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T-cell subsets: **Chemokine receptors guide the way**
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Recent results show that chemokine receptors and adhesion molecules can be differentially expressed on the different subsets of T helper cells, suggesting that regulated networks of gene expression may control tissue-specific migration of T helper cells.

Early events in an immune response stimulate the production of cytokines that direct the subsequent development of the different subsets of T helper (Th) cells which themselves have discrete patterns of cytokine production [1]. The development of these distinct Th subsets is strongly dictated by the type of microorganism invading a host, as well as by the genetic background of the host, probably as a result of the cytokines induced. It is likely that the dose of antigen and the route of antigen immunization may also be factors that determine the development of Th subsets [2–5].

Th1 cells, through their production of interferon-γ (IFN-γ) and tumour necrosis factor β, are responsible for directing cell-mediated immune responses leading to the eradication of intracellular pathogens [6,7] and may also cause diseases of the immune system, such as organ-specific autoimmune disease, if deregulated [8–10]. Th2 cells have been implicated in allergic inflammation, as cytokines produced by these cells, such as interleukin-4 (IL-4) and IL-5, can activate mast cells and eosinophils and, in addition, can result in elevated levels of immunoglobulin E [11]. The production of B-cell growth and differentiation factors by Th2 cells may in part explain why certain immune responses are predominantly antibody-mediated. However, it should be noted that delayed-type hypersensitivity responses, which are mediated by Th1 cells, are often accompanied by the production of complement-fixing antibodies such as immunoglobulin G2a [2,3,7]. The ability of cytokines to stimulate different effector mechanisms and thus different immune responses is also revealed by the fact that cytokines produced by each subset can regulate the function as well as the development of each subset [2,3,7].

An additional layer of control of the immune response depends on the regulated trafficking of lymphocyte subsets to the specialized microenvironments that control their differentiation and regulate their survival. Recruitment or homing of lymphocytes to these microenvironments is regulated by sequential engagement of adhesion and signaling receptors. This process can be separated into the following successive steps: primary, transient adhesion or tethering of the lymphocyte to the endothelium of the blood vessel; rolling of the cell along the endothelium; cellular activation through G-protein-coupled receptors; activation-dependent arrest; movement of the lymphocyte through the endothelium out of the blood vessel; and finally, further migration and localization within microenvironments in the tissue [12,13].

The exquisite specificity of lymphocyte homing is derived from the use of unique combinations of independently regulated receptor–ligand interactions at each of the sequential steps. Whereas the homing behavior of naive T cells — those that have not encountered antigen — is relatively homogeneous, the homing behavior of memory and effector T cells — those that have encountered antigen — is extremely heterogeneous with distinct subsets displaying restricted, tissue-selective patterns of recirculation [12]. Differential trafficking of naive and memory/effector cells implies that, during the naive to memory/effector transition, which is under the influence of the local environment, cells upregulate a unique set of homing receptors and chemokine receptors allowing them to selectively localize to appropriate effector sites outside the bloodstream and lymphoid organs.

Acquisition of a novel homing phenotype during memory/effector T-cell differentiation is temporally concomitant with the effector cytokine production associated with differentiation of Th effector cells such as Th1 and Th2 cells. Given their distinct effector functions, it seems likely that these Th effector cell subsets will be differentially recruited to sites of inflammation. The mechanisms responsible for the differential recruitment are now beginning to be elucidated. In this context, it has recently been shown that a percentage of Th1 cells, but not Th2 cells, is able to bind the adhesion molecule P-selectin and that these cells are selectively recruited to sites where Th1-mediated immune responses occur. Only Th1 cells can efficiently enter sites of inflammation where the immune response involves predominantly Th1 cells, for example sensitized skin or arthritic joints. This can be partially blocked by antibodies directed against P-selectin and further blocked by anti-E-selectin antibodies, even though soluble E-selectin binds poorly to Th1 cells [14]. Th2 cells that express the ligand for E-selectin do not home to the skin in these Th1-mediated immune responses [14]. However, it is possible that, under different immunizing regimens, Th2 cells may also be recruited.

Chemokines are pro-inflammatory cytokines that are involved in leukocyte recruitment and activation and have been divided into four classes — C–C, C–X–C, C–X$_3$–C and C — on the basis of the number and spacing of the conserved cysteine residues in their sequences [15]. The biological effects of chemokines on target cells are mediated by their interaction with seven-transmembrane G-protein-coupled receptors [15]. Given the heterogeneous localization of memory/effector cells, the distinct effector function of Th1 and Th2 subsets, and the model that lymphocyte recruitment is directed by a unique combination of adhesion molecules and chemokine receptors, it is perhaps not surprising that a rather striking pattern of expression of chemokine receptors on Th1 and Th2 subsets is emerging (Table 1). Whereas some of these receptors appear to be relatively stably expressed following Th1 and Th2 differentiation, the expression of other receptors appears to be more variable, potentially reflecting the activation state of the cells [15–21].

Effects of activation on the expression of chemokine receptors on T cells
Naive T lymphocytes do not respond to chemokines that are frequently produced as a result of inflammation. Chemotactic migration and the expression of CCR1, CCR2 and CCR5 have been shown to depend on the activation

Table 1

Differential expression of chemokine receptors.

Receptor	T cells	Other	Chemokine ligand
CCR1	+	Eosinophil	MIP-1α,1β, RANTES (MCP-3,4 low)
CCR2	+	Monocyte/basophil	MCP-1,2,3,4
CCR3	Th2	Eosinophil/basophil	MCP-3,4, eotaxin 1,2, RANTES
CCR4	Th2	Basophil	TARC, MDC
CCR5	Th1, Th2±	Monocyte, DC?	MIP-1α,1β, RANTES
CCR6	?	DC	MIP-3α
CCR7	+	DC	MIP-3β, 6Ckine
CCR8	Th2	Monocyte, thymocyte	I-309, TARC
CXCR1	?	?	IL-8, GCP-2
CXCR2	?	Neutrophil	IL-8, GCP-2, GROα,β,γ, ENA-78, NAP-2, LIX
CXCR3	Th1, Th2±	NK?	IP-10, MIG, 6Ckine
CXCR4	Naive, activated	–	SDF-1
CXCR5	–	B cell	BCA-1/BLC

± denotes that receptor expression was detectable in some but not all studies. NK, natural killer cell. DC, dendritic cell. For definitions of chemokines, see [15].

state of T cells and in particular on the presence of IL-2 [18,19,22,23]. In contrast, CXCR4 is expressed on both resting and activated T lymphocytes [15]. Of the receptors of the CXC class, naive T cells isolated from human peripheral blood lymphocytes have been shown to express only CXCR4, whereas other reports offer conflicting information on CXCR4 expression on polarized Th1 and Th2 cell lines, that is, cell lines that have been induced to differentiate down the Th1 or Th2 pathway [17,20]. Loetscher et al. [18] have shown that there is a significant variability in CCR5 expression and responsiveness to MIP-1β in T-cell lines generated from human donors by coculture with IL-2. They went on to show that, like CCR1 and CCR2 [18], CCR5 was rapidly lost in the absence of IL-2, and activation by antibodies against the T-cell accessory receptors CD3 and CD28 also resulted in CCR5 downregulation and loss of migration [19]. In this same study, they also showed that CXCR3 was present on Th1 and Th2 cells and T-cell lines cultured with IL-2. Whereas the majority of memory/effector T cells has been shown to express CXCR3, only a small proportion expresses CCR3 and CCR5 [17].

Th1-specific expression of chemokine receptors
CXCR3 has now been shown to be differentially expressed on lymphocytes and to bind the IFN-γ-inducible chemokines IP-10 and MIG [24]; moreover, it is expressed at much higher levels on Th1 cells and Th0 cells (which express both Th1-specific and Th2-specific cytokines) than on Th2 cells [17,20,25]. The migration of Th1 and Th0 cells to sites of inflammatory lesions in which high levels of IFN-γ are expressed may be favored by the responsiveness of the cells to IP-10 and MIG, together with their expression of functional ligands for P-selectin and E-selectin [14]. In fact, it has recently been reported that virtually all T cells that infiltrate joints affected by rheumatoid arthritis express CXCR3 [19]. CCR5, the receptor for RANTES, MIP-1α and MIP-1β, has been shown to be expressed at variable levels on T cells, unlike CXCR3: in some cases, CCR5 was detected on Th1 and Th2 cell lines [17] and, in other cases, it was found at higher levels on Th1 cell lines and clones than on Th2 cells [17,20]. This variability may be explained by the observation that CCR5 expression is influenced by the T-cell activation state and, like other receptors [18], is upregulated by IL-2 [22]. Expression of CXCR3 may not be affected significantly by the activation state, which may explain why it is detectable on a higher percentage of memory T cells [17]. Th1 lymphocytes expressing CCR5 as well as CXCR3 have been shown to predominate in the rheumatoid synovium [19].

Th2-specific expression of chemokine receptors
A number of studies have confirmed the initial observation [16] that CCR3 expression is restricted to Th2 cells [17,19,20]. Like CCR1, CCR2 and CCR5 [19], CCR3 is

downregulated on T-cell activation [16]. Levels of CCR3 have been shown to be moderate and variable in a number of studies [19,20], suggesting that CCR3-expressing lymphocytes might constitute a subpopulation of Th2 cells. However, Sallusto *et al.* [17], have demonstrated by flow cytometry that antibodies against CCR3 stain all of the cells within a population of Th2 cells, albeit with a heterogeneous pattern of expression, suggesting that different levels of CCR3 may be expressed on populations of Th2 cells but that all Th2 cells express CCR3. This idea is supported by the observation that there is enhanced CCR3 expression on Th2 cell lines that had been polarized through two rounds of stimulation in the presence of IL-4 and anti-IL-12 antibodies, in contrast to those obtained after one round of polarization [17]. The variability of CCR3 expression in Th2 populations may therefore represent various stages of polarization, as has been previously shown for differences in cytokine production by differentiating populations of Th2 cells [26]. In agreement with its expression on Th2 cells, the CCR3 receptor is a characteristic feature of T lymphocytes recruited into eosinophil-rich sites of allergic inflammation [27]. Furthermore, Th2 lymphocytes expressing CCR3 have been frequently found in allergic infiltrates [27].

Emerging studies now indicate that CCR3 is not the only chemokine receptor that is preferentially expressed on Th2 cells. Other such receptors expressed on Th2 but not on Th1 cells include CCR4 [16,19] as well as CCR8 [28]. Interestingly, both these receptors bind a common ligand termed TARC for thymus- and activation-regulated chemokine, although CCR4 and CCR8 each also bind MDC and I-309, respectively [16,28]. Furthermore, CCR3 expression is also observed on eosinophils and basophils, whereas CCR4 is expressed only on basophils (reviewed in [21]), and CCR8 is expressed only on monocytes [29]. This overlap in expression of chemokine receptors on Th2 cells and other effector cells involved in allergic responses is likely to represent a global mechanism for the recruitment of effector cells during allergic responses.

Ligands for CCR3, CCR4 and CCR8 may selectively attract not only eosinophils, basophils and monocytes, but also Th2 cells; upon activation with antigen, Th2 cells will produce IL-4 and IL-5 which induce the activation and survival of the key effector cells during allergic responses [2,3,7,11]. It will be of interest to determine whether these receptors are regulated by different stimuli or at discrete times in an allergic response. In this context, Sallusto *et al.* [17] have recently shown that polarization of T cells under conditions that favor the Th2 phenotype (treatment with IL-4 and anti-IL-12 antibodies) but in the presence of IFN-α not only inhibits differentiation of Th2 cells and IL-4 production, but also blocks the expression of both CCR3 and CCR4. In contrast, transforming growth factor β (TGF-β) inhibited Th2 development, IL-4 production

and CCR3 expression, but enhanced the expression of CCR4 [17]. In addition, TGF-β enhanced the expression of another chemokine receptor CCR7, which is also found on naive T cells [17].

Implicit in the differential trafficking and function of naive, memory and effector T-cell subsets is the concept that expression of adhesion molecules, cytokines, chemokines and chemokine receptors can be repeatedly regulated during the life span of the lymphocyte, not only during antigen-induced differentiation but also under the influence of the local microenvironment. These findings raise the possibility that the expression of certain chemokine receptors on Th cells may be developmentally regulated in accordance with the expression of key Th1 or Th2 cytokines, receptors for these cytokines and/or transcription factors. In contrast, the expression of other chemokine receptors may be less rigid and may be regulated independently of factors involved in the commitment of the Th1 and Th2 phenotype [17].

The differential expression of particular chemokine receptors on naive T cells as well as on Th1 and Th2 subsets suggests that regulated networks of chemokine receptor gene expression control the tissue-specific migration of Th effector cells [17]. It is possible that the expression of chemokines and their receptors by cells recruited to the site of an immune response may eventually provide a 'road map' for the identification of specific cell types participating in a given class of immune response. It is likely that the relative levels of cytokines may differ, however, often resulting in the development of Th1, Th2 and Th0 cells within an immune response. Thus, alternative levels of regulation may first differentially recruit subsets of cells and then may maintain a polarized reaction within a local site, allowing the possibility of an oppositely polarized reaction at a distinct site. Clearly, the kinetics of expression of cytokines as well as the expression of chemokines and their receptors will determine the activation, differentiation and recruitment of discrete Th effector subsets during an immune response.

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