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CME ARTICLE

Remodeling in small airways of asthma

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KEYWORDS
Remodeling;
Small airway;
Asthma;
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Summary
Airway inflammatory processes are pivotal as the pathological features of bronchial asthma. Standard therapy with inhaled corticosteroids markedly suppresses such inflammatory changes, resulting in clinical beneficial effects. However, it is now clear that several histological changes including goblet cell hyperplasia, subepithelial collagen deposits, increased capillary networks and smooth muscle hypertrophy can occur as a chronic consequence of this airway disorder even when asthma is treated with inhaled or oral steroids. These pathologic changes, the so-called remodeling, play an important role in increased airway obstruction, increased clinical severity and difficulty in controlling asthma, and eventually in the development of irreversible respiratory failure. Targeting these remodeling changes may become a new specific therapeutic strategy for controlling asthma.

Educational aims:

- To discuss histological evidence of small airway involvement in asthma.
- To discuss clinical significance of small airway remodeling in asthma.
- To discuss potential tools for evaluating small airway involvement in patients with asthma.
- To discuss the strengths and limitations of ultrafine particle inhaled corticosteroids and systemic anti-inflammatory agents in asthma control.

Introduction

Asthma is characterized by allergic inflammatory responses associated with airway hyper-responsiveness, and is increasing in prevalence and sociomedical burden in many countries.[1] Both clinical and experimental studies suggest that eosinophils and T cells play a key role in the induction of airway inflammation and mucosal injury, which closely links to non-specific airway hyper-responsiveness in asthma.[2] Inhaled corticosteroids markedly suppress the airway hyper-responsiveness and asthma symptoms along with decreased eosinophil infiltration in the airways. More targeted types of anti-inflammatory therapy such as anti-IL-5 antibody, however, have failed to show clinical improvement, despite apparent decrease in eosinophils in the peripheral blood, and hence posed some doubt about the critical role of this inflammatory cell.[3,4] It is now believed that asthma is a

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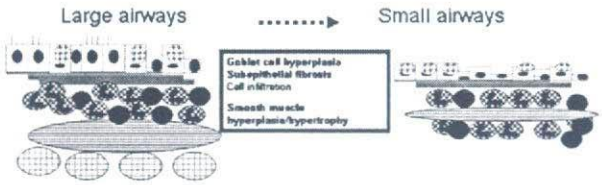

Figure 1 Involvement in large and small airways in asthma. There exist extensive changes with so-called remodeling as well as cell infiltration throughout the airways and lung parenchyma.

heterogeneous and complex airway disease that involves both inflammatory and "non-inflammatory" processes.[5,6] Airway structural change, the so-called "remodeling", generally includes subepithelial fibrosis, goblet cell hyperplasia, smooth muscle cells hypertrophy/hyperplasia and microvascular changes (Figure 1).

Most of the previous reports regarding the allergic inflammatory processes in asthma have been focused on the large airways.[7,8] Pathological as well as physiological findings reported during the past few years, however, suggest that the inflammatory processes involve not only the large airways but also the smaller peripheral airways and the lung parenchyma.[9,10] In fact, the small peripheral airways, those that are less than 2 mm in diameter,[11] are recognized as a predominant site of airflow obstruction in asthma.[12,13] The inflammation in these more distal airways has been described as more severe than large airway inflammation associated with the remodeling processes. Therefore, the peripheral airways should be considered a prime target in therapeutic strategies for treatment of asthma.

Are small airways involved in asthma?

Considerable information about the existence of small airway involvement in asthma has first come from post-mortem studies. Haley et al.[14] performed a morphometric analysis of postmortem lung specimens from patients who succumbed to asthma. They found that the inflammatory processes in distal airways appeared to be qualitatively different from that in large airways with different distributions of cellular infiltrates within the walls of large and small airways. In large airways with cartilaginous layers, relatively more T cells and eosinophils were found in the inner region of the airway wall (between basement membrane and smooth muscle), compared with the outer region (between smooth muscle and alveolar attachments). In the small airways, however, the pattern was reversed, with CD45+ T cells and eosinophils more abundant in the outer region. Faul et al.[15] studied inflammatory changes among patients with fatal asthma with sudden death. Large numbers of T cells and activated eosinophils were found in both proximal and distal lung tissue. In contrast to individuals with stable asthma, larger numbers of CD8+ T cells were found in those with fatal asthma.

Surgically obtained lung specimens from thoracic surgery on people with and without clinically defined asthma allow us to study larger samples than the specimens obtained during bronchoscopic procedures. Using these larger samples, Hamid et al.[16] were able to evaluate the inflammatory process throughout the entire length of the airways. They showed that increased numbers of T cells (CD3+), total eosinophils (major basic protein positive) and activated eosinophils (EG2 positive) were found in both the small (<2 mm internal diameter) and the large (>2 mm internal diameter) airways of sample taken from people with asthma compared to those without asthma. When comparing the large and small airways in those with asthma, there were an increased number of activated eosinophils (EG2 positive) in small airways, suggesting that a similar but more severe inflammatory process is present in the peripheral airways.

The inflammatory processes in the distal airways of patients with asthma have also been studied by using bronchoscopic techniques. Inflammatory infiltrates were observed in the airways of both untreated and patients receiving intensive steroid treatment, but with lesser inflammation in patients with mild-to-moderate asthma.[17] Evidence of distal lung inflammation was also reported by Vignola et al.[18] who compared several markers of inflammation in mucosal biopsy specimens and BAL fluid from healthy control subjects, patients with intermittent asthma, and patients with mild-to-moderate persistent asthma. They found that alveolar macrophage activation was significantly increased in patients with asthma compared with control patients.

Kraft et al.[19] studied biopsy samples from distal and proximal lung tissue, and found that the inflammatory changes that accompanied nocturnal asthma were more prominent in the alveolar tissue area and the number of inflammatory cells, especially eosinophils and macrophages, increased at night in the alveolar tissue area of patients with nocturnal asthma, whereas the number of inflammatory cells found in the proximal airways was similar in patients with asthma who did or did not experience nocturnal worsening. Specifically, they further found that the presence and extent of alveolar tissue eosinophils were correlated with nighttime decline in lung function, especially the nighttime decline in FEV_1. Taken together, this information shows that small airway involvement plays an important role in determining clinical features and severity of asthma.

Airway remodeling in small airways of asthma: pathological evidence

Asthma-related airway remodeling has been found to occur in both large small airways. From autopsy studies, Carroll et al.[20] found thickening of the inner wall of distal airways in individuals who died as a result of asthma as well as in people with asthma who died of nonrespiratory causes compared with airway measurements in nonasthmatic control subjects. The increased smooth muscle mass found in those with asthma suggests a mechanism for heightened response to broncho-constricting stimuli. Interestingly, the structural changes implicated in airway hyper-responsiveness were predominantly found in the distal airways of nonfatal asthma cases but were also found in both large and small airways in fatal asthma cases. These structural changes were not focal in their distribution but were observed throughout the distal lung.[21] In specimens from individuals who died of asthma,[22] the adventitial,

submucosal and muscle areas of the distal airways were greater than those measured in patients with chronic obstructive pulmonary disease (COPD) and control subjects. The smooth muscle layer in patients with asthma was 2 to 3 times thicker than that in control subjects and COPD, and was associated with disease severity. Muscle thickness was, in general, greater in patients with fatal asthma compared with those with nonfatal asthma.

Autopsy studies strongly support the existence of a degree of small airway remodeling in severe and fatal asthma, but did not provide clear evidence of whether or how often such structural changes were found in milder, well-controlled asthma. Recently, evidence of remodeling in peripheral airways of patients with mild-to-moderate asthma has been demonstrated by Bergeron et al.[23] Transbronchial and endobronchial biopsies were obtained from 12 patients with mild-to-moderate asthma before and after a 6-week course of small particle hydrofluoroalkane (HFA)-inhaled corticosteroid. Total collagen occupied 37.7% of the wall area of peripheral airways, compared with 54.5% of the wall area of central airways ($P = .04$). After inhaled corticosteroids therapy, there was no significant difference in central vs. peripheral airways for collagen III or α-smooth muscle actin immunoreactivity and in the number of TGF-β+ cells in the submucosa. The only significant effect of HFA-flunisolide was a decrease in α-smooth muscle actin area in peripheral airways that correlated with the percentage increase in forced expiratory flow at 25–75% of vital capacity. The researchers concluded that there is a considerable degree of airway remodeling in peripheral airways in patients with even mild asthma and that inhaled corticosteroid do not modulate collagen deposition and TGF-β expression. However, the treatment is associated with a significant decrease in the expression of α-smooth muscle actin in peripheral airways, which correlated with the improvement in peripheral airway function.

Airway remodeling in the peripheral airways of asthma: clinical implications

Physiologic consequences of small airway involvement in asthma

Since the volume and surface area of the lungs increases with increasing airway generations, the contribution of peripheral resistance to the total lung resistance was originally believed to be minimal. Ohrui et al.[24] demonstrated that there was a dose-dependent increase in both central and peripheral airway resistance in response to inhaled methacholine in patients with asymptomatic asthma using a catheter-tipped micromanometer wedged into the lower lobe of the bronchus in order to partition central and peripheral airway resistance. Wagner et al.[25] used the wedged bronchoscopic technique to measure airway resistance in the peripheral lung of patients with asymptomatic asthma who had near-normal spirometric values. Patients with asthma had significantly increased peripheral airway resistance compared with control subjects; there was more than a 7-fold increase in peripheral lung resistance in the asthma group. In addition, computational analyses based on quantitative histology[26] have shown the peripheral airways to account for the majority of airway hyper-responsiveness among asthmatic persons. Noninvasive methodologies for separating airway and parenchymal mechanics have been developed using the low-frequency forced oscillation technique in humans.[27] Hall et al.[28] showed the contribution of the small airways to the total lung resistance by this technique. These observations strongly suggest that contribution of the small airways to the total lung resistance has thus far been grossly underestimated.

Pathology–physiology correlations

Numerous studies have attempted to correlate clinical measures of standard lung function with the presence and degree of distal airway involvement. Those study suggest that it is likely that small airway involvement contributes to hyper-responsiveness by amplifying the effect of even slight amounts of muscle shortening or contraction. For example, the amount of airway smooth muscle shortening or contraction required for occluding an airway lumen is shown to be less in asthmatic than nonasthmatic airways.[29] Wagner et al.[30] examined the hyper-responsiveness in small airways by measuring changes in peripheral resistance after a histamine challenge by using a wedged bronchoscopic technique. Peripheral resistance doubled at lower average concentrations of histamine in patients with asthma compared with healthy control subjects, indicating an increased sensitivity to the provoking agent. Loss of lung elasticity is an overlooked, yet potentially significant, factor in the loss of lung function in asthma. Although the mechanism is unclear, the loss of elasticity is likely to reflect distal airway abnormalities, especially at the alveolar–lung parenchymal interface.[31]

Taken together, evidence from histologic as well as physiologic studies indicates that distal airways of individuals with asthma undergo pronounced and, perhaps, permanent structural changes along with long-standing inflammation that appears to affect coarse measures of lung performance. Structural changes, with wall thickening being the most pronounced, are likely to contribute to hyper-responsiveness. These structural changes in distal airways were observed even in patients receiving corticosteroid therapy, suggesting that this therapy did not completely control distal airway inflammation.

Assessment of small airway changes in asthma

As described, the small airways were once called the "silent zone of the lung". It is not easy to assess the disease processes of this area in clinical practice. While classic spirometry and flow-volume curves are routinely evaluated in daily practice, application of parameters of small airways, such as V25 or V50/V25, has been limited because of their lack of reproducibility. Low-frequency forced oscillation technique might be an alternative.[27,28] In addition, several research methods including CT, ultrathin bronchoscopy or microsampling have been proposed for evaluation of small airways in asthma. To date none of these are feasible for primary care or many non-academic specialty practices.

High-resolution CT

High-resolution CT (HRCT) studies can reveal anatomic details of the lung only as small as 200–300 μm, corresponding to wall thickness in airways 2 mm in diameter or larger.[32] At present, therefore, this method is inadequate for directly visualizing small airways. Alternatively, HRCT has proved to be useful to detect functional changes in the small airways by indirectly monitoring air trapping at distal sites.[33] Air trapped in the mid-zone airways at residual lung volume after methacholine challenge can be indirectly detected by HRCT, appearing as regions of decreased signal attenuation. Patients with moderate asthma exhibit an aberrant "mosaic" pattern of pulmonary perfusion, which is correlated with small airway obstruction as indicated by air trapping on HRCT and may therefore be useful for the evaluation of therapeutic effects (see below).

Other potentially available tools

As described above, application of fibroptic bronchoscopy in asthma has greatly facilitated understanding of the airway changes by obtaining biopsy samples from the large airways in patients with asthma. We successfully harvested living epithelial cells by brushing the small airway mucosa under direct vision by using a newly developed ultrathin bronchofiberscope BF-2.8T (the outer diameter: 2.7 mm with a biopsy channel of 0.8 mm in diameter).[34] It is well known that airway epithelial cells are potent sources of profibrotic growth factors and inflammatory cytokines. We recruited healthy non-smokers ($n = 8$) and mild asthmatics ($n = 8$), and recovered peripheral airway epithelial cells after informed consent. Among the factors studied, TGF-β-1 mRNA levels significantly increased and showed a significant inverse correlation with percent V25 and V50/V25 after bronchodilator treatment. These findings may highlight the role of airway epithelium-derived TGF-β-1 in the irreversible small airway obstruction found in chronic asthma, frequently observed as airway remodeling (manuscript in preparation).

Small airways as a therapeutic target in asthma

Although inhaled corticosteroids reduce airway inflammation in patients with asthma,[1] prolonged courses of inhaled steroids do not normalize hyper-responsiveness. Furthermore, it was demonstrated in deposition studies that most of the currently used inhaled corticosteroids are predominantly deposited in the large airways and not in the lung periphery. Therefore, therapeutic actions of inhaled corticosteroids at sites of small airway involvement must be improved.

Metered-dose inhaler (MDI) corticosteroids

Aerosol particle size is a key element in determining lung deposition and the regional distribution of inhaled medication within the lung. In general, smaller aerosol particles are more likely to be deposited in the lung. The fraction of particles emitted by a pressurized MDI is closely related to the percent of deposition in the lung; aerosol particles with a mass median of approximately 4.7 μm are respirable. In the lung, the smaller and less dense the particle, the more likely it is to reach the distal regions. However, particles between 0.6 and 0.3 μm mass median aerodynamic diameter are often exhaled.[12]

Deposition studies by scintigraphic techniques with technetium Tc 99m-radiolabeled HFA-beclomethasone (BDP) (mean diameter:1.1 μm) and classical chlorofluorocarbon (CFC)-BDP (mean diameter:3.5 μm) clearly showed that BDP in HFA showed an increased deposition compared with BDP with CFC (55–60% vs. 4–7%).[35] In addition to increasing lung deposition, the reduction in oropharyngeal deposition with HFA formulation can minimize local side effects.

Goldin et al.[36] used HRCT and regional air trapping to compare the efficacy of HFA– and CFC–corticosteroid formulations in corticosteroid-naive patients with mild-to-moderate asthma. Pretreatment functional CT studies before and after a methacholine challenge were performed at baseline and after 4 weeks of treatment. Post-treatment scanning indicated significant improvement in air trapping for both groups. However, after challenge with equal constrictor stimuli, smaller increases in air trapping were observed for subjects treated with the HFA formulation. Corren et al.[37] showed comparable efficacy between flunisolide HFA at one-third the dose of its CFC counterpart. However, trends favored flunisolide HFA on several parameters, including 40.4% fewer asthma exacerbations. Similar efficacy at lower doses was reported for HFA-BDP compared with CFC-BDP in a 6-week study by Busse et al.[38] Ciclesonide is delivered as a small-particle inhaled corticosteroid with HFA and improves lung function and airway hyper-responsiveness. Cohen et al.[39] assessed whether ciclesonide can specifically improve small airway function in asthma. Sixteen mild-to-moderate asthma patients (7 males, median age 39 (range 19–56) years, FEV1% predicted 89% (range 62–120)) were randomized to a 5-week treatment with placebo or 320 μg ciclesonide once daily. Both CT measurements of expiratory lung volume after methacholine challenge and alveolar eNO decreased significantly more with ciclesonide than with placebo. Ciclesonide did not significantly improve other small airways parameters such as $FEF_{25-75\%}$, percentage fall in FVC at PC20adenosine-'5-monophosphate (AMP) and at PC20methacholine. This recent report suggests that ciclesonide in HFA exerts anti-inflammatory effects on small airways. However, longer-term efficacy especially on small airway remodeling remains to be studied.

Dry powder inhalers

Dry powder inhalers (DPIs) represent another alternative to HFA-pMDIs. Data on regional lung deposition and distal lung access by DPI-delivered corticosteroids are limited. It has been demonstrated that deposition with DPIs is dependent on inspiratory flow rates (IFRs), with faster flow rates seeming to increase lung deposition. For example, scintigraphic imaging of budesonide delivered by DPI in healthy volunteers showed an overall pulmonary deposition of 14.8% and 27.7% at slower and faster IFRs, respectively.[40]

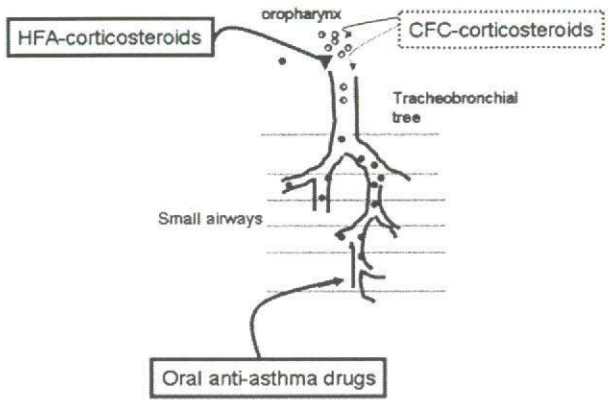

Figure 2 Therapeutic strategies for the treatment of small airways with asthma. Fine particle-corticosteroids in HFA formulations show increased deposition in small airways and are expected to inhibit inflammatory changes in these areas, but anti-remodeling effects remain to be clarified. Systemic anti-asthma drugs such as leukotriene antagonists would become alternative approaches.

Oral agents: an alternative route to the distal lung?

Oral agents would be other candidates that have access to this area of the lung through the circulation (Figure 2). The leukotriene receptor antagonists are oral anti-inflammatory agents with bronchodilating activity.[41] They are efficacious and safe for the treatment of asthma in adults and children.[1,41] In animal models of asthma, these agents are reported to prevent airway remodeling processes.[42] There is one clinical study which supports potential benefit of this class of agent.[43] However, specific studies are needed to define the potential action of anti-leukotrienes on distal airway inflammation and remodeling. Treatment of asthma as a systemic disease could become a new paradigm, and systemic anti-inflammatory and anti-remodeling therapy might be necessary for the complete control of this disease.[44,45]

Practice points: importance of small airway involvement in asthma

- Difficult to detect: limitation of techniques, the so-called Silent Zone
- Easy to be targeted: noxious agents such as tobacco, air pollutants, viruses, etc.
- Difficult to defend: lack of effective defense
- Disturbed lung function: remodeling causing irreversible obstructive changes
- Difficult to treat: needs strategy to drug treatment

Conclusion

There is accumulating evidence to suggest that small airway involvement plays a crucial role in the disease processes in asthma. It is anticipated that poorly controlled inflammation in small airways may contribute to accelerated decline in lung function and airway remodeling. The application of HRCT appears to allow indirect assessment of the morphological changes (resulting from air trapping and regional hyperinflation) in the small airways, still remains research tools. Less-invasive and concise techniques would be necessary for clinical evaluation of the disease at this site. Remodeling processes in this site would become important new therapeutic target in asthma.

CME Section

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Educational questions
Answer true or false to the following statements:

1. Asthma is an inflammatory disease that affects only large airways.
2. Airway inflammations show different patterns in large and small airways.
3. Airway remodeling is associated with increased smooth muscle thickness in both large and small airways.
4. Treatment with inhaled corticosteroids clear all findings of airway remodeling in patients with milder asthma.
5. In asthma airway resistance is not influenced by the smaller more peripheral airways.
6. HRCT scans can directly assess small airway remodeling.

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INTERNAL MEDICINE

□ CASE REPORT □

Intractable Desquamative Interstitial Pneumonia in a Tattooed Man

Toru Arai[1], Yoshikazu Inoue[1], Seiji Hayashi[1], Masanori Akira[1], Satoru Yamamoto[1], William D Travis[2] and Mitsunori Sakatani[1]

Abstract

A 20-year-old man with a 15 pack-year history of cigarette smoking had a tattoo outlined on his back with blue pigment. He noticed a dry cough and shortness of breath on exertion when the pigment of other colors was added at the age of 27. He visited our hospital two years later because of severe dyspnea. He was diagnosed with desquamative interstitial pneumonia by surgical lung biopsy. Steroid therapy with cessation of smoking was partially effective, however his disease worsened again and he died three and a half years after the diagnosis because of respiratory failure.

Key words: desquamative interstitial pneumonia, tattoo, foreign body, respiratory failure

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Introduction

Desquamative interstitial pneumonia (DIP) is histologically characterized by intraalveolar accumulation of macrophages and was first described by Liebow in 1965 (1). The etiology of this disease is unknown, but more than 90% of patients with DIP are smokers and have the potential for DIP to resolve spontaneously with cessation of smoking. Carrington et al (2) reported that patients with DIP show a better prognosis and response to steroid therapy than those with usual interstitial pneumonia (2-4). We report herein a patient with DIP, who developed shortness of breath simultaneously with tattooing and died from respiratory failure despite therapy with steroids as well as smoking cessation.

Case Report

A 20-year -old male clerical employee without dust exposure and no pets had his back tattooed using blue pigment. He noticed a dry cough and shortness of breath on exertion at the age of 27 when the pigment of other colors was started to be utilized for his tattoo. Dyspnea on exertion progressively worsened over about two years and he presented at our hospital at the age of 29. Although he had smoked one pack of cigarettes daily for 15 years, he stopped smoking because of dyspnea. Neither inspiratory crackles, digital clubbing nor cutaneous lesions were revealed by physical examinations. Pulmonary function tests demonstrated severe restrictive pattern with vital capacity of 1.18 L (27.9% predicted), and diffusing capacity of the lung for carbon monoxide was 18.5% of predicted. The findings of arterial blood gas analysis under 2 liters of oxygen supply were pH 7.413, $PaCO_2$ 41.1 Torr, PaO_2 63.3 Torr. His chest radiograph showed bilateral infiltrative shadows predominantly in the lower lung field and suggested loss of lung volume (Fig. 1). High-resolution computed tomography (HRCT) provided ground glass opacity (GGO) and traction bronchiectasis predominantly in bilateral lower lobes (Fig. 2). Laboratory examination showed white blood cells 8,700 /μl, C-reactive protein 0.30 mg/dl and elevation of lactate dehydrogenase to 534 IU/L. Peripheral blood eosinophilia was not observed throughout his clinical course.

Bronchoalveolar lavage fluid (BALF) was obtained from the left B^4 bronchus with 150 mL of saline and 23% of the fluid was recovered. Total cell count in BALF was 3.04×10^5 /mL and the differentiation of the cells was macrophages 34.3%, neutrophils 17.5%, lymphocytes 3.1% and eosino-

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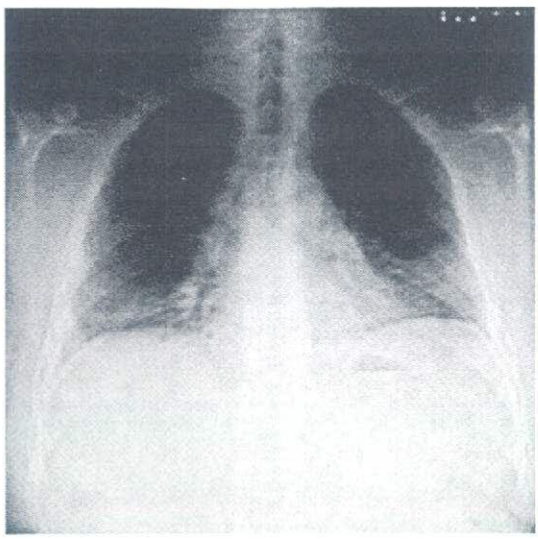

Figure 1. Chest radiograph on the first admission showed bilateral infiltrative shadows predominantly in lower lung field and suggested loss of lung volume.

phils 45.1%. The CD4+/CD8+ ratio of lymphocytes in BAL, determined by flow cytometry, was 0.79. Transbronchial lung biopsy proved to be nondiagnostic. The lung specimens obtained by video-assisted thoracoscopic surgery (VATS) showed temporal uniformity. Alveolar walls were thickened by lymphocytes and plasma cells, and alveolar spaces were filled with large numbers of pigmented macrophages, but with scant eosinophils, which were compatible with the DIP pattern (Fig. 3) (3). Findings of his HRCT and BALF were consistent with DIP (5, 6).

He underwent high-dose intravenous methylprednisolone therapy of 1 g daily for three days and after that 60 mg of oral prednisolone daily was started and reduced to 20 mg daily after 4 months. His cough and shortness of breath were resolved and findings of arterial blood gas analysis on room air improved to pH 7.405, $PaCO_2$ 43.3 Torr, PaO_2 89.6 Torr. After that he experienced four relapses of his lung disease in spite of smoking cessation and immunosuppressive therapy. He died from respiratory failure three and a half years after the diagnosis.

Discussion

Patients with DIP usually respond to steroid therapy and demonstrate a better prognosis than idiopathic pulmonary fibrosis (2-4). Although the patho-physiology of DIP is not precisely elucidated, it is suggested by many authors that DIP is related to smoking and spontaneous resolution is possible after smoking cessation (3, 7). However, unknown etiologies other than smoking are potential causes of DIP in some cases, and they often demonstrated a poor response to steroid and have an unfavorable prognosis (8, 9).

The relationship between DIP and a systemic disease has been suggested in some cases. King et al reported a female patient with progressive DIP (10). Although she discontinued smoking and was treated with steroid and cyclophosphamide, she became worse and received lung transplantation three years after diagnosis. Her disease recurred in the allograft and she died eight months after the transplant. Genetic factors are thought to be associated with some cases of DIP. Childhood cases of DIP (11, 12) often happened familiarly in patients under the age of 6 months old and the patients died in about three years despite the use of steroids and other immunosuppressive agents. Recently, it was shown that mutations in the surfactant protein-C (SP-C) gene are associated with familial DIP (13, 14) and the mutations are found in the adult cases (13, 14). Thus, we should keep in mind various factors possibly associated with DIP.

Cessation of smoking was ineffective in the present case and his unfavorable prognosis suggests an association with an unknown etiology other than smoking. It is difficult to prove that the cessation of the patient's smoking has been maintained. However, his lung disease did not improve spontaneously although he could not smoke in our hospital. No genetic background was indicated from his family history, or mutations of SP-C gene were not investigated. Infection was ruled out by examination of his surgical lung biopsy specimens and serological findings. If we consider something other than smoking may have caused his poor prognostic disease, we suppose a possibility is tattooing, because his cough and dyspnea occurred soon after he had his back tattooed with several pigments.

Pulmonary disease can be induced as a result of reaction to a foreign body outside the lungs. It is well known that silicone used for cosmetic breast-enlargement surgery can induce autoimmune diseases (15) and interstitial pneumonia (16). Tattooing can also arouse a foreign body reaction. The occurrence of granulomas in a tattoo is a well-known phenomenon (17, 18), and systemic granulomatous disease like sarcoidosis with uveitis and pulmonary lesions is also reported (19). Defects of local skin lesions cannot disallow a relationship between the tattoo and DIP in the present case, because pulmonary granulomas, associated with tattooing without remarkable skin lesion, is reported (20) and a silicone implant generally shows no local reaction in the breast.

We should also suppose the possibility of other diseases with increased eosinophils in BAL fluid. Chronic eosinophilic pneumonia (CEP) was characterized by intra-alveolar eosinophils, macrophages and amorphous proteinous exudates and CEP resembles DIP, especially in patients who have received steroids prior to the lung biopsy (21). However, eosinophil infiltration was not prominent in the VATS specimens of this case before the treatment. BAL eosinophilia has also been reported in DIP cases (22, 23). Thus, we suppose the histologic findings of this case are consistent with DIP rather than CEP, and he was finally diagnosed with DIP after our clinical, radiological and pathological conference.

Investigation of additional cases in the future may be necessary to make any more definite conclusions about the relationship, however, it is important to describe the possible

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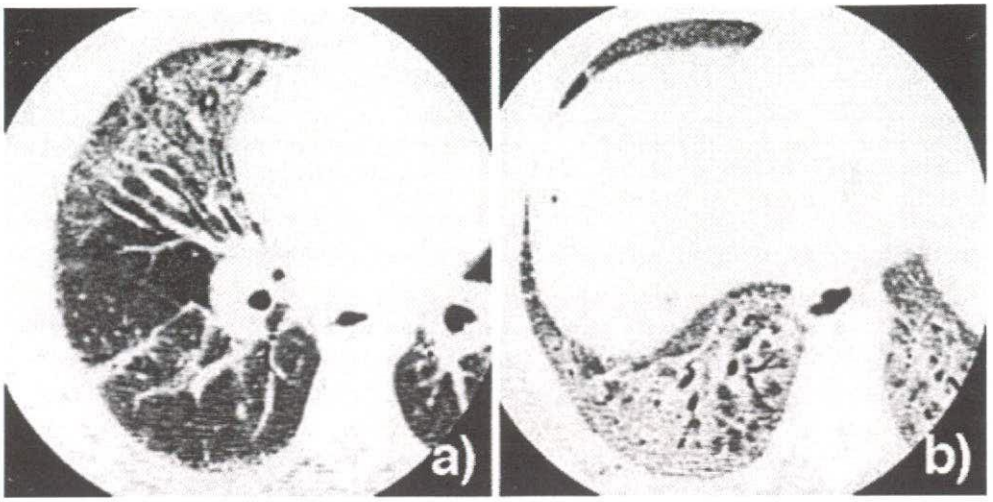

Figure 2. High-resolution computed tomography on the first admission. The ground glass opacity (GGO) was shown in the middle lobe (Fig. 2a) and GGO and traction bronchiectasis predominantly in the lower lobe (Fig. 2b).

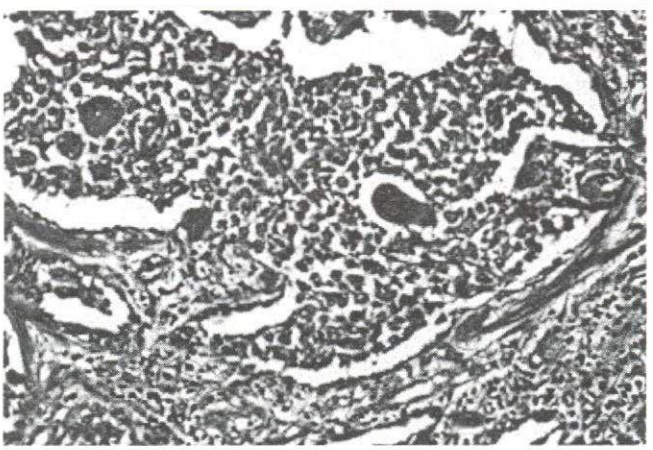

Figure 3. Microscopic findings of lung specimens obtained by video-assisted thoracoscopic surgery. HE staining. Alveolar walls were thickened with lymphocytes and plasma cells and alveoli were filled with pigmented alveolar macrophages. These findings were compatible with a desquamative interstitial pneumonia pattern.

cause of intractable cases of DIP.

Diagnosis of this case was discussed and ascertained in the Osaka Meeting on Diffuse Infiltrative Lung Diseases (August 28, 1999). We thank the members of the meeting for their useful discussion.

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Propionibacterium acnes in granulomas of a patient with necrotising sarcoid granulomatosis

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relationship between bronchial hyperresponsiveness (BHR) and physical activity. Several hypotheses are invoked to explain this association, including a suggestion that physical activity reduces bronchial inflammation by altering airway physiology.

Their major hypothesis is that obesity reduces physical activity and that it is this reduction in physical activity which causes, in some mysterious way, the increase in BHR observed. Their proposed mechanism—that this lack of exercise is associated with a decrease in deep inspiration—is truly breathtaking.

We suggest a much more obvious explanation, which is supported by the published evidence. In our recent survey reported in *Thorax*[2] we demonstrated a highly significant association of body mass index with chronic cough. Other associations observed in this study infer that the cough of obesity is reflux in nature. If obesity leads to reflux-related respiratory symptoms, can this form of upper airway reflux cause BHR?

Unfortunately, Shaaban *et al*[1] do not provide us with any information concerning the incidence of classic reflux symptoms in their population. In a study of patients with dyspepsia and endoscopically proven gastro-oesophageal reflux by Bagnato *et al*,[3] over one-third had significant BHR. These subjects had no personal/family history or symptoms suggestive of asthma.

However, about two-fifths of patients in the study by Shaaban *et al* had asthma-like symptoms, defined as wheeze and sedentary breathlessness. We suggest that these patients could still have reflux-related symptoms as one-third of patients with chronic reflux cough, as demonstrated by pH monitoring, complain of exertional wheeze and dyspnoea.[4]

With the rising levels of obesity in the population, the accurate recognition of the aetiology of the associated BHR is vital to avoid the spurious diagnosis of "late onset" asthma. Perhaps reflux asthma would be a better—but, as yet, unproven—term.

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Bronchial responsiveness and airway inflammation in trained subjects

We read with interest the paper by Shaaban and coworkers[1] on the protective effect of physical activity against bronchial hyperreactivity (BHR) in the general population. The authors suggest that a beneficial effect of deep inspirations during exercise could account for the lower prevalence of BHR in physically active subjects compared with sedentary subjects, while the accompanying editorial[2] favours an "anti-inflammatory" effect of exercise as the most plausible explanation.

We have studied lung function and airway cell biology in non-asthmatic amateur athletes[3 4] and found that both modulation of airway responsiveness and downregulation of airway inflammation occur with training. At rest, the response to single-dose methacholine inhalation in the absence of deep breaths was significantly lower in amateur runners than in age-matched sedentary controls.[3] Shortly after a marathon race the response to methacholine was further blunted, suggesting a causal relationship between endurance exercise and low bronchial responsiveness,[3] possibly mediated by ventilation at increased lung volumes.

We have previously reported large numbers of neutrophils in induced sputum of runners.[4] However, this finding was not associated with evidence of neutrophil activation after intense exercise, since expression of adhesion molecules by airway neutrophils decreased and the elastase concentration in sputum supernatants was unchanged after a marathon race compared with baseline.[4] Similarly, inflammatory cell infiltration in the airways was not associated with activation of the NFκB pathway in endurance-trained mice,[5] while airway inflammation was found to decrease strikingly in ovalbumin-sensitised trained mice compared with sedentary mice.[6] Exercise therefore appears as a model of tightly regulated airway inflammation, possibly secondary to exercise-induced mild bronchial epithelial damage.[5] Along the same line, physically active smokers appear to be protected against lung function decline and the risk of developing chronic obstructive pulmonary disease compared with sedentary smokers,[7] supporting a role for regular exercise in blunting airway inflammation.

We acknowledge that athletes, even at the amateur level, do not represent the general population. On the other hand, a publication bias may have favoured preferential reporting of exercise-associated BHR in athletes, especially those training under extreme environmental conditions (such as "ski asthma") or exposed to irritants (such as swimmers). It is time to reconsider the beneficial effects of regular exercise as a strategy to preserve respiratory health. Studies like that by Shaaban and coworkers will certainly help us to move in this direction.

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Competing interests: None.

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Propionibacterium acnes in granulomas of a patient with necrotising sarcoid granulomatosis

Necrotising sarcoid granulomatosis (NSG) was first described by Liebow[1] in 1973. It is defined by three pathological features: the presence of a conglomerate mass of sarcoid-like granulomas; varying degrees of necrosis within the confluent granulomas; and vasculitis with granulomas and giant cells involving the walls of muscular arteries and veins. The relationship between NSG and classic sarcoidosis is controversial. In NSG hilar lymphadenopathy is not seen as frequently as in sarcoidosis, extrapulmonary involvement is rare and serum levels of angiotensin-converting enzyme (ACE) are not necessarily raised.[2]

The cause of sarcoidosis is unknown, but it has been hypothesised that it results from exposure of a genetically susceptible individual to specific environmental agents. Abe *et al*[3] isolated *Propionibacterium acnes* (*P acnes*) in culture from sarcoidosis biopsy specimens, and recently the *P acnes* genome has been detected in sarcoid lymph nodes by

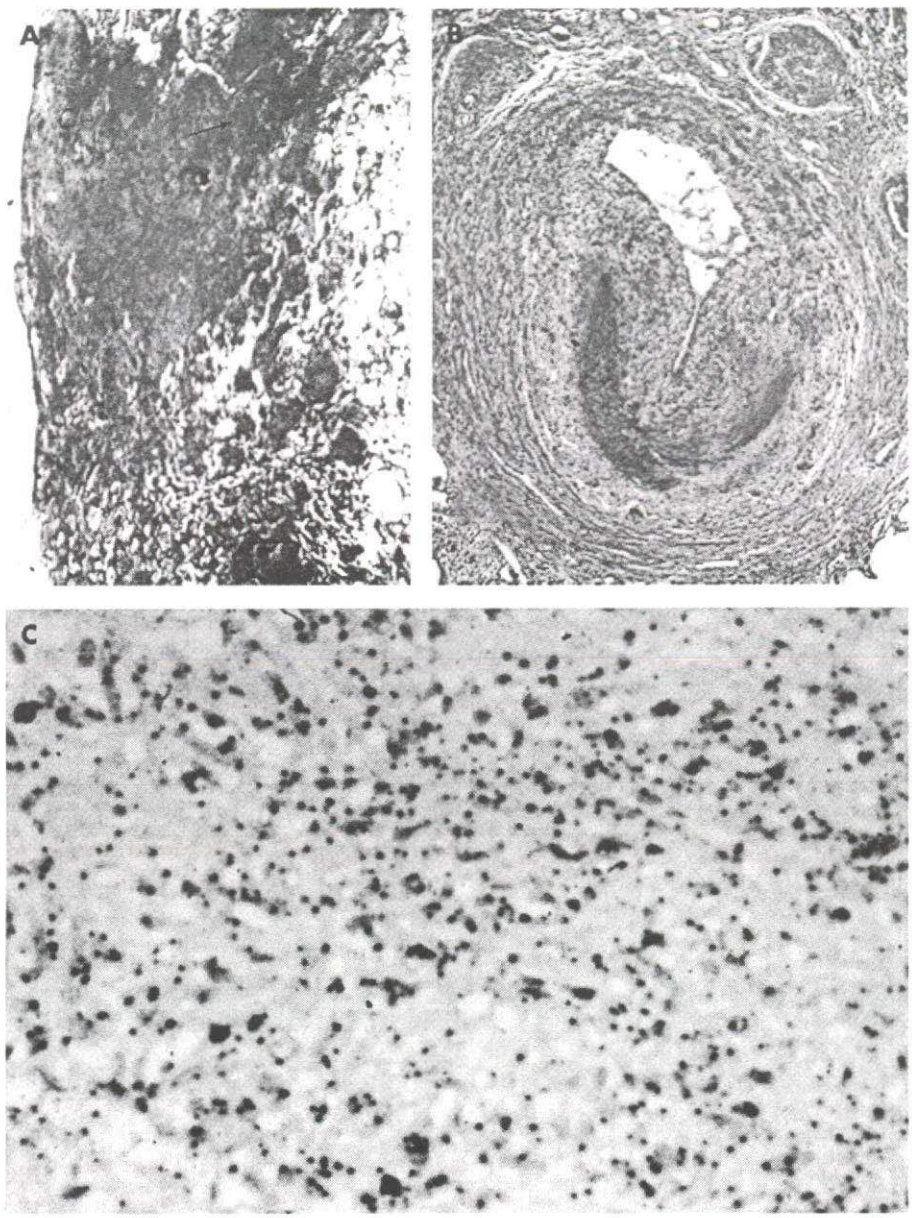

Figure 1 (A) Open lung biopsy specimen revealing necrotising granulomas (arrow) with giant cells aggregated in masses and distributed in a lymphangitic pattern. (B) Granulomatous vasculitis was also present: granulomas infiltrated the vascular walls and almost completely occluded the lumens of the vessels. (C) Genomic DNA of *Propionibacterium acnes* was detected in abundant amounts by in situ hybridisation in the lung tissue of a patient with necrotising sarcoid granulomatosis. Numerous dots indicate the existence of *P acnes* in the granulomas.

polymerase chain reaction[4] and in situ hybridisation.[5] The *P acnes* genome was less frequently and less abundantly detected in tuberculosis specimens.[4 5] Thus, an aetiological relationship between *P acnes* and sarcoidosis has been advocated. We report here the first case of a patient with NSG in whose lung specimens were found abundant *P acnes* genome.

A 65-year-old female non-smoker with no history of dust exposure or pet ownership was referred to our hospital with bloody sputum. The patient's superficial lymph nodes were not palpable. No abnormal findings were revealed by ophthalmological or otolaryngological examinations. Serum levels of C-reactive protein and lysozyme were raised to 2.62 mg/dl and 10.8 μg/ml, respectively. Antinuclear antibody, rheumatoid factor and antineutrophil cytoplasmic antibody were negative. The ACE level was within the normal range. A skin test with purified protein derivative was negative. Small mediastinal and hilar lymph nodes were detected on CT scanning. Multiple irregularly marginated consolidations with air bronchograms were distributed predominantly in peribronchovascular or subpleural lesions of both lungs on a high-resolution CT scan. Total cell count of bronchoalveolar lavage fluid was 9.7×10^5/ml with a cell population of 88% macrophages, 5% neutrophils, 5% lymphocytes and 2% eosinophils; the CD4+/CD8+ ratio was 11.1. Pathological findings of open lung biopsy specimens were consistent with NSG (fig 1A and B) and no pathogenic organisms (including mycobacteria and fungi) were detected in culture of the biopsy specimens. The patient was diagnosed with NSG. *P acnes* DNA was detected in abundant amounts in the granulomas by in situ hybridisation (fig 1C).[5]

This is the first report of NSG with *P acnes* DNA found in the granulomas of lung specimens. This may indicate an aetiological link between NSG and *P acnes*, and it also suggests that NSG is an atypical sarcoidosis with a common aetiology. The clinical and pathological differences between these diseases could be explained by variability in the host response to *P acnes* or the histological location of *P acnes*, although further study would be necessary to arrive at more definite conclusions.

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Symptoms limiting activity in cancer patients with breathlessness on exertion: ask about muscle fatigue

Rehabilitation is an integral part of cancer care and aims to maximise the functional

Characteristics of a Large Cohort of Patients with Autoimmune Pulmonary Alveolar Proteinosis in Japan

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Rationale: Acquired pulmonary alveolar proteinosis (PAP) is a syndrome characterized by pulmonary surfactant accumulation occurring in association with granulocyte/macrophage colony-stimulating factor autoantibodies (autoimmune PAP) or as a consequence of another disease (secondary PAP). Because PAP is rare, prior reports were based on limited patient numbers or a synthesis of historical data.

Objectives: To describe the epidemiologic, clinical, physiologic, and laboratory features of autoimmune PAP in a large, contemporaneous cohort of patients with PAP.

Methods: Over 6 years, 248 patients with PAP were enrolled in a Japanese national registry, including 223 with autoimmune PAP.

Measurements and Main Results: Autoimmune PAP represented 89.9% of cases and had a minimum incidence and prevalence of 0.49 and 6.2 per million, respectively. The male to female ratio was 2.1:1, and the median age at diagnosis was 51 years. A history of smoking occurred in 56%, and dust exposure occurred in 23%; instances of familial onset did not occur. Dyspnea was the most common presenting symptom, occurring in 54.3%. Importantly, 31.8% of patients were asymptomatic and were identified by health screening. Intercurrent illnesses, including infections, were infrequent. A disease severity score reflecting the presence of symptoms and degree of hypoxemia correlated well with carbon monoxide diffusing capacity and serum biomarkers, less well with pulmonary function, and not with granulocyte/macrophage colony-stimulating factor autoantibody levels or duration of disease.

Conclusions: Autoimmune PAP had an incidence and prevalence higher than previously reported and was not strongly linked to smoking, occupational exposure, or other illnesses. The disease severity score and biomarkers provide novel and potentially useful outcome measures in PAP.

Keywords: epidemiology; serum biomarkers; disease severity score; granulocyte/macrophage colony-stimulating factor; autoantibody

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Acquired pulmonary alveolar proteinosis (PAP) is a syndrome characterized by pulmonary surfactant accumulation occurring in association with granulocyte/macrophage colony-stimulating factor autoantibodies (autoimmune PAP) or as a consequence of another disease (secondary PAP). Because PAP is rare, prior reports were based on limited patient numbers or a synthesis of historical data.

What This Study Adds to the Field

Autoimmune PAP had an incidence and prevalence higher than previously reported and was not strongly linked to smoking, occupational exposure, or other illnesses.

Pulmonary alveolar proteinosis (PAP), first described in 1958 (1), is a rare syndrome characterized by the intraalveolar accumulation of surfactant lipids and proteins impairing gas exchange and resulting in progressive respiratory insufficiency. PAP, which has been reported in the medical literature under various terms (alveolar proteinosis, alveolar lipoproteinosis, alveolar phospholipidosis, pulmonary alveolar lipoproteinosis, and pulmonary alveolar phospholipoproteinosis), is recognized to occur in three distinct clinical forms: primary, secondary, and congenital (2, 3). Primary (idiopathic) PAP is a disorder of unknown etiology thought to represent approximately 90% of PAP cases. Secondary PAP occurs as a consequence of any one of a heterogeneous group of underlying clinical conditions (hematologic malignancies; inhalation of toxic dust, fumes, or gases; infectious or pharmacologic immunosuppression; or lysinuric protein intolerance) that impairs alveolar macrophage function, resulting in surfactant accumulation (4). Congenital PAP is a heterogeneous collection of disorders caused by homozygous mutation of the genes encoding surfactant protein (SP)-B, SP-C, and the ABCA3 transporter or by the absence of granulocyte/macrophage colony-stimulating factor (GM-CSF) receptor (5).

Our understanding of PAP pathogenesis has advanced rapidly over the past decade due to a series of contributions from basic, clinical, and translational research. The first real pathogenic clue was provided by the discovery that mice deficient in GM-CSF develop a lung phenotype biochemically, histologically, physiologically, and ultrastructurally indistinguishable from primary PAP (6, 7). Surfactant accumulation in these mice is not due to

increased production but rather to impaired catabolism of surfactant lipids and proteins by alveolar macrophages (8). This and numerous other alveolar macrophage defects were shown to be due to impaired terminal differentiation in mice caused by reduced levels of the GM-CSF–dependent transcription factor, PU.1 (9). GM-CSF also regulates PU.1 levels in human alveolar macrophages (10), which in patients with PAP have defects similar to those of GM-CSF–deficient mice (11). The observation of PAP in GM-CSF–deficient mice was quickly followed by the evaluation of GM-CSF therapy, first in single patient (12) and then in several small series (13–15). GM-CSF deficiency has not been reported in humans (3, 16). However, a second vital pathogenic clue was the observation that primary PAP is specifically and strongly associated with very high levels of GM-CSF autoantibodies (17) that eliminate GM-CSF bioactivity *in vivo* (18). Several methods for measuring GM-CSF autoantibodies have been developed, and an ELISA (19) has demonstrated excellent results (20).

Notwithstanding recent advances, current knowledge about PAP is based on small series and individual case reports. Although data from these studies have been synthesized into a comprehensive review (2), these data represent cases spanning nearly half a century. The incidence, prevalence, or the natural history of primary PAP have not been assessed in a large contemporaneous population. Furthermore, although diagnostic methods have evolved significantly over this period, the strong association of GM-CSF autoantibodies with primary PAP provides a potentially powerful new approach. Novel biomarkers of PAP lung disease also provide additional and potentially useful outcome measures for use in clinical trials to evaluate new therapies.

In July 1999, a national PAP registry was established to more accurately characterize the demographic, clinical, physiologic, radiologic, and serologic features of individuals with PAP in Japan. The present study was not designed to answer questions related to the natural history or responses to therapy; nor was it intended to report on secondary or congenital PAP. Rather, it is intended to provide a cross-sectional evaluation outlining the epidemiology and baseline characteristics of a large contemporaneous group of individuals with primary PAP in Japan at the time of enrollment into the registry. Some of the results of these studies have been previously reported in the form of an abstract (21) and in the proceedings of an international scientific meeting on PAP (22).

METHODS

Study Design

This study was conducted by the Japanese PAP Research Network, which includes nine primary clinical research centers and secondary clinical sites comprising a referral base encompassing the entirety of Japan, a steering committee comprised of the principal investigators at each of the nine primary clinical research centers, and a data collection and analysis center (National Hospital Organization Kinki-Chuo Chest Medical Center, Osaka, Japan). Primary clinical centers included Aichi Medical University, Chiba University, Hokkaido University, Kitazato University, National Kinki-Chuo Chest Medical Center, National Hospital Organization Sanyo Hospital, Nagasaki University, Niigata University Medical and Dental Hospital, and Tohoku University; secondary sites are listed in the APPENDIX. Some investigators in this research network (Y.I., K.N.) are also members of the Rare Lung Diseases Consortium supported by the United States National Institutes of Health. This trial was conducted in three phases: (*1*) study design and establishment of the Japanese PAP Research Network, (*2*) participant recruitment and data collection, and (*3*) data analysis. Three principle components of project were undertaken, including a cross-sectional study (reported here), a natural history study (ongoing), and an evaluation of GM-CSF inhalation therapy (to be reported elsewhere). An independent statistical evaluation of all data was done by the Data and Technology Coordinating Center of the Rare Diseases Clinical Research Network in the United States. The registry protocol was approved by the institutional review boards of each participating institution, and all participants gave informed consent. At enrollment, the treating physician for each participant completed a case report form and obtained a serum sample, both of which were sent to the data coordinating center for analysis.

Study Participants

Recruitment. Between July 1999 and July 2006, individuals diagnosed with PAP were recruited through the existing physician referral networks at primary or secondary clinical centers and pulmonary clinics and through letters to Japanese pulmonary physicians. Registration into the study was defined as the time of serum collection and completion of the case report form by the referring physician. All patient-related historical, physical examination, and other data were collected within 2 months of registration. To ensure the inclusion of individuals diagnosed with PAP independent of disease severity, patients with PAP were enrolled regardless of whether they were symptomatic. Asymptomatic individuals initially identified by a compatible chest radiograph obtained via mandatory annual health screening programs (students, employees of the government, and registered corporations) in whom a diagnosis of PAP was subsequently confirmed were included. As controls for the measurement of serum GM-CSF autoantibody levels, 24 individuals with other lung diseases (including idiopathic interstitial pneumonias [n = 10], sarcoidosis [n = 9], and collagen vascular disease [n = 5]) and 13 healthy control subjects were recruited into the study from the Kinki-Chuo Chest Medical Center in Osaka.

To evaluate recruitment efficiency, a secondary "intensive screening" was performed in the Niigata prefecture that took advantage of the close relationship among regional pulmonary physicians, 98% of whom trained at and remained affiliated with Niigata University and received weekly departmental communications. All physicians received a recruitment letter and follow-up phone calls as necessary to ensure participation.

Inclusion. All patients with a proven diagnosis of PAP were offered the opportunity to participate in the study regardless of whether they had been previously diagnosed and followed, were currently being followed, or were newly referred. Thus, prevalent and incident cases were enrolled. An acceptable PAP diagnosis was based on histopathologic findings of specimens obtained by open lung biopsy or transbronchial biopsy or on cytologic findings in BAL samples.

Exclusion. Individuals were excluded if they did not have a tissue-proven diagnosis of PAP or if serum was unavailable for analysis of GM-CSF autoantibody levels.

Composition of the study population. Two hundred forty-eight individuals with PAP were enrolled in the study; 11 cases of secondary PAP diagnosed at autopsy were identified during the study period but were excluded from the analysis because serum was unavailable. Of the participants enrolled, 223 had an elevated serum level of GM-CSF autoantibody and no underlying clinical condition known to cause PAP. Twenty-five individuals did not have an elevated serum level of GM-CSF autoantibody (*see below*) but had a condition known to cause PAP secondarily. One individual had neither elevated GM-CSF autoantibody levels nor an underlying explanation for the presence of PAP. These three groups of patients were categorized as autoimmune, secondary, and unclassified PAP, respectively. This report includes the data and analysis for individuals with autoimmune PAP. Data and results for secondary PAP will be reported elsewhere.

Diagnostic Criteria

The diagnosis of PAP was established by the presence of characteristic findings from high-resolution computed tomography (HRCT) of the chest (23) and pathologic and/or cytologic specimens obtained by video-assisted thoracoscopic lung biopsy (n = 16), transbronchial lung biopsy (n = 86), or bronchoalveolar lavage fluid (BALF)/cell cytology (n = 218) (24). The diagnosis of PAP was based on HRCT and bronchoalveolar lavage (BAL) cytology in 58.7% of patients; HRCT, BALF, and transbronchial lung biopsy (TBLB) in 34.1% of patients; and BAL cytology or TBLB and video-assisted thoracoscopic surgery (VATS) in 7.2% of patients. The radiologic features characteristic of PAP on HRCT used here included the presence of a diffuse, patchy, "geographic" pattern of ground glass opacification superimposed on interlobular septal thicken-

ing in multiple lobes (25). The diagnostic features of PAP in pathologic specimens included intraalveolar eosinophilic, periodic acid-Schiff–positive material and in cytology specimens included turbid, periodic acid-Schiff–positive, eosinophilic BALF, and intracellular surfactant inclusion bodies in alveolar macrophages (26, 27). Patients with PAP were enrolled irrespective of whether they were symptomatic at the time of registration.

Epidemiology

Because determining the incidence and prevalence of rare diseases is difficult, strict criteria were used. Only patients enrolled in full years of the study were included in calculations to avoid potential seasonal ascertainment bias. Incidence was determined for each full year of the study (2000–2006) and calculated by dividing the number of individuals newly diagnosed with PAP in a given year by the size of the Japanese population (129,180,548) taken from the governmental census report (WHLW Statistical Database, 2006, Ministry of Health, Labor and Welfare). Prevalence was calculated by dividing the total number of patients with PAP registered during all full years of the study period by the size of the Japanese population.

Pulmonary Function Methodology

Pulmonary function testing was performed by certified pulmonary function technicians. The data collected included FEV_1/FVC, FVC, VC, single-breath diffusing capacity of carbon monoxide ($D_{L\text{-}CO}$), and arterial blood gases. Acceptability and reproducibility criteria from the American Thoracic Society recommendations for standardization were used to judge the validity of each testing session (28). Pulmonary function data are presented as the percentage of predicted values. Arterial blood measurements were performed on samples obtained with the patient breathing room air at rest in the supine position.

Disease Severity Score

Participants were assigned a PAP disease severity score (DSS) based on the presence of symptoms and degree of reduction in Pa_{O_2} (both determined at registration) determined with the individual breathing room air in the supine position as previously described (22). The categories included DSS 1 = no symptoms and $Pa_{O_2} \geq 70$ mm Hg; DSS 2 = symptomatic and $Pa_{O_2} \geq 70$ mm Hg; DSS 3 = 60 mm Hg $\leq Pa_{O_2} < 70$ mm Hg; DSS 4 = 50 mm Hg $\leq Pa_{O_2} < 60$ mm Hg; DSS 5 = $Pa_{O_2} < 50$ mm Hg. Qualifying symptoms include dyspnea or cough related to PAP. Pa_{O_2} data were available for 215 of 223 registrants with autoimmune PAP. In the eight registrants in whom Pa_{O_2} was unavailable, oxygen saturation was used to estimate the Pa_{O_2} as follows: oxygen saturation values of 94%, 90%, and 85% were used as the values representing cutoff values of Pa_{O_2} of 70, 60, and 50 mm Hg, respectively.

GM-CSF Autoantibody Measurement

Sera from each participant (patients with PAP or control subjects) were stored frozen (−20°C or −80°C), and GM-CSF autoantibody concentration was measured by ELISA essentially as previously reported (19) with minor modifications as described in the online supplement. Standard antibody was kindly provided by Dr. K. Takada, Hokkaido University.

Serum Biomarker Measurements

Serum biomarkers were evaluated in duplicate samples in blinded fashion in a central laboratory (Y.I.). Serum KL-6, SP-A, and SP-D were measured by ELISA using commercial kits (ED046; Eizai Co. Ltd., Tokyo, Japan; SP-A Test Kokusai-F, International Reagents Co. Kobe, Japan; SP-D ELISA; Yamasa Co., Tokyo, Japan; respectively) as described previously (29–32). Serum carcinoembryonic antigen (CEA) level was measured by radioimmunoassay using monoclonal antibodies (Dainabott, Tokyo, Japan) as described previously (33). Normal serum ranges were KL-6 (<500 U/ml), SP-A (<43.8 ng/ml), SP-D (<110 ng/ml), and CEA (<2.5 ng/ml) (30–34). The normal range for lactate dehydrogenase (LDH) in the clinical laboratory used was <230 IU/L.

Statistics

Numeric data were evaluated for a normal distribution using the Kolmogorov-Smirnov test and for equal variance using the Levene median test. Parametric data are presented as means (±SE), and nonparametric data are presented as medians and interquartile ranges. Categorical data are presented as a percentage of the total or numerically, as appropriate. Statistical comparisons of parametric numeric data were made with Student's t test for two group comparisons if the assumption of equality of variance was satisfied or with the Scatterwaite test if not. Nonparametric numeric data were compared with the Wilcoxon test. Comparisons of categorical data were made with chi-square or Fisher's exact test. The correlation coefficient was obtained using Spearman's correlation method for all data. All tests were two sided, and $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Demographics

Of the 248 patients with histologically and/or cytologically proven PAP enrolled in the Japanese PAP registry, 223 (89.9%) had a positive GM-CSF autoantibody test and were considered to have autoimmune PAP (Figure 1). Patients with a negative GM-CSF autoantibody test had an underlying illness known to cause PAP and were considered to have secondary PAP (9.7%) or were unclassified (0.4%). This article focuses on patients with autoimmune PAP; the features of the latter two groups will be reported elsewhere.

Two-thirds of the patients with autoimmune PAP were men (Table 1). The median age at diagnosis was 51 years, and the mean duration of symptoms at enrollment was 10 months; neither differed according to gender. Patients younger than 10 years of age were rare (Figure 2). Two-thirds of the patients were symptomatic at enrollment, and the proportion did not differ by gender. The age at the time of diagnosis was skewed toward younger individuals due to skewing of the data for men (skewness = 0.0775; $P = 0.03$) but not for women, for whom it was distributed normally (skewness = −0.422; $P = 0.068$) (Figure 2). The bimodal distribution of age at diagnosis for women with PAP, with peaks at ages 25 and 40 years, as previously reported (2), was not observed in this cohort.

Although smoking is a suspected risk factor for PAP, at registration, more than one third of the patients (43%) were never-smokers, a proportion that differed significantly according to gender (83% of women and 24% of men were never-smokers; $P < 0.001$; Table 1). Exposure to dust inhalation, another suspected risk factor for acquired PAP, was present in the

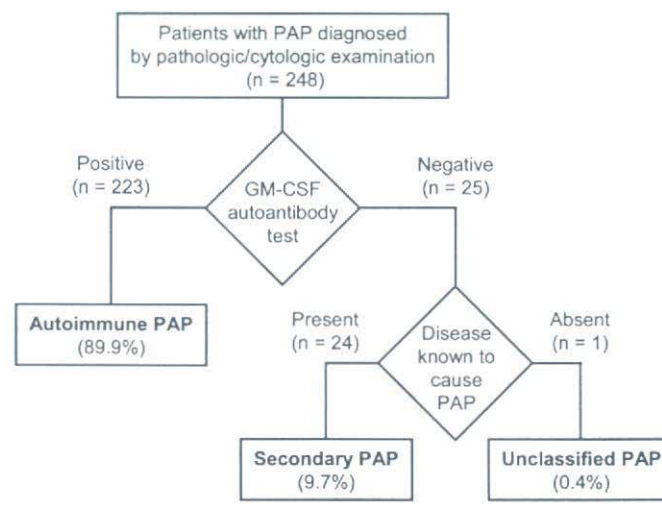

Figure 1. Disposition of the patients with pulmonary alveolar proteinosis (PAP) enrolled into the study. Participants were stratified according to the presence or absence of granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies and then by the presence or absence of an underlying disorder known to cause PAP.

TABLE 1. DEMOGRAPHICS AND DISEASE FEATURES OF PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS AT ENROLLMENT ACCORDING TO GENDER

Characteristic	All Patients (n = 223)			Male (n = 151)			Female (n = 72)			
	n	%	Median (IQR)* or Mean ± SD	n	%	Median (IQR)* or Mean ± SD	n	%	Median (IQR)* or Mean ± SD	*P* Value
Age, yr	223		53 (44–61)	151		52 (44–58)	72		55 (43.5–63)	0.08†
Age at diagnosis, yr	223		51 (41–58)	151		50 (15–89)	72		52 (9–85)	0.064†
Duration of symptoms CXR changes, mo	223		10 (4–36)	151		11 (4–48)	72		9 (4–24)	0.301†
Symptoms	220			147			70			0.754‡
Yes	150	68.4		103	69.6		47	65.3		
No	70	31.8		45	30.4		25	34.7		
Smoking status	217			147			70			<0.001‡
Current smoker	62	28.5		56	37		6	8.5		
Ex-smoker	62	28.5		56	37		6	8.5		
Never-smoker	93	43		35	24		58	83		
Dust exposure	199			137			62			0.001‡
Yes	52	26		44	32		8	13		
No	147	74		93	68		54	87		
Pulmonary function, mean ± SD										
FVC, % predicted	150		88.0 ± 18.9	101		90.2 ± 18.1	49		83.5 ± 19.9	0.04§
FEV_1/FVC	186		84.3 ± 11.2	128		84.7 ± 9.5	58		83.5 ± 14.4	0.57§
VC, % predicted	187		89.4 ± 19.3	129		90.4 ± 19.4	58		87.3 ± 19.1	0.31§
D_{LCO}, % predicted	154		68.6 ± 26.6	106		70.6 ± 29.0	48		64.3 ± 19.5	0.12§
Pa_{CO_2}, mm Hg	213		39.0 ± 3.91	145		39.2 ± 3.8	68		38.6 ± 4.2	0.31§
Pa_{O_2}, mm Hg	213		71.8 ± 13.5	144		71.6 ± 13.2	69		72.1 ± 14.1	0.83§
$[A\text{–}a]D_{O_2}$, mm Hg	211		31.14 ± 15.1	144		31.0 ± 14.9	67		31.3 ± 15.6	0.90§
GM-CSF autoantibody, μg/ml	223		15.29 (7.96–26.76)	151		14.91 (7.42-27.03)	72		15.58 (9.34-26.15)	0.51†

Definition of abbreviations: $(A\text{–}a)D_{O_2}$ = alveolar–arterial oxygen gradient; CXR = chest X-ray; D_{LCO} = carbon dioxide diffusing capacity; IQR = interquartile range; PAP = pulmonary alveolar proteinosis.

* Interquartile range is the range from the 25th to the 75th percentiles of the distribution.

† Calculated using the Wilcoxon rank sum test.

‡ Calculated using the chi-square test.

§ Calculated using Student's *t* test.

histories of only one-quarter (26%) of the patients and differed according to gender (32% of men and 13% of women had a history of exposure; n = 199; $P < 0.001$). No instances of autoimmune PAP were familial among any of the 223 individuals, consistent with the absence of a genetic predisposition.

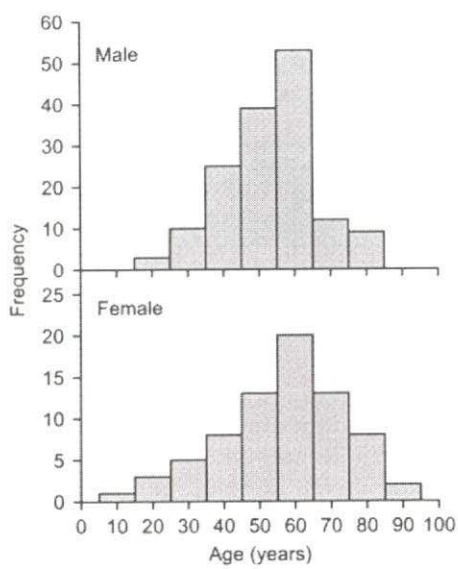

Figure 2. Histogram of the age at diagnosis of autoimmune pulmonary alveolar proteinosis in male (*top*) and female patients (*bottom*). Data are grouped into 10-year intervals. The age distribution in female subjects, but not male subjects, was normally distributed ($P = 0.03$ and 0.68, respectively; Kolmogorov-Smirnov test). The median age at diagnosis was similar in male and in female subjects (*see* Table 1).

Of the pulmonary functions evaluated in patients with autoimmune PAP, the median values for lung volumes (FVC % predicted, VC % predicted) and airflow (FEV_1/FVC) were within the normal range, and only gas transfer (D_{LCO} % predicted) was abnormal (Table 1). Arterial blood gas measurements revealed a similar degree of hypoxemia and elevation of alveolar-arterial oxygen gradient in male and female patients with autoimmune PAP (Table 1).

GM-CSF Autoantibody Levels

GM-CSF autoantibodies levels in the serum were elevated to a similar extent in male and female patients with autoimmune PAP ($P = 0.51$) and were below the level of detection in individuals with secondary PAP; individuals with other lung diseases; disease-free, healthy control subjects (Figure 3); individuals with congenital PAP (n = 5, not shown); or individuals with unclassified PAP (n = 1, not shown). GM-CSF autoantibody concentrations were skewed toward higher values (skewness = 2.03; $P < 0.001$), a pattern that was similar in men and women (Figure 4). Serum autoantibody concentrations were less than 35 μg/ml in most patients, a proportion that did not differ by gender (86% for men, 85% for women).

Levels of total serum immunoglobulins were not increased in autoimmune PAP; median (interquartile range [n]) values (in mg/dl) were IgG: 1,323 (1,091–1,468 [83]); IgA: 232 (178–284 [79]); IgM: 101 (71–149 [79]); IgE: 106 (27–219 [60]) mg/dl. In five of these patients, the level of serum IgG exceeded 2,000 mg/dl. Of these, three had intercurrent pulmonary aspergillosis, one had hepatitis C, and one had polymyalgia rheumatica.

Epidemiology

The incidence and prevalence of autoimmune PAP in Japan were evaluated using two approaches, one encompassing nine

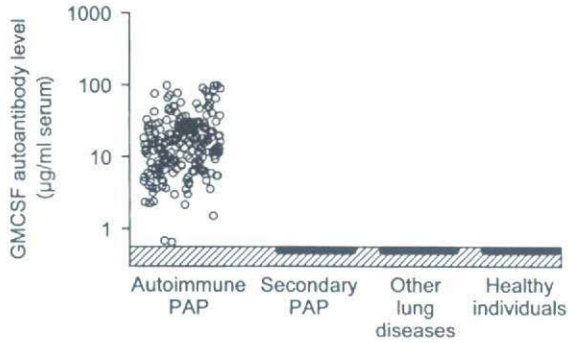

Figure 3. Concentration of granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies in the serum at enrollment of individuals with autoimmune pulmonary alveolar proteinosis (PAP) (n = 223), secondary PAP (n = 24), other lung diseases (n = 24), or healthy individuals (n = 14). In one individual with unclassified PAP, serum GM-CSF autoantibodies were undetectable (not shown).

nonoverlapping regions representing the entirety of Japan and a second focused to the Niigata prefecture.

In the first approach, incidence was estimated by enumerating individuals registered from all regions of Japan receiving a diagnosis of autoimmune PAP for each full year of the study from 2000 through 2005 (Table 2). Using the Japanese population size in 2005 (129,180,548) taken from the governmental census report (WHLW Statistical Database, 2006, Ministry of Health, Labor and Welfare), the mean (±SE) incidence of autoimmune PAP was 0.24 ± 0.03 cases per million population. The total number of patients with autoimmune PAP registered from each of the nine nonoverlapping regions (Hokkaido, Tohoku, Kanto, Hokushinetsu, Tokai, Kinki, Chugoku, Shikoku, and Kyushu) correlated closely with the size of the regional population (Figure 5). The mean (±SE) prevalence across all regions was 2.04 ± 0.31 cases per million. These geographic regions range in climate from temperate (Kyushu) to subarctic (Hokkaido), suggesting that climate may not influence the occurrence of autoimmune PAP.

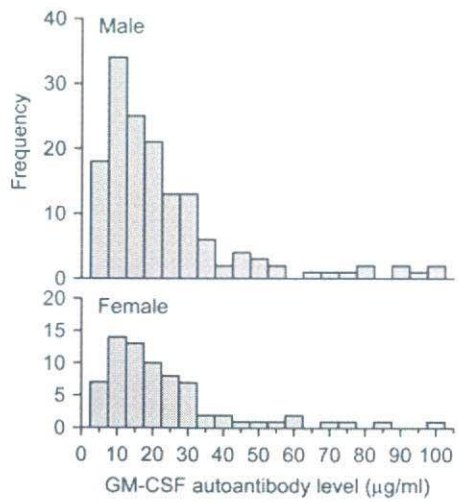

Figure 4. Histogram of serum granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibody concentrations in male (*top*) and female (*bottom*) patients with autoimmune pulmonary alveolar proteinosis. The concentrations were not normally distributed and were significantly skewed toward high values ($P < 0.001$ for both, Kolmogorov-Smirnov test). The distribution of serum GM-CSF autoantibody concentrations was similar in males and female subjects (*see* Table 1).

TABLE 2. ANNUAL INCIDENCE OF AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS AND ENROLLMENT DURING THE STUDY PERIOD

	Diagnosed		
Year*	Japan†	Niigata Prefecture‡	Enrolled§
1999	10	4	52
2000	30	2	15
2001	14	2	18
2002	26	1	23
2003	43	1	41
2004	43	1	34
2005	31	0	18
2006	22	4	22
Total	219	15‖	223

* Partial years of enrollment include 1999 (July 1 through December 31) and 2006 (January 1 through June 30).

† Includes patients diagnosed in all regions of Japan, the population of which was 129,180,548 as determined in the 2005 Japanese Census report.

‡ Includes patients diagnosed only in Niigata Prefecture, the population of which was 2,410,000 in 2005.

§ Enrollment in 1999 includes prevalent cases diagnosed before 1999.

‖ Includes 11 cases identified in the Japan-wide screen and four additional cases identified only in the intensive screen conducted in the Niigata prefecture.

A second approach used in the Niigata Prefecture took advantage of the especially close relationship and communication among regional pulmonary physicians, 98% of whom received their training at the Niigata University. Intensive screening in this region (population = 2.41 million) resulted in identification of 15 patients with autoimmune PAP during the study period (Table 2). This approach resulted in a mean (±SE) incidence of 0.49 ± 0.13 per year (in all full years of the study (2000–2005) and a prevalence of 6.2 cases per million of autoimmune PAP.

Clinical Characteristics

At the time of registration in the study, most patients with autoimmune PAP (69%) were symptomatic, with exertional dyspnea being most common (39%), followed by dyspnea and cough (11%) and cough only (10%) (Table 3). Fever was present in two cases, and weight loss was present in one case. Of the patients with autoimmune PAP, 31% were asymptomatic and were identified by mandatory or voluntary annual health screening programs.

Most patients with autoimmune PAP (65%) had no other intercurrent medical illnesses (Table 4). Among those that did, hypertension was most common (8.5%) and was commensurate with the frequency of hypertension in the Japanese population. Other autoimmune diseases were diagnosed in only three individuals and included polymyalgia rheumatica, hemolytic anemia, and Wegner's granulomatosis. Infections occurred in 12 individuals and included pulmonary aspergillosis in four, atypical mycobacteria in three, mycobacterial tuberculosis in two, pneumonia, hepatitis C, and tinea corporus. The proportion of individuals with intercurrent illness did not vary according to gender. COPD and asthma were underrepresented in our study population (Table 4).

Correlation of Disease Severity with Demographic, Clinical, and Biomarker Data

All patients with autoimmune PAP were stratified by disease severity score (DSS) categories at enrollment as defined in the methods from least severe (DSS-1) to most severe (DSS-5). Roughly one quarter of the patients fell into in each of DSS categories 1, 2, and 3, and one quarter were split between DSS categories 4 and 5 (Table 5).

Pulmonary gas transfer (D_{LCO}%) correlated well with DSS, showing a marked decrease at all successive DSS categories

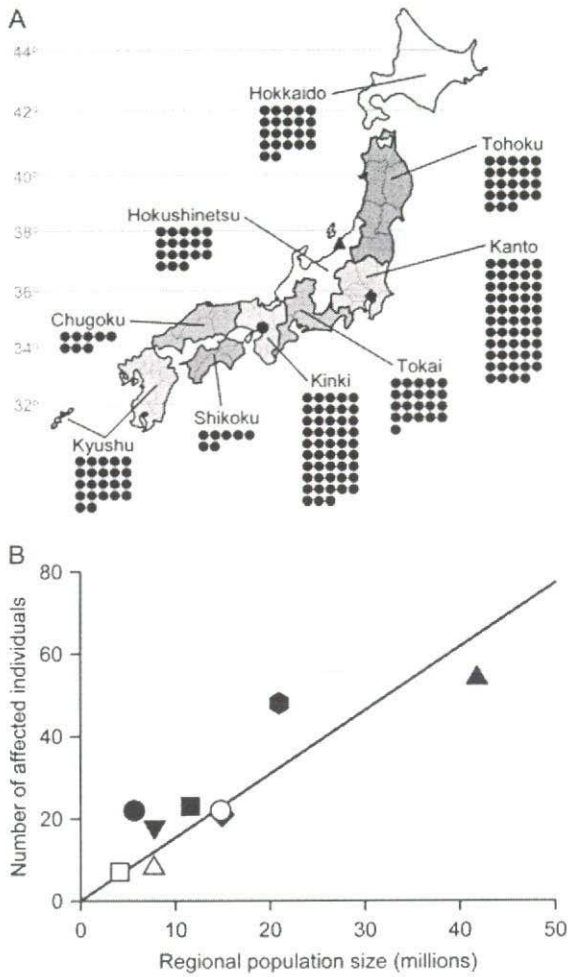

Figure 5. Distribution and regional prevalence of autoimmune pulmonary alveolar proteinosis (PAP) in Japan at the time of enrollment into the study. (*A*) Geographic locations of the patients. Shown are the nine separate regions (regional population size from the 2005 Japanese census report) representing all of Japan, including Hokkaido, (5,653,548), Tohoku (11,568,884), Kanto (41,760,805), Hokushinetsu (7,781,254), Tokai (14,894,128), Kinki (20,930,730), Chugoku (7,705,615), Shikoku (4,128,260), and Kyushu (14,757,324). Each *solid dot* represents one autoimmune PAP patient in the identified region. The Niigata prefecture, in which a second intensive screening for PAP cases was performed, is located within the Hokushinetsu region. The cities of Niigata (*triangle*), Osaka (*circle*) and Tokyo (*diamond*) and the global latitude (in degrees) for each region are indicated for orientation. (*B*) Correlation of the prevalence of autoimmune PAP cases and regional population size. Hokkaido (*solid circle*), Tohoku (*solid square*), Kanto (*solid triangle*), Hokushinetsu (*inverted solid triangle*), Tokai (*solid diamond*), Kinki (*solid hexagon*), Chugoku (*open triangle*), Shikoku (*open square*), Kyushu (*open circle*).

(Figure 6). Although PAP is often described as a lung disorder with restrictive physiology, the lung volumes (FVC% and VC%) were in the normal range in all patients except those with the most severe disease (DSS-5) (Table 5). Notwithstanding, the DSS correlated weakly with the mild reduction in lung volumes (FVC and VC). The DSS did not correlate with airflow limitation (FEV_1/FVC).

Infections were equally distributed in each of DSS categories 1 through 4, but none occurred in DSS-5. Thus, infections were infrequent in our population and did not correlate with DSS or the degree of impairment in pulmonary function (Table 5).

TABLE 3. SYMPTOMS OF THE PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS*

Symptom	Number	%
Asymptomatic	70	31.4
Dyspnea only	87	39.0
Cough only	22	9.9
Sputum only	0	0
Dyspnea + cough	24	10.8
Dyspnea + sputum	3	1.3
Dyspnea + cough + sputum	5	2.2
Other†	9	4.0
Not available	3	1.3

* Complete information was available for 220 individuals.
† Dyspnea and weight loss, or fever, or cough and fever.

The DSS correlated well with selected serum biomarkers, including LDH, SP-A, SP-D, KL-6, and CEA, the strongest of which were the latter two (Table 5). No correlation was observed between DSS and serum GM-CSF autoantibody titer or with antinuclear antibody titer. The DSS was only weakly correlated with age (Table 5) and not with gender, smoking status, history of occupational dust inhalation, or intercurrent medical illness (not shown).

Disease Severity and Progression

Although the present study was cross-sectional in design, data on disease severity and progression since disease onset were collected by the primary treating physician at the time of regis-

TABLE 4. INTERCURRENT MEDICAL ILLNESSES IN INDIVIDUALS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

Intercurrent Medical Illnesses	Total (*n* = 212)	Male (*n* = 143)	Female (*n* = 69)	*P* Value
None, n (%)	137 (65)	91 (63.6)	46 (66.7)	0.78
Hypertension, n (%)	18 (8.5)	11 (7.7)	7 (10.1)	0.74
Infections, n (%)*	12 (5.7)	7 (4.9)	5 (7.2)	ND
Hyperlipidemia, n (%)	9 (4.2)	6 (4.2)	3 (4.3)	ND
Diabetes mellitus, n (%)	8 (3.8)	6 (4.2)	2 (2.9)	ND
Inflammatory disorders of the gastrointestinal tract, n (%)‡	7 (3.3)	5 (3.5)	2 (2.9)	ND
Liver disease, n (%)§	7 (3.3)	6 (4.2)	1 (1.5)	ND
Asthma, n (%)	5 (2.4)	1 (0.1)	4 (5.8)	ND
Airflow limitation, n (%)‖	5 (2.4)	2 (1.4)	3 (2.1)	ND
Allergic rhinitis, n (%)	3 (1.4)	3 (2.1)	0 (0)	ND
Cancer, n (%)¶	4 (1.9)	4 (2.8)	0 (0)	ND
Autoimmune disorders, n (%)**	3 (1.4)	1 (0.1)	2 (2.9)	ND
Interstitial lung disease, n (%)††	3 (1.4)	3 (2.1)	0 (0)	ND
Stroke, n (%)	3 (1.4)	3 (2.1)	0 (0)	ND
Schizophrenia, n (%)	3 (1.4)	2 (1.4)	1 (1.5)	ND
Other disorders‡‡	(<1)	(<1)	(<1)	ND

Definition of abbreviation: ND = not determined.

* Diseases include pulmonary aspergillosis (n = 4), atypical mycobacteria (n = 3), tuberculosis (n = 2), pneumonia (n = 1), hepatitis C (n = 1), and tinea corporus (n = 1).

† Values shown are calculated using the chi-square test. For some diseases, the number of expected values was too low to be evaluated statistically.

‡ Diseases include oral ulceration (n = 1), esophagitis (n = 2), gastritis (n = 1), gastric ulcer (n = 1), duodenal ulcer (n = 1), and ulcerative colitis (n = 1).

§ Diseases include cirrhosis (n = 3), fatty liver (n = 3), and liver dysfunction (n = 1).

‖ Defined as individuals with FEV_1/FVC < 0.7.

¶ Diseases include cancer of the lung (n = 1), colon (n = 1), prostate (n = 1), and thyroid (n = 1).

** Diseases include polymyalgia rheumatica (n = 1), hemolytic anemia (n = 1), and Wegner's granulomatosis (n = 1).

†† Diseases include pulmonary fibrosis (n = 2) and interstitial pneumonitis (n = 1).

‡‡ Diseases include heart failure (n = 2), Alzheimer's (n = 1), obstructive sleep apnea (n = 1), monoclonal hypergammaglobulinemia (n = 1), hypothyroidism (n = 1), angina (n = 1), membranous nephropathy (n = 1), and Parkinson's disease (1).

TABLE 5. CORRELATION OF DISEASE SEVERITY SCORE WITH THE AGE AT DIAGNOSIS, SYMPTOMS, INFECTIONS, PULMONARY FUNCTION, AND SERUM BIOMARKERS IN PATIENTS WITH AUTOIMMUNE PULMONARY ALVEOLAR PROTEINOSIS

	Disease Severity Score*						
Characteristic	1 (*n* = 58)	2 (*n* = 60)	3 (*n* = 55)	4 (*n* = 37)	5 (*n* = 12)	*R*†	*P* Value‡
Age at diagnosis, yr	48 (34–55)	46 (36–56)	55 (45–59)	53 (46–59)	58 (53–65)	0.25	<0.001
Symptomatic, %	0	100.00	78.18	94.59	100.00	0.64	<0.001
Infection, n	3	3	3	3	0	0.01	0.887
Aspergillus spp., n	2	0	1	1	0		
Mycobacterium spp., n	1	1	1	2	0		
Other§	0	2	1	0	0		
Pulmonary function							
FVC, % predicted	97.1 ± 17.3	92.3 ± 17.2	81.9 ± 21.4	82.0 ± 13.1	76.4 ± 19.0	−0.36	<0.001
FEV_1/FVC	82.4 ± 7.9	84.8 ± 10.9	86.0 ± 8.3	85.7 ± 10.7	79.3 ± 27.3	0.16	0.034
VC, % predicted	98.8 ± 16.3	91.3 ± 20.6	87.4 ± 19.2	81.5 ± 14.4	76.1 ± 23.7	−0.35	<0.001
D_{LCO}, % predicted	86.8 ± 23.2	72.4 ± 24.5	64.9 ± 24.1	50.4 ± 18.0	43.1 ± 20.4	−0.56	<0.001
Biomarkers							
GM-CSF autoantibody, μg/ml	14.8 (7.78–25.62)	16.3 (7.49–27.03)	12.75 (6.46–27.38)	16.34 (11.82–25.77)	13.21 (7.70–25.88)	0.004	0.947
LDH, IU/ml	233 (199–365)	316 (247–454)	324 (253–445)	361 (266–474)	406 (247–538)	0.29	<0.001
CEA, ng/ml	1.6 (1.0–2.3)	3.0 (1.8–5.7)	4.7 (2.65–6.85)	5.6 (3.9–12)	17.1 (6–23)	0.53	<0.001
SP-A, ng/ml	48.4 (31.0–74.3)	80.1 (51.3–131)	106 (63.3–143)	133 (78.8–237)	162 (85.4–302)	0.45	<0.001
SP-D, ng/ml	126 (75.9–170)	193 (103–298)	211 (121–324)	268 (183–426)	233 (159–253)	0.37	<0.001
KL-6, U/ml	1,120 (739–2,480)	2,676 (1,585–8,200)	4,030 (2,000–8,330)	7,410 (4,290–12,600)	15,100 (2,150–24,400)	0.52	<0.001
ANA, 1/dilution	0 (0–20)	0 (0–20)	0 (0–40)	0 (0–20)	0 (0–40)	0.03	0.73

Definition of abbreviations: ANA = antinuclear antibody; CEA = carcinoembryonic antigen; GM-CSF = granulocyte/macrophage colony-stimulating factor; LDH = lactate dehydrogenase; SP-A = surfactant protein A; SP-D = surfactant protein D.

* Data are for all participants evaluated at enrollment and are presented as median (interquartile range) or mean ± SE or as percent affected or number of cases where indicated.

† Spearman correlation coefficient.

‡ Values calculated using the Spearman correlation test.

§ Includes hepatitis C (disease severity score [DSS]-3), unspecified pneumonia (DSS-2), and ringworm (DSS-2).

tration. To determine if the severity of autoimmune PAP at registration correlated with its duration, patients were stratified into three groups in which the time between onset and registration was as follows: (*1*) ≤1 year, (*2*) >1 year and ≤10 years, or (*3*) >10 years. No differences were observed among these three groups with respect to (*1*) the proportion of symptomatic individuals, (*2*) the proportion of individuals falling into each DSS category, (*3*) pulmonary function (FVC % predicted, VC % predicted, FEV_1/FVC, D_{LCO} % predicted) (Table 6), or (*4*) most serum biomarkers (GM-CSF autoantibody level, CEA, SP-A, KL-6, and antinuclear antibody) (not shown). A weak correlation of LDH and SP-D with the duration of disease in patients with autoimmune PAP was observed (not shown).

In two-thirds of the recently diagnosed individuals (group 1), symptoms were unchanged from the time of onset to registration (Table 6). In those with disease of intermediate duration (group 2), 42.5% had improved, 29.8% had worsened, and 27.7% were unchanged. In those with prolonged disease (group 3), nearly two-thirds had worsened, and one-quarter were unchanged. This result reflects the various and in some cases multiple treatment approaches used in these individuals.

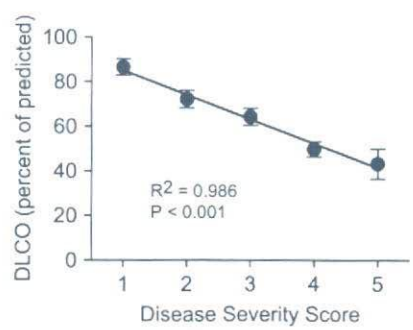

Figure 6. Correlation of the disease severity score with carbon monoxide diffusing capacity (D_{LCO}) in patients with autoimmune pulmonary alveolar proteinosis. Results are shown as mean (±SE) for individuals classified as disease severity score (DSS)-1 (n = 42), DSS-2 (n = 38), DSS-3 (n = 27), DSS-4 (n = 28), or DSS-5 (n = 12).

Our study design also permitted enrollment of asymptomatic individuals with autoimmune PAP. Among those asymptomatic at registration, 60% of those recently diagnosed, 74.1% with an intermediate duration of disease, and 33.3% with prolonged disease were unchanged since onset (Table 6). Despite the absence of whole-lung lavage therapy for PAP, 11 of 39 (28.2%) had subclinical disease or had undergone spontaneous improvement and were asymptomatic at the time of registration.

Correlations with Serum GM-CSF Autoantibody Concentration

To determine if the severity of the pulmonary abnormalities was correlated with the level of GM-CSF autoantibody, patients with autoimmune PAP were grouped into quartiles according to their serum GM-CSF autoantibody level (Q1–Q4 = <7.9, 8–15.2, 15.3–26.8, and >26.8 μg/ml, respectively). GM-CSF autoantibody levels in serum did not correlate with duration of disease, DSS, pulmonary function (FVC%, VC%, FEV_1%, D_{LCO}%), or serum biomarkers (LDH, SP-A, SP-D, CEA, KL-6) (*see* Table E1 in the online supplement). Even in individuals with GM-CSF autoantibody levels above 35 μg/ml (the top 13% of individuals), no correlations were observed in any of these parameters (not shown). The serum GM-CSF autoantibody also did not correlate with age, gender, smoking status, a history of environmental or occupational dust inhalation exposure, or duration of disease (not shown).

DISCUSSION

In this study, we report on the epidemiologic, demographic, clinical, pulmonary function, and serum biomarker data from