REVIEW

Chemokines, innate and adaptive immunity, and respiratory disease

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Chemokines, innate and adaptive immunity, and respiratory disease. I. Sabroe, C.M. Lloyd, M.K.B. Whyte, S.K. Dower, T.J. Williams, J.E. Pease. ©ERS Journals Ltd 2002.

ABSTRACT: Selective leukocyte trafficking and recruitment is primarily regulated by a specific family of small proteins called "chemokines". This extended family shepherds and guides leukocytes through their lives, facilitating their development, regulating their interactions with other leukocyte types, and guiding their recruitment to sites of inflammation.

Through the actions of chemokines, allergen sensitization is regulated in atopic asthma, through the controlled migration of dendritic cells, T- and B-lymphocytes, mast cells and basophils. Subsequently, atopic inflammation is driven by chemokine-directed recruitment of eosinophils, basophils and lymphocytes. Diseases from cancer to chronic obstructive pulmonary disease to interstitial fibrosis are all potential targets for chemokine receptor antagonism.

Innate immunity (the early pattern-recognition responses to stimuli such as lipopolysaccharide, viral proteins and bacterial DNA) needs to bridge the gap to specific immunity and antibody production and immunological memory. Again, chemokines are likely to be fundamental mediators of these responses.

Chemokines are fundamental regulators of leukocyte homeostasis and inflammation, and their antagonism by small molecule chemokine receptor antagonists may be of enormous importance in the future treatment of human respiratory disease.
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The last 15 yrs have seen an explosion in the number of small protein cytokines that have been demonstrated to regulate leukocyte trafficking. With sequencing of the human genome nearing completion, researchers may not be far away from knowing exactly how many of these chemotactic cytokines ("chemokines") are present, and how many receptors they may act through. However, science is a long way from understanding the detail of these systems, even though receptor antagonists for many of the receptors are currently in development.

The impact of a better understanding of these pathways on respiratory disease may be enormous. The functioning of the entire immune system depends upon the regulation of leukocyte trafficking, both at homeostatic levels (*e.g.* T-cell development, localization of mast cells and eosinophils to mucosal surfaces, immature dendritic cells to surfaces interfacing with the environment, such as the lung, skin and nasal mucosa) and at the level of active recruitment of cells to sites of inflammation.

Drug development in this field is geared towards the production of chemokine receptor antagonists [1–4]. These aim to prevent leukocyte migration and activation, blocking either homeostatic or pro-inflammatory pathways, or a combination of the two. Thus, chemokine receptor antagonists (CKRAs) will almost

inevitably be targeted to diseases where inflammation has crossed the boundary from the beneficial (*e.g.* the clearance of bacterial infection in pneumonia) to the detrimental. There are many such examples of these conditions in respiratory disease, including asthma, interstitial lung diseases, such as idiopathic pulmonary fibrosis and sarcoidosis, chronic obstructive pulmonary disease and acute respiratory distress syndrome, to name but a few. It is a theoretical possibility that drugs modifying chemokine function might also be used to enhance immune responses, *e.g.* by promoting antituberculous immune activity, either through the use of receptor agonists or CKRAs to modify T-helper cell types 1/2 (Th1/2) responses to pathogens. Cancer may be another viable target for CKRAs; angiogenesis of lung tumours is partly dependent upon chemokines (particularly acting through receptor CXCR2) and, very recently, metastasis of breast carcinoma cells has been shown to be dependent upon the chemokine receptor CXCR4 [5]. Modulation of angiogenesis may also target interstitial lung diseases such as idiopathic pulmonary fibrosis [6, 7].

Before the utility of CKRAs in these conditions can be predicted accurately, however, the chemokine pathways that they are dependent upon still need to be understood. Respiratory disease can be viewed from

a relatively reductionist perspective; inflammatory diseases all share many common features that suggest overlapping dependence on pathways that are subtly modified to influence the final outcome. However, whether this is true or not, the range of inflammatory diseases that can afflict the lung hints at a system of enormous complexity to support such a diversity of outcomes and processes. Thus, individual CKRAs may exhibit therapeutic selectivity, but may also have unexpected consequences on immune system function.

Inflammatory diseases are dependent upon multiple leukocyte types, from initiator cells (such as dendritic cells), effector cells (such as neutrophils), eosinophils and natural killer cells, regulatory cells (such as lymphocytes of various subtypes), to effector and "clear-up" cells (such as monocytes). All of these cells can be recruited individually, as part of carefully phased responses to tissue insults; for example, the recruitment of eosinophils to sites of allergic inflammation

is followed by later phases of T-cell accumulation [8, 9]. To accomplish this, separate chemokine and chemokine receptor pathways are required, often in combination with the selective control of other vital systems, such as specific adhesion molecule expression on the endothelium at the inflammatory site.

The basics of chemokine/chemokine receptor pathway organization are relatively clear (table 1). Chemokines are grouped by structure, which is dependent upon disulphide bonds between conserved cysteine residues. There are two major families, the CC and CXC, depending upon the organization of these cysteine residues. Naming of these molecules, previously descriptive (*e.g.* "eotaxin" which recruits eosinophils [12]) or, more occasionally, partly esoteric (*e.g.* regulated on activation of normal T-cell expressed and secreted (RANTES) [13]), has been rationalized, through numbering of CC and CXC chemokine ligands (*e.g.* eotaxin is now CC ligand 11,

Table 1.–Chemokines and their receptors

Systematic name	Previous name	Chemokine receptor(s)	Systematic name	Previous name	Chemokine receptor(s)
CXC chemokines			CC chemokines		
CXCL1	GRO-α/MGSA-α	CXCR2>CXCR1	(CCL9/10)	Unknown	Unknown
CXCL2	GRO-β/MGSA-β	CXCR2	CCL11	Eotaxin	CCR3
CXCL3	GRO-γ/MGSA-γ	CXCR2	(CCL12)	Unknown	CCR2 (mouse)
CXCL4	PF4	Unknown	CCL13	MCP-4	CCR2, CCR3
CXCL5	ENA-78	CXCR2	CCL14	HCC-1	CCR1
CXCL6	GCP-2	CXCR1, CXCR2	CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
CXCL7	NAP-2	CXCR2	CCL16	HCC-4/LEC	CCR1
CXCL8	IL-8	CXCR1, CXCR2	CCL17	TARC	CCR4
CXCL9	MIG	CXCR3	CCL18	DC-CK1/PARC	Unknown
CXCL10	IP-10	CXCR3		AMAC-1	
CXCL11	I-TAC	CXCR3	CCL19	MIP-3β/ELC/exodus-3	CCR7, CCR11
CXCL12	SDF-1α/β	CXCR4	CCL20	MIP-3α/LARC/exodus-1	CCR6
CXCL13	BLC/BCA-1	CXCR5	CCL21	6Ckine/SLC/exodus-2	CCR7, CCR11
CXCL14	BRAK/bolekine	Unknown	CCL22	MDC/STCP-1	CCR4
(CXCL15)	Unknown	Unknown	CCL23	MPIF-1	CCR1
CXCL16		CXCR6	CCL24	MPIF-2/Eotaxin-2	CCR3
CC chemokines			CCL25	TECK	CCR9, CCR11
CCL1	I-309	CCR8	CCL26	Eotaxin-3	CCR3
CCL2	MCP-1/MCAF	CCR2	CCL27	CTACK/ILC	CCR10
CCL3	MIP-1α/LD78α	CCR1, CCR5	CCL28	CCL28, MEC	CCR10, CCR3
CCL4	MIP-1β	CCR5	C chemokines		
CCL5	RANTES	CCR1, CCR3, CCR5	XCL1	Lymphotactin/SCM-1α/ATAC	XCR1
(CCL6)	Unknown	Unknown	XCL2	SCM-1β	XCR1
CCL7	MCP-3	CCR1, CCR2, CCR3	CXC chemokines		
			CX3CL1	Fractalkine/Neurotactin	CX3CR1
CCL8	MCP-2	CCR3			

Adapted from Zlotnik and Yoshie [10] and [11]. GRO: growth-related oncogene; MGSA: melanoma growth-stimulatory activity; PF4: platelet factor 4; ENA-78: epithelial-derived neutrophil attractant-78; GCP-2: granulocyte chemotactic protein-2; NAP-2: neutrophil activation protein-2; IL-8: interleukin-8; MIG: monokine induced by interferon-γ; IP-10: interferon-γ inducible protein-10; I-TAC: interferon-inducible T-cell alpha chemoattractant; SDF: stromal cell-derived factor; BLC/BCA-1: B-lymphocyte chemoattractant/B-cell attracting chemokine-1; BRAK: chemokine initially isolated from breast and kidney cells; I-309: human CC chemokine; MCP-1/MCAF: monocyte chemotactic protein/monocyte chemotactic and activating factor; MIP: macrophage inflammatory protein; RANTES: regulated upon activiation, normal T-cell expressed and secreted; HCC: haemofiltrate CC chemokine; Lkn-1: leukotactin-1; LEC: liver expressed chemokine; TARC: thymus- and activation-regulated chemokine; DC-CK1/PARC AMAC-1: dendritic cell-specific CC chemokine-1/pulmonary- and activation-regulated chemokine alternative macrophage activation-associated CC chemokine-1; ELC: Epstein Barr virus induced molecule 1 ligand chemokine; LARC: liver- and activation-regulated chemokine; SLC: secondary lymphoid-tissue chemokine; MDC/STCP-1: macrophage-derived chemokine/stimulated T-cell chemoattractant protein-1; MPIF: myeloid progenitor inhibitory factor; TECK: thymus-expressed chemokine; CTACK/ILC: interleukin-11 receptor alpha locus chemokine; MEC: mammary-enriched chemokine; SCM: single cysteine motif; ATAC: activation-induced, T-cell-derived and chemokine-related.

abbreviated to CCL11) [10]. The receptors to which they bind are also numbered; the receptor for eotaxin being CC chemokine receptor 3 (CCR3) [14, 15]. As a further illustration, the archetypal CXC chemokine and neutrophil chemoattractant, interleukin (IL)-8, has been redesignated CXCL8 and signals *via* receptors CXCR1 and CXCR2. Can researchers now start to fit these basic science pathways with clinical respiratory diseases?

Chemokines and asthma

All of the chemokine receptors discussed in this section are targets for drug development with the potential to modify disease. A fundamental mechanism of allergic asthma is the process of antigen sensitization, in which processed antigens are presented to naïve T-cells in lymph nodes. Dendritic cells are important professional antigen-presenting cells and there is evidence that immature dendritic cells may be recruited to tissues through the actions of chemokine CCL20 (macrophage inflammatory protein (MIP)-3α/liver- and activation-regulated chemokine) on CCR6. Once activated, they downregulate this receptor and in its place, express CCR7, whose ligand, CCL19 (MIP-3β), is expressed in lymph nodes [16–19], thus directing antigen-bearing, activated dendritic cells from skin/mucosal surfaces to lymphoid organs. CCL19 can also serve to recruit T-cells to lymph nodes [20], and may thus be responsible for colocalizing these cell types and facilitating the establishment of an antigen response. T-cell interactions with B-cells are then required to establish antigen-specific immunoglobulin formation (in the case of asthma, immunoglobulin-E), and the recruitment of B-cells to lymph nodes is also chemokine-regulated [21, 22].

In the course of an acute asthmatic attack, both neutrophils and eosinophils are recruited to the lung. The role of neutrophils in asthma is unclear, although some investigators have suggested that a predominantly neutrophilic recruitment pattern may be a feature of a subgroup of severe asthmatics [23]. Neutrophils express two main chemokine receptors, CXCR1 and CXCR2, with increasing evidence that CXCR2 is the major receptor involved in recruitment, whilst CXCR1 may have more of a role in neutrophil activation [2, 24, 25]. Eosinophils from most donors express only a single main chemokine receptor, CCR3, whose principal ligands are probably CCL11 (eotaxin) and CCL24 (eotaxin-2) [26]. Targeting of CXCR2 or CCR3 may therefore prevent the recruitment of potentially damaging granulocytes in asthma. Complicating this picture, however, are variations between donors in granulocyte chemokine receptor expression, for example, eosinophils from most subjects express CCR3 as the predominant receptor, but in some individuals, CCR1 may also have a role in eosinophil recruitment [26], and there are a few reports of other receptors on eosinophils and neutrophils of less clear significance [27–29].

In addition, other pathways, such as activated complement protein fragments and eicosanoid mediators, may be potent stimulators of granulocyte

recruitment in asthma [30], whilst the rumoured poor performance of other therapies targeting eosinophils (particularly anti-IL-5 drugs, although only one study on this has been published [31]) is reducing interest in CCR3 as an attractive drug target. This is probably an error, as it seems unlikely that eosinophils are simply a bystander in asthma inflammation, but it does suggest that targeting multiple cell types with CKRAs that block more than one chemokine receptor may be appropriate. Prototype drugs with these sorts of activities have already been described [3]. Also, as it is probably true that eosinophils evolved as part of our host defence to parasites, in the more developed world, in which parasitic diseases are less burdensome, therapies targeting eosinophil recruitment are perhaps less likely to have unwanted immunosuppressive side-effects than therapies targeting neutrophil recruitment.

However, if it is unconvincing that the eosinophil plays a large role in asthma, the blockade of T-cell recruitment may be of more interest. This area is still shrouded in some mystery. For example, if the objective is to interfere with T-cell function in asthma, which aspect needs to be manipulated? T-cells encounter antigen processed by cells such as the dendritic cell, usually in the environment of the draining lymph node [18]. This suggests that CKRAs that prevent T-cell (or dendritic cell) recruitment to the lymph node may need to be developed, preventing sensitization or restimulation, although this is not without potential risk, perhaps in the induction of nonspecific immune suppression. However, there is also increasing evidence that antigen presentation and B-cell aid can be initiated in the local lung microenvironment [32, 33]. Therefore, the recruitment of T-cells to the lung should perhaps be targeted and blocked. In both cases, the question of which sort of T-cell should be interfered with arises. There is ample evidence that asthma is largely dependent upon Th2-type T-cells, secreting cytokines such as IL-4, IL-5 and IL-13, which undoubtedly play a major role in asthma pathology [34]. There is also evidence that the Th1 cytokine, interferon-γ, is also produced in asthma [35–37], perhaps giving this Th2-dominated disease a potentially important Th1 component.

Whether antigen presentation occurs in the lung or lymph node, or both, it seems possible that there will be a nonselective recruitment of both Th1 and Th2 cells to the lung, with relative retention or activation of a Th2 group. Alternatively, there might be local generation of Th2-selective chemokines in allergic inflammation, resulting in the recruitment of Th2 cells only. Is there any evidence about which way around the system may work? T-cells can be divided *in vitro* into Th1 and Th2 type cells, which express selective patterns of chemokine receptors (CXCR3 and CCR5 for Th1 cells, CCR3, CCR4 and CCR8 for Th2 cells [38–40]). This suggests that chemokines may recruit specific subtypes of Th cells, but, *in vivo*, there is little evidence for CCR3 expression at sites of allergic inflammation and CCR4 has now been shown convincingly to be associated with both Th1 and Th2 cells outside the situations of very highly polarized cells *in vitro*. Other receptors may be relatively nonselective

for Th1 *versus* Th2 (*e.g.* CXCR4, CCR7, CXCR6, CCR1), favouring the hypothesis that lymphocyte recruitment is relatively unfocused, with the key issue being the retention of certain subsets in the tissue. Some animal models favour a role for nonspecific recruitment of T-cells, *e.g.* driven by CXCR4 [41], but in other models, antagonism of CCR3 and CCR4, CCR8, or neutralization of their ligands can modulate airways hyperreactivity [42–44]. CCR4 has been shown recently to have role in T-cell recruitment in asthma [45]. Therefore, the best current idea might be that sites of inflammation exhibit a chemokine bias, which favours, but does not absolutely specify, recruitment of certain subsets of T-cells. The chemokines themselves may also have other roles, *e.g.* potential enhancement of T-cell cytokine secretion within tissue [46]. Thus, selective CKRAs are likely to be useful in specific diseases, but definite prediction of the outcomes of early trials is difficult.

Chemokines and innate immunity

There is currently a resurgence of interest in how the innate immune system functions, that incredibly "old" evolutionary system that is inherited from bacteria, flies and plants. Innate immunity provides a rapid response to invasive stimuli (*e.g.* bacteria) mediated through pattern-recognition receptors such as the toll-like receptors (TLRs). These TLRs (there are currently ten described in the human genome) are the critical signalling proteins for bacterial lipopoly-saccharides (LPS; TLR4 [47, 48]), bacterial LPS/lipoproteins (TLR2 [49, 50]) and bacterial DNA (TLR9 [51]). Other members of the same receptor superfamily are also involved in the regulation of Th1/2 T-cell function [52]. Stimulation of TLR signalling pathways in tissues results in the rapid generation of an inflammatory response and the production of pro-inflammatory cytokines, such as IL-1, tumour necrosis factor-α and the chemokines. Leukocytes also express TLRs [53], which be involved in the regulation of their function. Signalling by LPS, perhaps predominantly mediated *via* TLR4, causes increased lifespan of neutrophils, possible upregulation of basophil histamine release, alterations in the expression of chemokine receptors on the surface of neutrophils and monocytes (which may modify processes of allergen sensitization), and chemokine generation from multiple cell types, including leukocytes and endothelial cells. Endotoxin responses may play an important role in the establishment of allergen sensitization in asthma, with variations in the LPS coreceptor CD14 having functional significance in the development of atopy (together with the current hygiene hypothesis theories) [54], and there is preliminary animal evidence that defects in TLR4 signalling can modify sensitization [55].

Bacterial CpG motifs in DNA, signalling *via* TLR9, can profoundly influence patterns of allergen sensitization, allergic disease, and mononuclear cell proliferation and survival [56, 57]. The sensitivity of effector cells, such as neutrophils and monocytes, to TLR agonists, suggests that these ancient pathways may be of considerable importance in the regulation of acute pulmonary inflammation. There is also growing evidence that TLR expression is regulated at sites of noninfective inflammation, such as in the myocardium [58]. Interactions between TLRs and chemokine receptors may prove to be a fascinating and fruitful field in the study of inflammation in the lung.

These new developments in chemokine and toll-like receptor science are creating new targets that will undoubtedly be of importance in the treatment of human disease, through the control of leukocyte recruitment and activation. Ultimately, it will be hard to predict the consequences of individual receptor antagonists until they are tried in human disease, but it is highly likely that they will be important additions to the current pharmacological armamentarium.

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