CCL18/DC-CK-1/PARC up-regulation in hypersensitivity pneumonitis

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Abstract: Hypersensitivity pneumonitis (HP) is a lung inflammatory disorder characterized by accumulation of T lymphocytes. However, the mechanisms implicated in this process remain undefined. We examined the expression of dendritic cell (DC)-derived CC chemokine 1 (CK1)/CCL18, a chemokine putatively involved in naive T cell recruitment, in lungs from 10 patients with HP, 9 patients with idiopathic pulmonary fibrosis (IPF), and 20 healthy lungs. CCL18 was measured by real-time quantitative PCR and localized in lungs by in situ hybridization and immunohistochemistry. CCL18 expression was significantly increased in lungs affected by HP in comparison with lungs affected by IPF (2,085±393 vs. 1,023±110; $P<0.05$) and controls (2,085±393 vs. 467±94; $P<0.01$). Macrophages, DCs, and alveolar epithelial cells were the main sources of CCL18. There was a direct correlation between the levels of tissue CCL18 and the number of lymphocytes in the bronchoalveolar lavage fluids. High levels of CCL18 were detected in the subacute rather than the chronic phase of HP. These findings suggest a role for CCL18 in the pathogenesis of HP. *J. Leukoc. Biol.* 70: 610–616; 2001.

Key Words: *chemokines · T lymphocytes · cell trafficking · allergic alveolitis*

INTRODUCTION

Hypersensitivity pneumonitis (HP) comprises a group of diffuse inflammatory disorders of the lung parenchyma provoked by exposure to a variety of organic particles and characterized by lymphocytic alveolitis [1]. One of the most frequent forms of HP is so-called pigeon breeder's disease, which is induced by the inhalation of avian antigens [2]. In general, HP is a serious lung disorder, which evolves into a diffuse, usually fatal fibrotic disorder in ~30% of patients with subacute/chronic disease [2, 3]. Although significant progress has been made recently in our understanding of the pathology of HP [3–6], the mechanisms responsible for lymphocyte recruitment in this ailment remain to be elucidated.

The chemokines are a superfamily of small, secreted proteins that regulate leukocyte migration. Many new members of this superfamily have been described in the last few years [7], and several of them have been associated with various diseases [8, 9]. Some chemokines have been reported to be expressed in the lung, including interleukin (IL)-8/CXCL8, eotaxin/CCL11, macrophage-inflammatory protein (MIP)-3α/CCL20, and DC-CK1/pulmonary- and activation-related chemokine (PARC)/alternative macrophage activation-associated CC-chemokine (AMAC)-1/CCL18 [9]. It is interesting that there is even one reported chemokine that is specifically expressed in the lung, lungkine/CXCL15 [10]. For this report, we used the new chemokine nomenclature recently proposed [7].

Because HP is characterized by a strong accumulation of lymphocytes in the lung parenchyma, we hypothesized that some chemokines play a critical role in this disease. To test this hypothesis, we performed a comprehensive analysis of the expression of chemokines and their receptors in HP-affected lungs using real-time PCR via TaqMan [9], and the results were compared with those obtained from lungs affected by idiopathic pulmonary fibrosis (IPF), a prototype of a chronic interstitial lung disease [11], and control lungs. Our findings indicate that one chemokine in particular, CCL18, is associated with the development of HP. CCL18 was originally reported as a DC product [12], but it has subsequently been shown to be produced by monocytes induced by IL-4 [13]. Another report explains that CCL18 is strongly expressed in the lung and is therefore called PARC by its authors [14]. However, the role of this chemokine in human lung diseases remains unexplored. Here we report that increased CCL18 expression was associated with HP and might represent an important mediator in the pathology of this disease.

MATERIALS AND METHODS

Study population

Nonsmoking female patients with subacute or chronic HP ($n=10$) [51±16 years old (mean±SD)] and patients with IPF ($n=9$; five women and four men; 60±8.3 years old) were included in this study. The study was approved by the ethics committee of our institute, Instituto Nacional de Enfermedades Respiratorias, and informed written consent was obtained from each subject. Diagnosis of HP was obtained as described elsewhere [2, 3] and based on the

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following criteria: a) pigeon exposure preceding the disease, along with positive serum antibodies against avian antigens; b) shortness of breath with partial improvement after avoidance of avian antigen exposure; c) clinical, radiological, and functional features of an interstitial lung disease; d) >40% lymphocytes in bronchoalveolar lavage (BAL) fluid; and e) lung histology compatible with HP [1–3]. Summarized briefly, the tissue samples showed diffuse interstitial inflammation of mononuclear predominance, mainly lymphocytes, and frequent multinucleated giant cells in terminal and respiratory bronchioles as well as in the alveolar walls. Small and loosely arranged granulomas were observed in the interstitium. Biopsy cultures were negative for bacteria, mycobacteria, and fungi.

Diagnosis of IPF was supported by clinical, radiological, and functional findings and by computerized-tomographic (CT) scans; diagnosis was further corroborated by open-lung biopsy [2, 11, 15]. Morphologic diagnosis was based on typical microscopic findings of usual interstitial pneumonia, and criteria included presence of patchy, nonuniform alveolar septal fibrosis and interstitial inflammation, consisting mostly of mononuclear cells but also of neutrophils and eosinophils. A variable macrophage accumulation in the alveolar spaces and cuboidalization of the alveolar epithelium were observed. Analysis of the biopsies by polarized-light microscopy indicated that they lacked granulomas, vasculitis, microorganisms, and inorganic material.

Lung samples were taken from patients with HP and IPF by open-lung biopsy, usually 1 week after hospital admission. None of the patients had been treated with corticosteroids or immunosuppressive drugs at the time of biopsy. A portion of the biopsy was immediately frozen in liquid nitrogen for RNA extraction and subsequent expression analysis by TaqMan.

In the TaqMan analyses, control lung tissues included lung parenchyma from transplant donors (n=11) and autopsy donors (n=2) or normal adjacent tissue from patients undergoing surgery for lung cancer (n=5). The available clinical history of the 13 transplant and autopsy donors indicated no known infections or lung diseases. One donor had a history of smoking. In addition, two RNAs from normal lungs were purchased from Clontech (Palo Alto, CA). One was from an individual, and the other was from a pool of RNA from five individuals. These control lung RNAs were from 10 females and 9 males, with a mean age of 39.2 ± 22.1 years old, whose ages ranged from 14 months to 76 years. The Clontech pool had lung RNA from both males and females, ranging from 14–40 years old. The control lung tissues for TaqMan analyses were obtained from the National Disease Research Interchange (Philadelphia, PA). For immunohistochemistry and in situ hybridization studies, control lung tissue samples were obtained from autopsies of patients who died from causes unrelated to lung disease (n=6; four males and two females; mean age, 43.2±11.1 years). Selected lung fragments that appeared macroscopically and microscopically normal were used.

Semiquantitative histological assessment

The amount of fibrosis present in lung samples was analyzed as described elsewhere [2, 3]. Briefly, this assessment was done on a slide, which was scanned completely in a zigzag fashion, first at 25× and then at 100× magnification. In each case, two slides, one stained with Masson's trichrome and the other with hematoxylin and eosin, were analyzed. We first determined the percentage of the lung biopsy that had abnormal tissue, indicating either inflammation or fibrosis or both (i.e., "extent of the lesion") and then the percentage of the abnormal lung with fibrosis. At a magnification of 25×, a slide comprised ~8–10 fields, and the extent of the lesion was evaluated. At a magnification of 100×, the percentage of the lung with fibrosis was evaluated in an average of 40 fields and was expressed in multiples of 10. The assessment of fibrotic changes included examination of young connective tissue rich in fibroblasts and relatively poor in mature collagen as well as areas with well-developed collagenization [16].

BAL

BAL was performed using a standard technique [17]. Briefly, a fiber-optic bronchoscope was wedged in two separate segments of the right middle lobe or lingula, and 300 mL of normal saline were instilled in 50-mL aliquots, with an average return of 65–70%. The recovered BAL fluid was filtered through sterile gauze, measured, and then centrifuged at 250 *g* for 10 min at 4°C. The cell pellet was resuspended in 1 mL of phosphate-buffered saline, and an aliquot was used to evaluate the total number of cells. Other aliquots were fixed in carbowax, and three slides per sample were stained with hematoxylin and eosin, Giemsa, and toluidine blue and used for differential cell count. The samples were counted in a double-blind fashion.

RNA Preparation and real-time PCR analysis (TaqMan)

RNA was extracted from lung tissue using guanidinium thiocyanate and then centrifugation in cesium chloride or RNA STAT-60 (Tel-Test Inc., Friendswood, TX). RNA quality was assessed with agarose gel electrophoresis. Total RNA (5 μg) was treated with RNase-free DNase I (Boehringer Mannheim, Indianapolis, IN) in First-Strand synthesis buffer in the presence of RNasin (Promega, Madison, WI). The samples were incubated for 20 min at 37°C, heated for 10 min at 70°C, and then immediately chilled on ice. A mixture of 2.5 μg of oligo(dT)$_{12–15}$ (Boehringer Mannheim) and 250 ng of random hexamers (Promega) were added to each sample. The samples were heated to 70°C for 10 min, rapidly chilled on ice, and then briefly spun in a microfuge (Gibco-BRL, Rockville, MD). cDNA was generated from the RNA using Superscript II reverse transcriptase (Gibco-BRL) according to the manufacturer's instructions in a final volume of 100 μL.

Ten nanograms of cDNA per sample were analyzed for expression of CCL18 and ubiquitin on a GeneAmp 5700 sequence detector (PE Applied Biosystems, Foster City, CA) in a 25-μL reaction mixture. CCL18 was detected using primers and probe with TaqMan Universal Master Mix (all from PE Applied Biosystems) or primers alone and SYBR Green PCR Master Mix (PE Applied Biosystems). Ubiquitin was detected using 200 nM primers (forward: CACTTGGTCCTGCGCTTGA; reverse: CAATTGGGAATGCAACAACTTTAT) with SYBR Green PCR Master Mix. The data were analyzed to calculate a cycle threshold value (C_t) for each sample, with GeneAmp 5700 SDS software (PE Applied Biosystems). The samples were assayed three times for CCL18 and twice for ubiquitin, and the average of the readings for both genes was used to calculate the relative level of CCL18 mRNA in the tissue by the following formula: $2^{(C_t \text{ of ubiquitin} - C_t \text{ of CCL18})} \times 10{,}000$ for each sample. The means and standard errors were calculated for each group.

Monoclonal antibody production

Mouse anti-CCL18 monoclonal antibodies were produced in BALB/c mice. Mice were immunized intraperitoneally with 25 μg of CCL18/immunoglobulin (Ig) fusion protein emulsified in complete Freund's adjuvant and then boosted every 2–3 weeks with 15 μg of the same protein in incomplete Freund's adjuvant. The final boost was performed with cleaved CCL18. Splenocytes from the immunized animals were fused with mouse myeloma SP2/0. Hybridoma supernatants were screened by enzyme-linked immunosorbent assay on cleaved CCL18-coated plates and by Western blot analysis. Positive hybridomas were cloned and rescreened.

Immunohistochemistry

Tissue sections were deparaffinized, rehydrated, and then blocked with 3% H_2O_2 in methanol for 30 min. Then antigen retrieval was performed with 10 mM citrate buffer (pH 6.0) for 5 min in a microwave. Tissue sections were then incubated with an antibody diluent with background-reducing components (Dako, Carpinteria, CA) diluted 1/100 in phosphate-buffered saline for 45 min. Mouse anti-CCL18 monoclonal antibody was applied and incubated at 4°C overnight. A secondary biotinylated anti-Ig and then horseradish peroxidase-conjugated streptavidin (BioGenex, San Ramon, CA) were used according to the manufacturer's instructions. 3-Amino-9-ethyl-carbazole (BioGenex) in acetate buffer containing 0.05% H_2O_2 was used as substrate [18]. The sections were counterstained with hematoxylin. The primary antibody was replaced by nonimmune serum for negative control slides. To identify macrophages and DCs, parallel sections were stained with HAM56 (7.5 μg/mL) and S-100 antibodies (11 μg/mL), respectively (DAKO) [19, 20].

In situ hybridization

Riboprobes for in situ hybridization were generated from human cDNA CCL18 cloned into pSPORT vector (Gibco-BRL). The plasmid was linearized before translation with *Kpn*I. An antisense 628-bp fragment was transcribed with T7, and a sense 150-bp fragment was transcribed with SP6 RNA polymerases. The transcription of sense and antisense transcripts was performed with a labeling mixture containing digoxigenin-UTP (Boehringer Mannheim, Germany).

In situ hybridization was performed on 4-μm sections as previously described [18, 19]. Briefly, the sections mounted on siliconized slides were incubated in 0.001% proteinase K (Sigma Chemical Co., St. Louis, MO) for 20

min at 37°C. After acetylation with acetic anhydride, the sections were prehybridized for 1 h at 45°C in hybridization buffer and then were incubated with the digoxigenin-labeled probes at 45°C overnight. Some sections were hybridized with digoxigenin-labeled sense RNA probe. The tissues were incubated with a polyclonal sheep anti-digoxigenin antibody coupled with alkaline phosphatase (Boehringer Mannheim Co., Indianapolis, IN) for 1 h at room temperature. The color reaction was performed by incubation with fast red chromogen (Biomeda Corp., Foster City, CA). The sections were lightly counterstained with hematoxylin.

Statistical analysis of TaqMan data

Statistical analysis was performed with JMP version 3.2.2 (SAS Institute, Inc., Cary, NC). The TaqMan data for the relative level of CCL18 were log transformed, and a one-way analysis of variance was performed. The log-transformed CCL18 level was used as the dependent variable, and results from the disease group were used as the independent variable. All pair-wise comparisons were made using Student's *t*-test. Correction for multiple comparisons was accomplished using the Bonferroni method. The association between the level of CCL18 and the percentage of lymphocytes present in the BAL fluid was assessed with Pearson's correlation. Finally, to evaluate a possible relationship between the CC18 level and the degree of lung fibrosis described by the pathologist, a Spearman correlation was used.

RESULTS

Patient characteristics

The baseline characteristics of the patients with HP and IPF are summarized in **Table 1**. All patients showed clinical and functional evidence of interstitial lung disease with variable degrees of dyspnea, decreased lung capacities, and hypoxemia at rest that worsens during exercise. In the HP group, differential cell counts in BAL fluid were characterized by marked lymphocytosis, usually well over 40%, but in lungs with IPF, most inflammatory cells in the BAL fluid were macrophages.

Quantification of CCL18 by TaqMan

To determine whether any chemokines were overexpressed in lung tissue from HP patients, we assayed cDNA prepared from RNA extracted from control and diseased lungs for the level of a panel of chemokines or their receptors using real-time PCR (TaqMan). Included on the panel were cDNAs made from 20 control lung samples, 9 biopsies from lungs with IPF, and 10 biopsies from lungs with HP. The biopsies were taken at the time of diagnosis. The initial series of analyses included the following chemokines and receptors: I-309/CCL1, monocyte chemoattractant protein (MCP)-1/CCL2, macrophage-inflammatory protein (MIP)-1α/CCL3, MIP-1β/CCL4, regulated on activation normal T expressed and secreted/CCL5, MCP-3/CCL7, eotaxin/CCL11, thymus- and activation-regulated chemokine/CCL17, DC-CK1/CCL18, MIP-3β/CCL19, MIP-3α/CCL20, monocyte chemoattractant protein 1/CCL22, very important chemokine/CCL28, epithelial neutrophil-activating peptide 78/CXCL5, interleukin (IL)-8/CXCL8, lymphotactin/XCL1, CC chemokine receptor (CCR) 3, CCR4, CCR5, CCR6, CCR7, CCR8, CXC chemokine receptor (CXCR) 3, CXCR4, and CXCR6/STRL33.

TABLE 1. Baseline Characteristics of the Study Populations

Characteristic	Population data (type of disease) HP	IPF
Number of patients	10	9
Age (years)	51 ± 16	60 ± 8.4
Time elapsed to first visit (months)	23 ± 20	28 ± 14
Dyspnea score[a]	2.3 ± 0.6	3.0 ± 0.7
FVC %[b]	55 ± 19	59 ± 16
TLC %[b]	63 ± 12	59 ± 12
PaO_2 (mm Hg)	49.5 ± 9	50.3 ± 7
BAL lymphocytes	66 ± 17	27 ± 18
BAL macrophages	32 ± 16	68 ± 17
BAL neutrophils	0.6 ± 1	2.3 ± 4.0
BAL eosinophils	1.6 ± 2	1.3 ± 2

[a] Dyspnea score (dyspnea at exercise): 1, slight; 2, moderate; 3, severe; 4, dyspnea at rest. [b] Percent of predicted. FVC, forced vital capacity; TLC, total lung capacity.

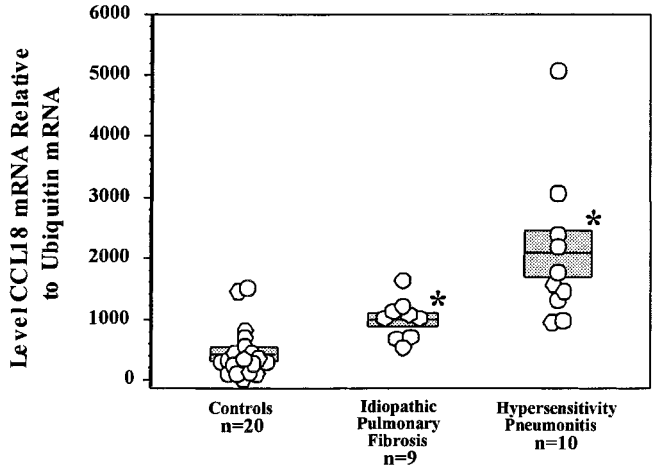

Fig. 1. mRNA for CCL18 is elevated in lung tissue from IPF and HP patients. Total RNA from lung parenchyma was reverse transcribed into cDNA and assayed for the relative abundance of CCL18 mRNA by real-time PCR. The amount of CCL18 was calculated relative to the level of ubiquitin mRNA present in each sample. Samples were from controls, lungs affected by IPF, or lungs affected by HP. The mean and SE for all samples in each of the groups are indicated. Both disease groups showed a statistically significant difference from the control group [$P<0.01$ for HP and $P<0.05$ for IPF, respectively (*)].

The level of each chemokine or chemokine receptor was calculated relative to the level of ubiquitin expressed in that sample. Of all the genes tested, CCL18 was the most strikingly and consistently increased chemokine in lung parenchyma from HP patients and, to a lesser degree, in IPF patients compared with the lung parenchyma from controls (2,085±392 vs. 1,023±110 and 466±94, respectively; $P<0.01$) (**Fig. 1**). In addition, CCL18 levels in the lungs of HP patients were significantly higher than those in the lungs of IPF patients ($P<0.05$).

Localization of CCL18 mRNA and immunoreactive protein in lung tissue

To examine the cellular source of CCL18, we performed both in situ hybridization and immunohistochemical studies on tissue sections taken from lungs of patients with HP and IPF and from control lungs. With in situ hybridization, CCL18 transcription was found primarily in isolated cells or clusters of interstitial inflammatory cells, as exemplified in **Figure 2A** in IPF-

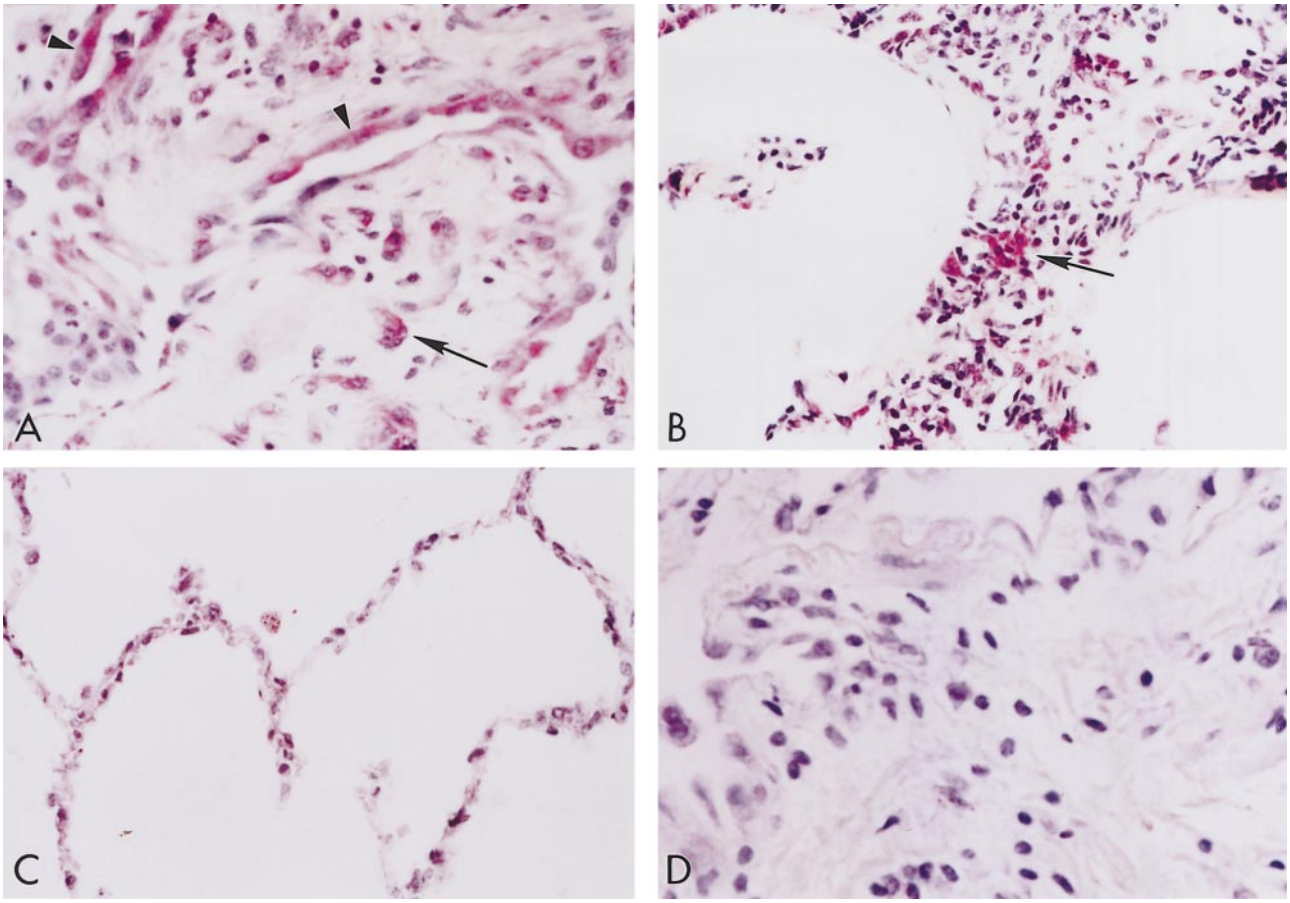

Fig. 2. Localization of CCL18 mRNA in tissue samples from lungs affected by HP and IPF. CCL18 mRNA is produced by reactive type 2 pneumocytes (arrowheads) and interstitial inflammatory cells (arrows) in tissue sections from biopsies from lungs affected by IPF (A) (60×) and HP (B) (40×). Control lungs did not express CCL18 mRNA (C) (60×). Analysis of controls using a sense riboprobe displayed no reactivity, as exemplified in panel D with a lung affected by IPF (60×). The slides are counterstained with hematoxylin.

affected lung tissue and in Figure 2B in HP-affected lung tissue. Occasionally, positive staining was also observed in reactive type 2 alveolar epithelial cells (Fig. 2A). CCL18 expression was noticed primarily in areas of more severe lung inflammation. Control tissues hybridized with antisense CCL18 probe were negative (Fig. 2C), as were HP- and IPF-affected tissues hybridized with the sense probe (Fig. 2D).

The expression pattern of the immunoreactive protein paralleled the mRNA observations. **Figures 3A–C** illustrate interstitial inflammatory cells revealing an intense staining for CCL18 in both HP- and IPF-affected lungs. Usually, more numerous positive cells were noticed in HP-affected tissues (Fig. 3A) than in IPF-affected tissues (Fig. 3C), and they were frequently found close to clusters of lymphocytes (Fig. 3B). By using HAM-56, positively stained interstitial inflammatory cells were identified mostly as macrophages (Fig. 3D), although some of them were also recognized as DCs (S-100 positive cells) (Fig. 3E). Immunohistochemical staining for CCL18 was negative in normal lungs (Fig. 3F). Lung tissue samples incubated with nonimmune sera were also negative (Fig. 3G).

Correlation of the level of CCL18 with the percentage of BAL lymphocytes in tissue

CCL18 has been shown to chemoattract naive T cells. In addition, it was identified in areas of severe lung inflammation by in situ hybridization and immunohistochemical staining in tissues affected by both HP and IPF. Therefore, we hypothesized that there might be a correlation between the level of CCL18 in lung tissue and the level of lymphocytes in the BAL fluid of these patients. In **Figure 4**, the log of the level of CCL18 is plotted against the percentage of lymphocytes present in the BAL fluid for all IPF and HP patients included in this study. The data show a significant correlation between these two parameters (;$r=0.765$; $P<0.01$). In addition, HP patients with the highest levels of CCL18 had subacute rather than chronic HP, as corroborated by the degree of tissue fibrosis. Thus, a significant negative correlation was found between the percentage of lung fibrosis and the levels of CCL18 ($r_s=-0.90$; $P<0.01$). **Figure 5** exemplified the higher CCL18 expression in subacute HP with minimal fibrosis compared with more chronic fibrotic lesions.

DISCUSSION

HP is a diffuse inflammatory disease caused by the inhalation of and sensitization to a wide variety of organic particles, and it is characterized by the accumulation of T lymphocytes within the bronchoalveolar structures of the lung. Although the etiology of the disease is known, the sequence of pathogenic events

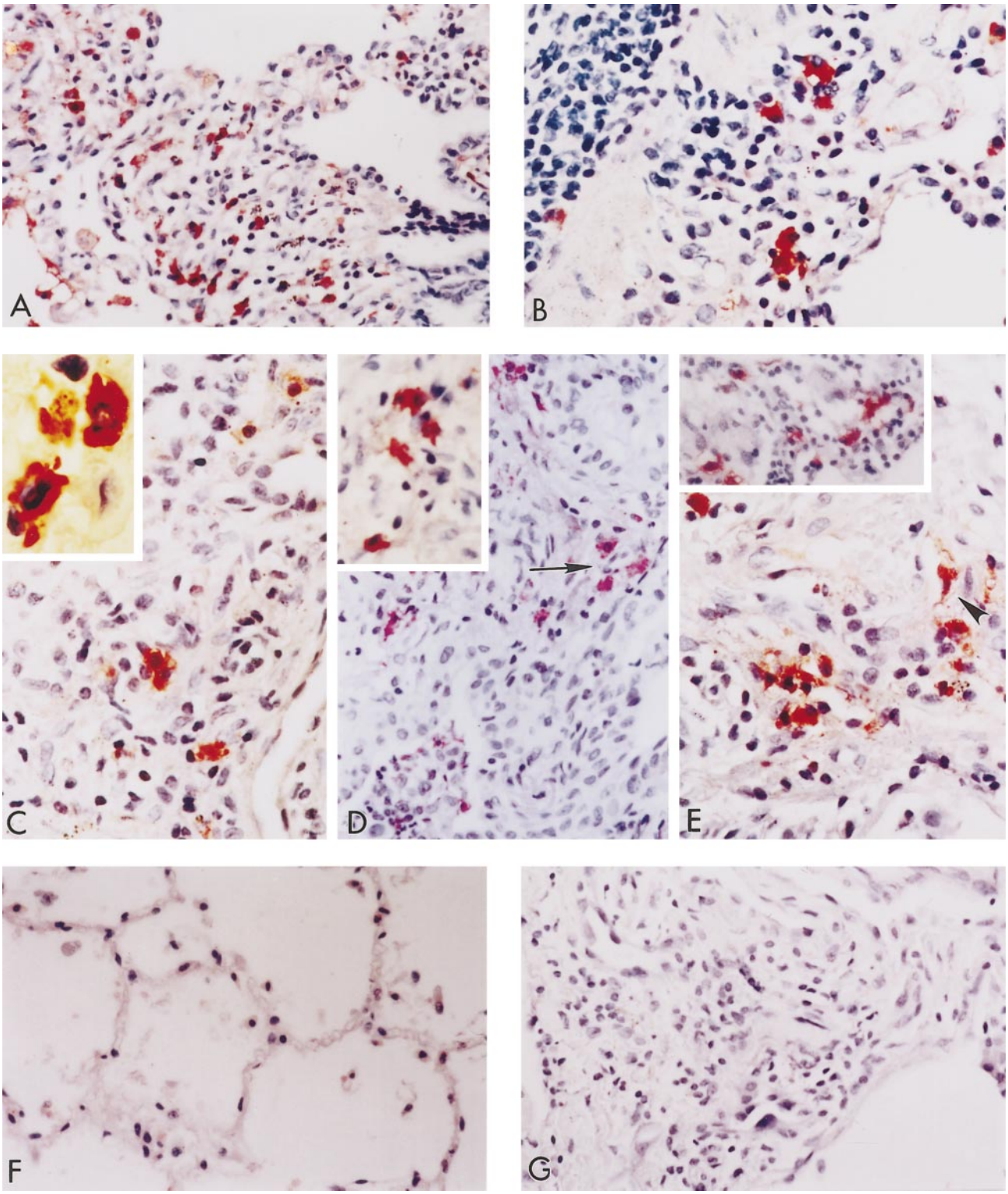

Fig. 3. Localization of CCL18-immunoreactive protein in lung tissue of patients with HP and IPF. CCL18 was produced by numerous interstitial inflammatory cells in tissue sections from HP [A (40×) and B (60×)] and IPF [C (60×)]. Positive cells were identified as macrophages (C, inset). Panel D shows numerous stained interstitial cells (magnification, 40×), indicating the presence of CCL18. Several of these cells were identified as macrophages (arrow) by HAM-56 [inset (60×)]. Panel E illustrates a tissue sample from tissue affected by IPF with CCL18-positive cells (60×), some of them identified as DCs (arrow) by S-100 staining [inset (40×)]. Control lungs did not show a detectable signal (F). Control antibody staining of samples taken from lungs with HP (G) and IPF revealed no immunoreactive protein.

leading to this state has not been clearly defined. Several mechanisms could be involved in the increased traffic and accumulation of T cells in lung parenchyma. Chemokines have been recognized in recent years as critical mediators that regulate trafficking of various leukocyte populations to specific anatomical locations [7]. It is therefore reasonable to hypothesize that they might be involved in the recruitment of pathogenic T cells in patients with HP. To test this hypothesis, we

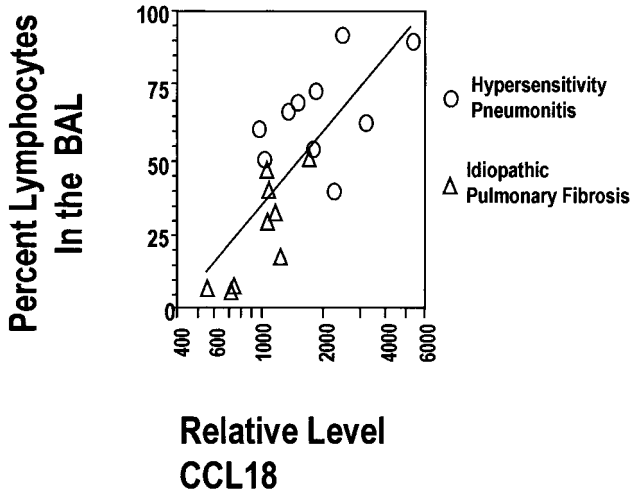

Fig. 4. Correlation between the level of CCL18 in lung parenchyma and lymphocyte infiltration. The level of CCL18 for all HP and IPF patients in the study was plotted versus the percentage of lymphocytes in the BAL fluid. The data show a significant correlation between these two parameters, (r=0.765; P<0.001).

undertook a systematic analysis of the expression of chemokine ligands and their receptors in various human lung diseases. Of the 16 chemokines tested, the most dramatic association was observed between HP and the chemokine now known as CCL18 [7], which was originally described as DC-CK1, AMAC-1, or PARC [12–14]. Although CCL18 was initially reported to be a DC product [12], its expression was subsequently found to be induced in macrophages by various cytokines including IL-4, IL-10, and IL-13 [13]. It is important that the high expression of CCL18 in the lung was also recognized in early studies [14]. However, no detailed analyses of the potential role of this chemokine in human lung diseases have been performed. It has recently been reported, however, that CCL18 is strongly expressed in the liver during hepatitis C infection [21].

CCL18 is known to act on some T cell subsets [11–13], and it is interesting that it exists in humans but not in mice. The latter observation is explained by the finding that CCL18 likely arose recently (in evolutionary terms) from the fusion of two MIP-1α/CCL3 genes [22]. Despite its strong similarity to MIP-1α/CCL3, CCL18 does not bind to CCR1, CCR3, or CCR5 [A. Zlotnick, DNAX Research Institute, unpublished results].

Here we demonstrated that lungs from patients with subacute/chronic HP strongly overexpress CCL18 compared with normal lung parenchyma. It is interesting that the expression of this chemokine was more abundant during the subacute state of the disease, which is characterized by severe inflammation affecting mainly the alveolar walls. These patients also exhibited the highest numbers of lymphocytes in their BAL fluid, supporting the hypothesis that CCL18 expression in the lung might mediate the recruitment of T lymphocytes during the development of the inflammatory process.

Regarding the cellular source, CCL18 was produced primarily by interstitial macrophages and occasionally by DCs and reactive type 2 pneumocytes, as shown by in situ hybridization and immunochemistry. Cells expressing this chemokine were often observed close to clusters of lymphocytes.

Compared with CCL18 in control lungs, CCL18 was also up-regulated in lungs affected by IPF, although the levels were significantly lower than in HP-affected lungs. IPF is a lung disorder in which the inflammatory process is usually moderate and mainly involves lymphocytes, although plasma cells, neutrophils, and eosinophils might also be present [11]. Actually, CCL18 levels in lungs with IPF were similar to those found in chronic (fibrotic) HP. Therefore, it can be hypothesized that there is a strong up-regulation of CCL18 primarily in lung

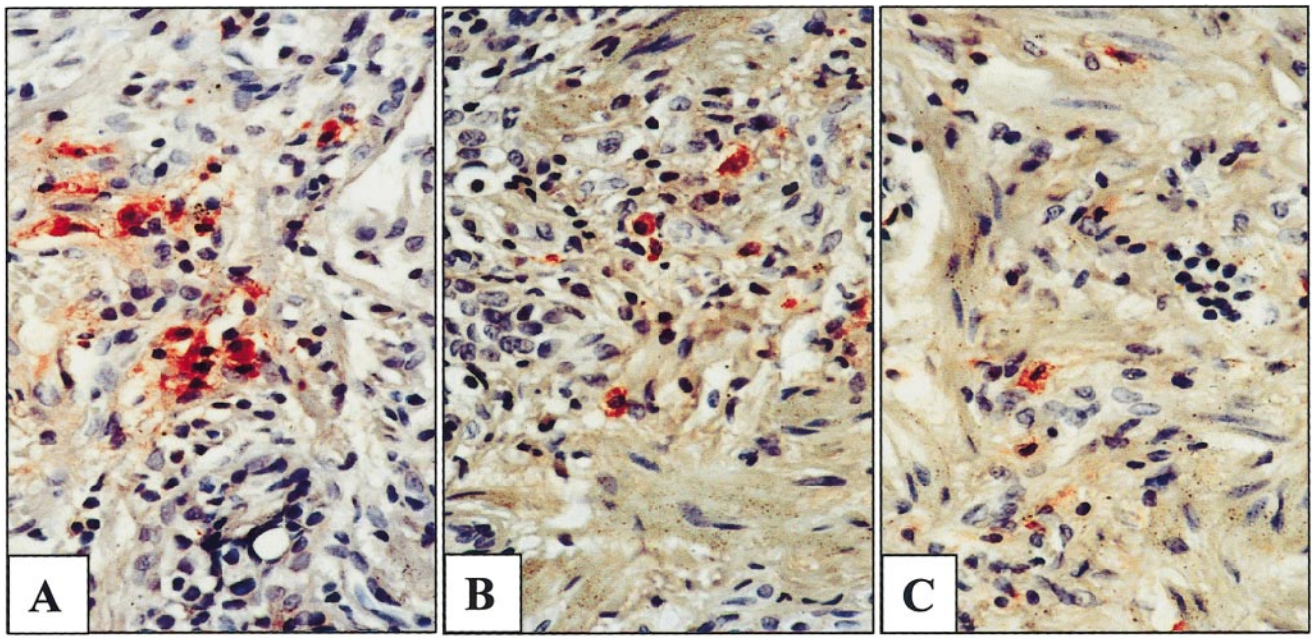

Fig. 5. A higher number of cells expressing CCL18 was observed in subacute HP lesions. (A) Light photomicrograph of subacute (inflammatory) HP (40×); (B and C) more chronic HP lesion, identifiable by the presence of numerous fibroblasts and the collagen deposit (40×).

diseases characterized by an exuberant T-lymphocytic alveolitis. Supporting this view, there was a strong correlation between the levels of CCL18 in the lungs of HP and IPF patients and the percentage of lymphocytes in BAL fluid. In patients with chronic hepatitis C, this chemokine was also expressed by mononuclear cells, and it was found in association with naive T cell infiltration [21].

On the other hand, HP is believed to be a predominantly T-helper (Th) type 1 lung disorder [23], whereas IPF is a Th2 lung disorder [24, 25]. In this context, our findings might also suggest that CCL18 has a role in regulating T cell function in the lung, because the correlation found between CCL18 and the number of lymphocytes in BAL fluid was positive in both groups of patients taken together. This concept is supported on one hand by the findings of Adema et al. [12], who determined that this chemokine is part of the immunostimulatory arsenal of DCs during the development of naive T cells into Th1 effector cells. On the other hand, results obtained by Kodelja et al. [13] suggest that CCL18 is induced, at least in vitro, in alternatively activated macrophages by Th2-associated cytokines.

Although the receptor for this chemokine is currently unknown [7], our results strongly suggest that lymphocytes found in the BAL fluid of HP patients are chemoattracted to the lung at least partially by CCL18. Thus, our data suggest that T cells in BAL fluid express the CCL18 receptor. Finally, these observations suggest that inhibitors of CCL18/receptor interaction might have therapeutic effects in patients with HP and perhaps in other interstitial lung diseases characterized by lymphocytic alveolitis such as sarcoidosis. Future experiments will explore in more detail the questions arising from these findings.

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