Pulmonary alveolar proteinosis in adults: pathophysiology and clinical approach

Anupam Kumar, Bassem Abdelmalak, Yoshikazu Inoue, Daniel A Culver

Pulmonary alveolar proteinosis (PAP) is a diffuse lung disease that results from the accumulation of lipoproteinaceous material in the alveoli and alveolar macrophages due to abnormal surfactant homeostasis. Identification of the granulocyte–macrophage colony-stimulating factor (GM-CSF) as an indispensable mediator of macrophage maturation and surfactant catabolism was the key discovery leading to the current understanding of the pathogenesis of most forms of PAP. Impaired GM-CSF bioavailability due to anti-GM-CSF autoimmunity is the cause of approximately 90% of adult PAP cases. Abnormal macrophage function due to endogenous or exogenous triggers, GM-CSF receptor defects, and other genetic abnormalities of surfactant production account for the remainder of causes. The usual physiological consequence of PAP is impairment of gas exchange, which can lead to dyspnoea, hypoxaemia, or even respiratory failure and death. Pulmonary fibrosis occurs occasionally in patients with PAP. For patients with moderate to severe disease, whole lung lavage is still the first-line treatment of choice. Supplemental GM-CSF is also useful, but details about indications, choice of agent, and dosing remain unclear. Other therapies, including rituximab, plasmapheresis, and lung transplantation have been described but should be reserved for refractory cases.

Introduction

Since its first description in 1958 by Rosen and colleagues,1 our understanding of the pathogenesis, natural history, and treatment of pulmonary alveolar proteinosis (PAP) has evolved considerably. PAP is characterised by the accumulation of surfactant in alveolar macrophages and alveoli, resulting in impaired gas exchange. Clinically, patients with PAP can be asymptomatic or present with progressive dyspnoea, worsening oxygen requirements, and have an increased susceptibility to infections. PAP has a variable clinical course, ranging from spontaneous resolution to death from infections or progressive respiratory failure. In this Review, we elucidate current knowledge of the pathogenesis of PAP, and also discuss the diagnosis, treatment, and future developments.

Pathogenesis and classification of PAP

The principal abnormalities in adult PAP result from poor surfactant clearance and altered surfactant homeostasis in the lung due to impaired alveolar macrophage function (figure 1). Attenuated alveolar macrophage maturation is typically caused by inadequate granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling, which is crucial for development of the full range of alveolar macrophage metabolic and immune functions.2 Autoimmune PAP, formerly known as idiopathic PAP, occurs when anti-GM-CSF antibodies lead to deficiency of bioavailable GM-CSF. Rarely, genetic defects in the GM-CSF receptor α or β chains (CSF2RA, CSF2RB) also lead to impaired macrophage maturation in the absence of autoimmunity (congenital or hereditary PAP).3 Finally, alveolar macrophage function might be abnormal because of several haematological or environmental factors that cause macrophage dysfunction (secondary PAP).4 Other genetic abnormalities involving surfactant proteins or lipid metabolism can also cause PAP, including mutations in the surfactant proteins B or C, ATP-binding cassette subfamily A member 3 (ABCA3), and thyroid transcription factor-1.5,6 Clinical disease due to these mutations typically manifests in neonates, often with concomitant pulmonary fibrosis, and will not be discussed further. PAP that does not match any of these types is called unclassified PAP. Autoimmune PAP accounts for the vast majority (90–95%) of adult cases, whereas secondary PAP comprises 5–10% of adult PAP.7 Although large-scale multicentre studies focusing on epidemiology are scarce, data from a Japanese registry reveal estimated incidence of 0·24–0·49 per million population and an estimated prevalence 2·04–6·2 cases per million population for autoimmune PAP.8

Key messages

- Pulmonary alveolar proteinosis (PAP) results from alteration of surfactant homeostasis in the lung, usually due to impaired alveolar macrophage function.
- Abnormalities involving granulocyte–macrophage colony-stimulating factor (GM-CSF)-mediated alveolar macrophage maturation constitute the most common pathway in the pathogenesis of PAP.
- Autoimmune PAP (accounting for more than 90% of adult PAP) occurs when anti-GM-CSF antibodies lead to deficiency of bioavailable GM-CSF.
- Secondary PAP results from alveolar macrophage dysfunction due to inhalational exposures (dust), haematopoietic disorders, infections, drugs, or disorders of immune regulation.
- In patients with clinically significant lung disease, whole-lung lavage remains the standard initial treatment method.
- Therapy with subcutaneous or inhaled recombinant GM-CSF as initial treatment or as sequel to whole-lung lavage could provide benefit for patients with PAP. However, there are no large-scale studies directly comparing its efficacy to that of whole-lung lavage.
Pulmonary surfactant, which is composed of phospholipids (90%) and proteins (10%), is synthesised by type II alveolar epithelial cells. The surfactant molecules are then secreted into the alveolar space, where they line the walls of the alveoli. Phospholipids, which are the major component of the surfactant, play a crucial role in reducing surface tension at the air–liquid interface, which prevents end-expiratory alveolar collapse, stabilises alveolar size, and reduces elastic recoil of the lung. Surfactant proteins A, B, C, and D are crucial for proper organisation of the surfactant, but are also important mediators of innate immunity of the lung. Surfactant is catabolised by uptake and recycling in the type II alveolar epithelial cells or alveolar macrophages. All recognised forms of adult PAP are thus a result of abnormal alveolar macrophage maturation or function.

Role of GM-CSF in PAP
One of the major breakthroughs in our understanding of the pivotal role of GM-CSF for alveolar macrophage function was the near fortuitous discovery that GM-CSF mediates maturation of a monocyte into a functional alveolar macrophage and degradation of surfactant. GM-CSF mediates macrophage maturation by binding to normal receptors, thus facilitating degradation of surfactant. Recombinant GM-CSF administration to patients with PAP results in an increase in alveolar macrophage counts and a decrease in surfactant protein levels and composition. These effects are dose-dependent and require several months to be observed.

Figure 1: Pathogenesis of pulmonary alveolar proteinosis
(A) The normal process of granulocyte–macrophage colony-stimulating factor-mediated maturation of a monocyte into a functional alveolar macrophage and degradation of surfactant. (B) The various mechanisms by which macrophage maturation or macrophage function is impaired, which results in development of pulmonary alveolar proteinosis; disorders of surfactant metabolism (blue box) result in accumulation of abnormal surfactant protein or lipids. Copyright of The Center for Medical Art & Photography (Cleveland Clinic, Cleveland, OH, USA).

Autoimmune PAP
Anti-GM-CSF antibody blocks macrophage maturation

Hereditary PAP
Mutation of α or β chain of GM-CSF receptor (CSF2RA, CSF2RB)

Secondary PAP
Toxins and dust exposure
Haematological disorders
Infections

Transcription factors
1 P-STAT5, 1 PU.1

Surfactant production disorders
Mutation of SFTPB, SFTPC, AICAJ, NKX2-1 (TTF-1)

Emerging data also show defective lipid component metabolism in alveolar macrophages as an important pathogenic mechanism, linking GM-CSF signalling with cholesterol homoeostasis in the lungs. Deficiency of GM-CSF might also contribute to the development of pulmonary fibrosis, which occurs in a minority of patients with PAP. Studies on type II alveolar epithelial cells of mice with bleomycin-induced fibrosis showed diminished expression of GM-CSF mRNA. Furthermore, increased fibrosis was noted after administration of neutralising anti-GM-CSF IgG to the bleomycin-treated rats. One of the proposed mechanisms for development of fibrosis in GM-CSF deficiency is the reduced synthesis of the potent anti-inflammatory prostaglandin, PGE2. However, proof that these observations are directly relevant to fibrosis in PAP remains to be established.
Correlation of biomarkers and serological tests with markers of PAP disease severity

<table>
<thead>
<tr>
<th>Correlation with biomarkers</th>
<th>Remarks</th>
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<tr>
<td>LDH</td>
<td>Moderate correlation with PaO₂, excellent correlation with high A-a gradient</td>
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<tr>
<td>Anti-GM-CSF antibody</td>
<td>Serum titre: poor correlation with PaO₂, (A-a gradient, pulmonary function; BAL titre: good correlation with high serum LDH, low PaO₂, high A-a gradient and DLCO)</td>
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<tr>
<td>KL-6</td>
<td>Elevated levels in BAL fluid and blood significantly correlates with higher serum LDH, low PaO₂, and high A-a gradient</td>
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<tr>
<td>Surfactant proteins (SP-A, SP-B, SP-D)</td>
<td>Significant correlation with high A-a gradient, low vital capacity, and low DLCO</td>
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<tr>
<td>CEA</td>
<td>Significant correlation with high LDH and low PaO₂</td>
</tr>
<tr>
<td>CYFRA 21-1</td>
<td>Significant predictor of disease severity and effectiveness of GM-CSF based therapy</td>
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<tr>
<td>Serum YKL-40</td>
<td>Increased in serum and BAL fluid of patients with PAP, correlates with DLCO and marker of disease progression</td>
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Panel: Type of PAP and causes

**Auto-immune PAP (most common in adults; 90-95% of cases)**
Caused by anti-GM-CSF antibody-mediated disruption of GM-CSF signalling pathway

**Secondary PAP (5-10% of adult cases)**
Caused by:
- Haemopoietic disorders (myelodysplasia [GATA 2 deficiency], plasma cell disorders, myeloid leukaemia, lymphoma);
- Immune dysregulation (severe combined immunodeficiency [SCID], hypogammaglobulinaemia, Fanconi anaemia, Behcet’s syndrome, bone-marrow transplantation, lung transplantation);
- Infections (nocardia, pneumocystis, cytomegalovirus, HIV);
- Inhalation (dust-silica [acute silicoproteinosis], cellulose fibres, aluminium, titanium dioxide, indium, agricultural, bakery flour, sawdust; chlorine, gasoline, nitrogen dioxide, plastic, varnish, or paint fumes);
- Lysinuric protein intolerance;
- Drug-induced or iatrogenic (busulfan, chemotherapy or antineoplastics, ciclosporin, desatinib, fentanyl, hydrofluoroc acid [inhaled], imatinib, lefunomide, mycophenolate mofetil, sirolimus, silicone breast implant, status after haemopoietic stem-cell or bone-marrow transplantation)

**Congenital or hereditary PAP (<1% of cases)**
Caused by recessive mutations with disruption of GM-CSF signalling (CSF2RA, CSF2RB mutations); surfactant production disorders (SFTPB, SFTPC, ABCA3, NXX2-1 [TTF-1] mutations)

**Unclassified PAP (<1% of cases)**
Caused by idiopathic or acquired cases and negative for anti-GM-CSF antibodies, without background causes of secondary PAP and congenital or hereditary PAP

**Differentiation of alveolar macrophages**
GM-CSF plays a key part in the terminal differentiation of alveolar macrophages, a process mediated by receptor ligation that induces phosphorylation of signal transducer and activator of transcription 5 (STAT5), which in turn stimulates multiple signalling pathways. GM-CSF also stimulates expression of PU.1, a myeloid maturation transcription factor that facilitates maturation and differentiation of alveolar macrophages. Alveolar macrophages from GM-CSF-deficient mice have reduced PU.1 expression. Similarly, PU.1 mRNA expression and PU.1-dependent terminal differentiation markers (eg, CD32, m-CSFR) were deficient in alveolar macrophages from people with autoimmune PAP.

**Lung immunity**
Aside from its role in surfactant metabolism, maturation induced by GM-CSF is required for immune function of myeloid cells. Despite possessing normal ultrastructure and differentiation markers, neutrophils from human beings with autoimmune PAP have impaired basal and antimicrobial functions. GM-CSF, via PU.1, also enhances phagocytosis by alveolar macrophages. GM-CSF-deficient mice have increased susceptibility to a wide range of microbial pathogens, including bacteria, viruses, fungi, and parasites. Human studies also show that GM-CSF therapy improves PU.1 expression and restores innate immune function. Clinical observations in human beings suggest that there is a higher than expected incidence of unusual infections in patients with PAP, including nocardiosis and mycobacterial infections. GM-CSF appears to be particularly crucial for control of Nocardia spp, and the presence of antibodies to GM-CSF have been identified as an independent risk factor for disseminated nocardiosis, even in seemingly healthy individuals.

Impaired alveolar macrophage function due to systemic or toxic causes is the hallmark of secondary PAP. Among the various causes of secondary PAP, haematological diseases (eg, myeloproliferative or haematological disorders) tend to be the most common. Other triggers include solid malignancies, infectious diseases, inhalational exposures, autoimmune diseases, and medications (panel). Occupational exposure to dust inhalation is directly toxic to alveolar macrophages, causing functional impairment that could lead to PAP. One of the first recognised occupational exposures associated with PAP was in silica workers, in whom the presentation can be fulminant, and is frequently referred to as acute silicoproteinosis. Since then, aluminium, titanium, and more recently, indium tin oxide, have also been implicated in development of PAP in exposed workers. A subset of these occupationally exposed individuals might also have anti-GM-CSF antibodies, human beings with PAP is insufficient; development of fibrosis is probably multifactorial.
but the pathophysiological relationship between the exaggerated autoimmune response and the environmental trigger is unclear.

**Clinical features**

PAP typically presents in the third to sixth decade of life. The disease is more common in men, with a prevalence ratio of approximately 2:1. However, women might have an earlier age of presentation than men. There is no known racial predilection. Although a causal relationship between tobacco use or environmental triggers and PAP has not been established, there is a higher than expected proportion of smokers (50–70%) and dust exposure (20–50%) in reported cohorts. It is possible that the apparent higher risk of PAP in men is due to historical differences in exposure rather than intrinsic sex differences.

Symptoms of PAP are nonspecific. 50–90% of patients with PAP report progressive dyspnoea. A substantial number of patients also develop cough, which can be productive. Other symptoms include fatigue, weight loss, chest discomfort, and arthralgias. Because of their increased susceptibility to infections, fever in patients with PAP should prompt further investigation, but fever can be a manifestation of PAP itself. Up to a third of patients can be asymptomatic. The physical examination is likewise nonspecific. Clubbing is found in up to 25% of individuals, and crackles might also be present but are not universal.

Pulmonary function tests typically reveal restrictive impairment, reduction of the diffusing capacity of the lungs for carbon monoxide (DLCO), or both. Metrics of gas transfer (eg, partial pressure of arterial oxygen \([\text{PaO}_2]\), alveolar-arterial gradient [A-a gradient]) are also commonly used surrogate markers for disease severity.

Inoue and colleagues used composite indices such as the Disease Severity Score, which categorises patients ordinally from 1 to 5 on the basis of \([\text{PaO}_2]\) obtained on room air in the prone position, as well as by symptoms. The Disease Severity Score has been a useful endpoint for routine surveillance of patients and for clinical trials.

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**Figure 2:** High resolution CT scan images of a patient with pulmonary alveolar proteinosis

Images show diffuse ground-glass opacification (A) and the so-called crazy paving pattern (B).

**Figure 3:** Characteristic milky or opalescent appearance of fluid return from whole-lung lavage in a patient with pulmonary alveolar proteinosis

L1 and R1 show the drained fluid after the first lavage cycle in the respective left and right lung; L2 and R2 bottles were collected after 9 L of lavage; and L3 and R3 show the clear drained fluid at the last lavage cycle, in this case after a total of 30 L per lung. The authors have used up to 56 L per lung in more severe cases.
in patients with PAP.44 A-a gradient has also been used as a sensitive primary endpoint in clinical trials.8

**Serological tests and biomarkers**

In the years after the first description of PAP, lactate dehydrogenase was investigated as a potential biomarker.47,48 Concentrations can be increased in up to 80% of patients with PAP, but it is not specific.4 In cases of suspected autoimmune PAP, antibodies to GM-CSF can be identified in almost all patients, both in the serum and bronchoalveolar lavage fluid.49,50 By contrast, serum autoantibodies to GM-CSF are not increased in patients with congenital or secondary PAP.8,20,22 The sensitivity and specificity of serum anti-GM-CSF antibody approaches 100%, making it the most useful diagnostic test.51 However, the concentration of serum anti-GM-CSF antibody does not correlate with disease severity.50 In individuals with CSF2RA or CSF2RB mutations, GM-CSF concentrations in the blood might be increased because of defective clearance by the mutated receptors.1 If there are no obvious exposures to secondary PAP risk factors, the combination of increased GM-CSF concentrations and no measurable anti-GM-CSF antibodies should prompt genetic testing for variant receptor genes. Several other serum analytes have been investigated as surrogate biomarkers of disease severity and progression, but none have become widely accepted, due in part to lack of specificity, limited clinical availability, and longitudinal insensitivity to change (table).52–57

**Radiology**

Chest radiographs typically show bilateral alveolar infiltrates. Classically, a bat-wing appearance with central hilar prominence is seen, which can progress to confluent infiltrates involving all five lobes. High-resolution chest CT (HRCT) scans usually reveal patchy ground-glass opacities.58 Interlobular septal thickening is present in up to 85% of patients (figure 2).59 The pattern of septal thickening frequently identified with PAP has been termed crazy paving, referring to the polygonal appearance of the secondary pulmonary lobules with interspersed ground glass attenuation.59 Crazy paving occurs in 83% of patients with PAP.58,61 HRCT characteristics could be useful for distinguishing secondary from autoimmune PAP: crazy paving is unusual in secondary PAP, and the distribution of ground-glass opacities is typically diffuse rather than geographically concentrated.18 Radiological findings similar to PAP can be seen in numerous other conditions, such as pulmonary oedema, alveolar haemorrhage, organising pneumonia, acute respiratory distress syndrome, pneumocystis pneumonia, and lipoid pneumonia.60,62 Architectural distortion of the lungs suggesting fibrosis is unusual in patients with PAP, with a frequency of 7–20%.61 Fibrosis on HRCT during initial presentation or at follow-up also portends a poor prognosis.62,63 Pleural effusion, enlargement of mediastinal lymph nodes, and evidence of air trapping are unusual and should prompt consideration of an alternate or concomitant diagnosis. Pulmonary nodules are also atypical, and should be assessed for malignancy or infection as warranted. Quantitative assessment of the intensity and extent of opacification on CT scans (CT grade score) has shown promise for evaluating therapeutic response in autoimmune PAP.64

**Diagnosis of PAP**

Although clinical presentation and HRCT pattern alone can be fairly suggestive in typical cases, the first step in confirming PAP is usually bronchoscopy. Broncho-alveolar lavage might demonstrate characteristic opalescent or milky-appearing fluid (figure 3). The milky consistency is due to the high lipoproteinaceous content of the amorphous
material that accumulates in the alveolar spaces. Under light microscopy, characteristic acellular oval bodies that are basophilic on May–Grünnwald–Giemsia staining can be observed. Histopathology shows diffuse dense acellular eosinophilic material in the airways with minimal interstitial inflammation (figure 4). The accumulated material is characteristically periodic acid Schiff-positive and oil red O-positive. Addition of transbronchial biopsy increases the diagnostic yield and should be considered, particularly if the radiology and gross appearance of bronchoalveolar lavage fluid are not characteristic. Addition of transbronchial biopsy also obviates the need for a surgical lung biopsy in most situations; less than 10–20% of patients require surgical biopsy for confirmation. There are no data about the safety and the role of transbronchial cryobiopsy specifically for the diagnosis of PAP. In patients with suspected secondary PAP, workup should be targeted towards identifying the underlying cause. Figure 5 is a proposed algorithm for diagnosis of suspected PAP.

**Treatment**

There are no international consensus guidelines for treatment of PAP. Conventionally, therapeutic decisions are largely based on disease severity and type of PAP. Patients with mild symptoms and no evidence of substantial physiological impairment can be monitored by serial assessment of symptoms, pulmonary function testing, oxygenation, and chest radiography. Patients with moderate-to-severe disease warrant a more aggressive approach. Similar to recommendations for patients with other chronic lung diseases, smoking cessation and preventive vaccination with influenza and pneumococcal immunisation are important. Figure 6 depicts a suggested algorithm for management of adult PAP.

**Therapeutic whole-lung lavage**

Whole-lung lavage (WLL) has been the cornerstone of therapy since it was first described in the 1960s. Since the first iterations of WLL, therapeutic WLL has evolved into the modern-day practice of single or sequential bilateral lavage by isolating each lung with a double-lumen endotracheal tube under general anaesthesia. Segmental lung lavage is also done through the bronchoscope (repeated bronchoalveolar lavage) in some centres, but the inferior adequacy of this technique, as well as the arduous nature of the procedure, has relegated it to situations in which WLL is not available.

WLL is done under general anaesthesia. On very rare occasions, when patients do not tolerate single-lung ventilation, extra-corporeal oxygenation has been used to facilitate the lavage procedure. In most situations, lavage of the two lungs is done in separate sessions (single-lung lavage) a few days to weeks apart. In select centres, lavaging both lungs (bilateral lung lavage) is done in a single setting, with the most affected lung being lavaged first. The use of a percussion device during the lavage process such as Vest (Hill-Rom, St Paul, MN, USA) can help clear the proteinaceous material. Recently, investigators removed up to 40% more protein and prolonged the time to relapse by modifying the classic lavage technique: they manually ventilated the lavaged lung with 300 mL of air using a bag five times, after instilling 500 mL of saline solution before draining it. In a survey of the global practice of WLL in PAP, fever was the most frequently reported adverse event (18%), followed by hypoxaemia (14%). Rates for pneumothorax were 0–8%. Despite the scarcity of rigorous prospective studies, data from the available literature generally show improved oxygenation and pulmonary function testing after WLL. In the comprehensive analysis by Seymour and Presnell, data in the 3-month period after WLL showed a mean increase in PaO₂ of 20 mm Hg (n=41; 95% CI 15·6–24·6, p<0·0001), forced expiratory volume in 1 s of 0·26 L (n=33; 0·09–0·42, p=0·0034), forced vital capacity of 0·50 L (n=40; 0·33–0·67, p<0·0001), and diffusing capacity of the lungs for carbon monoxide of 4·4 mL/mm Hg×min (n=25; 2·6–6·3, p<0·0001). There was a mean reduction in A-a gradient of –30·6 mm Hg (SD 15·6–24·6, p<0·0001). The median duration of benefit, defined as disease recurrence requiring repeat therapeutic lavage, was 15 months; 66% of the patients

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**Figure 5:** Diagnostic algorithm for patients with suspected PAP.

PAP=pulmonary alveolar proteinosis. HRCT=high-resolution chest CT. SP-D=surfactant protein D. SP-A=surfactant protein A. CEA=carcinoembryonic antigen. CYFRA 21-1=cytokeratin-19 fragments. LDH=lactate dehydrogenase.

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**Table 1:** Autoimmune PAP

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<tr>
<th>Criteria</th>
<th>PAP ruled out</th>
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<tr>
<td>Patient has been exposed to risk factors for secondary PAP (panel)</td>
<td>Elevated anti-GM-CSF autoantibody concentration</td>
</tr>
<tr>
<td>Elevated serum GM-CSF concentration, family history of PAP, genetic mutation, no secondary PAP, no other causes of elevated GM-CSF such as infections, malignancies, etc</td>
<td>Normal anti-GM-CSF autoantibody level</td>
</tr>
<tr>
<td>Autoimmune PAP</td>
<td>Secondary PAP</td>
</tr>
<tr>
<td>Congenital/hereditary PAP-confirm (GM-CSF α or β receptor mutation, etc)</td>
<td>Unclassifiable PAP</td>
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**Figure 6:** Suggested algorithm for management of adult PAP.
GM-CSF therapy

The finding that deficient GM-CSF bioavailability is the main pathophysiological defect in autoimmune PAP generated interest in the therapeutic use of recombinant GM-CSF. In the primary single-patient experience of its efficacy, use of subcutaneous recombinant human GM-CSF (rhGM-CSF) led to substantial improvement of oxygenation. Since then, several cohort studies, using subcutaneous or nebulised delivery, have generated the bulk of the available efficacy data.

In a prospective open-label trial in 25 adult patients with PAP, subcutaneous administration of escalating doses of exogenous GM-CSF improved oxygenation, radiographic findings, and symptoms in 48% of the patients who completed the trial. During follow-up, only 33% of patients who responded required further lavage or long-term oxygen supplementation, compared with 56% of those who did not respond. The regimen was well tolerated with a low incidence of local and systemic adverse effects. Although results from some earlier studies suggested that serum anti-GM-CSF antibody titre could predict response to rhGM-CSF therapy, data supporting this are limited. In a separate study, higher forced vital capacity and normal serum lactate dehydrogenase were found to be predictors of better response, although both probably reflect milder disease.

Nebulised rhGM-CSF (sargramostim) was studied in a prospective trial in 35 Japanese patients with autoimmune PAP. The study participants were observed for 12 weeks, and subsequently received nebulised rhGM-CSF over a total period of 24 weeks, using six cycles of a regimen of either high-dose therapy (250 μg inhaled twice a day for the first 8 days of each 2-week cycle, followed by once-daily dosing for 4 of 14 days for another 12 weeks) or low-dose therapy (125 μg inhaled twice a day for 4 of 14 days over six cycles). A majority (24 [62%] of 39) of the patients showed an improvement in both subjective (dyspnoea) and objective parameters (6-minute walk distance); 29 (83%) of 35 patients did not require further therapy for 1 year. Clinically significant side-effects were not observed. With extended follow up of 30 months from the end of inhalation therapy, 23 (66%) of 35 patients did not require additional treatments.

Limitations with use of GM-CSF-based therapies relate to the dearth of placebo-controlled randomised trials, and small sample sizes. The effect on A-a gradient is variable but generally moderate, and the responder rate is likewise suboptimal (figure 7). Fortunately, two clinical trials comparing inhaled rhGM-CSF with placebo are ongoing, using molgramostim (NCT02702180) and sargramostim (NCT02835742).

Therapies targeting autoimmunity

Corticosteroids

By analogy with other autoimmune diseases, corticosteroid therapy seems logical for treatment of autoimmune PAP. However, most data from patients with PAP treated with

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Followed-up for more than 1 year from diagnosis (median of 37 months) required more than one lavage. In more recent studies from China and France, the relapse rates ranged from 30–56%. In the French study, except for PaO₂, which improved by 6-4 mm Hg (p=0.0213) after WLL, there were no significant changes in other pulmonary function parameters. The discrepancy in efficacy estimates is probably due to differences in techniques across regions, centre experience, evolution of the technique over time, and the limitations of retrospective data collection.

Data from a worldwide survey of 368 patients reported that the mean number of procedures per patient was 2-5 (SD 1-5) in a 5-year period. There are no large-scale studies that have identified predictors of response to WLL. In the historic paper by Seymour and Fresnelli, the annual age was the only variable that predicted poor response to WLL. More recent experiences suggest that a percent predicted DLCO less than 42% might identify patients who are likely to require repeat WLL. Overall, WLL, despite being an invasive procedure, remains the foundation treatment for moderate to severe PAP. A detailed review of the technical aspects of WLL can be found elsewhere. The video shows the WLL procedure.
corticosteroids suggest more harm than benefit. In a retrospective study, patients treated with steroids had a dose-dependent worsening of their Disease Severity Scores. Although the exact explanation is unknown, it is likely that the negative effect corticosteroids exert on macrophage phagocytosis and catabolism account for the observations. Thus, corticosteroid therapy of PAP should generally be reserved only for those with concomitant connective tissue disease or other steroid responsive processes.

Rituximab
Pathological concentrations of the anti-GM-CSF auto-antibody that triggers autoimmune PAP are an obvious target for therapy directed at B cells. The anti-CD20 monoclonal antibody rituximab has been studied in small trials and several case reports. In an open-label, proof-of-concept trial in 10 patients with autoimmune PAP, a single treatment regimen of two doses of 1000 mg of rituximab, administered two weeks apart, improved gas exchange in seven patients. The therapy also led to an approximately 25% reduction in bronchoalveolar lavage anti-GM-CSF IgG concentrations, with evidence of improved lipid transport and surfactant catabolism. Although the data are promising, larger randomised studies must be initiated to substantiate a role for rituximab in the routine therapy of autoimmune PAP. At present, rituximab is employed mainly as salvage therapy in patients with refractory PAP. An ongoing prospective trial of rituximab therapy for anti-cytokine autoantibody-associated diseases, which will include patients with PAP, is underway in the USA (NCT01842386).

Plasmapheresis
Plasma exchange to reduce autoantibody levels has been used with marginal success in a few patients with autoimmune PAP that is refractory to conventional therapy. Experience in these cases has varied from using an extended protocol that spanned months, to an abbreviated version of 5 days, followed by rituximab. However, results with plasmapheresis in patients with PAP have not been consistent enough to warrant recommendation for routine use.

Lung transplantation
There is minimal experience with lung transplantation in patients with PAP. Recurrence in the lung allograft is one of the major concerns with transplantation in PAP. Recurrence in transplant recipients could be attributed to the immune dysregulation that is persistent in patients with PAP, particularly those with underlying genetic mutations. Secondary PAP can also develop de novo in some transplant recipients, and is probably related to disruption of the native immune system by immunosuppressant anti-rejection medications. In patients with PAP that is refractory to conventional treatment, lung transplantation might be offered but it would be prudent to rule out underlying genetic PAP.

Treatment of hereditary and secondary PAP
For patients with secondary PAP, the initial therapeutic focus should generally be on removing offending exposures or treating the underlying medical disorder. For individuals with myelodysplastic syndrome, the development of PAP is associated with poor survival. In patients with advanced respiratory compromise, WLL could be useful. Bone-marrow transplantation has been considered in myelodysplastic syndrome complicated by secondary PAP, but the data are limited to case reports. Bone-marrow transplantation, instead of lung transplantation, could also have a role in patients with abnormal GM-CSF signalling due to GM-CSF receptor variants.

Prognosis
The clinical course of PAP is highly variable, ranging from spontaneous resolution to death due to progressive respiratory failure or infection. Although observations from early cohorts suggested that spontaneous resolution occurs in 25–30% of patients, more recent studies observed resolution in only 7–8% of patients. There are no known reliable clinical features that can predict which patients will have spontaneous remission. In a large study starting in the 1950s by Seymour and Presneill, the disease-specific 5-year survival was estimated to be 88% (SD 4), with survival rates of 79% at 2 years, 75% at 5 years, and 68% at 10 years. No difference was observed between sexes. The use of WLL was associated with better 5-year survival compared with no treatment (94% vs 85%, p=0.04), but it is unclear whether the procedure itself or other patient factors were responsible for the difference. There are four studies that have reported 100% 5-year survival, with an infection rate of only 5%, probably reflecting advances in diagnosis,
treatment, and prevention of complications such as infections. Pulmonary fibrosis might occur in up to 20% of patients and is a poor prognostic factor. Prognosis for patients with secondary PAP is influenced by the underlying illness and generally tends to be poorer than for patients with autoimmune PAP.

Future research

One of the major impediments to estimating prevalence, ascertaining diagnosis, and defining the actual effect of therapies in PAP is the rarity of the condition. A US registry for patients with PAP is currently recruiting and should provide further insights into the prevalence and genetic risk factors for development of the disease. The registry also aims to demonstrate the feasibility of measuring GM-CSF antibodies with a diagnostic blood spot card (NCT02461615).

The indications and benefits of therapy need to be further delineated. WLL has remained the primary therapy for patients with PAP, but there are no large-scale randomised studies that have substantiated its long-term benefits. Randomised trials that have compared WLL with recombinant GM-CSF-based therapies have also not been done. Instances have been reported of use of one method when the other one is unsuccessful, but they are limited to case reports. Sequential GM-CSF inhalation followed by WLL, and not the other way around, has also been reported with remarkable results in a small number of patients. Thus, further studies will need to answer questions regarding the identification of appropriate candidates for these therapies, and determining efficacy, safety, and feasibility of combining these two treatment methods.

Impaired lipid metabolism, which is mediated by GM-CSF, has also been proposed as a fundamental pathogenic defect in PAP. This notion has spurred interest in the potential use of peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (eg, pioglitazone) in the treatment of PAP, given the crucial role of PPAR-γ in facilitating cholesterol export from macrophages through stimulation of ATP-binding cassette subfamily A member 1 and ATP-binding cassette subfamily G member 1. Preliminary studies in Csf2-deficient mice support the efficacy of PPAR-γ agonists in reducing cholesterol levels in alveolar macrophages and the turbidity of bronchoalveolar lavage fluid.

Transplanting Csf2rb gene-corrected pulmonary macrophages in Csf2rb-deficient mice was shown to be safe and effective. The administration of macrophages not only corrected the lung disease and secondary systemic manifestations, but also normalised disease-related biomarkers. One study successfully used a lentiviral vector (expressing a codon-optimised human CSF2RA-cDNA) to mediate transduction of cultured macrophage cell lines and primary human cells to reconstitute GM-CSF receptor expression, and thus restore GM-CSF signalling in hereditary PAP macrophages, possibly paving the way for macrophage transplantation in humans. Thus, the concept can be plausibly extended to human PAP when receptor defects are the cause, opening the path for future use of gene-corrected pluripotent stem-cell-derived monocytes and macrophages. However, it is worth mentioning that genetic mutation-driven disturbances of GM-CSF signalling constitute a substantial minority of patients with PAP. For congenital genetic defects, type II alveolar epithelial cells will be the requisite target. In this regard, preliminary studies on human amnion epithelial cells have shown that, in appropriate culture media, they can develop into alveolar epithelial phenotypes that might be able to secrete surfactant proteins. Jacob and colleagues also reported successful generation of type II alveolar epithelial cells from human pluripotent stem cells, again raising their potential use in lung tissue regeneration. Although this finding is promising for restoration of normal homeostasis, no such studies have been done specifically in patients with PAP.

Conclusions

Since the original description of PAP, our understanding of the pathogenesis and classification of the disorder have evolved substantially. Impaired alveolar macrophage maturation and surfactant clearance due to abnormal GM-CSF signalling are now recognised as the fundamental defects in autoimmune PAP, which constitutes the majority of adult cases. However, PAP can be secondary to other triggers, particularly bone-marrow disorders or inhalational exposures, and is rarely hereditary. Although WLL is an invasive treatment method, it seems to be safe, well tolerated, and confers symptomatic and physiological benefits, at least in centres with experience. GM-CSF-based therapies (subcutaneous and inhalation) are promising but there is a dearth of data to support GM-CSF supplementation as first-line therapy or how to optimally combine it with WLL. Future studies about PAP will focus on fine-tuning our knowledge of disease pathogenesis, and on identifying newer pathways.
mutations, and biomarkers. For genetic causes of PAP, cell-based therapies offer hope for a cure, but much work is necessary before their clinical application.

**Contributors**

All authors contributed equally to the design and writing of the manuscript.

**Declaration of interests**

We declare no competing interests.

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Review


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