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

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ABSTRACT

Background: A Th1 predominant immune response has been shown in acute hypersensitivity pneumonitis. Predominance of Th2 appears to favour the development of pulmonary fibrosis through the profibrotic process and has been described as crucial in the progression of idiopathic pulmonary fibrosis. Chronic bird fancier's lung (cBFL) can present with 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 chemokines (interferon-inducible protein, IP-10) and Th2-type chemokines (thymus- and activation-regulated chemokine, TARC) and their receptors (CXCR3 and CCR4) to the histological patterns of cBFL, 40 patients with cBFL who underwent surgical lung biopsies, 12 with acute BFL (aBFL) and 10 healthy volunteers were analysed. IP-10 and TARC levels in serum and bronchoalveolar lavage (BAL) fluid were measured by ELISA. Immunohistochemistry for CXCR3 and CCR4 was performed on surgical lung specimens.

Results: The ratio of TARC to IP-10 in the serum of patients with UIP-like lesions was significantly higher than in patients with cNSIP/OP-like lesions, aBFL and healthy volunteers. The ratio of CCR4 to CXCR3 in patients with UIP-like lesions was significantly higher than in those with cNSIP/OP-like lesions and fNSIP-like lesions. The ratio of CCR4-positive 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 idiopathic pulmonary fibrosis. A Th1 predominance may play a role in the development of cNSIP/OP-like lesions in cBFL.

Hypersensitivity pneumonitis (HP) is an immunologically mediated lung disease induced by inhalation of antigens contained in a variety of organic dusts. Bird fancier's lung (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 they present with chronic HP because exposure to birds tends to be chronic with small amounts of antigens.[1] Chronic BFL (cBFL) 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 those with 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 from patients with cBFL have various histological patterns such as usual interstitial pneumonia (UIP)-like lesions, fibrotic non-specific interstitial pneumonia (fNSIP)-like lesions, cellular NSIP (cNSIP)-like lesions and organising pneumonia (OP)-like lesions as seen in idiopathic interstitial pneumonias. The prognosis of patients with cBFL is variable based on each of their histological patterns.[3]

T helper types 1 and 2 cells (Th1 and Th2) are thought to play a crucial role in fibrogenesis.[4] Pulmonary Th1 cells, which produce interferon-γ (IFNγ), are likely to inhibit fibrosing processes[5] whereas a switch to Th2-type immune response with concomitant release of interleukin 4 (IL-4) is essential 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 idiopathic pulmonary fibrosis (IPF) since the CCR4/CXCR3 ratio of CD4 lymphocytes in bronchoalveolar lavage (BAL) fluid was higher in patients with IPF than in non-IPF patients, those with 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] IFNγ-inducible protein (IP-10, CXCL10) is a chemokine of the CXC subfamily and is the ligand for the receptor CXCR3. In acute HP, IFNγ 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] Thymus- and activation-regulated chemokine (TARC, CCL17) is a chemokine of the CC subfamily and is the ligand for the receptor CCR4; it is crucial in the development of pulmonary fibrosis in the bleomycin mouse model.[12]

We therefore hypothesised 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. We have evaluated the relevance of IP-10 and TARC levels in serum and BAL fluid and CXCR3- and CCR4-positive lymphocytes in surgical biopsy specimens to analyse 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

A retrospective review was conducted between April 1993 and March 2006 of the medical records of patients admitted to Tokyo Medical and Dental University; 40 patients with cBFL who underwent surgical lung biopsies, 12 patients with acute BFL (aBFL) and 10 healthy volunteers with no avian exposure were analysed.

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 CT scans; and either (6) progressive deterioration of a restrictive impairment on pulmonary function for 1 year or (7) respiratory symptoms related to HP for more than 6 months.[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 proposed by the joint ATS/ERS statement 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 BAL fluid to phosphodiesterase were measured by an enzyme-linked immunosorbent assey (ELISA) and the antigen-induced lymphocyte proliferation test was performed as previously described.[16]

High-resolution CT (HRCT) scoring

HRCT scans were reviewed independently by four experienced respiratory physicians (YM, MK, TJ, HF). 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 *et al*.[17] Each lobe of the lung was scored on a scale of 0–5. The scores for each lobe were averaged for all four readers for data analysis.

Bronchoalveolar lavage (BAL)

BAL was performed as previously described[18] using three 50 ml aliquots of sterile 0.9% saline. The cellular composition of the BAL fluid 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

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

Immunohistochemical analysis

Surgical lung biopsy specimens were analysed using an immunohistochemical double stain method as previously described[18] to determine the localisation of CXCR3 and IP-10, or CCR4 and TARC. Paraffin-embedded 4 μm sections in antigen unmasking solution (Vector Laboratories, Burlingame, USA) were autoclaved. Endogenous peroxidase was blocked by 3% hydrogen peroxide and endogenous avidin or biotin activity was blocked by Avidin/Biotin Blocking Kit (Vector Laboratories). The sections were incubated with anti-CXCR3 (mouse anti-human IgG; BD Biosciences, San Jose, USA diluted in 1.25 μg/ml) or anti-CCR4 (mouse anti-human IgG; a gift from Kyowa Hakko, Tokyo, Japan diluted in 2.5 μg/ml). The sections were then incubated with biotinylated secondary antibodies (horse anti-mouse IgG; Vector Laboratories). After incubation with ABC kit (Vector Laboratories), DAB (Nichirei, Tokyo, Japan) was added. For a second sequence, any unbound biotin activity resulting from the first step was blocked by the Avidin/Biotin Blocking Kit. The sections were then incubated with anti-IP-10 (rabbit anti-human IgG; Pepro Tech, London, UK diluted in 10 μg/ml) or anti-TARC (goat anti-human IgG; R&D Systems, diluted in 4 μg/ml) overnight at 4°C. Sections were then incubated with biotinylated secondary antibodies (goat anti-rabbit or horse anti-goat IgG; Vector Laboratories). After incubation with AP-ABC kit (Vector Laboratories), Vector Red Substrate Kit and levamisole (Vector Laboratories) was added. Tissue sections were counterstained with Mayer's haematoxylin and mounted with a coverslip.

Semiquantification of CCR4-positive and CXCR3-positive cells

The positive staining of infiltrating mononuclear cells for anti-CXCR3 and CCR4 was 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 (×400) fields (HPFs) of fibrosing areas and in 5 HPFs of lymphoid clusters. Quantification of immunohistochemistry was performed by MK. The positive cells were counted on three separate occasions to obtain the average.

Statistical analysis

Data were analysed using SAS Version 9.1 (SAS Institute, USA). When the data were normally distributed—such as the recovered volume, total cell counts, percentage of macrophages and lymphocytes in BAL fluid, age, percentage of vital capacity and total lung capacity, arterial oxygen tension (Pa_{O_2}), alveolar–arterial difference in oxygen delivery to the lungs ($A\text{–}aD_{O_2}$), fibrosis score, ground glass score and the ratio of CCR4-positive to CXCR3-positive cells—the Tukey-Kramer test was used. When the data were not normally distributed—such as the percentage of neutrophils and eosinophils, ratio of CD4 to CD8 in BAL fluid, serum and BAL fluid levels of IP-10, TARC levels and the ratio of TARC to IP-10—the Steel-Dwass test was used. Correlations between the serum TRAC to IP-10 ratio and the CCR4-positive to CXCR3-positive cell ratio were assessed with the Pearson's correlation coefficient. p Values <0.05 were considered significant. Data are presented as mean (SD) values in the text and in tables 1 and 2 and figs 1, 2, 3 and 5.

RESULTS

Clinical features of cBFL

Forty patients with cBFL were analysed (table 1), of whom 19 had UIP-like lesions (15 men and 4 women, 18 insidious BFL and

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

	UIP (n = 19)	fNSIP (n = 13)	cNSIP/OP (n = 8, 6 cNSIP, 2 OP)	p Value (UIP vs fNSIP)	p Value (UIP vs cNSIP/OP)	p Value (fNSIP vs cNSIP/OP)
M/F	15/4	5/8	3/5			
Age (years)	62.4 (7.3)	58.6 (9.8)	57.1 (10.7)	0.752	0.615	0.996
Percentage of smokers (number of current:ex:never)	63.2% (4:8:7)	30.8% (2:2:9)	50% (2:2:4)			
Onset	Insidious 18 cases; recurrent 1 case	Insidious 8 cases; recurrent 5 cases	Insidious 1 case, recurrent 7 cases			
Outcome	10 cases died	1 case died	All cases survived			
VC (%)	77.5 (25.6)	75.9 (18.0)	91.1 (27.8)	0.998	0.513	0.473
TLC (%)	69.6 (20.0)	85.4 (18.7)	98.9 (20.0)	0.196	0.031	0.603
Pa_{O_2} (kPa)	10.8 (1.2)	10.2 (1.6)	9.1 (1.2)	0.672	0.039	0.362
$A–aD_{O_2}$ (kPa)	2.51 (1.75)	2.56 (1.20)	4.35 (1.69)	0.999	0.049	0.111
Fibrosis score	2.19 (0.71)	2.21 (0.82)	1.29 (0.32)	0.997	0.011	0.015
Ground glass score	1.92 (0.75)	2.49 (0.71)	2.89 (0.98)	0.133	0.016	0.515

Data are shown as mean (SD).
$A–aD_{O_2}$, alveolar-arterial oxygen difference; NSIP, non-specific interstitial pneumonia; cNSIP, cellular NSIP; fNSIP, fibrotic NSIP; OP, organising pneumonia; Pa_{O_2}, arterial oxygen tension; TLC, total lung capacity; UIP, usual interstitial pneumonia; VC, vital capacity.

1 recurrent BFL), 13 had fNSIP-like lesions (5 men and 8 women, 8 insidious BFL and 5 recurrent BFL) and 8 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 between the three groups. Ten of the 19 patients with UIP-like lesions died of respiratory failure as a result of disease progression. The mean (SD) 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 with corticosteroids only. The percentage of total lung capacity (TLC) in patients with UIP-like lesions was significantly lower than that in patients with cNSIP/OP-like lesions (69.6 (20.0)% vs 98.9 (20.0)%, $p = 0.031$). No differences were detected in the percentage of VC. $A–aD_{O_2}$ in patients with cNSIP/OP-like lesions was significantly higher than in patients with UIP-like lesions (4.35 (1.69) kPa vs 2.51 (1.75) kPa, $p = 0.049$). Pa_{O_2} was significantly lower in patients with cNSIP/OP-like lesions than in patients with UIP-like lesions (9.1 (1.2) kPa vs 10.8 (1.2) kPa, $p = 0.039$). 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) vs 1.29 (0.32), $p = 0.011$; and 2.21 (0.82) vs 1.29 (0.32), $p = 0.015$). The ground glass score in patients with cNSIP/OP-like lesions was significantly higher than in patients with UIP-like lesions (2.89 (0.98) vs 1.92 (0.75), $p = 0.016$).

In BAL fluid 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 with patients with fNSIP-like lesions (69.5 (41.4) $\times 10^4$ vs 26.9 (12.0) $\times 10^4$, $p = 0.042$, table 2). The percentage of macrophages was significantly lower in patients with cNSIP/OP-like lesions and fNSIP-like lesions than in those with UIP-like lesions (29.9 (28.1)% vs 72.4 (18.0)%, $p<0.001$; and 51.2 (18.9)% vs 72.4 (18.0)%, $p = 0.027$). The percentage of lymphocytes was significantly higher in patients with cNSIP/OP-like lesions than in those with UIP-like lesions and fNSIP-like lesions (67.2 (30.5)% vs 21.7 (16.4)%, $p<0.001$; and 67.2 (30.5)% vs 40.8 (20.8)%, $p = 0.039$).

Serum and BAL fluid levels of IP-10

Levels of IP-10 and TARC in serum and BAL fluid were measured by ELISA in the four groups of patients (UIP-like lesions, fNSIP-like lesions, cNSIP/OP-like lesions and aBFL) and the healthy control volunteers. The mean (SD) serum IP-10 level was 93.8 (77.2) pg/ml in patients with UIP-like lesions, 113.7 (81.4) pg/ml in those with fNSIP-like lesions, 544.4 (536.3) pg/ml in those with cNSIP/OP-like lesions, 291.7 (147.1) pg/ml in patients with aBFL and 139.1 (94.4) pg/ml in healthy control volunteers. The mean (SD) IP-10 level in BAL fluid was 47.8 (43.5) pg/ml in those with UIP-like lesions, 179.1 (312.0) pg/ml in patients with fNSIP-like lesions, 506.9 (278.4) pg/ml in those with cNSIP/OP-like lesions, 518.1 (713.2) pg/ml in those with aBFL and 15.2 (13.9) pg/ml in healthy control volunteers. As shown in fig 1, the serum level of IP-10 in patients with cNSIP/OP-like lesions (544.4 (536.3) pg/ml) and those with aBFL (291.7 (147.1) pg/ml) was significantly higher than in patients with UIP-like lesions (93.8 (77.2) pg/ml) ($p = 0.017$ and

Table 2 Bronchoalveolar lavage fluid profiles

	UIP (n = 16)	fNSIP (n = 10)	cNSIP/OP (n = 6)	aBFL (n = 11)	Control (n = 7)	p Value (UIP vs fNSIP)	p Value (UIP vs cNSIP/OP)	p Value (fNSIP vs cNSIP/OP)
Recovered volume (ml)	86.0 (24.1)	89.6 (12.0)	93.0 (13.0)	89.6 (19.8)	107.4 (10.6)	0.989	0.943	0.997
Total cell counts ($\times 10^4$)	36.6 (17.0)	26.9 (12.0)	69.5 (41.4)	68.6 (37.1)	54.1 (32.2)	0.891	0.131	0.042
Macrophages (%)	72.4 (18.0)	51.2 (18.9)	29.9 (28.1)	14.5 (9.7)	90.1 (5.2)	0.027	<0.001	0.124
Lymphocytes (%)	21.7 (16.4)	40.8 (20.8)	67.2 (30.5)	81.9 (9.9)	8.8 (4.8)	0.063	<0.001	0.039
Neutrophils (%)	4.5 (6.8)	5.3 (7.1)	1.5 (2.0)	1.5 (2.6)	1.4 (1.7)	0.999	0.831	0.921
Eosinophils (%)	1.5 (3.3)	2.1 (3.0)	1.0 (2.0)	2.1 (3.3)	0.0 (0.0)	0.999	0.799	0.825
CD4/CD8 ratio	5.86 (5.9)	2.74 (3.1)	2.77 (4.6)	2.41 (2.7)	Not done	0.434	0.409	0.999

aBFL, acute bird fancier's lung; cNSIP, cellular NSIP; fNSIP, fibrotic NSIP; NSIP, non-specific interstitial pneumonia; OP, organising pneumonia; UIP, usual interstitial pneumonia.
Data are shown as mean (SD).

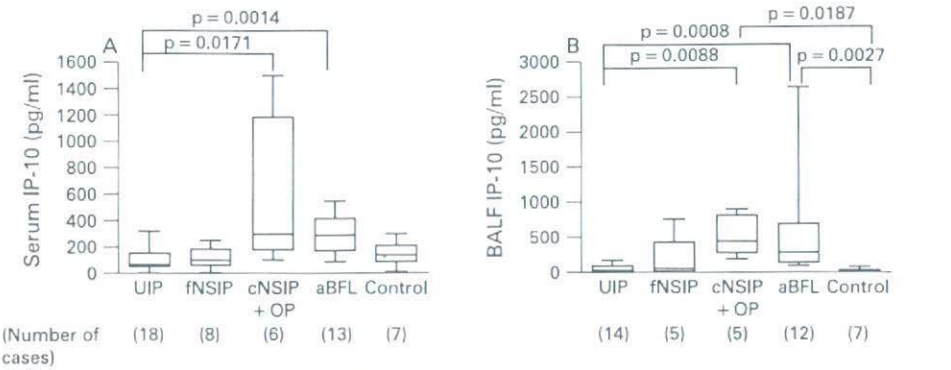

Figure 1 Levels of interferon-inducible protein (IP-10) in (A) serum and (B) bronchoalveolar lavage fluid (BALF). Data are presented as median with interquartile range represented by boxes and whiskers. Serum and BALF levels of IP-10 were higher in patients with cNSIP/OP-like lesions and aBFL. aBFL, acute bird fancier's lung; cNSIP, cellular non-specific interstitial pneumonia; fNSIP, fibrotic non-specific interstitial pneumonia; OP, organising pneumonia; UIP, usual interstitial pneumonia. Controls are healthy control volunteers.

Comparison	p value	Comparison	p value
UIP, fNSIP	0.9473	fNSIP, aBFL	0.0530
UIP, cNSIP/OP	0.0171	fNSIP, control	0.9663
UIP, aBFL	0.0014	cNSIP/OP, aBFL	0.9705
UIP, control	0.7121	cNSIP/OP, control	0.1901
fNSIP, cNSIP/OP	0.0948	aBFL, control	0.2074

Comparison	p value	Comparison	p value
UIP, fNSIP	0.9885	fNSIP, aBFL	0.3000
UIP, cNSIP/OP	0.0088	fNSIP, control	0.3339
UIP, aBFL	0.0008	cNSIP/OP, aBFL	0.7587
UIP, control	0.2429	cNSIP/OP, control	0.0187
fNSIP, cNSIP/OP	0.3650	aBFL, control	0.0027

p = 0.001, respectively). The BAL fluid level of IP-10 in patients with cNSIP/OP-like lesions (506.9 (278.4) pg/ml) and aBFL (518.1 (713.2) pg/ml) was significantly higher than in those with UIP-like lesions (47.8 (43.5) pg/ml) (p = 0.009 and p<0.001, respectively) and in healthy control volunteers (15.2 (13.9) pg/ml) (p = 0.019 and p = 0.003, respectively).

Serum and BAL fluid levels of TARC

The serum TARC level was 500.3 (319.9) pg/ml in patients with UIP-like lesions, 305.3 (395.5) pg/ml in those with fNSIP-like lesions, 129.1 (97.7) pg/ml in those with cNSIP/OP-like lesions, 193.0 (160.0) pg/ml in those with aBFL and 100.4 (60.4) pg/ml in healthy control volunteers. The BAL fluid TARC level was 9.3 (11.0) pg/ml in patients with UIP-like lesions, 8.6 (13.8) pg/ml in those with fNSIP-like lesions, 1.8 (4.1) pg/ml in those with cNSIP/OP-like lesions, 1.7 (6.1) pg/ml in those with aBFL and 0.0 (0.0) pg/ml in healthy control volunteers. As shown in fig 2, the serum level of TARC in patients with UIP-like lesions (500.3 (319.9) pg/ml) was significantly higher than in those with cNSIP/OP-like lesions (129.1 (97.7) pg/ml) and in healthy control volunteers (100.4 (60.4) pg/ml) (p = 0.046 and p = 0.013, respectively). The serum level of TARC in patients with fNSIP-like lesions was between the levels in patients with UIP-like lesions and those with cNSIP/OP-like lesions.

Ratio of TARC to IP-10 in serum and BAL fluid

The ratio of TARC to IP-10 was 8.351 (7.60) in the serum of patients with UIP-like lesions, 2.731 (2.20) in those with fNSIP-like lesions, 0.305 (0.26) in those with cNSIP/OP-like lesions, 0.775 (0.64) in patients with aBFL and 0.978 (0.69) in healthy control volunteers. The TARC to IP-10 ratio in the BAL fluid was 0.399 (0.73) in patients with UIP-like lesions, 0.114 (0.16) in those with fNSIP-like lesions, 0.005 (0.01) in those with cNSIP/OP-like lesions, 0.003 (0.01) in patients with aBFL and 0.000 (0.00) in healthy control volunteers. As shown in fig 3, the ratio of TARC to IP-10 in the serum of 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 in those with aBFL (0.775 (0.64)) and about 8.5 times higher than in healthy control volunteers (0.978 (0.69)) (p = 0.004, p<0.001, p = 0.020, respectively). The TARC to IP-10 ratio of serum in patients with fNSIP-like lesions (2.731 (2.20)) was about 9-fold increased over patients with cNSIP/OP-like lesions (0.305 (0.26), p = 0.034).

Ratio of CCR4-positive to CXCR3-positive cells

Intense IP-10 immunostaining was found in epithelial cells, macrophages, fibroblasts and endothelial cells in surgical biopsy tissues of UIP-like lesions, fNSIP-like lesions and cNSIP/OP-like

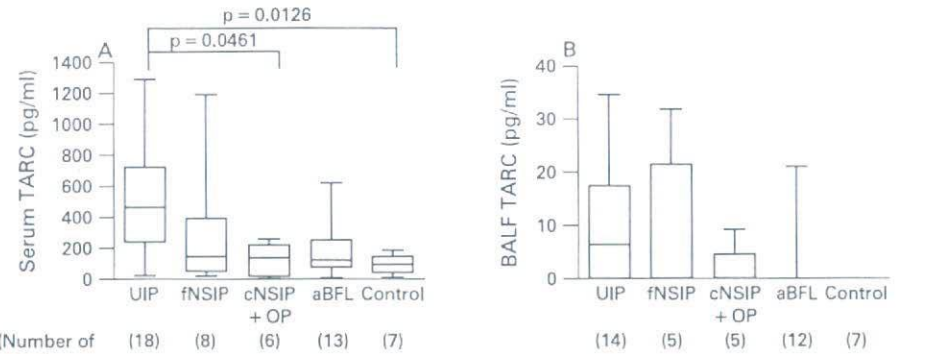

Figure 2 Levels of thymus- and activation-regulated chemokine (TARC) in (A) serum and (B) bronchoalveolar lavage fluid (BALF). Data are presented as median with interquartile range represented by boxes and whiskers. Serum and BALF levels of TARC were higher in patients with UIP-like lesions. aBFL, acute bird fancier's lung; cNSIP, cellular non-specific interstitial pneumonia; fNSIP, fibrotic non-specific interstitial pneumonia; OP, organising pneumonia; UIP, usual interstitial pneumonia. Controls are healthy control volunteers.

Comparison	p value	Comparison	p value
UIP, fNSIP	0.4380	fNSIP, aBFL	0.9999
UIP, cNSIP/OP	0.0461	fNSIP, control	0.8131
UIP, aBFL	0.0655	cNSIP/OP, aBFL	0.9834
UIP, control	0.0126	cNSIP/OP, control	0.9638
fNSIP, cNSIP/OP	0.9332	aBFL, control	0.4332

Comparison	p value	Comparison	p value
UIP, fNSIP	0.9905	fNSIP, aBFL	0.5379
UIP, cNSIP/OP	0.5788	fNSIP, control	0.3845
UIP, aBFL	0.2026	cNSIP/OP, aBFL	0.9780
UIP, control	0.1750	cNSIP/OP, control	0.7448
fNSIP, cNSIP/OP	0.8882	aBFL, control	0.9353

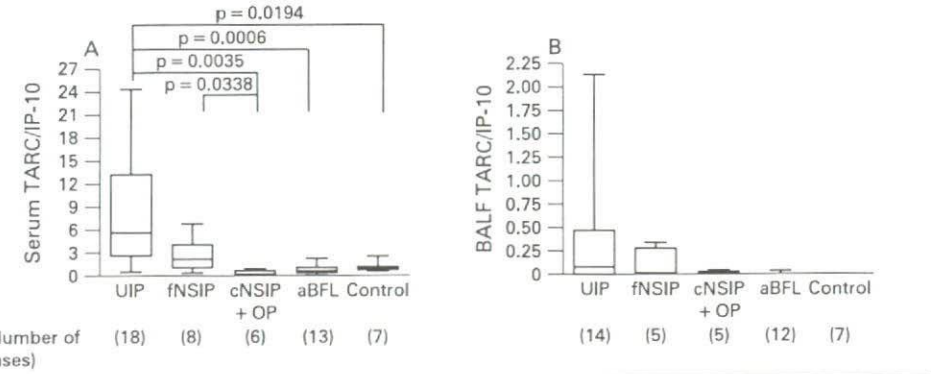

Figure 3 Ratio of thymus- and activation-regulated chemokine (TARC) to interferon-inducible protein (IP-10) in (A) serum and (B) broncholaveolar lavage fluid (BALF). Data are presented as medians with interquartile range represented by boxes and whiskers. The TARC to IP-10 ratios in serum and BALF were higher in patients with UIP-like lesions. aBFL, acute bird fancier's lung; cNSIP, cellular non-specific interstitial pneumonia; fNSIP, fibrotic non-specific interstitial pneumonia; OP, organising pneumonia; UIP, usual interstitial pneumonia. Controls are healthy control volunteers.

Comparison	p value	Comparison	p value
UIP, fNSIP	0.3073	fNSIP, aBFL	0.0774
UIP, cNSIP/OP	0.0035	fNSIP, control	0.2673
UIP, aBFL	0.0006	cNSIP/OP, aBFL	0.2862
UIP, control	0.0194	cNSIP/OP, control	0.1006
fNSIP, cNSIP/OP	0.0338	aBFL, control	0.8499

Comparison	p value	Comparison	p value
UIP, fNSIP	0.9906	fNSIP, aBFL	0.4357
UIP, cNSIP/OP	0.5793	fNSIP, control	0.3845
UIP, aBFL	0.1013	cNSIP/OP, aBFL	0.9780
UIP, control	0.1754	cNSIP/OP, control	0.7448
fNSIP, cNSIP/OP	0.8882	aBFL, control	0.9353

lesions (fig 4A–C). Marked TARC immunostaining was found in epithelial cells in surgical biopsy specimens of UIP-like lesions, fNSIP-like lesions and cNSIP/OP-like lesions (fig 4D–F). Many mononuclear cells showed as CXCR3-positive cells in both lymphoid clusters and fibrosing areas in each group (fig 4A–C). CXCR3-positive cells were also positive for CD3 (T cell lymphocytes) (data not shown). Many mononuclear cells were CCR4-positive in surgical biopsy specimens of UIP-like lesions (fig 4D) but few mononuclear cells were CCR4-positive in surgical biopsy specimens of fNSIP-like and cNSIP/OP-like lesions (fig 4E, F). CCR4-positive cells were CD3-positive lymphocytes (data not shown). In lymphoid clusters the ratio of CCR4-positive to CXCR3-positive cells 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)) ($p = 0.006$ and $p<0.001$, respectively). In fibrosing areas the ratio of CCR4-positive to CXCR3-positive cells in patients with UIP-like lesions (0.428 (0.183)) was also significantly higher than in those with fNSIP-like lesions (0.244 (0.134)) and cNSIP/OP-like lesions (0.082 (0.036)) ($p = 0.011$ and $p<0.001$, respectively, fig 5). The ratios of CCR4-positive to CXCR3-positive cells and serum TARC to IP-10 were normalised by conversion to logarithms. The logarithm of the ratio of CCR4-positive to CXCR3-positive cells correlated with the ratio of serum TARC to IP-10 in both lymphoid clusters ($r = 0.422$, $p = 0.035$) and fibrosing areas ($r = 0.600$, $p = 0.001$; fig 6).

DISCUSSION

This study has evaluated the relevance of serum and BAL fluid levels of IP-10 and TARC and the number of CXCR3- and CCR4-positive lymphocytes in histological patterns of tissue from patients with cBFL. Several factors were identified that may participate in the progression of the lesions of cBFL.

A higher TARC to IP-10 ratio in the serum and BAL fluid and a higher ratio of CCR4-positive to CXCR3-positive cells in lung specimens were observed in patients with UIP-like lesions compared with patients with cNSIP/OP-like lesions. On the other hand, a lower TARC to IP-10 ratio in the serum and BAL fluid and a lower ratio of CCR4-positive to CXCR3-positive cells in lung specimens were seen in patients with cNSIP/OP-like lesions compared with those with UIP-like lesions. These results suggest a shift to a Th2 immune response, and this response may have a role in the progression of UIP-like lesions. Moreover, a shift to a Th1 predominant immune response was seen 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 which were resistant to SR.[19] Mice with no expression of the gene coding for IFNγ developed minimal inflammation and no granulomas after exposure to SR.[20] IFNγ

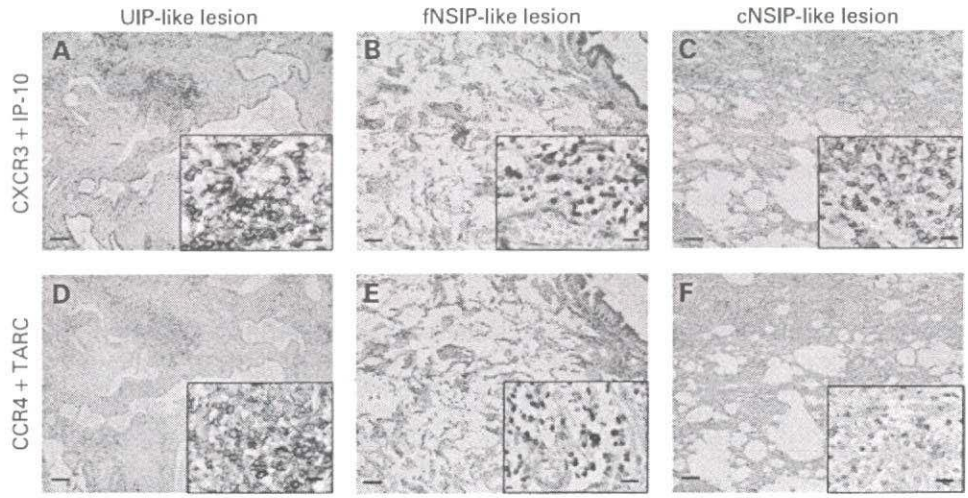

Figure 4 Localisation of CXCR3 and interferon-inducible protein (IP-10) using an immunohistochemical double staining in (A) UIP-like lesions, (B) fNSIP-like lesions, (C) cNSIP-like lesions and the localisation of CCR4 and thymus- and activation-regulated chemokine (TARC) using an immunohistochemical double staining in (D) UIP-like lesions, (E) fNSIP-like lesions, (F) cNSIP-like lesions. Inserts show 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. cNSIP, cellular non-specific interstitial pneumonia; fNSIP, fibrotic non-specific interstitial pneumonia; UIP, usual interstitial pneumonia.

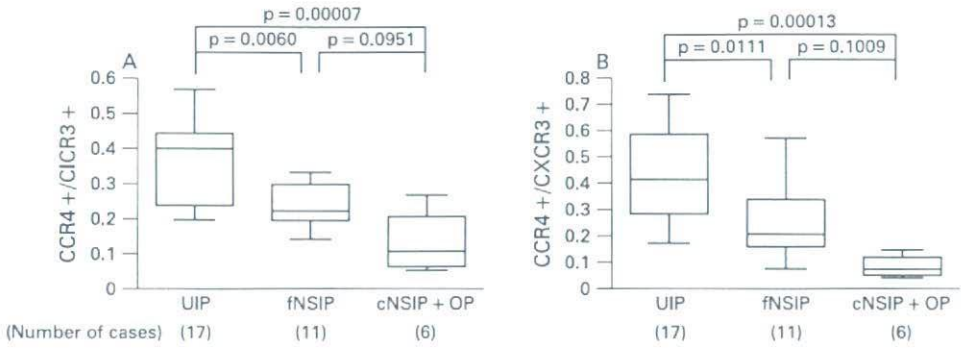

Figure 5 Ratio of CCR4-positive to CXCR3-positive cells in lung specimens: (A) lymphoid clusters; (B) fibrosing areas. Data are presented as medians with interquartile range represented by boxes and whiskers. The ratio of CCR4-positive to CXCR3-positive cells in patients with UIP-like lesions was significantly higher than in those with fNSIP-like lesions and cNSIP/OP-like lesions. cNSIP, cellular non-specific interstitial pneumonia; fNSIP, fibrotic non-specific interstitial pneumonia; OP, organising pneumonia; UIP, usual interstitial pneumonia.

may mediate the recruitment of CXCR3-positive lymphocytes into the lung via the production of IP-10 in mice and in patients with acute HP, resulting in Tc1 cell alveolitis and granuloma formation.[8 21] In a human study of gene expression profiles, IP-10 is thought to be essential for recruitment of 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 upregulation of IFNγ.[23] Th2 cells may have important anti-inflammatory properties in acute HP, as observed in a murine model exposed to SR which showed 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 the HRCT scan 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 the HRCT scan and histological specimens than UIP-like lesions. A shift to a Th1 predominant immune response may therefore 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 BAL fluid in UIP-like lesions than in cNSIP/OP-like lesions. We analysed the levels of IP-10 and TARC in serum and BAL fluid in 12 patients with IPF and found a higher ratio than in healthy control volunteers (serum: 11.28 (9.82) and 0.252 (0.40), respectively; $p = 0.014$) (BAL fluid: 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 fluid CD4 lymphocytes and reduced IP-10 levels in the BAL fluid in patients with IPF were pivotal in the progression of IPF.[7] Furthermore, a shift to a Th2 immune response has been described as crucial in the progression of IPF.[4–6]

TARC might be important for the development of pulmonary fibrosis. We found the fibrosis score on the HRCT scan in patients with UIP-like lesions was significantly higher than in those with cNSIP/OP-like lesions. As UIP-like lesions have more dense interstitial fibrosis than cNSIP/OP-like lesions on histological specimens, the fibrosis score is strongly correlated with the pathological fibrosis score.[17] These observations suggest that a shift to a 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 cells in IPF/UIP was significantly greater than 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. A shift to a Th2 immune response may play a critical role in the pathology of UIP-like lesions. The balance between Th1 and Th2 may contribute to the progression of lung inflammation and lung fibrosis leading to the individual histological patterns and clinical types in cBFL.

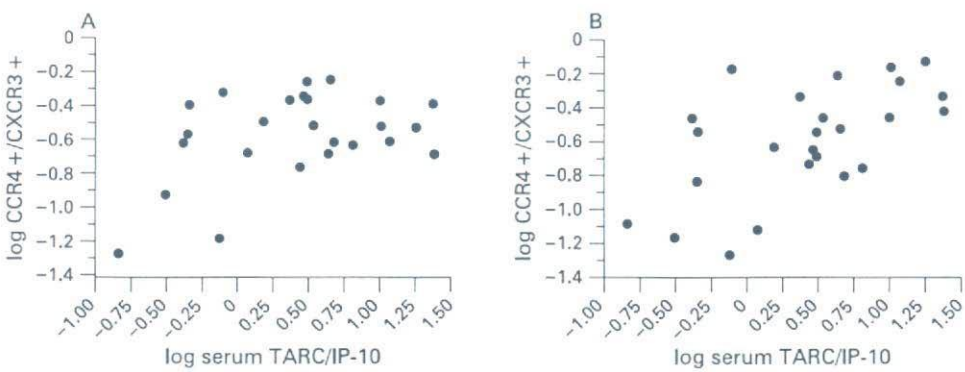

Figure 6 Correlations between logarithm of the CCR4-positive to CXCR3-positive cell ratio and the serum thymus- and activation-regulated chemokine (TARC) to interferon-inducible protein (IP-10) ratio. The logarithm of the CCR4-positive to CXCR3-positive cell ratio correlated with that of the serum TARC to IP-10 ratio in (A) lymphoid clusters ($r = 0.422$, $p = 0.035$) and (B) fibrosing areas ($r = 0.600$, $p = 0.001$).

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Competing interests: None.

Ethics approval: 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.

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CHEST

Original Research

HYPERSENSITIVITY PNEUMONITIS

Clinical Predictors and Histologic Appearance of Acute Exacerbations in Chronic Hypersensitivity Pneumonitis*

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Background: **Acute exacerbations (AEs) in idiopathic pulmonary fibrosis (IPF) are critical factors for its clinical course and prognosis. We have seen AEs and poor prognosis consequent to AE in patients with chronic hypersensitivity pneumonitis (HP), as has been seen in patients with IPF. The aim of this study was to evaluate the clinical features of the patients with AE in those with chronic HP.**

Methods: **We reviewed 100 consecutive patients with chronic bird fancier lung (BFL) from 1993 to 2006, and analyzed the clinical characteristics, including history, and laboratory and immunologic, imaging, BAL, and histologic findings.**

Results: **AE developed in 14 patients during this observation period (AE group), whereas 86 patients remained stable (non-AE [NAE] group). The 2-year frequency of AE among patients with chronic BFL having usual interstitial pneumonia (UIP)-like lesions seen on surgical lung specimens was 11.5%. Patients with AE were more likely to be smokers ($p = 0.003$). In pulmonary function test results, the mean total lung capacity (TLC) and diffusing capacity of the lung for carbon monoxide (DLCO) were lower in patients with AEs (TLC: AE patients, 63.0 ± 16.8%; NAE patients, 81.6 ± 20.0%; DLCO: AE patients, 41.9 ± 19.0%; NAE patients, 60.0 ± 19.4%). The mean number of lymphocytes in BAL fluid were lower (AE patients, 13.7 ± 7.5 lymphocytes; NAE patients, 37.2 ± 29.7 lymphocytes), while the number of neutrophils were greater in AE patients (AE patients, 10.7 ± 17.6 neutrophils; NAE patients, 3.6 ± 4.4 neutrophils). Histologic and/or radiologic findings revealed that all AE patients had UIP-like lesions. Diffuse alveolar damage was observed in six cases, whereas organizing pneumonia superimposed on preexistent fibrotic lesions was observed in two cases.**

Conclusions: **The present study showed several predictive factors for AE at the time of diagnosis. Low TLC and DLCO, low lymphocyte levels in BAL fluid, and a UIP-like pattern in histology at the time of diagnosis may be the risk factors for AE.** ***(CHEST 2008; 134:1265–1270)***

Key words: acute exacerbations; chronic hypersensitivity pneumonitis; diffuse alveolar damage; organizing pneumonia; risk factors

Abbreviations: AE = acute exacerbation; BFL = bird fancier lung; cNSIP = cellular nonspecific interstitial pneumonia; DAD = diffuse alveolar damage; DLCO = diffusing capacity of the lung for carbon monoxide; fNSIP = fibrotic nonspecific interstitial pneumonia; HP = hypersensitivity pneumonitis; HRCT = high-resolution CT; IPF = idiopathic pulmonary fibrosis; NAE = nonacute exacerbation; NSIP = nonspecific interstitial pneumonia; OP = organizing pneumonia; SLBx = surgical lung biopsy; TLC = total lung capacity; UIP = usual interstitial pneumonia

Recently, acute exacerbations (AEs) of idiopathic pulmonary fibrosis (IPF) are well-recognized conditions that are characterized by an acute worsening of dyspnea leading to hypoxemic respiratory failure, and accompanied by new infiltrates on radiologic images.[1,2] The histologic findings of lung biopsy specimens in IPF patients with AEs are diffuse alveolar damage (DAD) with or without hyaline membranes superimposed on usual interstitial pneumonia (UIP).[2–6] AEs have also been reported in patients with idiopathic nonspecific interstitial pneumonia (NSIP), interstitial pneumonia associated with collagen vascular diseases,[7] and in several case reports[8] of UIP-like lesions in patients with chronic hypersensitivity pneumonitis (HP).

Bird fancier lung (BFL) is an HP that is caused by the inhalation of bird-related antigens.[9] Patients can present with acute HP, but it is more likely to be a

chronic progressive disease, since exposure to birds tends to be long term with small amounts of antigens.[10] Chronic BFL is categorized into two subgroups according to the clinical features (recurrent and insidious).[11–13] Chronic BFL sometimes presents as an episode of AE, including case reports[4,14–17] of DAD superimposed on UIP-like lesions in patients with chronic HP. Little is known about the natural course of chronic BFL, especially in patients with AE.[13,18,19] In the present study, we reviewed 100 cases of patients with chronic BFL including 14 patients with AEs, and evaluated their clinical features including the frequency, predictive markers, and radiologic and pathologic characteristics.

Materials and Methods

Patients

A retrospective review of the medical records of patients with chronic BFL who were admitted to our hospital between April 1993 and November 2006 was undertaken. Most of the subjects were included in the previous article.[19] The diagnosis of chronic BFL was made based on clinical, radiologic, and histologic criteria, as was described previously.[11,13,20] We conducted inhalation provocation tests in 50 of 86 non-AE (NAE) patients and in 10 of 14 AE patients. The remaining patients for each group were positive for the reproduction of symptoms of HP by an environmental provocation or the improvement of symptoms by an avoidance of environmental antigen exposure. This study conformed to the Declaration of Helsinki and was approved by the institutional review board. Informed written consent was obtained for each subject.

Criteria of AEs

The criteria of Kondoh et al[4] were adopted to define an episode of AE. These criteria included the following: (1) aggravation of dyspnea within 1 month; (2) hypoxemia with an arterial oxygen tension/inspired oxygen tension ratio of < 225; (3) newly developing pulmonary infiltrates on chest radiographs; and (4) the absence of apparent infection or heart disease.

BAL

We performed BAL as previously described.[20] BAL was performed using three 50-mL aliquots of a sterile 0.9% saline solution. The cellular composition of the BAL fluid was determined using a cytospin smear with Wright stain by counting 200 cells. Lymphocyte phenotypes were performed by flow cytometry with monoclonal antibodies for CD4 and CD8.

Radiologic Assessment

A high-resolution CT (HRCT) scan was performed with standard technical parameters, both at the time of the initial diagnosis and the time of the AE. HRCT scans were reviewed independently by three experienced respiratory physicians. They scored ground-glass opacities for ground-glass scores and reticular opacities for fibrosis scores at the time of the initial diagnosis. The outlines of the scoring system used for the evaluation have been described previously by Kazerooni et al.[21] Each lobe of the lung was scored on a scale of 0 to 5. The scores for each lobe were averaged for all three readers for data analysis. Also HRCT scan findings during the episode of AE were classified as peripheral, multifocal, and diffuse parenchymal opacification, as previously reported by Akira et al.[5]

Pathologic Assessment

Histologic sections of surgical biopsy and/or autopsy materials were stained with hematoxylin-eosin and elastica van Gieson. Fifty-five patients underwent surgical biopsy at the time of the initial diagnosis. The histologic examinations were interpreted blindly by two pulmonary pathologist (T.A. and Y.O.). When the interpretations differed between the two pathologists, the final decision was reached by consensus. The background histologic patterns of chronic HP were classified according to the American Thoracic Society/European Respiratory Society international consensus classification as UIP-like lesions, NSIP-like lesions, and organizing pneumonia (OP)-like lesions based on the quality of fibrotic changes, including loose and dense fibrosis, and the temporal appearance. Patients with NSIP were subdivided into the following two groups: cellular NSIP (cNSIP) pattern group; and fibrotic NSIP (fNSIP) pattern group.[19,22,23] Then, we confirmed the presence of superimposed acute changes in the form of exudative DAD (with hyaline membranes), organizing DAD, or OP.[2]

Statistical Analysis

Data were analyzed using a statistical software package (StatView, version 5.0; SAS Institute, Cary, NC) and were described as the mean ± SD. The two groups were compared using the Mann-Whitney *U* test. Comparisons between groups were performed using a Fisher exact test for categoric variables. All statistical comparisons were two sided, and p values < 0.05 were considered as significant. For the frequency of AE, we evaluated the disease period for each patient at the end of November 2006, and the end point of the analysis was the time of the AE. Then, the frequency of AE was obtained from the Kaplan-Meier survival curve censoring AE, as was previously described by Park et al.[7]

Results

Clinical Features in Chronic BFL With AE

The characteristics of the 100 patients with chronic BFL are summarized in Table 1. Fourteen

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Table 1—*Demographics of the Patients

Characteristics	AE Group (n = 14)	NAE Group (n = 86)	p Value
Gender			0.07
Female	3	41	
Male	11	45	
Age, yr	64.7 ± 8.3	61.9 ± 10.8	NS
Smoking			0.003
Nonsmoker	4	40	
Ex-smoker	10	23	
Current smoker	0	21	
FVC, % predicted	63.5 ± 22.8	73.2 ± 18.6	0.117
VC, % predicted	72.3 ± 24.5	80.9 ± 21.4	0.183
TLC, % predicted	63.0 ± 16.8	81.6 ± 20.0	0.003
DLCO, %	41.9 ± 19.0	60.0 ± 19.4	0.002
Pao_2, mm Hg	75.2 ± 9.5	78.0 ± 11.8	NS
Fibrosis score	2.50 ± 0.57	2.10 ± 0.77	0.122
Ground-glass score	1.75 ± 0.59	2.44 ± 0.84	0.014
Histologic pattern			0.008
UIP	7	19	
fNSIP	1	19	
cNSIP	0	6	
OP	0	3	
Duration of illness, yr	7.9 ± 2.1	6.4 ± 1.8	NS

*Values are given as the mean ± SD or No., unless otherwise indicated. NS = not significant; VC = vital capacity.

patients (14%) were admitted to the hospital due to the acute deterioration of the disease, and those acute episodes were all consistent with the criteria of Kondoh et al[4] for AE (AE group), whereas the remaining 86 patients had experienced no AE during the observation period between April 1993 and November 2006 (NAE group). There was no significant difference in age between AE and NAE patients. AE tended to develop in male patients more often compared to female patients ($p = 0.07$). AE was less likely to develop in nonsmokers compared to smokers ($p = 0.003$). Pulmonary function test results showed more severe impairment in the AE group (total lung capacity [TLC]: AE group, 63.0 ± 16.8%; NAE group, 81.6 ± 20.0%; diffusing capacity of the lung for carbon monoxide [DLCO]: AE group, 41.9 ± 19.0%; NAE group, 60.0 ± 19.4), although no difference was observed in the duration of illness between the two groups. On HRCT scans at the time of the initial diagnosis, the ground-glass scores were lower in AE patients ($p = 0.014$) than in NAE patients, and fibrosis scores tended to be higher in AE patients ($p = 0.122$). Patients with AEs had more UIP-like lesions on histologic evaluation than did patients with NAE ($p = 0.008$). In findings from BAL fluid analysis, patients with AE had fewer lymphocytes and more neutrophils compared to patients with NAE ($p = 0.008$ and $p = 0.005$, respectively) (Table 2).

Table 2—*Profile of BAL

Variables	AE Group (n = 12)	NAE Group (n = 67)	p Value
Total cells, $\times 10^6$	35.1 ± 16.8	35.3 ± 23.9	NS
Macrophages, %	72.9 ± 20.5	57.3 ± 29.5	0.083
Lymphocytes, %	13.7 ± 7.5	37.2 ± 29.7	0.008
Neutrophils, %	10.7 ± 17.6	3.6 ± 4.4	0.005
Eosinophils, %	0.7 ± 0.9	2.2 ± 3.8	NS
CD4/CD8	4.1 ± 5.6	4.1 ± 4.2	NS

*Values are given as the mean ± SD, unless otherwise indicated. See Table 1 for abbreviation not used in the text.

Details of AE Patients: Treatments and Outcome

AEs were seen in 14 patients of 100 patients (14%) with chronic BFL during the observation period. All AE patients had the insidious type of chronic BFL, and no infectious agents were identified. Survivors tended to have a high Pao_2/fraction of inspired oxygen ratio and low lactate dehydrogenase levels (Table 3).

All 14 patients were treated with high-dose systemic corticosteroids (starting with methylprednisolone IV pulse, 500 to 1,000 mg/d for 3 days, then prednisolone, 0.5 to 1 mg/kg po, and gradually was tapered). Three patients were treated with cyclophosphamide, and five patients were treated with cyclosporine combined with a corticosteroid preparation (Table 3). Twelve of the 14 AE patients died of respiratory failure, and 2 patients survived for over 2 years, but the overall mortality rate was 85.7%. Eleven of the 12 deaths occurred within 1 month after the onset of an AE, and one patient survived for 104 days. We analyzed the frequency of AEs among 55 patients with chronic BFL who underwent surgical biopsy (Fig 1). Of the 55 patients, 26 patients had UIP-like lesions, 20 patients had fNSIP, 6 patients had cNSIP, and 3 patients had OP. Of the patients who underwent lung biopsy, AE developed in eight patients (UIP-like lesions, seven patients; fNSIP-like lesions, one patient). The 2-year frequency of AE patients who had UIP-like lesions was 11.5%, the 11-year frequency was 50% in patients with fNSIP-like lesions, and AEs did not develop in those who originally had cNSIP-like lesions and OP-like lesions (Fig 1). Of the 26 biopsied patients who had UIP-like lesions, AEs developed in 7 patients (63.6%) and 4 patients died of other causes, such as infection (27.3%) or chronic respiratory failure (9.1%). Of the 20 biopsied patients who had fNSIP-like lesions, 1 developed an AE (20%) and 4 died of other causes, such as infection (40%) or chronic respiratory failure (40%).

Table 3—*Clinical Data in Cases of Exacerbations**

Case/Age, yr/Gender	Subtype	PaO_2/FIO_2 Ratio	LDH	ES, pg/mL	β-D, pg/mL	Therapy	Duration Between Onset and Death, d	Outcome
1/75/M	Insidious	150	304	< 5	5.1	S, E	14	Dead
2/55/M	Insidious	155	370	7.8	13.3	S	10	Dead
3/57/M	Insidious	217	204	< 5	< 5	S	NA	Alive
4/67/M	Insidious	213	518	11.3	< 5	S, C	104	Dead
5/59/M	Insidious	122	375	< 5	< 5	S, E	30	Dead
6/72/M	Insidious	223	446	11.9	7.7	S, E	16	Dead
7/70/F	Insidious	60	332	< 5	9.1	S, C	30	Dead
8/67/M	Insidious	44	344	< 5	7.3	S, C	14	Dead
9/69/F	Insidious	59	402	< 5	< 5	S	14	Dead
10/67/M	Insidious	223	266	< 5	< 5	S	NA	Alive
11/74/M	Insidious	181	561	< 5	18.1	S	16	Dead
12/75/M	Insidious	62	720	< 0.8	< 5	S, C	7	Dead
13/66/F	Insidious	154	299	< 0.8	12	S	20	Dead
14/63/M	Insidious	77	550	< 0.8	< 6	S, C	14	Dead

*S = steroid; E = cyclophosphamide; C = cyclosporine; ES = endotoxin; β-D = β -D-glucan; LDH = lactate dehydrogenase; NA = not applicable; M = male; F = female; FIO_2 = fraction of inspired oxygen.

Pathologic and Radiologic Assessment

Seven of the 14 AE patients underwent initial surgical lung biopsy (SLBx) at the time of diagnosis, 1 AE patient underwent SLBx during an episode of AE, and autopsy materials were available in 7 patients (Table 4). Of the eight patients who underwent SLBx, one patient had granulomas, six patients had giant cells, and all eight patients showed peribronchiolar fibrosis and fibroblastic foci. We performed a SLBx at the time of AE only in the patient in case 10 (Fig 2), and intraalveolar exudative organization as seen in OP was observed to be superimposed on UIP-like lesions. We evaluated autopsy lung tissue samples from the six patients who died within 1 month of the onset of AE (six cases [cases 1, 2, 5, 6, 9, and 13]), and one patient who died at 104 days after the onset of AE (case 4) [Table 3]. DAD superimposed on UIP-like lesions was observed in six of the seven patients; OP and pulmonary alveolar hemorrhage were observed in one patient, and hyaline membranes were observed in six patients. Both the exudative and organizing phase of DAD were observed in five patients, and organizing DAD in one patient (Table 4).

On an HRCT scan obtained during the episode of AE (Table 4), three patterns of infiltrations (peripheral, diffuse, and multifocal) were observed to be superimposed on preexistent lung fibrosis, as previously described.[7] Two survivors of AEs showed a peripheral shadow on the HRCT scan during the AE episode.

Discussion

In this study, the 2-year frequency of AE among patients with chronic BFL who initially had UIP-like lesions was 11.5%, and the mortality rate was 86%. Low TLC and DLCO, fewer lymphocytes, and an increased percentage of neutrophils found in BAL fluid were the characteristics of the AE patients. Moreover, UIP-like lesions at the onset of the diagnosis were an additional risk factor for the development of AE in patients with chronic BFL. Patients who showed peripheral consolidations superimposed on the preexistent pulmonary fibrosis on HRCT scan at the onset of AE were likely to have a good prognosis.

AEs were initially reported in IPF patients who had an episode of accelerated deterioration.[1,2] However, several reports have documented that AEs have

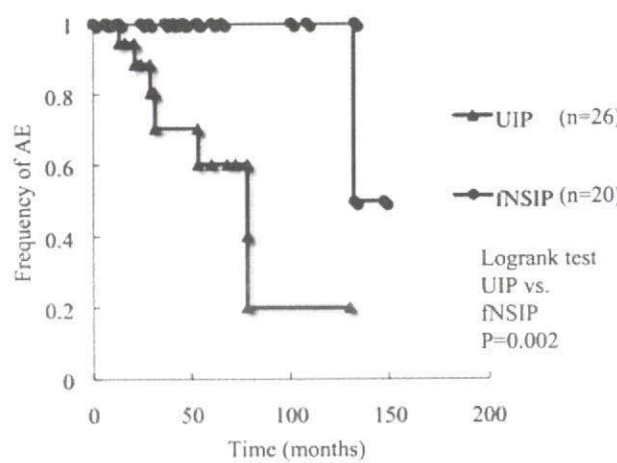

FIGURE 1. The frequency curve of AE for the two groups of patients having various underlying fibrosis lesions (total, 55 patients; UIP-like lesions, 26 patients; fNSIP-like lesions, 20 patients). The 2-year frequency of AE among patients with chronic BFL having UIP-like lesions found on SLBx specimens was 11.5%. The 5-year frequency of UIP-like lesions was 40%. The 11-year frequency of fNSIP-like lesions was 50%.

Table 4—*Pathology and Radiology of SLBx and Autopsies

Case	SLBx	Granuloma	Giant Cells	Peribronchiolar Fibrosis	Fibroblastic Foci	Autopsy/SLBx	Hyaline Membrane	Exudative DAD	Organizing DAD	Organizing Pneumonia (OP)	HRCT
1	UIP	−	−	+	−	UIP + DAD	+	+	−	−	Diffuse
2	UIP	−	−	+	−	UIP + DAD	+	+	+	−	Diffuse
3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Peripheral
4	NA	NA	NA	NA	NA	UIP + DAD	+	+	+	−	Diffuse
5	UIP	−	−	+	−	UIP + DAD	+	+	+	−	Diffuse
6	UIP	−	−	+	−	UIP + DAD	+	+	+	−	Diffuse
7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Diffuse
8	UIP	−	−	+	−	NA	NA	NA	NA	NA	Multifocal
9	UIP	−	−	+	−	UIP + OP	−	−	−	+	Diffuse
10	UIP + OP†	−	−	+	−	UIP + OP†	−	−	−	+	Peripheral
11	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Diffuse
12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Multifocal
13	fNSIP	+	−	+	−	UIP + DAD	+	+	+	−	Multifocal
14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Diffuse

*− = do not have this condition; + = have this condition. See Table 3 for abbreviations not used in the text.
†SLBx was performed at the time of AE.

developed in patients with collagen vascular disease-associated interstitial lung diseases[3] and HP.[4,14,16,17] In the present study, we found 14 episodes of AE defined by the criteria of Kondoh et al[4] among the 100 cases of chronic BFL.

Some reports[3] have suggested that AEs have occurred in the background fibrosis of NSIP-like lesions as well as of UIP-like lesions. We have reported[19] that chronic BFL could present as UIP-like, NSIP-like, and OP-like lesions as background fibrosis and the histology correlated with the prognosis. According to the frequency curve of AE in the various underlying fibrosis patterns seen in Figure 1, the 2-year frequency of AEs in the patients with UIP-like lesions was 11.5%; this percentage is similar to the frequency of AEs found in patients with IPF/UIP (9.6%) reported by Kim et al.[2] We identified only one patient with fNSIP-like lesions revealed by SLBx who later developed AE. The 11-year frequency of AE in the patients having fNSIP-like lesions was 50%, and this frequency is much lower than that in the patients having UIP-like lesions revealed by the log rank test (Fig 1). The autopsy of this patient with initially fNSIP-like lesions at the time of diagnosis showed DAD superimposed on UIP-like lesions. Therefore, the transition of fNSIP-like lesions to UIP-like lesions may have occurred during the 11 years of the clinical course.

In terms of the pathology superimposed on the underlying fibrosis, DAD of the organizing phase was predominant in previous reports.[2,3,6–8] However, at the time of AE, OP-like lesions superimposed on initially UIP-like lesions were observed in this study. Churg et al[8] have also found OP lesions superimposed on UIP in 5 of the 12 AE cases, and these patients all survived. During the episode of AE, OP may be another superimposed lesion as well as DAD.

AE was likely to develop in the smokers in this study. Previous reports[24] have shown a positive

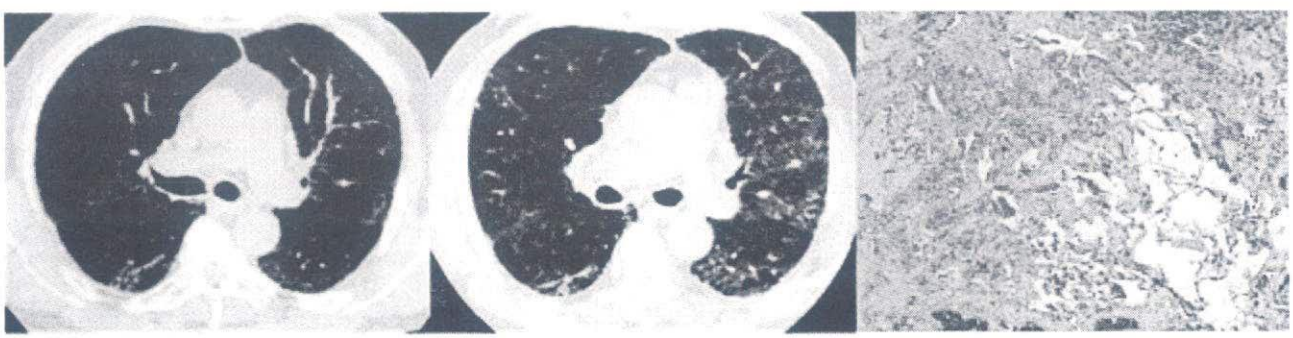

FIGURE 2. In the patient in case 10, a peripheral pattern was found on an HRCT scan during the AE, who survived after the AE. *Left*: HRCT scan at the time of the initial diagnosis. *Middle*: HRCT scan at the onset of AE. *Right*: a pathology analysis of an SLBx lung specimen during the episode of AE showed OP-like and DIP-like lesions (hematoxylin-eosin, original × 30).

correlation between the percentage of lung neutrophils and the severity of lung fibrosis, and the possible role of gelatinase B and collagenase-2 secreted from neutrophils in the fibrosing processes in HP lungs. In addition, increased numbers of neutrophils with few lymphocytes in cell found in BAL fluid were associated with the failure of steroid therapy.[25] These observations suggested that the increased numbers of neutrophils found in BAL fluid at the time of diagnosis among smokers were related to a poor prognosis.

Our data suggested that the patients who have UIP-like lesions are more likely to experience AEs than those who have fNSIP, cNSIP, and OP. In addition, our previous report showed that T-helper type 2 cell bias in the immune response played an important role in the development of UIP-like patterns[26] as seen in IPF patients,[27] and this T-helper type 2 cell bias is more prominent in the AE patients at the time of diagnosis (M.Y. and Y.Y; unpublished data). We are assuming that there is a common pathway between the pathophysiology of AE and the process of the formation of UIP lesions.

In conclusion, the present study showed several predictive factors for AE at the time of the initial diagnosis. Patients with UIP-like lesions have a poor prognosis. Further analysis of these factors will contribute to a better understanding of the pathophysiology of AE.

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Novel Strategies for the Treatment of Asthma

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Abstract: It is now clear that airway inflammatory processes characterized by eosinophils and Th2 lymphocytes are pivotal as the pathological features of asthma. Standard inhaled corticosteroids markedly suppress such inflammatory changes, resulting in clinical beneficial effects. However, it is also notified that airway wall remodeling including goblet cell hyperplasia, sub-epithelial collagen deposits, increased capillary networks and smooth muscle hypertrophy occur as a chronic consequence of this disorder even by the recommended strategies with steroid treatment. These pathologic changes play an important role in the increased airway obstruction and hyperresponsiveness, and eventually in the development of irreversible respiratory failure. Recent studies have elucidated that myofibroblasts and smooth muscle as well as mucosal epithelial cells play a vital role in these processes. Agents regulating proliferation, differentiation and activity of these cells, especially of low-molecular weight compounds, attract attention. Studies on molecular mechanisms of above processes, have led the development and patents of potential drugs including inhibitors of NF kappaB, statins, macrolides and phosphodiesterase-4 inhibitors.

Keywords: Asthma, mucosal epithelial cell, myofibroblast, smooth muscle cell, cytokines, chemokines, growth factor, signal transduction, transcription factor, statins.

INTRODUCTION

Asthma is characterized by allergic inflammatory responses with airway hyperresponsiveness, and its prevalence is increasing in many countries as one of the important socio-medical problems [1]. Both clinical and experimental studies suggest that eosinophils and Th2 type lymphocytes play a key role in the induction of airway inflammation and mucosal injury, which closely links to non-specific hyperresponsiveness in asthma [2]. In fact, inhaled corticosteroids markedly suppress the airway hyperresponsiveness and asthma symptoms along with decreased eosinophil infiltration in the airways. Clinical trials with anti-IL-5 antibody for the treatment of asthma, however, failed to show the clinical improvement despite apparent decrease in eosinophils of the peripheral blood, and hence, posed some doubt about the critical role of this inflammatory cell [3,4].

It is now proved that asthma is a heterogeneous and complex airway disease that involves both inflammatory and "non-inflammatory" processes [5,6]. It has been considered that asthma processes are affected by various interactions between airway epithelial cells and other mesenchymal cells such as myofibroblasts and smooth muscle cells. From viewpoints of the updated understanding, novel strategies are being proposed for better control of this disorder and drugs regulating not only inflammation, but also proliferation, differentiation and apoptosis of these cells attract attention.

UPDATED UNDERSTANDING OF ASTHMA PATHOGENESIS: ROLE OF AIRWAY REMODELING

Airway remodeling in asthma has been implicated as a significant pathological change that includes subepithelial fibrosis, goblet cell hyperplasia, smooth muscle cells prolixferation and microvascular changes (Fig. **1**). Such structural alterations observed in asthmatic airways are believed to be related to the severity and therapeutic outcomes of asthma [7,8].

1. Subepithelial Fibrosis and its Molecular Pathogenesis

Bronchial subepithelial fibrosis is considered to be an important part of airway remodeling. A significant correlation was found between subepithelial layer thickness and degree of airway hyper-reactivity [9]. Increased deposition of collagens type I, III and V is thought to be induced by fibroblasts under the basement membrane. Fibroblasts, especially so-called myofibroblasts, are increased in the airways of asthmatic patients, and the number of myofibroblasts is correlated with the degrees of airway hyperresponsiveness [10,11]. Abnormal extracellular matrix (ECM) deposition is induced by imbalance between matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs) [12]. Inflammatory cells such as neutrophils and eosinophils produce and release MMPs and TIMPs. Various ECM produced by myofibroblasts also act as growth and migration factors for fibroblasts. ADAM33 (A Disintegrin And Metalloproteinase33) has been identified as a gene that is linked to asthma in a Caucasian population [13]. ADAM33 is a membrane-anchored metalloproteinase [14]. The expression of this gene is abundant in airway fibroblasts and smooth muscles. Therefore, this protein may play an important role in airway remodeling.

Regulation of fibroblast proliferation is dependent on several growth factors via each specific as well as common signal transduction pathways. *In vitro* co-culture of bronchial epithelial cells and myofibloblasts as a model of airway remodeling of asthma has been reported, and when bronchial epithelial cells are damaged, growth factors [platelet-derived growth factor (PDGF), fibroblast growth

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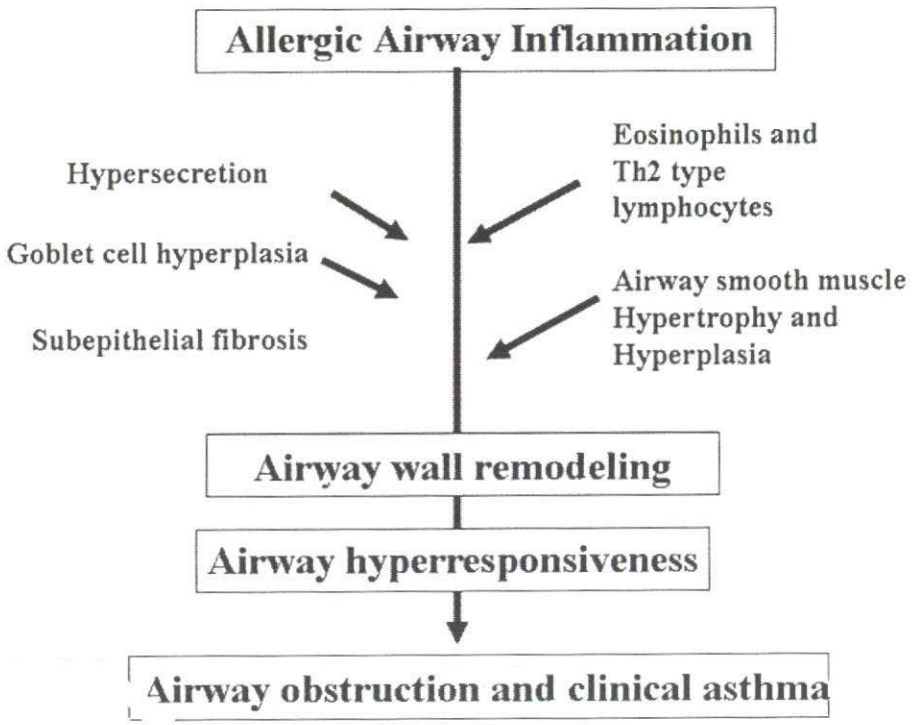

Fig. (1). Novel understanding of the pathogenesis of asthma.
Airway epithelial cells, fibroblasts and smooth muscle cells are capable of releasing various cytokines and growth factors which positively and negatively regulate mesenchymal cells, which are involved in airway remodeling.

factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), transforming growth factor (TGF)-β, endothelin-1 (ET-1)] are secreted by bronchial epithelial cells and these factors control myofibloblast proliferation and migration [15]. TGF-β 1 stimulates or inhibits proliferation depending on the condition of fibroblast cultures. In addition, prostanoids are involved in negative signals for fibroblast proliferation. We found that Th2 cytokines, IL-4 and IL-13 upregulated cell growth of normal lung fibroblasts, and a potent cyclooxygenase inhibitor indomethacin increased proliferation up to the level reached by the action of IL-4 or IL-13 [16].

Regulatory mechanisms of how fibroblasts differentiate into myofibroblasts have been studied. A potent fibrogenic growth factor TGF-β is known to induce myofibroblastic differentiation [17]. We found that IL-4 and IL-13 can directly induce differentiation of lung fibroblasts [16]. Importantly, such phenotypic differentiation was not attenuated by dexamethasone (DEX), a potent corticosteroid. As a Th1 type cytokine, interferon(IFN)-γ counteracted the effect of IL-4 and IL-13, and attenuated the expression of α-smooth muscle actin (SMA), a marker of myofibroblasts [16]. This result suggests that intervention to the Th1-Th2 imbalance in asthmatic airways can be beneficial for airway remodeling.

2. Airway Smooth Muscle Cells: Mechanisms of Proliferation and Hypertrophy

Airway narrowing in asthma is the result of spasms of airway smooth muscles (ASM). Hypertrophied ASM lead to the severe airway narrowing. ASM cell size is greater in patients with severe asthma as compared with that in control subjects [18]. Bronchial biopsies revealed that numbers and sizes of ASM cells were negatively associated with pre-bronchodilator and post-bronchodilator FEV1 values in asthma [18].

Regulatory mechanisms of proliferation of airway smooth muscle cells have been investigated. Nitric oxide and prostaglandin (PG)E2, which are released by bronchial epithelial cells, inhibit proliferation of smooth muscle cells [19,20]. PDGF, TGF-β, IGF-I have growth stimulating activity [21], which are also released by bronchial epithelial cells. ASM cells of bronchial asthma themselves produce connective tissue growth factor (CTGF) in response to stimulation with TGF-β [22]. CTGF release by smooth muscle cells may contribute to the increased production of fibronectin and collagen deposition in the remodeled airway wall. ASM in asthma release less endogenous PGE2 than normal smooth muscle cells [23].

Infiltrated inflammatory cells including eosinophils affect proliferation of smooth muscle cells via TGF-β, PDGF, and tumor necrosis factor (TNF) α [24]. Mast cell infiltration in airway smooth muscle layer is a unique feature in asthma [25]. Mast cell-derived histamine and tryptase induce proliferation of smooth muscle cells [26] whereas, mast cell chymase modifies cell matrix interactions and inhibits mitogen-induced proliferation of human ASM cells [27].

3. Goblet Cell Hyperplasia

In asthma, goblet cell hyperplasia and increased mucus production occur specially in the small airways. Goblet cell

hyperplasia is thought to be the result from the airway epithelial injury. Th2 cytokines interact with the repairing epithelium to promote goblet cell hyperplasia [28]. IL-13 induces the goblet cell hyperplasia by the production of heparin-binding-epidermal growth factor(EGF) [29]. Calcium-activated chloride channel-1 (CLCA1) gene may also be related to induce goblet cell hyperplasia in the airways of bronchial asthma [30].

4. Vascular Components

Airway remodeling also include changes of vascular components in the airway. Increased vascularity, vasodilation, and microvascular leakage occur, which are related to the increased airway wall thickness. Even among patients with mild asthma, increased number of small vessels in submucosal layer was reported, and this fact suggests that angiogenesis is a component of the chronic airway remodeling in asthma [31]. These changes of vascular components are thought to be induced by vascular endothelial growth factor (VEGF), which is induced by PDGF and platelet activating factor (PAF) [32].

AIRWAY REMODELING AS A RESULT OF DYSREGULATED EPITHELIAL MESENCHYMAL TROPIC UNITS

Above mentioned data strongly suggest that airway epithelial cell, fibroblast and smooth muscle cell closely interact with each other and exquisitely regulate the repair process and/or remodeling. In the asthmatic airways, dysregulated repair process might result in structural and functional changes known as remodeling. In the regeneration of epithelial cells, EGF plays an important role, and it is reported that the expression of EGFR (epidermal growth factor receptor) is upregulated in asthmatic bronchial epithelium, while proliferation of the epithelium dose not take place appropriately [33]. This observation suggests that bronchial epithelial cells in asthma lack in functional response to the binding of EGF.

In contrast, negative growth factors such as TGF-β seem to play an important role in epithelial repair of asthma. While intrinsic fragility and impaired proliferation of epithelial cells might be a cause of epithelial damage and subsequent profibrogenic growth factor production, much has yet to be clarified to explain the precise mechanism of remodeling.

Above mentioned progress in the understanding of the molecular mechanisms of airway inflammation and remodeling in asthma leads us to re-evaluate the present and future drugs for asthma treatment. Although complex and heterogeneous processes are involved, one can understand that certain signal transduction pathways play pivotal roles as final common processes, and thus, it is crucial to determine the target molecule(s) and route of administration for proper drug design (Fig. **2**).

POTENTIAL CHOICES FOR THE TREATMENT OF ASTHMA: A FUTURE PERSPECTIVE

1. Inhibitors of Transcription Factors

As mentioned above, increased expression of cytokines, chemokines, adhesion molecules and growth factors are the essential features of persistent, chronic asthma with inflammation and airway wall remodeling. Several transcription factors have been implicated in the pathogenesis of asthma, including the glucocorticoid receptor (GR), nuclear factor kappa B (NF kappaB), Activator Protein-1 (AP-1), Nuclear Factor of Activated T-cells (NF-AT), cyclic AMP Response Element Binding Protein (CREB) as well as signal transducer and activator of transcription(STAT)1 [34] and more recently, the CCAAT/Enhancer Binding Protein (C/EBP), STAT6, Peroxisome Proliferator-activated Receptor (PPAR) and the bZIP transcription factor, nuclear factor E2-related factor 2 (Nrf2) [35]. In clinical practice, inhaled corticosteroids and beta agonists are commonly used for the treatment in asthma and are often used together. Recent evidence suggests that many of the anti-inflammatory actions of corticosteroids are mediated by cross-talk between the activated GR and other transcription factors such as the pro-inflammatory NF kappaB. In a randomized, placebo controlled, crossover study of six weeks' treatment with inhaled budesonide (400 microg twice daily), terbutaline (1 mg four times daily), and combined treatment were recruited [36]. Biopsy samples of the bronchial mucosa were obtained after each treatment and analysed for the DNA binding activity of GR, CREB, and NF kappaB. Budesonide increased GR activity and decreased NF kappaB activity. No treatment combination altered CREB activity and terbuta-line had no significant effects on any transcription factor. Thus, effects of inhaled corticosteroids might be due to, at least in part, the dual effects on GR and NF kappaB activity in bronchial mucosa.

NF kappaB Inhibitors

NF kappaB is an inducible transcription factor that plays a central role in the regulation of many immune and inflammatory responses. While NF kappaB is required for cell survival and immunity, abnormal expression and/or activation of NF kappaB leads to the development of many pathological states, especially those involved in chronic and acute inflammation. A variety of signal transduction pathways, originating from various cellular stresses and stimuli, lead to the downstream molecular targets: the NF kappaB/IkappaB complex and its activating kinase (inhibitor of kappaB kinase, IKK). Several inhibitors of these processes have been patented. For examples, new pyrazoloisoquinoline derivatives are inhibitors of NF-kappa-B inducing kinase, especially IKK2 (also known as IKKß) [37], and can be useful in the treatment of disorders associated with inappropriate activity, such as rheumatoid arthritis, asthma, and COPD (chronic obstructive pulmonary disease). New Indazole carboxamide derivative or its salt is another inhibitor of IKK2 activity [38]. New crystalline monopotassium salt form of 2-((2-(2-methylamino-pyrimidin-4-yl)-1H-indole-5-carbonyl)-amino)-3-(phenylpyridin-2-ylamino)-propionic acid is IKK inhibitor [39]. New anilinopyrimidine derivatives are selective inhibitors of IKK, particularly IKK-2 [40].

Another invention is related to the co-administration of a dehydroepiandrosterone (DHEA) congener in combination with a parthenolide, a naturally occurring NkappaB inhibitor, to reduce inflammation [41].

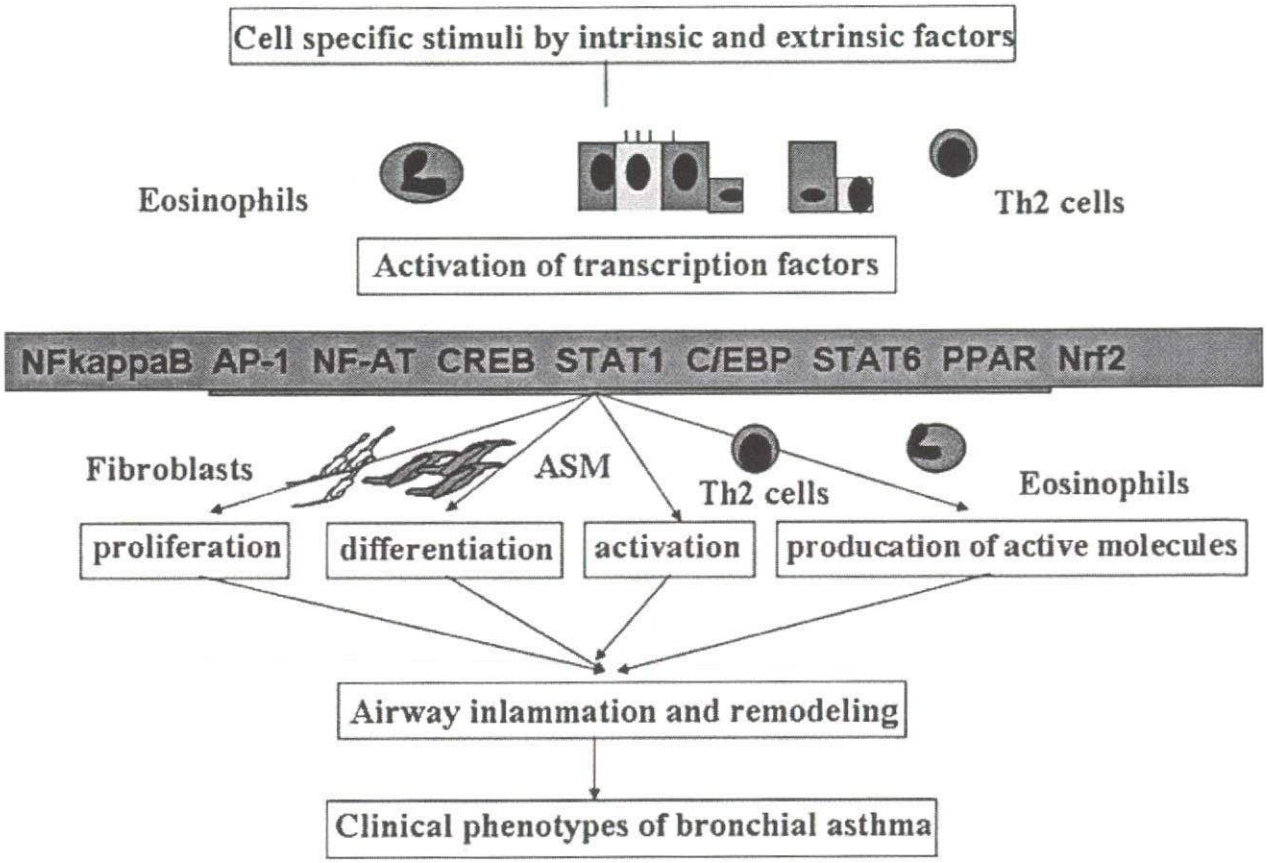

Fig. (2). Cell specific signal transduction in the molecular mechanisms of airway inflammation and remodeling in asthma.
Certain signal transduction pathways resulting in several transcription factors activation play pivotal roles as final common processes, and thus, it is crucial to determine the target molecule(s) and route of administration for proper drug design.

Although these low-molecular products seem to have a potential to be a novel choice for the treatment of intractable asthma and COPD, it is worrisome to suppress these pivotal transcription processes, since many transcription factors play a central role in tissue and organ homeostasis. Cell type specific application of decoy or antisense oligonucleotides or inhaled formulations to antagonize against NF kappaB, may help to control the inflammatory responses in the affected airways, with little adverse effects.

2. Statins

Statins reduce cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and have an established role in the treatment of atherosclerotic disease. Recent research has identified anti-inflammatory properties of statins. Statins appear to reduce the stability of lipid raft formation with subsequent effects on immune activation and regulation, and also inhibit signalling molecules with subsequent downregulation of gene expression. Both these effects result in reduced cytokine, chemokine, and adhesion molecule expression, with effects on cell apoptosis or proliferation. In allergic asthmatic models of mice, simvastatin reduced ovalbumin-specific IgE level, the number of total inflammatory cells, including macrophages, neutrophils, and eosinophils into bronchoalveolar lavage fluid, the expressions of CD40, CD40L or VCAM-1, the mRNA and protein levels of interleukin (IL)-4, IL-13 and TNF-alpha, the numbers of goblet cells, activities of MMPs, and further small G proteins, mitogen-activated protein kinases and NF-kappaB activities in bronchoalveolar lavage cells and lung tissues [42]. In clinical studies, lung transplant recipients with statin therapy had a better survival rate than those without it [43]. This result was probably reflected by down regulation of myofibroblast function with statin [44]. The important key cell signaling molecule affected by statins appears to be Ras, which is a small guanosine triphosphate (GTP) - binding protein and is a key signaling molecule acting downstream of growth factors. Lovastatin can inhibit the activation of Ras through a modification of Ras localization to the inner plasma membrane of fibroblast [44]. Studies have demonstrated that lovastatin potently induces apoptosis in fibroblasts constitutively expressing Myc, and that lung fibroblasts isolated from fibrotic lesions constitutively express growth-promoting genes [44]. Clinically achievable concentrations of lovastatin induce apoptosis in normal and fibrotic lung fibroblasts *in vitro*, as evidenced by acridine orange staining, terminal transferase nick end translation (TUNEL), and DNA laddering. Apoptosis of human lung fibroblasts was dose- and time-dependent, and blocked by exogenous mevalonic acid. Furthermore, apoptosis was associated with decreased levels of mature Ras, a molecule directly implicated in fibroblast rescue from apoptosis. The ability of lovastatin to induce fibroblast apoptosis *in vivo*

was ascertained using a guinea pig wound chamber model [44]. These findings support further study of statins as potential therapy for patients with fibroproliferative disorders.

It is also considered that mevalonate metabolites play an essential role in transducing EGF receptor (EGFR)-mediated signaling cascades. Targeting HMG-CoA reductase using lovastatin induces a potent apoptotic response in a variety of tumor types at therapeutically achievable levels of this drug [45]. As mentioned above, there exists a persistent activation of EGFR signaling in mesenchymal cells of asthmatic airways, and therefore, the effects of lovastatin on EGFR function might be applied to the prophylaxis and/or treatment of airway remodeling in chronic asthma [46].

A patented invention provides medicaments comprising combinations of bronchodilators, glucocorticosteroids and HMG-CoA reductase inhibitors in the treatment of respiratory disorders such as COPD as well as asthma [47].

3. Macro-Steroids

Erythromycin and its 14-member macrolide analouges have attracted attention for their effectiveness in a variety of airway diseases including diffuse panbronchiolitis (DPB), sinobronchial syndrome, chronic sinusitis and bronchial asthma. *in vitro* As well as *in vivo* studies strongly suggested that macrolides have potentials to inhibit expression of inflammatory cytokines and chemokines. In chronic airway inflammation, there is a prominent increase in a variety of cytokines such as IL-1, TNFα, and IL-8. Treatment with 14-ring member macrolide antibiotics resulted in decreased cytokine levels in the airway lining fluids, suggesting that they have potentials to inhibit cytokine production in the local milieu. *in vitro* Studies demonstrated that they have, indeed, inhibitory effects on cytokine /chemokine produc-tion by several kinds of cells [48]. Recent progress for the elucidation of molecular mechanisms of their unique and novel anti-inflammatory actions indicated that these agents inhibit activation of several transcription factors including NF kappaB and AP-1 [49]. They have also been shown to inhibit fibroblast proliferation, suggesting anti-remodeling activity. Recent reports [50,51] showing beneficial effects of anti-TNFalpha receptor antagonist on severe intractable asthma, further suggested therapeutic possibilities of this group of drugs. In this regards, macrolide conjugates with anti-inflammatory activity is interesting [52]. New macro-lide derivatives are glucocorticoid receptor antagonists which are expected to be useful to treat inflammatory diseases, disorders and conditions, and immune disorders associated with e.g. COPD, asthma and bronchitis. This invention might improve therapeutic action and the use in the treatment of inflammatory diseases and conditions in humans and animals. New decladinosyl macrolide deriva-tives are cytokine inhibitors used for treating inflammatory disorders e.g. asthma and adult respiratory distress synd-rome [53]. The present invention relates to novel semi-synthetic macrolides with anti-inflammatory activity. More particularly, the invention relates to 14- and 15-membered macrolides lacking cladinose sugar substituted at the C-3 position, to their pharmaceutically acceptable derivatives, to processes and intermediates for their preparation, to pharmaceutical compositions containing them and to their activity and use in the treatment of inflammatory diseases and conditions in humans and animals, especially those diseases associated with excessive secretion of TNF-alpha, IL-1, IL-6, IL-8, IL-2 or IL-5 [53].

4. PDE4 Inhibitors

Phosphodiesterase-4 (PDE4) is an important cyclic adenine monophosphate (cAMP)-metabolising enzyme in immune and inflammatory cells, airway smooth muscle and pulmonary nerves. PDE4 plays a significant role in modulating the activity of cAMP, an important second messenger that mediates the relaxation of airway smooth muscle and suppresses inflammatory cell function, thereby attenuating the inflammatory response. Selective inhibitors of this enzyme show a broad spectrum of activity in animal models of COPD and asthma [54]. These drugs block the hydrolysis of cAMP via inhibition of PDE4 and are attractive candidates for novel anti-inflammatory drugs. At present, two second-generation PDE4 inhibitors for the treatment of COPD and asthma patients are being tested in clinical Phase III trials. The first compound is the orally active, selective PDE4 inhibitor cilomilast (cis-4-cyano-4-[3-cyclopentyloxy-4-methoxyphenyl]-cyclohexanecarboxylic acid) [54]. Cilomilast shows high selectivity for cAMP-specific PDE4, an isoenzyme that predominates in pro-inflammatory and immune cells and that is 10-fold more selective for PDE4D than for PDE4A, -B or -C. *in vitro*, Cilomilast suppresses the activity of several pro-inflammatory and immune cells important in asthma and COPD. Moreover, it is highly active in animal models of these diseases [54]. Another PDE4 inhibitor, roflumilast was suggested to regulate the ECM and therefore processes of airway remodeling in asthma [55].

New pyrazolo-naphthyridinone compounds are PDE4 inhibitors useful for treating many diseases, including respiratory disease especially asthma [56]. New fluorene compounds are other group of PDE4 inhibitors [57], which down regulate or inhibit the production of TNF-alpha and therefore might be useful in the treatment of variety of allergic and inflammatory diseases including asthma and COPD.

5. Modulation of Th1/Th2 Balance

It has been documented that Th1/Th2 imbalance toward the predominance of Th2 cells play an important role in the airway inflammatory changes of asthma. As mentioned above, modulation of Th1/Th2 balance might also be a useful tool for the prophylactic treatment of airway remodeling. In fact, there is increasing body of literature suggesting that interferon may have a beneficial effect especially in severe persistent asthma [58-60]. A group of Th1 cytokines including interferon, IL-12 and other related substances, therefore, are worthy to be evaluated.

6. Other Anti-Inflammatory and Anti-Remodeling Drugs

Other groups of anti-inflammatory and anti-remodeling agents have been patented as potential promising compounds in the treatment of asthma and related inflammatory airway diseases.

MONOCYCLIC AROYLPYRIDINONES AS ANTI-INFLAMMATORY AGENTS

New monocyclic aroylpyridinones [61] useful for treating acute and chronic inflammatory processes e.g. asthma, chronic obstructive pulmonary disease.

PYRIMIDYL SULPHONE

New sulfonamidopyrimidine derivatives [62] are chemokine receptor modulators useful to treat asthma, rheumatoid arthritis, psoriasis and osteoporosis.

METALLOPROTEINASE INHIBITORS

New pyrrolidinone derivatives are metalloproteinase inhibitors [63] to be used for treating cancer, Alzheimer's disease, asthma, rhinitis and rheumatoid arthritis. These compounds are potent inhibitors of MMP12.

CURRENT AND FUTURE DEVELOPMENTS

Most of the drugs for the treatment of asthma have been developed based on the inhibitory activity on allergic airway inflammation and bronchial smooth muscle contraction. Recent progress in the understanding of molecular events in chronic asthma has proved that airway remodeling is a new potential target of asthma treatment. A variety of anti-inflammatory and anti-remodeling compounds have been attracting attention and patented. It would be vital to determine the target molecule(s) for re-evaluating each compound for novel drug design in this field [64].

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