Roles of chemokines in the regulation of leucocyte recruitment*

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ABSTRACT

In the search for new treatments for human inflammatory disease, antagonism of chemokine receptors by small molecules is an attractive goal. Although there are overlapping patterns of expression of chemokine receptors between leucocyte types, an investigation of the chemokine responsiveness of cells important in allergic inflammation, such as the eosinophil and the basophil, is beginning to uncover how selective recruitment may be regulated. The story of the eotaxin receptor, CCR3, and its central role in allergic inflammation illustrates that therapeutic antagonism of these pathways is imminently achievable.

The large, diverse range of human inflammatory diseases all have a common theme: that of leucocyte recruitment from the circulation into tissues. In respiratory disease, neutrophils accumulate in the lungs of patients with adult respiratory distress syndrome (ARDS) [1], and of some patients suffering from asthma [2]. The major characteristic of asthma is the strongly allergic nature of the inflammation, as shown by a dominant selective recruitment of eosinophils [3,4] with correlating regulatory lymphocytes [5]. Other cells associated with allergic reactions, such as basophils, are also recruited to the lung, although not as prominently as is observed in cutaneous allergic disease [6].

If drugs could be designed that blocked the migration of specific groups of leucocytes, what would be their therapeutic potential? There are many points in the pathogenesis of asthma that might be amenable to therapeutic interventions. These include sensitization to allergens (regulated in part by antigen-presenting cells such as dendritic cells [7,8]), the induction and maintenance of inflammation by T helper cells of the Th2 phenotype [9,10], and the possible role of both eosinophils and neutrophils in the tissue damage caused by acute allergic inflammation [2–4]. Modulation of the trafficking and recruitment of these leucocyte populations might therefore be beneficial [11].

In the last 12 years or so, major advances have been made in determining the signals that regulate the movement of inflammatory cells with the identification of the chemokines, a class of cytokines whose primary roles appear to be in the regulation of directed leucocyte migration. These small proteins act on a panel of at least 18 receptors [12,13] that are expressed on the surfaces of leucocytes in cell-specific patterns. These receptors are members of the large G-protein-coupled, seven-transmembrane superfamily that includes many previously pharmacologically targeted receptors, e.g. the β-adrenergic receptors. Numerous chemokines have now been identified, which are divided into the CC, CXC, CX3C and C structural subfamilies (based on the arrangement of conserved cysteine residues within the proteins). The receptor families show varying specificities for these chemokines, but each receptor usually only binds selected members of one of the chemokine families, and thus are likewise divided into CC, CXC, CX3C and C receptors. Our work has been principally concerned with the

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Key words: antagonist, basophil, chemokine, eosinophil, inflammation, receptor.

Abbreviations: MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein.

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Figure 1  Three patterns of leucocyte responses to chemo-
kines

How are leucocytes recruited selectively when there are both overlapping patterns of receptor expression between cell types and the ligands mostly act on more than one receptor? Clues may lie in our recent study of how chemokines can act on eosinophils (Eos), monocytes (Mono) and basophils (Baso). Eosinophils express CCR3, monocytes CCR2 and basophils both receptors. Depending on the concentration at which it is present, a single chemokine ligand may be able to selectively recruit different cells. Eotaxin can only act via CCR3, and is effective on eosinophils and basophils at high and low concentrations. MCP-1 acts via CCR2 at low concentrations, where it is an effective stimulator of monocytes and basophils. However, at high concentrations, CCR2 may be desensitized, and MCP-1 may then act via CCR3, thus stimulating eosinophils and basophils. MCP-4 shows an opposite pattern, stimulating CCR3 at low concentrations, hence, like eotaxin, acting on eosinophils and basophils. However, at high concentrations, CCR3 may be desensitized by MCP-4, and MCP-4 may then act via CCR2, thus stimulating basophils and monocytes.

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Intrapulmonary eosinophil recruitment is characteristic of asthma. In order to identify those mediators responsible for this, we have adopted an in vivo strategy working from a guinea pig model of allergic airway inflammation. Bronchoalveolar lavage fluid from sensitized guinea pigs that had been challenged with aerosolized allergen was shown to contain a single protein which caused eosinophil recruitment. On sequencing, this was found to be a chemokine belonging to the CC subfamily, which we named ‘eotaxin’ [14]. The DNA sequence was identified, and both eotaxin mRNA and protein have been shown to be expressed in human, guinea pig, mouse and rat [15–19]. Neutralization of eotaxin reduced eosinophil recruitment in the lungs of allergen-challenged mice in models of allergic airway inflammation [20], and inhibited basal trafficking of eosinophils into mucosal tissues [21], suggesting a major role across species for this chemokine in eosinophil recruitment.

It quickly became clear that eotaxin is an efficacious ligand at a single chemokine receptor, CCR3, which is present on only a small number of cell types, including eosinophils, mast cells, basophils and Th2-type T cells [22–26]. Eotaxin and its functional homologues, eotaxin-2 and eotaxin-3, are unusual in this respect, since most chemokines signal effectively via more than one receptor. Thus the eotaxin/CCR3 axis presents itself as an attractive target for the development of antagonists that might reduce eosinophil and Th2-type T-cell recruitment in human disease [11]. We cloned the guinea pig CCR3 receptor and produced blocking antibodies to it, which we found could prevent eosinophil recruitment in response to eotaxin in vivo [27]. We also investigated chemokine usage by human eosinophils in vitro. To facilitate this work, we developed a flow cytometry assay based on sensitive measurements of leucocyte shape change that enabled measurement of eosinophil chemokine responses simply, without the need for cell purification. We showed that eotaxins from the majority of individuals responded solely to CCR3-selective ligands, but also that eosinophils from a small percentage (perhaps 10%) of the general population also responded to CCR1-selective ligands, such as macrophage inflammatory protein (MIP)-1α [28]. These studies show population heterogeneity in eosinophil chemokine responses that are likely to be relevant in the rational design of chemokine receptor antagonists for the reduction of eosinophil...
recruitment. Recently, a small-molecule antagonist of CCR1 and CCR3 was described in the patent literature. In part, using the shape-change assay systems, we showed that this compound was an effective antagonist of eosinophil responses to MIP-1x and eotaxin [29]. Thus we have shown that small-molecule antagonists which can target more than one chemokine receptor may circumvent the problems of multiple active receptors on each cell type. We have since developed our flow cytometry systems to measure leucocyte shape change in whole blood, which will be useful in drug development and early clinical trials, as well as for measuring the effectiveness of drugs in individual patients (S. Bryan, T. J. Williams, T. T. Hansell and I. Sabroe, unpublished work).

Interestingly, the CCR1/CCR3 antagonist works partly via non-competitive mechanisms [29], suggesting that a viable mode of action for chemokine receptor antagonists may be by preventing receptor conformational change/signalling, as well as competition for ligand binding to the receptor. Other mechanisms of antagonism by small molecules that may be valid for the inhibition of chemokine-mediated signalling include the prevention of receptor cycling to and from the membrane, a process which appears to be a prerequisite for effective chemotaxis [30].

The complexity of the chemokine network raises important issues regarding redundancy: in particular, if one pathway is blocked does another one ‘take over’ without significant functional problems? ‘Knockout mouse’ studies, where single chemokines or receptors have been deleted from the genome, have yielded interesting results, with significant consequences in models of transplant rejection [31], the regulation of Th2-type T cell development [32] and the development of atherosclerosis [33]. However, roles for specific pathways can be subtle, and some chemokine/chemokine-receptor knockout mice have been associated with a relatively difficult search for a phenotype. Some human individuals are natural knockouts for the chemokine receptor CCR5, which is without any obvious clinical consequences, except a resistance of these individuals to infection by HIV (which uses CCR5 as a co-factor for cell entry [34,35]). We therefore developed the shape-change assay to study another cell with roles in allergic inflammation, the basophil. This cell is of interest, because it expresses many chemokine receptors that are also present on other cell types. For example, basophils express CCR3 at high levels, as do eosinophils; yet basophils show different temporal patterns of recruitment at sites of allergic inflammation compared with the eosinophil [36]. Until now, pathways that can regulate the selective recruitment of basophils have remained obscure. We showed that eosinophils (the main chemokine receptor of which is CCR3) and monocytes (expressing mainly CCR2) respond to monocyte chemotactic protein (MCP)-4 and MCP-1 respectively at low concentrations, but tend to show bell-shaped dose–response curves, with reduced responses at high concentrations of ligand. However, we found that in basophils, which express CCR2 and CCR3, MCP-1 and MCP-4 showed sequential usage of these chemokine receptors across a range of concentrations. For example, MCP-1 elicited shape-change responses at low concentrations in monocytes and basophils (via CCR2), but at high concentrations was an effective stimulus of eosinophil and basophil shape change (via CCR3). The ability of a chemokine to use different receptors in a sequential manner could allow a single chemokine to elicit more than one type of functional response within one cell type, or to mediate selective recruitment of more than one cell type in a concentration-specific manner [37] (Figure 1). These patterns of responses may begin to explain how the selective recruitment of different leucocytes may be mediated by the same chemokines, and introduces yet another layer of complexity in the regulation of cell recruitment by chemokines. These functional studies also suggest that subtle non-redundant pathways may regulate specific patterns of mixed leucocyte infiltrations in disease.

The discovery that low-molecular-mass compounds can selectively block different chemokine receptors opens up the opportunity for a generation of specifically targeted anti-inflammatory compounds that may become drugs of choice in, for example, asthma, rheumatoid arthritis and transplant rejection.

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