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EDITORIAL

Chemokines are important cytokines in the pathogenesis of interstitial lung disease

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Interstitial lung disease (ILD) is characterized by alveolar and interstitial inflammation, followed by the progressive derangement of alveolar architecture and deposition of extracellular matrix components. The disease is initiated by an unknown alveolar insult, which is followed by the influx of inflammatory cells to the lung. This recruitment phase results in a several fold increase in all cell types within the airspace, including neutrophils, eosinophils, lymphocytes and macrophages. In addition, substantial interstitial inflammation is apparent, consisting predominantly of newly recruited monocytes and lymphocytes. Alveolar and interstitial inflammation is followed by the loss of type I epithelial cells and endothelial cells, proliferation of type II cells, and expansion of the pulmonary interstitium *via* the proliferation of fibroblasts and deposition of collagen and other extracellular matrix components [1, 2].

Alveolar and interstitial inflammation in ILD is essential to the development of lung injury and subsequent fibrosis. Neutrophils and neutrophil products have been identified in increased amounts in the airspace of patients with ILD and in animal models of lung fibrosis [3]. These cells induce parenchymal injury by producing toxic radical oxygen species, and by secreting a variety of proteolytic enzymes, including elastase, collagenase, and other neutral proteases. Recruited monocytes contribute to pulmonary inflammation by elaboration of oxygen radical species, proteolytic enzymes, and factors that attract additional inflammatory cells. T-cells and B-cells, like monocytes, are present in increased numbers in the lung interstitium in ILD [4], and the recruitment of lymphocytes to the lung has been shown to precede the development of pulmonary fibrosis in models of lung fibrosis [5]. Furthermore, the development of pulmonary fibrosis in mice can be attenuated or completely abrogated by the selective depletion of CD4+ T-cells, CD8+ T-cells, or both [6]. Activated T-cells produce a number of cytokines that can modulate lung inflammation and fibrosis, including interleukin (IL)-2, IL-4, interferon-gamma (IFN-γ), and chemotactic factors both for leucocytes and fibroblasts. B-cells also contribute to the development of cellular activation and fibrosis in ILD *via* the overzealous secretion of immunoglobulin, leading to immune complex formation and deposition.

The immunopathological expression of ILD involves complex and dynamic interplay between immune effector cells, including neutrophils, macrophages and lymphocytes, and cellular constituents of the alveolar-capillary membrane, most importantly lung fibroblasts. The interaction of these lung cells and the mediators that they produce culminates in parenchymal cell injury, collagen deposition, and, ultimately, end-stage fibrosis. While insults that trigger the development of various forms of ILD differ, cellular mechanisms by which inflammatory leucocytes are recruited to the lung in ILD have not been fully characterized. A number of factors that possess leucocyte chemotactic activity have been identified in the lung of patients with ILD, including platelet-derived growth factor (PDGF) [7, 8], fibronectin [9], and transforming growth factor-beta (TGF-β) [10]. The alveolar macrophage is almost certainly involved in recruitment of inflammatory cells, as this cell produces a variety of specific and nonspecific leucocyte chemoattractants. Alveolar macrophages isolated from patients with ILD have been shown to spontaneously express several leucocyte chemotactic factors, including complement factor 5a (C5a) and leukotriene B_4 (LTB_4). In addition, interstitial and alveolar macrophages from patients with ILD constitutively express macrophage inflammatory protein-1alpha (MIP-1α) and monocyte chemoattractant protein-1 (MCP-1), and cells other than lung macrophages contribute to the production of these leucocyte chemotaxins [11–13]. The latter leucocyte chemoattractants belong to the supergene families of chemotactic and activating cytokines, which include CXC, CC and C chemokines [14–28]. These chemokines may be critically involved in the migration and activation of leucocytes in ILD.

The human CXC, CC and C chemokine families of chemotactic cytokines are three closely related polypeptide families that behave, in general, as potent chemotactic factors either for neutrophils, mononuclear cells or lymphocytes, respectively [14–28]. These cytokines, in their monomeric form, range from 7 to 10 kDa and are characteristically basic heparin-binding proteins. The chemokines display highly conserved cysteine amino acid residues: the CXC chemokine family has the first two NH_2-terminal cysteines separated by one nonconserved amino acid residue, the CXC cysteine motif; the CC chemokine family has the first two NH_2-terminal cysteines in juxtaposition, the CC cysteine motif; and the C chemokine has one lone NH_2-terminal cysteine amino acid, the C cysteine motif (table 1). CXC chemokines are clustered on human chromosome 4, and exhibit 20– 50% homology at the amino acid level. CC chemokines are clustered on human chromosome 17, and exhibit 28– 45% homology at the amino acid level. The one

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Table 1. – The CXC, CC and C chemokine families of chemotactic cytokines

CXC chemokines
Interleukin-8 (IL-8)
Epithelial neutrophil-activating protein-78 (ENA-78)
Growth-related oncogene-alpha (GRO-α)
Growth-related oncogene-beta (GRO-β)
Growth-related oncogene-gamma (GRO-γ)
Granulocyte chemotactic protein-2 (GCP-2)
Platelet basic protein (PBP)
Connective tissue activating protein-III (CTAP-III)
Beta-thromboglobulin (β-TG)
Neutrophil-activating protein-2 (NAP-2)
Platelet factor-4 (PF4)
Interferon-γ-inducible protein (IP-10)
Monokine induced by interferon-γ (MIG)
Stromal cell-derived factor-1 (SDF-1)
CC chemokines
Monocyte chemotactic protein-1 (MCP-1)
Monocyte chemotactic protein-2 (MCP-2)
Monocyte chemotactic protein-3 (MCP-3)
Monocyte chemotactic protein-4 (MCP-4)
Macrophage inflammatory protein-1alpha (MIP-1α)
Macrophage inflammatory protein-1beta (MIP-1β)
Regulated on activation normal T-cell expressed and secreted (RANTES)
Eotaxin
I-309
C chemokine
Lymphotactin

C chemokine, lymphotactin, is located on human chromosome 1 [14–28]. There is approximately 20–40% homology between the members of the three chemokine families [14–28]. For the purpose of this discussion, we will focus on the CC chemokines.

The genes for CC chemokines are clustered on human chromosome 17 (q11.2-q12) [14–28]. In general, the CC chemokine genes have three exons and two introns. The first and second introns of all the genes of this chemokine family are highly conserved [14–28]. The first, second and third exons of these genes encode the 5'-UTR and the signal peptide sequence, the NH_2-terminal half of the peptide, and the COOH-terminal half of the peptide and the 3'UTR, respectively [14–28]. The splice junctions between the second and third exons in all CC chemokine genes occur at precisely the same position [21], suggesting that the CXC and CC chemokine superfamily may have diverged from a common ancestral gene [14–28]. The 5'-flanking region of CC chemokines, with the exception of I-309, contain the usual "CCAAT" and "TATA" box-like structures [29, 30]. The 5'-flanking regions of CC chemokines contains a number of potential binding sites for several nuclear factors, such as NF-κB and NF-IL-6/C/EBPβ [29, 30]. In addition, the regulated on activation normal T-cell expressed and secreted (RANTES) promotor contains a transcriptional element similar to IL-2, that may be important for signalling through the cell surface accessory molecule, CD28 on T-cells [29].

The CC chemokine family genes encode complementary deoxyribonucleic acids (cDNAs) that are characterized by a short 5'-UTR, an ORF that encodes both the mature polypeptide and the amino terminal signal sequence, and a long 3'-UTR that contains variable numbers of "AUUUA" motifs [14–28]. The CC chemokines have been found to be produced by an array of cells, including monocytes, alveolar macrophages, neutrophils, platelets, eosinophils, mast cells, T-cells, B-cells, natural killer (NK) cells, keratinocytes, mesangial cells, epithelial cells, hepatocytes, fibroblasts, smooth muscle cells, mesothelial cells and endothelial cells [14–28]. These cells can produce CC chemokines in response to a variety of factors, including viruses, bacterial products, IL-1, tumour necrosis factor (TNF), C5a, LTB_4 and IFNs [14–28]. The production of CC chemokines both by immune and nonimmune cells supports the contention that these cytokines may play a pivotal role in orchestrating chronic inflammation in the lung during the pathogenesis of ILD.

In this issue of the Journal, PETREK *et al*. [31] provide evidence that the CC chemokine, RANTES, is present in bronchoalveolar lavage (BAL) cells from patients with sarcoidosis, cryptogenic fibrosing alveolitis (CFA), and fibrosing alveolitis associated with systemic sclerosis (FASSc). The expression of RANTES was determined by immunohistochemistry of protein, reverse transcriptase polymerase chain reaction (RT-PCR) and *in situ* hybridization of messenger ribonucleic acid (mRNA) from the BAL cells of the subjects. RANTES protein was found to be immunolocalized to 23.5, 12.5 and 7% of sarcoidosis, FA (combined CFA and FASSc groups), and control BAL cells, respectively. However, the percentages of immunopositive RANTES in BAL cells from control and FA groups were not statistically significant. In the sarcoidosis group, the alveolar macrophage appeared to be the major cellular source of RANTES. In contrast, in the FA group, both alveolar macrophages and eosinophils were found to be cellular sources of this chemokine. In the control group, the alveolar macrophage was found to be the only cellular source of RANTES. Interestingly, T-cells were not found to significantly express RANTES. The protein results for RANTES from the three groups were paralleled by the presence of mRNA, as determined either by RT-PCR or *in situ* hybridization. In subsequent studies, these investigators correlated the presence of RANTES mRNA to the presence of total and CD45RO+ (memory) subpopulations of T-cells, and found a positive correlation of total and CD45RO+ T-cells in the BALs that contained more than 13% lymphocytes. However, only a minor proportion of the total T-cells were CD45RA+ (naive). No correlation was found for the presence of neutrophils or eosinophils with the expression of RANTES mRNA in the BAL cells from these groups. While this study was limited to qualitative and nonfunctional analysis of RANTES from BAL, taken collectively, these results suggest that RANTES expression in sarcoidosis, as compared to the groups with FA or controls, may be associated with the accumulation of total and CD45RO+ T-cells in the bronchoalveolar compartment.

RANTES is a CC chemokine that was initially cloned from T-cells by subtractive hybridization, and was thought to be a T-cell specific sequence [32]. Recently, a number of cells have been found to express RANTES, including platelets, monocytes, B-cells, NK cells, epithelial cells, fibroblasts and endothelial cells [23]. While initially described to have selective ability to induce chemotaxis of monocytes and memory CD45RO+ T-cells [33], TAUB

et al. [34] have demonstrated that RANTES is not selective for CD45RO+ T-cells, and can induce chemotaxis of both CD45RO+ and CD45RA+ T-cells *in vitro*. Moreover, a number of studies have clearly demonstrated that RANTES is not a selective chemoattractant for T-cells, and that it has the ability *in vitro* to induce the chemotaxis of other leucocytes, including basophils, eosinophils, mast cells, dendritic cells, monocytes and B-cells [23].

Moreover, RANTES is not the only CC chemokine with the ability to recruit T-cells; the majority of CC chemokines have the ability to recruit these cells, including MCP-1 to MCP-4, MIP-1α, MIP-1β and I-309 [23]. While RANTES can induce migration of eosinophils, other CC chemokines have been found to be chemoattractants for eosinophils, including eotaxin, MIP-1α, MCP-3 and MCP-4 [23, 35]. This suggests a tremendous redundancy in the ability of CC chemokines to induce chemotaxis of T-cells or eosinophils. Furthermore, the discovery of at least five different CC chemokine receptors (CCR1–CCR5) [35], of which RANTES may potentially interact with four [35], suggests that the redundancy of CC chemokine-induced chemotaxis is now more complex; depending not only on the production of the ligand, but also on the pattern of expression and cellular sources of specific CCRs.

Thus, the study by PETREK *et al*. [31] provides additional important information on the role of chemokines in the recruitment of leucocytes in ILD, and underlines the need to further understand the biology of these cytokines and their receptors in models of pulmonary fibrosis. This strategy can then be used for translational and interventional approaches, to alter the pathogenesis of these often lethal pulmonary diseases.

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