



Lincoln's Inn Fields

## Leukocyte Adhesion

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### Group Leader **Nancy Hogg**

#### Postdoctoral Scientists

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Rachel Evans

#### Scientific Officers

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Paula Stanley

#### Visiting Worker

Meg Mathies

Our group focuses on how immune cells make use of the adhesion receptors known as integrins to leave the circulation and migrate into lymph nodes and infected tissues. We are particularly interested in how LFA-1, a member of the  $\beta 2$  integrin family, controls this migration. Another topic of investigation concerns the response of tissue macrophages to microbial stimuli that leads directly to the recruitment of neutrophils, one of the first events of an immune response. A third long term interest centres on the function of the S100A8/A9 proteins that are highly expressed by myeloid cells, but also have functions beyond the immune system.

#### **The behaviour of LFA-1 on migrating T lymphocytes: signaling and other intracellular processes that control migration**

The integrin LFA-1 is a promigratory adhesion receptor that immune cells utilise at many stages of an immune response. For example, when cells are recruited from the circulation, they use LFA-1 to migrate across the blood vessels into lymph nodes and infected tissues. How the integrin directs this migration has been a main focus of interest for the Leukocyte Adhesion Laboratory. We have discovered that different conformations of LFA-1 are organised into several distinctive activity zones on the membrane of migrating T cells. LFA-1 with high affinity for ligand ICAM-1 is located in

the mid-cell region and this 'focal zone' is maintained by linkage to the cytoskeletal protein talin (Smith *et al.*, *J. Cell Biol.* 170:141-151, 2005).

Recently, Paula Stanley found that LFA-1 of intermediate affinity forms new adhesions at the leading edge or lamellipodium of the T cell (Stanley *et al.*, *EMBO J.* 27, 62-75, 2008). This conformation of LFA-1 interacts with the actin binding protein,  $\alpha$ -actinin-1, and disruption of this link dissolves the LFA-1 attachments at the leading edge resulting in loss of cell spreading and migration. These different conformations of LFA-1 must cooperate for the cell to migrate. Our model is that high affinity LFA-1 attachments in the focal zone provide stability and support for intermediate affinity LFA-1 at the leading edge while the T cell scans surfaces, such as lymph node associated blood vessels. This study highlights a biological function for two active conformations of LFA-1 for the first time.

When T cells use LFA-1 to attach to ICAM-1, the resulting 'outside in' signalling causes cells in suspension to adhere and then migrate. We aim to dissect the signalling pathway that is immediately downstream of LFA-1. Lck and ZAP-70 are the main Src and Syk tyrosine kinase homologues in T cells and Rachel Evans finds that both are physically associated with LFA-1. These kinases are also essential for LFA-1-mediated migration as demonstrated by the use of pharmacological inhibitors and knockdown of Lck and ZAP-70. Rachel is interested in discovering where in the migrating T cell this signalling pathway operates and whether it is associated with a particular conformation of LFA-1.

Although our models of immune cell migration are very LFA-1-dependent, there may be other receptors involved. It is well known that the chemokines that stimulate the migration of naïve T cells signal through heterodimeric G proteins of the  $G_{\alpha i}$  class. Lena Svensson finds that T lymphoblasts are able to migrate randomly on ICAM-1 without direct chemokine

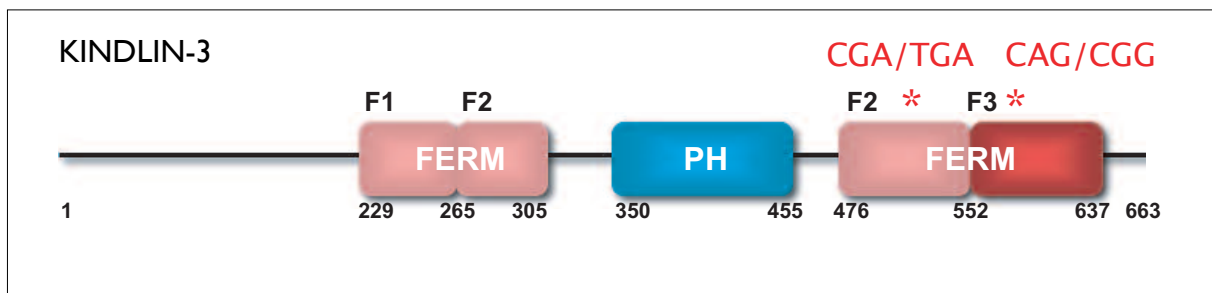


Figure 1. Diagrammatic representation of human kindlin-3 protein which is mutated in LAD-III patients. The positions of the two LAD-III mutations are indicated with red asterisks: one in exon 12 that results in a premature stop codon and the other affecting the splice acceptor site at exon 14 of the FERMT3 gene. Both mutations destabilise kindlin-3 mRNA leading to a lack of kindlin-3 protein in the LAD-III patients. A feature of the kindlin family is the characteristic intersecting of the FERM domains with a PH domain. The FERM F3 subdomain (darker red) binds to the  $\beta$  subunit of integrins.

stimulation and in a  $G\alpha i$ -independent manner. However other classes of G proteins, namely  $G\alpha q/11$  and  $G\alpha 12/13$  appear to be essential for migration and have distinctive roles. Lena is investigating the association of these G proteins with LFA-1-mediated migration.

### Leukocyte Adhesion Deficiency

The functional importance of integrins has been highlighted by Leukocyte Adhesion Deficiency-1 (LAD-1) patients who characteristically have mutations in the  $\beta 2$  subunit of the leukocyte integrins. The resulting lack of membrane-expressed  $\beta 2$  integrins on leukocytes is directly linked to recurrent bacterial infections experienced by the patients. The platelet integrin  $\alpha IIb\beta 3$  initiates the process of blood clotting through binding to fibrinogen and mutation in either  $\alpha$  or  $\beta$  subunit of  $\alpha IIb\beta 3$  leads to the bleeding disorder, Glanzmann thrombasthenia (GT). About 10 years ago an additional group of patients, named LAD-III, was identified that display symptoms of both LAD-1 and GT. Unlike these disorders, the haematopoietically-derived cells of the LAD-III patients express normal levels of the  $\beta 1$ ,  $\beta 2$  and  $\beta 3$  integrin subsets, but these integrins fail to function because of defective 'inside out' signalling (McDowall *et al.*, *J. Clin Invest.* 111, 51-60, 2003). The LAD-III lesion has been attributed to a C>A mutation in the CalDAG-GEF1 gene on chromosome 11q13.1 that codes for the protein. CalDAG-GEF1 is a good LAD-III candidate protein as it activates the GTPase Rap-1 that is important for integrin activation. However Lena Svensson, Alison McDowall and Irene Patzak in a collaboration with Ian Tomlinson's lab, have shown that this change is not responsible for the LAD-III disorder. Instead we have identified mutations in the nearby FERMT3 gene, that specifies the Kindlin-3 protein, as the cause of the LAD-III disorder (Svensson *et al.*, *Nat. Med.* In press, 2009). Two independent mutations in the FERMT3 gene in Maltese and Turkish patients result in decreased Kindlin-3 mRNA and loss of protein expression. Importantly, transfection of the patients' lymphocytes with Kindlin-3 cDNA, but not CalDAG-GEF1 restores adhesion and migration. There is evidence to suggest that Kindlin-3 enables talin to bind to integrin, but much remains

to be discovered about how this new 'player' promotes the activation of integrins on immune cells and platelets.

### Leukocyte migration *in vivo* and *in vitro*

Neutrophils are the first immune cells to migrate into infected tissue sites. It is therefore important to understand how they are recruited. In an *in vivo* study in mice, Katia De Filippo finds that resident tissue macrophages are the source of the major neutrophil chemoattractants, KC and MIP-2 (De Filippo *et al.*, *J Immunol.* 180: 4308-4315, 2008). The synthesis of these chemokines is rapidly regulated at the transcriptional level by signalling through the toll-like receptors TLR2, TLR3 and TLR4 that have diverse specificities for pathogens. Thus TLR signaling by tissue macrophages directly controls the synthesis of neutrophil-attracting chemokines that are essential for the earliest recruitment step in the innate immune response to microbial challenge.

### The S100 protein S100A8 is expressed by myeloid cells and, unexpectedly, also by early embryos

We have been interested for some time in two small  $Ca^{2+}$  binding proteins, termed S100A8 (MRP-8) and S100A9 (MRP-14), which form a heterodimeric complex in myeloid cells. Although these proteins are very abundant, making up ~40% of human neutrophil cytosolic protein, their function has long been uncertain. We have performed targeted disruption of both the S100A8 and S100A9 genes in mice. As the S100A9<sup>-/-</sup> mice are viable (Hobbs *et al.*, *Mol. Cell. Biol.* 23: 2564-2576, 2003), it was a surprise to find that S100A8 mice are embryonic lethal indicating that S100A8 has a function beyond heterodimer formation with S100A9. Jonathan Baker and Meg Mathies find S100A8 is required prior to the pre-implantation stage of embryo development and are presently attempting to pinpoint where it is required in this multi-step process. The hope is that an understanding of its function in the well-described stages of embryo development will shed light on its role in myeloid cells and highlight a new function for proteins of this class in embryo development.

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