during antigen T cell receptor signalling, no clear consensus has emerged as to whether the disease associated mutant is a gain or a loss-of-function phosphatase. We set out to examine how Lyp regulates integrin mediated function and to explore the phenotype of T cells expressing the autoimmune disease associated LypR620W mutant.

Materials and methods We used confocal microscopy to study the subcellular localisation of LFA-1, Lyp and its substrates ZAP-70 and Vav in primary human T cell blasts. Integrin mediated adhesion and migration were determined by culturing T cell blasts on Fc-ICAM coated plates. Images were captured by time–lapse microscopy every 15 s for 20 min in total, and mean speeds of each T cell calculated for single tracked cells. Lyp knockdown was performed using specific siRNA. Lyp overexpression was undertaken by transfecting HSB2 cells, which express very low levels of Lyp, with wild type Lyp620R or mutant Lyp620W, and adhesion and migration studied as above.

Results Lyp is expressed at the leading edge of T cells migrating on ICAM, and co-localises and co-immunoprecipitates with ZAP-70 and Vav. In contrast, phospho-ZAP-70 is expressed in discrete zones behind the leading edge. Knockdown of Lyp enhances migratory responses, while overexpresison of wild type Lyp in HSB2 cells leads to a dramatic reduction in migration. In contrast, expression of the disease associated LypR620W mutant fails to negatively regulate migratory responses.

Conclusions We show for the first time that Lyp is a negative regulator of integrin mediated T cell adhesion and migration, being expressed at the leading edge of migrating cells and serving to de-phosphorylate substrates that function to stabilise the LFA-1^{hi}/talin complex. Importantly, the expression of the disease associated Lyp mutant is associated with failure to regulate migratory responses.

A16 LYP/*PTPN22* IS A NEGATIVE REGULATOR OF INTEGRIN MEDIATED T CELL ADHESION AND MIGRATION; THE DISEASE ASSOCIATED *PTPN22* ALLELIC VARIANT IS A LOSS OF FUNCTION MUTANT THAT PERTURBS T CELL MIGRATION

Lena Svensson,[1] Garth Burn,[1] Cristina Sanchez-Blanco,[1] Rose Zamoyska,[2] Andrew Cope[1] *[1]Academic Department of Rheumatology, King's College School of Medicine, King's College London, London; [2]University of Edinburgh, UK*

10.1136/ard.2010.149096.16

Background and objectives A critical step in leucocyte adhesion and migration is the activation of integrins such as LFA-1. Engagement by its counterligand ICAM-1 leads to a cascade of signalling events that permit cell spreading, cytoskeletal rearrangement, cell migration and proliferation. Among intermediates that regulate integrin activation are src kinases such as Lck. The protein tyrosine phosphatase Lyp, which negatively regulates src kinases, has received much attention in recent years following reports of a strong association between a missense single nucleotide polymorphism in the gene (*PTPN22*) and autoimmune diseases such as rheumatoid arthritis, lupus and type I diabetes. While much work has focused on the regulatory effects of Lyp on Lck activation