Dynamic Mass Redistribution (DMR) as a Method to Enable Real-time Investigation of CKR Signalling in Human T Cells.

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Objective. Interaction of chemokines with conventional chemokine receptors (cCKR) promote specific trafficking of leukocytes. Here, we describe the use of label-free dynamic mass redistribution (DMR) to enable real-time investigation of cCKR responses in human T cells. This method allows increased throughput and reproducibility when compared to conventional chemotaxis assays.

Methods. T cells were isolated from human PBMCs (EasySep™ Human T Cell Isolation Kit), activated (Immunocult™ CD3/CD28/CD2) and cultured in IL-2 supplemented medium. Cell-surface expression of T cell markers and 15 cCKR were investigated using flow cytometry over 16 days. T cells (+/- PTX) were seeded, allowed to settle for 2 hrs and stimulation with antagonist or agonist prior to reading on a Corning® Epic® BT System. Following a 1 hr incubation, T cells were stimulated with predetermined EC80 CXCL11 concentration and inhibition of maximum response fitted.

Results. CXCR3, CXCR4, CCR4 and CCR7 were detected on >60% of CD3+ T cells and CCR10, CCR6, CXCR5 and CCR8 on >25% at a given timepoint. CCR1, CCR2, CCR3, CCR5, CCR9, CX3CR1 and CXCR6 were not detected. T cells from multiple donors demonstrated reproducible, concentration dependent, PTX sensitive responses to CXCR3 agonists (CXCL11>CXCL10>CXCL9: pEC50 10.03 ±0.1, 9.26 ±0.1 and 8.30 ±0.2, respectively). Previously described CXCR3 antagonists[1] were able to inhibit the CXCL11 stimulated response (pIC50 9.37 ±0.36 and 9.24 ±0.38, respectively).

Conclusion. T cells isolated and activated from PBMCs can be cultured and used to investigate CXCR3, CXCR4, CCR4 and CCR7 signalling in vitro. Flow cytometry identified the correct time-point should be used to achieve a T cell population with the highest cCRK expression. Importantly, DMR offers an alternative tool to determine the potency of CKR antagonists in a native, label free setting; a challenge for traditional chemotaxis assays.

[1] Chen *et al.* Bioorg. Med. Chem. Lett 22(1), 357-362 (2012)

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