

## EDITORIAL

# Clinical & Experimental Allergy

## Is there a role for CCR8 in the pathogenesis of asthma?

This editorial discusses the findings of the paper by K. Mutalithas et al. [9] pp. 1175–1185.

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Under both homeostatic and inflammatory conditions, the spatial organization of leucocytes is chiefly controlled by a family of around 40 proteins known as chemokines. These proteins bind to specific cell surface receptors on the leucocyte surface and direct the migration of the cell to the source of the chemokine, a process known as chemotaxis. The chemokine receptors belong to the superfamily of proteins known as G protein-coupled receptors, which historically have proved amenable to pharmacological intervention by small molecule agonists and antagonists. Because the recruitment of T-helper type 2 (Th2) cells from the circulation to the allergic lung is known to play a key role in the orchestration of the resulting inflammatory response, there has been much interest in the chemokines and receptors crucial for T cell migration, with a view to their therapeutic blockade.

Early work using human T cell subsets polarized *in vitro* documented IL-4 producing Th2 cells as expressing mRNA for the chemokine receptors CCR3, CCR4 and CCR8, which correlated with functional responses to their respective ligands CCL11/Eotaxin-1, CCL17/TARC and CCL1/I-309 [1]. While several studies have documented increased expression of CCR3 and CCR4 ligands in asthmatic airways (reviewed in [2]) a role for the CCR8 : CCL1 axis in asthma pathogenesis has proved difficult to establish. Conflicting reports exist in the literature as to whether or not increased expression of CCL1 is observed in the lungs of asthmatics compared with healthy controls [3–7] and also whether or not increased numbers of CCR8<sup>+</sup> T cells are recruited to the allergic lung. In the earliest reported study by Panina-Bordignon et al. [3], immunostaining of bronchial biopsies with a goat anti-

serum recognizing CCR8 identified large numbers of CCR8<sup>+</sup> Th2 cells infiltrating the airway mucosa, which correlated with the decrease in forced expiratory volume in 1 s observed during the late asthmatic response. These findings were supported in part by *in situ* hybridization studies reporting significantly increased numbers of CCR8<sup>+</sup> cells within the asthmatic airways compared with healthy volunteers [6]. In contrast to both these reports, two studies have failed to show any difference between the levels of CCR8<sup>+</sup> cells in asthmatic and control biopsies [4, 7] using a polyclonal CCR8-specific antisera from the same source as that used by Panina-Bordignon et al. [3]. Although CCR8-specific monoclonal antibodies are available, these have not always proved reliable indicators of functional levels of CCR8 expression. For example, in a recently published study of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells, CCR8 surface staining was undetectable using a commercially available antibody, although actin polymerization in response to CCL1 was readily observed, which could be blocked by a CCR8-specific antagonist [8].

In this issue of *Clinical and Experimental Allergy*, Mutalithas and colleagues describe the use of a CCR8-specific monoclonal antibody named 433H, which robustly stains over 20% of IL-4<sup>+</sup> polarized human T cells [9]. The antibody was generated by scientists at ICOS Corporation by immunizing mice with transfectants expressing human CCR8, a proven methodology for the generation of monoclonal antibodies directed against chemokine receptors [10]. Data provided in their paper suggest that 433H is a blocking antibody, as pre-incubation of polarized T cells with the antibody ablates their chemotactic responses to a fixed 100 ng/mL concentration of CCL1. Interestingly, flow cytometry suggests that the antibody and ligand may bind to overlapping sites on CCR8; fluorescently labelled CCL1 identified a similar proportion of CCR8<sup>+</sup> T cells as did staining with 433H and pre-incubation of cells with either CCL1 or 433H abrogated any staining with the heterologous reagent. Because ligand-occupied receptor remains undetected by the mAb 433H, one pitfall with the use of this reagent may be that CCR8 levels are underestimated in samples where both ligand and receptor are present.

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Mutalithas and colleagues subsequently used the 433H antibody to ascertain the relative expression of CCR8 on T cells obtained from asthmatic individuals. Data presented show that significantly higher percentages of CCR8<sup>+</sup> T cells (4.7%) were found in PBMCs isolated from the venous blood of asthmatics compared with those of control subjects (3.0%), the vast majority of these T cells expressing both CD4 and the memory subset marker CD45RO. As might be anticipated, a significant proportion of these CCR8<sup>+</sup> T cells from either asthmatic or control subjects, produced the Th2 cytokines IL-4 and IL-13. Notably, there was no significant association between asthma severity (measured by serum IgE levels or blood eosinophil counts) and CCR8 expression, suggesting that CCR8 may be of limited use as a biomarker of disease progression.

Subsequent recovery of T cells from the BAL of asthmatic subjects painted a similar picture to the PBMC data, with a significantly higher percentage of CCR8<sup>+</sup> T cells (8.6%) found in the BAL of asthmatics compared with those of controls (3.9%), with CCR8 expression again enriched among IL-4 and IL-13 expressing cells. Worthy of mention is the observation that there was also a significant increase in the numbers of CCR8<sup>+</sup> BAL T cells compared with those found in peripheral blood within the same asthmatic subjects (9.0% vs. 5.7%). Immunostaining of bronchial biopsies from asthmatics with the 433H mAb revealed intra-epithelial and sub-epithelial CCR8<sup>+</sup> cells and a threefold increase in the number of CCR8<sup>+</sup> cells within the sub-epithelium compared with controls. These cells are presumed to be T cells in light of the earlier data from PBMCs and BAL, although data demonstrating a direct co-localization of CCR8<sup>+</sup> and CD3<sup>+</sup> cells is not presented in the paper. One potential caveat is that all the patients who underwent bronchoscopy and BAL were severe asthmatics undergoing treatment with oral steroids alone or in combination with high doses of inhaled steroids. Thus it remains possible that the steroids themselves were responsible for the increased levels of CCR8 expression observed, because dexamethasone-induced up-regulation of the chemokine receptor CXCR4 on T cells has been reported previously [11].

What then accounts for the increased numbers of CCR8<sup>+</sup> cells observed in asthmatic lung? Are lung resident T cells simply proliferating or are they recruited from the circulation? Although an earlier report suggested that the Th2-associated chemokine CCL17 might be a ligand for CCR8 [12], this has been debated by others and the general consensus is that CCL1 is the principal CCR8 ligand [13]. Supportive of CCL1:CCR8-mediated recruitment of T cells to the allergic lung is the finding that almost threefold higher levels of CCL1 were found in the BAL of asthmatic subjects compared with controls (36 pg/mL vs. 13 pg/mL). It should be noted that these levels are fivefold lower than previously reported by

Montes-Vizuet et al. [5] and may reflect the different purification procedures used. In this regard, it would be interesting to see if elevated levels of CCL1 are also found in induced sputum samples from asthmatics. The source of the CCL1 found in the BAL is also unclear, although previous reports have proposed that bronchial epithelia [5] or mast cells may represent the source of CCL1 responsible for T cell recruitment to the lung [6].

So does CCR8 represent a bonafide target for therapeutic intervention aimed at impairing the trafficking of Th2 cells to the allergic lung? Although small molecule antagonists with nanomolar affinity for CCR8 have been reported in the literature (reviewed in [14], as the authors of this manuscript point out, the precise contribution of CCR8<sup>+</sup> Th2 cells to asthma pathogenesis is still far from established. Although CCR8 expression was substantially enriched on IL-4<sup>+</sup> T cells, single staining protocols were used, leaving the possibility that the increased numbers of CCR8<sup>+</sup> T cells observed in asthmatics also includes Th0 and Tc2 populations. Similarly, a previous study using fluorescently labelled-CCL1 to stain CCR8<sup>+</sup> human PBMCs reported that FOXP3<sup>+</sup> T regulatory cells (Tregs) comprised around 20% of the entire CCR8<sup>+</sup> CD4 memory T cell population. It will be interesting to determine the relative numbers of CCR8<sup>+</sup> Th2 cells and CCR8<sup>+</sup> Tregs in asthmatic individuals and whether an imbalance in these populations is associated with allergic disease. The availability of the 433H mAb should enable this and many other interesting questions to be addressed.

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