Insurmountable CCR4 antagonists are less effective at inhibiting CCR4-agonist induced actin polymerisation in regulatory T cells than other T cell phenotypes

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The chemokine receptor CCR4 is expressed on several subpopulations of T cell including regulatory T cells (Treg) and type 2 helper T cells (which are thought to be involved in allergic inflammation). A characteristic of Treg is high expression of CD25 (IL-2 receptor alpha-chain). In this study, we have compared the effects of CCR4 agonists and antagonists on human Treg ($CD4^+CD25^{high}$) and other $CD4^+CD25^+$ cells ($CD25^{lo}$) in actin polymerisation assays.

Increases in the F-actin content of human T cells were measured in whole blood or isolated peripheral blood mononuclear cell preparations (PBMC) as previously described (Weston & Hall, 2008). Statistical significance was determined using the paired *t*-test. All donors gave informed consent prior to blood donation. The blood collection was approved by North Hertfordshire Research Ethics Committee.

Macrophage derived chemokine (MDC) and Thymus and Activation Regulated Chemokine (TARC) induced concentration-dependent increases in the F-actin content of both Treg and $CD25^{lo}$. In whole blood, the response of Treg to MDC was significantly more potent than that of $CD25^{lo}$. The pEC_{50}s were 9.88±0.05 and 9.53±0.06, respectively ($p<10^{-5}$, n=9). The potency of TARC was also significantly higher in Treg (9.98±0.04 vs 9.64±0.08, p=0.016, n=5) and its intrinsic activity, relative to MDC, was greater (1.03±0.02 vs 0.95±0.03, p=0.002). These observations indicate that CCR4 has a higher receptor reserve in Treg than in $CD25^{lo}$. Several CCR4 antagonists are insurmountable inhibitors of CCR4 agonist-induced actin polymerisation. We therefore investigated the effects of various CCR4 antagonists, following 30 min incubation with the cells, on TARC-induced increases in $CD4^+CD25^+$ cell F-actin content in PBMC preparations. Under these conditions, the Treg also responded to TARC more potently than $CD25^{lo}$ (pEC_{50} = 9.82±0.10 cf 9.69±0.12, n=4, p=0.004). A surmountable CCR4 antagonist (**1** in Weston & Hall, 2008; 3μM) showed no significant difference in its effects on TARC in these two cell types (pA_2=6.58 ±0.08 in Treg vs 6.46±0.05 in $CD25^{lo}$, n=4). However, two insurmountable CCR4 antagonists (**3** in Weston & Hall, 2008, 30nM, and an analogue, 300nM) caused a significantly greater inhibition of the maximal response to TARC in $CD25^{lo}$ than in Treg (**3**: 41±8% cf 22±6%, p=0.03, n=4; analogue: 43±8% cf 25±8%, n=4, p=0.001).

These data indicate that insurmountable CCR4 antagonists are less effective at inhibiting CCR4 mediated responses in Treg than in $CD25^{lo}$. This suggests that such compounds may not simply inhibit the recruitment of T cells to sites of allergic inflammation but may do this in a Treg sparing fashion. Such antagonists may therefore have stronger anti-inflammatory effects than those of surmountable CCR4 antagonists.

Weston & Hall, (2008) Proceedings of the British Pharmacological Society
http://www.pa2online.org/abstracts/vol6issue4abst066P