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## Pulmonary Alveolar Proteinosis

### A Bench-to-Bedside Story of Granulocyte-Macrophage Colony-Stimulating Factor Dysfunction

Sara R. Greenhill, MD; and Darrell N. Kotton, MD

**Pulmonary alveolar proteinosis (PAP) is a rare disorder characterized by ineffective clearance of surfactant by alveolar macrophages. Through recent studies with genetically altered mice, the etiology of this idiopathic disease is becoming clearer. Functional deficiency of granulocyte-macrophage colony-stimulating factor (GM-CSF) appears to contribute to disease pathogenesis because mutant mice deficient in GM-CSF or its receptor spontaneously develop PAP. Recent human studies further suggest a connection between PAP and defective GM-CSF activity because inactivating anti-GM-CSF autoantibodies are observed in all patients with idiopathic PAP, and additional rare cases of PAP in children have been accompanied by genetic defects in the  $\alpha$  chain of the GM-CSF receptor. In patients and mouse models of PAP, deficient GM-CSF activity appears to result in defective alveolar macrophages that are unable to maintain pulmonary surfactant homeostasis and display defective phagocytic and antigen-presenting capabilities. The most recent studies also suggest that neutrophil dysfunction additionally contributes to the increased susceptibility to lung infections seen in PAP. Because the phenotypic and immunologic abnormalities of PAP in mouse models can be corrected by GM-CSF reconstituting therapies, early clinical trials are underway utilizing administration of GM-CSF to potentially treat human PAP. The development of novel treatment approaches for PAP represents a dramatic illustration in pulmonary medicine of the “bench-to-bedside” process, in which basic scientists, translational researchers, and clinicians have joined together to rapidly take advantage of the unexpected observations frequently made in the modern molecular biology research laboratory.**

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**Abbreviations:** AM = alveolar macrophage; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; PAP = pulmonary alveolar proteinosis; PAS = periodic acid-Schiff

**P**ulmonary alveolar proteinosis (PAP) is an idiopathic lung disease resulting from the accumulation of surfactant within the alveolar spaces. Initially described by Rosen et al<sup>1</sup> in 1958, PAP is characterized by pulmonary infiltrates with varying degrees of hypoxemia. The disease is rare, with a prevalence of only 0.37 per 100,000 people and a median age at

diagnosis of 39 years.<sup>2</sup> Patients present most commonly with symptoms of dyspnea and fatigue, although 30% of cases may be asymptomatic.<sup>2</sup> In the years since Rosen's initial description, multiple clinical forms of PAP have been described according to presumed etiology, including the following: (1) secondary PAP resulting from conditions in which alveolar macrophage (AM) function is suppressed, such as hematologic malignancies, exposure to inorganic dusts (*eg*, silica), or pharmacologic impairment; and (2) idiopathic (primary) PAP, the most common form diagnosed in adults. This review summarizes the basic science of PAP and describes how the remarkable and serendipitous observations made in mouse models have led to a better understanding of this once mysterious disease in humans. This body of work, spanning 14 years and incorporating the full

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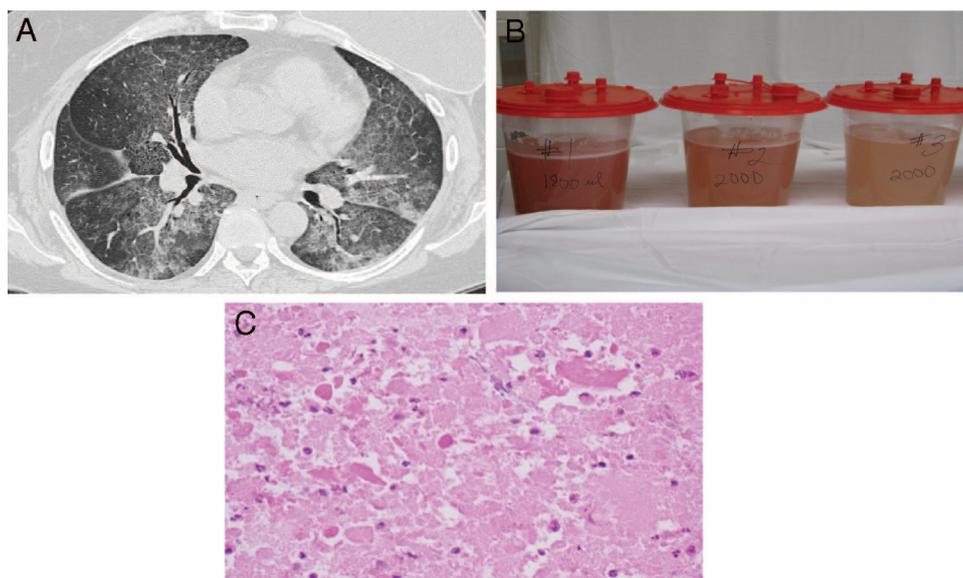


FIGURE 1. Radiologic and pathologic specimens from a patient with PAP. A representative CT scan of the chest in a patient with PAP, showing normal structural lung architecture with patchy areas of ground-glass opacities, consistent with alveolar filling (A). A right lung lavage of 22 L performed 2 days after a left lung lavage of 21 L in a patient with PAP, demonstrating the initial lavage return consisting of dark proteinaceous material. (B) Microscopic analysis of the lavaged fluid aspirated from the alveolar spaces demonstrates positively staining mucin (PAS, original  $\times 400$ ) consistent with the diagnosis of PAP (C).

spectrum of laboratory bench work and bedside clinical studies, has suggested that PAP arises from subtly altered pulmonary surfactant homeostasis due to the defective function of the AM and the growth factor necessary for its maturation, granulocyte-macrophage colony-stimulating factor (GM-CSF). Most intriguing is recent compelling evidence suggesting that idiopathic PAP may be an autoimmune disease involving autoantibodies against GM-CSF, and, as a consequence, may be treatable by GM-CSF augmentation therapy.

Thirty years after the initial description of PAP as being characterized by “alveolar proteinosis,” it was determined that the material accumulating within alveoli of these patients was surfactant.<sup>3</sup> The biochemical and cellular characteristics of the alveolar fluid obtained by BAL from PAP patients have now been well characterized. This fluid consists of elevated levels of surfactant lipids and proteins (including phospholipids and surfactant proteins A, B, C, and D), tubular myelin, membranous vesicles, and structures resembling lamellar bodies.<sup>4,5</sup> The cellular component of PAP BAL fluid consists predominantly of enlarged, foamy macrophages. In lung biopsy specimens (used to definitively make the diagnosis of PAP histopathologically), these foamy AMs can be observed in a distinctive background of eosinophilic, granular, periodic acid-Schiff (PAS) positive material filling alveoli.<sup>2,5</sup> The alveolar filling process is easily detected on

chest CT scans as patchy airspace disease, sometimes described as “crazy paving,” an appearance that is characteristic but not entirely specific for PAP<sup>2</sup> (Fig 1).

#### GENETICALLY ALTERED MICE REVEAL THAT GM-CSF IS CRITICAL FOR SURFACTANT HOMEOSTASIS

The first suggestion that GM-CSF may be central to PAP pathogenesis came from an unanticipated yet striking observation made by investigators originally interested in understanding the role of growth factors in hematopoiesis. Because *in vitro* studies<sup>6</sup> suggested a wide variety of hematologic effects depended on GM-CSF, Dranoff and colleagues<sup>7</sup> as well as Stanley et al<sup>8</sup> in the same year engineered a “knockout” mouse deficient in GM-CSF (with homozygous null GM-CSF alleles; hereafter GM<sup>-/-</sup>). GM-CSF is a hematopoietic growth factor known, *in vitro*, to stimulate differentiation, proliferation, and survival of myeloid cells, including monocytes, macrophages, eosinophils, neutrophils, and dendritic cells.<sup>7,8</sup> Surprisingly the genetically engineered GM<sup>-/-</sup> mice displayed a normal life span, normal bone marrow hematopoietic progenitors, and normal circulating numbers of RBCs and WBCs despite being unable to produce any GM-CSF protein. Unexpectedly, the predominant abnormality in

these mice, found as early as 3 weeks of age, appeared to be lung alveolar spaces filled with granular, eosinophilic, PAS-positive material, and large, foamy macrophages—lesions highly reminiscent of PAP in humans. Thus, these results demonstrated for the first time that GM-CSF is not an essential growth factor for basal hematopoiesis but is critical for pulmonary homeostasis.

Careful work<sup>7–9</sup> further confirmed that lack of GM-CSF resulted in a phenotype in mice that was virtually indistinguishable from human PAP. Analysis of BAL fluid from GM<sup>-/-</sup> mice via immunoblot, enzyme-linked immunosorbent assay, and electron microscopy all established the overabundance of surfactant lipid and proteins<sup>7</sup> and confirmed the presence of tubular myelin and multilamellated structures in the fluid filling alveoli, all of which are the defining characteristics of human PAP. The critical role of GM-CSF in lung tissue was further confirmed when additional genetically engineered mice lacking one chain of the GM-CSF receptor (GM R<sub>βc</sub><sup>-/-</sup>) had PAP develop.<sup>2,4,10</sup>

Follow-up studies next revealed that the altered surfactant homeostasis observed in GM<sup>-/-</sup> and GM R<sub>βc</sub><sup>-/-</sup> mice resulted from impaired surfactant catabolism by AMs, rather than from increased surfactant production by type II alveolar epithelial cells.<sup>11,12</sup> The levels of surfactant messenger RNA in the GM<sup>-/-</sup> mice were identical to the levels found in wild-type mice.<sup>7</sup> Furthermore, immunohistochemical analysis of pathologic sections of the GM<sup>-/-</sup> lung did not show increased amounts of surfactant proteins or their precursors in type II epithelial cells. However, the AMs, which were abnormal in appearance but present in normal numbers, were found to have a significantly increased amount of surfactant protein and lipids, and degradation of these elements was severely impaired.<sup>11,12</sup>

This consequence of GM-CSF deficiency in mice led investigators<sup>2</sup> to hypothesize that PAP in humans might also result from defective GM-CSF. Analysis of BAL and blood specimens from PAP patients, however, revealed normal levels of GM-CSF protein and no detectable defects in the gene encoding GM-CSF.<sup>2,5</sup> Based on these findings, it was initially suspected that no etiologic relationship existed between the phenotypically similar lung disease found in GM<sup>-/-</sup> mice and that in humans. A potential etiologic connection between the mouse model and human disease did not arrive for 5 more years, until Kitamura and colleagues<sup>13</sup> made the striking observation that 11 of 11 patients with idiopathic PAP appeared to have circulating autoantibodies to GM-CSF (Fig 2). This IgG antibody, detected in both the serum and BAL fluid of these subjects, was found neither in healthy control subjects nor those with

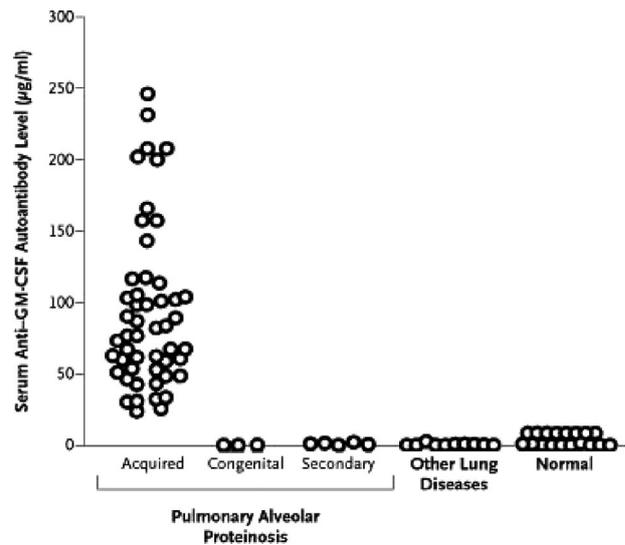


FIGURE 2. Presence of autoantibodies against GM-CSF within the BAL fluid and serum of patients with acquired PAP. No autoantibodies were present in persons with congenital or secondary PAP or other lung diseases or in normal persons. Each point represents an individual patient. Some of these data are from Kitamura et al.<sup>13</sup> Reproduced with permission from Trapnell et al<sup>2</sup> (Fig 5).<sup>4</sup>

secondary forms of the disease. The presence of anti-GM-CSF autoantibodies in patients with idiopathic PAP has since been confirmed in numerous studies.<sup>13–15</sup> Most importantly, these antibodies in lung tissue have been shown to bind to GM-CSF with high affinity and high specificity, reacting with its superstructure and neutralizing GM-CSF functional activity.<sup>14</sup> Thus, analogous to murine PAP models, it now appears that autoantibodies in humans reduce GM-CSF activity, resulting in AM dysfunction and surfactant accumulation.<sup>13,14</sup>

It should be noted that anti-GM-CSF antibodies are not 100% specific for PAP. Broad epidemiologic surveys<sup>5</sup> have now documented very low rates of GM-CSF antibodies in apparently healthy control subjects (4 of 1,258), although testing for GM-CSF neutralization was not performed. In addition, autoantibodies to GM-CSF have been reported to develop in 41 of 425 other patients surveyed, most of whom had myasthenia gravis. However, only three of these patients had GM-CSF neutralizing antibodies (two with myasthenia gravis and one with multiple sclerosis).<sup>5</sup> Taken together, accumulating literature supports the presence of neutralizing GM-CSF autoantibodies as the most specific biomarker of PAP yet described in humans.

In addition to the neutralizing autoantibodies, case reports<sup>16,17</sup> were recently published demonstrating the development of PAP in children who were found to have genetic mutations in the gene encoding the  $\alpha$

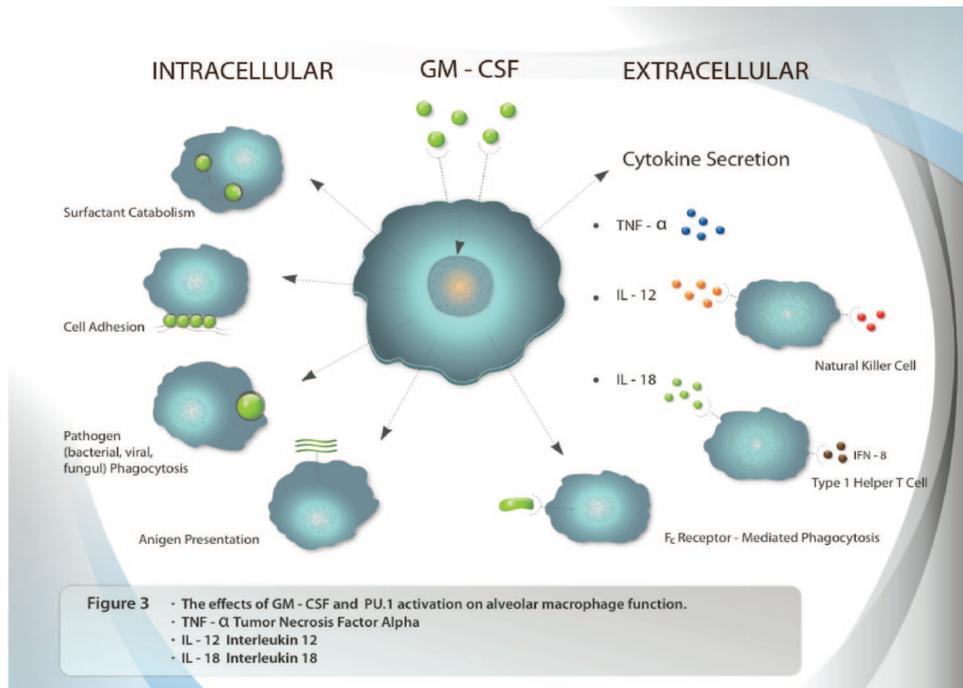


FIGURE 3. The effects of GM-CSF and PU.1 activation on AM function. TNF- $\alpha$  = tumor necrosis factor  $\alpha$ .

chain of the GM-CSF receptor located on the X chromosome. These cases displayed radiographic and histopathologic findings similar to the cases of idiopathic PAP described thus far in adults. These initial demonstrations in humans that GM-CSF receptor mutations may be accompanied by PAP further suggest that defective GM-CSF signaling is likely a key component of PAP pathogenesis.

#### AM AND NEUTROPHIL DYSFUNCTION IN PAP

The seminal discovery that GM-CSF deficiency results in defective surfactant catabolism by AMs has helped to quickly advance our understanding of AM biology. In addition to maintaining surfactant homeostasis, AMs play a central role in the host defense and innate immunity of the lung. Through release of interleukin (IL)-18 and IL-12, AMs also enhance type I helper T cells and natural killer cells, respectively, setting off a cascade of reactions resulting in stimulation of interferon- $\gamma$  and B cells, creating a link between innate and adaptive immunity. GM-CSF is now known to play a significant role in the terminal differentiation of AMs and their ability to provide adequate host defense, including adequate cytokine synthesis and phagocytic capacity.<sup>2</sup> Although GM-CSF also aids in granulocytic and monocytic cell growth, differentiation, and activa-

tion, it is clearly not essential for these other cell types *in vivo*, as evidenced by normal basal hematopoiesis in GM<sup>-/-</sup> and GM R<sub>bc</sub><sup>-/-</sup> mice.

To achieve maturation of AMs, GM-CSF activates the transcription factor PU.1 in the AM nucleus (Fig 3). PU.1 is a member of the *ets* family of transcription factors and has been shown to regulate myeloid and B-cell lineage development,<sup>18</sup> promoting proliferation and differentiation. Although PU.1 is expressed in multiple hematopoietic lineages, including hematopoietic stem cells,<sup>18</sup> in macrophages it appears to be present only in AMs. It is not found in other tissue macrophages,<sup>4,18</sup> suggesting a unique differentiation pathway in AMs and potentially explaining why macrophage dysfunction has not been found outside the lung in the setting of GM-CSF deficiency.<sup>19</sup>

*In vivo* studies demonstrate that GM-CSF stimulates PU.1 expression, and many lines of evidence support PU.1 as the transcriptional regulator mediating the effects of GM-CSF on AMs.<sup>2,18</sup> When analyzed *in vitro*, AMs from GM<sup>-/-</sup> mice show significant deficiencies in surfactant catabolism, cell adhesion, phagocytosis, bacterial killing, and inflammatory cytokine signaling.<sup>18</sup> These deficiencies are all completely corrected with retroviral-mediated expression of PU.1 in the AMs from GM<sup>-/-</sup> mice. Expression of PU.1 also corrected the the inability of

AMs to defend against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, and yeast *in vitro*.<sup>18</sup>

Although GM-/- mice were originally observed to exhibit normal basal hematopoiesis with predominant abnormalities in AM function, more recently neutrophil dysfunction has also been detected. This finding further explains the defective immune function and increased susceptibility to a wide variety of infections in mice or humans with PAP. *In vitro* studies<sup>20</sup> demonstrate an increased number of opsonized bacteria per neutrophil in the presence of GM-CSF as well as increased numbers of active neutrophils. GM-CSF increases the antimicrobial ability of neutrophils via priming, a mechanism that increases levels of the adhesion molecule, CD11b. *In vivo* studies<sup>21</sup> with human PAP patients as well as GM-/- mice confirm neutrophil dysfunction in the presence of GM-CSF autoantibodies or absence of GM-CSF, respectively. Neutrophils in PAP patients have normal structure and levels of PU.1 expression; however, anti-GM-CSF autoantibodies appear to cause reduced levels of CD11b cell surface expression, along with reduced oxidative burst and bactericidal capability.<sup>21</sup>

To further understand the subtleties of immune dysregulation in PAP, investigators have assessed a wide variety of cytokines and growth factors.<sup>22-25</sup> For example, in the absence of GM-CSF, elevated levels of macrophage colony-stimulating factor have been found. Increased macrophage colony-stimulating factor in turn leads to increased levels of matrix metalloproteinase-2 and metalloproteinase-9 in BAL fluid from PAP patients.<sup>22</sup> These metalloproteinases have been implicated in animal models of pulmonary fibrosis and asthma, although the lung parenchymal architecture of PAP patients and GM-/- mice appears to be normal.

Other biomarkers for disease activity in PAP, in addition to the levels of GM-CSF autoantibodies, include KL-6 and activin A. Case reports<sup>23</sup> have demonstrated elevated levels of KL-6, a mucin-like glycoprotein lining the intraalveolar epithelial surface of PAP patients. KL-6 has also been described as a potential serum marker for interstitial lung disease.<sup>23,24</sup> In PAP, elevated KL-6 serum levels have been suggested to decrease with improved radiographic changes.<sup>24</sup> RNA and protein analyses of BAL fluid cells from PAP patients have also shown decreased levels of activin A, a 28-kd cytokine and member of the transforming growth factor- $\beta$  family. Activin-A has been linked to B-cell proliferation and reduction of foam-cell formation.<sup>25</sup> *In vitro* studies<sup>25</sup> have suggested that Activin A levels may improve in response to GM-CSF augmentation or with decreased GM-CSF autoantibody levels.

## TREATMENT STRATEGIES DEVELOPED IN MOUSE MODELS OF PAP

If deficient GM-CSF activity is an etiology of PAP, treatments designed to reconstitute GM-CSF signaling in lung tissue should be able to ameliorate or reverse PAP. Initial proof-of-concept studies aimed at correcting GM-CSF deficiency in mouse models of PAP were achieved by generating a second line of genetically altered mice.<sup>26,27</sup> These transgenic mice had homozygous null GM-CSF alleles (GM-/-); however, production of GM-CSF was reconstituted in lung tissue using a GM-CSF complementary DNA transgene under control of a surfactant protein C promoter active specifically in lung epithelial cells.<sup>26,27</sup>

Other novel therapies aimed at correcting alveolar proteinosis in GM-/- mice employed gene therapy. Although the defect in GM-/- is a systemic deficiency of GM-CSF, the effects are seen only in the lungs. GM-/- mice were given intratracheal instillations of adenoviral vectors expressing GM-CSF and analyzed 1, 3, and 5 weeks after treatment.<sup>28</sup> Results from BAL fluid demonstrated the expression of the GM-CSF gene product 1 week after infection and decreased levels of surfactant protein B by 5 weeks, suggesting successful treatment of PAP using this localized gene therapy approach.<sup>28</sup>

Most importantly, localized drug delivery to the respiratory tract of GM-/- mice has been achieved using aerosolized recombinant mouse GM-CSF protein.<sup>29</sup> Again, decreased levels of surfactant lipids and proteins were observed. However, these changes were not sustained after cessation of GM-CSF therapy because surfactant levels returned to pretreatment levels after 5 weeks off therapy. Treatment with intraperitoneal or systemic injection of mouse GM-CSF did not reverse the PAP in the GM-/- mice.<sup>29</sup>

Even though localized GM-CSF therapy in the GM-/- mice was effective in reversing alveolar proteinosis, not surprisingly this treatment was ineffective in mice lacking the GM-CSF receptor (GM R<sub>βc</sub>-/- mice). However, reversal of PAP in these mice was achieved with bone marrow transplanted from normal donor mice.<sup>2,10</sup> In this case, the AMs derived from the normal donor marrow expressed normal GM-CSF receptors, thus demonstrating that correcting GM-CSF signaling in the hematopoietic compartment (and more specifically in AMs) rather than correcting GM-CSF signaling in the alveolar epithelium was sufficient to reverse the disease in mice.

## ADVANCES IN TREATMENT OF HUMAN DISEASE

Although GM-CSF therapy in GM-/- mice ameliorates PAP, it is not obvious whether GM-CSF therapy in humans would be effective because PAP

patients have autoantibodies to GM-CSF rather than abnormal protein levels. To date, whole-lung lavage remains the standard of care for patients with symptomatic PAP (Fig 1). It is not without risk, however, requiring hours of general anesthesia and intubation with a dual-lumen endotracheal tube, along with lavage of upward of 50 L of saline solution. Thus, during the past 8 years investigators have worked to apply the findings from PAP mouse models to develop a less invasive therapy for the human disease. Although the human and murine diseases are of differing etiologies, similar treatments using supplemental GM-CSF have shown promising results.<sup>30–35</sup>

There are currently three published, open-label studies using subcutaneous administration of GM-CSF for treatment of PAP.<sup>30–32</sup> All patients in each study had elevated levels of circulating GM-CSF autoantibodies prior to treatment. The initial safety study enrolled four patients to receive escalating doses of GM-CSF.<sup>30</sup> Three of the four experienced symptomatic and radiographic improvement, with once-daily self-injection of 3 to 9  $\mu\text{g}/\text{kg}/\text{d}$  (dose escalation depending on response) for 12 weeks.<sup>30</sup> Response was based on symptom score, arterial blood gas, pulmonary function tests, and radiographs. There was no increase in the peripheral WBC count, as was expected. A second study evaluated 14 patients administered daily subcutaneous GM-CSF in doses of 5  $\mu\text{g}/\text{kg}/\text{d}$  for 6 to 12 weeks, with dose escalation if there was a lack of response.<sup>31</sup> Five of 14 patients (35%) responded, with responses lasting a median of 39 weeks, and responses were reproducible on retreatment.<sup>31</sup> The only finding predictive of response was treatment-related eosinophilia. The third and most recent study, a follow-up to the initial trial of four patients, showed an improvement in the same evaluated parameters in 12 of 25 patients (48%) after treatment with subcutaneous GM-CSF daily for 3 to 12 months, at a starting dose of 250  $\mu\text{g}/\text{d}$  with gradual increases based on clinical response.<sup>32</sup> In this latest trial,<sup>32</sup> the level of serum GM-CSF autoantibody correlated with disease activity and could be used as an inverse correlate of treatment response. Nonresponders had higher baseline titers of GM-CSF autoantibody.<sup>33</sup> Additional case reports of GM-CSF therapy describe twice-daily inhaled or aerosolized GM-CSF to treat adolescents with PAP.<sup>34,35</sup> In one report after 1 year on therapy, a 13-year-old girl with purportedly idiopathic PAP exhibited improved symptoms, pulmonary function test results, growth parameters, and decreased autoantibody titers.<sup>34</sup> Her improvement was maintained for at least 3 months off therapy.<sup>34</sup> A second report of two adolescents treated for 9 months with aerosolized GM-CSF twice daily for 2

weeks of each month demonstrated mixed results.<sup>35</sup> One patient exhibited almost immediate symptomatic response to therapy; the second case responded more slowly, requiring a whole lung lavage during the 9 months of treatment. At the end of the course of treatment, both patients showed an increase in FEV<sub>1</sub> and a decrease in ground-glass opacities on CT scan imaging.

Although these trials of GM-CSF augmentation are compelling, the efficacy of this treatment approach will remain unproven until randomized, controlled trials have been conducted. However, these innovative potential therapies may help to reverse the putative inactivating effects of anti-GM-CSF autoantibodies and offer less invasive treatment options aimed at ameliorating PAP and improving endogenous AM function. To date, it remains to be seen if therapies with GM-CSF targeted directly to the lungs, such as aerosolized therapy similar to that employed in the GM-/- mouse, will be more beneficial than systemic therapy. Perhaps most important of all, the etiology of the development of the autoantibodies observed in PAP patients remains a mystery, and whether plasmapheresis to remove these antibodies can reverse PAP remains an intriguing, yet untested, treatment approach.

#### SUMMARY

Although rare, PAP can cause significant morbidity and mortality. In addition to dyspnea, hypoxemia, and restrictive lung disease, patients experience a susceptibility to a wide array of life-threatening infections. The GM-CSF knockout mouse (GM-/-) has lead serendipitously to an animal model of PAP and has greatly advanced our understanding of normal surfactant homeostasis as well as PAP disease pathogenesis. A better understanding of AM biology as well as the immunologic defects associated with a complete or relative absence of GM-CSF has also emerged. The development of novel treatment approaches for PAP based on GM-CSF augmentation represents a dramatic illustration in pulmonary medicine of the bench-to-bedside process, where basic scientists, translational researchers, and clinicians have joined together to rapidly take advantage of the frequently unexpected observations made during the course of carrying out the scientific method in the modern molecular biology research laboratory.

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## REFERENCES

- 1 Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. *N Engl J Med* 1958; 258:1123–1142
- 2 Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med* 2003; 349:2527–2539
- 3 Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. *Am J Respir Crit Care Med* 2002; 166:215–235
- 4 Trapnell BC, Whitsett JA. GM-CSF regulates pulmonary surfactant homeostasis and alveolar macrophage-mediated innate host defense. *Annu Rev Physiol* 2002; 64:775–802
- 5 Mazzone PJ, Thomassen MJ, Kavuru MS. Pulmonary alveolar proteinosis: recent advances. *Semin Respir Crit Care Med* 2002; 23:115–126
- 6 Gough NM, Metcalf D, Gough J, et al. Structure and expression of the mRNA for murine granulocyte-macrophage colony stimulating factor. *EMBO J* 1985; 4:645–653
- 7 Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* 1994; 264:713–716
- 8 Stanley E, Lieschke GJ, Grail D, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci U S A* 1994; 91:5592–5596
- 9 Dranoff G, Mulligan RC. Activities of granulocyte-macrophage colony-stimulating factor revealed by gene transfer and gene knockout studies [abstract]. *Stem Cells* 1994; 12(suppl):173
- 10 Robb L, Drinkwater CC, Metcalf D, et al. Hematopoietic and lung abnormalities in mice with a null mutation of the common  $\beta$  subunit of the receptors for granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5. *Proc Natl Acad Sci U S A* 1995; 92:9565–9569
- 11 Yoshida M, Ikegami M, Reed JA, et al. GM-CSF regulates protein and lipid catabolism by alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2001; 280:L379–L386
- 12 Ikegami M, Jobe AH, Huffman Reed JA, et al. Surfactant metabolic consequences of overexpression of GM-CSF in the epithelium of GM-CSF-deficient mice. *Am J Physiol* 1997; 273:L709–L714
- 13 Kitamura T, Tanaka N, Watanabe J, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1999; 190:875–880
- 14 Uchida K, Nakata K, Trapnell BC, et al. High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis. *Blood* 2004; 103:1089–1098
- 15 Bonfield TL, Russell D, Burgess S. Autoantibodies against granulocyte macrophage colony-stimulating factor are diagnostic for pulmonary alveolar proteinosis. *Am J Respir Cell Mol Biol* 2002; 27:481–486
- 16 Suzuki T, Sakagami T, Rubin BK, et al. Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. *J Exp Med* 2008; 205:2703–2710
- 17 Martinez-Moczygemba M, Doan ML, Elidemir O. Pulmonary alveolar proteinosis caused by deletion of the GM-CSFR $\alpha$  gene in the X chromosome pseudoautosomal region 1. *J Exp Med* 2008; 205:2711–2716
- 18 Shibata Y, Berclaz PY, Chroneos ZC, et al. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity* 2001; 15:557–567
- 19 Nakata K, Kanazawa H, Watanabe M. Why does the autoantibody against granulocyte-macrophage colony-stimulating factor cause lesions only in the lung? [abstract]. *Respirology* 2006; 11(suppl):S65–S69
- 20 Fleischmann J, Golde DW, Weisbart RH, et al. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by neutrophils. *Blood* 1986; 68:708–711
- 21 Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med* 2007; 356:567–579
- 22 Bonfield TL, Swaisgood CM, Barna BP, et al. Elevated gelatinase activity in pulmonary alveolar proteinosis: role of macrophage-colony stimulating factor. *J Leuk Biol* 2006; 79:133–139
- 23 Kobayashi M, Takeuchi T, Ohtsuki Y. Differences in the immunolocalization of surfactant protein (SP)-A, SP-D, and KL-6 in pulmonary alveolar proteinosis. *Pathol Int* 2008; 58:203–207
- 24 Nara M, Sano K, Ogawa H, et al. Serum antibody against granulocyte-macrophage colony-stimulating factor and KL-6 in idiopathic pulmonary alveolar proteinosis [abstract]. *Tohoku J Exp Med* 2006; 208:349
- 25 Bonfield TL, Barna BP, John N, et al. Suppression of activin A in autoimmune lung disease associated with anti-GM-CSF. *J Autoimmun* 2006; 26:37–41
- 26 Huffman JA, Hull WM, Dranoff G, et al. Pulmonary epithelial cell expression of GM-CSF corrects the alveolar proteinosis in GM-CSF-deficient mice. *J Clin Invest* 1996; 97:649–655
- 27 Huffman Reed JA, Rice WR, Zsengeller ZK, et al. GM-CSF enhances lung growth and causes alveolar type II epithelial cell hyperplasia in transgenic mice. *Am J Physiol* 1997; 273:L715–L725
- 28 Zsengeller ZK, Reed JA, Bachurski CJ, et al. Adenovirus-mediated granulocyte-macrophage colony-stimulating factor improves lung pathology of pulmonary alveolar proteinosis in granulocyte-macrophage colony-stimulating factor-deficient mice. *Hum Gene Ther* 1998; 9:2101–2109
- 29 Reed JA, Ikegami M, Cianciolo ER, et al. Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 1999; 276:556–563
- 30 Kavuru MS, Sullivan EJ, Piccin R, et al. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med* 2000; 161:1143–1148
- 31 Seymour JF, Presneill JJ, Schoch OD, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. *Am J Respir Crit Care Med* 2001; 163:524–531
- 32 Venkateshiah SB, Yan TD, Bonfield TL, et al. An open-label trial of granulocyte-macrophage colony-stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. *Chest* 2006; 130:227–237
- 33 Bonfield TL, Kavuru MS, Thomassen MJ. Anti-GM-CSF titer predicts response to GM-CSF therapy in pulmonary alveolar proteinosis. *Clin Immunol* 2002; 105:342–350
- 34 Price A, Manson D, Cutz E, et al. Pulmonary alveolar proteinosis associated with anti-GM-CSF antibodies in a child: successful treatment with inhaled GM-CSF. *Pediatr Pulmonol* 2006; 41:367–370
- 35 Robinson TE, Trapnell BC, Goris ML, et al. Quantitative analysis of longitudinal response to aerosolized granulocyte-macrophage colony-stimulating factor in two adolescents with autoimmune pulmonary alveolar proteinosis. *Chest* 2009; 135:842–848

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