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REVIEW

Role of IL-4 in delayed type hypersensitivity

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SUMMARY

IL-4 plays a key role in the contact sensitivity skin reaction. This has several implications. First, the view that contact sensitivity (CS) is only mediated by cells with a Th1 profile of cytokine secretion needs modification, in the light of the essential role of IL-4 at the effector stage. Second, the concept of a single cell involved in the systemic transfer of CS is no longer tenable, as it is known that both $\alpha\beta$ and $\gamma\delta$ cells are required. Studies with the cell lines (which contain both $\alpha\beta$ and a few $\gamma\delta$ cells) suggest that this double requirement may involve the action of IL-4 on $\gamma\delta$ cells, which bear receptors for IL-4. Finally, the view that T cell lines only transfer CS when injected locally, but not when injected intravenously (systemic transfer), is correct but incomplete, as T cell lines actually give systemic transfer of CS, providing the cell line or the recipient is treated with IL-4.

Keywords IL-4 contact sensitivity delayed hypersensitivity picryl $\gamma\delta$ T cells

Contact sensitivity (CS) to simple haptens such as picryl chloride (TNP) provides a useful tool to study cell-mediated immune responses *in vivo*. There are at least two components to the effector phase of CS. The first is the initial arrival of cells at the site of challenge. In one formulation, this step is mediated by antigen-specific initiator cells that release antigen-specific factors needed for the arrival of the antigen-specific effector T cells [1]. In any event, the second stage is the interaction of these antigen-specific effector T cells with antigen associated with MHC. This liberates a range of cytokines, which cause further cell arrival. These two stages were recognized about 30 years ago, when it was realized that most of the cells arriving at a passively transferred delayed hypersensitivity (DH) reaction were of recipient, and not of donor, origin [2,3].

The first component, namely the initial arrival of cells, may have several different mechanisms. First, a number of contact sensitizers induce liberation of cytokines, such as IL-1β and tumour necrosis factor-alpha (TNF-α) when they interact with Langerhans cells and keratinocytes [4]. These, in turn, induce adhesion molecules which cause leucocytes to adhere to, and then to penetrate, capillary endothelium. In some cases there is a role for mast cells which acquire antibody or antigen-specific T cell factors and liberate various factors on contact with antigen [1,5,6]. In the light of the role of IL-4 and TNF-α in CS, it may be relevant that mast cells are an important source of preformed cytokines [7,8].

CS is often regarded as a Th1 phenomenon. This view is based on the correlation between the ability of T cell clones and lines to produce interferon-gamma (IFN-γ) and their ability to give local passive transfer *in vivo* [9,10]. However, the detailed mechanism of the effector phase of CS remains poorly defined, mainly because of the lack of studies at a clonal level. In fact, although many Th1 cell lines and clones show *in vivo* CS or DH activity when injected into the same site as the eliciting antigen (local passive transfer), they are in general inactive when given intravenously (systemic transfer), unless large numbers of cells are used [9–15].

Bianchi *et al.* [11] explained this contrast by postulating 'changes in recirculation and homing properties brought about by long-term *in vitro* propagation', and Cher & Mosmann [9] raised the question whether IL-4 and/or IL-5 might be critical for the systemic transfer of DH.

This question was investigated using a panel of picryl-specific T cell lines obtained by long-term *in vitro* culture of picryl-immune lymph node cells with specific antigen and IL-2 [15]. These T cell lines showed a Th1 pattern of cytokine production, i.e. they produced IL-2, IFN-γ, IL-3, TNF-α, but failed to produce IL-4 and IL-5. The lines transferred CS locally in an antigen-specific and MHC-restricted fashion, but in no case was systemic transfer obtained, even when high numbers of cells were injected intravenously. However, treatment of recipient mice with low doses of IL-4 or incubation of the cell lines in IL-4 allowed systemic transfer of CS. Dose–response analysis showed that as little as 10 pg per mouse *in vivo* or 10 pg/ml *in vitro* allowed transfer of CS by T cell lines. Kinetic experiments showed that IL-4 was effective when given to the recipient within 2 h before transfer of the cell lines, while no transfer occurred when IL-4 was given to recipient mice 1–5 days beforehand [15].

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Table 1. Effects of IL-4 on contact sensitivity (CS) to picryl chloride

1.	IL-4 allows systemic passive transfer of CS by T cell lines
2.	Injection of anti-IL-4 MoAb inhibits CS and its passive transfer
3.	Treatment of cells with antisense to IL-4 inhibits transfer of CS
4.	Injection of anti-IL-4 MoAb does not alter picryl-specific proliferation and IL-2 and IFN-γ production *in vitro*

The crucial role of IL-4 at the effector phase of CS was confirmed using two other systems: CS in actively sensitized mice and the systemic passive transfer of CS by immune cells. In mice sensitized to picryl chloride, MoAb to IL-4 given 3 days after sensitization blocked CS on challenge the subsequent day, and also reduced the histological changes typical of CS. Study of systemic passive transfer showed that the production of IL-4 by donor cells and its action in the recipient mouse are critical for systemic transfer. In fact, treatment of 4 day immune lymph node cells with an antisense oligonucleotide to IL-4 blocked their ability to transfer CS to recipient mice, and this was reversed by injection of IL-4. Moreover, injection of 4 day immune cells into mice treated with MoAb to IL-4 blocked the transfer of CS, and this was reversed by giving IL-4 [16]. Table 1 shows the effects of IL-4 in the CS reaction.

Overall, the results obtained using three different experimental systems clearly indicate that IL-4 is a critical cytokine at the effector phase of CS in the mouse. This is strengthened by the observation (unpublished) that IL-4 knockout mice develop only a weak CS reaction to picryl chloride.

IFN-γ [10], and TNF-α [17] in some systems, are also critical cytokines. IL-1β is known to be a key cytokine at the induction phase [4]. It is not known whether it is also critical at the effector phase of CS.

In general, the passive transfer of the CS reaction by immune lymph node cells is virtually confined to days 4 and 5 after immunization [18]. This is the time at which IL-4 gene expression has been detected by polymerase chain reaction (PCR) and IL-4 biological activity found in supernatants [19–22]. This raises the question whether the time course of the ability of lymph node cells to transfer CS is determined by the time course of IL-4 production. Interestingly, preliminary data from our laboratories have shown that skin-derived, Vγ3-positive $\gamma\delta$ cells expand in the lymph nodes of immunized mice at days 4 and 5 after immunization and bear IL-4 receptors. Similarly, $\gamma\delta$ cells, not $\alpha\beta$ cells, in the picryl-specific T cell lines bear IL-4 receptor and bind it [15], and there is evidence that $\gamma\delta$ cells are needed for the systemic transfer of CS [15,23].

This finding is consistent with recent reports indicating a role for $\gamma\delta$ cells in several cell-mediated responses. $\gamma\delta$ cells influence the outcome of bacterial infections [24–27] and contribute to disease pathogenesis [28,29]. Because $\gamma\delta$ cells can recognize native, unprocessed ligands or a limited number of non-polymorphic molecules, it is possible that these cells may be recruited into inflamed tissues as a consequence of either cytokine production or the different expression of tissue- specific vascular addressins which are up-regulated during inflammation.

This suggests that IL-4 may exert its effect through the induction of the production of IL-4 in an autocrine way, or of an unknown cytokine by $\gamma\delta$ cells, In any event, the nature of the signal provided by IL-4 to $\gamma\delta$ cells and the functional consequences of this interaction, remain unknown. As IL-4 enables cell lines which give local passive transfer only, to transfer systemically, the strong likelihood is that IL-4 acts on the ability of cells to move from the blood stream to the site of challenge. This requires initial adherence to and spreading on the surface of the endothelium, penetration of the endothelium and subsequently the basement membrane, and dissolution of and movement through the extracellular matrix. It is known that IL-4 increases leucocyte adhesion to vascular endothelium [30] and induces endothelial vascular cell adhesion molecule-1 (VCAM-1) expression [31,32]. The counterligands of VCAM-1 include the β1 integrin VLA-4 and fibronectin. These interactions are important in CS as MoAb to VLA-4 and a rigid analogue of a key motif of fibronectin block the effector phase of CS [33,34]. IL-4 also augments endothelial production of a monocyte chemotactic protein. However, there is no evidence that IL-4 induces adhesion molecules on lymphocytes [35]. Figure 1 summarizes some of the explanations of the role of IL-4 in CS.

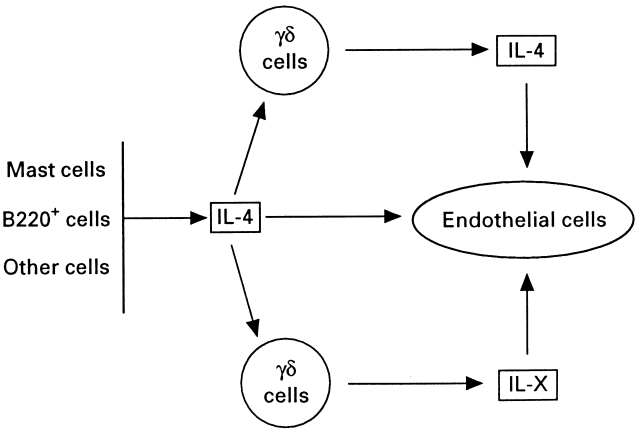

Fig. 1. Possible mechanisms of action of IL-4 in the contact sensitivity reaction. The figure illustrates that IL-4 may come from mast cells, B220$^+$ cells or other cells. IL-4 binds to $\gamma\delta$ cells and induces them to produce IL-4 in an autocrine fashion or a different (unknown) cytokine (IL-X). IL-4 or IL-X then increase the expression of certain adhesion molecules on endothelial cells which are important in contact sensitivity.

There are contrasting data on the role of IL-4 in T cell-mediated reactions leading to tissue damage and destruction. The anti-inflammatory effect of IL-4 has been demonstrated *in vivo* in various animal models [36–38], and it has been suggested that IL-4 may prove useful in the management of chronic inflammatory diseases by blocking the production of proinflammatory cytokines and the development of effector T cells. However, *in vivo* [39,40] and *in vitro* [31,41] studies have suggested a potential proinflammatory function of IL-4. IL-4 has been shown to enhance the development of virus-specific cytotoxic T cells [42] and administration of soluble IL-4 receptor to mice inhibits an allogeneic response *in vivo* and enhances heart allograft survival [43]. Finally, administration of IL-4 failed to inhibit DH to *Leishmania major* in mice immune to Leishmania, and optimal inhibition of DH required a combination of IL-4 and IL-10 [44].

Thinking more generally, the important role of IL-4 at the effector stage of the CS reaction raises the question of whether one of the factors influencing the chronicity of tissue damage is the production of IL-4 and its interaction with $\gamma\delta$ cells, as might occur in atopic eczema [45], which is a variety of CS, and in the rejection of kidney allografts [46].

Note added in press

There is an important new reference to the antigen-non-specific role of $\gamma\delta$ cells: Askenase PW, Szczepanik M, Ptak M *et al*. γ/δ T cells in normal spleen assist immunized α/β T cells in the adoptive cell transfer of contact sensitivity. Effect of *Bordetella pertussis*, cyclophosphamide, and antibodies to determinants on suppressor cells. J Immunol 1995; **154**:3644–53.

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