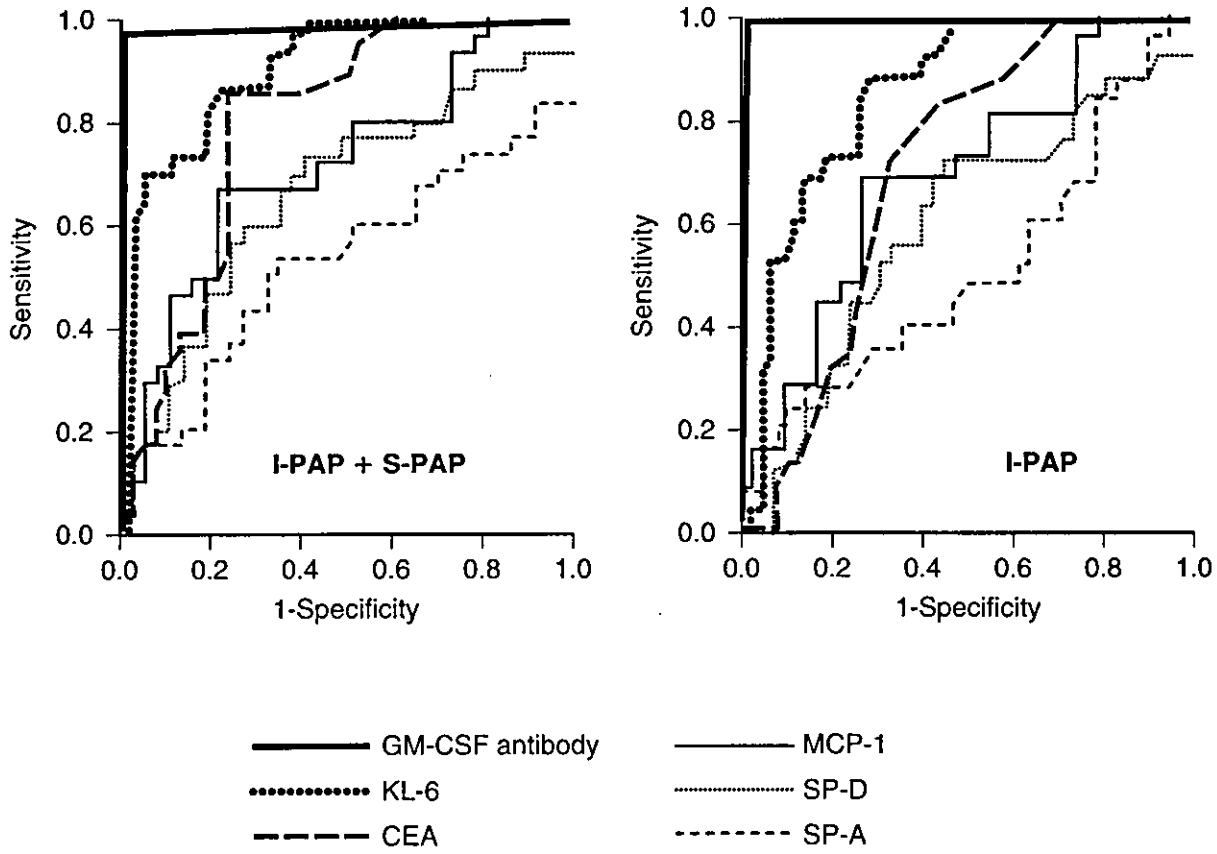

Fig. 6. Comparison of serum markers (GM-CSF antibody, KL-6, CEA, SP-A, SP-D, MCP-1) as diagnostic tests for PAP ("idiopathic", I-PAP; secondary, S-PAP) by receiver operator characteristic curves.

are normally low, the presence of neutralizing autoantibodies might have an important effect on GM-CSF bioactivity levels in the lung [156].

Analogous to murine PAP models, it is plausible that the autoantibodies reduce GM-CSF activity, which results in alveolar macrophage dysfunction and surfactant accumulation. Estimation of the neutralizing activity of the autoantibody in the lung of patients and characterization of their biologic properties revealed that GM-CSF bioactivity was completely abrogated in BALF of patients with idiopathic PAP but not in normal subjects. The autoantibodies were present in the alveoli in high concentrations and co-localized with GM-CSF. The autoantibody recognizes human GM-CSF with high avidity (K_{AV} = 20.0 ± 7.5 pM) and high specificity, reacting with its superstructure. Although target epitopes varied among patients, GM-CSF amino acids 78 to 94 were consistently recognized. The autoantibodies bind GM-CSF with high specificity and high affinity, abundantly exist in the lung, and effectively block GM-CSF binding to its receptor, which inhibits alveolar macrophage differentiation and function.

Granulocyte-macrophage colony-stimulating factor as therapy for pulmonary alveolar proteinosis

Although the enormous body of data that clarified the pathogenetic link between GM-CSF neutralizing antibodies and PAP was not known at the time, clinical trials that explored the therapeutic role of GM-CSF commenced in the mid 1990s. The first treated patient in the world responded favorably, with improved oxygenation, which prompted early reporting of this new phenomenon [157]. At the completion of this initial study using 5 μg/kg/day subcutaneous GM-CSF with the potential for later dose escalation, considering the initial treatment and the subsequent dose-escalation schedule, 6 of 14 evaluable patients responded (43% response rate; 95% confidence interval, 18%–71%), with responses lasting a median of 39 weeks (range, 6–67 weeks) [158]. Responses were generally slow to be manifest, with improvements in $[A - a]O_2$ not evident until 4 to 6 weeks, with maximal improvements not achieved until 6 to 10 weeks of therapy. Among treatment-related variables, only the peak eosinophil count predicted re-

sponse (median 0.63 versus 0.27 × 10^9/L; P=0.01). This study also noted the markedly attenuated hematopoietic response among treated patients [159], a phenomenon attributable to the neutralizing activity of the GM-CSF antibody. Subcutaneous GM-CSF treatment was easily deliverable, with only one patient ceasing treatment because of an adverse effect. Toxicities at 5 μg/kg were infrequent and mild, the most common being local erythema and induration in 36% of patients. Patients with PAP also seem to have a lower incidence of adverse effects than anticipated [44,45]. The presence of GM-CSF–neutralizing antibodies likely results in a reduced effective biologic dose.

Other independent studies have replicated many of these findings and support the therapeutic activity of GM-CSF in patients with acquired PAP. A prospective trial was run through the Cleveland Clinic, using 5 to 18 μg/kg/day for 10 to 48 weeks (median, 26 weeks). Using the criteria to define clinical benefit of a minimum 10 mm Hg improvement in $[A-a]D_{O_2}$, they have seen responses in 6 of 13 patients (46%) [160–162] that were accompanied by "symptomatic, physiologic, and radiographic improvement" [160]. Among responding patients, the mean arterial P_{O_2} improved from 69.1 ± 3.4 mm Hg at baseline to 84.0 ± 2.0 at the completion of therapy [162]. The overall response rate from these two prospective studies combined is 44% (95% confidence intervals, 26%–65%). There have been six published individual case reports of subcutaneous, GM-CSF using that they used 3 to 6 μg/kg/day [163–168], with five patients responding. The cumulative response rate among all published cases is 52% (17/33; 95% confidence intervals, 34%–69%), although there is likely to be publication bias among single case reports. This frequency of response to GM-CSF is significantly greater than the highest suggested rates of "spontaneous remission" in any series, and, together with the favorable responses to retreatment, clearly establish that SC GM-CSF has therapeutic activity in a proportion of patients with acquired PAP.

Given the detection of GM-CSF antibodies in all of the treated patients, it is not apparent why therapeutic responses were variable. Although limited data are available, the effective antibody titer, which reflects total GM-CSF–neutralizing capacity, may be lower in responders [21,162]. Improvements in oxygenation are achieved more rapidly after lavage than with GM-CSF; however, issues such as resource availability, comorbid conditions, and symptom severity also influence treatment considerations. Although it seems that within the dose range of 5 to 18 μg/kg/day of GM-CSF the proportion of patients responding to lavage may be higher, the magnitude of the therapeutic effect displayed by responding patients with either approach may be similar. From the available reports that provide specific data on pre- and post-lavage arterial P_{O_2} levels [1], the mean improvement in arterial P_{O_2} is 12 to 19 mm Hg with lavage (overall mean 14.5 mm Hg), whereas the mean improvements in arterial P_{O_2} for all patients who responded to GM-CSF were 23 mm Hg [158] and 14.9 mm Hg [162].

Although these studies have demonstrated the reproducible therapeutic activity of GM-CSF in patients with idiopathic acquired PAP, many aspects of this novel therapy require further investigation. Even within the limited number of patients treated to date, some patients required significant dose escalation to attain therapeutic effects. The sequential application of increasing doses seeking evidence of biologic activity, such as eosinophilia, may identify appropriate dose ranges for further evaluation. Although the subcutaneous route was used in most patients, based on its proven safety and efficacy in other settings, recent animal work suggests that inhaled GM-CSF may have greater pulmonary effects [77] but conversely it may not ameliorate any extrapulmonary aspects of the disease. Although aerosolized GM-CSF is well tolerated in humans [134,169] and this route of GM-CSF delivery has been used successfully in three patients with acquired PAP [170,171], the issues of pharmacokinetics and reliable drug delivery remain unresolved.

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Serum neutralizing capacity of GM-CSF reflects disease severity in a patient with pulmonary alveolar proteinosis successfully treated with inhaled GM-CSF

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Summary Existence of anti-granulocyte-macrophage colony-stimulating factor (GM-CSF) neutralizing antibody and treatment with recombinant GM-CSF are new topics in idiopathic pulmonary alveolar proteinosis (PAP). We have hypothesized inhaled GM-CSF is effective and neutralizing capacity of GM-CSF, not concentration of anti-GM-CSF antibody in serum reflect disease severity.

A 57-year-old female smoker with idiopathic PAP was treated with inhaled GM-CSF. The response to the treatment was evaluated by diffusing capacity for carbon monoxide (*DL*CO), alveolar-arterial oxygen gradient ($[A\text{-}a]DO_2$). Conventional serum markers, including KL-6, surfactant apoprotein (SP)-A, SP-D, carcino-embryonic antigen and cytokeratin fragment 19 (CYFRA), and concentration of anti-GM-CSF antibody were examined. The neutralizing capacity of GM-CSF in serum was evaluated using a GM-CSF dependent cell line, TF-1.

Ground glass opacity disappeared at the end of the treatment. Her *DL*CO, $[A\text{-}a]DO_2$ remarkably improved after treatment. The neutralizing capacity of GM-CSF declined in line with disease remission and it correlated significantly with *DL*CO ($P = 0.0137$). The concentration of anti-GM-CSF antibody had no significant relation with disease severity and serum markers including neutralizing capacity. Conventional serum markers other than CYFRA showed no significant correlation with *DL*CO.

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Inhaled GM-CSF was effective for idiopathic PAP. Serial measurement of neutralizing capacity of GM-CSF was useful to evaluate disease severity and the anti-GM-CSF antibody was proved to be a causative factor for PAP. In the future, inhaled GM-CSF may replace whole lung lavage and response to GM-CSF and its optimal amount may be decided by the capacity.

Introduction

The pathogenesis of pulmonary alveolar proteinosis (PAP) is supposed to be through impaired alveolar macrophage function caused by deficiency of granulocyte-macrophage colony-stimulating factor (GM-CSF) in alveolar spaces, induced by anti-GM-CSF antibody.[1–4] In 1996, Seymour et al. [5] reported that subcutaneous administration of GM-CSF was effective for PAP, and the result was confirmed in two published prospective phase II trials.[6,7] We hypothesize that inhaled GM-CSF in a patient with severe PAP are effective through a result of the experiment using GM-CSF knockout mouse [8] and that neutralizing capacity of GM-CSF in serum is a novel and useful serum marker to reflect disease severity.

Methods

Patient and clinical course before GM-CSF inhalation

A 57-year-old female smoker suffered from cough with white sputum and progressive shortness of breath on exertion for 4 months. High-resolution computed tomography (HRCT) of the chest showed crazy paving appearance. Diffusing capacity for carbon monoxide (*DL*CO) was reduced at 34.2% of the predicted value and the arterial oxygen tension (PaO_2) on room air was 43 Torr. She was diagnosed with idiopathic PAP from the findings of bronchoalveolar lavage and transbronchial lung biopsy.[2] Anti-GM-CSF antibody was detected in the serum and bronchoalveolar lavage fluid. One month later, her disease did not improved and she was treated with GM-CSF inhalation.

GM-CSF inhalation

Bacterially- synthesized recombinant GM-CSF (Leucomax; Schering-Plough, Baulkham Hills, Australia) of 125 mcg was nebulized with a PARI LC PLUS nebulizer set (Pari Respiratory Equipment, Inc., Richmond, VA, USA) twice-a-day, 7 days a week, followed by a treatment-free week, repeated for 24 weeks. This method is based on the early study for cancer patients.[9]

Assay of the neutralizing capacity of GM-CSF and concentration of anti-GM-CSF antibody in the serum

GM-CSF dependent TF-1 cells were cultured with or without recombinant human GM-CSF, in the presence of serum from idiopathic PAP or control serum. Survival of TF-1 cells was evaluated using MTT assay as described previously.[2] The neutralizing capacity was defined as the following equation:

Neutralizing capacity $(\%)=[1-(A-B)/(C-D)] \times 100$, where *A*, *B*, *C* and *D* are the absorbance of TF-1 cells grown with serum from PAP and GM-CSF, with serum from PAP and no GM-CSF, with control serum and GM-CSF, and with control serum and no GM-CSF, respectively.

Concentration of anti-GM-CSF antibody in serum and BALF was measured by the enzyme-linked immunosorbent assay previously described.[10]

Statistical methods

Correlation of variables was assessed using the Spearman rank correlation coefficient. Linear regression lines were calculated using the least-squares method. The *P* value is significant, if it is less than 0.05.

Results

Response of GM-CSF inhalation and serial change of neutralizing capacity, anti-GM-CSF antibody level and the other serum markers

After the first 2 weeks of the treatment $[A\text{-}a]DO_2$ and *DL*CO improved (Fig. 1a). Ground glass opacity on HRCT almost completely disappeared at the final inhalation. This therapy was tolerable without apparent toxicity.

The levels of serum cytokeratin fragment 19 (CYFRA), KL-6, carcino-embryonic antigen (CEA), surfactant apoprotein (SP)-A, SP-D and lactate dehydrogenase (LDH), conventional serum markers

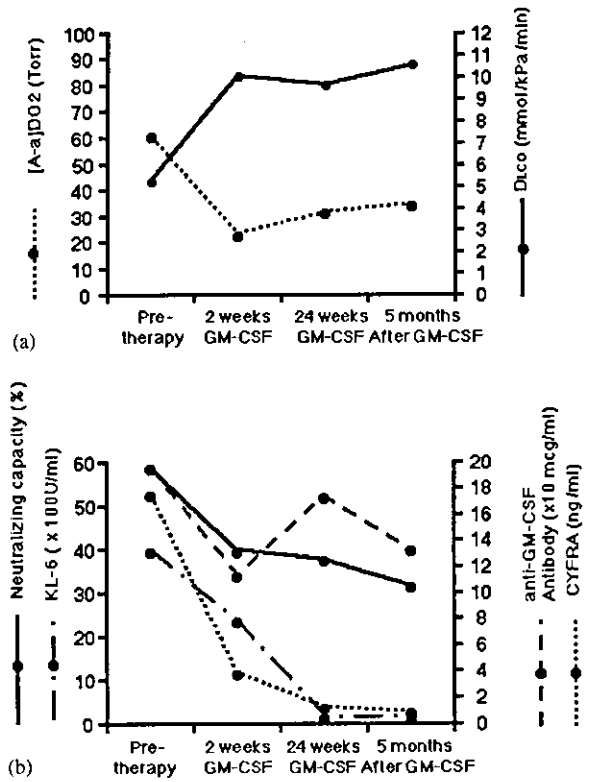

Figure 1 (a) Diffusing capacity of carbon monoxide (*DLCO*) increased and alveolar-arterial oxygen gradient ($[A\text{-}a]DO_2$) decreased after the GM-CSF inhalation. (b) The levels of serum cytokeratin fragment 19 (CYFRA) and KL-6 declined rapidly during the first two weeks of the granulocyte macrophage colony-stimulating factor (GM-CSF) inhalation therapy. Neutralizing capacity of GM-CSF in serum apparently decreased gradually, although concentration of anti-GM-CSF antibody did not decline remarkably. The cut off level for abnormal elevation was 500 U/ml for KL-6 and 2.8 ng/ml for CYFRA.

for PAP, rapidly decreased two weeks after the first inhalation (Fig. 1b). Neutralizing capacity of GM-CSF in serum apparently decreased gradually, although concentration of anti-GM-CSF antibody did not decline remarkably (Fig. 1b).

Correlation between neutralizing capacity and disease severity

Correlation between serum markers and disease severity markers, $[A\text{-}a]DO_2$ and *DLCO*, was analyzed (data not shown). The neutralizing capacity had significant correlation with *DLCO* ($n = 7$, rho $= -0.8571$, $P = 0.0137$) and levels of SP-A, KL-6, CEA and CYFRA, but not with $[A\text{-}a]DO_2$. Serum anti-GM-CSF antibody concentration was correlated neither with the disease severity markers nor with serum markers including neutralizing capacity of GM-CSF. Conventional serum markers other than CYFRA ($n = 7$, rho $= -0.8571$, $P = 0.0137$) showed no significant correlation with *DLCO*.

Discussion

The concentration of anti-GM-CSF antibody and neutralizing capacity of GM-CSF were evaluated as new serum markers in the patient successfully treated with inhaled GM-CSF. The anti-GM-CSF antibody level did not decline significantly and it was not correlated with any of the serum and disease severity markers in our case. The neutralizing capacity of GM-CSF kept decreasing gradually and it was significantly correlated with *DLCO*, and with serum markers, KL-6, SP-A, CEA and CYFRA. These results suggest that it is useful to assess the disease severity of individual PAP patients during the clinical courses and neutralization of GM-CSF is fundamental for pathogenesis of PAP.

Seymour [10] also showed that the concentration of anti-GM-CSF antibody is not correlated with the respiratory functions of PAP patients, suggesting that the neutralizing capacity of the antibody is variable in the individual cases. If so, concentration of anti-GM-CSF antibody should correlate with neutralizing capacity of GM-CSF in each PAP patient, but we could not find significant correlation between these two markers in the presented case. Anti-GM-CSF antibodies are known to be polyclonal [1] in each case, and the binding capacity of each antibody should be different. The antibody with strong binding capacity to GM-CSF will be present more as a form of immune-complex with GM-CSF and be cleared more rapidly through opsonization by mature macrophage activated by administered GM-CSF than one with weak binding capacity. Thus the antibody with weak binding capacity persisted longer than the one with strong capacity and neutralizing capacity of GM-CSF in serum gradually decreased, even if the concentration of the antibody did not decrease significantly.

We conclude that inhaled GM-CSF is effective treatment for idiopathic PAP, and that the measurement of the neutralizing capacity is useful to evaluate disease severity as a new serum marker reflecting the activity of a causative factor, anti-GM-CSF antibody. We anticipate that the response to GM-CSF and its optimal amount in each PAP patient may be decided using this new marker in addition to the other serum markers.

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REVIEW ARTICLE

MECHANISMS OF DISEASE

Pulmonary Alveolar Proteinosis

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PULMONARY ALVEOLAR PROTEINOSIS IS A RARE DISORDER IN WHICH lipoproteinaceous material accumulates within alveoli.[1] The clinical course of the disease is variable, ranging from respiratory failure to spontaneous resolution. An important feature of the disease is susceptibility to pulmonary infections, sometimes with opportunistic organisms.

Pulmonary alveolar proteinosis occurs in three clinically distinct forms: congenital, secondary, and acquired. The congenital form comprises a heterogeneous group of disorders[2] caused by mutations in the genes encoding surfactant protein B or C or the β_C chain of the receptor for granulocyte–macrophage colony-stimulating factor (GM-CSF).[3-7] Secondary pulmonary alveolar proteinosis develops in association with conditions involving functional impairment or reduced numbers of alveolar macrophages. Such conditions include some hematologic cancers, pharmacologic immunosuppression, inhalation of inorganic dust (e.g., silica) or toxic fumes, and certain infections.[8-15] Acquired (or idiopathic) pulmonary alveolar proteinosis has been an enigmatic and fascinating disorder since its initial description, in 1958.[1] Recent observations in transgenic mice and humans, however, have provided important clues to its pathogenesis. In this review, we highlight the ways in which these studies led to the concept that acquired pulmonary alveolar proteinosis is an autoimmune disease targeting GM-CSF and the ways in which the critical role of GM-CSF in the lung was identified.

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EPIDEMIOLOGY

The prevalence of acquired pulmonary alveolar proteinosis has been estimated to be 0.37 per 100,000 persons.[16] It is a primary acquired disorder in more than 90 percent of cases.[7,17-19] The median age at the time of diagnosis is 39 years; most patients are men, and 72 percent have a history of smoking.[19] The male predominance may be linked to the more frequent use, historically, of tobacco by men.[19]

CLINICAL, RADIOGRAPHIC, AND LABORATORY MANIFESTATIONS

CLINICAL PRESENTATION

Most patients with acquired pulmonary alveolar proteinosis present with progressive exertional dyspnea of insidious onset and cough.[1,17-20] Less commonly, fever, chest pain, or hemoptysis also occurs, especially if secondary infection is present. The history does not include clinically significant environmental pulmonary exposures or other potential causes. The findings on physical examination can be unremarkable, but there are inspiratory crackles in 50 percent of patients, cyanosis in 25 percent, and digital clubbing in a small percentage. Several reviews,[17-19] including an excellent analysis of data from 410 patients, accounting for most if not all of the published cases,[19] provide further details on the clinical presentation, demographics, and clinical course of patients with acquired pulmonary alveolar proteinosis.

In uncomplicated pulmonary alveolar proteinosis, the chest radiograph usually reveals bilateral air-space disease with an ill-defined nodular or confluent pattern, often with a perihilar predominance suggestive of the "bat wing" appearance of pulmonary edema but without other radiographic signs of left-sided heart failure (Fig. 1A).[1,21,22] Notably, the extent of radiographic abnormalities is often disproportionately increased relative to the severity of the symptoms and physical findings. High-resolution computed tomography shows patchy, ground-glass opacifications with superimposed interlobular septal and intralobular thickening, a pattern commonly referred to as "crazy paving" (Fig. 1B).[23,24] Though not specific for pulmonary alveolar proteinosis,[24] the extent and severity of these radiographic findings correlate with the degree of impairment in pulmonary function as measured by spirometry or arterial blood gas analysis.[23]

LABORATORY FINDINGS

In acquired pulmonary alveolar proteinosis, routine blood counts and the results of routine blood chemical analysis and urinalysis are usually normal.[18,22,25] The serum level of lactate dehydrogenase is frequently slightly elevated[26] and may be a useful marker of the severity of the disease.[19,26] Elevations in the serum levels of carcinoembryonic antigen,[27] cytokeratin 19,[28] mucin KL-6,[29] and surfactant proteins A, B, and D[30,31] are of unclear prognostic value.

PULMONARY FUNCTION

The results of tests of pulmonary function can be normal, but typically they show a restrictive ventilatory defect with slight impairments in the forced vital capacity and total lung capacity and a disproportionate, severe reduction of the carbon monoxide diffusing capacity.[19,32] Hypoxemia is caused by ventilation–perfusion inequality and intrapulmonary shunting, resulting in a widened alveolar–arteriolar diffusion gradient.[19,33]

CHARACTERISTICS OF BRONCHOALVEOLAR-LAVAGE FLUID

Clinical and radiographic findings often suggest the diagnosis of pulmonary alveolar proteinosis[1,22,34]; in about 75 percent of suspected cases, findings on examination of a bronchoalveolar-lavage specimen can establish the diagnosis.[22] The lavage fluid in patients with this disorder has an opaque, milky appearance (Fig. 2A). It contains large and foamy alveolar macrophages (Fig. 2B) or monocyte-like alveolar macrophages and increased numbers of lymphocytes[35,36] but relatively few inflammatory cells of other types. There are also large, acellular,

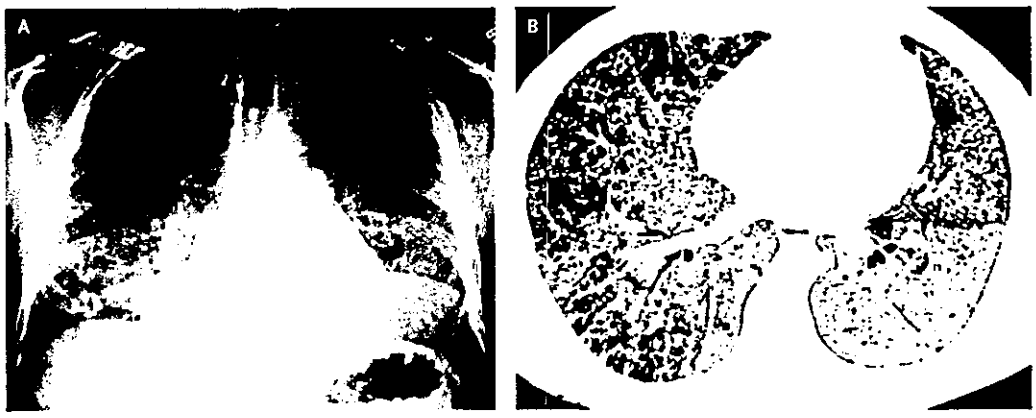


Figure 1. **Radiographic Appearance of Pulmonary Alveolar Proteinosis.**

A posteroanterior chest radiograph shows the typical features of pulmonary alveolar proteinosis, including widespread, bilateral air-space disease that is patchy and asymmetric in nature and that is not accompanied by evidence of cardiomegaly, adenopathy, or effusion (Panel A). A high-resolution computed tomographic scan of the chest shows patchy areas of ground-glass opacification and interlobular septal thickening, a pattern commonly characterized as "crazy paving" (Panel B).

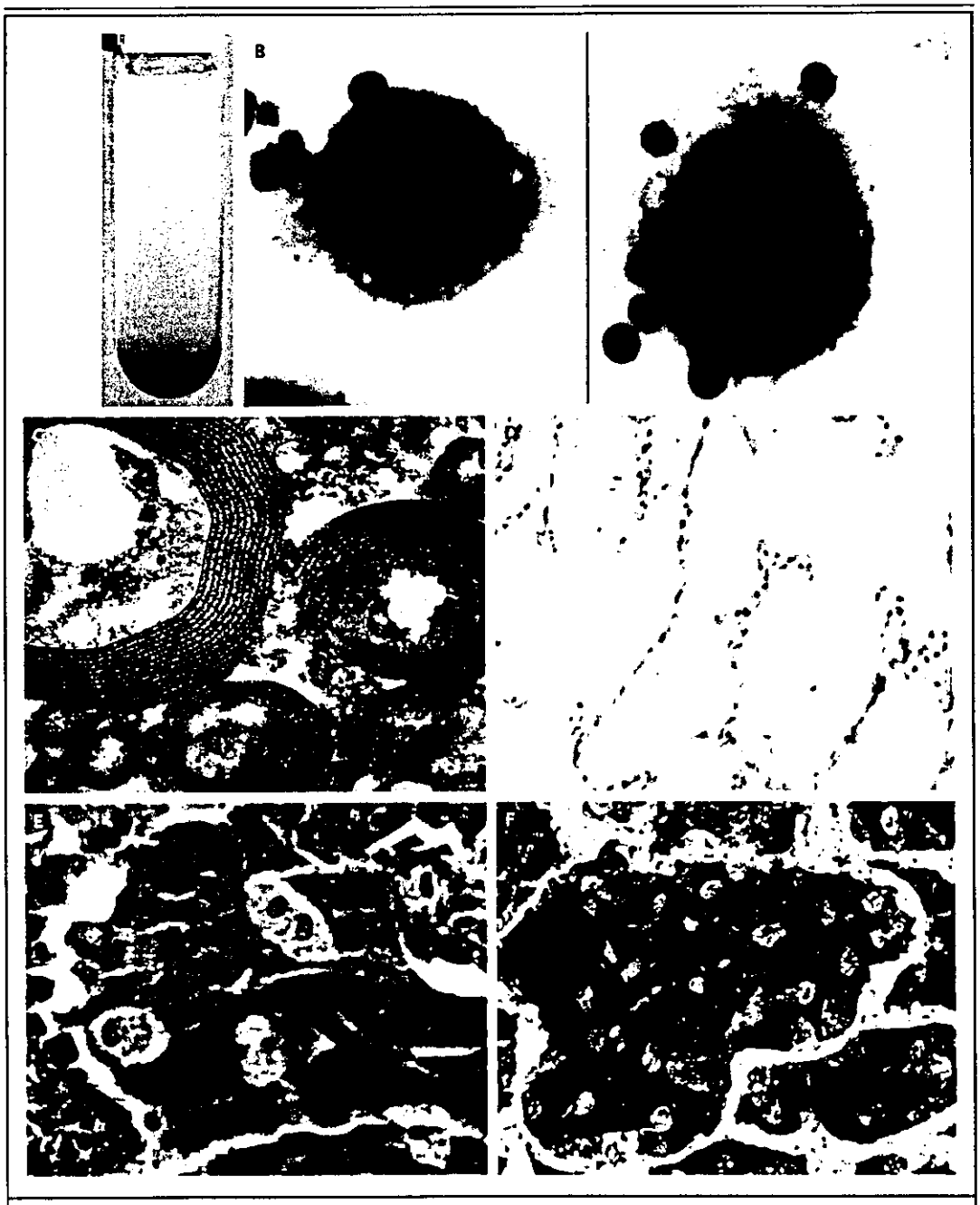


Figure 2. **Appearance of the Lipoproteinaceous Material Accumulating in the Lungs in Acquired Pulmonary Alveolar Proteinosis.**

Opalescent, viscous, milky material removed from the lungs by lavage settles in a culture flask (Panel A). Cytologic preparations of bronchoalveolar-lavage fluid from two patients show "foamy" alveolar macrophages (Panel B; buffered eosin and azure B, ×480). Comparison with the brown-staining red cells also visible in these preparations shows that the macrophages are two to three times their normal size. On ultrastructural examination, sediment from the bronchoalveolar-lavage fluid shows fused membrane structures and amorphous debris (Panel C; uranyl acetate, ×30,000). A lung-biopsy specimen contains alveoli filled with eosinophilic material; there is relative preservation of the parenchymal architecture and no inflammatory response (Panel D; hematoxylin and eosin, ×100). Another lung-biopsy specimen shows abundant intraalveolar material that stains with periodic acid–Schiff (Panel E, ×400). On immunohistochemical analysis, abundant accumulation of surfactant protein A can be seen in the intraalveolar space (Panel F; human anti–surfactant protein A immunostain, ×200).

eosinophilic bodies in a diffuse background of granular material that stains with periodic acid–Schiff, as well as elevated levels of surfactant proteins.[22,36] Electron microscopy shows that the intraalveolar material consists of amorphous, granular debris containing numerous osmiophilic, fused membrane structures with a periodicity of 4.7 nm and resembling lamellar bodies and tubular myelin (Fig. 2C).

PATHOLOGICAL FEATURES

Open-lung biopsy is the gold standard for the diagnosis of pulmonary alveolar proteinosis, but it is not always required and can be complicated by false negative results due to sampling error.[1,22,37] On light-microscopical examination, the architecture of the lung parenchyma is preserved unless there is infection. The walls of transitional airways and alveoli are usually normal (Fig. 2D), but sometimes they are thickened by lymphocytic infiltration or, less commonly, fibrosis. Alveoli are filled with granular, eosinophilic material that stains with periodic acid–Schiff (Fig. 2D and 2E) and within which intact and degenerating macrophages are usually evident. Immunohistochemical staining reveals abundant accumulation of surfactant protein (Fig. 2F). A useful serologic test for the disease (discussed below) has been developed.[38]

NATURAL HISTORY

In any given case of acquired pulmonary alveolar proteinosis, the clinical course falls into one of three categories: stable but with persistent symptoms, progressive deterioration, or spontaneous improvement.[1] A retrospective analysis of 303 cases[19] found clinically significant spontaneous improvement in 24 (8 percent). In a retrospective analysis of 343 cases, the five-year survival rate was about 75 percent.[19] Of the deaths in that study, 72 percent were directly due to respiratory failure from pulmonary alveolar proteinosis and 20 percent were due to pulmonary alveolar proteinosis with uncontrolled infection.

Patients with acquired pulmonary alveolar proteinosis are at risk for infections from a variety of pathogens.[18,19,39] Although such infectious agents include common respiratory pathogens, opportunistic pathogens (especially nocardia) are common.[18,19,40] Interestingly, infections in pulmonary alveolar proteinosis frequently occur at sites outside the lung, suggesting systemic defects in host defense.[19,41-43]

SURFACTANT HOMEOSTASIS

Surfactant plays a vital part in reducing surface tension at the air–liquid interface of the alveolar wall, thus preventing alveolar collapse and transudation of capillary fluid into the alveolar lumen.[44] About 90 percent of surfactant is lipid (predominantly phospholipid), 10 percent is protein, and less than 1 percent is carbohydrate. Surfactant proteins A, B, C, and D contribute to the surface-active properties and structural forms of intraalveolar surfactant,[45] participate in surfactant metabolism,[46] opsonize microbial pathogens,[47] and stimulate the defensive functions of alveolar macrophages.[48] Surfactant lipids and proteins are synthesized, stored, and secreted into the alveoli by alveolar type II epithelial cells and are cleared by uptake into alveolar type II cells and alveolar macrophages (Fig. 3A). The size of the surfactant pool is tightly regulated by mechanisms controlling the synthesis, recycling, and catabolism of surfactant.[49]

In their initial description of pulmonary alveolar proteinosis,[1] Rosen et al. established that the eosinophilic material within the alveoli was rich in lipids and that it contained proteins and carbohydrates. Similarities between this material[1] and the substance lining the normal alveolar wall[50,51] suggested an abnormality in the production, degradation, or structure of this surface-active material[52] in the disorder.[53] These similarities and the identification of defects in the clearance, but not the synthesis, of alveolar phospholipid pointed to an underlying defect in the clearance of surfactant.[54] The results of ultrastructural,[37,55] biochemical,[56,57] and functional[58] investigations, together with the results of studies in genetically modified mice (discussed below), strongly support the concept that the alveolar material in pulmonary alveolar proteinosis is in fact surfactant, which accumulates due to reduced clearance rather than to overproduction.[59]

MOUSE MODELS

An important clue to the pathogenesis of pulmonary alveolar proteinosis came in 1994, with the discovery that a pulmonary disorder similar to the acquired form of the disease in humans developed in knockout mice that were deficient in GM-CSF.[60,61] GM-CSF, a 23-kD hematologic growth factor,[62] is encoded by a gene the structure and pattern of expression of which are similar in humans and

mice.[63,64] The biologic effects of GM-CSF are initiated when it binds to cell-surface receptors on various hematopoietic cells, including monocytes and macrophages, and other cells, including alveolar type II epithelial cells.[65-69] Until 1994, the principal biologic effects of GM-CSF were thought to be stimulation of the production of myeloid cells from hematopoietic precursors and enhancement of some immune functions in mature myeloid cells.[64,70] Indeed, GM-CSF is used to ameliorate chemotherapy-induced neutropenia and to hasten hematopoietic recovery after bone marrow transplantation.[67,71]

GM-CSF AND SURFACTANT HOMEOSTASIS

Targeted disruption of the gene encoding GM-CSF or the gene encoding the β_C chain of the GM-CSF receptor in mice ($GM^{-/-}$ and $\beta_C^{-/-}$ mice, respectively) causes accumulations of eosinophilic lipoproteinaceous material and large, foamy macrophages in the alveoli.[60,61,72,73] The alveolar material contains tubular myelin and lamellar bodies as well as surfactant phospholipids and surfactant proteins at dramatically increased levels.[74] Except for a reduction in the number of eosinophils in the blood, these mice had no base-line hematologic abnormalities.

Studies of the lungs of $GM^{-/-}$ mice disclosed that levels of messenger RNA for surfactant proteins A, B, and C were not altered relative to those in control mice, suggesting that the biosynthesis of these proteins was not increased.[60] The secretion of surfactant phospholipids into the alveolar space also was not increased, but pulmonary phospholipid clearance was severely impaired, resulting in an increase in the size of the alveolar phospholipid pool by a factor of 6.3.[74] Pulmonary clearance of surfactant protein A was also impaired. The abnormal accumulation of surfactant phospholipids and proteins in pulmonary alveolar proteinosis in both humans and mice suggested that there was a defect in the catabolism of surfactant by alveolar macrophages. This hypothesis was supported by findings on examination of alveolar macrophages recovered from $GM^{-/-}$ mice. Despite increased uptake by the alveolar macrophages of surfactant phospholipids and proteins, the catabolism of these molecules was severely impaired.[75]

Effect of GM-CSF Replacement

The efficacy of GM-CSF replacement was assessed in $GM^{-/-}$ mice by three methods: administration of GM-CSF,[76] expression of the GM-CSF gene in the lungs of double-transgenic mice with the use of a lung-specific promoter from the gene encoding surfactant protein C (SPC-$GM^{+/+}$/$GM^{-/-}$ mice),[77] and expression of GM-CSF in the lungs of $GM^{-/-}$ mice after adenovirus-mediated transfer of the GM-CSF gene.[78] Each of these distinct approaches resulted in resolution of the pulmonary alveolar proteinosis. The site of action of GM-CSF must have been within the lung, because the GM-CSF levels were high in the lungs but undetectable in the blood of SPC-$GM^{+/+}$/$GM^{-/-}$ mice.[77] Moreover, pulmonary, but not systemic, administration of GM-CSF resulted in resolution of the disorder in $GM^{-/-}$ mice.[76]

Cellular Target of GM-CSF

Notwithstanding, these studies did not identify the cellular target of GM-CSF: was it the alveolar macrophage or the alveolar type II epithelial cell? This question was answered during studies in the $\beta c^{-/-}$ mouse, in which both cell types are unresponsive to GM-CSF because of the absence of the high-affinity GM-CSF receptor.[66,73] Transplantation of bone marrow from normal mice corrected the defective metabolism of surfactant in the $\beta c^{-/-}$ mice.[72] Since the alveolar macrophages, but not the alveolar type II epithelial cells, in the recipient mice were of donor origin, we can conclude that bone marrow–derived alveolar macrophages are the principal target of GM-CSF replacement.[72]

IMMUNE FUNCTIONS OF ALVEOLAR MACROPHAGES AND GM-CSF

Prompted by the high risk of infections in acquired pulmonary alveolar proteinosis, investigators examined host defenses in $GM^{-/-}$ mice. These mice are susceptible to pulmonary infection by group B streptococcus[79] and *Pneumocystis carinii* (after CD4+ depletion)[80] and have severely impaired pulmonary clearance of bacterial, fungal, and viral pathogens.[79-81] Of note, primary and cultured alveolar macrophages from $GM^{-/-}$ mice have defects in cellular adhesion, expression of pathogen-recognition receptors, phagocytosis, superoxide production, microbial killing, and secretion of proinflammatory cytokines.[79-84] All these abnormalities were corrected by restoring pulmonary expression of GM-CSF. Hence, it could be concluded that this factor has a critical role in protecting the lung against infection and that it carries out this role by acting locally, within the lung itself.

ROLE OF THE TRANSCRIPTION FACTOR PU.1

The diversity of the abnormalities in alveolar macrophages in $GM^{-/-}$ mice suggested that the maturation of these macrophages was defective. Indeed, pulmonary GM-CSF stimulates the production of high levels of PU.1 in alveolar macrophages.[84] PU.1 is a transcription factor that promotes the growth and differentiation of myeloid progenitors and that is required for the production of macrophages.[85-92] Transfection of the PU.1 gene into cultured alveolar macrophages from $GM^{-/-}$ mice corrected all the alveolar-macrophage abnormalities described above and, it is important to note, also corrected abnormalities in the catabolism of surfactant lipids and protein[59,81,82,84] (Fig. 4).

LESSONS FROM ANIMAL MODELS

Thus, studies in mouse models of pulmonary alveolar proteinosis revealed the critical roles of GM-CSF in surfactant homeostasis and in alveolar-macrophage–mediated protection of the lung against infection. GM-CSF acts within the lung by stimulating the terminal differentiation of alveolar macrophages, principally by raising the levels of PU.1. The accumulation of alveolar surfactant in $GM^{-/-}$ mice is due to a defect in surfactant clearance by alveolar macrophages, and not to an increase in production.

PATHOGENESIS IN HUMANS

Initially, it was thought that an inhaled irritant (e.g., silica) or infectious agent that increased the production of the natural material lining the alveoli caused pulmonary alveolar proteinosis.[25,53] However, the inability to find such agents in the lung-biopsy specimens of most patients with the disorder failed to support this idea. The strong association between acquired pulmonary alveolar proteinosis and smoking suggests that there is a link between the two, but nothing more is known about this association.

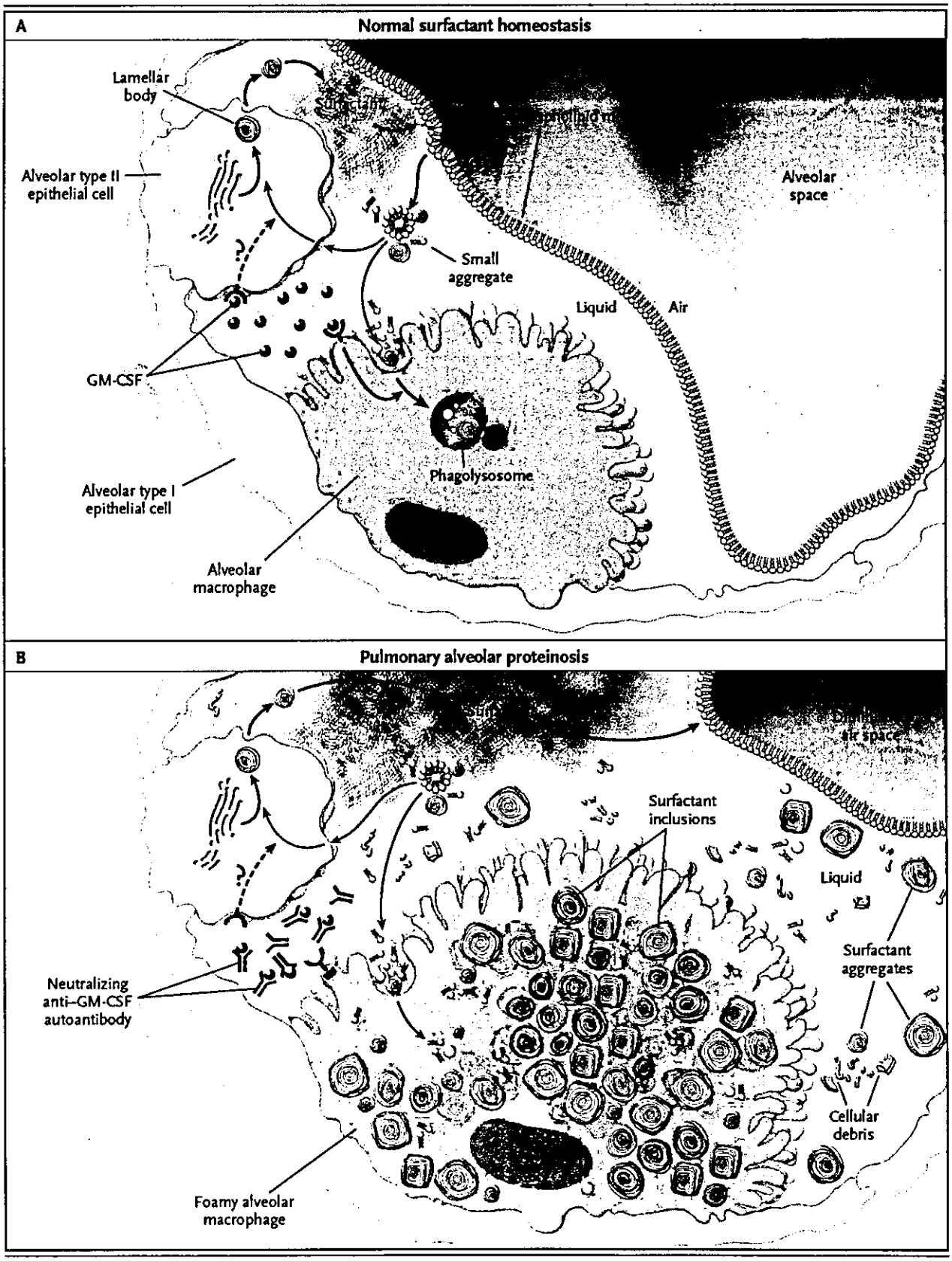
ROLE OF AUTOIMMUNITY

Inhibition of Alveolar Macrophages

The alveolar macrophages in acquired pulmonary alveolar proteinosis contain giant secondary lysosomes filled with the same material that accumulates within the alveoli,[93] and they have defects in chemotaxis,[93] adhesion,[93] phagocytosis,[94] microbicidal activity,[93] and phagolysosome fusion.[95] This puzzling array of abnormalities was initially attributed to excessive ingestion of lipoproteinaceous material.[96] However, that idea was difficult to reconcile with the discovery of a substance, found in bronchoalveolar-lavage fluid from patients with pulmonary alveolar proteinosis, that caused normal alveolar macrophages to acquire some of those abnormalities.[97,98] Furthermore, a factor found in both pulmonary-lavage fluid and serum from patients with pulmonary alveolar proteinosis blocked mitogen-stimulated proliferation of normal mono-

Figure 3 (facing page). **Surfactant Homeostasis and Impaired Surfactant Catabolism in Pulmonary Alveolar Proteinosis.**

Granulocyte–macrophage colony-stimulating factor (GM-CSF) has a critical role in surfactant homeostasis in the normal lung (Panel A). Interruption of GM-CSF signaling in the lung results in pulmonary alveolar proteinosis (Panel B). Surfactant lipids and surfactant proteins A, B, C, and D are produced by alveolar type II epithelial cells (solid black arrows). Surfactant protein precursors are processed in the Golgi network. Some (surfactant proteins B and C) are then assembled, along with surfactant phospholipids, in lamellar bodies; surfactant proteins A and D are secreted by other secretory vesicles. After exocytosis into the alveolar surface liquid, the lamellar bodies assemble into surfactant structures known as tubular myelin as well as into large and small aggregates. Phospholipids from extracellular surfactant structures form continuous monolayers and multilayers of phospholipids that line the alveolar spaces and airways, with their polar heads oriented toward the liquid and their acyl chains toward the air. The large aggregates, extracellular lamellar bodies, and tubular myelin all have surface-active properties. Normally (Panel A), surfactant is inactivated by mechanical and biologic processes and converted into small, surface-inactive aggregates. Approximately 70 to 80 percent of the small aggregates are taken up by alveolar type II cells, transported to phagolysosomes, and reused (green arrows) or catabolized (red arrows). Alveolar macrophages internalize and catabolize the remaining surfactant pool, a process critically dependent on GM-CSF. Although GM-CSF stimulates lung growth and causes alveolar type II epithelial-cell hyperplasia, a potential role for GM-CSF in surfactant recycling by these cells has not been defined (dashed black arrows). In pulmonary alveolar proteinosis (Panel B), interruption of GM-CSF signaling (small red bar) in the alveolar macrophage — for example, by targeted ablation of the gene encoding GM-CSF or its receptor in mice or, presumably, by neutralizing anti–GM-CSF autoantibodies in humans — impairs the catabolism of surfactant by alveolar macrophages without impairing its uptake. This results in the intracellular buildup of membrane-bound, concentrically laminated surfactant aggregates. Progressive expansion of the extracellular surfactant pool and accumulation of cellular debris due to the impaired catabolism eventually cause filling of the alveoli, thus reducing the size of the available gas-exchange surface and eventually leading to the clinical syndrome.


A
Normal surfactant homeostasis
Lamellar body
Alveolar type II epithelial cell
Alveolar space
Small aggregate
Liquid
Air
GM-CSF
Phagolysosome
Alveolar type I epithelial cell
Alveolar macrophage
B
Pulmonary alveolar proteinosis
Surfactant inclusions
Liquid
Surfactant aggregates
Neutralizing anti–GM-CSF autoantibody
Cellular debris
Foamy alveolar macrophage