Pulmonary alveolar proteinosis

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Abstract | Pulmonary alveolar proteinosis (PAP) is a syndrome characterized by the accumulation of alveolar surfactant and dysfunction of alveolar macrophages. PAP results in progressive dyspnoea of insidious onset, hypoxaemic respiratory failure, secondary infections and pulmonary fibrosis. PAP can be classified into different types on the basis of the pathogenetic mechanism: primary PAP is characterized by the disruption of granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling and can be autoimmune (caused by elevated levels of GM-CSF autoantibodies) or hereditary (due to mutations in *CSF2RA* or *CSF2RB*, encoding GM-CSF receptor subunits); secondary PAP results from various underlying conditions; and congenital PAP is caused by mutations in genes involved in surfactant production. In most patients, pathogenesis is driven by reduced GM-CSF-dependent cholesterol clearance in alveolar macrophages, which impairs alveolar surfactant clearance. PAP has a prevalence of at least 7 cases per million individuals in large population studies and affects men, women and children of all ages, ethnicities and geographical locations irrespective of socioeconomic status, although it is more-prevalent in smokers. Autoimmune PAP accounts for >90% of all cases. Management aims at improving symptoms and quality of life; whole-lung lavage effectively removes excessive surfactant. Novel pathogenesis-based therapies are in development, targeting GM-CSF signalling, immune modulation and cholesterol homeostasis.

Pulmonary alveolar proteinosis (PAP) is a syndrome defined by progressive accumulation of surfactant in pulmonary alveoli, which results in hypoxaemic respiratory failure and an increased risk of secondary infections and/or pulmonary fibrosis[1–3]. PAP occurs in a heterogeneous group of mechanistically distinct diseases caused by either impaired surfactant clearance or abnormal surfactant production (BOX 1). Disorders in which surfactant clearance is impaired are divided into primary and secondary PAP. In primary PAP, disruption of signalling by granulocyte–macrophage colony-stimulating factor (GM-CSF) results in dysfunction of alveolar macrophages and neutrophils; primary PAP can be further classified as autoimmune or hereditary. Secondary PAP occurs as a consequence of a disease, environmental exposure or pharmaceutical agent that reduces the numbers and/or functions of alveolar macrophages. Finally, congenital PAP comprises surfactant production disorders (also known as pulmonary surfactant metabolic dysfunction disorders), a group of diseases caused by mutations in genes encoding surfactant proteins or proteins involved in surfactant production or lung development. In rare cases, the aetiology of PAP cannot be attributed to a known cause and the patient is diagnosed with unclassified PAP.

The classification of PAP into primary, secondary or congenital (BOX 1) is based on similarities and differences in pathogenesis, clinical manifestations, diagnosis, management and treatment options[4]. The discovery that GM-CSF-deficient mice develop PAP in 1994 (REFS[5,6]) and of GM-CSF autoantibodies in patients with PAP in 1999 (REF.[7]) stimulated intense research on a global scale that transformed our understanding of PAP and led to the recognition that what had been previously considered idiopathic PAP is an autoimmune disease specifically targeting GM-CSF signalling[3]. Basic, clinical and translational research advances since the 1990s have raised PAP from obscurity through improvement in our knowledge of pathogenetic mechanisms, epidemiology, diagnosis and therapy. Importantly, this ongoing research has informed a crucial role of GM-CSF in alveolar macrophage ontogeny, pulmonary surfactant homeostasis, alveolar stability, lung function, host defence, pulmonary and systemic inflammation and autoimmunity.

In this Primer, we discuss the causes and epidemiology of PAP, describe the pathogenesis and outline the diagnostic strategies. We also summarize the available and emerging therapeutic options and ongoing research in the field. Finally, we discuss the potential future

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advancements that could improve the outlook and quality of life for patients with PAP.

Epidemiology

The analysis of reports on PAP in the medical literature is complicated by variable and inconsistent use of multiple terms, including PAP, alveolar lipoproteinosis, alveolar phospholipidosis and alveolar phospholipoproteinosis. Notwithstanding, in a comprehensive review of 410 identifiably separate cases of PAP described in 241 separate initial publications between 1958 and 1998, 90% of patients were diagnosed with acquired PAP of unknown aetiology that was not associated with any familial predisposition and was characterized by abnormal alveolar surfactant accumulation and a variable natural history[2]. PAP occurs in men, women and children of all ages, ethnicities and geographical locations independent of socioeconomic status; there seems to be no substantial evidence of global variation in the epidemiology of PAP. The overall prevalence of PAP has been measured to be at least 7 cases per million individuals in the general population of the United States and Japan, where the largest population studies have been conducted[8,9]. However, this figure should be considered a minimum estimate, as PAP often remains undiagnosed for long periods of time either because the disease is mild or it is misdiagnosed as another more-common disease. In a Japanese national PAP registry study initially reporting on 223 patients with autoimmune PAP[9], cumulative enrolment between 1999 and 2016 resulted in the identification of 952 patients, of whom 877 (92%) had primary PAP, 71 (7.5%) had secondary PAP and 4 (<1%) had unclassified PAP; congenital PAP was not represented in this cohort. Of the patients with primary PAP, 872 had autoimmune PAP and 5 had hereditary PAP caused by GM-CSF receptor defects, representing 91.6% and <1% of all cases, respectively. On the basis of enrolment in the Japanese national PAP registry over a decade, the annual incidence of autoimmune PAP was 1.65.

Autoimmune PAP is the best studied PAP-causing disease and accounts for ~90% of all patients[8,9]. In a 2008 report[9] from the Japanese national PAP registry, the annual incidence and prevalence of autoimmune PAP were reported at 0.49 and 6.2 cases per million individuals, respectively, the median age at diagnosis was 51 years and the male:female ratio was 2.1:1.0. However, 2018 US data from a large population-based study reported more female patients than male patients for all PAP, with a male:female ratio of 1:1.17 (REF.[10]). Although smoking is considered to increase the risk of developing PAP[2], only 124 (57%) of 217 patients with autoimmune PAP previously reported[9] had a history of smoking. Similarly, although occupational inhalation exposure to dust and/or particulates is associated with autoimmune PAP, such exposure was present in only 52 (26%) of 199 patients with autoimmune PAP[9]; hence, the underlying aetiology of autoimmune PAP remains obscure.

Hereditary PAP is caused by mutations in *CSF2RA* or *CSF2RB* (encoding GM-CSF receptor subunit-α (also known as CDw116) and cytokine receptor common subunit-β (also known as GM-CSF/IL-3/IL-5 receptor common β-subunit or CD131), respectively) and has been described in a small series of reports[11–18]. In one study reporting the initial cohort of 8 patients identified, the median age at symptom onset was 4.8 ± 1.6 s.e. years, although two patients of 5 and 8 years of age were asymptomatic[13]. Furthermore, several individuals have been reported to have disease onset as adults. The sample sizes are very small in these studies, as the prevalence of hereditary PAP is extremely low and accounts for <5% of all cases of PAP[8].

Secondary PAP has been reported in association with various underlying diseases[19–32] that are thought to cause PAP by reducing the numbers and/or functions of alveolar macrophages[3]. In one study reporting 31 cases of secondary PAP, the median age at diagnosis was 53 and 58 years in men and women, respectively, and the male:female ratio was 1.14:1.0 (REF.[22]). Among the various underlying diseases associated with the development of secondary PAP, haematological diseases, in particular myelodysplastic syndromes, are the most common cause.

Box 1 | **Classification of PAP**

Primary pulmonary alveolar proteinosis (PAP): caused by disruption of granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling and can be divided into

- Autoimmune PAP, due to GM-CSF autoantibodies
- Hereditary PAP, due to mutations in genes encoding GM-CSF receptor subunits

Secondary PAP: caused by reduced functions and/or numbers of alveolar macrophages as a result of

- Haematological disorders
- Malignancies
- Immune deficiency syndromes
- Chronic inflammatory syndromes
- Chronic infections
- Toxic inhalation syndromes
- Mutations affecting functions or numbers of mononuclear phagocytes

Congenital PAP: caused by surfactant production disorders, which result from

- Mutations causing deficiency in surfactant proteins
- Mutations causing deficiency in the lipid transporter ATP-binding cassette subfamily A member 3 (ABCA3)
- Mutations affecting lung development

Congenital PAP has been reported in neonates, infants, children, adolescents and adults in association with mutations in the genes required for normal surfactant production, including *SFTPB* and *SFTPC* (encoding pulmonary surfactant-associated protein B (SP-B) and SP-C), *ABCA3* (encoding ATP-binding cassette subfamily A member 3 (ABCA3)) and *NKX2-1* (encoding thyroid transcription factor 1 (TTF1))[33–40]. The incidence of surfactant production disorders, on the basis of data from the United States and Denmark, has been estimated to vary from ~1 in 3,000 individuals per year for ABCA3 deficiency to 1 in 1.7 million individuals per year for SP-B deficiency[41].

Environmental risk factors

PAP has been associated with inhalation exposure to various environmental agents such as cigarette smoke, fumes (for example, from electric welding), gases (for example, nitrogen dioxide), organic and inorganic dusts, aluminium, cellulose fibres, titanium dioxide and indium-tin oxide[1,20,26,42,43]. Most reports on environmental exposures other than cigarette smoke are single cases or small retrospective series and, in general, have not established a causal relationship between the exposure and the development of PAP. A 1969 report on 138 patients with PAP found that the most common environmental exposure was silica, which occurred in 10 individuals[44]. With the introduction of protective equipment in the workplace, this form of 'acute silicoproteinosis'[31,45] is rare. The link between silica and PAP is supported by studies in rats demonstrating that inhalation of silica particles disrupts surfactant homeostasis and results in increased surfactant accumulation[46,47].

Mechanisms/pathophysiology

Alveolar homeostasis

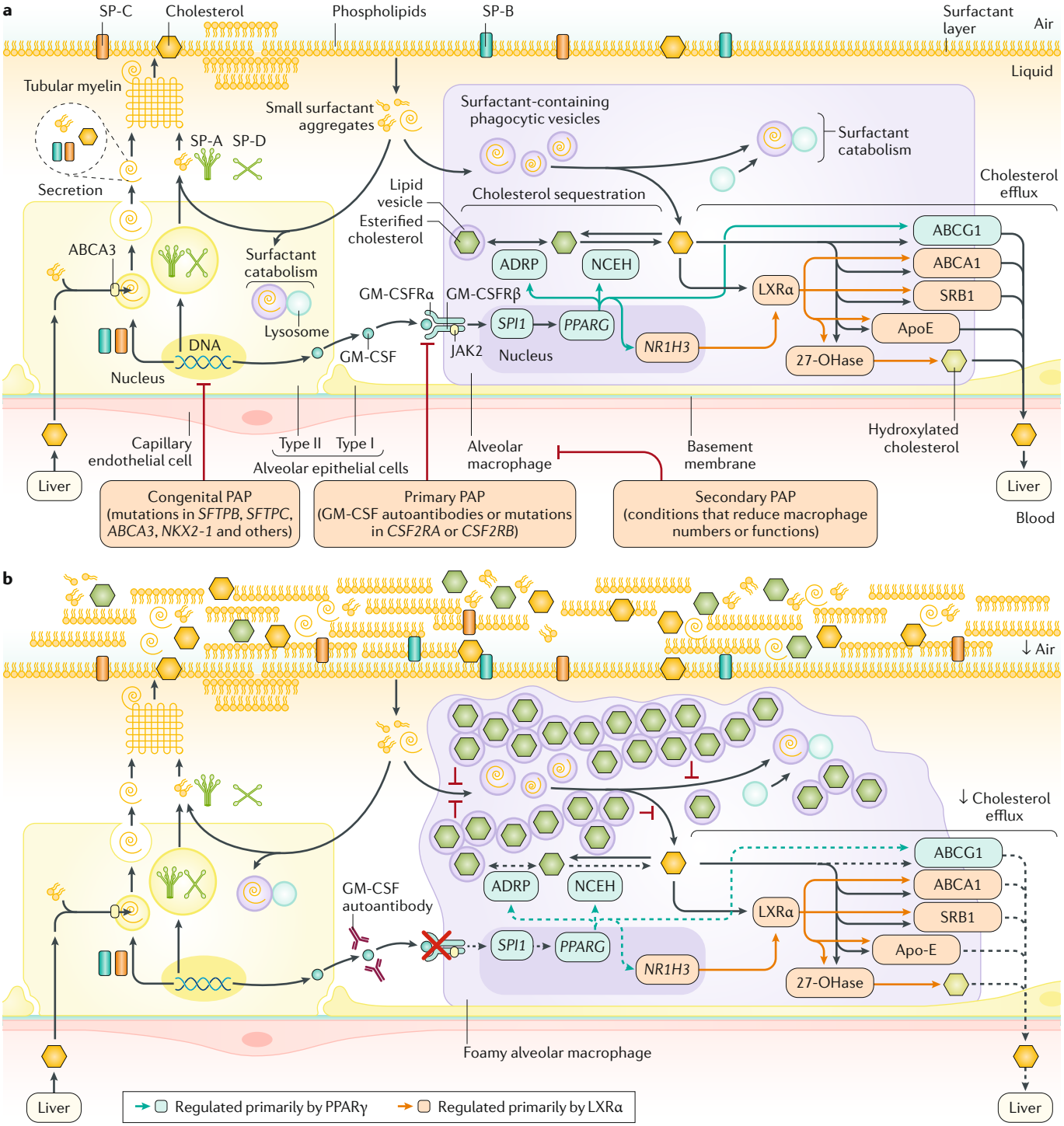
Surfactant composition, function and metabolism. The alveolar wall is covered by a fluid film known as pulmonary surfactant, which functions by reducing surface tension and preventing alveolar collapse during respiration; pulmonary surfactant is also important to host defence against microbial pathogens[48]. Surfactant consists of 80% polar phospholipids (primarily saturated phosphatidylcholine and other less-abundant phospholipid species), 10% neutral lipids (primarily free cholesterol with trace amounts of triglycerides and free fatty acids) and 10% surfactant proteins[49,50]. Maintenance of surfactant as a thin layer on the alveolar surface (that is, surfactant homeostasis) is tightly regulated by balanced production in type II alveolar epithelial cells, secretion into the alveolar space and clearance by either recycling or catabolism in type II alveolar epithelial cells or uptake and clearance by alveolar macrophages (FIG. 1a). Numerous studies in animals and humans that informed our current concepts of PAP pathogenesis also identified GM-CSF as a pulmonary hormone crucial to surfactant homeostasis, alveolar stability, lung function and innate and adaptive immune host defence.

GM-CSF. GM-CSF, initially named for its ability to stimulate the formation of neutrophil and macrophage colonies, is a 23 kDa glycoprotein cytokine produced by type II alveolar epithelial cells, among others, that binds to cell-surface receptors comprising a low-affinity GM-CSF binding subunit, CDw116, and a nonbinding, affinity-enhancing CD131 subunit[51,52]. Although neither subunit has intrinsic signalling activity[53], CD131 constitutively binds tyrosine-protein kinase JAK2[53], which is involved in cytokine signalling. GM-CSF binding causes activation of JAK2 and initiation of signalling via multiple pathways[53–55], including activation of signal transducer and activator of transcription 5 (STAT5)[56], transcription factor PU.1 (encoded by *SPI1*)[57], peroxisome proliferator-activated receptor-γ (PPARγ)[58] and others. GM-CSF signalling via PU.1 (REF.[58]) and PPARγ[59,60] is required constitutively for alveolar macrophage specification and numerous functions, including cholesterol export, surfactant clearance, host defence and others[61].

Pathogenesis of primary PAP

Animal studies. The pathogenesis of PAP remained obscure until the serendipitous discovery that mice deficient in GM-CSF owing to ablation of the colony-stimulating factor 2 (granulocyte–macrophage) gene (*Csf2*$^{-/-}$ mice) developed a disease identical to PAP in humans, the manifestations of which included progressive surfactant accumulation[5,6], functional impairment of alveolar macrophages[57,62–64] and neutrophils[65] and an increased risk of secondary infections by a broad range of microbial pathogens[63,66–77]. In *Csf2*$^{-/-}$ mice, accumulation of surfactant in alveoli results from reduced clearance of surfactant by alveolar macrophages without an increase in surfactant production by type II alveolar epithelial cells[78] or an intrinsic inability of alveolar macrophages to internalize surfactant[79]. Knockout mice deficient in the colony-stimulating factor 2 (granulocyte–macrophage) receptor β-chain (*Csf2rb*$^{-/-}$ mice)[80,81] or in colony-stimulating factor 2 (granulocyte–macrophage) receptor α-chain (*Csf2ra*$^{-/-}$ mice)[82,83] also develop PAP identical to PAP caused by *CSF2RA* or *CSF2RB* mutations observed in children[11,13–15,17,18] (see below). Correction of PAP in *Csf2rb*$^{-/-}$ mice, by transplantation of wild-type bone marrow[84] or by instillation of wild-type bone-marrow-derived macrophages directly into the lungs[85] (pulmonary macrophage transplantation), confirmed alveolar macrophages as the cellular component in the pathogenesis of PAP caused by disruption of GM-CSF signalling in mice. *IL3*/*CSF2* knock-in mice, which express human IL-3 and GM-CSF and develop PAP owing to elimination of mouse GM-CSF, permit engraftment of human CD34^{+} haematopoietic cells and support development of human alveolar macrophages that partially rescued the PAP phenotype[86].

Pulmonary GM-CSF, via the activation of transcription factor PU.1, is a crucial regulator of the terminal differentiation of murine alveolar macrophages[57]. GM-CSF activation of transcription factor *Pparg* is also crucial for the differentiation of alveolar macrophages in mice[58]. Alveolar macrophages from *Csf2*$^{-/-}$ mice also had reduced expression of *Pparg* and its target *Abcg1*, which encodes ATP-binding cassette subfamily G member 1 (ABCG1), a transmembrane lipid transporter protein important in cholesterol efflux from macrophages[87–89].



Thus, this finding implicates the dysregulation of cholesterol homeostasis in the pathogenesis of PAP[90–92]. *Abcg1*$^{-/-}$ mice also developed a PAP-like pulmonary phenotype characterized by cholesterol accumulation in alveolar macrophages and type II alveolar epithelial cells and progressive alveolar surfactant accumulation, thereby confirming a crucial role for ABCG1 in pulmonary surfactant homeostasis[93,94]. Alveolar macrophages from *Csf2rb*$^{-/-}$ and *Csf2ra*$^{-/-}$ mice also had decreased expression of *Pparg* and *Abcg1*, which resulted in massive accumulation of esterified cholesterol (esterification is a protective mechanism used by cells to sequester cholesterol) within intracytoplasmic lipid droplets in alveolar macrophages; this accumulation results in a foamy appearance of macrophages and a secondary reduction in the uptake and clearance of surfactant[61,83]. Studies in these GM-CSF signalling-deficient mice indicate that GM-CSF regulates cholesterol efflux in a constitutive, reversible and dose-dependent fashion[61]. Autoimmune PAP has been reproduced in non-human primates by passive immunization with GM-CSF autoantibodies derived from patients with PAP[95,96] (see below).

◀ Fig. 1 | **Alveolar surfactant homeostasis and its disruption in PAP. a** | Alveolar surfactant homeostasis. Pulmonary surfactant is normally maintained as a thin layer of polar lipids (mostly phospholipids), low amounts of neutral lipids (mostly cholesterol) and surfactant-associated protein A (SP-A), SP-B, SP-C and SP-D located at the alveolar air–liquid interface. Homeostasis is tightly regulated by balanced secretion and removal of surfactant components. Type II alveolar epithelial cells synthesize, assemble and secrete surfactant components resulting in the formation of tubular myelin, which unravels to form the surfactant layer and then removes the surfactant components through recycling and catabolism after they are expelled from the surfactant layer. Alveolar macrophages remove approximately half of the expelled surfactant by catabolism of phospholipids and efflux and reverse transport of cholesterol to the liver. Alveolar macrophages require constitutive granulocyte–macrophage colony-stimulating factor (GM-CSF) stimulation to maintain an adequate rate of cholesterol efflux, which occurs through five pathways that are mainly regulated by *PPARG* (encoding peroxisome proliferator-activated receptor-γ (PPARγ)) or *NR1H3* (encoding oxysterols receptor LXRα). Intracellular cholesterol levels are also tightly regulated, and when levels increase, cholesterol is esterified and stored in intracellular lipid droplets via adipose differentiation-related protein (ADRP), which facilitates the passage of esterified cholesterol into and out of the vesicle. Esterified cholesterol can be reverted to cholesterol by neutral cholesterol ester hydrolase (NCEH) and can then be secreted. **b** | Pathogenesis of autoimmune pulmonary alveolar proteinosis (PAP). An increase in polyclonal, neutralizing GM-CSF autoantibodies blocks GM-CSF signalling in the blood and tissues; thus, GM-CSF autoantibodies impair multiple functions (dashed lines) by altering expression of multiple genes, including expression of the macrophage cholesterol transporter ABCG1 (ATP-binding cassette subfamily G member 1), resulting in a primary reduction in cholesterol efflux from alveolar macrophages and a secondary reduction in surfactant clearance from the alveolar surface. Increased intracellular cholesterol stimulates increases in ATP-binding cassette subfamily A member 1 (ABCA1) and other LXRα-mediated cholesterol clearance pathways that attempt, albeit unsuccessfully, to compensate for the loss of ABCG1-mediated clearance. In response to reduced cholesterol efflux, lipid droplets containing esterified cholesterol accumulate, resulting in the formation of foam cells. 27-OHase, sterol 27-hydroxylase; ApoE, apolipoprotein E; GM-CSFRα, GM-CSF receptor subunit-α (also known as CDw116); GM-CSFRβ, GM-CSF receptor common β-subunit (also known as CD131); JAK2, tyrosine-protein kinase JAK2; SRB1, scavenger receptor class B member 1.

Human studies. Alveolar macrophages from human patients with primary PAP also had decreased expression of *SPI1* (REF.[59]), *PPARG* (REF.[90]) and *ABCG1* (REF.[90]) and a foamy appearance owing to the marked accumulation of esterified-cholesterol-containing intracytoplasmic lipid droplets with a minimal increase in accumulation of surfactant phospholipid[10], similar to results from *Csf2*$^{-/-}$ and *Csf2rb*$^{-/-}$ mice. In both patients with primary PAP and GM-CSF-signalling-deficient mice, the cholesterol:phospholipids ratio in pulmonary surfactant was abnormally increased[10,61], which has important implications for the surface-tension-lowering biophysical properties of surfactant[97–99].

In summary, human and murine studies indicate that the GM-CSF–PU.1–PPARγ–ABCG1 axis in alveolar macrophages is crucial for maintenance of surfactant homeostasis and that GM-CSF regulates cholesterol efflux from macrophages in a constitutive, dose-dependent and reversible fashion but is not essential for clearance of surfactant phospholipids (FIG. 1a). Furthermore, in both human and murine PAP associated with disruption of GM-CSF signalling, the primary disturbance in lipid metabolism in alveolar macrophages is a marked reduction in the rate of GM-CSF-dependent cholesterol efflux, not impaired catabolism of phospholipids; the reduction in surfactant clearance is a secondary consequence of reduced surfactant uptake by foamy alveolar macrophages engorged with esterified-cholesterol-rich intracytoplasmic lipid droplets[100] (FIG. 1a). Finally, the loss of GM-CSF signalling in both human and murine PAP is associated with a marked increase in the cholesterol:phospholipids ratio in pulmonary alveolar surfactant, which is relevant to surfactant function.

Pathogenesis of autoimmune PAP. Autoimmune (previously idiopathic) PAP is mediated by autoantibodies targeting GM-CSF (FIG. 1b). This mechanism is supported by multiple lines of evidence. First, neutralizing GM-CSF autoantibodies are present at high levels in patients with autoimmune PAP[101,102] but not in those with hereditary, secondary or congenital PAP, other lung diseases or in healthy people[2,3,9,103,104]. Second, passive immunization of healthy non-human primates with highly purified, GM-CSF autoantibodies derived from patients with autoimmune PAP[65] reproduced the cardinal features of PAP[95,96]. Third, GM-CSF autoantibodies isolated directly from patients with PAP or from passively immunized primates have the same GM-CSF-neutralizing capacity[95,96]. Fourth, a 'critical threshold' value of GM-CSF autoantibody concentration exists such that concentrations above this value disrupt GM-CSF-stimulated functions in alveolar macrophages and blood leukocytes and increase the risk of PAP[105–107]. This finding has diagnostic importance and reconciled these initially puzzling observations: GM-CSF autoantibody levels do not correlate with disease severity in patients with autoimmune PAP[103,106], and low levels of GM-CSF autoantibodies are ubiquitously present in people without autoimmune PAP[105,106,108]. In non-human primates passively immunized with highly purified, GM-CSF autoantibodies derived from patients with PAP[96], as well as in patients with autoimmune PAP and healthy controls[104], GM-CSF signalling correlates inversely with serum GM-CSF autoantibody concentration at values <5 μg ml^{-1} and is undetectable at concentrations ≥5 μg ml^{-1}; thus, 5 μg ml^{-1} is the minimum concentration needed to block GM-CSF signalling. In studies to refine the diagnostic value of the serum GM-CSF autoantibody test (see below), concentrations ≥5 μg ml^{-1} are strongly associated with an increased risk of autoimmune PAP[109]. Together, these results provide strong evidence that GM-CSF autoantibodies mediate the pathogenesis of autoimmune PAP and are not an epiphenomenon of the disease[95,105].

The aetiology of the autoimmune response in patients with autoimmune PAP has been evaluated by characterizing GM-CSF autoantibodies. They are composed of polyclonal immunoglobulin G (predominantly subclasses 1 and 2 with trace amounts of subclasses 3 and 4), target multiple epitopes distributed throughout the GM-CSF molecule, have high binding affinity (20 pM, s.d. 7.5 pM), neutralize GM-CSF at concentrations higher than those present physiologically and effectively block GM-CSF signalling in vivo[101,103,104]. The observation that the autoantibodies are polyclonal excludes that a single clone of autoantibodies is responsible for the pathogenesis in each patient. Utilization of multiple immunoglobulin variable region (V) genes excludes a preferred V-gene use as the underlying pathological mechanism. Immune targeting of multiple non-overlapping GM-CSF epitopes suggests that

autoantibody formation is driven by GM-CSF and not by a pathogen-related, cross-reacting epitope. Finally, the presence of numerous somatic mutations in GM-CSF autoantibodies suggests that T cells are involved and that the GM-CSF-specific memory B cells have re-entered germinal centres and undergone somatic mutation and affinity selection multiple times during development of the autoimmune response[110]. The polyclonal nature of GM-CSF autoantibodies may also be important to pathogenesis, as administration of a monoclonal anti-human CDw116 antibody or a monoclonal anti-GM-CSF antibody to humans as experimental therapy of rheumatoid arthritis did not result in the development of PAP[111] among hundreds of recipients, whereas administration of monoclonal GM-CSF autoantibodies derived from patients with PAP neutralized human GM-CSF signalling in a humanized mouse model but only when at least three non-cross-competing antibodies were co-injected[112].

Studies demonstrating that GM-CSF-dependent neutrophil functions are reduced in patients with autoimmune PAP and GM-CSF-deficient mice provide an explanation for the increased risk of infection in PAP[65]. Additional studies show that isolated monoclonal GM-CSF autoantibodies derived from patients with PAP block GM-CSF-stimulated expression of αM integrin (also known as CD11b) on neutrophils in whole blood[110], which is expected to alter cellular adhesion and recruitment. The observation of an inverse correlation between GM-CSF autoantibody concentration and neutrophil phagocytosis in healthy individuals suggests a physiological role in which GM-CSF autoantibodies might function to limit the pro-inflammatory effects of this cytokine[106]. This concept is supported by the observations that >99% of the GM-CSF present in healthy individuals and patients with PAP is bound to GM-CSF autoantibodies in vivo[106].

Pathogenesis of hereditary PAP. Several reports established hereditary PAP as a genetic disease caused by autosomal recessive mutations in *CSF2RA* or *CSF2RB*, which result in reduced protein expression on the cell surface[11–16,113]. In some cases, the mutated genes have been cloned and the defects reproduced in vitro, leading to detailed studies of signalling abnormalities and development of several novel biomarker-based diagnostic tests capable of identifying patients with defects in either receptor chain[12–14]. Importantly, variability in disease severity across family members with identical mutations suggests that other factors in addition to GM-CSF signalling may be important[13].

Pathogenesis of secondary PAP

Various medical conditions can cause secondary PAP[2,3]. Systemic disorders associated with secondary PAP include malignant and non-malignant haematological diseases, non-haematological malignancies, immune deficiency syndromes, chronic inflammatory syndromes and chronic infections. Chronic myeloid leukaemia and myelodysplastic syndromes are the most frequently reported haematological disorders associated with PAP syndome[2,19,22,24,27,28,30,114,115]. Immune deficiency syndromes associated with secondary PAP include thymic alymphoplasia (deficiency of lymphocytes in the thymus)[116], immunoglobulin A deficiency[76], immunosuppression following solid organ transplantation[117] and AIDS[23]. Secondary PAP has also been reported in lysinuric protein intolerance (a metabolic disorder caused by *SLC7A7* mutations resulting in an inability to digest the amino acids lysine, arginine and ornithine)[118,119] and in individuals with mutations in methionyl-tRNA synthetase (*MARS*)[120,121], although the underlying mechanisms have not been established.

Although the mechanisms responsible for secondary PAP are poorly studied, conditions reducing either the numbers or functions of alveolar macrophages would be expected to reduce the capacity of resident alveolar macrophages to clear surfactant from the lung surface, thereby promoting secondary PAP. A study in animals supports this concept, with evidence of increased surfactant pool size (that is, the content of pulmonary surfactant in the lungs of an individual) following reduction in alveolar macrophage numbers induced by chemical depletion[122].

Pathogenesis of congenital PAP

Mutations in *SFTPB, SFTPC* and *ABCA3* disrupt the production and function of surfactant and cause respiratory disease that can manifest in neonates, children and adults and is invariably associated with pulmonary fibrosis and varying levels of surfactant accumulation[48]. SP-B and SP-C are hydrophobic peptides that reside within the surfactant phospholipid layer[123]. ABCA3 is a membrane protein involved in lipid transport into lamellar bodies in type II alveolar epithelial cells, where the surfactant complex is assembled, processed and stored[124].

Infants homozygous for recessive loss-of-function mutations in *SFTPB* develop respiratory failure and die shortly after birth[33]. By contrast, individuals heterozygous for recessive loss-of-function *SFTPB* alleles have normal lung function[125]. Known autosomal dominant mutations in *SFTPC* are associated with interstitial lung disease (a group of disorders in which the interstitium is affected in various ways, including increased inflammation and/or fibrosis) in neonates, children and adults[36,37,126–128]. Infants homozygous for recessive loss-of-function mutations in *ABCA3* also develop fatal surfactant deficiency and die shortly after birth[39,129]. However, some *ABCA3* mutations produce dysfunctional ABCA3 in association with dysfunctional surfactant that is deficient in phosphatidylcholine, resulting in chronic respiratory disease in older children and adults[40,130]. These *ABCA3* mutations indicate an important role for ABCA3 in surfactant phospholipid homeostasis[131]. The transcription factor TTF1 is essential for lung development as well as expression of SP-B, SP-C and ABCA3. Haploinsufficiency of *NKX2-1* causes a complex phenotype in neonates that can include hypothyroidism, brain abnormalities and acute and chronic lung disease[132,133].

Murine knockout models of *Sftpb*, *Sftpc* and *Abca3* all develop lung diseases, which faithfully recapitulate their corresponding disease in humans and are histologically distinct from PAP in GM-CSF-deficient mice[123,124,134–137]. Additionally, mice with severe combined

immunodeficiency[138], pulmonary overexpression of IL-4 (REF.[139]) or IL-13 (REF.[140]) and pulmonary SP-D deficiency[141] also develop PAP; together, these animal models provide further insight into surfactant homeostasis and the related fibrotic processes.

Pulmonary fibrosis

Pulmonary fibrosis can occur in PAP-causing diseases but is, perhaps, the least well-studied manifestation of PAP and remains poorly understood in terms of frequency of occurrence, aetiology and pathogenesis. It occurs in a small but undefined proportion of patients with autoimmune PAP and may represent a manifestation of advanced-stage or end-stage lung disease[142] but has been proposed to be a treatment-related adverse effect of whole-lung lavage (WLL), GM-CSF augmentation or oxygen administration[138,139,143,144]. Interestingly, fibrosis has been reported in the lungs and liver of GM-CSF-deficient mice, suggesting it may be related to disruption of GM-CSF signalling rather that a treatment-related toxicity[145]. Pulmonary fibrosis occurs more commonly in surfactant production disorders caused by mutations in *ABCA3*, *SFTPB* and *SFTPC*[35–38,40].

Secondary infections

Infections are a fairly common complication of autoimmune PAP, can be the presenting manifestation at onset and account for 18–20% of the attributable mortality[2]. Multiple mechanisms underlie the predisposition to systemic and local infections in patients with PAP[146]. In primary autoimmune PAP, the presence of GM-CSF autoantibodies affects the terminal differentiation of alveolar macrophages and their acquisition of normal innate immune functions, including adhesion, phagocytosis, expression of pathogen recognition receptors, microbial killing and chemokine secretion[57]. Although neutrophil differentiation seems unaffected by disruption of GM-CSF signalling, basal functional capacity of circulating neutrophils, including phagocytosis, cellular adhesion, reactive oxygen species production and bactericidal activity, are impaired similarly in patients with autoimmune PAP and GM-CSF-deficient mice, presumably owing to the loss of GM-CSF-dependent, constitutive, in vivo priming[65].

In patients with secondary PAP, a reduction in the numbers or functions of alveolar macrophages increases the susceptibility to secondary infections, which occur frequently and substantially contribute to death[21,22,25,28].

Diagnosis, screening and prevention

Clinical presentation

The age of onset, symptoms and clinical manifestations at initial presentation depend on which PAP-causing disease is present and, for diseases with a genetic basis, on the zygosity and specific mutation. Furthermore, clinical severity also varies widely with the specific PAP-causing disease, ranging from asymptomatic disease in elderly patients with mild primary PAP to death within the first days of life in neonates with some surfactant production disorders associated with congenital PAP.

Most patients with autoimmune PAP present as adults in the third to fifth decade of life with exertional dyspnoea of insidious onset with or without nonspecific respiratory symptoms (cough and/or production of white frothy sputum) or systemic symptoms (fatigue and/or weight loss), often lasting many months before evaluation or initial diagnosis[4,147]. Fever and haemoptysis (coughing up blood) are less common and usually present only in the context of superimposed infection[9]. Importantly, in the large Japanese national PAP registry study, one-third of patients with autoimmune PAP were asymptomatic and were identified only through mandatory health screening programmes[9]. The physical examination is generally unremarkable, but crackles (clicking or rattling lung noises) and cyanosis have been reported in a small proportion of patients. Digital clubbing is not a manifestation of autoimmune PAP. Chest radiography typically demonstrates symmetrical infiltrates in the mid-lung and lower-lung fields, and chest CT typically reveals diffuse, ground-glass opacities with septal thickening and subpleural sparing. Patients are usually misdiagnosed with pneumonia on the basis of radiological findings or asthma (especially in children) on the basis of nonspecific symptoms until the failure to respond to 'appropriate' therapy (bronchodilator therapy for a diagnosis of asthma or multiple courses of antibiotics for a diagnosis of pneumonia) prompts reconsideration of the diagnosis; in such cases, an accurate diagnosis is delayed on average by 18 months[3,9,18,21,22,147,148].

Patients with hereditary PAP present similarly to patients with autoimmune PAP, except usually (but not always) in late infancy or childhood[13]. Patients with secondary PAP present in the context of the underlying environmental exposure or other underlying clinical condition[19,21,24,25,27–30,149]. Patients with congenital PAP present variably depending on the nature of the genetic defect[150,151].

Principles of diagnosis

A clinical history of slowly progressive dyspnoea with or without cough and fatigue together with the characteristic high-resolution CT findings should prompt the consideration of PAP (FIG. 2). The presence of PAP syndrome can be identified by the characteristic opaque, milky appearance of bronchoalveolar lavage (BAL) fluid or by histological evaluation of a lung biopsy sample[4]. However, these methods are unable to identify the specific PAP-causing disease, and biopsy samples — even from surgical lung biopsies — can have a substantial false-negative rate[148]. The demonstration of high levels of GM-CSF autoantibodies in serum is used to differentiate autoimmune PAP from other PAP-causing diseases (see below), which are indistinguishable on the basis of clinical presentation, radiology and histopathology. A detailed medical, occupational and environmental exposure history in every patient suspected of having PAP is essential to determine any possible causes of secondary PAP in the event of normal GM-CSF autoantibody levels and genetic testing.

Patients with PAP are at increased risk of secondary infection with common and opportunistic pathogens[2]. Such infections are a fairly common complication, occurring in ~13% of cases[2], and can also be present at disease onset. All PAP-causing diseases can be complicated by

systemic or pulmonary infections over time, and infections account for 18–20% of deaths related to PAP[2]. Data from the Japanese national PAP registry study have shown that patients with autoimmune PAP experienced an infection rate of 5%, owing mostly to respiratory infections with *Aspergillus* species[9]. Respiratory infections in PAP in European cohorts have been reported to occur in 11–16% of patients[147,152]. Infections can be pulmonary or extrapulmonary or both. Opportunistic pathogens often include *Nocardia*, *Mycobacterium* or fungal species; infections occur on average within 16 months of PAP diagnosis and are associated with a poor prognosis and increased mortality[153].

Although the presence of pulmonary fibrosis seems to be uncommon and may appear only in advanced or end-stage disease in patients with primary (autoimmune[142] or hereditary (B.C.T., unpublished observation)) PAP, congenital PAP is usually accompanied by marked parenchymal distortion, fibrosis and respiratory insufficiency[146,154]. Hence, an awareness of the possibility of concomitant fibrosis should be part of the routine follow-up of all patients with PAP. Fibrosis should be suspected when deteriorating pulmonary function test results indicate that a restrictive ventilatory pattern has developed or worsened. In this case, chest CT should be performed to exclude or identify fibrosis. Subpleural honeycombing and nonspecific interstitial pneumonia accompanied by minor traction bronchiectasis (irreversible dilatation of the bronchi caused by altered parenchymal structure) and emphysema (damage leading to destruction of alveoli) have all been described[142].

Radiological findings

Chest radiography typically shows diffuse bilateral symmetrical infiltrates in a perihilar distribution (that is, around the hila, the areas where the major blood vessels and bronchi enter the lungs)[155,156]. Chest CT should be performed and usually shows characteristic findings of PAP including ground-glass opacities with intralobular lines and interlobular septal thickening, often in polygonal shapes (FIG. 3), a pattern described as 'crazy paving'[156,157]. Occasionally, areas of consolidation with air bronchograms (areas of the lung in which surfactant-filled alveoli (with a dense white appearance) surround air-filled bronchial tubes (which have a black appearance)) are present in addition to the ground-glass opacification[156].

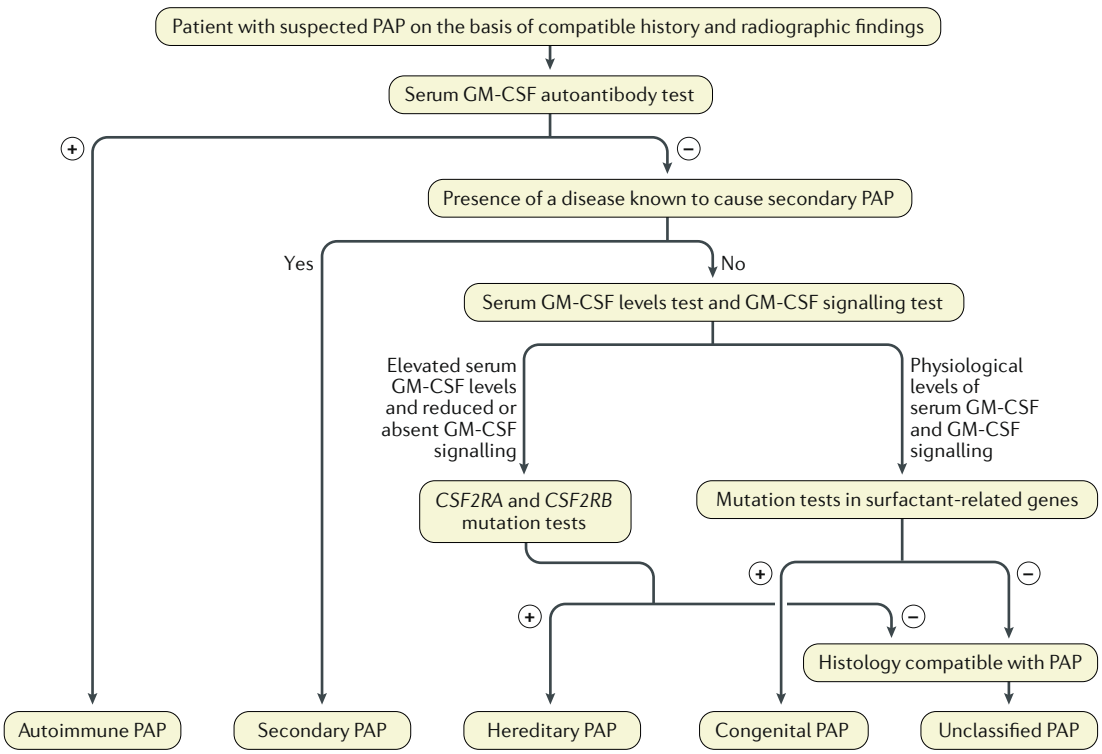


Fig. 2 | **Algorithm for the differential diagnosis of PAP.** The presence of pulmonary alveolar proteinosis (PAP) is suspected on the basis of a compatible history, typical radiological findings and bronchoalveolar lavage cytology findings. A granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibody test should be performed first: a positive test confirms the diagnosis of autoimmune PAP. Patients with a negative GM-CSF autoantibody test who have a disease known to cause PAP are diagnosed with secondary PAP. If an underlying causative condition cannot be found, patients should undergo a blood-based GM-CSF signalling test and serum GM-CSF levels test; high concentrations of serum GM-CSF and no or reduced GM-CSF signalling should prompt further tests for *CSF2RA* and *CSF2RB* mutations to identify hereditary PAP. Patients with physiological levels of serum GM-CSF and GM-CSF signalling should undergo further tests for other gene mutations to diagnose congenital PAP. If no PAP-causing mutation can be found, the patient is diagnosed with unclassified PAP and a transbronchial or surgical lung biopsy for lung parenchymal histopathological examination may be needed to confirm diagnosis. This diagnostic algorithm reflects an ideal setting in which physicians have unrestricted access to the appropriate diagnostic tools and tests.

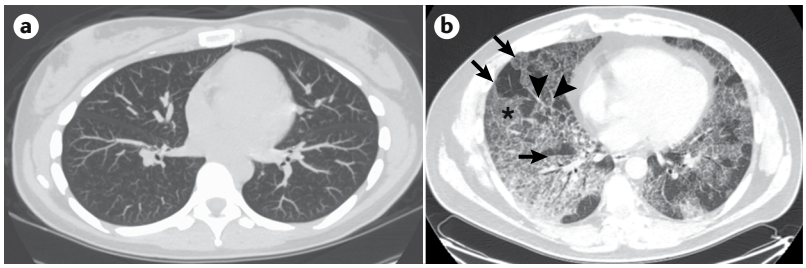


Fig. 3 | **Radiological findings in PAP.** CT scans of the chest in a healthy person (panel **a**) and a patient with autoimmune pulmonary alveolar proteinosis (PAP) (panel **b**). Note the ground-glass opacification (asterisk) and superimposed, angulated septal thickening (arrowheads), a pattern referred to as 'crazy paving', which is characteristic but not diagnostic of autoimmune PAP. Note also the geographical appearance (arrows), which arises from the juxtaposition of severely affected (lighter-appearing) secondary lobules adjacent to more-normal secondary lobules (darker-appearing).

This 'crazy paving' pattern is not specific for PAP and can be found in a variety of idiopathic, infectious, neoplastic and inhalational disorders of the lung[157]. Moreover, it is impossible to distinguish between PAP-causing diseases on the basis of high-resolution CT appearance[158].

Bronchoscopy and BAL

Bronchoscopy with BAL is a minimally invasive procedure that can be used to identify the presence of PAP (but not to identify the PAP-causing disease) in adults and children. The BAL fluid has a characteristic milky, opaque gross appearance and usually contains large amounts of sediment. The microscopic appearance typically comprises acellular globules that appear basophilic after May-Grünwald-Giemsa and periodic acid–Schiff (PAS) staining, alveolar macrophages that appear foamy and red after staining with oil-red-O and a substantial amount of cell debris that stains only weakly with PAS staining[2,9,159,160]. Fungal, mycobacterial and other infectious aetiologies should be ruled out by appropriate special stains and microbial cultures. Because highly specific and sensitive blood tests are now available for the accurate diagnosis of most adult patients (see below), such tests should be performed first, and bronchoscopy with BAL and/or transbronchial biopsy should be reserved for those cases in which the blood tests do not result in a disease-specific diagnosis.

Pulmonary physiology

Pulmonary function tests, outside of the diffusion capacity (a measure of the transfer of gas from alveolar air to blood), are of limited use in diagnosing or determining the severity of PAP. Spirometry (which measures airflow while breathing) is generally within normal limits, but some patients show decreased forced vital capacity (the total volume of air that a person exhales during a forced expiratory breath), consistent with restrictive physiology[2,161]. However, frequently the diffusion capacity of the lung for carbon monoxide (DLCO) is reduced and this reduction correlates with disease severity[9]. Oxygen desaturation and increased alveolar-arterial oxygen gradient ($A\text{-}aDO_2$), indicating reduced gas exchange in the alveoli, correlate better than DLCO with disease activity and are useful indicators of the need for treatment[2].

Laboratory tests

Most routine laboratory investigations are typically within physiological ranges in PAP, except in cases of secondary PAP due to haematological or immunodeficiency disorders. Serum lactate dehydrogenase levels are usually elevated and often correlate with $A\text{-}aDO_2$ (REF.[2]). Several biomarkers have been shown to variably correlate with disease severity, such as serum tumour antigens carcinoembryonic antigen[162], CYFRA 21-1 (cytokeratin 19 fragments)[147,163] and neuron-specific enolase (also known as γ-enolase)[164]; lung-epithelium-derived proteins (mucin 1 (MUC1; also known as KL6)[165], SP-A, SP-B and SP-D[166,167]); CC-chemokine ligand 2 (CCL2; also known as MCP1)[168]; and chitinase 3-like protein 1 (CHI3L1; also known as YKL40)[169]. None of these biomarkers is specific for or diagnostic of PAP or any of the PAP-causing diseases, and they remain in exploratory and research phases.

Serum GM-CSF autoantibody test. When PAP is suspected, a serum GM-CSF autoantibody test should be the first diagnostic test performed because it is highly accurate for diagnosis of autoimmune PAP[109] and because this disease comprises >90% of all patients with PAP from any cause[4,9,148]. Although GM-CSF autoantibodies are detectable at low concentrations (usually $<1\ \mu g\ ml^{-1}$) in serum from people without autoimmune PAP, including healthy people[106] and those with malignancies[170], inflammatory conditions[171] and secondary PAP[43], the serum GM-CSF autoantibody level is high ($>9\ \mu g\ ml^{-1}$) in patients with autoimmune PAP[3,65,109]. This diagnostic test has been standardized, thoroughly evaluated and reported to have a sensitivity and specificity of 100% for autoimmune PAP[109]. Receiver operating characteristic (ROC) curve analysis identified a test result of $5\ \mu g\ ml^{-1}$ as the critical threshold for diagnosis in individuals with autoimmune PAP[109]. This diagnostic test is now offered by an increasing number of laboratories, including centres in the United States, Japan, Germany and China; affiliated clinical centres in other countries (for example, Italy, Netherlands and others) offer this test through clinical research collaboration with testing centres. Current information about the availability of diagnostic testing for PAP is available from the PAP Foundation and EuPAPNet, the European network for PAP. It is important to note that measuring GM-CSF autoantibody concentration by enzyme-linked immunosorbent assay using a patient-derived polyclonal, neutralizing GM-CSF autoantibody as the reference standard[3] returns a value higher than that obtained when using a cloned, non-neutralizing, monoclonal GM-CSF antibody as the reference standard[9]. A method is available to convert results obtained in laboratories using these two different reference standards[109].

Serum GM-CSF concentration and blood-based GM-CSF signalling tests. In patients suspected of having PAP who have a normal serum GM-CSF autoantibody level and no underlying disease or condition known to cause secondary PAP, the next diagnostic tests performed should be measurement of serum GM-CSF concentration and/or GM-CSF signalling. Serum GM-CSF

concentration is elevated in patients with hereditary PAP caused by *CSF2RA* or *CSF2RB* mutations (owing to reduced clearance by dysfunctional receptors)[11–14,16,172]. Serum GM-CSF concentration is considered a screening test because GM-CSF can also be increased by serious infection in patients without hereditary PAP. The concentration of GM-CSF in serum is typically undetectable or below the lower limit of quantification (7 pg ml^{-1}) in healthy individuals and elevated (>10 pg ml^{-1}) in patients with hereditary PAP[13].

GM-CSF signalling can be measured by quantifying the increase in the level of intracellular phosphorylated STAT5 (REFS[12–14]) (with the STAT5 phosphorylation index test) or cell-surface CD11b[173] (with the CD11b stimulation index test) in neutrophils in response to incubation of heparinized whole blood with GM-CSF and flow cytometry. These tests are used to confirm GM-CSF receptor dysfunction in patients suspected of having hereditary PAP and to confirm the diagnosis of autoimmune PAP when the serum GM-CSF autoantibody is close to the critical threshold value (between 5 and 9 μg ml^{-1}).

Genetic testing for causes of congenital and secondary PAP. When the GM-CSF autoantibody test, serum GM-CSF concentration test and GM-CSF signalling tests are normal and no disease or condition known to cause secondary PAP is present, the next step in diagnostic work-up should be to screen for mutations in genes required for production of surfactant (for example, *SFTPB*[34,174,175], *SFTPC*[175–177], *ABCA3* (REFS[178,179]) or *NKX2-1* (REF.[180])) or genes associated with the development of secondary PAP (for example, *SLC7A7* and *MARS*[118–121]).

Unclassified PAP

In the small number of individuals in whom PAP is suspected but the aetiology remains uncertain, a careful occupational history should be obtained, infectious and/or inflammatory conditions should be considered and a transbronchial or surgical lung biopsy sample may be obtained to confirm the presence of PAP and/or further evaluate lung histopathology if PAP cannot be confirmed. The classical histological findings of PAP early in the disease course include alveoli with well-preserved wall architecture that are filled with PAS-positive, granular material; enlarged, foamy-appearing alveolar macrophages; and positive immunohistochemical staining for SP-A (which is usually detected only in small amounts in the absence of PAP)[3].

Screening and prevention

Universal screening for PAP is not routinely performed in either newborn babies or adults owing to low prevalence of PAP and the existence of a number of mechanistically distinct diseases associated with development of PAP. When PAP is suspected in a child, screening for mutations known to cause hereditary PAP or congenital PAP should be considered. Additionally, any paediatric or adult patient with ground-glass opacification observed on a chest CT scan and who has dyspnoea of insidious onset should have a GM-CSF autoantibody test to screen for autoimmune PAP, as this disease can present in individuals as young as 3 or as old as 90 years of age (B.C.T., unpublished data).

There are no proven disease prevention approaches for most PAP-causing diseases. Because cigarette smoking increases the risk of autoimmune PAP[2], its avoidance could theoretically reduce the risk. Similarly, avoidance of inhalation exposure to environmental toxins may reduce the risk of developing secondary PAP; use of protective equipment at all times while working with gases, fumes or other toxins should be encouraged. Moreover, once PAP is identified, prevention of complications, in particular the occurrence of secondary infections, should be of paramount importance as this can improve survival.

Disease course

In a comprehensive meta-analysis of 343 patients with any type of PAP-causing disease, the actuarial survival from the date of diagnosis was 78%, 75% and 68% at 2, 5 and 10 years; among the 69 deaths that occurred, 65 (94%) were attributable to PAP either directly owing to respiratory failure in 47 (72%) or indirectly owing to uncontrolled infection in 12 (18%) (including predominantly cerebral foci in 4) or cardiac arrest during WLL in 1 (1.5%)[2]. In autoimmune PAP, although longitudinal studies have not been performed, several large cross-sectional cohort studies have informed disease progression[2,9,147,152,181]. The clinical course of lung disease in autoimmune PAP follows one of three patterns: progressive deterioration, stable but unremitting disease and spontaneous resolution[3]. In the Japanese national PAP registry study, among 223 patients with autoimmune PAP, no deaths occurred over the 5-year period of the study[9]. Spontaneous improvement of PAP was noted in the initial description of PAP[1] and, in the large meta-analysis cited above, was noted to have occurred in 7.9% of 410 patients with any type of PAP[2]. In reports of patient cohorts in Japan, China, Germany and Italy, collectively comprising 735 patients, spontaneous resolution has been reported to occur in 5–7% of patients[9,147,152,181]. In hereditary PAP, first reported in 2008, the overall survival has not been estimated but the clinical course is similar to that of patients with autoimmune PAP (both are types of primary PAP caused by GM-CSF signalling deficiency)[11–14,16,172]. In patients with secondary PAP, the prognosis is markedly worse; 2-year survival has been reported to be 40%[21] and the median survival <20 months[22]. Again, although not formally studied prospectively, the poor survival in patients with secondary PAP seems to be linked more to the underlying disease leading to the development of PAP than to the manifestations of PAP itself[19,21,22,24,25,27,29,30]. The clinical course in patients with congenital PAP is highly dependent on the genetic mutation present and ranges from death in the first hour of life for patients with deficiency of SP-B or ABCA3 to insidious onset of pulmonary fibrosis presenting in childhood or adulthood in patients with mutations in *SFTPC*[48,150].

Management

Therapeutic management of PAP depends on the PAP-causing disease present and its severity and ranges from no treatment for patients with asymptomatic autoimmune PAP to nonspecific WLL therapy; pathogenesis-based

medicinal, gene or cell therapies for some diseases associated with primary or secondary PAP; and lung transplantation when progression leads to end-stage respiratory failure. The goals of management are to alleviate symptoms, improve oxygenation and improve quality of life.

Whole-lung lavage

The current standard of care in primary PAP and some causes of secondary PAP (but not congenital PAP) is WLL, an invasive procedure requiring general anaesthesia and endotracheal intubation of each lung in which one lung is mechanically ventilated while the other is repeatedly filled with saline and drained (up to 50 litres per lung), to physically remove the surfactant sediment[182–184]. WLL is typically administered when respiratory symptoms intensify and impair the quality of life, oxygen treatment is required or the DLCO declines, indicating worsening gas transfer[2,147,152,181,185]. Despite widespread use of WLL since its introduction in the 1960s and some procedural improvements, the procedure has not yet been standardized, remains highly operator-dependent and has not been proved successful in many of the more-rare PAP-causing diseases[32]. Questions remain regarding the volume of saline used, the use of mechanical percussion (physically tapping the chest wall to mobilize the surfactant sediment), the indications for WLL use, how to evaluate efficacy and the frequency of repeated procedures. Although there has not been any systematic evaluation of the clinical efficacy of WLL, it is widely considered by practitioners to improve symptoms, radiological abnormalities and oxygenation in patients[186–188]. In one report on 231 individuals, WLL was associated with an increase in overall 5-year survival and there was a marked improvement in arterial partial pressure of oxygen (PO_2) and A-aDO_2 (REF.[2]). The median duration of benefit, or time to next WLL, was 15 months, and the median number of WLL procedures was 2 per patient (note that this is the largest cohort described over 44 years of reported data)[2]. Most patients had a clinical response (defined as improvement in oxygenation) to WLL; however, ~5% of patients failed to respond[2].

Although WLL is generally a safe procedure, it is not without morbidity; known complications include hypoxia, pneumothorax (collapsed lung), hydrothorax (fluid in the pleural cavity), superimposed infection and acute respiratory distress syndrome. If there is evidence of concurrent active bacterial pneumonia, a WLL should not be performed, as this increases the risk of disseminated infection and sepsis. In children, if available double lumen tubes do not fit into the airways, special techniques are used for WLL[189]. Complications are infrequent but include transient hypoxia, hydrothorax, minor bleeding from airway injury, balloon rupture and pneumothorax[190]. In high-risk adult patients with multiple comorbidities or severe hypoxaemia, several procedures can be used to reduce the risk of intraoperative complications during WLL; these procedures include the use of extracorporeal membrane oxygenation or hyperbaric conditions[191,192] during WLL. Bronchoscopy with segmental and lobar BAL procedures have also been reported[181,193]; however, more data are needed before segmental lavage can be judged as a possible alternative to WLL[181].

Secondary infections

Although there seems to be a preponderance for opportunistic pathogens, in particular *Nocardia* spp., mycobacteria and fungi, a wide variety of microorganisms have been reported to cause both pulmonary and systemic infections[153]. Individual secondary infections should be treated appropriately. Experimental treatment of PAP-causing diseases with immunosuppressive drugs, including glucocorticoids, is of uncertain value and may increase the risk of infection[21,113].

Other therapies

All patients with PAP should be closely monitored with pulmonary function tests on a regular basis, and chest CT in the case of functional and clinical worsening, to detect interstitial lung abnormalities early. Although anti-fibrotic agents have emerged for use in idiopathic pulmonary fibrosis[194,195], their utility is unclear in other fibrotic lung diseases, including fibrosis associated with PAP. Hence, lung transplantation remains the only therapeutic option for fibrotic lung disease. Double lung transplantation has been used as a treatment option in several patients with a range of PAP-causing diseases who were unresponsive to WLL or other experimental therapies, resulting in severe functional impairment[196,197]. However, there have been reports of recurrence of PAP in autoimmune, hereditary and secondary PAP[197–199]. Moreover, solid organ transplantation is a reported cause of secondary PAP, and this includes cases of PAP following lung transplantation for another disease[29]. Lung transplantation has been used successfully as therapy for congenital PAP[200,201].

Emerging pathogenesis-based therapies

GM-CSF augmentation. The first use of GM-CSF as therapy of PAP was in 1996, in a single patient who received GM-CSF by subcutaneous administration, with marked improvement in symptoms and arterial oxygenation[202]. Follow-up studies in patients with autoimmune PAP receiving subcutaneous GM-CSF in escalating doses for 3 or 6–12 months resulted in overall response rates of 43% and 48%, respectively[167,203]. Several other case studies reported similar findings with objective improvements in ~50% of cases, with variable therapeutic responses among patients that seemed dependent on dose and treatment duration[204,205]. In one of the initial studies, 85% of patents reported local reactions at the site of subcutaneous GM-CSF injection[203], and despite mixed treatment responses, no studies of subcutaneous GM-CSF therapy have demonstrated a consistent change in the GM-CSF autoantibody level.

The most promising therapy to date has been the use of aerosolized GM-CSF in autoimmune PAP, as several small studies assessing a range of doses have shown positive results[206]. In the largest study to date, 35 patients with autoimmune PAP received aerosolized GM-CSF, with an initial high dose followed by a maintenance lower dose[207]. Importantly, only patients with unremitting or

progressive disease were included in the study, and 62% of patients demonstrated an improvement in the A-aDO_2 gradient, whereas serum GM-CSF autoantibody levels were unchanged[207]. After a 30-month follow-up observation period, 66% demonstrated a durable response to therapy requiring no further additional dose, and a low baseline vital capacity (the largest volume of air that can be expelled from the lung after a deep breath) may be a prognostic marker for disease recurrence and treatment response[208]. Most importantly, unlike the reactions to subcutaneously administered GM-CSF, no treatment-related adverse effects have been noted in any of the trials of inhaled GM-CSF[204–210]. Additionally, non-human toxicology studies have been conducted to better define the safety of inhaled GM-CSF therapy (B.C.T. and K.N., unpublished results). Currently, a multi-national phase III randomized, double-blind, placebo-controlled trial is ongoing assessing the efficacy and safety of inhaled recombinant GM-CSF (molgramostim), in which the primary outcome measure is a reduction in the A-aDO_2 gradient. This trial will be the first of its kind in autoimmune PAP, and with a planned recruitment of 135 patients it will be the largest study to date[211].

Therapy targeting GM-CSF autoantibodies. Since the discovery of the pathogenetic nature of GM-CSF autoantibodies, different therapeutic strategies aimed at reducing the level of the autoantibodies have been employed in autoimmune PAP[212]. Retrospective studies on the effects of corticosteroids suggest that these drugs are more harmful than beneficial in autoimmune PAP[213]. Other therapies include plasmapheresis to remove the autoantibodies and B lymphocyte depletion using rituximab (an anti-B cell monoclonal antibody)[214–217]. One open-label phase II study of rituximab evaluated the efficacy of a single cycle of therapy consisting of two infusions 15 days apart. In 7 of 9 patients there was a small improvement in A-aDO_2 gradient, but this improvement was minimal at the 6-month follow-up[214]. A retrospective report of 13 patients treated with rituximab demonstrated that no patient showed any improvement 6 months after treatment and concluded that the data were insufficient to support or rule out the use of rituximab as therapy for autoimmune PAP[218]. Further prospective studies are needed before any conclusions can be drawn about the potential utility of this theoretically attractive strategy.

Pulmonary macrophage transplantation. GM-CSF therapy is ineffective in hereditary PAP owing to GM-CSF receptor deficiency; thus, other therapeutic options are needed. Animal studies in *Csf2rb*$^{-/-}$ mice have demonstrated that bone marrow transplantation of congenic, wild-type[84] or gene-corrected *Csf2rb*$^{-/-}$ (REFS[82,83,219]) bone marrow can restore surfactant homeostasis and correct hereditary PAP in mice. Bone marrow transplantation has been attempted as therapy in children with hereditary PAP and has had limited success. However, in one case[11], the patient died of overwhelming infection before engraftment, and in another patient graft versus host disease complicated the successful engraftment of haematopoietic stem cells[220]. Importantly, bone marrow transplantation is limited by the substantial morbidity and mortality associated with myeloablation, and secondary PAP can itself be a rare complication of bone marrow transplantation[221,222]. Studies in mice have demonstrated the potential utility of an alternative cell transplantation approach, pulmonary macrophage transplantation, in which wild-type or gene-corrected bone-marrow-derived macrophages are administered directly to the lungs of *Csf2rb*$^{-/-}$ (REF.[85]) or *Csf2ra*$^{-/-}$ (REFS[82,83]) mice. In mice, this procedure is well tolerated, corrects PAP and reduces PAP-related mortality. Macrophages persisted for >1 year after transplantation and retained therapeutic efficacy throughout this period[85]. It is possible to generate genetically corrected macrophages from patient-derived induced pluripotent stem cells[223,224], and a clinical trial employing these techniques to conduct pulmonary macrophage transplantation in human patients with hereditary PAP is being planned.

Targeting pulmonary cholesterol homeostasis. GM-CSF signalling deficiency in PAP results in decreased cholesterol clearance from alveolar macrophages, and this impaired clearance is the primary macrophage defect driving the pathogenesis of PAP[10,61]. The foamy alveolar macrophages in patients with PAP have markedly increased cholesterol content, and there is an increase in the cholesterol:phospholipids ratio present in the BAL fluid in PAP caused by disruption of GM-CSF signalling in humans[10] and mice[61]. These results have led to the consideration of small-molecule therapy targeting cholesterol homeostasis as a novel approach in PAP. In GM-CSF signalling-deficient mice, oral PPARγ agonist therapy increased cholesterol clearance from macrophages and ameliorates PAP[61], and these findings have translated to a first-in-human trial of pioglitazone (a PPARγ agonist)[225]. In mice, oxysterols receptor LXRα agonists demonstrated therapeutic efficacy by increasing expression of cholesterol transporters; however, there are no clinically safe LXRα agonists available for humans[61]. Moreover, therapy with oral statin (which increases cholesterol clearance in macrophages) in *Csf2rb*$^{-/-}$ mice increases cholesterol efflux from alveolar macrophages, reduces cholesterol accumulation in alveolar macrophages and reduces PAP disease severity. In patients with autoimmune PAP, statin therapy was associated with significant resolution of PAP measured by improvements in quantitative CT densitometry (to quantify the abnormally accumulated surfactant), oxygenation and symptoms[10]. These reports support the possibility of statins and other drugs targeting cholesterol homeostasis as novel pathogenesis-based therapeutic approaches for all types of PAP (FIG. 4).

Quality of life

Few studies have addressed quality of life in patients with PAP, and the quality of life varies widely among patients depending on the PAP-causing disease present, clinical course and disease severity. Many factors are potentially relevant, including pulmonary symptoms as well as the need for medical interventions such as home oxygen therapy and WLL. The majority of patients are symptomatic with exertional dyspnoea and frequently cough[9]. Apart from secondary infections, comorbidities are not

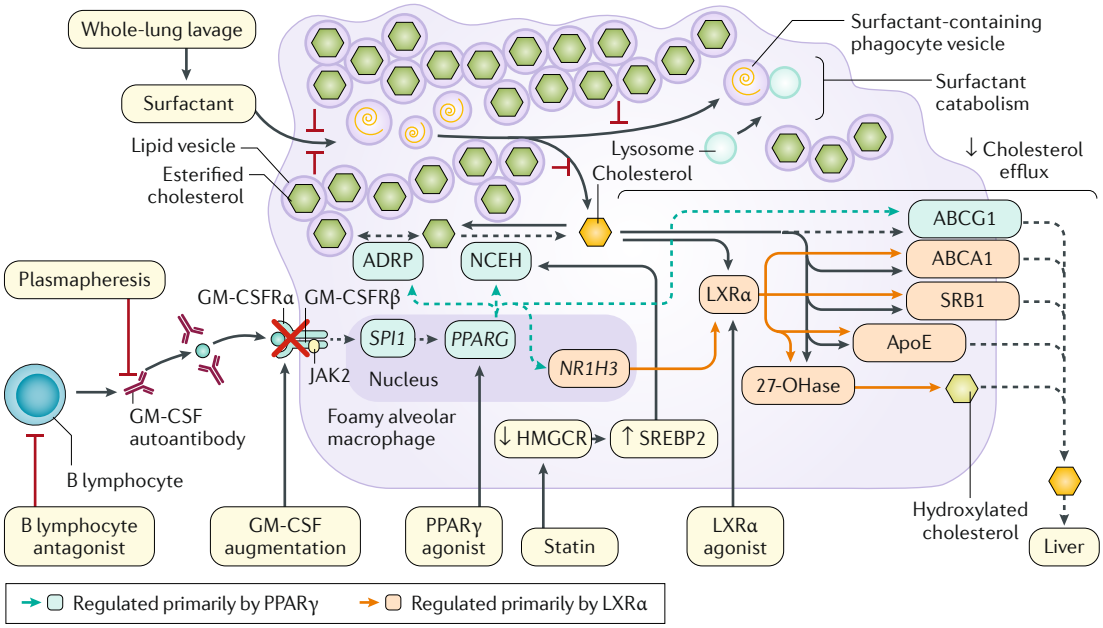


Fig. 4 | **Currently available and emerging therapies to treat patients with PAP.** Current therapy for pulmonary alveolar proteinosis (PAP) is focused on removing the excess surfactant by physically washing it out of alveoli by whole-lung lavage. Emerging, pathogenesis-based therapies target the abnormal production of granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibodies (plasmapheresis), the lymphocytes producing them (B lymphocyte antagonists) or the restoration of cholesterol homeostasis (peroxisome proliferator-activated receptor-γ (PPARγ) agonists, oxysterols receptor LXRα (encoded by *NR1H3*) agonists and statins). Approaches targeting restoration of the GM-CSF–PU.1 (encoded by *SPI1*)–PPARγ (encoded by *PPARG*) signalling axis are aimed at restoring multiple pathways that are impaired (dashed lines). 27-OHase, sterol 27-hydroxylase; ABCA1, ATP-binding cassette subfamily A member 1; ABCG1, ATP-binding cassette subfamily G member 1; ADRP, adipose differentiation-related protein; ApoE, apolipoprotein E; GM-CSFRα, GM-CSF receptor subunit-α (also known as CDw116); GM-CSFRβ, GM-CSF receptor common β-subunit (also known as CD131); HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; JAK2, tyrosine-protein kinase JAK2; NCEH, neutral cholesterol ester hydrolase; SRB1, scavenger receptor class B member 1; SREBP2, sterol regulatory element-binding protein 2.

very common, but patients with PAP have a significantly increased Charlson Comorbidity Index compared with the general population[8], and the most common comorbidities are depression, cardiovascular disease, diabetes mellitus and hypertension[8,147]. To determine disease severity and symptoms in an objective manner, a PAP Disease Severity Score (DSS) has been devised that is based on the presence of symptoms including dyspnoea and cough and the severity of hypoxaemia. The DSS ranges from 1 (least severe) to 5 (most severe), and in the initial descriptive study ~25% of patients were in the most severe categories (DSS 4–5)[9]. This score has been used in previous trials and is used by some centres to guide treatment.

The effects of subcutaneous GM-CSF therapy on quality of life, measured with the 36-item Short Form survey (SF-36), have been determined in an open-label trial[203]. The SF-36 is a well-validated patient survey with eight scaled scores examining factors including physical, emotional and social functioning, health perceptions, pain and mental health[226]. An improvement in all elements of the SF-36, except pain (which is rarely reported in PAP), was demonstrated after 6 months of GM-CSF augmentation therapy, and dyspnoea scores also improved[203]. In a separate trial of inhaled GM-CSF, treatment was associated with an improvement of >1 point in DSS, and this improvement was maintained long-term (30 months) for approximately two-thirds of patients[208]. These studies suggest that GM-CSF therapy does improve quality of life; however, the lack of placebo arms in these trials limits the conclusions that can be drawn from the studies.

WLL possibly improves quality of life measures as it is known to convey beneficial effects on oxygenation and pulmonary function[2]; however, no quality of life studies have been reported. Interestingly, spontaneous remission has been reported in PAP, and the majority of patients who achieve remission have undergone ≥1 WLL procedures[147]. The reported remission rate varies widely, as high as 70% in one series compared with much lower rates of ≤10% in others[2,147]. It remains unclear whether these reports represent true disease-free remission as opposed to periods of quiescent subclinical disease. In the future, the focus should be placed on research that examines quality of life in patients with PAP and the effect of emerging therapies. Survival and the incidence of spontaneous resolution should also be re-examined as therapies continue to evolve and improve for patients with PAP.

Outlook

Notwithstanding major advances in our understanding of PAP over the past several decades, a number of important questions remain unanswered and will serve to drive future research regarding aetiology, pathogenesis, current and emerging therapies and management.

Aetiology

Although the pathogenesis of autoimmune PAP has been elucidated, its aetiology (what induces the excessive production of GM-CSF autoantibodies) remains obscure. By contrast, secondary PAP is less well studied in terms of pathogenesis, but in some cases, the aetiology (for example, loss of alveolar macrophages in haematological disorders and after chemotherapy) seems clear. The extreme rarity of this disorder makes its study difficult. The aetiology of congenital PAP is also clear and continues to provide important information regarding surfactant production and processing.

Pathogenesis

The disease-specific mechanisms of the various conditions that cause secondary PAP remain largely unproved. Additionally, although the genes in which mutations result in the development of congenital PAP have been identified, the mechanisms by which these molecular defects lead to intense fibrosis and abnormal surfactant accumulation are poorly understood.

Emerging pharmacotherapeutic approaches

Clinical trials show that inhaled GM-CSF in patients with autoimmune PAP is safe and effective; however, the mechanism underlying this therapeutic effect has not been identified, nor it has been explained why administration of GM-CSF to these patients does not result in increased levels of GM-CSF autoantibodies[207]. Moreover, the optimal dose, timing or duration of administration have not been defined, and these findings could potentially improve the current response rate. Further research is needed to evaluate both inhaled GM-CSF and other therapies, including cholesterol-targeted therapy, plasmapheresis, anti-B lymphocyte therapy and combination therapies. Combination therapies include combining WLL with aerosolized GM-CSF (to reduce time to clearing) and combining plasmapheresis and anti-B lymphocyte therapy (to deplete GM-CSF autoantibodies)[227].

Clinical practice guidelines

Standardized clinical practice guidelines for PAP are needed. Objective outcome measures to determine treatment efficacy and disease severity are required, and consensus guidelines will probably be the best way to determine which measures to utilize. Similarly, WLL has been in use for ~50 years, but there have been no prospective studies to monitor the outcomes and evaluate them in relation to WLL indications, timing or methods. Furthermore, the procedure itself has not been standardized.

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