LYMPHOCYTE MIGRATION

Restricted entry into lymph nodes

The ectodomain of the adhesion molecule L-selectin — which is crucial for T-cell migration from the bloodstream into peripheral lymph nodes (PLNs) — is proteolytically shed from the cell surface after cross-linking. A new study by Galkina *et al.* has now defined that L-selectin shedding shapes the migration pattern of T cells after antigen activation, but that it is not required for naive T-cell trafficking.

L-selectin mediates tethering and rolling of naive T cells in high endothelial venules (HEVs) prior to their migration to the PLNs. To investigate the importance of L-selectin shedding in this process, Ann Ager and colleagues generated endogenous L-selectin-deficient mice transgenic for either wild-type L-selectin (WT) or uncleavable L-selectin (LAP) in T cells. The percentage of T cells in the PLNs of these animals was not statistically different, providing preliminary

evidence that naive T-cell trafficking is not regulated by L-selectin shedding.

The authors further investigated the importance of L-selectin shedding in T-cell rolling *in vitro*, using a hydrodynamic flow model, and in T-cell trafficking *in vivo* after T-cell transfer to normal or recombinase-activating gene 1 (Rag1)-deficient mice. *In vitro*, the number of rolling LAP and WT T cells, as well as their rolling velocity and behaviour, was the same. *In vivo*, transferred WT and LAP naive T cells migrated in equal numbers to the PLNs. Together, these studies indicate clearly that naive T-cell homing to the PLNs is independent of L-selectin shedding.

After activation, T cells downregulate the expression of L-selectin and their migration patterns change. Antigen-specific L-selectin downregulation was shown to be mediated by L-selectin shedding using offspring of

T-CELL SIGNALLING

A new adaptor in the LIME-light

Several transmembrane adaptor proteins found in lipid rafts have been shown to influence the outcome of immunoreceptor signalling in lymphocytes. Two studies in *The Journal of Experimental Medicine* have now defined a new transmembrane adaptor molecule — LIME (LCK-interacting membrane molecule) — which is involved in regulating LCK-transduced signals in T cells.

Using a tyrosine phosphorylation-dependent yeast two-hybrid system, Hur *et al.* identified LIME as a protein that associates with the tyrosine kinase LCK. This association was confirmed in immunoprecipitation studies and was shown to require LCK-mediated phosphorylation of LIME.

In mice, LIME was detected in resting peripheral T cells and its expression levels were upregulated after stimulation with CD3-specific and CD28-specific antibodies. Confocal microscopy showed that LIME was distributed throughout the plasma membrane of resting T cells and that it relocated to the immunological synapse after T-cell receptor (TCR) recognition of antigen. These data indicate that LIME is a component of lipid rafts and this cellular localization was shown to be a result of palmitoylation of the membrane-proximal region of LIME.

Several signalling molecules, including the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and growth factor receptor-bound protein 2 (GRB2), were found to associate with LIME, and LIME-specific signals were shown to induce the phosphorylation of JUN N-terminal kinase (JNK) and extracellular signal-regulated kinase 1 (ERK1)/ERK2. Together, the results presented by Yun and colleagues indicate that through its association with LCK, LIME has a role in several signalling pathways that are activated after TCR stimulation.

In a separate study, Brdičková *et al.* identified mouse LIME by database searching for molecules that contain the domains characteristic of lipid-raft-associated transmembrane adaptor proteins, and then cloned the human homologue. As in mice, LIME protein was shown to be expressed by human peripheral blood T cells, in which it was palmitoylated and present in lipid rafts. However, a decrease in the expression level of LIME was detected after CD3-specific antibody stimulation, and removal of the TCR signal induced LIME re-expression, indicating that TCR signals regulate LIME expression by human peripheral blood T cells.

Stimulation of human peripheral blood T cells with CD4-specific antibodies induced tyrosine phosphorylation of LIME by protein tyrosine kinases, including LCK. Phosphorylation of distinct tyrosine residues mediated the association of LIME with LCK and the negative regulator of SRC kinases, CSK kinase, indicating that LIME phosphorylation can direct LCK and CSK recruitment to lipid rafts.

LIME-associated LCK was phosphorylated at both the negative and positive regulatory tyrosine residues, and overexpression of LIME induced an enhanced calcium response to TCR triggering by CD3-specific antibodies. These observations led Hořejší and colleagues to suggest that after TCR and CD4 co-ligation, LIME might potentiate TCR-mediated signals by binding LCK and so recruiting it to the lipid rafts and blocking its switch to the inactive form.

These two studies have identified a new transmembrane adaptor protein — LIME — that regulates T-cell signalling through the tyrosine kinase LCK; however, further studies will be required to define its role fully.

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References and links

ORIGINAL RESEARCH PAPERS Brdičková, N. *et al.* LIME: a new membrane raft-associated adaptor protein involved in CD4 and CD8 coreceptor signalling. *J. Exp. Med.* 10 November 2003 (doi: 10.1084/jem.20031484) | Hur, E.M. *et al.* LIME, a novel transmembrane adaptor protein associates with p56lck and mediates T cell activation. *J. Exp. Med.* 10 November 2003 (doi: 10.1084/jem.20030232)