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# T lymphocytes on the move: chemokines, PI 3-kinase and beyond

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**The ordered, directional migration of T lymphocytes is a key process in development, immune surveillance and the immune response. Chemokines have an important role in the guidance of T lymphocytes and activate several members of the phosphoinositide 3-kinase (PI 3-kinase) family, which contribute to various aspects of the migratory machinery in many cell systems. However, the role of PI 3-kinase in T-cell movement is unclear, and its importance has been largely dismissed. Over the past two years, there has been exciting progress in our appreciation not only of the finer details of PI 3-kinase involvement in T-cell migration, but also of other signalling events that probably influence T-cell migration in response to recognized chemoattractants. These aspects of T-cell migration are the subject of this review.**

The coordinated trafficking of T lymphocytes in lymphoid and peripheral tissues is pivotal to immunosurveillance and the immune response. This process involves a multistep adhesion cascade: lymphocytes first roll along the surface of the blood vessel, adhering as a result of interactions between selectin and integrin and their respective vascular ligands; then integrins are activated, a process mediated by chemokines; firm integrin-mediated adhesion of lymphocytes to the endothelium of the microvasculature then occurs; the lymphocytes transmigrate through the vessel wall; and, finally, they migrate further into extravascular tissue [1,2].

Because of the central role of lymphocytes in inflammation and autoimmune disease, pharmacological interference with adhesion molecules and chemokine receptors in the recruitment cascade has been a popular strategy for therapeutic intervention in inflammatory disorders [3,4]. With a few exceptions, the outcome of clinical trials with adhesion molecule blockers has been variable and largely disappointing [5,6]. Nevertheless, the concept of pharmacological interference with lymphocyte cell migration remains a common avenue for development of novel immunosuppressants, as well as of anti-inflammatory drugs. This requires a complete understanding of the chemoattractant signals and biochemical mechanisms that T cells use to navigate to their intended destination. Here, we discuss the role of the phosphoinositide 3-kinase (PI 3-kinase)-dependent signalling cascade in leukocyte migration, which has attracted intense interest in recent years. Of the several different catalytic isoforms of PI 3-kinase (Box 1), there is strong evidence that the βγ-regulated p110γ catalytic subunit is a key signalling molecule for neutrophil migration that is driven by chemoattractants acting through G-protein-coupled receptors (GPCRs) [7]. This has led to the concept that pharmacological blockade of p110γ might offer an innovative rationale-based therapeutic strategy for several inflammatory diseases. Indeed, recent evidence indicates the effectiveness of p110γ inhibitors in several models of chronic inflammatory diseases, primarily resulting from inhibition of neutrophils, as well as $CD4^+$ T-cell migration [8,9].

## PI 3-kinase and lymphocyte migration: first impressions can be misleading

For a cell to migrate to a chemoattractant source, the cell must be polarized, which means that the molecular processes at the front (leading edge) and the back (uropod) of a moving cell are different. Establishing and maintaining cell polarity in response to extracellular stimuli appear to be mediated by a set of interlinked positive-feedback loops, involving PI 3-kinases, the Rho family of small GTPases, integrins, microtubules and vesicular transport. The relative contributions of the various signals depend on the cell type and the specific stimulus (reviewed in Ref. [10]). These intracellular signals result in reorganization of the cytoskeleton and cell adhesion, causing the cells to send out pseudopodia and crawl up the chemoattractant gradient. In some cell systems, PI 3-kinase-dependent signalling events contribute to several aspects of the migratory machinery, including gradient sensing, signal amplification and actin reorganization and, hence, cell motility [10–12].

Most of what we understand about biochemical events in T cells that are moving towards a chemoattractant gradient was derived from studying chemokine signal transduction (Box 2), although it is now clear that several lipid chemoattractants have a prominent role in directed migration of T lymphocytes. Chemokines mediate at least two processes: chemokine-mediated integrin activation (which mediates cell adhesion) and chemokine-induced chemotaxis (which is probably integrin independent). Interaction of chemokines with GPCRs on lymphocytes is dependent predominantly on Gi proteins [13]. This has led to the assumption that these receptors are coupled to the βγ-dependent p110γ isoform of PI 3-kinase; this is indeed the case, although several chemokine receptors can

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**Box 1. The class I and II PI 3-kinase families**

The class I phosphoinositide 3-kinases (PI 3-kinases) are divided into class IA and class IB PI 3-kinases. Class IA enzymes include the α, β and δ catalytic isoforms. The single class IB PI 3-kinase consists of the p110γ catalytic subunit, which is activated by G-protein βγ subunits and signals downstream of G-protein-coupled receptors (GPCRs). Some GPCRs, including chemokine receptors, activate class IA PI 3-kinases (reviewed in refs. [14,24]). Class I PI 3-kinases show broad substrate specificity towards PtdIns, PtdIns(4)P and PtdIns(4,5)$P_2$. The resulting 3′-phosphorylated lipids have important biological functions that rely on interaction with effector proteins containing lipid-binding domains, such as the pleckstrin homology (PH) and phox homology (PX) domains [29,56]. The effects of PtdIns(3,4,5)$P_3$ are counteracted by the lipid phosphatases PTEN and SHIP, which convert the lipid to PtdIns(4,5)$P_2$ and PtdIns(3,4)$P_2$, respectively. In some leukocytes (e.g. neutrophils), PtdIns(3,4,5)$P_3$ is located at the leading edge of a migrating cell; the cell responds to a chemotactic agent through the localized action of PI 3-kinase(s), which reside at the leading edge, and the positioning of PTEN, which is located at the margins and rear of the cell. This arrangement has not been seen in T lymphocytes [10,11].

Class II PI 3-kinases are structurally distinct and are thought to use only PtdIns and PtdIns(4)*P* as substrates. Mammalian class II PI 3-kinases predominantly include the ubiquitously expressed PI3KC2α and PI3KC2β. Whereas class I PI 3-kinases reside mainly in the cytoplasm until recruited to active signalling complexes, class II PI 3-kinases are mainly associated constitutively with membrane structures (including plasma and intracellular membranes) and with nuclei [46]. Chemokine receptors and integrins all stimulate class II PI 3-kinase activity [15,46], although the mechanism by which class II PI 3-kinases are activated is unclear. Class I PI 3-kinases have extended N and C termini, which might help facilitate protein–protein interactions in absence of a recognized specialized adaptor protein analogous to p85 and p101, which serve the class I PI 3-kinases. The N terminus has both coiled-coil and proline-rich motifs that probably facilitate protein–protein interactions; the C terminus has tandem PX and C2 domains that probably facilitate membrane-lipid binding and protein–protein interactions. Pharmacological tools, such as wortmannin and LY294002, have limited actions on class II PI 3-kinases [57,58]; the wide use of such tools, coupled with their relative weakness against class II PI 3-kinases compared with class I PI 3-kinases, means that the biological role of class II PI 3-kinases might have been seriously underestimated.

activate other PI 3-kinase isoforms [14,15]. PI 3-kinase activation seems to be a signalling event shared by most chemokine receptors expressed on T cells; however, paradoxically, it is now clear that activation of PI 3-kinase by chemokines can be a dispensable signal for directional migration of T cells [14,16–18].

Significant progress has been made recently in unravelling the confusion concerning the role of PI 3-kinase in lymphocyte migration. This was made possible by closer examination of mice that were deficient in the p110γ catalytic isoform of PI 3-kinase, as well as analysis of mice that expressed a mutant, catalytically inactive, class IA p110δ isoform of PI 3-kinase. The *in vitro* migration of p110γ-deficient $CD4^+$ and $CD8^+$ T cells towards chemokine CC-motif ligand (CCL) 19, chemokine CXC-motif ligand (CXCL) 12 and CCL21 is significantly decreased compared with cells from wild-type mice. By contrast, T-cell responses were largely unaffected by deficiency of the p110δ isoform of PI 3-kinase [19]. Hence, in settings where T-cell migration required PI 3-kinase activation, p110γ appears to be the predominant isoform required. This correlates with recent observations that p110γ-selective inhibitors reduce the number of $CD4^+$ memory T cells in models of systemic lupus [9].

Interestingly, B-cell migration to chemokines was not significantly affected by p110γ deficiency, thus implicating the involvement of other PI 3-kinase isoforms or signalling pathways in B-cell migration [19,20]. In this regard, analysis of p110δ-deficient B cells showed a defect in B-cell chemotaxis towards CXCL13, although responses to chemokine CC-motif receptor (CCR) 7 and chemokine CXC-motif receptor (CXCR) 4 ligands were less affected. Adoptive-transfer experiments with B cells that expressed inactive p110δ revealed diminished CXCR5-mediated homing to Peyer's patches and splenic white pulp cords. The ability of p110δ to function downstream of chemokine receptors in the chemotactic response of lymphocytes is consistent with the finding that a broad-spectrum,

**Box 2. Chemokines and lipid chemoattractants orchestrate T-lymphocyte migration**

Originally identified because of their important roles in recruiting innate immune cells and effector T cells to sites of inflammation, certain members of the chemokine superfamily (most notably CCL19 and CCL21, which both bind CCR7, CXCL13 and its receptor CXCR5, and CXCL12 and its receptor CXCR4) are now recognized as having key roles in promoting the organization and function of secondary lymphoid tissues (SLOs) [59]. Within SLOs, antigen-loaded dendritic cells present antigen–peptide–MHC complexes to clonotypic T cells and provide co-stimulatory signals for initiation of the immune response [59]. As a result, effector and memory T cells are generated, and they migrate to the periphery for participation in the immune response and immune surveillance. Relocation of effector and memory T cells is nonrandom because of tissue-specific address codes, which are mediated by unique combinations of adhesion molecules and chemokine receptors that enable proper tissue homing [2,60]. However, it is apparent that patterns of adhesion molecules and chemokine receptors might not necessarily provide the complete navigational apparatus for T-cell homing. Indeed, there is growing appreciation that lipid chemoattractants, such as sphingosine 1-phosphate (S1P; Box 4) and eicosanoids, which also engage GPCRs, have a prominent role at different stages of the immune response in navigation of distinct T-cell subsets to their intended destinations at sites of infection and inflammation.

Members of the eicosanoid family appear to contribute to the orchestrated trafficking of effector T cells at sites of infection or inflammation. The leukotriene B4 (LTB4) receptor BLT1 is expressed on type 1 T-helper (Th1) cells and type 2 T-helper (Th2) cells, $CD8^+$ effector T cells, and in the spleen and lymph nodes. The prostaglandin D2 (PGD2) receptor (DP2) is expressed on Th2 cells and in the thymus [61]. Experiments using mice deficient of the BLT1 receptor and the other PGD2 receptor DP1 have revealed important and unexpected roles of these receptors and their eicosanoid ligands in T-cell trafficking that occurs during immune activation and the inflammatory response [61,62]. LTB4 and PGD2 are produced in large quantities during mast-cell degranulation and probably provide an essential link between the innate and adaptive effector responses to inflammation. One model suggests that LTB4 and PGD2 direct the early phases of T-cell recruitment to the airways immediately after exposure to allergens, whereas chemokines direct the subsequent phases of T-cell recruitment that amplify and/or maintain airway inflammation in asthma [61].