Pathogenesis of cBFL in common with IPF? Correlation of IP-10/TARC ratio with histological patterns

Masato Kishi, Yasunari Miyazaki, Torahiko Jinta, Haruhiko Furusawa, Yoshio Ohtani, Naohiko Inase, Yasuyuki Yoshizawa
The Department of Integrated Pulmonology, Tokyo Medical and Dental University, Tokyo, Japan

Correspondence to:
Yasuyuki Yoshizawa, M.D., Ph.D.
Professor and Chairman
The Department of Integrated Pulmonology,
Tokyo Medical and Dental University,
1-5-45, Bunkyo-ku, Tokyo, 113-8519, Japan
Telephone: 81-3-5803-5950
Facsimile: 81-3-5803-0167
E-mail: yoshizawa.pulm@tmd.ac.jp

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ABSTRACT

Background: A Th1 predominant immune response has been shown in acute hypersensitivity pneumonitis. A Th2 predominance appears to favor the development of pulmonary fibrosis through the profibrotic process, and has been described as crucial in the progression of idiopathic pulmonary fibrosis (IPF). Chronic bird fancier’s lung (cBFL) can present a histological pattern of usual interstitial pneumonia (UIP)-like lesions. Little is known about the Th1/Th2 balance in the pathogenesis of cBFL.

Methods: To evaluate the relevance of Th1-type (IP-10) and Th2-type (TARC) chemokines, and their receptors (CXCR3 and CCR4) with reference to histological patterns of cBFL, we analyzed 40 patients with cBFL who underwent surgical lung biopsies, 12 acute BFL (aBFL) and 10 healthy volunteers. We measured IP-10 and TARC levels in serum and bronchoalveolar lavage fluid (BALF) by ELISA. Immunohistochemistry for CXCR3 and CCR4 was performed on surgical lung specimens.

Results: The ratio of TARC to IP-10 in serum of patients with UIP-like lesions was significantly higher than that with cNSIP/OP-like lesions, aBFL, and healthy volunteers. The ratio of CCR4 to CXCR3 in UIP-like lesions was significantly higher than that in cNSIP/OP-like lesions and fNSIP-like lesions. The ratio of CCR4- to CXCR3-positive cells correlated with the ratio of TARC to IP-10 in serum.

Conclusions: A Th2 predominant immune response may play an important role in the development of UIP-like lesions as already observed in IPF. A Th1 predominance may play a role in the development of cNSIP/OP-like lesions in cBFL.
INTRODUCTION

Hypersensitivity pneumonitis (HP) is an immunologically-mediated lung disease induced by inhalation of antigens contained in a variety of organic dusts. BFL is a type of HP and develops in individuals who are susceptible to avian antigens. Patients with BFL sometimes present with acute HP but more frequently present with chronic HP, because exposure to birds tends to be chronic with small amounts of antigens.[1] Chronic BFL is further subgrouped into recurrent and insidious BFL. Patients with recurrent BFL often breed dozens of pigeons in lofts. They tend to inhale much more avian antigens than insidious BFL. In contrast, patients with insidious BFL are often exposed indoors to small birds, such as budgerigars, with long-term exposure to much smaller amounts of avian antigens.[2] Surgical lung specimens in cBFL present various histological patterns, such as usual interstitial pneumonia (UIP)-like lesions, fibrotic nonspecific interstitial pneumonia (fNSIP)-like lesions, cellular NSIP (cNSIP)-like lesions and organizing pneumonia (OP)-like lesions as seen in idiopathic interstitial pneumonias. Prognosis of patients with cBFL is variable based upon each of their histological patterns.[3]

T-helper type1 (Th1) and T-helper type2 (Th2) cells are thought to play a crucial role in fibrogenesis.[4] Pulmonary Th1 cells, which produce IFN-gamma are likely to inhibit fibrosing processes,[5] whereas a switch to Th2 type immune response with concomitant release of IL-4 is mandatory for the expression of mesenchymal matrix components in the surrounding environment.[6] A Th2 predominant immune response has been described as crucial in the progression of IPF since the CCR4/CXCR3 ratio of CD4 lymphocytes in BAL was higher in patients with IPF than non-IPF, sarcoidosis and healthy control subjects.[7] Although a Th1 predominant immune response has been shown to be pivotal in acute HP,[8] little is known about the Th1/Th2 balance in the pathogenesis of ‘chronic’ HP. The Th1 and Th2 balance can be evaluated by the detection of chemokine receptors on T-cells. CXCR3 is expressed on Th1 cells, whereas CCR4 is expressed on Th2 cells.[9][10] IP-10 (IFN-gamma-induced protein; CXCL10) is a chemokine of the CXC subfamily; is the ligand for the receptor CXCR3. In acute HP, IFN-gamma mediates the recruitment of lymphocytes into the lung via the production of IP-10.[8] In addition, CXCR3 exhibits a significant role in attenuating pulmonary fibrosis in the bleomycin mouse model.[11] TARC (thymus- and activation-regulated chemokine; CCL17) is a chemokine of the CC subfamily and is the ligand for the receptor CCR4, and is crucial in the development of pulmonary fibrosis in the bleomycin mouse model.[12]

Therefore, we hypothesized that Th1 chemokine (IP-10) plays a role in the inhibition of fibrosing processes, whereas Th2 chemokine (TARC) is involved in the acceleration of fibrosis. If this is the case, cBFL may present various histological patterns such as UIP-like lesions, fNSIP-like lesions, cNSIP and OP-like lesions according to the Th1/Th2 balance.

In this study, we evaluated the relevance of IP-10 and TARC levels in both serum and BALF, and CXCR3- and CCR4-positive lymphocytes in surgical biopsied specimens to analyze the Th1/Th2 chemokine balance involved in the pathogenesis of cBFL with UIP-like lesions, fNSIP-like lesions, cNSIP-like lesions and OP-like lesions.

METHODS

Study population
We conducted a retrospective review of medical records between April 1993 and March 2006 admitted to Tokyo Medical and Dental University. We analyzed 40 patients with cBFL who underwent surgical lung biopsies, 12 patients with acute BFL (aBFL), and 10 healthy volunteers with no avian exposure.

The diagnostic criteria for cBFL included 1) a history of avian contact, 2) antibodies and/or lymphocyte proliferation to avian antigen, and 3) reproduction of symptoms of HP by environmental provocation or laboratory-controlled inhalation of avian antigen;[13] either 4) evidence of pulmonary fibrosis with or without granulomas on histological analysis, or 5) honeycombing on computed tomographic scans; and either 6) progressive deterioration of a restrictive impairment on pulmonary function throughout 1 year or 7) more than 6 months’ duration of respiratory symptoms related to HP.[14] Histological patterns were subgrouped by three pulmonary pathology specialists without knowledge of the patient’s clinical course into UIP-like lesions, fNSIP-like lesions and cNSIP/OP-like lesions according to the international classification of idiopathic interstitial pneumonias that has been proposed by the joint statement of ATS and ERS in 2002.[15]

The study conformed to the declaration of Helsinki and was approved by the internal review board of our institution. Informed written consent was obtained for each subject.

**Inhalation challenge and Immunological findings**

Antigen inhalation provocation tests were conducted as previously described.[13] Antibodies in serum and BALF to PDE were measured by an enzyme-linked immunosorbent assay (ELISA) and the antigen-induced lymphocyte proliferation test was performed as previously described.[16]

**High Resolution Computed Tomography (HRCT) scoring**

HRCT scans were reviewed independently by four experienced respiratory physicians (Y.M., M.K., T.J., H.F.). They scored ground glass opacity for ground-glass scores and reticular opacity for fibrosis scores. The outlines of the scoring system used for the evaluation have been described previously by Kazerooni.[17] Each lobe of the lung was scored on a scale of 0 to 5. The scores for each lobe were averaged for all four readers for data analysis.

**Bronchoalveolar lavage**

We performed BAL as previously described.[13] BAL was performed using the three 50-ml aliquots of sterile 0.9% saline. The cellular composition of the BAL was determined using a cytospun smear with Wright stain by counting 200 cells. Lymphocyte phenotypes were performed by flow cytometry with monoclonal antibodies for CD4 and CD8.

**Measurement of IP-10 and TARC**

We measured levels of IP-10 and TARC in serum and BALF by ELISA using commercial ELISA kits (DuoSet: R&D Systems, Minneapolis, USA) following the manufacturers’ instructions for undiluted BALF samples and serum samples diluted 1:2.

**Immunohistochemical analysis**

We performed immunohistochemistry on lung specimens of surgical lung biopsies. We examined localization of CXCR3 and IP-10, or CCR4 and TARC, using an immunohistochemical double-stain method as previously described.[18] Paraffin-embedded 4-µm sections in antigen unmasking solution (Vector Laboratories, Burlingame, USA) were autoclaved. Endogenous peroxidase was blocked by 3% H2O2. Endogenous avidin or biotin activity was
blocked by Avidin/Biotin Blocking Kit (Vector Laboratories, Burlingame, USA). Sections were incubated with anti-CXCR3 (mouse anti-human IgG; BD Biosciences, San Jose, USA; diluted in 1.25µg/ml), or anti-CCR4 (mouse anti-human IgG; a gift from Kyowa Hakko, Tokyo, JAPAN; diluted in 2.5µg/ml). Then sections were incubated with biotinylated secondary antibodies (horse anti-mouse IgG; Vector Laboratories, Burlingame, USA). After incubation with ABC kit (Vector Laboratories, Burlingame, USA), DAB (Nichirei, Tokyo, Japan) was added. For a second sequence, any unbound biotin activity resulting from the first step was blocked by Avidin/Biotin Blocking Kit. Subsequently, sections were incubated with anti-IP-10 (Rabbit anti-human IgG; Pepro Tech, London, UK; diluted in 10µg/ml), or anti-TARC (Goat anti-human IgG; R&D Systems, Minneapolis, USA; diluted in 4µg/ml) overnight at 4°C. Then sections were incubated with biotinylated secondary antibodies (goat anti-rabbit or horse anti-goat IgG; Vector Laboratories, Burlingame, USA). After incubation with AP-ABC kit (Vector Laboratories, Burlingame, USA), Vector Red Substrate Kit and levamisole (Vector Laboratories, Burlingame, USA) was added. Tissue sections were counterstained with Mayer’s hematoxylin, and mounted with a covership.

**Semiquantification of CCR4- and CXCR3-positive cells**

The positive staining of infiltrating mononuclear cells for anti-CXCR3 and CCR4 were separately evaluated blinded to the diagnosis in fibrosing areas and in lymphoid clusters of surgical lung biopsy specimens by immunohistochemistry. The positive cells were counted in 20 random high-power (x400) fields (HPFs) of fibrosing areas and in 5 HPFs of lymphoid clusters. Quantification of immunohistochemistry was done by the first author. The positive cells were counted on separate three occasions to obtain the average.

**Statistical analysis**

Data were analyzed using SAS 9.1 (SAS Institute Inc., USA). When the data were normally distributed, such as the recovered volume, total cell counts, the percentage of macrophages and lymphocytes in BALF, age, the percentage of vital capacity and total lung capacity, PaO2, A-aDO2, the fibrosis score, the ground-glass score, and the CCR4- to CXCR3-positive cell ratio, we performed the Tukey-Kramer test. When the data were not normally distributed, such as the percentage of neutrophils and eosinophils, the ratio of CD4/8 in BALF, the levels of serum or BALF IP-10, the levels of TARC and the TARC to IP-10 ratio, we performed the Steel-Dwass test. Correlations between serum TRAC to IP-10 ratio and CCR4- to CXCR3-positive cell ratio were assessed with the Pearson’s correlation coefficient. P values less than 0.05 were considered as significant. The data are presented by mean ± SD in Tables 1 and 2, Figures 1, 2, 3 and 5 and text.

**RESULTS**

**Clinical features of cBFL**

We analyzed 40 patients with cBFL in this study (Table 1). Of 40 patients, 19 patients had UIP-like lesions (15 men and 4 women, 18 insidious BFL and 1 recurrent BFL); 13 patients had fNSIP-like lesions (5 men and 8 women, 8 insidious BFL and 5 recurrent BFL), and 8 patients had cNSIP/OP-like lesions (6 cNSIP and 2 OP, 3 men and 5 women, 1 insidious BFL and 7 recurrent BFL). There were no significant differences in age among these three groups. Ten of 19 cases with UIP-like lesions died of respiratory failure as a result of disease progression. The average time from
diagnosis to death was 32±17.5 months. Only one of 13 patients with fNSIP-like lesions died of respiratory failure due to disease progression even though this patient had tried to avoid the causal antigen and had been treated by corticosteroids alone, or with either cyclosporine or cyclophosphamide. All patients with cNSIP/OP-like lesions are alive since they have avoided the causal antigen and have been treated by corticosteroids only. The percentage of total lung capacity (TLC) in patients with UIP-like lesions was significantly decreased compared to that in patients with cNSIP/OP-like lesions (69.6±20.0% and 98.9±20.0%, respectively; p=0.031). No differences were detected in the percentage of VC. A-aDO₂ in patients with cNSIP/OP-like lesions were significantly increased compared to patients with UIP-like lesions (4.35±1.69kPa and 2.51±1.75kPa, respectively; p=0.049). PaO₂ in patients with cNSIP/OP-like lesions were significantly decreased compared to patients with UIP-like lesions (9.1±1.2kPa and 10.8±1.2kPa, respectively; p=0.039). The fibrosis score in patients with UIP-like lesions and fNSIP-like lesions was significantly higher than in patients with cNSIP/OP-like lesions (2.19±0.71 and 1.29±0.32, respectively; p=0.011) (2.21±0.82 and 1.29±0.32, respectively; p=0.015). The ground-glass score in patients with cNSIP/OP-like lesions was significantly higher than patients with UIP-like lesions (2.89±0.98 and 1.92±0.75, respectively; p=0.016). In BALF profiles, no differences were detected in volume of fluid returned among all groups. The number of total cell counts was significantly increased in cNSIP/OP-like lesions compared to patients with fNSIP-like lesions (69.5±41.4x10⁶ and 26.9±12.0x10⁶, respectively; p=0.042) (Table 2). The percentage of macrophages was significantly decreased in cNSIP/OP-like lesions and fNSIP-like lesions compared to UIP-like lesions (29.9±18.0% and 72.4±18.0%, respectively; p=0.027). The percentage of lymphocytes was significantly increased in cNSIP/OP-like lesions compared to UIP-like lesions and fNSIP-like lesions (67.2±30.5% and 40.8±20.8%, respectively; p=0.039).

Table 2. Profiles of BALF

<table>
<thead>
<tr>
<th>Pathological patterns</th>
<th>UIP</th>
<th>fNSIP</th>
<th>cNSIP/OP</th>
<th>p value (UIP vs. fNSIP)</th>
<th>p value (UIP vs. cNSIP/OP)</th>
<th>p value (fNSIP vs. cNSIP/OP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>recovered volume (ml)</td>
<td>86.0±24.1</td>
<td>89.6±12.0</td>
<td>93.0±13.0</td>
<td>89.6±19.8</td>
<td>107.4±10.6</td>
<td>0.989</td>
</tr>
<tr>
<td>Total cell counts (x10⁶)</td>
<td>36.6±17.0</td>
<td>26.9±12.0</td>
<td>69.5±41.4</td>
<td>68.6±37.1</td>
<td>54.1±32.2</td>
<td>0.891</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of patients, pulmonary function tests, radiographic findings

<table>
<thead>
<tr>
<th>Pathological patterns</th>
<th>UIP</th>
<th>fNSIP</th>
<th>cNSIP/OP</th>
<th>p value (UIP vs. fNSIP)</th>
<th>p value (UIP vs. cNSIP/OP)</th>
<th>p value (fNSIP vs. cNSIP/OP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (male / female)</td>
<td>19 (15/4)</td>
<td>13 (5/8)</td>
<td>8 (cNSIP 6, OP 2) (3/5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>62.4±7.3</td>
<td>58.6±9.8</td>
<td>57.1±6.7</td>
<td>0.752</td>
<td>0.615</td>
<td>0.996</td>
</tr>
<tr>
<td>Percentage of smokers (Number of current : ex : never)</td>
<td>63.2% (4 : 8 : 7)</td>
<td>30.8% (2 : 2 : 9)</td>
<td>50% (2 : 2 : 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>Insidious 18 cases</td>
<td>Insidious 8 cases</td>
<td>Insidious 1 case</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td>10 of 19 cases were died</td>
<td>1 of 13 cases was died</td>
<td>All cases have been survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC (%)</td>
<td>77.5±25.6</td>
<td>75.9±18.0</td>
<td>91.1±27.8</td>
<td>0.998</td>
<td>0.513</td>
<td>0.473</td>
</tr>
<tr>
<td>TLC (%)</td>
<td>69.6±20.0</td>
<td>85.4±18.7</td>
<td>98.9±20.0</td>
<td>0.196</td>
<td>0.031</td>
<td>0.603</td>
</tr>
<tr>
<td>PaO₂ (kPa)</td>
<td>10.8±1.2</td>
<td>10.2±1.6</td>
<td>9.1±1.2</td>
<td>0.672</td>
<td>0.039</td>
<td>0.362</td>
</tr>
<tr>
<td>A-aDO₂ (kPa)</td>
<td>2.51±1.75</td>
<td>2.56±1.20</td>
<td>4.35±1.69</td>
<td>0.999</td>
<td>0.049</td>
<td>0.111</td>
</tr>
<tr>
<td>Fibrosis score</td>
<td>2.19±0.71</td>
<td>2.21±0.82</td>
<td>1.29±0.32</td>
<td>0.997</td>
<td>0.011</td>
<td>0.015</td>
</tr>
<tr>
<td>Ground-glass score</td>
<td>1.92±0.75</td>
<td>2.49±0.71</td>
<td>2.89±0.98</td>
<td>0.133</td>
<td>0.016</td>
<td>0.515</td>
</tr>
</tbody>
</table>

UIP: usual interstitial pneumonia, NSIP: nonspecific interstitial pneumonia, fNSIP: fibrotic NSIP, cNSIP: cellular NSIP, OP: organizing pneumonia, VC: vital capacity, TLC: total lung capacity, A-aDO₂: alveolar-arterial oxygen difference. Data are shown as mean±SD.
Serum and BALF levels of IP-10 were higher in patients with cNSIP/OP-like lesions and aBFL

We measured levels of IP-10 and TARC in serum and BALF by ELISA among four groups (UIP-like lesions, fNSIP-like lesions, and cNSIP/OP-like lesions), aBFL, and healthy control volunteers. The serum IP-10 level of UIP-like lesion was 93.8±77.2pg/ml, fNSIP-like lesion 113.7±81.4pg/ml, cNSIP/OP-like lesion 544.4±536.3pg/ml, aBFL 291.7±147.1pg/ml, and healthy control volunteers 139.1±94.4pg/ml, respectively. The BALF IP-10 level of UIP-like lesion was 47.8±43.5pg/ml, fNSIP-like lesion 179.1±312.0pg/ml, cNSIP/OP-like lesion 506.9±278.4pg/ml, aBFL 518.1±713.2pg/ml, and healthy control volunteers 15.2±13.9pg/ml, respectively.

As shown in fig 1, the serum level of IP-10 in patients with cNSIP/OP-like lesions (544.4±536.3pg/ml) and aBFL (291.7±147.1pg/ml) was significantly higher than that with UIP-like lesions (93.8±77.2pg/ml) (p=0.0171, p=0.0014, respectively). BALF level of IP-10 in patients with cNSIP/OP-like lesions (506.9±278.4pg/ml) and aBFL (518.1±713.2pg/ml) was significantly higher than that with UIP-like lesions (47.8±43.5pg/ml) (p=0.0088, p=0.0008, respectively) and that in healthy control volunteers (15.2±13.9pg/ml) (p=0.0187, p=0.0027, respectively).

Serum and BALF levels of TARC were higher in patients with UIP-like lesions

The serum IP-10 level of UIP-like lesion was 500.3±319.9pg/ml, fNSIP-like lesion 305.3±395.5pg/ml, cNSIP/OP-like lesion 129.1±97.7pg/ml, aBFL 193.0±160.0pg/ml, and healthy control volunteers 100.4±60.4pg/ml, respectively. The BALF IP-10 level of UIP-like lesion was 9.3±11.0pg/ml, fNSIP-like lesion 8.6±13.8pg/ml, cNSIP/OP-like lesion 1.8±4.1pg/ml, aBFL 1.7±6.1pg/ml, and healthy control volunteers 0.0±0.0pg/ml, respectively.

As shown in Fig 2, the serum level of TARC in patients with UIP-like lesions (500.3±319.9pg/ml) were significantly higher than that with cNSIP/OP-like lesions (129.1±97.7pg/ml) and that in healthy control volunteers (100.4±60.4pg/ml) (p=0.0461, p=0.0126, respectively). The serum level of TARC in patients with fNSIP-like lesions was between that with UIP-like lesions and cNSIP/OP-like lesions.

The TARC to IP-10 ratio in BALF of UIP-like lesion was 0.399±0.73, fNSIP-like lesion 0.114±0.16, cNSIP/OP-like lesion 0.005±0.01, aBFL 0.003±0.01, and healthy control volunteers 0.000±0.00, respectively. As shown in Fig 3, the TARC to IP-10 ratio of serum in patients with UIP-like lesions (8.351±7.60) was about 27.4 times higher than in patients with...
cNSIP/OP-like lesions (0.305±0.26), about 10.8 times than that in aBFL (0.775±0.64), and about 8.5 times higher than that in healthy control volunteers (0.978±0.69) (p=0.0035, p=0.0006, p=0.0194, respectively). The TARC to IP-10 ratio of serum in patients with fNSIP-like lesions (2.731±2.20) was about 9.0-fold increased over patients with cNSIP/OP-like lesions (0.305±0.26) (p=0.0338).

**CCR4- to CXCR3-positive cell ratio in UIP-like lesions was significantly higher than that in fNSIP-like lesions and cNSIP/OP-like lesions**

Intense IP-10 immunostaining was found in epithelial cells, macrophages, fibroblasts, and endothelial cells in surgical biopsied tissues of UIP-like lesions, fNSIP-like lesions, and cNSIP/OP-like lesions (Fig 4A, 4B, 4C). Marked TARC immunostaining was found in epithelial cells in surgical biopsied specimens of UIP-like lesions, fNSIP-like lesions, and cNSIP/OP-like lesions (Fig 4D, 4E, 4F). Many mononuclear cells showed as CXCR3 positive cells in both lymphoid clusters and fibrosing areas in each groups (Fig 4A, 4B, 4C). CXCR3-positive cells were also positive for CD3 (T cells lymphocytes) (data not shown). Many mononuclear cells were CCR4-positive in surgical biopsied specimens of UIP-like lesions (Fig 4D), but few of mononuclear cells were CCR4 positive in surgical biopsied specimens of fNSIP-like and cNSIP/OP-like lesions (Fig 4E, 4F). CCR4-positive cells were CD3-positive lymphocytes (data not shown). In lymphoid clusters, the CCR4- to CXCR3-positive cell ratio in UIP-like lesions (0.362±0.124) was significantly higher than that in fNSIP-like lesions (0.231±0.062) and cNSIP/OP-like lesions (0.123±0.078) (p=0.0060, p=0.00007, respectively). In fibrosing areas, the CCR4- to CXCR3-positive cell ratio in UIP-like lesions (0.428±0.183) was significantly higher than that in fNSIP-like lesions (0.244±0.134) and cNSIP/OP-like lesions (0.082±0.036) (p=0.0111, p=0.00013, respectively), too (Fig 5). The ratio of CCR4- to CXCR3-positive cell and serum TARC to IP-10 were normalized by conversion to logarithms. The logarithm of CCR4- to CXCR3-positive cell ratio correlated with that of serum TARC to IP-10 ratio in both lymphoid clusters (r=0.422, p=0.0349) and fibrosing areas (r=0.600, p=0.0011) (Fig 6).

**DISCUSSION**

In further studies on the pathology of cBFL, this study evaluates the relevance of IP-10 and TARC levels in both serum and BALF, and also the number of CXCR3- and CCR4-positive lymphocytes in histological patterns of tissue from patients. The present study demonstrated several factors that are likely to participate in the progression of the lesions of cBFL.

The higher ratio of TARC to IP-10 in serum and BALF, and CCR4- to CXCR3-positive cells in lung specimen were observed in patients with UIP-like lesions compared to patients with cNSIP/OP-like lesions. On the contrary, the lower ratio of TARC to IP-10 in serum and BALF, and the lower ratio of CCR4 to CXCR3 in lung specimen were revealed in patients with cNSIP/OP-like lesions compared to those with UIP-like lesions. These results suggest a shift to a Th2-immune response, and that this response plays a role in the progression of UIP-like lesions. Moreover, a shift to a Th1-predominant immune response was shown in the progression of cNSIP/OP-like lesions.

Acute HP has been considered as a immunological disease predominated by Th1. C57BL/6 mice, which are genetically Th1-prone, were susceptible to *Saccharopolyspora rectivirgula* (SR) and developed an acute disease, in
contrast Th2-prone DBA/2 mice were resistant to SR.[19] Mice with no expression of the gene coding for interferon-gamma developed minimal inflammation and no granulomas after exposure to SR.[20] IFN-gamma may mediate the recruitment of CXCR3-positive lymphocytes into the lung via the production of IP-10 in mice and in patients of acute HP resulting in T1-cell alveolitis and granuloma formation.[8][21] In a human study of gene expression profiles, IP-10 is thought to be essential to recruit activated T cells through the chemokine receptor CXCR3 and has been associated with Th1 immune responses in acute HP.[22] IL-10-deficient mice exposed to SR resulted in an increase in alveolitis associated with the up-regulation of IFN-gamma.[23] Th2 cells may have important anti-inflammatory properties in acute HP as observed in a murine model exposed to SR, showing that inflammatory responses were attenuated by infusing IL-4.[24] Severe alveolitis and granuloma formation of the lung are common lesions in aBFL and cNSIP/OP-like lesions, whereas they are few in UIP-like lesions of cBFL. Granuloma formation was found in 42.9% of cNSIP/OP-like lesions, but only in 25.0% of fNSIP-like lesions, and was not observed in UIP-like lesions.[3] The ground-glass score on HRCT in patients with cNSIP/OP-like lesions was significantly higher than in patients with UIP-like lesions. As the ground-glass score correlated with the pathological inflammatory score as previously described.[17] cNSIP/OP-like lesions showed more severe interstitial inflammation on HRCT and histological specimens compared to UIP-like lesions. Therefore a shift to a Th1-predominant immune response may play an important role in the pathology of aBFL and cNSIP/OP-like lesions.

TARC (Th2-type chemokine) and its receptor CCR4 have a crucial role in the development of pulmonary fibrosis in the bleomycin mouse model and in the model of radiation pneumonitis in rats.[12][25] The present study showed a higher ratio of TARC to IP-10 in serum and BALF in UIP-like lesions compared to cNSIP/OP-like lesions. We analyzed the levels of IP-10 and TARC in serum and BALF in 12 patients with IPF, showing a higher ratio as compared to those in healthy control volunteers (Serum; 11.28±9.82 and 0.252±0.40, respectively; p=0.014) (BALF; 0.978±0.69 and 0.000±0.00, respectively; p=0.119). The ratio of TARC to IP-10 was similar to that in UIP-like lesions of cBFL in this study. An imbalance in CXCR3/CCR4 expression on BAL CD4 lymphocytes and reduced IP-10 levels of BAL in patients with IPF were pivotal in the progression of IPF.[7] Furthermore, a shift to a Th2 of immune response has been described as crucial in the progression of IPF.[4][5][6] TARC might be important for the development of pulmonary fibrosis. We found the fibrosis score on HRCT in patients with UIP-like lesions were significantly higher than that with cNSIP/OP-like lesions. As UIP-like lesions have more dense interstitial fibrosis compared to cNSIP/OP-like lesions on histological specimens, the fibrosis score strongly correlated with the pathological fibrosis score.[17] These observations suggest that a shift to Th2 immune response plays a critical role in the pathology of UIP-like lesions. It has been shown that TARC was detectable in patients with IPF, but not in control subjects and the ratio of CCR4-positive to CXCR3-positive cell in IPF/UIP was significantly greater than that in idiopathic NSIP and in IP associated with collagen vascular diseases.[7][26-28] Not only similar pathological findings but also a shift to a Th2 immune response observed in patients with IPF is likely to occur in patients with UIP-like lesions of cBFL.

In conclusion, a shift to a Th1 immune response may play an important role in the pathology of cNSIP/OP-like lesions. On the contrary, a shift to a Th2 immune response may play a critical role in the pathology of UIP-like lesion.
The Th1 and Th2 balance may contribute to the progression of lung inflammation and lung fibrosis leading to the each histological pattern and the clinical type in cBFL.

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REFERENCES


LEGEND FOR FIGURES

Figure 1. Levels of IP-10 in serum and BALF. (A) Serum levels of IP-10. (B) BALF levels of IP-10. Data are presented as median with interquartile range represented by boxes and whiskers. Control: healthy control volunteers. Serum and BALF levels of IP-10 were higher in patients with cNSIP/OP-like lesion and aBFL.

Figure 2. Levels of TARC in serum and BALF. (A) Serum levels of TARC. (B) BALF levels of TARC. Data are presented as median with interquartile range represented by boxes and whiskers. Control: healthy control volunteers. Serum and BALF levels of TARC were higher in patients with UIP-like lesion.

Figure 3. TARC to IP-10 ratio of serum and BALF. (A) TARC to IP-10 ratio of serum. (B) TARC to IP-10 ratio of BALF. Data are presented as median with interquartile range represented by boxes and whiskers. Control: healthy control volunteers. TARC to IP-10 ratio of serum and BALF were higher in patients with UIP-like lesion.

Figure 4. The localization of CXCR3 and IP-10 using an immunohistochemical double-staining in UIP-like lesion (A), fNSIP-like lesion (B), cNSIP-like lesion (C), and the localization of CCR4 and TARC using an immunohistochemical double-staining in UIP-like lesion (D), fNSIP-like lesion (E), cNSIP-like lesion (F). Inserts; high-power fields of each tissue section. CXCR3 was stained with brown, IP-10 was stained with red. CCR4 was stained with brown, TARC was stained with red. Scale bars: 150 µm , and 15 µm in inserts.

Figure 5. CCR4- to CXCR3-positive cell ratio in lung specimens. (A) lymphoid clusters. (B) Fibrosing areas. Data are presented as median with interquartile range represented by boxes and whiskers. CCR4- to CXCR3-positive cell ratio in UIP-like lesion was significantly higher than in fNSIP-like lesion and cNSIP/OP-like lesion.

Figure 6. Correlations between a logarithm of CCR4- to CXCR3-positive cell ratio and serum TARC to IP-10 ratio. The logarithm of CCR4- to CXCR3-positive cell ratio correlated with that of serum TARC to IP-10 ratio in both lymphoid clusters (r=0.422, p=0.0349) (A) and fibrosing areas (r=0.600, p=0.0011) (B).
Figure 1.
Figure 2.
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Figure 6.
Pathogenesis of cBFL in common with IPF?
Correlation of IP-10/TARC ratio with histological patterns

Masato Kishi, Yasunari Miyazaki, Torahiko Jinta, Haruhiko Furusawa, Yoshio Ohtani, Naohiko Inase and Yasuyuki Yoshizawa

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