a reduction of contact hypersensitivity (CH) responses. $CD8^+$ T cells, which are the more abundant T cells infiltrating both normal and inflamed epidermis, were found to predominate in ACD to urushiol, as well as in other hapten-related diseases, such as drug hypersensitivity reactions. In the case of ACD to nickel, specific $CD8^+$ responses are restricted to allergic individuals and correlate with the expression of the disease. By contrast, peripheral blood of both allergic and nonallergic subjects shows comparable frequencies of nickel-reactive $CD4^+$ T cells, with a higher percentage of IL-10-producing cells in the latter group[7]. These data might explain why AIDS patients can be affected by ACD despite the low number of $CD4^+$ cells. The higher propensity of haptens, compared with soluble proteins, to activate $CD8^+$ cells might be explained by their capacity to interact directly with peptides bound to both MHC class I and class II to form immunogenic hapten–peptide–MHC tri-molecular complexes. MHC class II-deficient mice, lacking in functional $CD4^+$ cells, display either upregulated or unaltered CH reactions and fail to mount oral tolerance to haptens, a process thought to be mediated by active induction of suppressor T cells. Together, these findings suggest that $CD4^+$ T cells play a multifunctional role in the expression of ACD, and exert both effector and regulatory functions.

Although IL-4 has been suggested to downmodulate immune responses to haptens, CH expression is reduced or unmodified in *IL-4*$^{-/-}$ mice and decreased in signal transducer and activator of transcription 6 (STAT-6)-deficient mice[8], in which Th2 responses are impaired. Recently, it has been shown that perforin and Fas/Fas ligand (FasL) double-knockout mice cannot develop CH reactions to haptens, indicating that an efficient T-cell cytotoxic machinery is required for CH expression[9]. In humans, it has been demonstrated that keratinocytes are susceptible to T-cell-induced apoptosis, with both a perforin- and Fas/FasL-mediated pathway, and this mechanism could be involved in the expression of several T-cell mediated skin diseases, including ACD (Refs 10,11). Nickel-loaded keratinocytes are highly susceptible to nickel-specific cytotoxicity induced by skin-infiltrating $CD8^+$ T cytotoxic 1 (Tc1) and Tc2 cells, and to a lesser extent by Th1 cells, but are resistant to Th2-mediated cytotoxicity[11]. Th1-mediated killing requires prior treatment of keratinocytes with IFN-γ to stimulate the expression of MHC class II molecules and ICAM-1. Moreover, IFN-γ upregulates Fas expression and renders keratinocytes susceptible to the FasL-mediated cytotoxicity pathway that predominates in Th1 cells. By contrast, pretreatment with IFN-γ is not necessary for Tc1- and Tc2-mediated killing. Thus, distinct T-cell subsets exhibit a different propensity to induce keratinocyte apoptosis, with $CD8^+$ T cells probably responsible for the initiation of the tissue damage, and Th1 cells involved in a later phase, when IFN-γ released by activated lymphocytes renders keratinocytes sensitive to the $CD4^+$ T-cell attack. Th2 cells, although not directly involved in the induction of keratinocyte apoptosis, might contribute to the amplification of the immune response by releasing IL-4, which acts in concert with IFN-γ to augment the recruitment of effector Th1 cells (Fig. 1).

Regulatory T cells in ACD

Intervention of regulatory mechanisms to limit excessive tissue damage and promote the termination of ACD is essential for maintaining the skin integrity. The typical fluctuation of the disease severity in time and the kinetics of the immune reaction

with a self-healing outcome indicate a tight and complex regulation. Cytokines are profoundly implicated in the modulation of immune responses and IL-10 has been shown to suppress murine CH potently. Specialized regulatory T cells have been described that modulate immune responses through the release of IL-10 (Ref. 12). These lymphocytes, called T regulatory (Tr) cells, are thought to be crucially involved in the hapten tolerance induced by ultraviolet radiation in mice[13]. In humans, nickel-reactive $CD4^+$ cells that produce high levels of IL-10, low levels of IFN-γ and no IL-4 have been isolated from the blood and skin of allergic individuals and, at higher frequency, from nonallergic subjects. These $Th^{IL\text{-}10}$ cells produce IL-10 as the predominant cytokine, and with earlier and more sustained kinetics compared with that of Th1 and Th2 cells. Functionally, $Th^{IL\text{-}10}$ cells block in an IL-10-dependent manner the maturation of dendritic cells including IL-12 release, thus impairing their capacity to activate specific Tc1 and Th1 effector lymphocytes, as well as alloreactive T cells[14]. Interestingly, $Th^{IL\text{-}10}$ cells display a broad array of chemokine receptors, and characteristically high levels of CCR8 (Ref. 15). CCR8 and I-309 mRNA precede IL-10 appearance at skin sites of hapten-challenge, suggesting a role for I-309 in the recruitment of regulatory T-cell populations in the skin.

Concluding remarks

The outcome of the immune response towards skin-applied haptens is the result of a balance between effector and regulatory mechanisms. Both type 1 and type 2 T cells contribute to the development of the inflammatory skin reaction, which can be prevented or terminated by IL-10-producing regulatory T cells. Important to the expression and the regulation of ACD is the crosstalk between infiltrating T cells and other resident cell populations, keratinocytes in particular, which are both targets and modulators of T-cell functions. Dissecting the cellular and molecular mechanisms underlying the expression of ACD might afford new and more effective preventive and therapeutic strategies.

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