

EDITORIAL

Mechanisms of T cell migration to the lung

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The lung is a tertiary lymphoid organ

The lung, like other organs directly exposed to the outside environment such as the gut and skin, is an important site of antigen entry. Consistent with this lung lymphatics drain to a large network of associated lymph nodes where T and B cell memory to lung presented antigens develops. In addition, the adult lung contains large numbers of predominantly memory α/β T cells in various compartments. These include the bronchial submucosa (sampled by endobronchial biopsies), intra-epithelial lymphocytes (bronchial brushings), the bronchial lumen and alveolar compartment (bronchoalveolar lavage (BAL)) and the lung interstitium (dispersed lung cells from lung resection material). B cells in contrast are relatively scanty in the lung mucosa [1]. The infant lung contains only small numbers of lymphocytes, suggesting that as memory to inhaled antigens develops T cells with specificity for lung antigen start to migrate into, and in part reside, in the lung, which becomes a tertiary lymphoid organ [2]. Bronchial associated lymphoid tissue (BALT) are aggregates of secondary lymphoid tissue usually found at carinal bifurcations. Like Peyer's Patches, they are covered by specialized epithelium that facilitates antigen entry. They are found in the lung during childhood but not in the normal adult lung [3].

Lymphocyte homing

Lymphocytes re-circulate from blood to tissue. Naïve cells are only able to enter secondary lymphoid tissue through specialized high endothelial venules whereas memory cells, as a result of expression of a larger array of adhesion and chemokine receptors, are also able to enter other tissues through post-capillary endothelium. T cell re-circulation is a highly regulated process controlled by organ-specific expression of chemokines and adhesion receptors. This directs not only migration into different organs but also micro-compartmentalization within those organs [4]. Migration into tissue through the systemic circulation follows the established multi-step paradigm of an initial capture step mediated by selectins and their ligands as well as the α_4 integrins, followed by an activation step thought to be mediated largely by endothelially expressed chemokines, which results in integrin-mediated arrest and chemokine-mediated directional migration [5]. In the lung whereas migration through the systemic bronchial circulation follows this rule, migration into the alveolar compartment and interstitium takes place through the low-pressure pulmonary circulation in part through the capillary network where the capture and in some circumstances the arrest step are not required [6].

A well-studied model of lymphocyte trafficking are the mechanisms controlling migration into peripheral secondary

lymph nodes in the mouse [7]. The arrest step is mediated by L-selectin binding to a number of sialylated, sulphated, O-glycosylated glycoproteins that come under the collective name of peripheral lymph node addressin (PNAd) that are collectively recognized by an antibody called MECA 79. These include, in humans, specific glycoforms of CD34 and podocalyxin [8]. The activation step is mediated predominantly by CCL21 a ligand for CCR7, a receptor that is expressed by all naïve cells and a subset of memory cells. The arrest step is mediated by lymphocyte function-associated antigen-1 (LFA-1) binding to intercellular adhesion molecule (ICAM)-1 and ICAM-2, a relatively non-specific step. Once in the lymph node, localized expression of another CCR7 ligand CCL19 directs migration to T cell zones whereas B cells are directed into germinal zones by localized expression of CXCL13, the ligand for CXCR5. Migration of naïve cells into mesenteric lymph nodes and Peyer's Patches in the mouse also involves L-selectin probably binding to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) rather than PNAd as well as $\alpha_4\beta_7$ rather than LFA-1. In BALT, again in the mouse, L-selectin binding to PNAd and LFA-1 binding to ICAM-1 are involved as are very late antigen (VLA)-4 and vascular cell adhesion molecule-1 (VCAM-1) but not $\alpha_4\beta_7$ [9].

A second pattern of migration involves organ-specific homing of memory cells, which results in their preferential return to the organ in which they encountered their cognate antigen, a process known as lymphocyte homing. Teleologically, this is thought to increase the chance of a T cell encountering its specific antigen as antigen entry can be relatively organ specific, i.e. influenza virus always infects through the respiratory tract and rotavirus through the gut. Organ-specific migration has been described for the small intestine and skin as a result of the definition of T cell subsets in peripheral blood that express specific patterns of adhesion and chemokine receptors and preferentially contain memory for antigen presented through those organs. Gut homing is thought to be mediated by $\alpha_4\beta_7^{\text{HI}}$ binding to MAdCAM-1, which is selectively expressed on small intestinal endothelium, and CCR9, which is expressed on a subset of $\alpha_4\beta_7^{\text{HI}}$ circulating T cells and whose ligand CCL25 (TECK) is expressed on small (but not large) intestinal endothelium and epithelium. Virtually all small intestinal T cells express CCR9 [10]. Memory for rotavirus is contained within the $\alpha_4\beta_7^{\text{HI}}$ subset of T cells in peripheral blood [11]. Skin homing T cells express cutaneous lymphocyte antigen (CLA) [12], a glycoform of P-selectin glycoprotein-1 (PSGL-1) that binds well to E-selectin that is well expressed on inflamed skin endothelium. CLA-expressing cells in peripheral blood express high levels of CCR4 and a subset express CCR10. CCR4 and CCR10 are thought to be involved in the arrest and transmigration steps as a result of expression of their ligands CCL17 (TARC) and CCL27 (CTACK) on skin endothelium [13]. Recently, CCR8

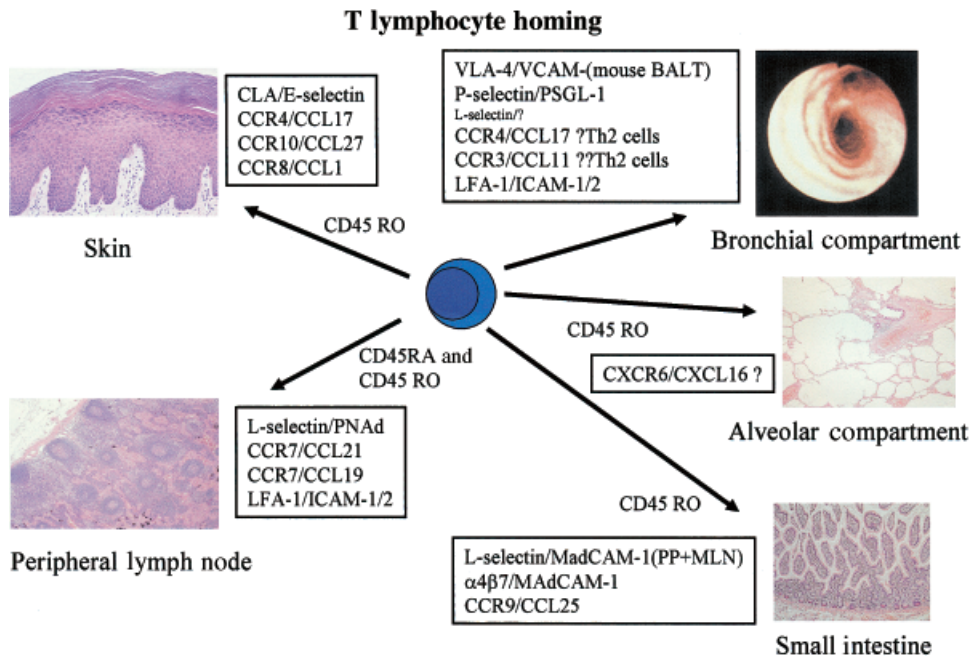


Fig. 1. Patterns of T cell homing. Naïve T cells (CD45RA) can only migrate to secondary lymphoid tissue such as peripheral lymph nodes (PLNs), Peyer's Patches (PPs), mesenteric lymph nodes (MLNs) and bronchial-associated lymphoid tissue (BALT) through high endothelial venules. Memory T cells (CD45RO) can also migrate into tissue via post-capillary venules. Migration to lymphatic tissue is controlled by L-selectin binding to peripheral lymph node addressin (PNAd) in PLN and MAdCAM-1 in PP and MLN and very late antigen-4 (VLA-4)/vascular cell adhesion molecule-1 (VCAM-1) in BALT. Organ-specific homing is controlled by cutaneous lymphocyte antigen (CLA)/E-selectin and a combination of CCR4:10 and 8 and their ligands in the skin and α₄β₇/MAdCAM-1 and CCR9:CCL25 in the small intestine. In the bronchial mucosa, PSGL-1/P-selectin plays a major role as does lymphocyte function-associated antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1). However, neither of these is very specific. In the alveolar: - peripheral compartment, CXCR6/CXCL16 may be important. The possibility that T-helper type 2 (Th2) cell migration may be under different control to Th1 cell migration has been suggested by studies of *in vitro* polarized cells with CCR3: CCR4 and CCR8 potentially important for Th2 cells. However, the evidence that this is important *in vivo* remains weak.

and its ligand CCL1 (I-309) have also been implicated in skin homing to healthy skin [14]. Memory for house dust mite allergen in patients with atopic dermatitis was found preferentially in the CLA-expressing blood T cells [15]. Induction of expression of organ-specific homing receptors by memory cells appears to be regulated by dendritic cells in the associated secondary lymph nodes [16].

It is generally assumed that T cells migrate into tissue and then, via the efferent lymphatics, to the organ-associated lymph nodes. If they do not encounter antigen-presenting cells expressing their cognate antigen they then pass back into the blood. However, the kinetics of this process are poorly understood. Tissue T cells including those of the lung express receptors such as VLA-1 and α₄β₇, which are not found on peripheral blood T cells to any extent and are associated *in vitro* with prolonged antigen stimulation. This suggests that tissue T cells expressing these receptors must be long-term residents in the lung mucosa, a hypothesis supported by the finding from organ transplantation that donor T cells can be found in the transplanted tissue and host blood for several months after transplantation. The mechanisms involved in the retention of T cells in the lung is a poorly explored and understood aspect of lymphocyte migration. A diagrammatic representation of patterns of T cell homing is shown in the accompanying figure (Fig. 1).

Migration of T-helper type 1 vs. T-helper type 2 cells

As well as organ-specific patterns of T cell trafficking, there is evidence for differential regulation of T-helper types 1 and 2 (Th1 and Th2) cell migration. Naïve T cells polarized *in vitro* towards a Th1 and Th2 phenotype by culture in either IL-4 or IL-12 demonstrate distinct patterns of adhesion and chemokine receptor expression with Th1 cells expressing functional

PSGL-1 (the major ligand for P- and E-selectin), CCR5 and CXCR3, whereas Th2 cells were shown to be unable to bind P-selectin and expressed CCR3, CCR4 and CCR8 [17, 18]. The extent to which this occurs physiologically is less certain. CCR8-expressing cells in the skin are not biased towards a Th2 phenotype and although there was a bias towards CCR4-expressing cells secreting IL-4 and CXCR3 cells secreting IFN-γ in blood and inflamed synovium this was far from absolute [14, 19]. Whether peripheral blood T cells expressing functional PSGL-1 have a Th1 bias has not been reported but P-selectin expression on endothelium is induced by Th2 rather than Th1 cytokines [20].

T cell migration to the lung

Whereas animal studies have been very useful in studying lymphocyte migration to other organs, interpretations of studies of lung trafficking in mice are complicated by the different anatomy, for example mice appear to only have a pulmonary circulation, the tendency for T cells to become lodged in the pulmonary capillary bed in *in vivo* trafficking studies and the lack of any significant number of resident T cells in the lung mucosa in experimental mice kept in pathogen-free conditions. The mouse ovalbumin challenge model has been used to study T cell migration to the lung in the context of Th2-mediated inflammation [21]. While there is a tendency for the findings to depend on the precise experimental details, it can be reasonably firmly concluded that there is limited evidence for a role for CCR8 in Th2 cell recruitment in the mouse [22, 23]. One study using the CCR3 KO mouse demonstrated a mast cell-associated increase in airway hyper-responsiveness (AHR) whereas another using a different sensitization protocol showed decreased AHR and inflammation [24, 25]. A study using adoptive transfer did

demonstrate that CCR3 and CCR4 were involved in trafficking of Th2 cells to the lung, but these cells were *in vitro* polarized so the physiological relevance is uncertain [26]. Gene deletion of CCR4 had no effect on the mouse model of allergic inflammation [27].

Two important questions for the allergist are whether there is an organ-specific pathway for lung homing T cells in general and a disease-specific pathway in asthma. With regard to the lung homing pathway, the limited studies undertaken in humans suggest that P-selectin (with a minor role for L-selectin) rather than E-selectin is the key receptor involved in the capture step in migration into the bronchial mucosa, with LFA-1 binding to both ICAM-1 and ICAM-2 involved in the arrest step [28, 29]. Lung-derived T cells from both BAL and peripheral lung do express a pattern of adhesion and chemokine receptors distinct from the small intestine and skin, suggesting that there is a lung-specific pathway. Thus, they are L-selectin negative, $\alpha_4\beta_7^{LO}$, CCR9 negative, CLA negative, CCR10 negative and CCR4^{LO} [30]. A chemokine receptor involved in lung recruitment should be enriched on lung T cells compared with blood. Kallinich and colleagues in this month's *Clinical and Experimental Allergy* report a comprehensive analysis of chemokine receptor expression on BAL T cells comparing expression with peripheral blood in three normal subjects [31]. CXCR3 and CCR5 were enriched but these receptors appear to be highly expressed on all tissue T cells. CCR4, CCR2 and CCR6 were also shown to be modestly enriched on BAL cells but the study did not correct for the higher percentage of memory cells (which preferentially express these receptors) in lung compared with blood. The current published literature therefore points to a specific lung homing pathway without defining the molecular basis for this. One receptor not investigated by Kallinich et al. was CXCR6, which we have found to be greatly enriched in BAL T cells from patients with interstitial lung disease (Morgan, Guillen and Wardlaw, submitted). High concentrations of its ligand CXCL16 were also found in BAL apparently derived from alveolar macrophages so that CXCR6 is an interesting candidate for a lung homing receptor involved in migration into the peripheral lung compartment.

The inflammation in asthma is considered to be driven by allergen-specific Th2 cells. There has therefore been considerable interest in whether CCR3, CCR4 and CCR8 binding chemokines could be involved in T cell recruitment into the lung in asthma. Most studies have shown undetectable or very low concentrations of Th2-related chemokines in BAL in clinical asthma, although eotaxin can be detected in sputum in more severe disease [32, 33]. After allergen challenge increased amounts of TARC, macrophage-derived chemokine and a number of CCR3 binding chemokines (but not CCL1:I-309) have been reported in BAL although concentrations have been no higher than the CXCR3 binding (Th1 related) chemokine IP-10 [33–35]. Are the number of CCR3, CCR4 and CCR8 expressing T cells increased in asthmatic airways and do these cells preferentially express IL-4 compared with IFN- γ ? No clear comment can be made regarding CCR8 as there are no good antibodies available. CCR3 is expressed on less than 10% of BAL T cells in asthma and normals, with no difference between the two groups. As shown by Kallinich et al. and ourselves, CCR4 is expressed by between 20% and 40% of BAL T cells with no increase in

mild asthma [30, 31]. Two studies have investigated the numbers of CCR4 expressing cells in the bronchial mucosa before and after allergen challenge [36, 37]. Panina-Bordignon observed a marked increase in CCR4 cells after allergen challenge, almost all of which were reported as expressing IL-4. This is in marked contrast to BAL and peripheral lung tissue T cells where the majority of T cells are non-cytokine producing and the numbers of IL-4-producing cells in both normals and mild asthmatics are less than 10% even after stimulation with a much greater number of IFN- γ -producing cells [38, 39]. This group observed about 100 times more T cells in the bronchial mucosa than other allergen challenge studies have reported. Nouri-Aria et al. did not observe any increase in CCR4 expressing CD3⁺ cells by immunohistochemistry after segmental allergen. Kallinich et al. have extended these observations by studying CCR4 expression on BAL T cells by flow cytometry, a more precisely quantifiable technique than immunohistochemistry, after allergen challenge. They again did not see any significant increase in CCR4-expressing cells, although their observations were underpowered and the three out of six patients who had the most marked eosinophilia did show an increase. Do the CCR4 and CCR3 expressing cells in the lung contain the IL-4-secreting (putative Th2) population? We have found that CCR3 and CCR4 were indeed preferentially expressed on IL-4 as opposed to IFN- γ -producing T cells in the lung. However, because the majority of BAL T cells express CCR5 and CXCR3, the majority of IL-4 producing T cells also express these receptors, emphasizing that *in vivo* the situation is more complex than in the *in vitro* polarized cells (Morgan and Wardlaw, submitted).

Conclusion

In conclusion, there is increasing evidence for an organ-selective pathway of constitutive T cell lung migration with the possibility that trafficking into the bronchial and alveolar compartments may be differentially regulated. No specific lung receptor has yet been identified although P-selectin is likely to be a key receptor involved in migration into the airways. There is evidence for a bias towards lung Th2 cells preferentially expressing CCR4 and possibly CCR3, but this is far from absolute and there is little hard evidence from either animal models or human studies that CCR4, CCR3 or CCR8 are involved in directing migration of T cells into the lung in asthma. Whether antagonists of these receptors with the aim of blocking Th2 cell migration will have any therapeutic benefit therefore remains open to question.

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