

P004 Evidence for PI3K-Independent Directional T Lymphocyte Migration Following CCR4 Ligation.

Darran Cronshaw, *Charles Owen, *Zarin Brown and Stephen Ward.

Department of Pharmacology, University of Bath, Bath BA2 7AY, UK

*Novartis Horsham Research Centre, Horsham RH12 5AB, UK

Little is known about CCR4-mediated signal transduction pathways and their roles in functional responses such as chemotaxis. Ligation of CCR4 with its ligand, MDC (CCL22), leads to PIP3 accumulation in a leukaemic T cell line (CEM) which is abrogated with the use of the PI3K inhibitors, LY294002 (3-30 μ M) and Wortmannin (30-300nM). Additionally, IVLK assays demonstrate MDC is able to instigate the activation of the PI3K isoforms, p110 δ and p110 γ (both Wortmannin/LY294002 *sensitive*), but no detectable activation of the class II PI3K isoforms. Furthermore, we demonstrate the ability of MDC to flux calcium in CEMs and polarised human Th2 cells. This calcium flux is attenuated with the IP₃R antagonist, 2-APB, and the PLC inhibitor, U73122. Studies have advocated a role for PI3K in chemokine-mediated cell migration for a number of chemokine receptors. Surprisingly, CCR4-mediated chemotaxis of CEM/Th2 cells is *insensitive* to the PI3K inhibitors, LY294002 and Wortmannin. However, this chemotaxis is *sensitive* to U73122 and RO-32-0432 (PKC inhibitor) but 2-APB has no effect. This implicates a role for a novel PKC isoform, with recent experiments suggesting PKC δ . This data supports the notion that PI3K activation is insufficient to drive CCR4-mediated T cell migration.