State of the Art

Pulmonary Alveolar Proteinosis
Progress in the First 44 Years

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Pulmonary alveolar proteinosis is a rare clinical syndrome that was first described in 1958. Subsequently, over 240 case reports and small series have described at least 410 cases in the literature. Characterized by the alveolar accumulation of surfactant components with minimal interstitial inflammation or fibrosis, pulmonary alveolar proteinosis has a variable clinical course ranging from spontaneous resolution to death with pneumonia or respiratory failure. The most effective proven treatment—whole lung lavage—was described soon after the first recognition of this disease. In the last 8 years, there has been rapid progress toward elucidation of the molecular mechanisms underlying both the congenital and acquired forms of pulmonary alveolar proteinosis, following serendipitous discoveries in gene-targeted mice lacking granulocyte-macrophage colony-stimulating factor (GM-CSF). Impairment of surfactant clearance by alveolar macrophages as a result of inhibition of the action of GM-CSF by blocking autoantibodies may underlie many acquired cases, whereas congenital disease is most commonly attributable to mutations in surfactant protein genes but may also be caused by GM-CSF receptor defects. Therapy with GM-CSF has shown promise in approximately half of those acquired cases treated, but it is unsuccessful in congenital forms of the disease, consistent with the known differences in disease pathogenesis.

Keywords: pulmonary alveolar proteinosis; granulocyte-macrophage colony-stimulating factor; pulmonary surfactants; bronchoalveolar lavage; autoantibodies

Pulmonary alveolar proteinosis (PAP), also referred to as alveolar proteinosis, alveolar lipoproteinosis, alveolar phospholipidosis, pulmonary alveolar lipoproteinosis, and pulmonary alveolar phospholipoproteinosis, is a rare and enigmatic disorder that is characterized by abnormal intraalveolar surfactant accumulation and a variable natural history. With an estimated annual incidence and prevalence of 0.36 and 3.70 cases per million population, respectively (1), it is difficult for any one clinician or treatment center to accumulate significant experience with the disorder, and single case reports or small case series comprise more than 75% of all described instances of PAP. There are only five published series of 10 or more cases reported (2–6). Over the last 8 years, there has been a revolution in the understanding of the pathogenesis of PAP, which has led to the investigation of innovative treatment approaches. There are no randomized studies of any interventions yet available, and an interpretation of the outcome of uncontrolled studies would be improved by a better understanding of the natural history of the disease and the efficacy of current standard therapies. This review aims to provide an overview of aspects of this field in the light of these recent scientific advances and to describe management options for this puzzling disease. A number of excellent up-to-date and comprehensive reviews have discussed the clinical features (2, 7–9), diagnostic techniques (10, 11), and radiologic appearances

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(12, 13) of PAP and also the procedural aspects of therapeutic whole-lung lavage (7). The reader is referred to these publications for more detailed discussion of the important areas that are not addressed in detail here.

Throughout this article, we refer to data synthesized from reported cases of acquired PAP in the literature over the first 40 years following its initial description, up to 1998 when granulocyte-macrophage colony-stimulating factor (GM-CSF), a new biologic therapy potentially able to alter the natural history of the disease, emerged as a treatment option (14). The details of the methodology of the literature analysis used are provided (*see* online data supplement) and include information from 410 identifiably separate cases of PAP, described in 241 separate initial publications.

DESCRIPTION OF A "NEW" DISEASE

PAP has a relatively short history. Remarkably for what is now considered such a distinctive disorder, its existence was only recognized in 1958 through the seminal report of Rosen and colleagues (15). Dr. Benjamin Castleman of the Massachusetts General Hospital recognized the first case of this series in July 1953, and the remaining 26 cases were accumulated over the subsequent 4 years. The authors commented that the histologic appearance of the Periodic Acid Schiff (PAS)-positive proteinaceous alveolar deposits in the absence of a cellular infiltrate and normal interalveolar septa was "so characteristic and similar from one case to another that it seems highly unlikely that it could have escaped description previously" (15).

In retrospect, with interpretation sharpened by the clear description provided by Rosen and colleagues (15), there are at least two earlier publications of cases of probable PAP (16, 17). Linell and associates from Sweden described the case of a 57-year-old man who had been symptomatic since 1946 and died of disseminated cryptococcosis in June 1951 (17). At autopsy, pulmonary changes characteristic of PAP were found but were attributed to the cryptococcosis while noting that "no such observations are on record." The true nature of the underlying condition in this case was subsequently acknowledged in 1961 (18). The other report predating that of Rosen and colleagues was the 1957 description of a woman with marked thrombocytosis caused by an underlying myeloproliferative disorder, where acellular eosinophilic "intra-alveolar coagulum" was found but erroneously attributed to platelet deposition (16, 19). Again, with the benefit of hindsight, these features were recognized as manifestations of PAP (20) and represent the first described case of PAP occurring as a "secondary" phenomenon to an underlying hematologic malignancy. Doyle and colleagues first postulated in 1963 that this association was not purely fortuitous (21) and rightly suggested that "careful hematologic studies would appear to be indicated in pulmonary alveolar proteinosis." Mechanistic explanations for this association between PAP and hematologic disorders have only recently been forthcoming (*see* PATHOGENESIS AND CLASSIFICATION subsequently here).

NATURE OF THE ACCUMULATED ALVEOLAR MATERIAL

At the time of the initial characterization of PAP, the existence and physical characteristics of the normal alveolar lining fluid were newly established (22, 23). Using a series of special stains, histochemical processes, and direct chemical analysis, Rosen and colleagues skillfully demonstrated the high lipid content of the accumulated material and established that protein and carbohydrate were also present (15). They suggested that "exfoliated alveolar septal cells" (type II pneumocytes) could be the source of this material. In 1965, based on similarities of chemical composition, Larson and Gordinier first proposed that the material was surfactant (24) and commented that the abnormal accumulation could be due to "an overproduction . . . , impairment of removal, or [an] abnormal type of surfactant." This "surfactant" hypothesis for PAP was temporarily weakened when the material obtained by lavage failed to demonstrate the expected surface-active properties (25, 26) and the hypothesis that it was derived primarily from the plasma transiently gained support (27). However, over the next 15 years, sequential reports have demonstrated the restoration of normal surface activity after ethyl alcohol extraction (28), consistent electron microscopic features (29, 30), and immunohistochemical confirmation of the presence of surfactant proteins (SPs) (31), cumulatively confirming the material as surfactant derived. The issue raised in 1965 of a potential structural abnormality of the accumulated surfactant material continues to be debated (32, 33), with most investigators now suggesting that the observed abnormalities are secondary to altered stoichiometric conditions, rather than representing the primary defect (34).

PUBLISHED FEATURES OF PATIENTS WITH PAP

Presentation

Acquired PAP (the most common type, *see* PATHOGENESIS AND CLASSIFICATION subsequently here) usually presents as progressive dyspnea of gradual onset, at times associated with a minimally productive cough or fatigue (2, 7). Other variably associated features may include weight loss and low-grade fever, although marked fever usually indicates the presence of a complicating infection. Physical examination is often normal or reveals relatively minor and nonspecific pulmonary findings, and digital clubbing is uncommon (9, 35). Described biochemical abnormalities may include elevated serum levels of lactate dehydrogenase (LDH), other protein products of pulmonary epithelial cells, including carcinoembryonic antigen (36), cytokeratin 19 (37), and the mucin KL-6 (38, 39), and levels of the SP-A, SP-B, and SP-D (40, 41), although none of these findings are specific for PAP.

Demographic Features

From an analysis of 410 published cases (Table 1), the median duration of symptoms before diagnosis was 7 months. The median age at diagnosis was 39 years, but this differed significantly according to gender (39 years for males and 35 for females, $p = 0.001$). Most patients were men (male:female ratio = 2.65:1.0). As has been described repeatedly, most patients (72%) were smokers at the onset of symptoms, although this varied significantly according to gender (85% for males and 39% for females, $p < 0.0001$). There was no male predominance among nonsmokers (male:female ratio = 0.69:1.0), suggesting that the high proportion of males among PAP patients may be explained by their higher frequency of tobacco use in most societies. In addition, there are also seven patients reported (1.7%, two male and five female) who had co-existing autoimmune disorders or positive autoimmune serology (29, 42–47). The autoimmune disorders comprised rheumatoid arthritis in two cases, positive smooth-muscle antibodies in two cases (with positive rheumatoid factor in the absence of clinical arthritis in one), and immunoglobulin A nephropathy, multiple sclerosis, and possible celiac disease in one case each. There are no available data on any possible human leukocyte antigen associations.

Serum immunoglobulin levels have been reported to be reduced in 4% of patients tested (44, 48, 49); however, two of

TABLE 1. DEMOGRAPHIC AND DISEASE FEATURES AMONG PUBLISHED CASES OF ACQUIRED PAP ACCORDING TO GENDER

	All Patients (*n = 410*)			Male (*n = 292*)			Female (*n = 110*)			
Characteristic	n	%	Median (*I.Q. range*)*	n	%	Median (*I.Q. range*)*	n	%	Median (*I.Q. range*)*	p Value†
Age, years	408		39 (30–46)	292		39 (32–47)	109		35 (22–45)	0.001
Duration of symptoms/CXR changes, mo	288		7 (3–19)	216		7 (3–23)	72		8 (3–14.5)	0.5
African American race‡	144	17		111	15		33	24		0.2
Nonsmoker§	168	28		114	15		46	61		< 0.0001
Mode of diagnosis‖	360			266			94			0.3
Autopsy		11			11			10		
Open biopsy		71			70			72		
Transbronchial biopsy		10			11			10		
BAL		4			3			7		
Elevated hemoglobin¶	97	21		78	19		19	26		0.5
Elevated LDH**	77	82		53	81		24	83		0.8
Pa_{O_2}, mm Hg	159		60 (46–70)	116		60 (48–69)	42		54 (41–72)	0.2
$[A–a]D_{O_2}$, mm Hg††	131		48 (34–60)	96		49 (33–60)	35		45 (37–61)	0.9
Therapeutic lavage,‡‡	312	54		214	52		90	56		0.6

Definition of abbreviations: BAL = bronchoalveolar lavage; CXR = chest x-ray; I.Q. = interquartile; LDH = lactate dehydrogenase; PAP = pulmonary alveolar proteinosis.

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

† Values shown are calculated using the Mann-Whitney U-test or Kruskall-Wallis test for numeric data, and the χ^2 test for categorical data, as appropriate.

‡ Distribution of race applied only to those cases reported from the United States.

§ At the time of onset of symptoms, patients 10 years of age or less at diagnosis were assumed to be nonsmokers.

‖ Percentages do not total 100%, as 11 patients had the diagnosis established by other means (sputum examination in three and percutaneous needle biopsy in eight).

¶ Eighteen or greater g/dl for males, ⩾ 17 g/dl for females.

** Serum level of lactate dehydrogenase above upper limit of cited reference range.

†† Alveolar–arterial oxygen gradient; where the actual figure is not provided, this has been calculated assuming BTPS conditions.

‡‡ As stated within the follow-up available at the date of most recent publication for those patients potentially eligible to receive such a procedure (cases published after 1963 and diagnosed ante-mortem).

these were siblings with immunoglobulin A deficiency (49), and there was also a single instance of a low-level immunoglobulin M paraprotein without proven hematologic malignancy (15). Elevated cholesterol levels have been described in 19% of the patients tested. There were 52 cases with data provided for absolute neutrophil counts, and all but one were normal; one patient had mild neutropenia of 1.04×10^9 per L (43). Bone marrow examinations were reported in 13 patients, and these showed no definite abnormalities other than erythroid hyperplasia attributable to chronic hypoxia (17, 43, 44, 50–58).

A number of these clinical features resemble those seen among patients with another autoantibody-mediated systemic disease with pulmonary manifestations, Goodpasture's syndrome (antiglomerular basement membrane disease). In this disorder, there is also a marked male predominance (approximately 6:1) (59) and a high proportion of smokers (up to 80% [60, 61]). However, in contrast to PAP, there is a bimodal age peak seen in the incidence of Goodpasture's syndrome (59). In Goodpasture's syndrome, the target antigen is a domain of the α3 chain of type IV collagen, preferentially contained within glomerular and pulmonary alveolar basement membrane. The predominance of smokers suggests that inhaled toxins may either expose or lead to conformational alterations of the type IV collagen, rendering these immunogenic, or may alternatively increase the permeability of lung capillaries, allowing access of preformed antibodies to the alveolar basement membrane (60). Similar hypothetical models can be proposed for the role of smoking in the pathogenesis of PAP, but the anatomic localization of GM-CSF is far less restricted. These similarities between PAP and Goodpasture's syndrome suggest other possible areas for future investigation, including the possible HLA associations with the development of PAP (62), the role of antibody titers in prognosis and therapeutic monitoring (63), and the possible utility of plasmapheresis and immunosuppressive therapies targeting antibody production (59).

Smoking

Excluding patients younger than 10 years of age who were effectively nonsmokers by allocation, disease and demographic features have been reported for 121 smoking patients and 35 nonsmoking patients. Apart from the gender imbalance described previously here and a tendency for smokers to more often be reported from North America (66% of smokers versus 49% of nonsmokers, $p = 0.08$), there were no differences in age, duration of symptoms, hemoglobin, serum level of LDH, Pa_{O_2}, or alveolar–arterial oxygen gradient ($[A–a]D_{O_2}$) evident between these two groups (each $p \geqslant 0.2$).

Age

Age at diagnosis is approximately normally distributed with a mean ± SD of 37.8 ± 13.3 years (Figure 1). There is a minor under-representation of patients 70 years of age or more, possibly attributable to less intensive investigation or reluctance to seek medical attention in this age group. Conversely, there is a small over-representation of cases below the age of 10 years. This may be due to a reporting or publication bias of cases occurring in this group, given the extreme rarity of this event. Alternatively, it may represent misclassification of truly congenital cases (*see* PATHOGENESIS AND CLASSIFICATION subsequently here).

The distribution of age at diagnosis among males (mean ± SD, 39.6 ± 12.3 years) closely resembled that of the population as a whole. However, there were notable differences in the pattern of age at diagnosis for females (Figure 2). First, females were diagnosed an average of almost 6 years earlier than males (33.7 ± 14.8, $p = 0.001$ versus males). Also, the distribution among female cases was non-normal (D-score 0.093,

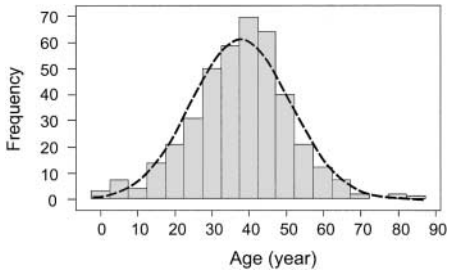


Figure 1. Histogram of the age at diagnosis for published cases of acquired PAP. Data are grouped into 5-year intervals and are shown with a superimposed comparative normal distribution curve of identical mean and SD (mean = 37.8, SD = 13.3, n = 408).

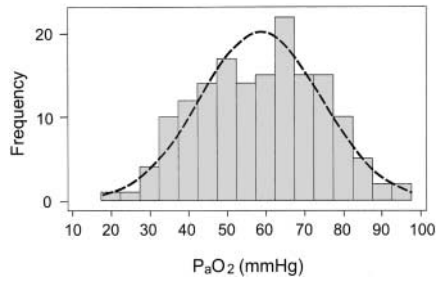


Figure 3. Arterial P_{O_2} measurements at the time of diagnosis of acquired PAP.

p = 0.03), instead displaying a bimodal pattern with peak frequencies at the ages of approximately 25 and 40 years. This pattern may represent the agglomeration of cases with two distinct pathogenetic mechanisms affecting different age groups or alternatively a relative protection against a common mechanism during the 25- to 40-year age interval, which may relate to the peak reproductive period in Western societies (64). Consistent with this suggestion, there is just one reported case of a woman with PAP presenting during pregnancy (65). The only difference evident among female patients according to age was a higher frequency of smoking among women aged 35 years or more at diagnosis (56% versus 21%, p = 0.03).

Arterial Oxygen Pressure

The mean ± SD partial Pa_{O_2} at diagnosis was 58.6 ± 15.8 mm Hg (Figure 3), and this was approximately normally distributed, without any difference in level or distribution according to gender.

Serum LDH

Data on LDH values and applicable normal ranges were only reported in 36 cases (36, 43, 49, 56, 66–84) and are expressed as a percentage of the upper limit of the applicable normal reference range. PAP patients had a mean ± SD LDH level that was 168 ± 66% of the upper limit of the normal range. There was no difference in LDH level according to gender (p = 0.91), nor was there any correlation between LDH and age at diagnosis (p = 0.75). Serial measurements of serum LDH levels in individual cases have suggested that the level of this enzyme may be useful as an indicator of disease severity (70, 85). There were 27 patients reported who had values for both serum LDH and concurrent Pa_{O_2} (in 24 cases the $[A–a]D_{O_2}$ was also known). LDH and Pa_{O_2} were moderately correlated (r^2 = 0.372, p = 0.001), but the correlation between LDH and $[A–a]D_{O_2}$ was more significant (r^2 = 0.489, p = 0.0001) (Figure 4).

Spirometric and Radiographic Features

On pulmonary function testing, the most common pattern seen is that of a restrictive defect, with a disproportionate reduction in diffusing capacity relative to modest impairment of vital capacity (2, 7, 9, 12). By plain chest radiograph, widespread bilateral patchy and asymmetrical airspace consolidation may be seen, without predilection for central or peripheral distributions, but many patterns are possible (9). The extent of radiologic abnormalities is frequently disproportionate to the relatively modest pulmonary symptoms and physical findings. High-resolution computed tomography scanning has a characteristic appearance of patchy or geographic air-space "ground-glass" opacities or consolidation with some thickening of the interlobular septa, resulting in a "crazy-paving" pattern (12, 13) (Figure 5), with the extent and severity of these changes showing correlations with the degree of impairment of spirometric function and pulmonary gas exchange (12). There is no definite lobar or zonal predominance. Although this pattern is characteristic for PAP, it is not specific (86, 87). In rare cases, a significant component of interstitial fibrosis can be present (75, 88–90), more typically developing late in the clinical course of the disease.

DIAGNOSTIC PROCEDURES

Initially, most cases required open-lung biopsy for diagnosis, and this remains the "gold standard," although false negatives are possible because of sampling error (11). Open-lung biopsy is less commonly required now (7, 10), as a diagnosis of PAP can be established in approximately 75% of clinically suspected cases by the classic findings of a "milky" effluent from

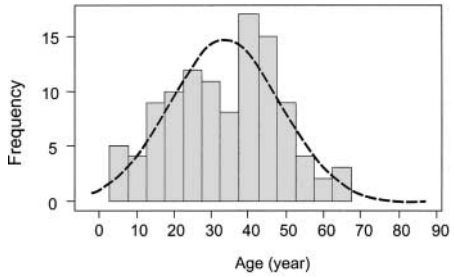


Figure 2. Histogram of the age at diagnosis for published cases of acquired PAP in females. Data are grouped into 5-year intervals and are shown with a superimposed comparative normal distribution curve of identical mean and SD (mean = 33.7, SD = 14.8, n = 109).

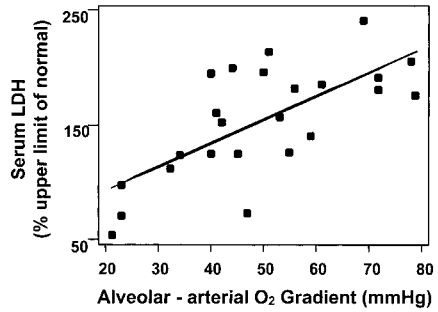


Figure 4. Correlation with superimposed regression line (r^2 = 0.489, p = 0.0001) between the serum level of LDH and the alveolar–arterial oxygen gradient among 24 patients with acquired PAP where concurrent values were reported.

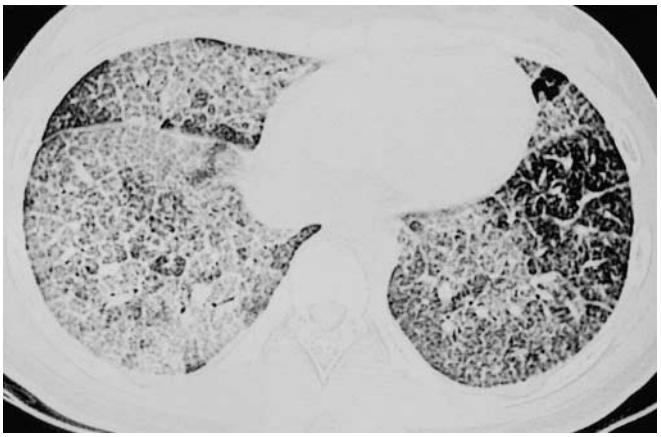


Figure 5. Thin-section, high-resolution computed tomography scan of the thorax showing features typical of acquired PAP. Extensive opacity involves most of the lungs. The areas of ground-glass opacity demonstrate thickening of the interlobular septa leading to the characteristic "crazy paving" appearance typical of PAP.

bronchoalveolar lavage (BAL). This fluid contains large amounts of granular acellular eosinophilic proteinaceous material with morphologically abnormal "foamy" macrophages engorged with diastase-resistant PAS-positive intracellular inclusions (10, 11, 91) (Figure 6A), which also display characteristic features following Papanicolaou staining (92, 93). The presence of concentrically laminated phospholipid structures called lamellar bodies on electron microscopic examination of BAL fluid can be confirmatory (29, 94) (Figure 6B).

HISTOPATHOLOGY

The characteristic features of PAP on light microscopy of lung biopsy specimens (Figure 7) are the near-complete filling of the alveolar space and terminal bronchioles with PAS-positive acellular surfactant. There may be a mild interstitial lymphocytic infiltrate (15, 95); however, this is not a prominent feature, and the alveolar architecture is usually well preserved, except in those cases where pulmonary fibrosis has developed (96), typically late in the natural history of the disorder. Again, electron microscopic examination can be confirmatory in difficult cases (29, 94).

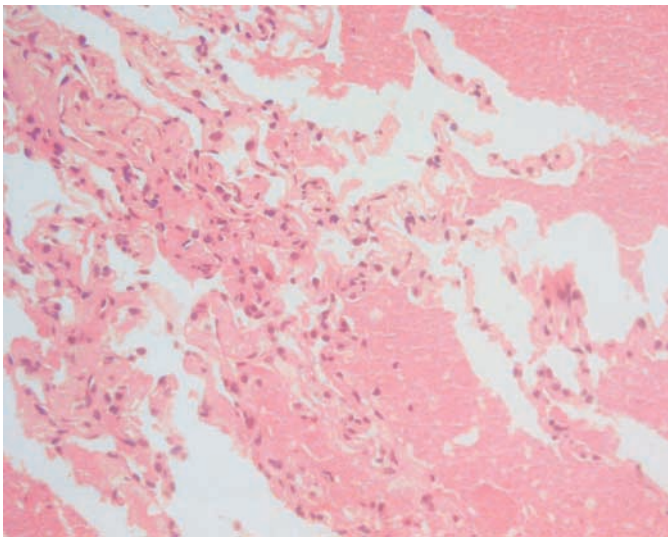
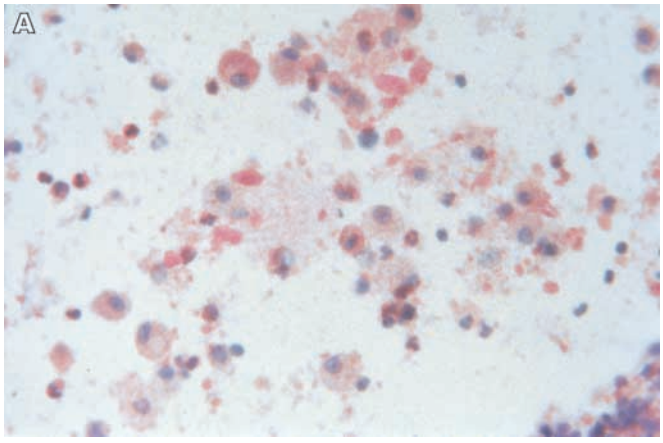


Figure 7. Lung biopsy appearance of acquired PAP, showing alveoli filled with eosinophilic lipoproteinaceous material with otherwise relatively preserved lung architecture (hematoxylin and eosin, ×40).

PATHOGENESIS AND CLASSIFICATION

Novel insights over the last 8 years into the pathogenesis of PAP have lead to a greater understanding of the spectrum of disease processes that may lead to this clinical syndrome. For more than 30 years following the initial description of PAP, the pathogenesis remained unclear, with a commonly held view being that of enhanced surfactant secretion in response to an unknown inhaled irritant. Recognizing some histologic similarities with PAP, acute inhalation of silica (97, 98) and other particulate substances were suspected causes (30). Animal models were developed (99–103), but these did not accurately reproduce the clinical features of PAP; lung biopsy specimens from patients rarely contained the quantities of particulate matter predicted (104, 105). An alternative hypothesis, which gained some support through the 1960s and 1970s, was that of an abnormal pulmonary response to an unusual infectious agent, such as *Pneumocystis carinii* (15, 20, 106) or *Cryptococcus neoformans* (18). However, the vast ma-



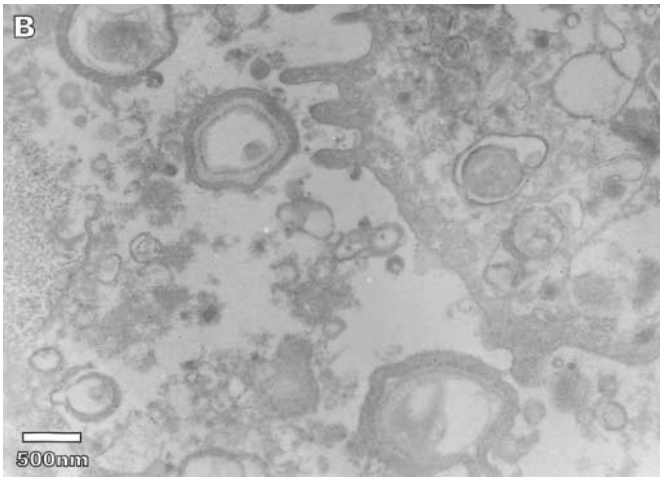


Figure 6. (*A*) Cytological preparation of BAL showing alveolar macrophages containing abundant PAS-positive material in a background of PAS-positive granular lipoproteinaceous material (PAS, ×400). (*B*) Ultrastructural appearance of BAL fluid showing cellular debris and characteristic concentrically laminated phospholipid lamellar bodies.

jority of lung lavage fluid samples are microbiologically sterile, and it is now recognized that most cases of infection encountered are a secondary event rather than the initiating process.

An important conceptual shift that facilitated advances in the understanding of the pathogenesis of PAP was the gradual recognition that there were three distinct classes of disease with a somewhat similar spectrum of histologic findings, namely acquired PAP, congenital PAP, and secondary PAP. Each of these is discussed in turn.

Acquired PAP

More than 90% of all cases of PAP occur as a primary acquired disorder of unknown etiology (2, 4, 15, 96), not associated with any familial predisposition. The first step toward understanding the possible pathogenesis came unexpectedly in 1994 from the field of experimental hematology through the development of gene-knockout mice lacking the hematopoietic growth factor GM-CSF (107, 108).

GM-CSF had been chemically purified in the late 1970s (109) and in 1984 was one of the first human cytokines to be cloned (110). GM-CSF became an intense focus of investigation through this period because of its potent capacity to stimulate the proliferation and differentiation of neutrophilic and monocyte/macrophage lineage hematopoietic cells *in vitro*, an action that had remained unexplained since its first recognition in 1964 (111). This capacity provides the basis for the clinical application of GM-CSF (112–114). GM-CSF shares some, but not all, of its actions with granulocyte colony-stimulating factor, the other major neutrophilic hematopoietic regulator currently in clinical usage (112, 113). The pharmacologic administration of recombinant GM-CSF consistently leads to a dose-dependent stimulation of myeloid hematopoiesis resulting in peripheral blood neutrophilia, monocytosis, and eosinophilia (112). Each of these actions requires engagement of GM-CSF with its high-affinity receptor complex, which comprises a GM-CSF–specific α chain and a common β chain (β_c). In both the human and the mouse, this β_c is also a component of the receptor complexes for interleukin-3 and interleukin-5 (115) and is expressed on both alveolar macrophages and alveolar type II epithelial cells (116). Pulmonary epithelial cells also produce GM-CSF (34).

To explore the innate physiologic role of GM-CSF, investigators used gene-targeting methods to generate mice lacking either GM-CSF ($GM^{-/-}$) (107, 108) or β_c ($\beta_c{}^{-/-}$) (117, 118). Surprisingly, these animals had no detectable abnormality of steady-state hematopoiesis but had impaired surfactant clearance by alveolar macrophages (119–121), leading to a condition that resembled human PAP, including a prominent lymphocytic infiltrate. In contrast to the diminished rate of surfactant clearance, the rates of synthesis of surfactant phospholipid and proteins were unperturbed (119–121). This abnormality of surfactant clearance in $GM^{-/-}$ mice could be corrected by the local delivery of GM-CSF and did not require a systemic effect (122–125). Although type II alveolar epithelial cells are responsible for synthesis, secretion, and recycling of all surfactant components (126, 127), surfactant catabolism involves approximately equal contributions from both type II cells and alveolar macrophages (34, 128). Although current evidence suggests that the primary defect in surfactant clearance in the absence of GM-CSF activity is alveolar macrophage dysfunction (119, 121, 129), alternative non-GM-CSF–dependent surfactant clearance pathways, perhaps involving type II cells, may explain the less severe surfactant accumulation in $\beta_c{}^{-/-}$ compared with $GM^{-/-}$ mice (120). These conclusions are based on the demonstration of impaired surfactant catabolic capacity in isolated $GM^{-/-}$ alveolar macrophages (121) together with complete correction of the PAP phenotype in $GM^{-/-}$, but not $\beta_c{}^{-/-}$ mice, by bone marrow transplantation, which would not be expected to influence any type II cell defect (129, 130). Other incompletely investigated influences on surfactant homeostasis that may modulate the severity of PAP include the levels of alveolar SP-D (131–133) and activity of the transcription factor PU.1, through which the GM-CSF signal is transduced (134). Thorough overviews of the metabolic profiles of $GM^{-/-}$ and $\beta_c{}^{-/-}$ mice have been published recently (34, 120, 121, 135).

Although some naturally occurring immunodeficient mouse strains such as CB.17 scid/scid and the beige mouse have been reported to develop PAP (136, 137), in contrast to the $GM^{-/-}$ and $\beta_c{}^{-/-}$ animals, the penetrance of this phenotype is low, and the lung abnormalities occur late in the animals' life. To date, the cellular mechanisms for these observations have not been elucidated, and their relevance to the human condition of PAP remains unclear.

In addition to the PAP, $GM^{-/-}$ mice manifest a number of more subtle, but important, extrapulmonary abnormalities. These include disturbed macrophage function (138–140), a propensity to develop systemic infections (141, 142), reduced fertility and impaired ovarian follicle maturation (141, 143–145), T-cell dysfunction (146, 147), a reduced number of cutaneous Langerhans cells (148), and ultimately reduced survival (141).

These observations provided an impetus to explore the possible existence of defects in either GM-CSF or its receptor and the potential therapeutic activity of exogenous GM-CSF in patients with PAP. A clinical study of GM-CSF therapy that began in August 1995 provided two important early observations (14). First, in contrast to expectations, GM-CSF administration did not result in any increase in peripheral blood neutrophil or monocyte counts, the only apparent hematopoietic response being a mild eosinophilia (149). Second, there was apparent improvement in the severity of PAP in the first treated patient (14). This attenuated hematopoietic response to GM-CSF has subsequently been observed in all patients with confirmed PAP treated by our own group (41), investigators at the Cleveland Clinic (150, 151), and other groups (152) (Ana Romero, personal communication, July 2001).

A series of experiments performed by Nakata and colleagues in Tokyo provided the likely explanation for this attenuated hematopoietic response to GM-CSF. They identified a GM-CSF–neutralizing autoantibody in the serum and BAL fluid of patients with acquired PAP that was not present in patients with congenital or secondary PAP (153–155). Other investigators have confirmed these findings (156). No clear defects in either the GM-CSF β_c receptor (41, 157) or GM-CSF gene sequence itself (157) have yet been identified in patients with acquired PAP. Although a study of two patients suggested normal basal GM-CSF secretion by alveolar macrophages (158), there have been some *in vitro* observations of impaired responsiveness to GM-CSF (41) or reduced GM-CSF secretion in response to various agonists (156, 159, 160), but these results are difficult to interpret in light of the likely presence of neutralizing GM-CSF antibody and the use of antigenic assays such as enzyme-linked immunosorbent assay rather than functional assays specific for biologically active GM-CSF. On present evidence, it is likely that the anti–GM-CSF antibody is pathogenic in the development of the disease through its ability to inhibit the activity of endogenous GM-CSF, leading to a state of functional GM-CSF deficiency, recapitulating the findings in the $GM^{-/-}$ mouse.

Antibodies capable of binding, and in some cases neutralizing, GM-CSF have been reported to occur in low titer in some immunocompetent patients treated with recombinant GM-CSF (161–163). The period of persistence of these therapy-induced antibodies and any possible *in vivo* activity remains unclear. In the absence of exposure to recombinant GM-CSF, spontaneous GM-CSF binding antibodies are far less common, being detected in only 4 of 1258 (0.3%) healthy volunteers (164, 165). There is a case report of the association of a GM-CSF–neutralizing immunoglobulin G antibody in a patient with acquired amegakaryocytic thrombocytopenia (166), but the causal relationship originally suggested is called into doubt by our current understanding of the redundancy of GM-CSF in steady-state hematopoiesis (107, 108). However, there does appear to be a higher frequency of GM-CSF–neutralizing antibodies among patients with autoimmune disorders (0.7%), specifically myasthenia gravis (1.9%), with at least one patient followed for more than 2 years without any pulmonary symptoms manifest (167).

This important discovery of GM-CSF–neutralizing antibodies in patients with PAP potentially also explains a number of earlier observations. It had been shown 20 years ago that the BAL fluid and serum from patients with PAP had "immunoinhibitory" activity *in vitro*, blocking the response of mononuclear cells to mitogens (168–170). Similarly, although high concentrations of surfactant are inhibitory to a number of macrophage functions (92), the observations of impaired phagocytic function (171, 172), chemotaxis (173), and microbial killing (174, 175) by alveolar macrophages derived from patients with PAP may be partly attributable to the actions of the anti–GM-CSF antibody. Furthermore, the systemic production of the antibody now provides an explanation for the recurrence of PAP following double-lung transplantation (176).

Congenital PAP

Although cases of PAP in infants had been sporadically reported in the 1950s and 1960s (15, 177–179), including cases in families with multiple affected siblings (177, 180), they were not considered initially to represent a process distinct from adult cases. Following the recognition of PAP as a rare cause of immediate-onset neonatal respiratory distress (181–183), the description in 1981 of a consanguineous family with four affected siblings (184) established "congenital" PAP as a distinct familial and likely genetic disorder.

It has been shown subsequently that most cases of congenital PAP are transmitted in an autosomal recessive manner (184, 185), most often caused by homozygosity for a frame shift mutation (121ins2) in the SP-B gene (186, 187), which leads to an unstable SP-B mRNA, reduced protein levels, and secondary disturbances of SP-C processing (188). The estimated gene frequency of the 121ins2 mutation is one per 1,000 to 3,000 persons in the United States (189). However, there is increasing recognition of molecular genetic heterogeneity among infants with congenital SP-B deficiency (187, 190–194), which may have phenotypic and prognostic correlations (195). Importantly, heterozygotes for the most commonly recognized SP-B mutation (121ins2) appear to have normal respiratory function into their 4th decade of life (196). If the mouse model of this condition is an accurate predictor of the human condition, such heterozygotes may be at risk for the development of reduced lung compliance and gas trapping with aging (197) and could be at increased risk of hyperoxic lung injury (198). It also has been recognized recently that mutations in SP-C can lead to similar forms of neonatal respiratory distress (199). In animal models, the deletion of SP-D also leads to accumulation of alveolar macrophages and increased surfactant pool size (131), but in both of these settings, the histopathology is distinct from that seen in congenital SP-B deficiency (200). Also, the SP-C processing defect of SP-B deficiency is not manifest in mice with SP-D deficiency (132).

There are a proportion of infants with the syndrome of neonatal onset PAP who do not have any recognized disturbance of SP-B expression, and abnormalities of the GM-CSF receptor β_c have been implicated in some of these cases (201). In a single report, four infants with congenital PAP were shown to have dramatically reduced β_c expression on peripheral blood mononuclear cells, greatly reduced binding of GM-CSF and impaired *in vitro* responsiveness to both GM-CSF and interleukin-3 (201). In one of these patients, a putative mutation in the GM-CSF β_c gene, which would lead to an amino acid substitution, was identified (201). To date, other investigators have been unable to identify any similar cases of β_c mutations (149, 202, 203), and five infants with congenital PAP in the absence of SP-B mutations have been treated with GM-CSF, all manifesting a normal hematopoietic response, which excludes the possibility of such a receptor defect (149). Conversely, preliminary findings from a British group have identified high levels of expression of a novel truncated form of β_c lacking the usual transmembrane domain, suggesting that this may function as a soluble inhibitory receptor (202). It is too early to be sure what proportion of otherwise unexplained cases of congenital PAP is due to defects in GM-CSF receptor expression, but these results clearly demonstrate that defects in this pathway can be associated with human disease states.

Secondary PAP

Although uncommon among adult PAP patients, there exist a number of recognized underlying causes for the secondary development of PAP. These conditions include lysinuric protein intolerance, acute silicosis and other inhalational syndromes, immunodeficiency disorders, and malignancies and hematopoietic disorders.

The rare genetic disorder "lysinuric protein intolerance" is attributable to a mutation in the "y+L amino acid transporter-1" gene (204–206), resulting in defective plasma membrane transport of dibasic amino acids and multisystem manifestations, including hematopoietic abnormalities leading eventually to PAP in a high proportion of the cases (207–210).

A rare acute-onset form of silicosis recognized in the 1930s (97, 211) was subsequently called "acute silico-proteinosis" to emphasize the histologic resemblance to PAP (98, 212, 213). This was associated with heavy short-term exposure to high concentrations of respirable free silica. With improved occupational health and safety standards, this condition has been reported rarely since the 1980s (42, 214, 215), except for cases of intentional inhalation of domestic scouring products (216). Very rarely, other inhaled environmental or industrial materials such as cement dust (217), cellulose fibers (218), aluminum dust (78), or titanium dioxide (219) have been associated with the development of PAP. Whether such associations are truly causal is not entirely clear in each of these circumstances.

Alveolar proteinosis also develops rarely as a complication of either underlying immunodeficiency disorders, such as thymic alymphoplasia (220), severe combined immunodeficiency disorder (221), or immunoglobulin A deficiency (49), or in the context of iatrogenic immunosuppression such as following solid-organ transplantation (222, 223). One patient has been reported to develop PAP in the setting of dermatomyositis, but this patient also had received prolonged steroid therapy (224). A single study has suggested that patients with the ac-

quired immunodeficiency syndrome complicated by *P. carinii* pneumonia may have some features of PAP (225), although this observation has not been reproduced, and described cases of PAP associated with acquired immunodeficiency syndrome remain extremely rare (226, 227).

PAP also occurs in association with underlying malignancies, almost exclusively of hematopoietic origin (228–231). In these cases, the development of secondary PAP probably reflects numerical deficiency and/or functional impairment of alveolar macrophages, which are derived from blood monocytes (228, 232–234). In the myeloid leukemias and myelodysplastic syndromes, where secondary PAP is most commonly encountered, alveolar macrophages may be derived from the malignant clone itself and in some circumstances have been shown to carry specific defects that may explain the observed functional impairment of surfactant clearance (234). This suggestion is supported by resolution of the pulmonary process following restoration of normal hematopoietic function (230, 234). Similarly, there is some evidence implicating defects in GM-CSF signaling in patients with secondary PAP complicating acute myeloid leukemia. Three patients have been described where their leukemic cells lacked expression of β_c and were unresponsive to GM-CSF, with similar defects shown to be present in BAL-derived cells (234). These defects, together with the underlying PAP, were corrected in each of two cases where normal hematopoiesis was successfully re-established, suggesting that the leukemic clone with its impaired responsiveness to GM-CSF was responsible for replacing/displacing the functionally normal alveolar macrophages and potentially contributing to the manifestations of secondary PAP (234).

DEVELOPMENT OF EFFECTIVE TREATMENT

Without knowing the source, nature, or cause of the accumulated material in PAP, initial therapies were empirical. These included antibiotics, corticosteroids, and attempts at physical dissolution through the administration of potassium iodide, streptokinase, trypsin, heparin, and acetylcysteine (30, 106), all without manifest benefit. Following recognition of the nature of the accumulated material as surfactant, pharmacological manipulation of the surfactant system was attempted (235), again without reproducible benefit.

The first advance in the treatment of PAP came in November 1960, when Dr. José Ramirez-Rivera at the Veterans' Administration Hospital in Baltimore applied repeated "segmental flooding" as a means of physically removing the accumulated alveolar material (52, 66, 85, 236). Following 30 mg of oral codeine, and without other sedation or anesthesia, a percutaneous transtracheal endobronchial catheter of 1.17-mm external diameter was positioned "blindly." Through this catheter, aliquots of 100 ml of warmed saline were instilled at a rate of 50–60 drops per minute. This usually initiated a bout of "45 to 70 minutes of violent coughing," which typically produced "30–40 ml of white viscid material," and was repeated four times a day for 2–3 weeks using physical positioning to direct the saline sequentially into different lung segments (237). The procedure was prolonged, distressing for patients, and burdensome but provided the first therapy with reproducible and functionally significant improvements in symptoms and pulmonary function. In the early 1960s, the application of such a procedure was truly radical and on the basis of available animal experimental data (238) was viewed as potentially harmful (237) even though Garcia-Vincente had initially proposed the concept of pulmonary lavage in 1929 (239).

Such "segmental flooding" provided proof that physical removal of adequate amounts of the material provided functional improvement, but as initially described, this was clearly an impractical therapy for broad application. Although a number of influential colleagues were reportedly "not very encouraging" of the concept (237), in July 1964, Ramírez-Rivera proceeded with a trial of whole-lung lavage, using up to 3 L of saline with added heparin or acetylcysteine, initially under local anesthesia (240). This human trial built on the earlier physiologic studies of lung degassing of Coryllos and Birnbaum (241) and the smaller scale canine experimental work of Kylstra (242), suggesting such a procedure was potentially feasible and safe. Over the next 4 decades, this original procedure has been sequentially refined through the routine use of general anesthesia (67, 240), increased lavage volumes (67, 68), the use of saline alone (243–245), the addition of concomitant chest percussion (244, 246), and the successful completion of bilateral sequential whole lung lavage in the same treatment session (7). If required by the severity of hypoxia, this procedure can be performed with the assistance of partial extracorporeal membrane oxygenation (247). Whole-lung lavage remains the current standard of care for PAP (5, 96), and the physiologic changes associated with this procedure have been thoroughly reviewed elsewhere (6, 248–250).

Application and Efficacy of Therapeutic Lavage

In the absence of a randomized trial or even a formal prospective study, the true impact of the advent of therapeutic lavage on the natural history of acquired PAP is difficult to ascertain. Furthermore, any comparison of survival rates for those patients who did, or did not, undergo lavage makes no allowance for the relative severity of the disease process itself, although it seems likely that those patients with more severe disease would have been more likely to undergo lavage. Nevertheless, from the analyzed literature, those patients who underwent lavage at any time during the course of their disease had a superior survival, with a 5-year actuarial survival rate ± SE from diagnosis of 94 ± 2% compared with 85 ± 5% for those not receiving such treatment ($p = 0.04$) (Figure 8).

Patients who were treated with lavage were more likely to have been reported after 1969, reflecting increasing use of the newly described therapeutic procedure (Table 2). They were also more likely to have an elevated serum LDH at diagnosis and a higher $[A–a]D_{O_2}$, findings that are closely correlated in PAP patients.

Timing of Lavage

In the literature cases, the interval between the diagnosis of PAP and the first application of therapeutic whole-lung lavage ranged from 0 (immediate lavage) to 210 months, with a me-

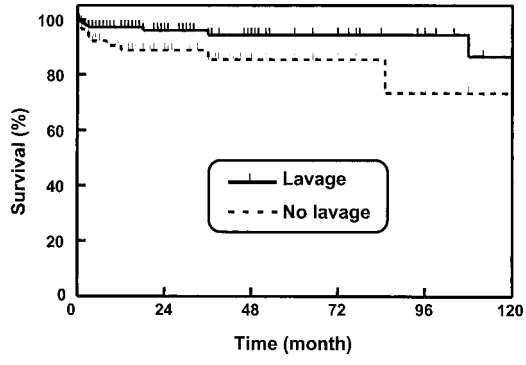


Figure 8. Overall survival from the time of diagnosis of acquired PAP was significantly improved if patients had received therapeutic lavage at any time during their disease course (lavage, n = 146; no lavage, n = 85, p = 0.044).

TABLE 2. DEMOGRAPHIC AND DISEASE FEATURES AT DIAGNOSIS AMONG PUBLISHED CASES OF ACQUIRED PULMONARY ALVEOLAR PROTEINOSIS, ACCORDING TO REPORTED TREATMENT WITH THERAPEUTIC LAVAGE

	Lavage (*n = 146*)			No Lavage (*n = 85*)			
Feature	n	%	Median (*I.Q. Range*)*	No.	%	Median (*I.Q. Range*)*	p Value†
Age, years	146		39 (30–45)	85		37 (29–46)	0.7
Male, sex	140	69		85	78		0.2
Smoker‡	59	76		38	82		0.6
Duration of symptoms, mo	111		7 (3–15)	39		4 (3–27)	0.7
Publication 1970 or later	146	85		85	60		< 0.0001
Elevated LDH§	42	93		15	60		0.007
North American source	146	73		85	69		0.7
[A–a]Do_2, mm Hg‖	60		54 (4–68)	34		42 (34–55)	0.006

Definition of abbreviations: BTPS = body temperature, ambient pressure, and saturated with water vapor; I.Q. = interquartile; LDH = lactate dehydrogenase.

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

† Values shown are calculated using the Mann-Whitney U-test or Kruskall-Wallis test for numeric data and the χ^2 test for categorical data, as appropriate.

‡ At the time of onset of symptoms, patients 10 years of age or younger at diagnosis were assumed to be nonsmokers.

§ Serum level of lactate dehydrogenase above upper limit of cited reference range.

‖ Alveolar–arterial oxygen gradient; where the actual figure is not provided, this has been calculated assuming BTPS conditions.

dian of 2 months (n = 92). The majority of patients who underwent lavage did so within 12 months of diagnosis (79%), but there was a continuing increase in the proportion of patients having received such therapy. In the era of availability of lavage after 1964, the likelihood of a patient with PAP remaining free from therapeutic lavage was only 37% at 5 years (Figure 9).

Repeat Lavage

Among patients who had undergone therapeutic lavage, the median total number of procedures performed was two (range, 1 to 22), and the number of procedures performed was proportional to the duration of follow-up, such that 66% of patients followed for more than 1 year from diagnosis (median, 37 months) had required more than one lavage.

RESPONSE TO THERAPEUTIC LAVAGE

Although there are no established response criteria for therapeutic lavage, significant clinical, physiologic, and radiologic improvements were claimed following the first therapeutic lavage in 84% of the evaluable published cases (5, 29, 66, 69, 72, 77, 249, 251–259).

Duration of Response Following Lavage

In 55 instances of reported response to lavage, there was information provided on the duration of benefit. The definition of disease recurrence varied between reports but included any of the following: the recurrence, or significant progression of respiratory symptoms attributable to PAP, or the application of further therapeutic interventions such as repeated lavage. Episodes of infection were not considered to represent disease recurrence. The median duration of clinical benefit from lavage was 15 months, with less than 20% of those patients followed beyond 3 years remaining free of recurrent PAP manifestations (Figure 10).

Predictors of Response to Lavage

Comparing the demographic and disease-related features of patients who did (n = 92) or did not (n = 18) respond to therapeutic lavage, there were no differences seen in gender, region of origin, duration of symptoms, smoking status, time from diagnosis to lavage, [A–a]Do_2, serum LDH, or year of publication (each $p \geq 0.3$). There was a tendency for nonresponding patients to be younger (median 35 versus 39 years, p = 0.1). When response rates to lavage were calculated within cohorts for age at diagnosis (20 years or less, 21–39 years, and 40 years or more), there was a significant difference observed: 58% (7 of 12), 84% (42 of 50), and 90% (43 of 48), respectively (p = 0.03).

Prognostic Impact of "Response" to Lavage

To reduce "lead-time bias" associated with those patients who survived long enough to undergo therapeutic lavage many

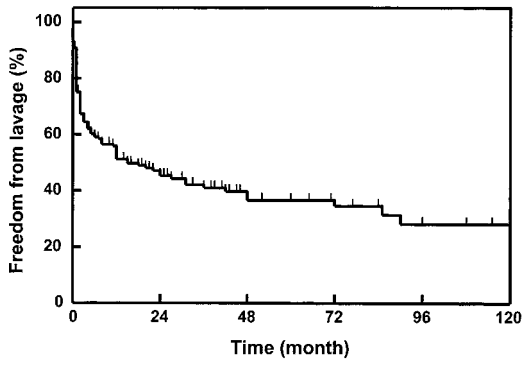


Figure 9. Proportion of patients surviving without receiving therapeutic lung lavage following the diagnosis of acquired PAP (37% [SE 4%] at 5 years).

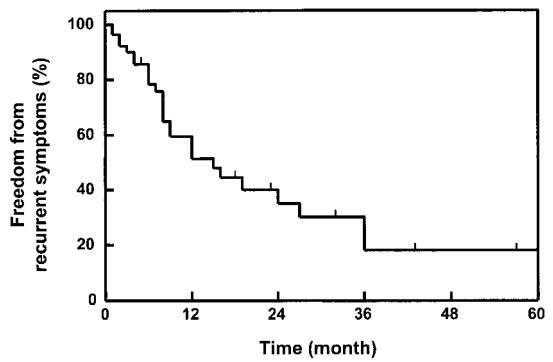


Figure 10. Duration of response to therapeutic lavage for patients with acquired PAP (median = 15 months; n = 55).

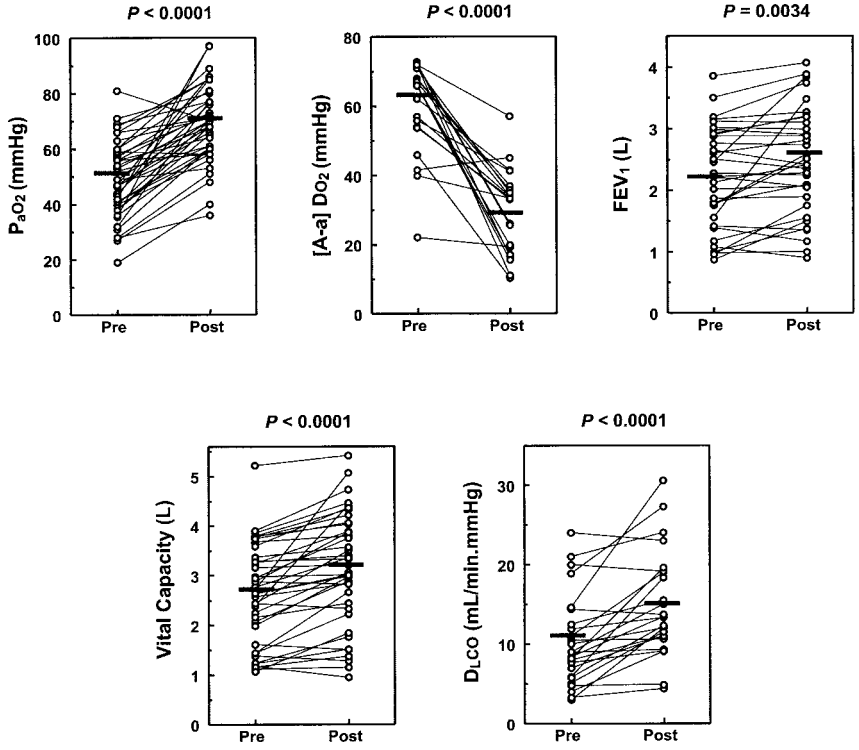


Figure 11. Paired prelavage and postlavage pulmonary function data from patients with acquired PAP. The p values shown are for the comparison of prelavage versus postlavage data for individual patients for each parameter using a two-sample *t* test. The *heavy horizontal lines* show the mean values. The number of evaluable patients for each parameter were: Pa_{O_2} (41), $[A–a]D_{O_2}$ (21), FEV_1 (33), vital capacity (40), and diffusion capacity for carbon monoxide (25).

years after diagnosis, patients who underwent lavage more than 12 months after diagnosis were excluded from this analysis. Although there was a greater early mortality among the nonresponders (survival at 6 months was 87 ± 9% versus 97 ± 2%), overall survival did not differ significantly (actuarial 5-year survival rate 87 ± 9% versus 92 ± 4%, p = 0.3).

Improvement in Pulmonary Parameters Following Lavage

There were 47 patients reported with paired data prelavage and postlavage for Pa_{O_2}, $[A–a]D_{O_2}$, $FEV_{1.0}$, vital capacity, or diffusion capacity for carbon monoxide. A favorable therapeutic response was claimed for 85% of these patients. Overall, following therapeutic lavage there was a clear and significant improvement in all parameters analyzed (Figure 11 and Table 3) when the best result reported within 3 months following lavage was considered. There was no correlation between the magnitude of change of any of these parameters and gender, age, region of origin, or year of publication. There were too few nonsmokers to explore the influence of cigarette use on responses obtained with lavage. When compared with males, females had both a lower prelavage vital capacity, despite the lack of difference in other parameters (1.61 versus 3.16 L, $p < 0.0001$), and a smaller mean absolute increment in vital capacity (0.22 versus 0.54 L, p = 0.02). However, there was no difference in the magnitude of response according to gender, when change in vital capacity was standardized by expression as a percentage of baseline values (mean change 13.0% versus 15.5%, p = 0.1).

The degree of change of each of the previously mentioned lung function parameters following lavage was not closely correlated. For example, there was no significant correlation between the increment in $[A–a]D_{O_2}$ and either Δ diffusion capacity for carbon monoxide ($r^2 = 0.011$, p = 0.8) or Δ vital capacity ($r^2 = 0.074$, p = 0.3), nor was there a significant correlation between the Δvital capacity and Δ diffusion capacity for carbon monoxide ($r^2 = 0.059$, p = 0.2). One report also measured changes in pulmonary shunt fraction following lavage in 14 patients, and this improved from a mean ± SE of 20 ± 1% to 11 ± 1% ($p < 0.001$) (249).

Although the number of patients evaluable for some parameters was very small, those patients described in the original reports as "responders" had numerically greater improvements in each of the parameters analyzed than "nonresponders," supporting the validity of the self-reported response categories (each $p \leq 0.08$). The median changes for "responding" patients were Pa_{O_2} +20.5 mm Hg (n = 34), $[A–a]D_{O_2}$ −33.0 mm Hg (n = 17), $FEV_{1.0}$ +0.21 L (n = 27), vital capacity +0.52 L (n = 33), and diffusion capacity for carbon monoxide +4.5 ml/min·mm Hg (n = 20).

Additional Individual Institutional Reports

In addition to the previously mentioned efficacy data derived from published reports with individual patient data, there are a small number of single institutional reports that have evaluated the efficacy of lavage without providing individual patient data.

Harbour General Hospital, California

Through the period 1966 to 1976, Dr. Wasserman had performed therapeutic lavage on 19 patients, with efficacy data

TABLE 3. PRELAVAGE AND POSTLAVAGE PULMONARY PARAMETERS FOR PATIENTS WITH ACQUIRED PAP

Parameter	n	Mean Change (*SD*)	95% CI of the Mean	p Value*
Arterial Po_2, mm Hg	41	20.1 (14.3)	15.6 to 24.6	< 0.0001
$[A–a]D_{O_2}$, mm Hg	21	−30.6 (18.0)	−38.8 to −22.4	< 0.0001
$FEV_{1.0}$, L	33	0.26 (0.47)	0.09 to 0.42	0.0034
Vital capacity, L	40	0.50 (0.54)	0.33 to 0.67	< 0.0001
$D_{L_{CO}}$, mL/mm Hg · min	25	4.4 (4.5)	2.6 to 6.3	< 0.0001

Definition of abbreviations: CI = confidence interval; $D_{L_{CO}}$ = diffusing capacity for carbon monoxide; PAP = pulmonary alveolar proteinosis.

* p Value is for the comparison of prelavage versus postlavage data for individual patients for each parameter for only those patients with available data using a two-sample *t* test.

available from 11 of these (6). Reporting all results as median values for the evaluable patients, vital capacity improved from 78 to 89% of predicted normal values, diffusion capacity for carbon monoxide from 44% of predicted to 71%, and Pa_{O_2} from 66 to 85 mm Hg (each $p \leq 0.005$). In a subsequent publication from the same institution evaluating 21 patients (96), the median time to repeated lavage was 22 months, and after 5 years, 62% (13%) of patients had required a repeated procedure.

Mayo Clinic, Rochester

Through the period 1957 to 1983, a total of 29 patients with acquired PAP were seen, and 21 of these underwent some form of lavage procedure (8 transtracheal and 13 using more recent isolated whole-lung procedures) (4). Only three of these 21 patients (14%) were described as not obtaining any response to the lavage (type not specified). A major weakness in this report is that "postlavage" studies were performed many months after the lavage procedure, such that the mean interval between preprocedure and postprocedure tests was 30 ± 21 months (range, 2–135).

Cleveland Clinic, Cleveland

A review of 24 patients was recently published (2), 13 (54%) of whom underwent lavage on a median of two occasions (range, 1–8). There were limited objective response data presented for the first lavage, with the median vital capacity for three evaluable patients being unimproved, 71% and 66% of predicted, prelavage, and postlavage, respectively.

GM-CSF THERAPY IN ACQUIRED PAP

There are two published prospective phase II studies of subcutaneous GM-CSF treatment of patients with acquired PAP (41, 150). In an American study, which commenced accrual in March 1998 using 5–9 μg/kg/day, formal response criteria were not specified, but three of four treated patients (75%) attained "symptomatic, physiologic, and radiographic improvement" (150) with their mean ± SD $[A–a]D_{O_2}$ improving from 48.3 ± 20.1 mm Hg at baseline to 18.3 ± 4.2 mm Hg at Week 16 of treatment. A subsequent abstract has updated this study (151), with a cumulative response rate of 71% (five of seven patients). In a larger multinational study conducted between August 1995 and September 1998, 5 of 14 patients (36%) showed a response to initial therapy with 5 μg/kg/day (41), despite pre-existing GM-CSF–neutralizing antibodies (41, 155). Among these five responding patients, the mean improvement in $[A–a]D_{O_2}$ was 23.2 mm Hg (range 13.1 to 46.2 mm Hg). One further patient responded after dose escalation to 20 μg/kg/day (improvement in $[A–a]D_{O_2}$ from 52.0 to 28.8 mm Hg). Taken together, these studies demonstrate an overall response rate of 10/21 (48%; 95% CI, 26–70%) to GM-CSF at 5–9 μg/kg/day; however, there are clearly some additional patients who respond only to higher doses. Two other case reports also support a therapeutic effect of subcutaneous GM-CSF (152) (Ana Romero, personal communication, July 2001). This frequency of apparent response to GM-CSF is clearly lower than that obtained with therapeutic lavage and needs to be interpreted in the context of the known variability in the natural history of the disorder and the reported occurrence of apparent "spontaneous" remissions in approximately 10% of patients (*see* later here).

It is important to note that the duration of follow-up of patients treated with GM-CSF to date is less than 5 years, although there have not been reports of any late toxicity (41, 150, 151). Patients with PAP infrequently develop late pulmonary fibrosis (96), and the development of pulmonary fibrosis in an adenovirus-mediated GM-CSF transgenic mouse model (260) raises the concern that GM-CSF therapy may enhance this risk. However, the fibrosis in this transgenic model is likely due to the vector used, as other GM-CSF transgenic animals have no such propensity (124, 125, 260, 261).

From early studies among cancer patients with pulmonary metastases, it is known that GM-CSF can be delivered safely as an aerosol (262), and the successful use of this form of GM-CSF delivery has been described in a single PAP patient (263). However, such a route of delivery may not correct any systemic manifestations of GM-CSF deficiency in PAP patients.

SECONDARY INFECTIONS

It has long been recognized that patients with PAP are at risk of secondary infections with a variety of organisms. Although certainly reported, the common bacteria responsible for many respiratory infections in community and hospital patients, such as *Streptococcus* (257, 264–267), *Klebsiella* (257), *Haemophilus* (257, 268), *Staphylococcus* (268, 269), *Pseudomonas* (269), *Serratia* (71), *Proteus* (270–272), and *Escherichia coli* (273), do not predominate. Although there is likely to be some degree of publication bias favoring the reporting of "unusual" organisms, opportunistic pathogens are over-represented among patients with acquired PAP, being reported in 13% of patients overall (Table 4). One institutional series reported the isolation of atypical *Mycobacteria* from the lavage material of 42% of patients who underwent therapeutic lavage between 1984 and 1992; however, five of these eight instances were in very low colony numbers of dubious clinical significance, and clinical infection was not felt to be present in any of these patients (274).

Notably, a number of infections among patients with PAP were disseminated, particularly involving the central nervous system, either caused by *Aspergillus* spp. (275) or *Nocardia* spp. (251, 276–281). This phenomenon suggests that the predisposition to infection among patients with acquired PAP is systemic in nature, rather than simply reflecting local environmental changes in the lung. This may be important in light of the suggested pathogenetic role of the systemic neutralization of GM-CSF activity.

No disease- or patient-related factors could be identified that were associated with the occurrence of opportunistic infection. There was no difference in reported neutrophil counts (median 6.7 versus 5.2×10^9 per L, $p = 0.3$). Patients with opportunistic infections had a shorter duration of symptoms before the diagnosis of PAP (median 5 versus 8 months, $p = 0.05$), perhaps consistent with their presentation being precipitated by the development of the infection itself, and were more likely to be diagnosed at autopsy (21% versus 9%, $p = 0.01$). Patients who developed opportunistic infections had not been treated with corticosteroids more frequently (16% versus 18%, $p = 0.6$) and were no more likely to be smokers (75% versus 73%, $p = 1.0$). This does not support the earlier contention that clinically significant exposure to organisms such as *Mycobacterium avium-intracellulare* may be acquired from cigarettes (274, 282). The frequency of opportunistic infections in published cases has not changed over time.

SURVIVAL AND CAUSE OF DEATH

Data on duration of survival from the date of diagnosis of PAP, including those diagnosed at autopsy, were available for 343 patients. The median period of observation for patients still alive at last reported follow-up is 18 months, with the longest follow-up available being 26 years (25, 66, 85, 236,

TABLE 4. OVERVIEW OF OPPORTUNISTIC PATHOGENS REPORTED IN PATIENTS WITH ACQUIRED PAP

Opportunistic Pathogen	References	n	Comments
Mycobacterium tuberculosis	(25, 287, 303, 306–308)	6	No cases reported since 1987; one case of tuberculous meningitis
MAIC	(309)	1	
Streptomyces spp.	(310)	1	
Cryptococcus spp.	(15, 17, 71, 264, 311)	4	Two patients had received corticosteroids
Nocardia spp.	(15, 42, 49, 53, 168, 276–281, 288, 289, 312–324)	34	Twenty-nine cases from North America; prior corticosteroids in four cases; Cerebral involvement in seven; Infection fatal in five instances; Nocardia preceded diagnosis of PAP by 4 years in one case
Mucorales	(30)	1	No prior steroids reported
Histoplasma spp.	(325)	3	All three cases from endemic region of Venezuela
Coccidiodies immitis	(254)	1	From nonendemic region of United States
Aspergillus spp.	(275, 326, 327)	3	Prior steroids in one, and one case of fatal cerebral involvement
Blastomyces dermatitidis	(328)	1	From endemic region of United States
Acinetobacter spp.	(329)	1	

Definition of abbreviations: MAIC = mycobacterium avium-intracellulare complex; PAP = pulmonary alveolar proteinosis.

283). The actuarial survival rates ± SE at 2, 5, and 10 years are 78.9 ± 8.2%, 74.7 ± 8.1%, and 68.3 ± 8.6%, respectively (Figure 12).

There were a total of 69 deaths reported, 65 of which were attributable to PAP. The cause of death was directly due to respiratory failure resulting from PAP in 47 cases (72%). In an additional 13 cases (20%), the cause of death was indirectly related to PAP through uncontrolled infection in 12 (including predominantly cerebral foci in four) (15, 24, 271, 275, 281–289) and cardiac arrest during lavage in one case (253). In the remaining five cases (8%), the deaths were attributable to unrelated causes, including one case each of bladder cancer (290), bowel cancer (291), acute myocardial infarction (101), pancreatitis (273), and gastrointestinal bleeding (50). These incidental causes of death account for two of the seven deaths observed beyond 2 years from diagnosis.

Disease-specific survival of those patients diagnosed during life was assessed to reduce potential biases introduced by cases diagnosed at autopsy and intercurrent events (Figure 13). The actuarial 5-year disease-specific survival rate was 88 ± 4%. More than 80% of the loss of life attributable to PAP seen during the first 5 years of observation occurs during the first 12 months following diagnosis. The risk of death declined significantly beyond 12 months from diagnosis. When data from those patients diagnosed at autopsy were included, by calculating survival from the first recorded onset of symptoms or radiographic changes, the 5-year actuarial survival rate was 70% (n = 254).

POTENTIAL PROGNOSTIC FACTORS FOR SURVIVAL

Gender and Age

There was no difference in overall survival from the date of diagnosis according to gender (5-year survival rate 74% for males and 76% for females, $p > 0.5$). Also, there was no difference among women according to age, with a 5-year survival rate of 80 ± 6% for those aged above the median for women of 34 years and 71 ± 9% for those aged 34 years or less ($p = 0.4$).

However, within the entire cohort, using the median age of the entire group (39 years) as a cut point, there was evidence of inferior outcome for older patients ($p = 0.006$), attributable to both a greater direct mortality from PAP and a greater incidence of death from incidental causes. The risk of death from opportunistic infections did not differ appreciably between the two age groups. Excluding incidental causes of death, the magnitude of the survival difference (actuarial rates of 84 ± 3% versus 70 ± 5% at 5 years, $p = 0.055$) was reduced.

Given the higher than expected incidence of cases among young children less than 5 years of age, this subgroup was examined, revealing a poor outlook. The actuarial 5-year survival rate was 14 ± 13%, with just one of the seven patients surviving beyond 10 months.

Cases Less Than 5 Years of Age

There were just seven patients diagnosed at less than 5 years of age in the period before 1998. The adverse outlook in this age group appeared to be attributable in part to the reduced efficacy of therapeutic lavage. There were five such children

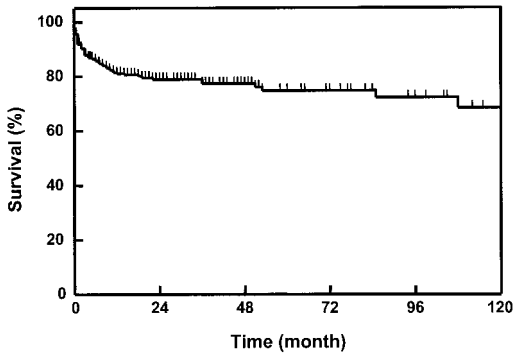


Figure 12. Overall survival from the date of diagnosis based on published all-cause mortality data for 343 patients with acquired PAP (78% [SE 8%] at 5 years). Patients remaining alive at the last date of follow-up are indicated as censored.

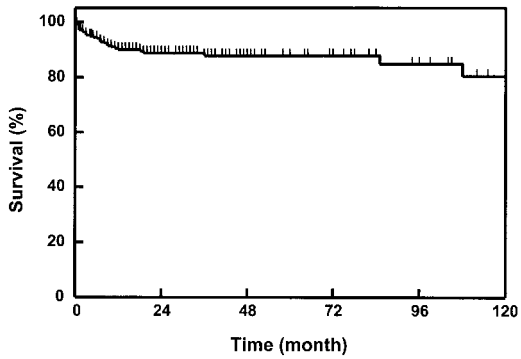


Figure 13. Disease-specific survival of the 307 patients diagnosed with acquired PAP during life. The actuarial 5-year survival rate ± SE was 88 ± 4%.

diagnosed during the era of lavage availability (44, 177, 201, 292, 293), and three were treated (one repeatedly) without any evident benefit (44, 177, 293). Recent reports confirm this adverse experience (294, 295). This diminished effectiveness of therapeutic lavage mirrors that reported for patients with congenital disease where lavage also has limited efficacy (181, 185, 201, 296, 297) and suggests that some of these cases may have been misdiagnosed forms of congenital PAP. Some of these infants were also treated with corticosteroids, N-acetylcysteine, and ambroxol without any evidence of benefit.

Within the limitations of the small number of patients available in published case reports, there are similarities in the clinical presentation of apparent PAP in children below the age of 5 years with those of congenital disease. Four of the reported cases described prominent gastrointestinal symptoms at presentation (food intolerance, failure to thrive, progressive weight loss, and vomiting) (44, 177, 287, 293). Also, one infant presenting at the age of 4 years had a sibling who died at the age of 2 and a half months from a respiratory illness labeled as "viral pneumonia" without a lung biopsy or autopsy being performed, and another family had two affected children, suggesting a possible familial basis (293, 295). The majority of these cases were reported before the recognition of SP-B deficiency as a cause of congenital PAP, although in one instance, this diagnosis was excluded by the finding of SP-B in BAL fluid (201) and another by normal polymerase chain reaction for SP-B mRNA (295).

Smoking

There were 103 patients with available survival data who were smokers at the time of onset of symptoms (including three who then ceased prior to the confirmation of the diagnosis) and 24 who were nonsmokers at the first onset of symptoms (including six who had ceased between 1 and 10 years earlier). The actuarial 5-year survival rate from the time of diagnosis was identical at 82% for these two groups. There are insufficient data available to comment on any possible influence of continued smoking on infection risk or survival.

Arterial Oxygenation

In the 159 patients where arterial oxygenation data were reported, Pa_{O_2} at presentation, using either the median of the cohort (58 mm Hg) or quartiles, was not associated with any differences in disease-specific survival ($p \geq 0.2$). However, there was a trend for an improved outcome among patients with an $[A–a]D_{O_2}$ below the median of 50 mm Hg, where 5-year actuarial survival rates were 98 ± 2% compared with 90 ± 4% for those 50 mm Hg or more ($p = 0.09$).

LDH Level

Serum LDH was not predictive of survival, whether expressed as a percentage of the upper limit of normal or simply as elevated compared with normal.

Year of Publication

Grouping patients in 10-year cohorts by year of initial publication, there has been a dramatic and consistent improvement in survival with time (trend $p < 0.0001$). The 5-year actuarial survival rates for patients reported in the years 1958–1967, 1968–1977, 1978–1987, and 1988–1997 are 52%, 72%, 93%, and 100%, respectively. This improvement, at least in part, is due to a major reduction in the number of patients diagnosed at autopsy. However, the same trend is evident using disease-specific survival and restricting the analysis to patients diagnosed antemortem, with 5-year actuarial survival rates of 77 ± 6%, 91 ± 4%, 96 ± 3%, and 100%, respectively (trend $p = 0.0002$).

Geographic Region

There was no statistically significant difference observed between cases reported from North America (n = 140), Europe (n = 41), or Asia (n = 15); 5-year actuarial survival rates were 89 ± 3%, 87 ± 5%, and 100%, respectively ($p = 0.4$).

SPONTANEOUS RESOLUTION

The concept of possible "spontaneous resolution" of the manifestations of acquired PAP was initially proposed in the series of Rosen and colleagues (15). While acknowledging the brief period of follow-up since diagnosis for their patients, they described the patients as either having stable persistent symptoms, progressive deterioration in their disease process, or spontaneous improvement. A total of 26 patients were so classified: 19%, 31%, and 50% in these three categories, respectively. Although five patients were claimed to have "shown definite improvement," in only one instance was this accompanied by clearance of all previous radiological abnormalities (Case 19).

Other authors also have made similar observations. Kariman and colleagues described "spontaneous remission" of the disease process occurring 1 to 3 years after diagnosis in 24% of a series of 23 patients (5). In the retrospective review of the Mayo clinic experience (4), 29% of patients showed apparently spontaneous resolution after an unspecified period of observation.

Overall, from all available individual patient reports collected, 24 of 303 (7.9%) were described as manifesting a significant degree of spontaneous improvement (5, 15, 24, 30, 53, 82, 257, 267, 298–305). The features of these patients are compared in Table 5 with those of patients not claimed to have manifest such spontaneous improvement. There were no features able to identify clearly those patients who would subsequently manifest "spontaneous resolution," although such patients tended to have a shorter duration of symptoms before diagnosis, and had been followed for a longer period of time. Interestingly, 33% of all claimed cases of "spontaneous resolution" had been published before 1965. In their retrospective review, Kariman and colleagues suggested that spontaneous remitters had higher Pa_{O_2} levels (72 ± 5 versus 57 ± 4 mm Hg, $p < 0.05$) and lower $[A–a]D_{O_2}$ (38 ± 3 versus 51 ± 3 mm Hg, $p < 0.05$) at diagnosis than those patients who eventually required therapeutic lavage (5). However, in this comparison, they did not account for the lead-time bias necessary to allow the manifestation of such spontaneous resolution (19 patients underwent lavage 3 months or less after initial assessment) nor compensate for the fact that low Pa_{O_2} levels were one of the criteria for the institution of therapeutic lavage.

Among the 24 claimed cases of spontaneous resolution, the median time from diagnosis to resolution was 20 months (range, 3 to 61 months; n = 19) and from the onset of symptoms was 24 months (range, 10 to 76 months; n = 19). None of the reported patients had subsequently manifest deterioration, or recurrence, and none of these patients had died. At the time of most recent reporting the median duration of these ongoing remissions was 14 months (range, 1 to 144 months; n = 20).

Although such reports have clearly established that the severity of symptoms, radiographic abnormalities, and functional defects may diminish over time in a minority of patients with acquired PAP, it is not clear that this represents complete resolution of the disease process and restoration of entirely

TABLE 5. FEATURES OF PATIENTS WITH ACQUIRED PAP WHO CLAIMED TO HAVE MANIFEST "SPONTANEOUS RESOLUTION"

	Spontaneous remitters (*n* = *24*)			Nonremitters (*n* = *279*)			
Characteristic	n	%	Median (*I.Q. range*)*	n	%	Median (*I.Q. range*)*	p Value†
Age at diagnosis	24		38 (25–45)	278		38 (30–45)	0.6
Gender, % male	24	71		273	73		0.8
Publication date, % pre-1965	24	33		279	20		0.1
Duration of symptoms	20		4 (3–14)	178		8 (3–24)	0.1
Smoker, %‡	17	88		105	73		0.2
Serum LDH, % elevated§	5	60		56	80		0.3
Arterial Pa_{O_2}, mm Hg	12		62 (52–69)	123		60 (48–70)	0.9
$[A–a]D_{O_2}$, mm Hg‖	11		47 (33–57)	102		47 (33–57)	0.8
Duration of follow-up, mo	24		33 (12–76)	218		22 (10–48)	0.1

Definition of abbreviations: BTPS = body temperature, ambient pressure, and saturated with water vapor; I.Q. = interquartile; LDH = lactate dehydrogenase; PAP = pulmonary alveolar proteinosis.

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

† p Values shown are calculated using the Mann-Whitney U-test or Kruskall-Wallis test for numeric data and the χ^2 test for categorical data, as appropriate.

‡ At the time of onset of symptoms, patients 10 years of age or less at diagnosis were assumed to be nonsmokers.

§ Serum level of lactate dehydrogenase above upper limit of cited reference range.

‖ Alveolar–arterial oxygen gradient; where the actual figure is not provided, this has been calculated assuming BTPS conditions.

normal lung function and surfactant homeostasis. Closer examination of the details of the individual cases discloses that comprehensive objective studies to document disease resolution (such as radiographic studies, spirometry, and arterial blood gases) were either not repeated (15, 30, 257, 298, 300–302) or when repeated documented persisting abnormalities (15, 24, 53, 82, 299–301, 303, 304). The cases from the large series of Kariman and colleagues were classified as spontaneous remitters if "diffuse pulmonary infiltrates cleared and pulmonary function returned to normal levels" without providing individual patient data to confirm this assessment (5).

Although it is clear that the disease process of PAP eventually enters a quiescent state in most surviving patients, either without therapeutic intervention, or following a period of variable duration where therapeutic lavage is required, it is unclear whether the underlying pathophysiologic process is reversed, or simply reduced in severity to such a degree that clinical, radiographic, and functional consequences are minimized. Without a simple, dichotomous, noninvasive diagnostic test, it has not been possible yet to distinguish definitively between a true pathophysiologic "cure" and the persistence of subclinical disease.

Conclusion

Analysis of 241 published articles describing over 400 separate individual cases of PAP has revealed a number of important features of this rare lung condition. The great majority of PAP cases are of the acquired variety occurring in adults. From these reports, a "typical patient" was a male smoker aged 30 to 50 years, and females aged 25–40 are under-represented. PAP is also a congenital disorder in a minority of cases, whereas other cases are secondary to other conditions, notably hematologic malignancy.

A number of important milestones mark the key advances of medical insight into PAP. Early among these was the 1965 description of whole-lung lavage, which remains today's standard therapy. Within 5 years from diagnosis, almost two-thirds of PAP patients had received such lavage, with more than 80% attaining significant, but transient, benefit.

More recently, major advances since 1994 have resulted in the discovery of animal models of PAP based on deletion of genes for GM-CSF itself or the GM-CSF receptor. Thus deficiency of GM-CSF has become a strongly suspected pathogenetic mechanism for adult acquired PAP, and GM-CSF administration with therapeutic intent has been evaluated in several small studies with benefit in approximately 50% of all patients so far treated. In the last 2 years, the demonstration of neutralizing anti–GM-CSF antibodies in all cases of acquired PAP has suggested that the disease may have an autoimmune pathogenesis, with the pulmonary disease resulting from a blockade of endogenous GM-CSF action. That GM-CSF therapy may reverse both pulmonary and extrapulmonary abnormalities found to be present in PAP suggests that these blocking antibodies, even if pathogenic, may in some cases be overcome or circumvented by pharmacologic administration of GM-CSF.

Spontaneous clinical resolution clearly occurs in a small minority of adult PAP patients, although the available evidence makes the existence of spontaneous cure less certain. There are no simple biochemical or clinical parameters so far described that are of use as prognostic variables applicable to most patients, although serum LDH levels correlate with the degree of impairment in oxygenation. Fortunately, only 10–15% of PAP patients may die directly from PAP-induced pulmonary failure. This mortality risk is perhaps less among those diagnosed in the last decade, probably because the prognosis of PAP patients with respiratory impairment is improved by whole-lung lavage. Those rare children aged less than 5 years diagnosed with apparently acquired PAP have a very adverse prognosis, and these cases may represent atypical manifestations of congenital PAP.

Much remains to be learned about this fascinating disease, and it is likely that we are on the threshold of a new era in the understanding of PAP. With the recent recognition of its possible autoimmune nature, it is to be hoped that improved pathophysiologic insights will translate rapidly into targeted therapeutic strategies, which may render redundant the physical removal by lung lavage of accumulated surfactant.

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References

1. Ben Dov I, Kishinevski Y, Roznman J, Soliman A, Bishara H, Zelligson E, Grief J, Mazar A, Perelman M, Vishnizer R, *et al.* Pulmonary alveolar proteinosis in Israel: ethnic clustering. *Isr Med Assoc J* 1999; 1:75–78.
2. Goldstein LS, Kavuru MS, Curtis-McCarthy P, Christie HA, Farver C, Stoller JK. Pulmonary alveolar proteinosis: clinical features and outcome. *Chest* 1998;114:1357–1362.
3. Du Bois RM, McAllister WAC, Branthwaite MA. Alveolar proteinosis: diagnosis and treatment over a 10-year period. *Thorax* 1983;38: 360–363.
4. Prakash UBS, Barham SS, Carpenter HA, Dines DE, Marsh HM. Pulmonary alveolar lipoproteinosis: experience with 34 cases and a review. *Mayo Clin Proc* 1987;62:499–519.
5. Kariman K, Kylstra JA, Spock A. Pulmonary alveolar proteinosis: prospective clinical experience in 23 patients for 15 years. *Lung* 1984; 162:223–231.
6. Selecky PA, Wasserman K, Benfield JR, Lippmann M. The clinical and physiological effect of whole-lung lavage in pulmonary alveolar proteinosis: a ten-year experience. *Ann Thorac Surg* 1977;24:451–461.
7. Shah PL, Hansell D, Lawson PR, Reid KB, Morgan C. Pulmonary alveolar proteinosis: clinical aspects and current concepts on pathogenesis. *Thorax* 2000;55:67–77.
8. Sherter CB, Mark EJ. Case 2-2001: a 42-year-old woman with acute worsening of chronic dyspnea and cough. *N Engl J Med* 2001;344: 212–220.
9. Mazzone P, Thomassen MJ, Kavuru M. Our new understanding of pulmonary alveolar proteinosis: what an internist needs to know. *Cleve Clin J Med* 2001;68:977–984.
10. Wang BM, Stern EJ, Schmidt RA, Pierson DJ. Diagnosing pulmonary alveolar proteinosis: a review and update. *Chest* 1997;111:460–466.
11. Maygarden SJ, Iacocca MV, Funkhouser WK, Novotny DB. Pulmonary alveolar proteinosis: a spectrum of cytologic, histochemical, and ultrastructural findings in bronchoalveolar lavage fluid. *Diagn Cytopathol* 2001;24:389–395.
12. Lee KN, Levin DL, Webb WR, Chen D, Storto ML, Golden JA. Pulmonary alveolar proteinosis: high-resolution CT, chest radiographic, and functional correlations. *Chest* 1997;111:989–995.
13. Holbert JM, Costello P, Li W, Hoffman RM, Rogers RM. CT features of pulmonary alveolar proteinosis. *AJR Am J Roentgenol* 2001;176: 1287–1294.
14. Seymour JF, Dunn AR, Vincent JM, Presneill JJ, Pain MC. Efficacy of granulocyte-macrophage colony-stimulating factor in acquired alveolar proteinosis. *N Engl J Med* 1996;335:1924–1925.
15. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. *N Engl J Med* 1958;258:1123–1143.
16. Levinson B, Jones RS, Wintrobe MM. Un cas de thrombocythémie avec polyglobulie chez une jeune femme avec envahissement pulmonaire par une substance éosinophile hyaline. *Sang* 1957;28:224–225.
17. Linell F, Magnusson B, Nordén Å. Cryptococcosis: review and report of a case. *Acta Dermatovener* 1953;33:103–122.
18. Bergman F, Linell F. Cryptococcosis as a cause of pulmonary alveolar proteinosis. *Acta Path Microbiol Scand* 1961;53:217–224.
19. Levinson B, Jones RS, Wintrobe MM, Cartwright GE. Thrombocythemia and pulmonary intra-alveolar coagulum in a young woman. *Blood* 1958;13:959–971.
20. Plenk HP, Swift SA, Chambers WL, Peltzer WE. Pulmonary alveolar proteinosis: a new disease? *Radiology* 1960;74:928–938.
21. Doyle AP, Balcerzak SP, Wells CL, Crittenden JO. Pulmonary alveolar proteinosis with hematologic disorders. *Arch Intern Med* 1963;112: 940–946.
22. Pattle RE. Properties, function and origin of the alveolar lining layer. *Nature* 1955;175:1125–1126.
23. Pattle RE, Thomas LC. Lipoprotein composition of the film lining the lung. *Nature* 1961;189:844.
24. Larson RK, Gordinier R. Pulmonary alveolar proteinosis: report of six cases, review of the literature, and formulation of a new theory. *Ann Intern Med* 1965;62:292–312.
25. Kuhn C, Györkey F, Levine BE, Ramirez-Rivera J. Pulmonary alveolar proteinosis: a study using enzyme histochemistry, electron microscopy, and surface tension measurement. *Lab Invest* 1966;15:492–509.
26. Ramirez J, Harlan WR Jr. Pulmonary alveolar proteinosis: nature and origin of alveolar lipid. *Am J Med* 1968;45:502–512.
27. Hawkins JE, Savard EV, Ramirez-Rivera J. Pulmonary alveolar proteinosis: origin of proteins in pulmonary washings. *Am J Clin Pathol* 1967;48:14–17.
28. McClenahan JB, Mussenden R. Pulmonary alveolar proteinosis. *Arch Intern Med* 1974;133:284–287.
29. Costello JF, Moriarty DC, Branthwaite MA, Turner-Warwick M, Corrin B. Diagnosis and management of alveolar proteinosis: the role of electron microscopy. *Thorax* 1975;30:121–132.
30. Davidson JM, Macleod WM. Pulmonary alveolar proteinosis. *Br J Dis Chest* 1969;63:13–28.
31. Singh G, Katyal SL, Bedrossian CWM, Rogers RM. Pulmonary alveolar proteinosis: staining for surfactant apoprotein in alveolar proteinosis and in conditions simulating it. *Chest* 1983;83:82–86.
32. Hattori A, Kuroki Y, Katoh T, Takahashi H, Shen HQ, Suzuki Y, Akino T. Surfactant protein A accumulating in the alveoli of patients with pulmonary alveolar proteinosis: oligomeric structure and interaction with lipids. *Am J Respir Cell Mol Biol* 1996;14:608–619.
33. Voss T, Schäfer KP, Nielsen PF, Schäfer A, Maier C, Hannappel E, Maassen J, Landis B, Klemm K, Przybylski M. Primary structure differences of human surfactant-associated proteins isolated from normal and proteinosis lung. *Biochim Biophys Acta* 1992;1138:261–267.
34. Trapnell BC, Whitsett JA. GM-CSF regulates pulmonary surfactant homeostasis and alveolar macrophage-mediated innate host defence. *Annu Rev Physiol* 2002;64:775–802.
35. Hazouard E, Jacquemain C, Rivoire B, Besnier JM, de Muret A, Diot P. Digital clubbing associated with primary alveolar proteinosis: possible implication of growth factors. *Presse Med* 2000;29:999.
36. Fujishima T, Honda Y, Shijubo N, Takahashi H, Abe S. Increased carcinoembryonic antigen concentrations in sera and bronchoalveolar lavage fluids of patients with pulmonary alveolar proteinosis. *Respiration* 1995;62:317–321.
37. Minakata Y, Kida Y, Nakanishi H, Nishimoto T, Yukawa S. Change in cytokeratin 19 fragment level according to the severity of pulmonary alveolar proteinosis. *Intern Med* 2001;40:1024–1027.
38. Takahashi T, Munakata M, Suzuki I, Kawakami Y. Serum and bronchoalveolar fluid KL-6 levels in patients with pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 1998;158:1294–1298.
39. Nakajima M, Manabe T, Niki Y, Matsushima T. Serum KL-6 level as a monitoring marker in a patient with pulmonary alveolar proteinosis. *Thorax* 1998;53:809–811.
40. Kuroki Y, Takahashi H, Chiba H, Akino T. Surfactant proteins A and D: disease markers. *Biochim Biophys Acta* 1998;1408:334–345.
41. Seymour JF, Presneill JJ, Schoch OD, Downie GH, Moore PE, Doyle IR, Vincent JM, Nakata K, Kitamura T, Langton D, *et al.* Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. *Am J Respir Crit Care Med* 2001;163:524–531.
42. Rubin E, Weisbrod GL, Sanders DE. Pulmonary alveolar proteinosis: relationship to silicosis and pulmonary infection. *Radiology* 1980;135: 35–41.
43. Warner TFCS, Beirn PO. Pulmonary alveolar proteinosis: report of a case. *J Ir Med Assoc* 1973;66:563–566.
44. Gray ES. Autoimmunity in childhood pulmonary alveolar proteinosis. *BMJ* 1973;4:296–297.
45. Cardillo MR. Pulmonary alveolar proteinosis: a cytomorphological histochemical and ultrastructural study of one case. *Arch Anat Cytol Pathol* 1989;37:259–261.
46. Stoebner P, Miech G, Lefakis P, Maier A, Witz JP, Le Gal Y. Ultrastructure du poumon dans la protéinose alvéolaire: a propos de deus cas. *Ann Anat Pathol (Paris)* 1967;12:287–310.
47. Varpela E, Toivonen S, Teppo L. Pulmonary alveolar proteinosis. *Ann Chir Gynaecol Fenn* 1966;55:10–14.
48. Anonymous. Dearborn, Michigan (9/29/58): case report: pulmonary alveolar proteinosis. *Quarterly Progress Report of the Veterans' Administration - Armed Forces Study on the Chemotherapy of Tuberculosis* 1958;13:25.
49. Webster JR Jr, Battifora H, Furey C, Harrison RA, Shapiro B. Pulmonary alveolar proteinosis in two siblings with decreased immunoglobulin A. *Am J Med* 1980;69:786–789.
50. Schoen I, Reingold IM, Meister L. Relapsing nodular nonsuppurative panniculitis with lung involvement: clinical and autopsy findings, with notes on pathogenesis. *Ann Intern Med* 1958;49:687–698.
51. Payseur CR, Konwaler BE, Hyde L. Pulmonary alveolar proteinosis: a progressive, diffuse, fatal pulmonary disease. *Am Rev Tuberc* 1958; 78:906–915.
52. Moertel CG, Woolner LB, Bernatz PE. Pulmonary alveolar proteinosis: report of case. *Proc Mayo Clin* 1959;34:152–157.
53. Burbank B, Morrione TG, Cutler SS. Pulmonary alveolar proteinosis and nocardiosis. *Am J Med* 1960;28:1002–1007.
54. Fraimow W, Cathcart RT, Taylor RC. Physiologic and clinical aspects

of pulmonary alveolar proteinosis. *Ann Intern Med* 1960;52:1177–1194.
55. Spevak-Marinkovic L, Rogulja P, Ilic V. Plucna alveolna proteinoza. *Am Rev Respir Dis* 1967;95:295–297.
56. Mork JN, Johnson JR, Zinneman HH, Bjorgen J. Pulmonary alveolar proteinosis associated with IgG monoclonal gammopathy. *Arch Intern Med* 1968;121:278–283.
57. Case records of the Massachusetts General Hospital, weekly clinicopathological exercises: case 34-1974. *N Engl J Med* 1974;291:464–469.
58. Riker JB, Wolinsky H. Trypsin aerosol treatment of pulmonary alveolar proteinosis: case report. *Am Rev Respir Dis* 1973;108:108–113.
59. Kluth DC, Rees AJ. Anti-glomerular basement membrane disease. *J Am Soc Nephrol* 1999;10:2446–2453.
60. Donaghy M, Rees AJ. Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. *Lancet* 1983;2:1390–1393.
61. Herody M, Duvic C, Noel LH, Nedelec G, Grunfeld JP. Cigarette smoking and other inhaled toxins in anti-GBM disease. *Contrib Nephrol* 2000;130:94–102.
62. Phelps RG, Rees AJ. The HLA complex in Goodpasture's disease: a model for analyzing susceptibility to autoimmunity. *Kidney Int* 1999; 56:1638–1653.
63. Savage CO, Pusey CD, Bowman C, Rees AJ, Lockwood CM. Anti-glomerular basement membrane antibody mediated disease in the British Isles 1980-4. *Br Med J (Clin Res Ed)* 1986;292:301–304.
64. Births, marriages, divorces, and deaths: provisional data for 1999. *Natl Vital Stat Rep* 2001;48:1–2.
65. Crocker HL, Pfitzner J, Doyle IR, Hague WM, Smith BJ, Ruffin RE. Pulmonary alveolar proteinosis: two contrasting cases. *Eur Respir J* 2000;15:426–429.
66. Ramirez RJ, Campbell GD. Pulmonary alveolar proteinosis: endobronchial treatment. *Ann Intern Med* 1965;63:429–441.
67. Wasserman K, Blank N, Fletcher G. Lung lavage (alveolar washing) in alveolar proteinosis. *Am J Med* 1968;44:611–617.
68. Ramirez J. Pulmonary alveolar proteinosis: treatment by massive bronchopulmonary lavage. *Arch Intern Med* 1967;119:147–156.
69. Frank ST, Maloney TR, Weg JG. Pulmonary alveolar proteinosis: report of two cases and comments on its pathogenesis. *South Med J* 1969;62:867–870.
70. Fountain FF Jr. Lactate dehydrogenase isoenzymes in alveolar proteinosis. *JAMA* 1969;210:1283.
71. Sunderland WA, Campbell RA, Edwards MJ. Pulmonary alveolar proteinosis and pulmonary cryptococcosis in an adolescent boy. *J Pediatr* 1972;80:450–456.
72. Ramirez J. Alveolar proteinosis: importance of pulmonary lavage. *Am Rev Respir Dis* 1971;103:666–678.
73. Lathan SR Jr, Williams JD Jr, McLean RL, Ramirez J. Pulmonary alveolar proteinosis: treatment of a case complicated by tuberculosis. *Chest* 1971;59:452–454.
74. Farca A, Maher G, Miller A. Pulmonary alveolar proteinosis: home treatment with intermittent positive pressure breathing. *JAMA* 1973; 224:1283–1285.
75. Hudson AR, Halprin GM, Miller JA, Kilburn KH. Pulmonary interstitial fibrosis following alveolar proteinosis. *Chest* 1974;65:700–702.
76. Freedman AP, Pelias A, Johnston RF, Goel IP, Hakki HI, Oslick T, Shinnick JP. Alveolar proteinosis lung lavage using partial cardiopulmonary bypass. *Thorax* 1981;36:543–545.
77. Matuschak GM, Owens GR, Rogers RM, Tibbals SC. Progressive intrapartum respiratory insufficiency due to pulmonary alveolar proteinosis: amelioration by therapeutic whole-lung bronchopulmonary lavage. *Chest* 1984;86:496–499.
78. Miller RR, Churg AM, Hutcheon M, Lam S. Pulmonary alveolar proteinosis and aluminum dust exposure. *Am Rev Respir Dis* 1984;130:312–315.
79. Dawkins SA, Gerhard H, Nevin M. Pulmonary alveolar proteinosis: a possible sequel of NO_2 exposure. *J Occup Med* 1991;33:638–641.
80. Honda Y, Takahashi H, Shijubo N, Kuroki Y, Akino T. Surfactant protein-A concentration in bronchoalveolar lavage fluids of patients with pulmonary alveolar proteinosis. *Chest* 1993;103:496–499.
81. Murphy TR, Sullivan EJ, Stoller JK. Nonresolving alveolar infiltrates in a 43-year-old woman. *Cleve Clin J Med* 1997;64:21–25.
82. Haddad PG, Pankey GA. Pulmonary alveolar proteinosis: a case with spontaneous resolution. *J La State Med Soc* 1969;121:365–376.
83. Honke R, Barlow JF. Thirty-six year old Caucasian male with dyspnea and a bilateral pulmonary infiltrate. *S D J Med* 1980;33:7–11.
84. Ramírez-Rivera J, Halprin G. Pulmonary alveolar proteinosis: physiologic observations during and after pulmonary lavage. *Bol Asoc Med P R* 1973;65:183–197.
85. Ramirez RJ, Nyka W, McLaughlin J. Pulmonary alveolar proteinosis: diagnostic techniques and observations. *N Engl J Med* 1963;268: 165–171.
86. Johkoh T, Itoh H, Muller NL, Ichikado K, Nakamura H, Ikezoe J, Akira M, Nagareda T. Crazy-paving appearance at thin-section CT: spectrum of disease and pathologic findings. *Radiology* 1999;211: 155–160.
87. Murayama S, Murakami J, Yabuuchi H, Soeda H, Masuda K. "Crazy paving appearance" on high resolution CT in various diseases. *J Comput Assist Tomogr* 1999;23:749–752.
88. Clague HW, Wallace AC, Morgan WKC. Pulmonary interstitial fibrosis associated with alveolar proteinosis. *Thorax* 1983;38:865–866.
89. Kaplan AI, Sabin S. Case report: interstitial fibrosis after uncomplicated pulmonary alveolar proteinosis. *Postgrad Med* 1977;61:263–265.
90. Miller PA, Ravin CE, Walker-Smith GJ, Osborne DRS. Pulmonary alveolar proteinosis with interstitial involvement. *Am J Roentgenol* 1981;137:1069–1071.
91. Costabel U, Guzman J. Bronchoalveolar lavage in interstitial lung disease. *Curr Opin Pulm Med* 2001;7:255–261.
92. Shen HQ, Duan CX, Li ZY, Suzuki Y. Effects of proteinosis surfactant proteins on the viability of rat alveolar macrophages. *Am J Respir Crit Care Med* 1997;156:1679–1687.
93. Chou CW, Lin FC, Tung SM, Liou RD, Chang SC. Diagnosis of pulmonary alveolar proteinosis: usefulness of papanicolaou- stained smears of bronchoalveolar lavage fluid. *Arch Intern Med* 2001;161:562–566.
94. Gilmore LB, Talley FA, Hook GER. Classification and morphometric quantification of insoluble materials from the lungs of patients with alveolar proteinosis. *Am J Pathol* 1988;133:252–264.
95. Divertie MB, Brown AL Jr, Harrison EG Jr. Pulmonary alveolar proteinosis: two cases studied by electron microscopy. *Am J Med* 1966; 40:351–359.
96. Wasserman K, Mason GR. Pulmonary alveolar proteinosis. In: Murray JF, Nadel JA, editors Textbook of respiratory medicine, 5th ed. Philadelphia: Saunders; 1994. p. 1933–1946.
97. Cabot RC, Painter FM. Case records of the Massachusetts General Hospital: case 20102. *N Engl J Med* 1934;210:551–554.
98. Buechner HA, Ansari A. Acute silico-proteinosis: a new pathologic variant of acute silicosis in sandblasters, characterized by histologic features resembling alveolar proteinosis. *Dis Chest* 1969;55:274–284.
99. Gross P, deTreville RTP. Alveolar proteinosis: its experimental production in rodents. *Arch Pathol* 1968;86:255–261.
100. Corrin B, King E. Pathogenesis of experimental pulmonary alveolar proteinosis. *Thorax* 1970;25:230–236.
101. Heppleston AG, Young AE. Alveolar lipo-proteinosis: an ultrastructural comparison of the experimental and human forms. *J Pathol* 1972;107:107–117.
102. Heppleston AG, Wright NA, Stewart JA. Experimental alveolar lipoproteinosis following the inhalation of silica. *J Pathol* 1970;101:293–307.
103. Heppleston AG, Fletcher K, Wyatt I. Changes in the composition of lung lipids and the "turnover" of dipalmitoyl lecithin in experimental alveolar lipo-proteinosis induced by inhaled quartz. *Br J Exp Pathol* 1974;55:384–395.
104. McEuen DD, Abraham JL. Particulate concentrations in pulmonary alveolar proteinosis. *Environ Res* 1978;17:334–339.
105. Abraham JL, McEuen DD. Inorganic particulates associated with pulmonary alveolar proteinosis: SEM and X-ray microanalysis results. *Appl Pathol* 1986;4:138–146.
106. De Sanctis PN. Pulmonary alveolar proteinosis: a review of the findings and theories to date with a digression on *Pneumocystis carinii* pneumonia. *Boston Med Q* 1962;13:19–35.
107. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JAM, Naher DW, Cebon J, Sinickas V, Dunn AR. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoeisis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci USA* 1994;91:5592–5596.
108. Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, Dickerson GR, Bachurski CJ, Mark EL, Whitsett JA, *et al.* Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* 1994;264:713–716.
109. Burgess AW, Camakaris J, Metcalf D. Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. *J Biol Chem* 1977;252:1998–2003.
110. Gough NM, Gough J, Metcalf D, Kelso A, Grail D, Nicola NA, Burgess AW, Dunn AR. Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor. *Nature* 1984;309:763–767.
111. Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. *Aust J Exp Biol Med Sci* 1966;44:287–299.

112. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (1). *N Engl J Med* 1992;327:28–35.
113. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (2). *N Engl J Med* 1992;327:99–106.
114. Armitage JO. Emerging applications of recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1998;92:4491–4508.
115. Miyajima A, Mui AL, Ogorochi T, Sakamaki K. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. *Blood* 1993;82:1960–1974.
116. Huffman Reed JA, Rice WR, Zsengellér ZK, Wert SE, Dranoff G, Whitsett JA. GM-CSF enhances lung growth and causes alveolar type-II epithelial cell hyperplasia in transgenic mice. *Am J Physiol Lung Cell Mol Physiol* 1997;273:L715–L725.
117. Robb L, Drinkwater C, Metcalf D, Li R, Köntgen F, Nicola NA, Begley CG. Hematopoietic and lung abnormalities in mice with a null mutation of the common β subunit of the receptors for granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5. *Proc Natl Acad Sci USA* 1995;92:9565–9569.
118. Nishinakamura R, Nakayama N, Hirabayashi Y, Inoue T, Aud D, McNeil T, Azuma S, Yoshida S, Arai K, Miyajima A, *et al.* Mice deficient for the IL-3/GM-CSF/IL-5 bc receptor exhibit lung pathology and impaired immune response, while b_{IL3} receptor-deficient mice are normal. *Immunity* 1995;2:211–222.
119. Ikegami M, Ueda T, Hull W, Whitsett JA, Mulligan RC, Dranoff G, Jobe AH. Surfactant metabolism in transgenic mice after granulocyte macrophage-colony stimulating factor ablation. *Am J Physiol* 1996;270:L650–L658.
120. Reed JA, Ikegami M, Robb L, Begley CG, Ross G, Whitsett JA. Distinct changes in pulmonary surfactant homeostasis in common beta-chain- and GM-CSF-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L1164–L1171.
121. Yoshida M, Ikegami M, Reed JA, Chroneos ZC, Whitsett JA. GM-CSF regulates protein and lipid catabolism by alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L379–L386.
122. Reed JA, Ikegami M, Cianciolo ER, Lu W, Cho PS, Hull W, Jobe AH, Whitsett JA. Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. *Am J Physiol* 1999;276:L556–L563.
123. Ikegami M, Jobe AH, Huffman Reed JA, Whitsett JA. Surfactant metabolic consequences of overexpression of GM-CSF in the epithelium of GM-CSF-deficient mice. *Am J Physiol* 1997;273:L709–L714.
124. Zsengellér ZK, Reed JA, Bachurski CJ, LeVine AM, Forry-Schaudies S, Hirsch R, Whitsett JA. Adenovirus-mediated granulocyte-macrophage colony-stimulating factor improves lung pathology of pulmonary alveolar proteinosis in granulocyte-macrophage colony-stimulating factor-deficient mice. *Hum Gene Ther* 1998;9:2101–2109.
125. Huffman JA, Hull WM, Dranoff G, Mulligan RC, Whitsett JA. Pulmonary epithelial cell expression of GM-CSF corrects the alveolar proteinosis in GM-CSF-deficient mice. *J Clin Invest* 1996;97:649–655.
126. Wright JR. Clearance and recycling of pulmonary surfactant. *Am J Physiol* 1990;259:L1–L12.
127. Wright JR, Youmans DC, Pison U. Metabolism and degradation of alveolar surfactant. *Prog Respir Res* 1994;27:69–73.
128. Gurel O, Ikegami M, Chroneos ZC, Jobe AH. Macrophage and type II cell catabolism of SP-A and saturated phosphatidylcholine in mouse lungs. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L1266–L1272.
129. Nishinakamura R, Wiler R, Dirksen U, Morikawa Y, Arai K, Miyajima A, Burdach S, Murray R. The pulmonary alveolar proteinosis in granulocyte macrophage colony-stimulating factor/interleukins 3/5 βc receptor-deficient mice is reversed by bone marrow transplantation. *J Exp Med* 1996;183:2657–2662.
130. Cooke KR, Nishinakamura R, Martin TR, Kobzik L, Brewer J, Whitsett JA, Bungard D, Murray R, Ferrara JLM. Persistence of pulmonary pathology and abnormal lung function in IL-3/GM-CSF/IL-5 βc receptor-deficient mice despite correction of alveolar proteinosis after BMT. *Bone Marrow Transplant* 1997;20:657–662.
131. Korfhagen TR, Sheftelyevich V, Burhans MS, Bruno MD, Ross GF, Wert SE, Stahlman MT, Jobe AH, Ikegami M, Whitsett JA, *et al.* Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. *J Biol Chem* 1998;273:28438–28443.
132. Ikegami M, Whitsett JA, Jobe A, Ross G, Fisher J, Korfhagen T. Surfactant metabolism in SP-D gene-targeted mice. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L468–L476.
133. Ikegami M, Hull WM, Yoshida M, Wert SE, Whitsett JA. SP-D and GM-CSF regulate surfactant homeostasis via distinct mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L697–L703.
134. Shibata Y, Berclaz PY, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity* 2001;15:557–567.
135. Reed JA, Whitsett JA. Granulocyte-macrophage colony-stimulating factor and pulmonary surfactant homeostasis. *Proc Assoc Am Physicians* 1998;110:321–332.
136. Jennings VM, Dillehay DL, Webb SK, Brown LAS. Pulmonary alveolar proteinosis in SCID mice. *Am J Respir Cell Mol Biol* 1995;13:297–306.
137. Gross NJ, Barnes E, Narine KR. Recycling of surfactant in black and beige mice: pool sizes and kinetics. *J Appl Physiol* 1988;64:2017–2025.
138. Scott CL, Hughes DA, Cary D, Nicola NA, Begley CG, Robb L. Functional analysis of mature hematopoietic cells from mice lacking the β_c chain of the granulocyte-macrophage colony-stimulating factor receptor. *Blood* 1998;92:4119–4127.
139. Zhan Y, Basu S, Lieschke GJ, Grail D, Dunn AR, Cheers C. Functional deficiencies of peritoneal cells from gene-targeted mice lacking G-CSF or GM-CSF. *J Leuk Biol* 1999;65:256–264.
140. Scott CL, Roe L, Curtis J, Baldwin T, Robb L, Begley CG, Handman E. Mice unresponsive to GM-CSF are unexpectedly resistant to cutaneous Leishmania major infection. *Microbes Infect* 2000;2:1131–1138.
141. Seymour JF, Lieschke GJ, Grail D, Quilici C, Hodgson G, Dunn AR. Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte-macrophage CSF have impaired reproductive capacity, perturbed neonatal granulopoiesis, lung disease, amyloidosis, and reduced long-term survival. *Blood* 1997;90:3037–3049.
142. Zhan Y, Lieschke GJ, Grail D, Dunn AR, Cheers C. Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. *Blood* 1998;91:863–869.
143. Robertson SA, Roberts CT, Farr KL, Dunn AR, Seamark RF. Fertility impairment in granulocyte-macrophage colony- stimulating factor-deficient mice. *Biol Reprod* 1999;60:251–261.
144. Gilchrist RB, Rowe DB, Ritter LJ, Robertson SA, Norman RJ, Armstrong DT. Effect of granulocyte-macrophage colony-stimulating factor deficiency on ovarian follicular cell function. *J Reprod Fertil* 2000;120:283–292.
145. Jasper MJ, Robertson SA, Van der Hoek KH, Bonello N, Brännström M, Norman RJ. Characterization of ovarian function in granulocyte-macrophage colony-stimulating factor-deficient mice. *Biol Reprod* 2000;62:704–713.
146. Noguchi Y, Wada H, Marino MW, Old LJ. Regulation of IFN-γ production in granulocyte-macrophage colony-stimulating factor-deficient mice. *Eur J Immunol* 1998;28:3980–3988.
147. Wada H, Noguchi Y, Marino MW, Dunn AR, Old LJ. T cell functions in granulocyte/macrophage colony-stimulating factor deficient mice. *Proc Natl Acad Sci USA* 1997;94:12557–12561.
148. Burnham K, Robb L, Scott CL, O'Keeffe M, Shortman K. Effect of granulocyte-macrophage colony-stimulating factor on the generation of epidermal Langerhans cells. *J Interferon Cytokine Res* 2000;20:1071–1076.
149. Seymour JF, Begley CG, Dirksen U, Presneill JJ, Nicola NA, Moore PE, Schoch OD, van Asperen P, Roth B, Burdach S, *et al.* Attenuated hematopoietic response to granulocyte-macrophage colony-stimulating factor in patients with acquired pulmonary alveolar proteinosis. *Blood* 1998;92:2657–2667.
150. Kavuru MS, Sullivan EJ, Piccin R, Thomassen MJ, Stoller JK. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 2000;161:1143–1148.
151. Mazzone PJ, Sullivan EJ, Piccin R, Stoller JK, Thomassen MJ, Kavuru MS. Granulocyte macrophage-colony stimulating factor therapy for pulmonary alveolar proteinosis [abstract]. *Am J Respir Crit Care Med* 2000;161:A888.
152. Barraclough RM, Gillies AJ. Pulmonary alveolar proteinosis: a complete response to GM-CSF therapy. *Thorax* 2001;56:664–665.
153. Tanaka N, Watanabe J, Kitamura T, Yamada Y, Kanegasaki S, Nakata K. Lungs of patients with idiopathic pulmonary alveolar proteinosis express a factor which neutralizes granulocyte-macrophage colony stimulating factor. *FEBS Lett* 1999;442:246–250.
154. Kitamura T, Tanaka N, Watanabe J, Uchida K, Kanegasaki S, Yamada Y, Nakata K. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte-macrophage colony stimulating factor. *J Exp Med* 1999;190:875–880.

155. Kitamura T, Uchida K, Tanaka N, Tsuchiya T, Watanabe J, Yamada Y, Hanaoka K, Seymour JF, Schoch OD, Doyle IR, *et al.* Serological diagnosis of idiopathic pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 2000;162:658–662.
156. Thomassen MJ, Yi T, Raychaudhuri B, Malur A, Kavuru MS. Pulmonary alveolar proteinosis is a disease of decreased availability of GM-CSF rather than an intrinsic cellular defect. *Clin Immunol* 2000; 95:85–92.
157. Bewig B, Wang XD, Kirsten D, Dalhoff K, Schafer H. GM-CSF and GM-CSF beta c receptor in adult patients with pulmonary alveolar proteinosis. *Eur Respir J* 2000;15:350–357.
158. Poulakis N, Androutsos G, Voucouti N, Paterakis G, Loukides S, Kontozoglou T, Bastas A, Bitsakou C, Provata A, Polyzogopoulos D, *et al.* Cytokine production by monocytes/macrophages is normal in patients with alveolar proteinosis: a report of two cases. *Respiration* 2001;68:224–225.
159. Carraway MS, Ghio AJ, Carter JD, Piantadosi CA. Detection of granulocyte-macrophage colony-stimulating factor in patients with pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 2000;161:1294–1299.
160. Tchou-Wong KM, Harkin TJ, Chi C, Bodkin M, Rom WN. GM-CSF gene expression is normal but protein release is absent in a patient with pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 1997;156:1999–2002.
161. Ragnhammar P, Friesen H, Frödin J, Lefvert A, Hassan M, Österberg A, Mellstedt H. Induction of anti-recombinant human granulocyte-macrophage colony-stimulating factor (*Escherichia coli*-derived) antibodies and clinical effects in nonimmunocompromised patients. *Blood* 1994;84:4078–4087.
162. Wadhwa M, Skog AL, Bird C, Ragnhammar P, Lilljefors M, Gaines-Das R, Mellstedt H, Thorpe R. Immunogenicity of granulocyte-macrophage colony-stimulating factor (GM-CSF) products in patients undergoing combination therapy with GM-CSF. *Clin Cancer Res* 1999; 5:1353–1361.
163. Wadhwa M, Bird C, Fagerberg J, Gaines-Das R, Ragnhammar P, Mellstedt H, Thorpe R. Production of neutralizing granulocyte-macrophage colony-stimulating factor (GM-CSF) antibodies in carcinoma patients following GM-CSF combination therapy. *Clin Exp Immunol* 1996;104:351–358.
164. Revoltella RP, Laricchia-Robbio L, Moscato S, Genua A, Liberati AM. Natural and therapy-induced anti-GM-CSF and anti-GCF antibodies in human serum. *Leuk Lymphoma* 1997;26:29–34.
165. Svenson M, Hansen MB, Ross C, Diamant M, Rieneck K, Nielsen H, Bendtzen K. Antibody to granulocyte-macrophage colony-stimulating factor is a dominant anti-cytokine activity in human IgG preparations. *Blood* 1998;91:2054–2061.
166. Hoffman R, Briddell RA, van Besien K, Srour EF, Guscar T, Hudson NW, Ganser A. Acquired cyclic amegakaryocytic thrombocytopenia associated with an immunoglobulin blocking the action of granulocyte-macrophage colony-stimulating factor. *N Engl J Med* 1989;321: 97–102.
167. Meager A, Wadhwa M, Bird C, Dilger P, Thorpe R, Newsom-Davis J, Willcox N. Spontaneously occurring neutralizing antibodies against granulocyte-macrophage colony-stimulating factor in patients with autoimmune disease. *Immunology* 1999;97:526–532.
168. Müller-Quernheim J, Schopf RE, Benes P, Schulz V, Ferlinz R. A macrophage-suppressing 40-kD protein in a case of pulmonary alveolar proteinosis. *Klin Wochenschr* 1987;65:893–897.
169. Carre PC, Didier AP, Pipy BR, Forgue MF, Beraud MF, Meeus EP, Caratero AL, Leophonte PJ. The lavage fluid from a patient with alveolar proteinosis inhibits the *in vitro* chemiluminescence response and arachidonic acid metabolism of normal guinea pig alveolar macrophages. *Am Rev Respir Dis* 1990;142:1068–1072.
170. Stratton JA, Sieger L, Wasserman K. The immunoinhibitory activities of the lung lavage materials and sera from patients with pulmonary alveolar proteinosis. *J Clin Lab Immunol* 1981;5:81–86.
171. Nugent KM, Pesanti EL. Macrophage function in pulmonary alveolar proteinosis. *Am Rev Respir Dis* 1983;127:780–781.
172. Gonzalez-Rothi RJ, Harris JO. Pulmonary alveolar proteinosis: further evaluation of abnormal alveolar macrophages. *Chest* 1986;90:656–661.
173. Hoffman RM, Dauber JH, Rogers RM. Improvement in alveolar macrophage migration after therapeutic whole lung lavage in pulmonary alveolar proteinosis. *Am Rev Respir Dis* 1989;139:1030–1032.
174. Golde DW, Territo M, Finley TN, Cline MJ. Defective lung macrophages in pulmonary alveolar proteinosis. *Ann Intern Med* 1976;85:304–309.
175. Harris JO. Pulmonary alveolar proteinosis: abnormal *in vitro* function of alveolar macrophages. *Chest* 1979;76:156–159.
176. Parker LA, Novotny DB. Recurrent alveolar proteinosis following double lung transplantation. *Chest* 1997;111:1457–1458.
177. Wilkinson RH, Blanc WA, Hagstrom JWC. Pulmonary alveolar proteinosis in three infants. *Pediatrics* 1968;41:510–515.
178. Neimann N, Rauber G, Duprez A. Protéinose alvéolaire pulmonaire chez un nourrisson de un an. *Arch Anat Pathol* 1964;12:231–234.
179. Gómez LH, Sangüeza P. Sobre un probable caso de proteinosis pulmonar alveolar. *Arch Pediatr Urug* 1965;36:701–704.
180. Seard C, Wasserman K, Benfield JR, Cleveland RJ, Costley DO, Heimlich EM. Simultaneous bilateral lung lavage (alveolar washing) using partial cardiopulmonary bypass. *Am Rev Respir Dis* 1970;101: 877–884.
181. Mazyck EM, Bonner JT, Herd HM, Symbas PN. Pulmonary lavage for childhood pulmonary alveolar proteinosis. *J Pediatr* 1972;80:839–842.
182. Spock A, Lanning CF, Kylstra JA. Lavage of both lungs of a nine month old infant with alveolar proteinosis. *Clin Res* 1977;25:84A.
183. Coleman M, Dehner LP, Sibley RK, Burke BA, L'Heureux PR, Thompson TR. Pulmonary alveolar proteinosis: an uncommon cause of chronic neonatal respiratory distress. *Am Rev Respir Dis* 1980;121: 583–586.
184. Teja K, Cooper PH, Squires JE, Schatterly PT. Pulmonary alveolar proteinosis in four siblings. *N Engl J Med* 1981;305:1390–1392.
185. Nogee LM, deMello DE, Dehner LP, Colten HR. Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. *N Engl J Med* 1993;328:406–410.
186. Nogee LM, Garnier G, Dietz HC, Singer L, Murphy AM, deMello DE, Colten HR. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. *J Clin Invest* 1994;93:1860–1863.
187. Nogee LM. Genetics of the hydrophobic surfactant proteins. *Biochim Biophys Acta* 1998;1408:323–333.
188. Beers MF, Hamvas A, Moxley MA, Gonzales LW, Guttentag SH, Solarin KO, Longmore WJ, Nogee LM, Ballard PL. Pulmonary surfactant metabolism in infants lacking surfactant protein B. *Am J Respir Cell Mol Biol* 2000;22:380–391.
189. Cole FS, Hamvas A, Rubinstein P, King E, Trusgnich M, Nogee LM, deMello DE, Colten HR. Population-based estimates of surfactant protein B deficiency. *Pediatrics* 2000;105:538–541.
190. Nogee LM, Wert SE, Profitt SA, Hull WM, Whitsett JA. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. *Am J Respir Crit Care Med* 2000;161:973–981.
191. Wallot M, Wagenvoort C, deMello D, Muller KM, Floros J, Roll C. Congenital alveolar proteinosis caused by a novel mutation of the surfactant protein B gene and misalignment of lung vessels in consanguineous kindred infants. *Eur J Pediatr* 1999;158:513–518.
192. Lin Z, deMello DE, Wallot M, Floros J. An SP-B gene mutation responsible for SP-B deficiency in fatal congenital alveolar proteinosis: evidence for a mutation hotspot in exon 4. *Mol Genet Metab* 1998; 64:25–35.
193. Lin Z, deMello DE, Batanian JR, Khammash HM, DiAngelo S, Luo J, Floros J. Aberrant SP-B mRNA in lung tissue of patients with congenital alveolar proteinosis (CAP). *Clin Genet* 2000;57:359–369.
194. Mildenberger E, deMello DE, Lin Z, Kossel H, Hoehn T, Versmold HT. Focal congenital alveolar proteinosis associated with abnormal surfactant protein B messenger RNA. *Chest* 2001;119:645–647.
195. Dunbar AE, Wert SE, Ikegami M, Whitsett JA, Hamvas A, White FV, Piedboeuf B, Jobin C, Guttentag S, Nogee LM. Prolonged survival in hereditary surfactant protein B (SP-B) deficiency associated with a novel splicing mutation. *Pediatr Res* 2000;48:275–282.
196. Yusen RD, Cohen AH, Hamvas A. Normal lung function in subjects heterozygous for surfactant protein-B deficiency. *Am J Respir Crit Care Med* 1999;159:411–414.
197. Clark JC, Weaver TE, Iwamoto HS, Ikegami M, Jobe AH, Hull WM, Whitsett JA. Decreased lung compliance and air trapping in heterozygous SP-B-deficient mice. *Am J Respir Cell Mol Biol* 1997;16:46–52.
198. Tokieda K, Iwamoto HS, Bachurski C, Wert SE, Hull WM, Ikeda K, Whitsett JA. Surfactant protein-B-deficient mice are susceptible to hyperoxic lung injury. *Am J Respir Cell Mol Biol* 1999;21:463–472.
199. Nogee LM, Dunbar AE, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 2001;344:573–579.
200. deMello DE, Lin Z. Pulmonary alveolar proteinosis: a review. *Pediatr Pathol Mol Med* 2001;20:413–432.
201. Dirksen U, Nishinakamura R, Groneck P, Hattenhorst U, Nogee L, Murray R, Burdach S. Human pulmonary alveolar proteinosis associated with a defect in GM-CSF/IL-3/IL-5 receptor common β chain expression. *J Clin Invest* 1997;100:2211–2217.

202. Slater N, Rothwell DG, DeBlic J, Kotecha S, Chopra R. Congenital alveolar proteinosis is associated with mRNA expression of a soluble GMCSFRβ subunit [abstract]. *Blood* 2000;96:84a.
203. Slater N, Chopra R, Kotecha S, de Blic J. Congenital pulmonary alveolar proteinosis is not associated with GM-CSF-R alpha or beta chain point mutations [abstract]. *Hematol J* 2000;1:5.
204. Torrents D, Mykkänen J, Pineda M, Feliubadaló L, Estévez R, de Cid R, Sanjurjo P, Zorzano A, Nunes V, Huoponen K, *et al.* Identification of SLC7A7, encoding y+LAT-1, as the lysinuric protein intolerance gene. *Nat Genet* 1999;21:293–296.
205. Borsani G, Bassi MT, Sperandeo MP, De Grandi A, Buoninconti A, Riboni M, Manzoni M, Incerti B, Pepe A, Andria G, *et al.* SLC7A7, encoding a putative permease-related protein, is mutated in patients with lysinuric protein intolerance. *Nat Genet* 1999;21:297–301.
206. Lauteala T, Mykkanen J, Sperandeo MP, Gasparini P, Savontaus ML, Simell O, Andria G, Sebastio G, Aula P. Genetic homogeneity of lysinuric protein intolerance. *Eur J Hum Genet* 1998;6:612–615.
207. Parenti G, Sebastio G, Strisciuglio P, Incerti B, Pecoraro C, Terracciano L, Andria G. Lysinuric protein intolerance characterized by bone marrow abnormalities and severe clinical course. *J Pediatr* 1995;126:246–251.
208. Incerti B, Andria G, Parenti G, Sebastio G, Ghezzi M, Strisciuglio P, Sperli D, Di Rocco M, Borrone C, Parini R, *et al.* Lysinuric protein intolerance: studies on 17 Italian patients. *Am J Human Genet* 1993; 53(Suppl)908.
209. Simell O. Lysinuric protein intolerance and other cationic aminoacidurias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors The metabolic basis of inherited disease, 6th ed. New York: McGraw-Hill; 1989. p. 2497–2513.
210. Santamaria F, Parenti G, Guidi G, Rotondo A, Grillo G, Larocca MR, Celentano L, Strisciuglio P, Sebastio G, Andria G. Early detection of lung involvement in lysinuric protein intolerance: role of high resolution computed tomography and radioisotopic methods. *Am J Respir Crit Care Med* 1996;153:731–735.
211. Chapman EM. Acute silicosis. *JAMA* 1932;98:1439–1441.
212. Xipell JM, Ham KN, Price CG, Thomas DP. Acute silicoproteinosis. *Thorax* 1977;32:104–111.
213. Ziskind M, Jones RN, Weill H. Silicosis. *Am Rev Respir Dis* 1976; 113:643–665.
214. Owens MW, Kinasewitz GT, Gonzalez E. Case report: sandblaster's lung with mycobacterial infection. *Am J Med Sci* 1988;295:554–557.
215. Esteban PJ, Champeaux A. Silicoproteinosis masquerading as community-acquired pneumonia. *J Am Board Fam Pract* 2000;13:376–378.
216. Dumontet C, Biron F, Vitrey D, Guerin JC, Vincent M, Jarry O, Meram D, Peyramond D. Acute silicosis due to inhalation of a domestic product. *Am Rev Respir Dis* 1991;143:880–882.
217. McCunney RJ, Godefroi R. Pulmonary alveolar proteinosis and cement dust: a case report. *J Occup Med* 1989;31:233–237.
218. McDonald JW, Alvarez F, Keller CA. Pulmonary alveolar proteinosis in association with household exposure to fibrous insulation material. *Chest* 2000;117:1813–1817.
219. Keller CA, Frost A, Cagle PT, Abraham JL. Pulmonary alveolar proteinosis in a painter with elevated pulmonary concentrations of titanium. *Chest* 1995;108:277–280.
220. Haworth JC, Hoogstraten J, Taylor H. Thymic alymphoplasia. *Arch Dis Child* 1967;42:40–54.
221. Fisher M, Roggli V, Merten D, Mulvihill D, Spock A. Coexisting endogenous lipoid pneumonia, cholesterol granulomas, and pulmonary alveolar proteinosis in a pediatric population: a clinical, radiographic, and pathologic correlation. *Pediatr Pathol* 1992;12:365–383.
222. Yousem SA. Alveolar lipoproteinosis in lung allograft recipients. *Hum Pathol* 1997;28:1383–1386.
223. Vethanayagam D, Pugsley S, Dunn EJ, Russell D, Kay JM, Allen C. Exogenous lipid pneumonia related to smoking weed oil following cadaveric renal transplantation. *Can Respir J* 2000;7:338–342.
224. Samuels MP, Warner JO. Pulmonary alveolar lipoproteinosis complicating juvenile dermatomyositis. *Thorax* 1988;43:939–940.
225. Tran Van Nhieu JT, Vojtek AM, Bernaudin JF, Escudier E, Fleury-Feith J. Pulmonary alveolar proteinosis associated with *Pneumocystis carinii*: ultrastructural identification in bronchoalveolar lavage in AIDS and immunocompromised non-AIDS patients. *Chest* 1990;98: 801–805.
226. Israel RH, Magnussen CR. Are AIDS patients at risk for pulmonary alveolar proteinosis? *Chest* 1989;96:641–642.
227. Ruben FL, Talamo TS. Secondary pulmonary alveolar proteinosis occurring in two patients with acquired immune deficiency syndrome. *Am J Med* 1986;80:1187–1190.
228. Ladeb S, Fleury-Feith J, Escudier E, Tran Van Nhieu JT, Bernaudin JF, Cordonnier C. Secondary alveolar proteinosis in cancer patients. *Support Care Cancer* 1996;4:420–426.
229. Hildebrand FL Jr, Rosenow ECI, Habermann TM, Tazelaar HD. Pulmonary complications of leukemia. *Chest* 1990;98:1233–1239.
230. Cordonnier C, Fleury-Feith J, Escudier E, Atassi K, Bernaudin JF. Secondary alveolar proteinosis is a reversible cause of respiratory failure in leukemic patients. *Am J Respir Crit Care Med* 1994;149:788–794.
231. Du EZ, Yung GL, Le DT, Masliah E, Yi ES, Friedman PJ. Severe alveolar proteinosis following chemotherapy for acute myeloid leukemia in a lung allograft recipient. *J Thorac Imaging* 2001;16:307–309.
232. Winston DJ, Territo MC, Ho WG, Miller MJ, Gale RP, Golde DW. Alveolar macrophage dysfunction in human bone marrow transplant recipients. *Am J Med* 1982;73:859–866.
233. Springmeyer SC, Altman LC, Kopecky KJ, Deeg HJ, Storb R. Alveolar macrophage kinetics and function after interruption of canine marrow function. *Am Rev Respir Dis* 1982;125:347–351.
234. Dirksen U, Hattenhorst U, Schneider P, Schroten H, Göbel U, Böcking A, Müller KM, Murray R, Burdach S. Defective expression of granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 receptor common β chain in children with acute myeloid leukemia associated with respiratory failure. *Blood* 1998;92:1097–1103.
235. Diaz JP, Manresa Presas F, Benasco C, Guardiola J, Munoz L, Clariana A. Response to surfactant activator (ambroxol) in alveolar proteinosis. *Lancet* 1984;i:1023.
236. Ramirez J, Schultz RB, Dutton RE. Pulmonary alveolar proteinosis: a new technique and rationale for treatment. *Arch Intern Med* 1963; 112:419–431.
237. Ramírez-Rivera J. The strange beginnings of diagnostic and therapeutic bronchoalveolar lavage. *P R Health Sci J* 1992;11:27–32.
238. Huber GL, Finley TN. Effect of isotonic saline on alveolar architecture. *Anesthesiology* 1965;26:252–253.
239. Garcia Vincente S. Le lavage des poumons. *Press Med* 1929;78:1266–1268.
240. Ramirez J, Kieffer RF Jr, Ball WC Jr. Bronchopulmonary lavage in man. *Ann Intern Med* 1965;63:819–828.
241. Coryllos PN, Birnbaum GL. Studies in pulmonary gas absorption in bronchial obstruction. II. The behaviour and absorption times of oxygen, carbon dioxide, nitrogen, hydrogen, helium, ethylene, nitrous oxide, ethylchloride and ether in the lung. *Am J Med Sci* 1932;183: 326–347.
242. Kylstra J. Lavage of the lung. *Acta Physiol Pharmacol Neerl* 1958; 7:163–221.
243. Wasserman K. Evaluation of solutions used for lung lavage in alveolar proteinosis. *Rounds of the Teaching Staff of Wadsworth Hospital Centre* 1968;11:217–222.
244. Kao D, Wasserman K, Costley D, Benfield JR. Advances in the treatment of pulmonary alveolar proteinosis. *Am Rev Respir Dis* 1975; 111:361–363.
245. Sunderland WA, Klein RL. Heparin absorption during heparin-saline lavage in a patient with pulmonary alveolar proteinosis. *Chest* 1973; 63:1033–1034.
246. Hammon WE, McCaffree DR, Cucchiara AJ. A comparison of manual to mechanical chest percussion for clearance of alveolar material in patients with pulmonary alveolar proteinosis (phospholipidosis). *Chest* 1993;103:1409–1412.
247. Cohen ES, Elpern E, Silver MR. Pulmonary alveolar proteinosis causing severe hypoxemic respiratory failure treated with sequential whole-lung lavage utilizing venovenous extracorporeal membrane oxygenation: a case report and review. *Chest* 2001;120:1024–1026.
248. Rogers RM, Szidon JP, Shelburne J, Neigh JL, Shuman JF, Tantum KR. Hemodynamic response of the pulmonary circulation to bronchopulmonary lavage in man. *N Engl J Med* 1972;286:1230–1233.
249. Rogers RM, Levin DC, Gray BA, Moseley LW Jr. Physiologic effects of bronchopulmonary lavage in alveolar proteinosis. *Am Rev Respir Dis* 1978;118:255–264.
250. Cohen E, Eisenkraft JB. Bronchopulmonary lavage: effects on oxygenation and hemodynamics. *J Cardiothorac Anesth* 1990;4:609–615.
251. Robertson HE. Pulmonary alveolar proteinosis. *Can Med Assoc J* 1965;93:980–983.
252. Charpin J, Payan H, Longefait H, Lebreuil G, Ohresser P, Valade G, Boutin C. La protéinose alvéolaire: a propos de deux nouvelles observations. *Poumon Coeur* 1966;22:911–924.
253. Bhagwat AG, Wentworth P, Conen PE. Observations on the relationship of desquamative interstitial pneumonia and pulmonary alveolar proteinosis in childhood: a pathologic and experimental study. *Chest* 1970;58:326–332.

254. Herger PC. A case study: anesthetic considerations for pulmonary lavage. *AANA J* 1975;43:398–400.
255. Brach BB, Harrell JH, Moser KM. Alveolar proteinosis: lobar lavage by fiberoptic bronchoscopic technique. *Chest* 1976;69:224–227.
256. Smith LJ, Katzenstein AL, Ankin MG, Shapiro BA. Management of pulmonary alveolar proteinosis: clinical conference in pulmonary disease from Northwest University McGaw Medical Center, Chicago. *Chest* 1980;78:765–770.
257. Lee MG, Spencer H, Clarke WF, Rao BN, Lowe M, Nelson M. Pulmonary alveolar proteinosis in Jamaica. *West Indian Med J* 1982;31:103–110.
258. Wilson DO, Rogers RM. Prolonged spontaneous remission in a patient with untreated pulmonary alveolar proteinosis. *Am J Med* 1987;82: 1014–1016.
259. Paul K, Müller KM, Oppermann HC, Nützenadel W. Pulmonary alveolar proteinosis in a seven-year-old girl: a follow-up over six years. *Acta Paediatr Scand* 1991;80:477–481.
260. Lang RA, Metcalf D, Cuthbertson RA, Lyons I, Stanley E, Kelso A, Kannourakis G, Williamson DJ, Klintworth GK, Gonda TJ, *et al.* Transgenic mice overexpressing a hematopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. *Cell* 1987;51:675–686.
261. Johnson GR, Gonda TJ, Metcalf D, Hariharan IK, Cory S. A lethal myeloproliferative syndrome in mice transplanted with bone marrow cells infected with a retrovirus expressing granulocyte-macrophage colony stimulating factor. *EMBO J* 1989;8:441–448.
262. Anderson PM, Markovic SN, Sloan JA, Clawson ML, Wylam M, Arndt CAS, Smithson WA, Burch P, Gornet M, Rahman E. Aerosol granulocyte-macrophage colony-stimulating factor: a low toxicity, lung-specific biologic therapy in patients with lung metastases. *Clin Cancer Res* 1999;5:2316–2323.
263. Wylam ME, Ten RM, Katzmann JA, Clawson M, Prakash UBS, Anderson PM. Aerosolized GM-CSF improves pulmonary function in idiopathic pulmonary alveolar proteinosis [abstract]. *Am J Respir Crit Care Med* 2000;161:A889.
264. McCook TA, Kirks DR, Merten DF, Osborne DR, Spock A, Pratt PC. Pulmonary alveolar proteinosis in children. *Am J Roentgenol* 1981; 137:1023–1027.
265. Gumpert BC, Nowacki MR, Amundson DE. Pulmonary alveolar lipoproteinosis: remission after antibiotic treatment. *West J Med* 1994; 161:66–68.
266. Mahaffey DE, Rush EN, Allen JD. Pulmonary alveolar proteinosis treated with varidase. *J Kentucky State Med Assoc* 1959;57:1230–1254.
267. Gilligan DM, McCabe MM, FitzGerald MX. Pulmonary alveolar proteinosis: primary and secondary, a report of three cases. *Ir J Med Sci* 1989;158:14–17.
268. Wilson JW, Rubinfeld AR, White A, Mullerworth M. Alveolar proteinosis treated with a single bronchial lavage. *Med J Aust* 1986;145: 158–160.
269. Jenkins DW, Teichner RL, Griggs GW, Byrd RB. Alveolar proteinosis: lavage in the presence of bronchopleural fistula. *JAMA* 1975;234:74–75.
270. Rodi G, Iotti G, Galbusera C, Mencherini S, Raimondi F, Braschi A. Whole lung lavage. *Monaldi Arch Chest Dis* 1995;50:64–66.
271. Jones CC. Pulmonary alveolar proteinosis with unusual complicating infections. *Am J Med* 1960;29:713–722.
272. Wolman L. The cerebral complications of pulmonary alveolar proteinosis. *Lancet* 1961;2:733–736.
273. Preger L. Pulmonary alveolar proteinosis. *Radiology* 1969;92:1291–1295.
274. Witty LA, Tapson VF, Piantadosi CA. Isolation of *Mycobacteria* in patients with pulmonary alveolar proteinosis. *Medicine (Baltimore)* 1994;73:103–109.
275. Björkholm B, Elgefors B. Cerebellar aspergilloma. *Scand J Infect Dis* 1986;18:375–378.
276. Fried J, Hinthorn D, Ralstin J, Gerjarusak P, Liu C. Cure of brain abscess caused by *Nocardia asteroides* resistant to multiple antibiotics. *South Med J* 1988;81:412–413.
277. Supena R, Karlin D, Strate R, Cramer PG. Pulmonary alveolar proteinosis and *Nocardia* brain abscess: report of a case. *Arch Neurol* 1974;30:266–268.
278. Walker DA, McMahon SM. Pulmonary alveolar proteinosis complicated by cerebral abscess: report of a case. *J Am Osteopath Assoc* 1986;86:447–450.
279. Wongthim S, Charoenlap P, Udompanich V, Punthumchinda K, Suwanagool P. Pulmonary nocardiosis in Chulalongkorn hospital. *J Med Assoc Thai* 1991;74:271–277.
280. Taleghani-Far M, Barber JB, Sampson C, Harden KA. Cerebral nocardiosis and alveolar proteinosis. *Am Rev Respir Dis* 1964;89:561–565.
281. Oerlemans WGH, Jansen ENH, Prevo RL, Eijsvogel MMM. Primary cerebellar nocardiosis and alveolar proteinosis. *Acta Neurol Scand* 1998;97:138–141.
282. Slutzker B, Perryman PH. Pulmonary alveolar proteinosis: response to nebulized enzyme therapy. *Arch Intern Med* 1962;109:406–413.
283. Green D, Criner GJ. Twenty-five year follow-up of a patient treated with lung lavage for pulmonary alveolar proteinosis. *N Engl J Med* 1987;317:839–840.
284. Hall GFM. Pulmonary alveolar proteinosis. *Lancet* 1960;1:1383–1385.
285. Andriole VT, Ballas M, Wilson GL. The association of nocardiosis and pulmonary alveolar proteinosis: a case study. *Ann Intern Med* 1964; 60:266–275.
286. Craighead CC. Lung biopsy for obscure pulmonary lesions. *J La State Med Soc* 1959;111:256–261.
287. Udani PM, Mukerji S. Pulmonary alveolar proteinosis. *India J Child Health* 1963;12:256–258.
288. Raich RA, Casey F, Hall WH. Pulmonary and cutaneous nocardiosis: the significance of the laboratory isolation of *Nocardia. Am Rev Respir Dis* 1961;83:505–509.
289. Saltzman HA, Chick EW, Conant NF. Nocardiosis as a complication of other diseases. *Lab Invest* 1962;11:1110–1117.
290. Rogers RM, Tantum KR. Bronchopulmonary lavage: a "new" approach to old problems. *Med Clin North Am* 1970;54:755–771.
291. Kittredge RD. Alveolar proteinosis. *Am J Roentgenol Radium Ther Nucl Med* 1968;103:519–521.
292. Mahut B, Delacourt C, Scheinmann P, de Blic J, Mani TM, Fournet JC, Bellon G. Pulmonary alveolar proteinosis: experience with eight pediatric cases and a review. *Pediatrics* 1996;97:117–122.
293. Danigelis JA, Markarian B. Pulmonary alveolar proteinosis: including pulmonary electron microscopy. *Am J Dis Child* 1969;118: 871–875.
294. Ito T, Sato M, Okubo T, Ono I, Akabane J. Infantile pulmonary alveolar proteinosis with interstitial pneumonia: bilateral simultaneous lung lavage utilizing extracorporeal membrane oxygenation and steroid therapy. *Tohoku J Exp Med* 1999;187:279–283.
295. Sakai Y, Abo W, Yoshimura H, Sano H, Kuroki Y, Satoh M, Kaimori M. Pulmonary alveolar proteinosis in infants. *Eur J Pediatr* 1999;158: 424–426.
296. Moulton SL, Krous HF, Merritt TA, Odell RM, Gangitano E, Cornish JD. Congenital pulmonary alveolar proteinosis: failure of treatment with extracorporeal life support. *J Pediatr* 1992;120:297–302.
297. Hamvas A, Nogee LM, Mallory GB Jr, Spray TL, Huddleston CB, Auguust A, Dehhner LP, deMello DE, Moxley M, Nelson R, *et al.* Lung transplantation for treatment of infants with surfactant protein B deficiency. *J Pediatr* 1997;130:231–239.
298. McDowell C, Williams SE, Hinds JR. Pulmonary alveolar proteinosis. *Australas Ann Med* 1959;8:137–142.
299. Sanders M, Kahan M, Sbar S. Pulmonary alveolar proteinosis: a review of the literature, report of two cases. *Dis Chest* 1962;42:437–441.
300. Mather CL, Hamlin GB. Pulmonary alveolar proteinosis: a case followed from diagnosis to recovery. *N Engl J Med* 1965;272:1156–1159.
301. Oka S, Kanagami H, Nasu S, Miyakawa K, Seda K, Koike T, Handa T. Pulmonary alveolar proteinosis: report of a case from Japan. *Am Rev Respir Dis* 1961;83:878–885.
302. Dobson MB, Karlish AJ. Pulmonary alveolar proteinosis. *Proc R Soc Med* 1975;68:88–89.
303. Morinari H, Terashi R, Okuba S, Homma S, Tanaka M. Remission of pulmonary alveolar proteinosis during antituberculous chemotherapy. *Eur J Respir Dis* 1987;71:54–55.
304. Martínez-López MA, Gómez-Cerezo G, Villasante C, Molina F, Diaz S, Cobo J, Medraño C. Pulmonary alveolar proteinosis: prolonged spontaneous remission in two patients. *Eur Respir J* 1991;4:377–379.
305. Grall F, Larroque J, Hardel P, Personne C. Protéinose alvéolaire pulmonaire: à propos d'une observation. *J Franc Med Chir Thor* 1967; 21:233–244.
306. García Río F, Alvarez-Sala R, Caballero P, Prados C, Pino JM, Villamor J. Six cases of pulmonary alveolar proteinosis: presentation of unusual associations. *Monaldi Arch Chest Dis* 1995;50:12–15.
307. Buechner HA. The differential diagnosis of miliary diseases of the lung. *Med Clin North Am* 1959;43:89–112.
308. Schoen D, Aly FW. Die alveoläre lungenproteinose. *Fortsch Roentgenstr* 1968;108:449–457.
309. Bakhos R, Gattuso P, Arcot C, Reddy VB. Pulmonary alveolar proteinosis: an unusual association with *Mycobacterium avium-intracellulare* infection and lymphocytic interstitial pneumonia. *South Med J* 1996;89:801–802.
310. Manfredi F, Rosenbaum D, Behnke RH, Williams JF Jr. Pulmonary al-

veolar proteinosis, a report of 2 cases: the diagnostic value of percutaneous needle lung biopsy. *Am J Med Sci* 1961;242:51–64.
311. Lee YC, Chew GT, Robinson BWS. Pulmonary and meningeal crytococcosis in pulmonary alveolar proteinosis. *Aust NZ J Med* 1999;29:843–844.
312. Andersen BR, Ecklund RE, Kellow WF. Pulmonary alveolar proteinosis with systemic nocardiosis: a case report. *JAMA* 1960;174:28–31.
313. Summers JE. Pulmonary alveolar proteinosis: review of the literature with follow-up studies and report of two new cases. *Calif Med* 1966; 104:428–436.
314. Viroslav J, Williams TW Jr. Nocardial infection of the central nervous system: successful treatment with medical therapy. *South Med J* 1972; 64:1382–1385.
315. Chodkowska S, Krakówka P, Kazimierz T, Kubit S, Serafin R, Pawlicka L. Proteinosis pecherzyków plucnych. *Gruzlica* 1961;29:895–901.
316. Harris JO, Castle JR, Swenson EW, Block AJ. Lobar lavage: therapeutic benefits in pulmonary alveolar filling disorders. *Chest* 1974;65:655–659.
317. Clague HW, Harth M, Hellyer D, Morgan WKC. Septic arthritis due to *Nocardia Asteroides* in association with pulmonary alveolar proteinosis. *J Rheumatol* 1982;9:469–472.
318. Anonymous. Case records of the Massachusetts General Hospital, weekly clinicopathological exercises: case 19–1983. *N Engl J Med* 1983;308: 1147–1156.
319. Zapol WM, Wilson R, Hales C, Fish D, Castorena G, Hilgenberg A, Quinn D, Kradin R. Venovenous bypass with a membrane lung to support bilateral lung lavage. *JAMA* 1984;251:3269–3271.
320. Rubinstein I, Mullen JBM, Hoffstein V. Morphologic diagnosis of idiopathic pulmonary alveolar lipoproteinosis-revisited. *Arch Intern Med* 1988;148:813–816.
321. Goodwin JD, Müller NL, Takasugi JE. Pulmonary alveolar proteinosis: CT findings. *Radiology* 1988;169:609–613.
322. Anonymous. Case records of the Massachusetts General Hospital, weekly clinicopathological exercises: case 18–1988. *N Engl J Med* 1988; 318:1186–1194.
323. Pascual J, Gómez Aguinaga MA, Vidal R, Maudes A, Sureda A, Gómez Mampaso E, Fogúe L. Alveolar proteinosis and nocardiosis: a patient treated by bronchopulmonary lavage. *Postgrad Med J* 1989; 65:674–677.
324. Maderazo EG, Quintiliani R. Treatment of nocardial infection with trimethoprim and sulfamethoxazole. *Am J Med* 1974;57:671–675.
325. Hartung M, Salfelder K. Pulmonary alveolar proteinosis and histoplasmosis: report of three cases. *Virchows Arch A Pathol Anat Histol* 1975;368:281–287.
326. Egidy HV, Bässler R, Tilling W. Beitrag zur alveolarproteinose der lungen. *Beitr Klin Tuberk* 1967;134:365–380.
327. Groniowski J, Walski M, Szymanska D. Electron microscopic observations on pulmonary alveolar lipoproteinosis. *Ann Med Sect Pol Acad Sci* 1974;19:109–110.
328. Kellar SL, Harshfield DL, Grigg KG. Radiological case of the month. *J Ark Med Soc* 1995;92:307–308.
329. Geppert EF. Recurrent pneumonia. *Chest* 1990;98:739–745.