The C-terminus of the CC chemokine receptor CCR4 is crucial for receptor turnover and function

CC chemokine receptor 4 (CCR4) is a G protein-coupled chemokine receptor expressed by both Th2 and T regulatory cells [1,2]. As such, it has been implicated in the pathogenesis of allergic diseases including asthma and atopic dermatitis [1]. Expression of CCR4 on both pro-inflammatory and regulatory cell types makes targeting this receptor in inflammatory conditions a challenge. Evidence indicating that the two endogenous chemokine ligands of CCR4, CCL17 and CCL22, act as biased agonists of the receptor complicates this further [3]. Recently, gain-of-function CCR4 mutations affecting the C-terminus of the receptor were discovered in patients with adult T cell lymphoma/leukemia (ATLL), hinting at the importance of this region for the regulation of CCR4 expression and function [4].

The role of the C-terminus of CCR4 was examined by truncation, with CCR4 expression and function investigated in the murine pre-B lymphoma cell line, L1.2. Cells were transiently transfected with plasmids encoding either wild-type (WT) CCR4 or a truncation mutant, CCR4-Δ40, which lacked the final 40 amino acids of the receptor C-terminus. Receptor expression and function was assessed by flow cytometry, Western blotting and modified Boyden chamber chemotaxis assays. Although the WT-CCR4 and CCR4-Δ40 constructs were expressed at identical levels on the cell surface, as deduced by flow cytometry, cells expressing the CCR4-Δ40 construct had significantly more efficacious chemotactic responses to CCL17 and CCL22 than cells expressing WT-CCR4, with a 2-3 fold enhancement observed at the optimal concentration of ligand (CCL17, $p<0.001$, CCL22, $p<0.05$, repeated measures ANOVA, n=5). Treatment of transfectants with 100nM CCL17 or 100nM CCL22, resulted in significant endocytosis of WT-CCR4, with a 60% reduction of cell surface levels ($p<0.001$, 2-way ANOVA, n=5). In contrast, cell surface levels of the CCR4-Δ40 cell construct were not significantly reduced following treatment with either chemokine. Constitutive internalization of CCR4 was also affected by truncation, with endocytosis of the CCR4-Δ40 construct significantly impaired compared to that of WT-CCR4 ($p<0.05$, repeated measures ANOVA, n=5). CCR4 was readily degraded following constitutive endocytosis, as deduced by Western blot analysis. Degradation was sensitive to the inhibitor MG132, suggesting involvement of the proteasome. In conclusion, truncation of the C-terminus of CCR4 results in an imbalance in receptor turnover that leads to sustained CCR4 expression at the cell surface and an enhanced chemotactic response. Targeting the C-terminus of CCR4 therefore presents a novel opportunity for drug discovery at this receptor in the context of allergic inflammation and cancer.

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3. Anderson *et al* (2016) *Journal of Leukocyte Biology.* In press.
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