

## REVIEW ARTICLE

## MECHANISMS OF DISEASE

## Pulmonary Alveolar Proteinosis

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**P**ULMONARY ALVEOLAR PROTEINOSIS IS A RARE DISORDER IN WHICH lipoproteinaceous material accumulates within alveoli.<sup>1</sup> 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<sup>2</sup> caused by mutations in the genes encoding surfactant protein B or C or the  $\beta_C$  chain of the receptor for granulocyte-macrophage colony-stimulating factor (GM-CSF).<sup>3-7</sup> 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.<sup>8-15</sup> Acquired (or idiopathic) pulmonary alveolar proteinosis has been an enigmatic and fascinating disorder since its initial description, in 1958.<sup>1</sup> 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.<sup>16</sup> It is a primary acquired disorder in more than 90 percent of cases.<sup>7,17-19</sup> The median age at the time of diagnosis is 39 years; most patients are men, and 72 percent have a history of smoking.<sup>19</sup> The male predominance may be linked to the more frequent use, historically, of tobacco by men.<sup>19</sup>

CLINICAL, RADIOGRAPHIC,  
AND LABORATORY MANIFESTATIONS

## CLINICAL PRESENTATION

Most patients with acquired pulmonary alveolar proteinosis present with progressive exertional dyspnea of insidious onset and cough.<sup>1,17-20</sup> 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,<sup>17-19</sup> including an excellent analysis of data from 410 patients, accounting for most if not all of the published cases,<sup>19</sup> 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).<sup>1,21,22</sup> 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).<sup>23,24</sup> Though not specific for pulmonary alveolar proteinosis,<sup>24</sup> 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.<sup>23</sup>

#### LABORATORY FINDINGS

In acquired pulmonary alveolar proteinosis, routine blood counts and the results of routine blood chemical analysis and urinalysis are usually normal.<sup>18,22,25</sup> The serum level of lactate dehydrogenase is frequently slightly elevated<sup>26</sup> and may be a useful marker of the severity of the disease.<sup>19,26</sup> Elevations in the serum levels of carcinoembryonic an-

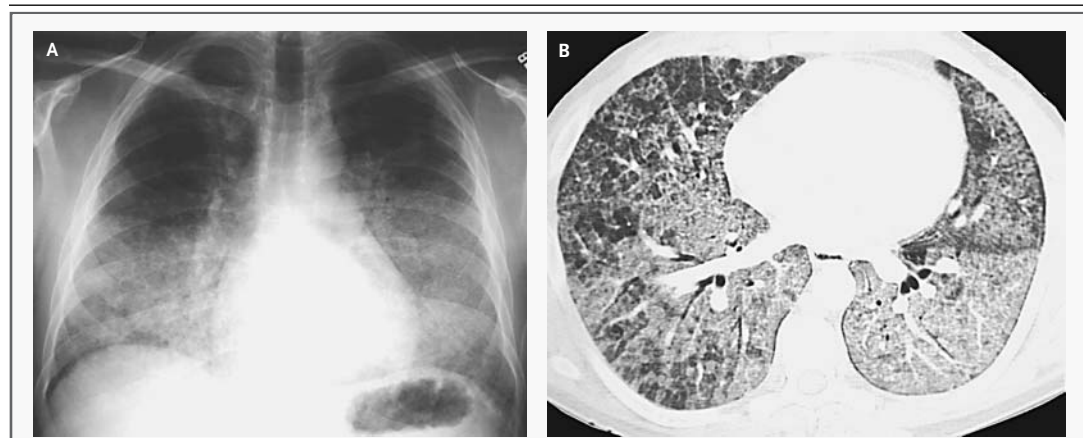
tigen,<sup>27</sup> cytokeratin 19,<sup>28</sup> mucin KL-6,<sup>29</sup> and surfactant proteins A, B, and D<sup>30,31</sup> are of unclear prognostic value.

#### 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.<sup>19,32</sup> Hypoxemia is caused by ventilation–perfusion inequality and intrapulmonary shunting, resulting in a widened alveolar–arteriolar diffusion gradient.<sup>19,33</sup>

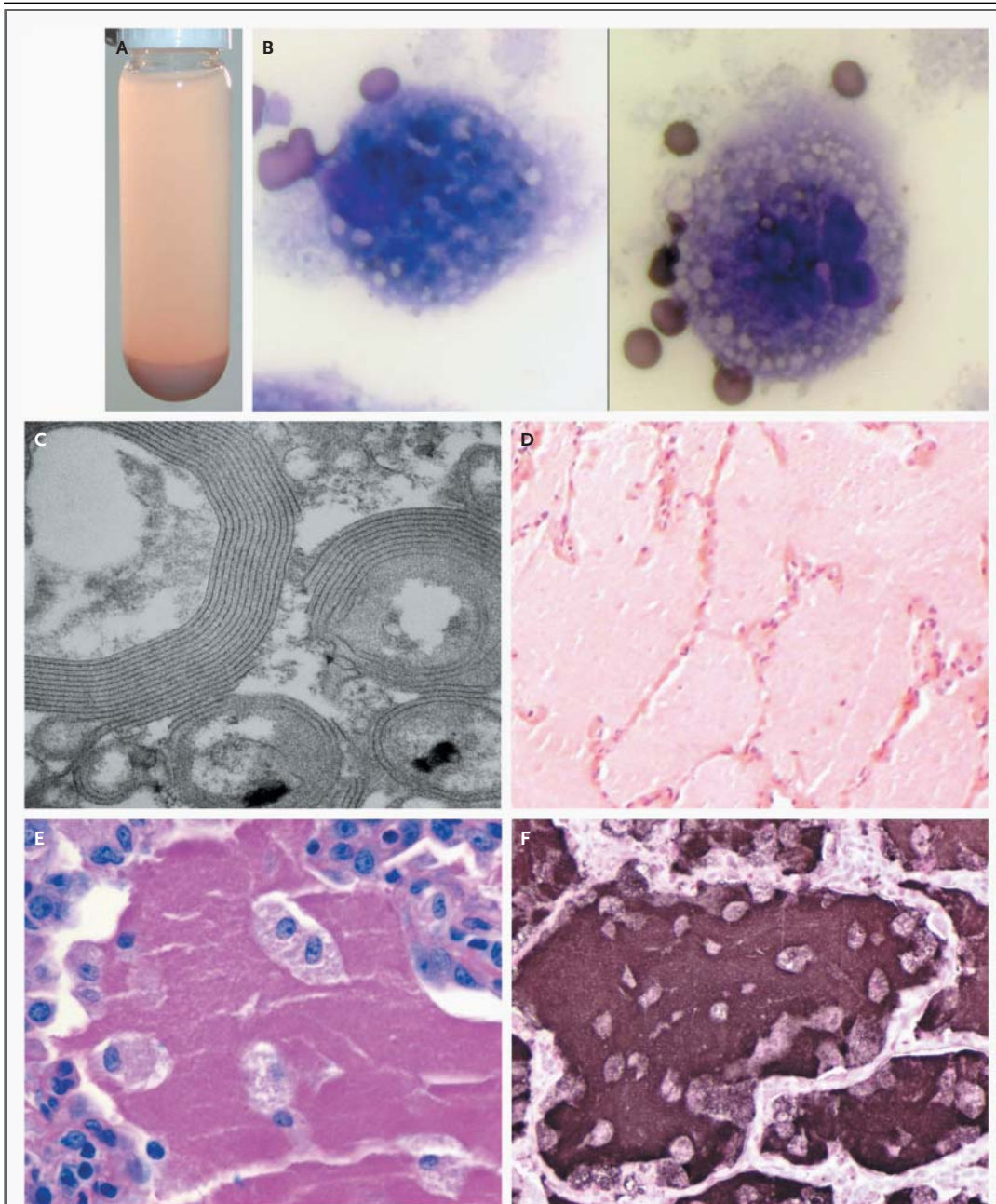
#### CHARACTERISTICS OF BRONCHOALVEOLAR-LAVAGE FLUID

Clinical and radiographic findings often suggest the diagnosis of pulmonary alveolar proteinosis<sup>1,22,34</sup>; in about 75 percent of suspected cases, findings on examination of a bronchoalveolar-lavage specimen can establish the diagnosis.<sup>22</sup> 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<sup>35,36</sup> 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,  $\times 480$ ). 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,  $\times 30,000$ ). 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,  $\times 100$ ). Another lung-biopsy specimen shows abundant intraalveolar material that stains with periodic acid-Schiff (Panel E,  $\times 400$ ). On immunohistochemical analysis, abundant accumulation of surfactant protein A can be seen in the intraalveolar space (Panel F; human anti-surfactant protein A immunostain,  $\times 200$ ).

eosinophilic bodies in a diffuse background of granular material that stains with periodic acid–Schiff, as well as elevated levels of surfactant proteins.<sup>22,36</sup> 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.<sup>1,22,37</sup> 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.<sup>38</sup>

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#### NATURAL HISTORY

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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.<sup>1</sup> A retrospective analysis of 303 cases<sup>19</sup> 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.<sup>19</sup> 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.<sup>18,19,39</sup> Although such infectious agents include common respiratory pathogens, opportunistic pathogens (especially nocardia) are common.<sup>18,19,40</sup> Interestingly, infections in pulmonary alveolar proteinosis frequently occur at sites outside the lung, suggesting systemic defects in host defense.<sup>19,41-43</sup>

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#### SURFACTANT HOMEOSTASIS

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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.<sup>44</sup> 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,<sup>45</sup> participate in surfactant metabolism,<sup>46</sup> opsonize microbial pathogens,<sup>47</sup> and stimulate the defensive functions of alveolar macrophages.<sup>48</sup> 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.<sup>49</sup>

In their initial description of pulmonary alveolar proteinosis,<sup>1</sup> 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<sup>1</sup> and the substance lining the normal alveolar wall<sup>50,51</sup> suggested an abnormality in the production, degradation, or structure of this surface-active material<sup>52</sup> in the disorder.<sup>53</sup> 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.<sup>54</sup> The results of ultrastructural,<sup>37,55</sup> biochemical,<sup>56,57</sup> and functional<sup>58</sup> 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.<sup>59</sup>

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#### MOUSE MODELS

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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.<sup>60,61</sup> GM-CSF, a 23-kD hematologic growth factor,<sup>62</sup> is encoded by a gene the structure and pattern of expression of which are similar in humans and

mice.<sup>63,64</sup> 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.<sup>65-69</sup> 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.<sup>64,70</sup> Indeed, GM-CSF is used to ameliorate chemotherapy-induced neutropenia and to hasten hematopoietic recovery after bone marrow transplantation.<sup>67,71</sup>

#### GM-CSF AND SURFACTANT HOMEOSTASIS

Targeted disruption of the gene encoding GM-CSF or the gene encoding the  $\beta_C$  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.<sup>60,61,72,73</sup> The alveolar material contains tubular myelin and lamellar bodies as well as surfactant phospholipids and surfactant proteins at dramatically increased levels.<sup>74</sup> 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.<sup>60</sup> 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.<sup>74</sup> 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.<sup>75</sup>

#### *Effect of GM-CSF Replacement*

The efficacy of GM-CSF replacement was assessed in  $GM^{-/-}$  mice by three methods: administration of

GM-CSF,<sup>76</sup> 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),<sup>77</sup> and expression of GM-CSF in the lungs of  $GM^{-/-}$  mice after adenovirus-mediated transfer of the GM-CSF gene.<sup>78</sup> 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.<sup>77</sup> Moreover, pulmonary, but not systemic, administration of GM-CSF resulted in resolution of the disorder in  $GM^{-/-}$  mice.<sup>76</sup>

#### *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.<sup>66,73</sup> Transplantation of bone marrow from normal mice corrected the defective metabolism of surfactant in the  $\beta_C^{-/-}$  mice.<sup>72</sup> 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.<sup>72</sup>

#### 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<sup>79</sup> and *Pneumocystis carinii* (after CD4+ depletion)<sup>80</sup> and have severely impaired pulmonary clearance of bacterial, fungal, and viral pathogens.<sup>79-81</sup> 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.<sup>79-84</sup> 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<sup>-/-</sup> 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.<sup>84</sup> PU.1 is a transcription factor that promotes the growth and differentiation of myeloid progenitors and that is required for the production of macrophages.<sup>85-92</sup> Transfection of the *PU.1* gene into cultured alveolar macrophages from GM<sup>-/-</sup> 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<sup>59,81,82,84</sup> (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<sup>-/-</sup> 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.<sup>25,53</sup> 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.

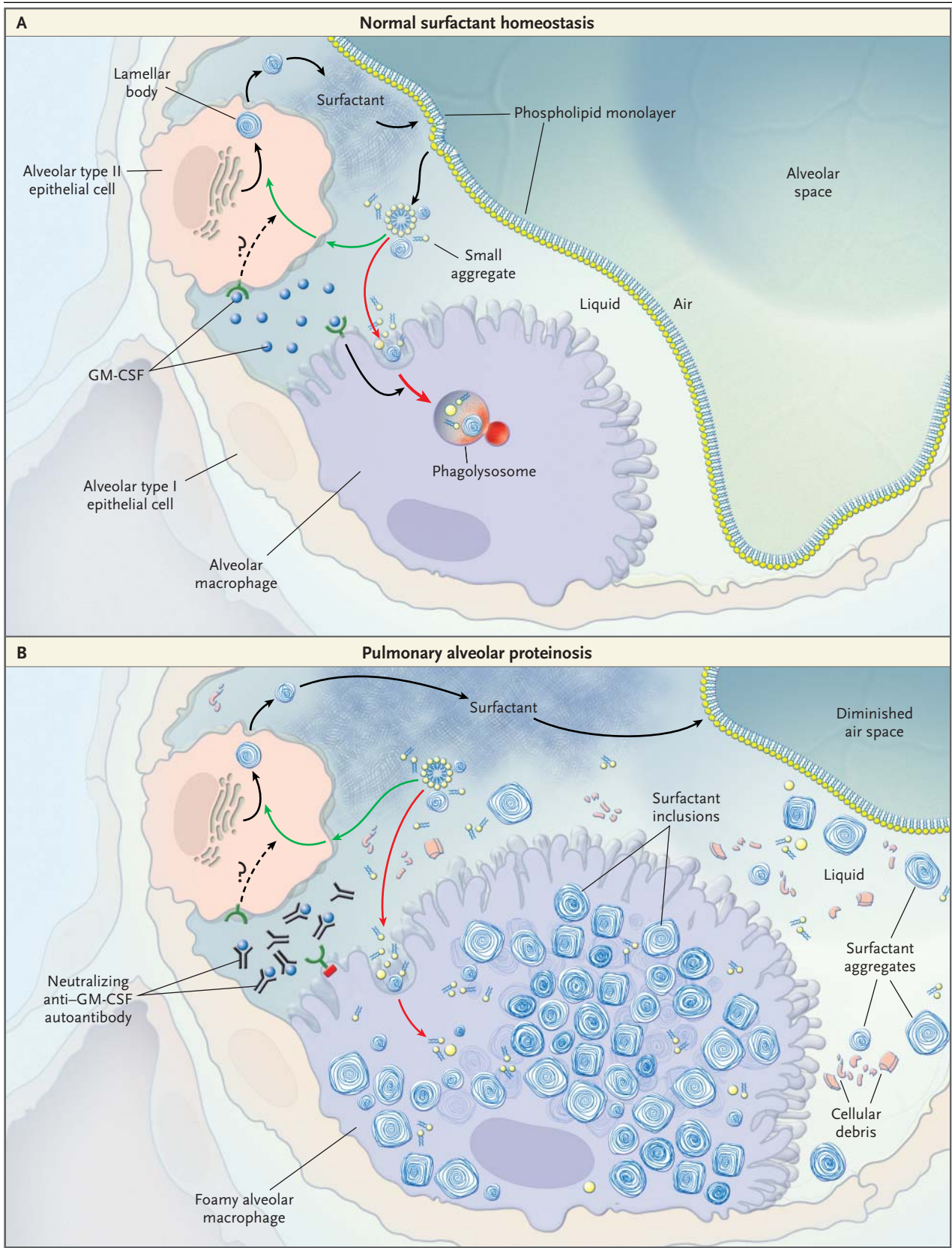
**ROLE OF AUTOIMMUNITY***Inhibition of Alveolar Macrophages*

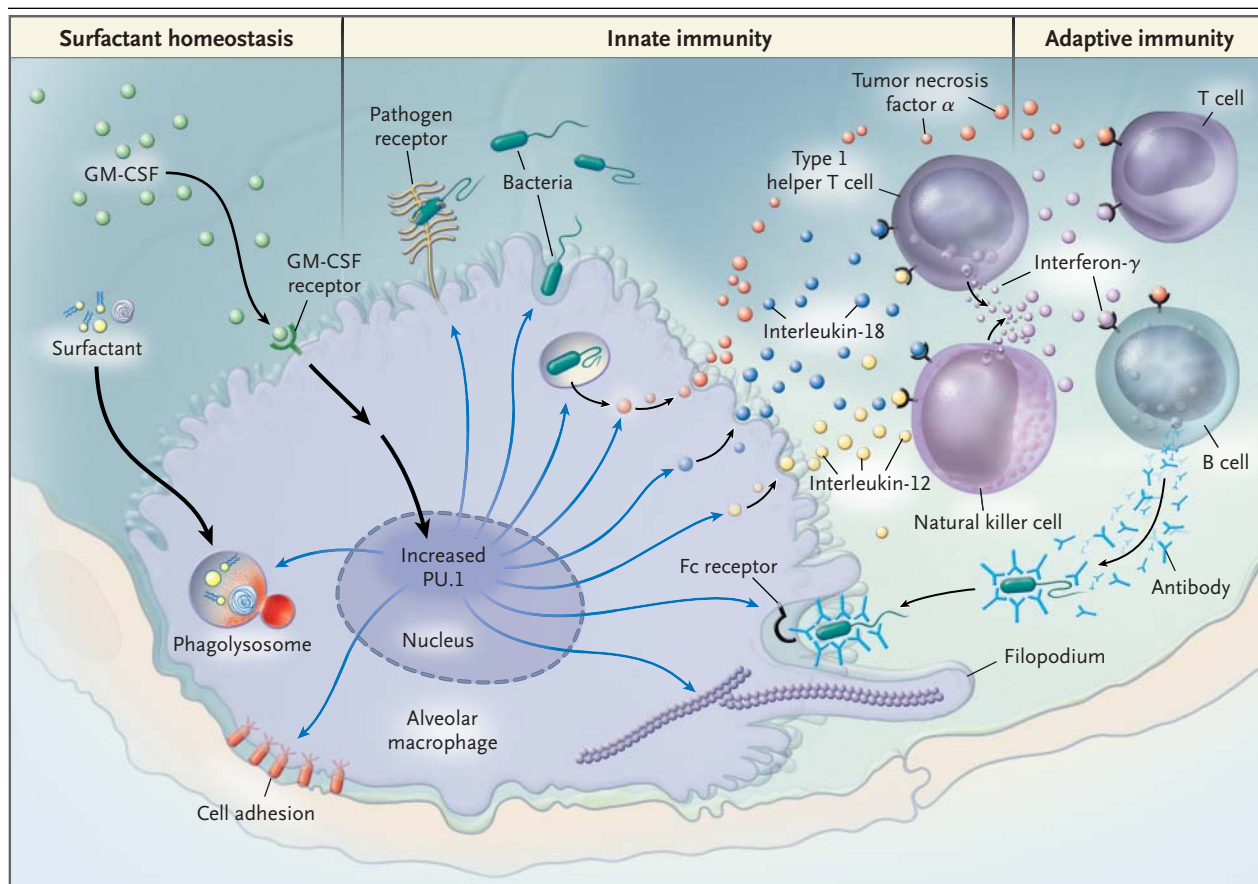
The alveolar macrophages in acquired pulmonary alveolar proteinosis contain giant secondary lysosomes filled with the same material that accumulates within the alveoli,<sup>93</sup> and they have defects in chemotaxis,<sup>93</sup> adhesion,<sup>93</sup> phagocytosis,<sup>94</sup> microbicidal activity,<sup>93</sup> and phagolysosome fusion.<sup>95</sup> This puzzling array of abnormalities was initially attributed to excessive ingestion of lipoproteinaceous material.<sup>96</sup> However, that idea was difficult to recon-

**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.

cile 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.<sup>97,98</sup> 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 4. Role of Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF) in Modulating the Function of Alveolar Macrophages in Mice.**

In vivo, pulmonary GM-CSF stimulates an increase in the level of PU.1, a transcription factor, in alveolar macrophages in the lung. In vitro, alveolar macrophages from knockout mice without the GM-CSF gene have a number of functional defects, including defects in cellular adhesion, catabolism of surfactant proteins and surfactant lipids, expression of pathogen-associated molecular pattern receptors (e.g., toll-like receptors and the mannose receptor), toll-like–receptor signaling, phagocytosis of pathogens (bacteria, fungi, and viruses), intracellular killing of bacteria (independent of uptake), pathogen-stimulated secretion of cytokines (tumor necrosis factor  $\alpha$ , interleukin-12, and interleukin-18), and Fc receptor–mediated phagocytosis. Cytoskeletal organization is abnormal and may in part account for defects in phagocytosis. The inability of alveolar macrophages to release interleukin-12 and interleukin-18 severely impairs the interferon- $\gamma$  response to pulmonary infection, thus impairing an important molecular connection between innate and adaptive immunity in the lung. Retroviral-vector–mediated, constitutive expression of PU.1 in alveolar macrophages from GM-/- mice corrects all these defects, suggesting that GM-CSF stimulates terminal differentiation of the macrophages through the global transcription factor PU.1. The blue arrows represent the functions of PU.1 that are affected by the absence of GM-CSF.

cytes, suggesting the involvement of a circulating inhibitor in the pathogenesis of the disease.<sup>99</sup>

**Role of GM-CSF**

The demonstration that GM-CSF deficiency caused pulmonary alveolar proteinosis in mice prompted a reevaluation of the pathogenesis of the acquired form of the disease in humans. A clue came from a report that the systemic administration of recombinant human GM-CSF had produced radiographic,

physiological, and symptomatic improvement in one affected patient.<sup>100</sup> Similar treatment of additional patients (discussed below) failed to produce the expected neutrophilia<sup>100</sup> — a curious finding that was confirmed in subsequent studies.<sup>30,101</sup> Attempts to identify mutations in the genes encoding GM-CSF and its receptor in acquired pulmonary alveolar proteinosis have been unsuccessful to date,<sup>102</sup> in contrast to findings in the congenital form of the disease.<sup>15</sup> Furthermore, the levels of GM-CSF in



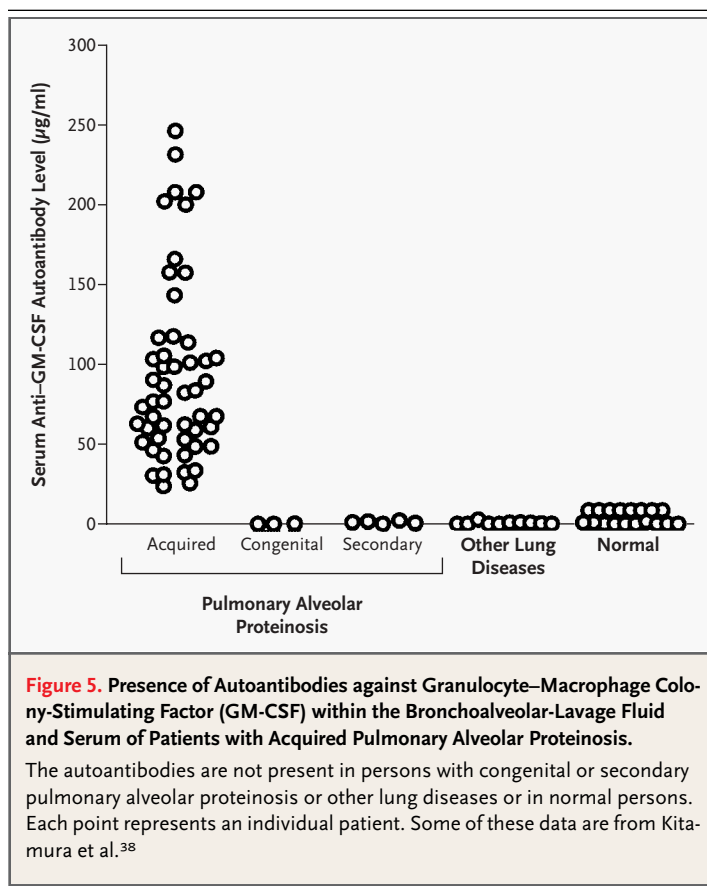
bronchoalveolar-lavage fluid and plasma are actually elevated in the acquired form, thus ruling out the possibility that the disease is due to the absence of GM-CSF itself.<sup>103</sup>

#### Autoantibodies against GM-CSF

An immunologic explanation for these observations was revealed by a reexamination of the inhibitory factor in pulmonary alveolar proteinosis. Bronchoalveolar-lavage fluid from subjects with the disease, but not that from control subjects, inhibited the ability of GM-CSF to stimulate the proliferation of normal monocytes and a GM-CSF-dependent cell line and competitively inhibited the binding of GM-CSF to cells bearing GM-CSF receptors.<sup>104</sup> This inhibitory activity was due to a neutralizing IgG antibody against GM-CSF.<sup>105</sup> The antibody was present in bronchoalveolar-lavage fluid and serum from all patients with acquired pulmonary alveolar proteinosis but not those with the congenital or secondary form of the disorder, those with several other lung disorders, or normal controls (Fig. 5).<sup>105,106</sup> The specific association between neutralizing anti-GM-CSF autoantibodies and acquired pulmonary alveolar proteinosis<sup>38,105,106</sup> strongly supports the view that in this disorder, a neutralizing autoantibody against GM-CSF causes defects in the functioning of alveolar macrophages, including impairment of the catabolism of surfactant lipids and proteins and disruption of surfactant homeostasis. Further strong support for this concept comes from the recent demonstration that the presence of these antibodies is correlated with the elimination of GM-CSF bioactivity in the lungs of patients with pulmonary alveolar proteinosis.<sup>107</sup> The finding of this autoantibody has led to the development of a latex-agglutination test with high sensitivity (100 percent) and specificity (98 percent) for diagnosing the acquired disease.<sup>38</sup>

#### PULMONARY CYTOKINES

Similarities between pulmonary alveolar proteinosis in mice and the acquired form of the disease in humans also include abnormalities of pulmonary cytokines. For example, the level of macrophage colony-stimulating factor, which is elevated in the lungs of GM<sup>-/-</sup> mice,<sup>84</sup> is also elevated in the lungs of humans with acquired pulmonary alveolar proteinosis.<sup>106</sup> Similarly, the level of monocyte chemoattractant protein 1 is elevated in the lungs of both GM<sup>-/-</sup> mice and humans with the acquired disease.<sup>35,83</sup> The mechanism of these cytokine changes is not



**Figure 5.** Presence of Autoantibodies against Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) within the Bronchoalveolar-Lavage Fluid and Serum of Patients with Acquired Pulmonary Alveolar Proteinosis.

The autoantibodies are not present in persons with congenital or secondary pulmonary alveolar proteinosis or other lung diseases or in normal persons. Each point represents an individual patient. Some of these data are from Kitamura et al.<sup>38</sup>

known, but the latter may explain the increased numbers of lymphocytes in the lungs of mice<sup>60</sup> and patients with pulmonary alveolar proteinosis.<sup>35</sup>

## THERAPEUTIC APPROACHES

### CURRENT APPROACHES

The treatment of pulmonary alveolar proteinosis depends on the underlying cause. Current therapy for the congenital form of the disorder is supportive,<sup>7</sup> although successful lung transplantation has been reported.<sup>108</sup> Therapy for secondary pulmonary alveolar proteinosis generally involves treatment of the underlying condition; for example, when the disorder is associated with a hematologic cancer, successful chemotherapy or bone marrow transplantation corrects the associated pulmonary disorder.<sup>12</sup>

Acquired pulmonary alveolar proteinosis has been treated successfully since the early 1960s by whole-lung lavage, and this procedure remains the standard of care today.<sup>109-113</sup> Although it has not been tested in prospective, randomized trials,

whole-lung lavage improves clinical, physiological, and radiographic findings. A retrospective analysis of 231 cases found clinically significant improvement in arterial oxygen tension and in measures of pulmonary function (forced expiratory volume in one second, vital capacity, and diffusing capacity for carbon monoxide).<sup>19</sup> Such therapy also improves survival: in a group of 146 patients, the mean ( $\pm$ SD) rate of survival at five years was  $94\pm 2$  percent with lavage, as compared with  $85\pm 5$  percent without lavage ( $P=0.04$ ).<sup>19</sup> The median duration of clinical benefit from lavage has been reported to be 15 months.<sup>19</sup> Interestingly, therapeutic whole-lung lavage improves defects in the migration<sup>114</sup> and phagocytosis<sup>115</sup> of alveolar macrophages. Successful treatment of pulmonary alveolar proteinosis by lobar lavage through fiberoptic bronchoscopy has also been reported, although the practical clinical utility of this approach is unclear.<sup>116</sup>

#### GM-CSF THERAPY

Several prospective phase 2 trials of GM-CSF therapy for acquired pulmonary alveolar proteinosis have been undertaken. The first, conducted from 1995 through 1998, evaluated the effectiveness of subcutaneous GM-CSF (at a dose of 5  $\mu$ g per kilogram of body weight per day) for 6 to 12 weeks in 14 patients.<sup>30</sup> Five patients had a response to this dose, with a mean improvement in the alveolar–arteriolar diffusion gradient of 23.2 mm Hg; four of the patients who did not have a response then received 20  $\mu$ g per kilogram per day and had a response to that dose. The remaining five patients did not have a

response at the higher dose. An ongoing study, initiated in 1998, reported a response in three of four initial patients who received daily subcutaneous injections of GM-CSF in escalating doses over a period of 12 weeks.<sup>101,117</sup> These three patients had symptomatic, physiological, and radiographic improvement as well as a reduction in the mean alveolar–arteriolar diffusion gradient from 48.3 mm Hg at base line to 18.3 mm Hg after 16 weeks of treatment. These initial results are encouraging, but the mechanism of the effect of GM-CSF treatment is unclear. The observation of a reduction in pulmonary levels of anti-GM-CSF antibody in association with clinical improvement suggests that desensitization to GM-CSF may be involved.<sup>36</sup>

#### CONCLUSIONS

Clinical investigations, research in transgenic mice, and translation of findings from the bench to the bedside have considerably changed our concepts of the pathogenesis and treatment of pulmonary alveolar proteinosis. In addition to illuminating the mechanism of this disorder, research has revealed critical roles for GM-CSF in the regulation of mature alveolar macrophages in the lung, the regulation of surfactant homeostasis, and the stimulation of multiple mechanisms that protect the lung against microbial invasion.

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