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The Role of Interleukin-8 and its Receptors in Inflammatory Lung Disease

Implications for Therapy

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Abstract

Neutrophils have been implicated in the pathogenesis of many inflammatory lung diseases, including the acute respiratory distress syndrome, chronic obstructive pulmonary disease and asthma. The CXC chemokine interleukin (IL)-8, is a potent neutrophil recruiting and activating factor and the detection of IL-8 in clinical samples from patients with these diseases has led clinicians to believe that antagonism of IL-8 may be a practicable therapeutic strategy for disease management.

Work over the last decade has concentrated on both the molecular mechanisms by which IL-8 is produced in the inflammatory setting and also on the manner in which IL-8 activates the neutrophil. Expression of the IL-8 gene appears to be controlled by several components of the inflammatory milieu. Whilst lipopolysaccharide, IL-1β and tumor necrosis factor-α are capable of augmenting IL-8 production, IL-10 is a potent inhibitor of IL-8 synthesis and appears to play an auto-regulatory role. Regulation of the IL-8 gene is under the control of nuclear factor κB which appears to be a primary target for corticosteroid-mediated repression of IL-8 production.

IL-8 exerts is effects on neutrophils by binding with high affinity to two receptors on its cell surface, the chemokine receptors CXCR1 and CXCR2. These closely related receptors belong to the superfamily of G-protein coupled receptors, proteins that historically have proved amenable to antagonism by small molecules. The recent descriptions in the literature of highly potent small molecule antagonists of CXCR2 and their success in blocking *in vivo* trafficking of neutrophils suggest that antagonsim of IL-8 at the receptor level is a viable therapeutic strategy. Clinical trials of such compounds will ultimately provide crucial information currently lacking and will define whether or not IL-8 blockade provides future therapy in pulmonary disease.

1. Chemokines

The discovery of chemokines and their corresponding receptors on the surface of leukocytes, has paved the way for an understanding of the mechanisms by which immune system cells are recruited to an inflammatory site. Chemokines are a structurally related family of more than 50 small basic proteins which act on distinct subsets of leukocytes via specific G-protein coupled receptors (GPCRs) and govern multiple aspects of host defense and inflammation, such as leukocyte trafficking, hematopoiesis, and angiogenesis.[1,2] The chemokine family can be conveniently divided into two major subsets by examination of their four conserved cysteine residues. The CXC or α class have a single amino acid interspersed between the amino-terminal two cysteines and are mainly active on neutrophils and T-lymphocytes, whilst the CC or β class has adjacent amino terminal cysteines and are active on monocytes, basophils, eosinophils and lymphocytes but do not target neutrophils. Two minor subsets also exist, the CX3C and C chemokines, which possess only single members, namely fractalkine and lymphotactin.[3,4] Fractalkine contains three amino acids between the first amino-terminal two cysteines, whilst lymphotactin contain only two cysteines, corresponding to the first and third cysteines in the other three groups. All the chemokines share a common protein fold, the Greek key motif, composed of three anti-parallel β-pleated sheets overlaid by a carboxy terminal α helix (figure 1).

2. Interleukin (IL)-8 and its Receptors, CXCR1 and CXCR2

Interleukin (IL)-8 is a secretory product of stimulated macrophages and other cells and was the first CXC chemokine to be

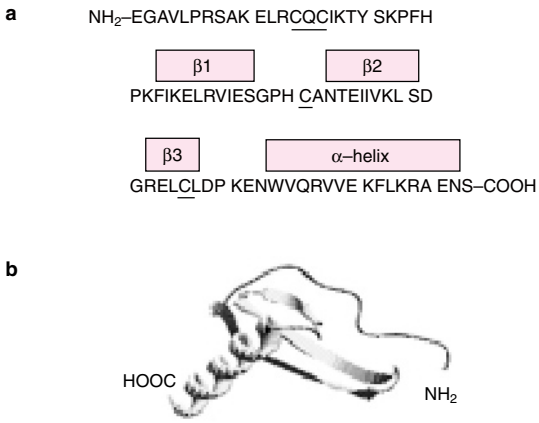

Fig. 1. (**a**) represents the primary sequence of the mature interleukin (IL)-8 protein. Conserved cysteine residues are underlined. The location of the first, second and third β-pleated sheets and the α helix within the sequence are indicated overhead. (**b**) illustrates the solution structure of monomeric IL-8 as deduced by Clore et al.[5] and was produced by the package SwissPdb Viewer v3.51[6] (URL: http://www.expasy.ch/spdbv/) using the file PDB ID: 1IL8 deposited in the protein data bank[7] (URL: http://www.rcsb.org/pdb/).

discovered and characterized.[8-10] It is a potent chemoattractant for neutrophils both *in vitro* and *in vivo* and comprises part of the inflammatory exudate induced by microbial infection *in vivo*.[11,12] Truncation of the IL-8 amino-terminus region results in a receptor antagonist, suggesting that this portion of the chemokine is crucial for activation of the receptor.[13] IL-8 is produced by numerous cell types including monocytes, neutrophils, basophils, eosinophils,[14,15] fibroblasts,[16] alveolar macrophages,[17] bronchial epithelial cells,[18] pulmonary microvascular endothelial cells[19] and airway smooth muscle cells.[20] Its expression by monocytes is up-regulated by lipopolysaccharide (LPS), IL-1β, or tumour necrosis factor (TNF)-α[21] and is inhibited by IL-4[22] and IL-10.[23] Additionally, recent reports have demonstrated that complement (C)3a and IL-18 can also induce IL-8 expression by epithelial cells[24] and eosinophils, respectively.[25]

The glucocorticoid dexamethasone can suppress IL-8 gene expression in many cell types including airway smooth muscle,[20] cultured airway epithelial cells[26] and eosinophils.[27] IL-8 production is controlled at the activation step of the transcription factors nuclear factor (NF)-κB and intranuclear activator protein (AP)-1,[28] the former of which is the target for dexamethasone induced suppression of IL-8 gene expression.[29]

IL-8 exerts its effects on neutrophils via two different cell surface receptors initially named as IL-8 receptors type A[30] and B[31] and now known as CXCR1 and CXCR2, respectively.[2] CXCR1 and CXCR2 were the first chemokine receptors to be identified and are the major chemokine receptors on neutrophils, sharing 77% identify at the amino acid level and differing significantly only in their amino and carboxy-terminal regions. Whilst both receptors can bind IL-8 with high affinity, they are also receptors for other CXC chemokines. CXCR2 is relatively promiscuous and in addition to IL-8 can also bind the CXC chemokines growth-related oncogene-α, neutrophil activating peptide-2, epithelial cell-derived neutrophil activating peptide-78 and granulocyte chemotactic protein (GCP)-2 (figure 2). In contrast, CXCR1 binds only IL-8 and GCP-2.[32] CXCR1 and CXCR2 have also been reported to be present on the surface of microvascular endothelial cells[33] and activation of CXCR2 has been shown to induce cytoskeletal reorganization and retraction of endothelial cells which may contribute to the increased vascular permeability observed in acute inflammation.[34]

In terms of chemotaxis, there appears to be a division of labor amongst the receptors. CXCR2 responds to picomolar concentrations of IL-8 and is thought to initiate the migration of neutrophils distant from the site of inflammation, whilst CXCR1 mediates a signal in response to higher concentrations of IL-8 such as those encountered at the inflammatory site, presumably those involved in activation of the neutrophil.[35] IL-8 engagement of either CXCR1 or CXCR2 on neutrophils results in coupling to signals such as intracellular calcium flux, and phosphatidylinositol-3-kinase activation. Cross-linking of CD28 induces CXCR1 expression and increases migration of neutrophils in response to IL-8[36] whilst CD45 signaling down modulates both CXCR1 and CXCR2[37] suggesting a role for this co-stimulatory molecule in modulating IL-8 receptor expression.

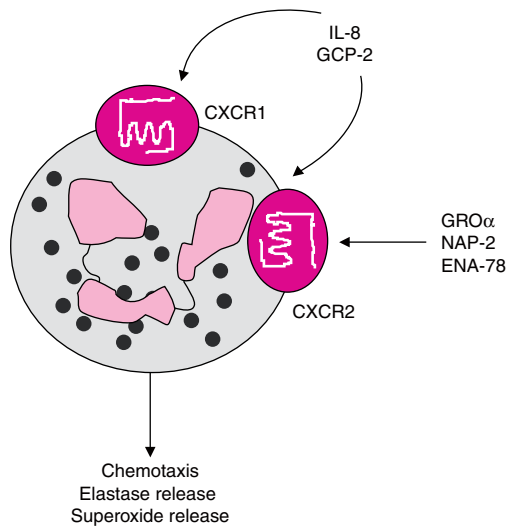

Fig. 2. The promiscuity of CXCR2 compared with CXCR1. Both receptors couple to intracellular pathways leading to activation of the neutrophil and a resultant microbicidal response. **ENA-78 =** epithelial cell-derived neutrophil activating peptide-78; **GCP-2** = granulocyte chemotactic protein-2; **GROα** = growth-related oncogene-α; **IL-8** = interleukin-8.

3. The Role of IL-8 in Asthma

Although most often thought of as a disease dependent upon eosinophils and T lymphocytes, there is ample evidence for the recruitment of neutrophils into the lungs of patients with acute asthma. In patients with severe asthma, there may be a separate subgroup who show pronounced neutrophilic but not eosinophilic inflammation[38] and some post mortem studies of acute asthma deaths have linked these to a marked neutrophilic infiltrate. The history of IL-8 and asthma is relatively long in such a new field as chemokine biology. An initial link between neutrophils and the pathogenesis of asthma arose with the discovery by Atkins and colleagues[39] in 1977 of a neutrophil chemotactic activity present in the plasma and associated with an asthmatic reaction, following a ragweed-inhalation challenge of susceptible individuals. This activity was never completely purified, so its identity remains unknown. A later study of exercise-induced late asthmatic reactions resulted in the observation of enhanced neutrophil chemotactic activity, accompanied by reductions in forced expiratory volume in 1 sec (FEV_1) after treadmill exercise.[40] Following the identification of IL-8, and the development of suitable reagents, IL-8 was subsequently identified as an inflammatory factor implicated in the pathogenesis of asthma. In support of this, IL-8 can be detected in the sera of patients with severe atopic asthma and the levels correlate with disease activity[41,42] raising the possibility of a role for the neutrophil in patients with severe persistent asthma. Since asthma that is characterized by non-eosinophilic airway inflammation is generally less responsive to corticosteroid treatment[43] it may be that treatment of such individuals with a regimen directed at the activity of IL-8 proves beneficial. The use of serum levels of IL-8 as a marker for asthma remains controversial as others have argued that it is a poor indicator of disease activity.[44]

IL-8 levels are also raised in the tracheal aspirates of patients with acute severe asthma, where they correlate with high levels of neutrophils.[45] Analysis of induced sputum from patients with persistent asthma also identified a pattern of non-eosinophilic inflammation which is associated with a neutrophil influx and activation thought to be mediated by IL-8 secretion.[46] Transient increases in IL-8 levels appear to precede the exacerbation of acute asthma in such attacks.[47] The glucocorticoid budesonide, has recently been shown to reduce the circulating levels of IL-8, which may be a further contributory mechanism by which these drugs are effective in the therapy of asthma.[48]

IL-8 is expressed by both the bronchial epithelial cells[49] and the peripheral blood eosinophils of patients with asthma.[50] Although peripheral blood eosinophils express IL-8, it appears that these cells do not release IL-8 into the circulation, although they do release it into the lung.[51] The eosinophil granule protein, major basic protein (MBP), may serve to stimulate IL-8 production either in an autocrine or hormonal fashion, acting on the eosinophil itself[52] or the neutrophil.[53] Of interest are the findings that IL-8 does not induce chemotaxis of eosinophils from healthy volunteers, but is chemotactic for eosinophils purified from patients with either a blood eosinophilia,[54] birch pollen allergy[55] or patients with asthma who have undergone bronchoprovocation with a relevant allergen.[56] Collectively, the findings are suggestive of an eosinophilia-associated priming mechanism which may induce CXCR1/2 expression on eosinophils, although we showed that in normal and atopic donors, IL-8 was ineffective at inducing eosinophil shape change, which is generally thought to be a pre-requisite for chemotaxis.[57] Whilst some reports have found no CXCR1 or CXCR2 on eosinophils from normal volunteers either by reverse transcription polymerase chain reaction or flow cytometry,[58] we were able to show low levels of non-functional CXCR2 on eosinophils.[57] Additionally, Bonecchi et al.[59] recently showed that T helper 2 cytokines such as IL-4 and IL-13 could induce the expression of functional CXCR1 and CXCR2 on human monocytes, macrophages, and dendritic cells, so although IL-8 has a principal role in the regulation of neutrophil recruitment, within the context of asthma it may also regulate the recruitment of other cell types. There is also an appreciation that the IL-8 signal might originate from multiple etiologies involved in acute and chronic asthma. For example, viral infections such as respiratory syncytial virus can trigger asthma exacerbations and induce IL-8 generation.[60,61] Additionally, endotoxin, which is a component of environmental dust and a major stimulus of NF-κB driven IL-8 generation, may regulate some of the responses perceived to be due to environmental allergen. Michel et al.[62] showed that asthma severity correlated better with endotoxin levels in house dust than with levels of house dust mite allergen.

4. The Role of IL-8 in Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is associated with chronic inflammation of both the airways and lung parenchyma. In comparison with asthma, less is known about the inflammatory response during acute COPD exacerbation. Cigarette smoking is the most important etiological factor in the development of COPD and is associated with increased amounts of airway IL-8.[63] Although the monocyte may have a significant role in COPD, neutrophils are found in high numbers in lung tissue and sputum in COPD[64] and it is believed that activation of these cells, resulting in the release of activated oxygen species and proteases, such as elastase, may be important in lung damage

and chronic dysfunction. IL-8 has been detected by enzyme-linked immunosorbent assay in lung tissue and in the circulation of patients with COPD[65,66] and elevated levels of IL-8 are observed in sputum obtained from patients with COPD during disease exacerbation.[67] The best use of corticosteroids in the treatment of COPD remains a subject of much debate.[68] In contrast to *in vitro* findings, a recent report suggests that *in vivo*, short-term corticosteroid treatment does not reduce sputum levels of IL-8 in patients with chronic bronchitis.[69] Further clinical studies on the effects of corticosteroid on IL-8 production in COPD patients would be timely.

5. IL-8 in the Adult Respiratory Distress Syndrome

The adult respiratory distress syndrome (ARDS) comprises a neutrophilic response to acute lung injury resulting in severe pulmonary inflammation associated with a very high mortality (40 to 60%).[70,71] ARDS is rare in the neutropenic patient, and experimental data confirm that the neutrophil plays a central role in the development of this condition.[72] Hopes were high in the early 1990s of improving outcomes in ARDS by anti-TNF treatments but the results of clinical trials have been disappointing.[73-75] Suggestive of a role for IL-8 in the pathology of ARDS, is the reported correlation between the level of circulatory IL-8 and clinical outcome in ARDS patients.[76] In keeping with this, amelioration of lung injury in a rabbit model of lung ischemia/reperfusion injury was achieved using anti–IL-8,[77] and neutrophils have been shown to play a role in other animal models of lung injury.[78,79] Furthermore, a bronchoscopic study of patients at risk of developing ARDS found a significant correlation between IL-8 levels in the bronchoalveolar lavage fluid and the likelihood of subsequent disease.[72,80] A significant portion of IL-8 in lung fluids from patients with ARDS is associated with either anti–IL-8 auto-antibodies or α2-macroglobulin which may act as a carrier, protecting it from proteolysis and thereby enhancing its activity.[81,82] Studies from the same group using a rabbit model of ARDS have shown that whilst IL-8 complexed with α2-macroglobulin retained chemotactic activity *in vitro*, its ability to induce an influx of neutrophils into the rabbit lung *in vivo* was impaired.[83] Thus, conjugation of IL-8 *in vitro* may afford a clearance mechanism to remove IL-8 from the inflamed lung.

6. The Development of IL-8 Receptor Antagonists

The observations outlined above provide the basis for the development of low molecular weight compounds aimed at selectively preventing the recruitment of neutrophils during inflammation of the lung by the antagonism of the specific IL-8 receptors CXCR1 and CXCR2. This pursuit is facilitated considerably

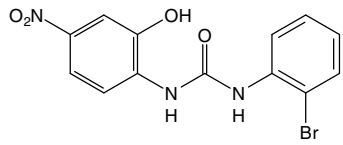

Fig. 3. The chemical structure of the potent small molecule CXCR2 antagonist SB 225002 as described by White and colleagues.[84]

by the vast library of compounds that are already available to the pharmaceutical industry following years of GPCR study. Indeed, such compounds either, agonists or antagonists, provide a large proportion of drugs currently in use. For example, antagonists of the α and β adrenergic receptors are used in the treatment of angina, arrhythmias, and hypertension, β-agonists are a vital component in the management of asthma, whilst histamine H_1 and H_2 receptor antagonists are used in the treatment of allergic reactions and gastric ulceration. In terms of CXCR1/2 antagonism, progress has been hampered by the fact that although orthologues of IL-8 have been isolated from nine species, the gene appears to absent in the rat and mouse.[84] Similarly, whilst both CXCR1 and CXCR2 orthologues have been found in several species including the rat, no murine CXCR1 orthologue has been forthcoming, hindering the development of *in vivo* models.

Despite these obstacles, the first published low molecular weight compound to be developed against a chemokine receptor was a CXCR2 antagonist.[85] This compound, SB 225002 (figure 3) is a potent and selective antagonist that blocks IL-8 binding to CXCR2 (IC_{50} 22nmol/L) and possesses over 150-fold selectivity for CXCR2 over CXCR1. Whilst the majority of small antagonist programs have been blighted with the inability of compounds to cross the species barrier, thus preventing *in vivo* testing in relevant disease models, this is not the case with SB 225002. The compound is a potent inhibitor of IL-8 mediated rabbit neutrophil chemotaxis and *in vivo* in the same species, selectively blocks IL-8–induced neutrophil margination. It is feasible that such a CXCR2 antagonist could be developed to target the many actions of IL-8 and related chemokines acting upon CXCR2. However, such effects would have to be monitored carefully as neutrophils are essential for host defense against microbial pathogens, and undesired immunosuppression is surely the most worrying potential adverse effect of administration of these compounds to humans.

7. Conclusion

Over the last two decades, a combination of clinical observations in both *in vitro* cell biology and *in vivo* animal modeling has highlighted potentially important mechanisms underlying the

associated lung inflammation in a variety of pulmonary diseases including asthma, COPD and ARDS. These extensive studies have generated novel targets for the development of future therapeutic compounds. It is now clear that chemokines such as IL-8 play a fundamental role in regulating leukocyte trafficking in these diseases. Early studies with a CXCR2 specific antagonist provide the proof of principle that IL-8 receptors can be blocked both *in vitro* and *in vivo* with a low molecular weight antagonist. Clinical trials with this compound will provide the crucial information that will define whether or not IL-8 blockade will provide future therapy in pulmonary disease.

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