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Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis

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ABSTRACT: Recently, granulocyte-macrophage colony-stimulating factor (GM-CSF) auto-antibodies have been found in many patients with pulmonary alveolar proteinosis (PAP). The present study reports a retrospective case series of patients who used aerosolised GM-CSF in the treatment of idiopathic PAP. Between 1999 and 2003, 12 patients elected to receive aerosolised GM-CSF (250 μg *b.i.d.* every other week) in lieu of whole-lung lavage or observation.

Patient characteristics, pulmonary function tests, arterial blood gas analysis, laboratory values and chest radiographs were extracted from the patient's medical records. Of the six patients tested, all had GM-CSF neutralising antibodies. Additionally, abnormalities in GM-CSF gene expression (one patient), receptor expression (two patients) and ability to upregulate adhesion molecules (one patient) were found.

All patients except one had a positive response (mean improvements in arterial oxygen tension, alveolar–arterial oxygen gradient, carbon monoxide diffusing capacity of the lung and forced vital capacity were 17.1 mmHg, 18.4 mmHg, 16.6% pred and 13.5% pred, respectively). Two patients made a complete recovery and were disease free 1 and 2 yrs after discontinuing treatment. Four patients showed complete response to both the initial course or when treated again for recurrence after discontinuation of treatment. One patient required dose escalation (500 μg *b.i.d.*) with complete response. GM-CSF was well tolerated without late toxicity after median (range) follow-up of 30.5 (3–68) months.

In conclusion, aerosolised granulocyte-macrophage colony-stimulating factor is safe and effective in treating pulmonary alveolar proteinosis providing an alternative to whole-lung lavage or subcutaneous granulocyte-macrophage colony-stimulating factor.

KEYWORDS: Granulocyte-macrophage colony-stimulating factor, pulmonary alveolar proteinosis, surfactant

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Pulmonary alveolar proteinosis (PAP) was first described by ROSEN *et al*. [1] as a rare interstitial lung disease characterised by an excess accumulation of lipoproteinaceous-rich materials within the alveoli. Congenital PAP may occur as a mutation in surfactant protein (SP)-B or SP-C genes [2, 3], or the β-chain gene of the receptor for granulocyte-macrophage colony-stimulating factor (GM-CSF) [4, 5], as well as mutations of the SLC7A7 gene resulting in lysinuric protein intolerance [6]. Although PAP may develop in association with haematological malignancies, dust exposure (*e.g.* silica) or immunodeficiency disorders [2, 7, 8], >90% of all reported cases of PAP have previously been considered ''idiopathic PAP'' [2].

Recently, abnormal GM-CSF activity has been considered pathogenic in idiopathic PAP [3]. Briefly, two groups [9, 10] independently demonstrated PAP pulmonary pathology in mice with a null GM-CSF allele. Moreover, the PAP pathology was ameliorated by GM-CSF replacement using either overexpression of GM-CSF gene coupled to a lung-specific promoter [11, 12], adenovirus-mediated expression of GM-CSF administered intratracheally [13], or aerosolised GM-CSF [14]. These findings taken together suggested that the influence of GM-CSF on the development of PAP was a localised pulmonary phenomenon due to lack of GM-CSF activity within the alveolus, resulting in alveolar macrophage dysfunction and surfactant protein accumulation. In humans, reduced GM-CSF activity has been recently reported due to the presence of a neutralising auto-antibody against GM-CSF protein [15–18].

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Subsequently, limited clinical studies have noted variable improvement in patients with PAP following subcutaneous administration of GM-CSF [19–22]. As the safety and efficacy of aerosolised GM-CSF in the treatment of lung metastases has previously been reported [23], this treatment is now being extended to idiopathic PAP and a response rate potentially greater than when GM-CSF is systemically administration is noted.

METHODS

The present authors identified 12 patients with idiopathic PAP who were treated with aerosolised GM-CSF at the Mayo Clinic (Rochester, MN, USA) between January 1, 1999 and December 31, 2003. The retrospective medical record review of these patients was approved by the Institutional Review Board. All patients had pathological tissue reviewed (five transbronchial biopsy and seven open lung biopsy) by Mayo Clinic pathologists. The following data were abstracted from clinical records: age, sex, smoking history, vital status, physical examination, pathology reports for lung biopsies, prior whole lung lavage, side effects of treatment, and follow-up information. Typically, the following tests were carried out at initial evaluation and at every follow-up visit (usually every 3 months): 1) chest radiographs and/or computed tomography (CT) scans; 2) spirometry and diffusion capacity; 3) rest and exercise arterial blood gases; 4) serum lactate dehydrogenase (LDH); 5) liver enzymes; and 6) complete blood count. Chest radiographs were reviewed by two of the authors during treatment and graded as completely normal, moderately improved, slightly improved, no change, slightly worse and moderately worse.

Pulmonary function variables were expressed as percentage predicted and included: total lung capacity using plethysmography; forced vital capacity (FVC); forced expiratory volume in one second (FEV1); carbon monoxide diffusing capacity of the lung ($D_{L,CO}$); and alveolar volume (VA). The carbon monoxide single-breath method (1995 American Thoracic Society recommendations) was used. Test-to-test reproducibility of FVC was 250±2 mL and of $D_{L,CO}$ was 3.2 mL·s·mmHg^{-1}. Reproducibility in partial pressure of oxygen (P_{O_2}) measurement was assessed with quality control testing administered by the College of American Pathologists (Northfield, IL, USA) in compliance with the Clinical Laboratories Improvement Act.

GM-CSF gene transcription, GM-CSF receptor expression, GM-CSF function was determined in 10 patients. In six patients neutralising anti-GM-CSF antibody was determined.

GM-CSF gene transcription

Peripheral blood mononuclear cells (PBMC) were obtained from PAP patients or normal controls in a Ficoll-Hypaque gradient. Lymphocytes were purified by depletion of monocytes by overnight plastic adherence in tissue culture flasks and stimulated or not with 100 μg·mL^{-1} of lipopolysaccharide (LPS), a known inducer of GM-CSF gene transcription, for 2 h. Total RNA was extracted by the RNAzol method and complementary DNA (cDNA) produced from 10 μg of RNA by standard procedures. RT-PCR was performed with the cDNA, specific GM-CSF primers (Stratagene, La Jolle, CA, USA), dNTP, $MgCl_2$ and Taq-polymerase under the following conditions: 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 60°C for 1 min, 72°C for 1 min and then 72°C for 7 min. The amount of GM-CSF amplification was compared between patients with PAP and normal controls, as well as between basal and LPS-induced in patients with PAP (fig. 1). As a control for equal cDNA loading in each reaction, RT-PCR was performed with specific primers for β-actin.

GM-CSF receptor expression

To investigate the surface expression of the GM-CSF receptor (GM-CSFR) in the PBMC of patients with PAP, specific antibodies that recognise either the receptor α-chain (CD116; Immunotech, Fullerton, CA, USA) or the β-chain (CDw131; Pharmingen, San Diego, CA, USA) were used by flow cytometry in a fluorescence activated cell sorter. The expression of the individual subunits of the GM-CSFR was detected with a phycoerythrin-labelled rabbit anti-mouse antibody and compared with normal human controls.

GM-CSF regulated adhesion molecules

To determine whether patients had functional expression of GM-CSF-regulated adhesion molecules/receptors, leukocyte function associated antigen-1 (LFA-1; CD18; BD Biosciences, CD11a; Immunotech), FcγRI (CD64; Immunotech) and intercellular adhesion molecules (CD54; Immunotech) were studied by flow cytometry in PBMC of PAP patients and normal human controls. As a negative control, isotype-matched conjugated immunoglobulin-G1 was used (BD Biosciences).

Neutralising anti-GM-CSF antibody

To determine whether PAP is an autoimmune disease, the presence of neutralising anti-GM-CSF antibody was studied in the sera of patients with PAP before aerosol GM-CSF treatment. Samples were stored at -80°C and sent to K. Nakata (Kanagawa, Japan) for analysis by ELISA, as described previously [15].

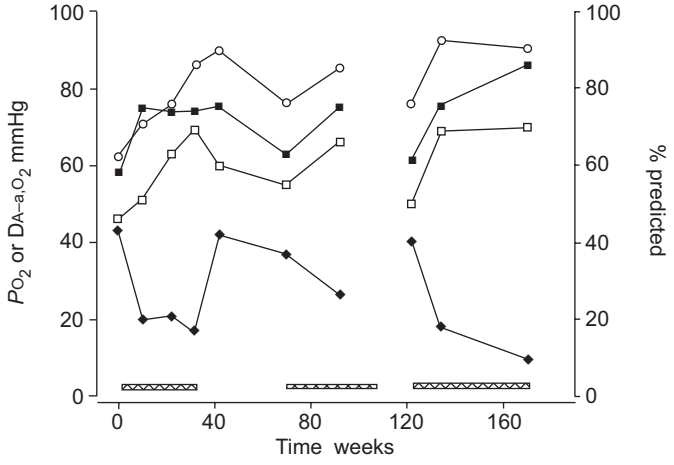

FIGURE 1. Time course showing onset of improvement and relapse during, and following, discontinuation of aerosolised granulocyte-macrophage colony-stimulating factor (GM-CSF) for pulmonary alveolar proteinosis in one patient. P_{O_2}: partial pressure of oxygen; D_{A-a,O_2}: alveolar–arterial oxygen-tension gradient. ■: P_{O_2}; ♦: D_{A-a,O_2}; □: carbon monoxide diffusing capacity of the lung; ○: forced vital capacity; ▨: aerosolised GM-CSF.

Treatment

All patients were treated by one of the authors. The first patient refused whole-lung lavage and was then informed of the possible pathogenic role of GM-CSF in the development of idiopathic PAP including, at that time, the only therapeutic report of subcutaneously administered GM-CSF in PAP [19], as well as previous limited experience with aerosolised yeast-derived recombinant GM-CSF in metastatic lung cancer [23]. Following successful use in this patient [24], subsequent patients self-determined to either undergo whole-lung lavage observation, or aerosolised GM-CSF. Those that chose the latter form the basis of the present study. None of these patients had severe hypoxaemia, evidence of secondary PAP, or severe dyspnoea, which would have influenced a clinical decision to urgently perform whole-lung lavage. GM-CSF (Leukine; Immunex, Seattle, WA, USA) was administered at a dose of 250 μg *b.i.d.* every other week. GM-CSF was diluted with 2.0 mL of bronchosaline and administered using a Pari LC Plus nebuliser (PARI Respiratory Equipment, Midlothian, VA, USA) set with an interrupter valve. Dose escalation (maximum of 500 μg *b.i.d.* every other week) was used if no response was noted to the initial dose after at least 12 weeks of treatment. As the binding of GM-CSF to its receptor on normal haematopoietic cells resulted in a down-modulation of both G-CSF and M-CSF receptors on normal cells [25], and to minimise potential toxicity an intermittent 1 week on/1 week off schedule was chosen which had previously been deemed to be safe [23]. Note that systemically administered doses of recombinant-human granulocyte-CSF >100 $\mu g \cdot kg \cdot day^{-1}$ have been given without dose-limiting toxicity (Amgen, data on file). A positive response was considered an improvement in one or more objective parameters including arterial oxygen tension (P_{a,O_2}), alveolar–arterial oxygen gradient (D_{A-a,O_2}), $D_{L,CO}$ or FVC (an increase of 10 mmHg, 12 mmHg, 12% predicted or 7% pred, respectively). These variables and criterions are similar to data from a previous study using subcutaneously administered GM-CSF [20]. Relapse after discontinuation of treatment was noted and data at time of relapse and best response to resuming treatment was collected.

Statistical methods

Pre- and post-treatment data were analysed with paired t-tests. Results are presented as two-sided comparisons, with a p-value of <0.05 regarded as significant. All data are expressed as mean±SD. In some studies linear regression analysis was performed using Origin 7.0 (OriginLab, Northampton, MA, USA).

RESULTS

Patient characteristics

Medical records and studies from 12 patients treated with aerosolised GM-CSF were reviewed. In all patients the diagnosis of idiopathic PAP was established by biopsy (open surgical lung biopsies, n=7; transbronchial lung biopsies, n=5; table 1). All diagnoses were new, except two patients. Specifically, in those cases the patients had previously been treated repetitively with whole lung lavage (eight and 14 times, respectively) with the most recent lavages performed 12 and 24 weeks prior to beginning aerosolised GM-CSF.

Results of basal and lipopolysaccharide (LPS)-stimulated GM-CSF production from PBMCs, GM-CSFR assays, GM-CSF regulation of adhesion molecules, and GM-CSF antibody studies are reported in table 2. In patient one, CDw131 (β-subunit of GM-CSFR) was absent on peripheral blood monocytes and granulocytes. In patient two, LFA-1 expression, specifically CD18, was reduced compared with controls. In patient five, basal GM-CSF production in PBMCs was significantly reduced compared with control patients. However, GM-CSF was appropriately upregulated following PBMC incubation with LPS. In patient 10, CD116 (α-subunit of GM-CSFR) was absent on granulocytes, but not lymphocytes and monocytes. Finally, in six out of 10 patients GM-CSF neutralising antibody concentration was determined. In each case, GM-CSF neutralising antibody concentration (mean (range) 115.7±113.2 (28.2–321.0) $\mu g \cdot mL^{-1}$) was significantly >3 $\mu g \cdot mL^{-1}$, a value previously determined to be maximal in normal sera (personal communication K. Nakata, Dept of Respiratory Diseases, International Medical Center of Japan, Tokyo, Japan) [16].

TABLE 1 Baseline characteristics of the 12 treated patients with aerosolised granulocyte-macrophage colony-stimulating factor (GM-CSF)

Characteristics	Data
Age yrs	42.8 (22–63)
Males	7
Females	5
Smoking	
Previous	2 (16.7)
Current	6 (50.0)
Never	4 (33.3)
Symptoms#	
Dyspnoea	10 (83.3)
Cough	11 (75.0)
Fatigue	6 (50.0)
Elevated LDH	6 (50)
Previous whole-lung lavage	2 (16.7)
Approximate time between symptom onset and diagnosis months	19.2 (2–48)
Time between diagnosis to initial treatment with GM-CSF months	10.1 (0.5–84)

Data are presented as median (range), n or n (%). LDH: lactate dehydrogenase. #: other reported symptoms included fever, chills, sweats (4), chest pain (1), haemoptysis (1), or weight loss (3).

Effect of aerosolised GM-CSF in PAP

The results from the first patient treated with aerosolised GM-CSF encouraged the present authors to offer this therapy to others with idiopathic PAP. In this index case, despite continuation of cigarette smoking evident by carboxyhaemoglobin levels, PAP related symptoms resolved and were consistent with improved pulmonary function, gas exchange (fig. 1) and chest radiographs (fig. 2). As carboxyhaemoglobin levels rose from 4.2 to 6.4% the apparent clinical improvement cannot be attributed to smoking cessation. Specifically, following 16 cycles (32 weeks) of treatment with aerosolised GM-CSF, FVC and $D_{L,CO}$ improved from 62 to 86% and 45 to 69% pred, respectively. Pulmonary function and gas exchange

TABLE 2 Granulocyte-macrophage colony-stimulating factor (GM-CSF) molecular studies and neutralising antibody in 10 patients treated with aerosolised GM-CSF for pulmonary alveolar proteinosis

Patient	GM-CSF expression[#]		GM-CSF receptors[¶]		GM-CSF Activity	Anti-GM-CSF serum Ab μg·mL^{-1}
	Basal	LPS stimulated	α CD116	β CDw131	LFA-1 upregulation CD18/CD11a	
1	+	+	+	[f]	+	26.54
2	+	+	+	+	-	73.23
3	+	+	+	+	+	169.33
4	NA	NA	+	+	+	NA
5	-	+	+	+	+	321.03
6	+	+	+	+	+	75.65
7	+	+	+	+	+	28.16
8	+	+	+	+	+	NA
9	+	+	+	+	+	NA
10	+	+	±[§]	+	+	NA

LPS: lipopolysaccharide; LFA-1: leukocyte function associated antigen; Ab: antibody; NA: not available. +: normal; -: qualitatively decreased compared with controls. [#]: normalised to β-actin expression content; [¶]: compared to same day controls; [§]: lymphocyte and monocyte staining for CD116 were normal compared with controls, however, the granulocytes gate was negative for CD116 (α chain); [f]: CDw131 (β chain) was absent on monocytes and granulocytes compared with normal controls.

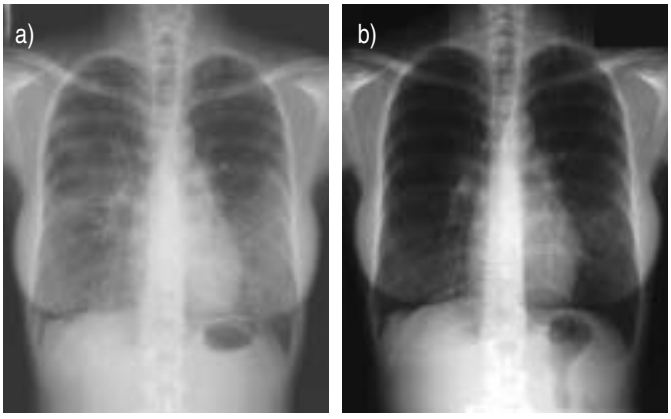

FIGURE 2. Chest radiographs at a) baseline and b) following 12 cycles of aerosolised granulocyte-macrophage colony-stimulating factor (24 weeks) in an index pulmonary alveolar proteinosis case.

worsened following discontinuation of aerosolised GM-CSF, but improved again following reinstitution of therapy (fig. 1).

In total, 12 cases had analysable data concerning clinical symptoms, pulmonary function and chest radiographs. Eleven subjects had analysable data concerning arterial blood gas analysis. As such, 11 out of the 11 patients had significant resolution of symptoms, particularly resolution of cough following aerosolised GM-CSF. Pulmonary function improved significantly in 11 out of 12 patients and gas exchange (fig. 3, table 3) improved significantly in 10 out of 11 patients following aerosolised GM-CSF. In the single patient where pulmonary function improved less than criterion for a positive response, P_{O_2} and D_{A-a,O_2} worsened (decreased 10 mmHg and increased 7 mmHg, respectively) and $D_{L,CO}$ and FVC improved (increased 9% pred and 5% pred, respectively). In the latter case the baseline FVC and $D_{L,CO}$ were already 88% pred. The diagnosis followed 6 yrs of chronic respiratory symptoms prior to treatment and the lack of improvement was possibly due to pre-existing fibrosis evident by chest CT. Though most patients reported symptomatic improvement within the first 4 weeks of treatment, the retrospective nature of the present study did not permit precise determination of the magnitude of symptom improvement, nor the precise timing of benefit following the onset of treatment. Baseline and maximal improvement in pulmonary function and gas exchange during treatment are summarised in table 3. Although the time to follow-up was not tightly standardised due to the retrospective nature of the data, the best pulmonary function test values were achieved after 34.5±18.2 weeks (median (range) 30.6 (9–56)) and best gas exchange values were achieved after 24.1±13.2 weeks (median (range) 24.0 (8–56)). The greatest improvements were noted in D_{A-a,O_2}, resting room air P_{a,O_2} and $D_{L,CO}$. Lesser, but nonetheless significant, improvement was noted in lung volumes and airflow. Using linear regression analysis there was no correlation between change in P_{O_2}, D_{A-a,O_2}, $D_{L,CO}$ and FVC and the magnitude of neutralising antibody to GM-CSF (p=0.86, 0.86, 0.28, and 0.61 respectively).

Following a modest improvement in a single patient using the current conventional GM-CSF dose, a further improvement followed an increased dose of GM-CSF (250 μg *b.i.d.* alternating with 500 μg *b.i.d.* every other week for 64 weeks). Subsequently the patient resumed the conventional GM-CSF dosing and remained stable.

Radiological assessment

Three patients' radiograms completely cleared and eight patients' chest radiograms moderately improved. A single patient whose pulmonary function improved less than criterion for a positive response had no radiographical change. The latter patient improved clinically and radiographically following an increase in GM-CSF dose (500 μg·*b.i.d.*$^{-1}$) *vide infra*.

Side effects of GM-CSF

No patient experienced bone or joint pain while receiving aerosolised GM-CSF. No systemic symptoms, cough, fatigue,

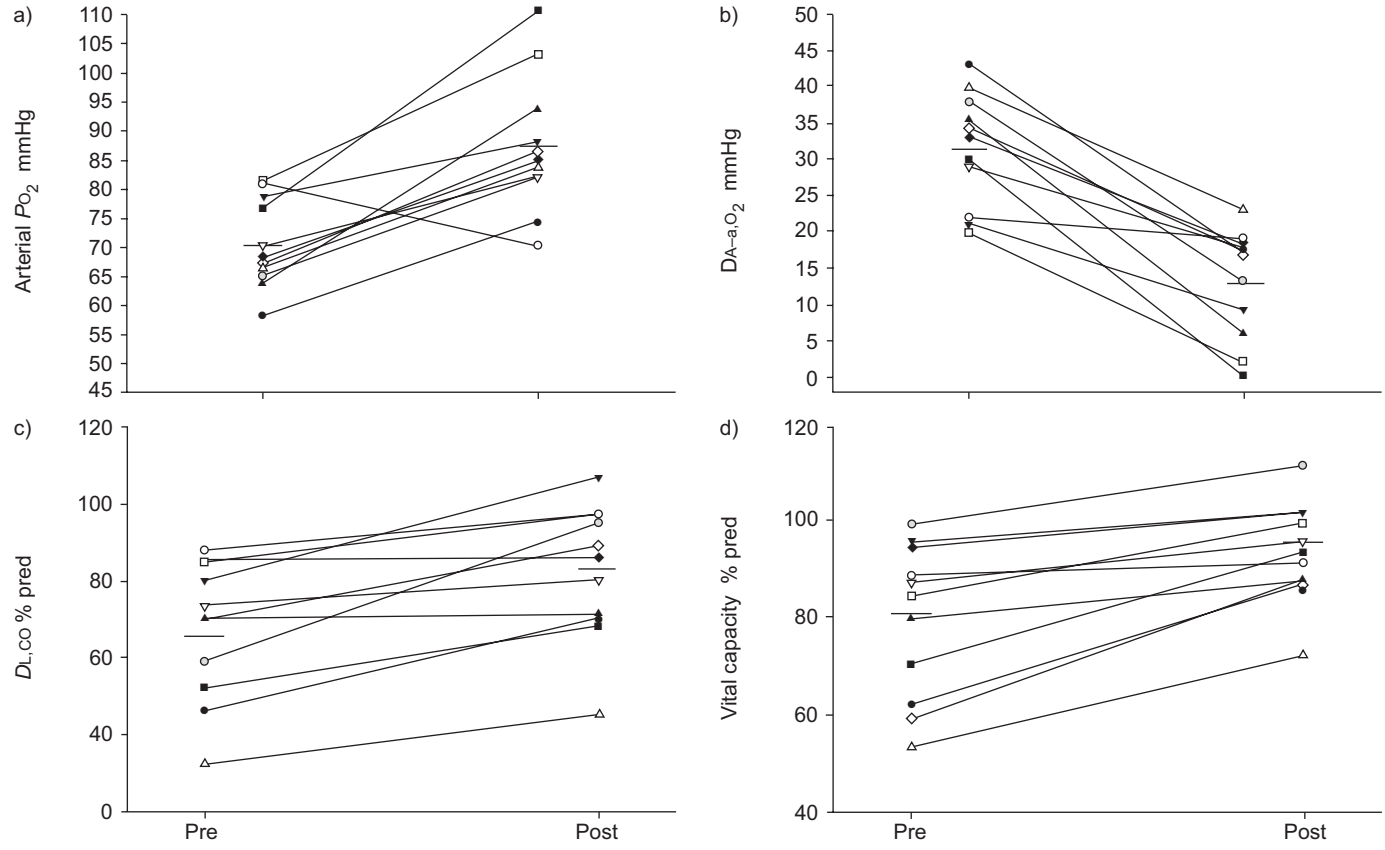

FIGURE 3. Pre- and post-treatment values of patients treated with aerosolised granulocyte-macrophage colony-stimulating factor (GM-CSF) for pulmonary alveolar proteinosis. P_{O_2}: partial pressure of oxygen; [A-a]DO_2: alveolar-arterial oxygen-tension gradient; $D_{L,CO}$; carbon monoxide diffusing capacity of the lung. ○: single patient who did not have a positive pulmonary function or gas exchange response to GM-CSF treatment. a) p=0.0007; b) p=0.0003; c) p=0.0004; and d) p=0.0002.

fever, myalgia or bone pain were reported during aerosolised GM-CSF use. White blood cell counts did not increase or decrease with GM-CSF aerosol (baseline values of 6.1 ± 1.1, median (range) 6.0 (4.8–7.7), $10^9\cdot L^{-1}$; and at maximum response 5.6 ± 1.4, median (range) 5.0 (3.9–8), $10^9\cdot L^{-1}$; p=0.52). No patient developed neutropenia or thrombocytopenia. There was no significant change in the FEV1/FVC or forced mid-expiratory flow, nor did any patient develop symptoms of airway disease during follow-up. All patients with elevated baseline LDH values normalised during GM-CSF aerosol treatment (baseline: 221.6 ± 47.2 U·L^{-1}, median (range) 215.5 U·L^{-1} (172–289); during GM-CSF aerosol: 171.6 ± 26.1 U·L^{-1} median (range) 163 (147–229); p=0.016). After a median (range) follow-up of 30.5 (13–68) months, no haematopoietic malignancy, detectable pulmonary fibrosis, airway disease or mortality occurred.

Duration of clinical improvement in idiopathic PAP following aerosolised cessation of aerosolised GM-CSF

During the course of observation in these patients, five had recurrence of symptoms as well as a measurable decline in spirometry and measured gas exchange after elective discontinuation of GM-CSF aerosol treatment. Follow-up data were available on four out of five of these patients and indicated complete resolution of their symptoms following re-treatment with GM-CSF aerosol. In these four patients, mean time to relapse varied (mean (range) 6.3 (5.5–12) months). The baseline magnitude of decline in pulmonary function tests and arterial blood gases was not different than when patients first started treatment with aerosolised GM-CSF. All parameters normalised following reinstitution of aerosolised GM-CSF after a mean (range) of 24 (8–56) weeks. Four other patients have been examined and remained asymptomatic with stable pulmonary function and gas exchange for 12 to 41 months following discontinuation of GM-CSF aerosol treatment. In two cases, the durable response was associated with complete normalisation of pulmonary function, gas exchange and normal chest radiograms.

DISCUSSION

Since the original descriptions [26, 27] whole-lung lavage has remained the conventional treatment for PAP. Although its role is developed from clinical case series in academic centres, its optimal use, effect on the disease progression, morbidity and mortality in nontertiary centres are relatively unknown. Recently, BECCARIA *et al*. [28] reported on the safety and long-term follow-up of 21 patients treated with whole-lung lavage. They noted that at a median of 5 yrs, recovery of $D_{L,CO}$ was incomplete (75 ± 19% pred value) and there were residual gas exchange abnormalities (D_{A-a,O_2} 3.6 ± 1.5 kPa (27 ± 11 mmHg)) and exercise limitation, probably explained by engorgement of lymphatic vessels. However, in that series the mean baseline values of FVC, $D_{L,CO}$ and P_{a,O_2} (8.24, 5.85 and 7.31 kPa, respectively) were less than in the current study patients (10.64, 8.76 and 9.31 kPa, respectively). In the present study of

TABLE 3 Pulmonary function tests[#] and arterial blood gas analysis[¶]

	Baseline values before treatment		Best values after treatment	p-value
	n	Mean±SD	Mean±SD	
TLC %	7	79.3±11.1	90.6±11.9	0.1
FVC %	12	80.4±15.7	93.8±10.2	0.0001
FEV_1 %	12	80.4±15.7	91.3±14.1	0.00002
$D_{L,CO}$ %	12	65.9±17.9	82.6±16.9	0.0005
V_A %	6	87.2±11.5	95.6±17.0	0.035
P_{a,O_2} mmHg				
Rest	12	70.0±7.4	87.2±11.7	0.0007
Exercise	9	60.3±10.3	78.2±8.5	0.009
O_2 sat %				
Rest	12	92.1±2.8	94.5±2.9	0.008
Exercise	9	88.9±3.9	93.9±2.8	0.017
D_{A-a,O_2} mmHg				
Rest	12	31.3±7.4	12.9±7.6	0.00003
Exercise	9	45.4±11.7	25.3±8.6	0.007

TLC: total lung capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; $D_{L,CO}$: diffusing capacity of carbon monoxide; V_A: alveolar volume; P_{a,O_2}: arterial oxygen tension; O_2 sat: oxygen saturation; D_{A-a,O_2}: alveolar–arterial oxygen-tension gradient. [#]: best pulmonary function test values were achieved after mean±SD (34.5±18.2), median (range) 30.6 (9–56) weeks; [¶]: best arterial blood gas values were achieved after mean±SD (24.1±13.2) median (range) 24.0 (8–56) weeks.

clinical data on 12 patients with idiopathic PAP treated with aerosolised GM-CSF, all improved both symptomatically and by objective measurement of pulmonary function. Though radiological improvement was variable, all but one patient had a significant improvement in gas exchange.

Recently, several causes of idiopathic PAP in adults have been determined including, high affinity auto-antibodies [18], failure of PBMCs to release GM-CSF following LPS stimulation (possibly due to mutation in the GM-CSF complementary DNA) [29, 30], as well as expression defects in the GM-CSF/interleukin (IL)-3/IL-5 receptor common β-chain in children with PAP [4]. In secondary PAP, such as haematopoietic malignancies numerical deficiency and/or functional impairment of alveolar macrophages may be causal [8]. Although the present retrospective study is small and there was no intention to thoroughly investigate disease mechanisms, in those patients tested, measurable antibody to GM-CSF was demonstrated. Additionally, for clinical purposes, and prior to studies by others [31], three patients had either reduced expression or activity of GM-CSF, or abnormal GM-CSF receptor expression (in PBMCs) which may, in addition, have contributed to the clinical disease. Though these findings are inconclusive, the possibility remains that distinct mechanisms altering GM-CSF activity, other than high affinity auto-antibody, may be responsible for some clinical cases of acquired/idiopathic PAP. Nonetheless, neither the magnitude of neutralising antibody to GM-CSF nor these variations prevented, nor correlated, with the magnitude of improvement following aerosolised GM-CSF. The present authors did not measure neutralising capacity against GM-CSF in BALF before or during treatment. However, results by TAZAWA *et al*. [32] show that inhalation of GM-CSF restores the bioactivity in the lung of patients with PAP by reducing the neutralising capacity against GM-CSF in proportion to the reduction in the amount of auto-antibody detected in the BALF.

Although a retrospective series is not intended to be directly compared with other forms of treatment, the clinical response rate to aerosolised GM-CSF appears to be better than in idiopathic PAP patients treated with subcutaneous GM-CSF [20, 31]. Aerosol delivery provides a means to achieve local effects in the lung with minimal systemic drug exposure [33]. Estimates of the current authors nebulisation system suggests that it deposits 12–20% of output to the 17th–23rd generation of the bronchials, the transitory zones leading to terminal alveolated lung [33]. Thus, the current authors calculate that ~15–20% of the aerosolised protein would deliver ~100 μg of $GM\text{-}CSF \cdot day^{-1}$. In addition, GM-CSF delivered to the lung by aerosol is likely to be trapped within the bronchial and pulmonary lymphatics by high-affinity receptors on resident immune cells limiting systemic activity. Prior studies of subcutaneous GM-CSF administration for idiopathic PAP reported clinical response rates of six out of 11 (54%) [31] and six of 14 (43%) [20] in patients to both the initial and subsequent dose escalation compared with the present response of 11 out of 12 (91.69%). Moreover, in the study by SEYMOUR and PRESNEILL [2], there was no treatment effect on spirometric values, whereas all the current study patients showed significant improvement as well as two of the patients achieving a complete response (normal predicted pulmonary function, gas exchange and radiograph). It has been suggested that a normal serum LDH level predicted a higher likelihood of response to subcutaneous GM-CSF [2]. Although the ultimate value of LDH determination is not known, in the present study, in those patients in whom LDH was determined, all

values normalised with treatment and were significantly less than baseline values ($p=0.016$).

Unregulated chronic GM-CSF production in transgenic mice has been associated with eosinophilia, monocytosis and fibrotic reactions in the lung by transforming growth factor-β1 and myofibroblast accumulation [34, 35]. As reported by MONICK *et al*. [36] disease-related cytokines, such as GM-CSF, may modulate activator protien-1, DNA binding activity in alveolar macrophages and promote tissue fibrosis. However, GM-CSF transgenic mice do not show such propensity [12, 13] and patients with idiopathic PAP untreated with GM-CSF may develop pulmonary fibrosis [2]. In addition, GM-CSF may have a critical role in the development of asthma as it prolongs the survival of eosinophils [37], activates antigen presenting cells, and increases smooth muscle function collagen-1 and fibronectin expression [38]. Conversely, prior studies suggest that the administration of GM-CSF auto-antibodies in a murine model of asthma significantly reduces airway hyperresponsiveness [39]. Prior studies using aerosol GM-CSF in intubated, anaesthetised, nonhuman primates given ~80 μg on one or two occasions were without pulmonary toxicity [40], as well as in a prior study in seven patients with lung metastases given dose escalation to 240 $mg \cdot dose^{-1}$ *b.i.d.* for 7-day cycles [23]. Recently, TAZAWA *et al*. [32] reported favourable clinical responses without apparent toxicity in three patients with PAP treated for 24 weeks according to the original protocol [23]. Thus, despite potential mechanisms for GM-CSF to elicit untoward effects, in all patients treated so far with aerosolised GM-SCF following prolonged use no immediate or late toxicity was seen. Thus, aerosolised GM-CSF appears to be a safe and effective alternative to whole lung lavage as well as to subcutaneous GM-CSF administration for the treatment of idiopathic PAP.

GM-CSF is a 23-kDa glycoprotein originally identified by its ability to promote *in vitro* proliferation and differentiation of haematopoietic progenitors to neutrophils and macrophages [41]. It has been suggested that GM-CSF is a previously unsuspected modulator of surfactant metabolism probably by effects on alveolar macrophage function [42]. Moreover GM-CSF and its receptor are constitutively produced and located by or on type II and other respiratory epithelial cells, as well as human airway myocytes. However, GM-CSF-deficient mice had surfactant clearance problems [9, 10], reduced fertility and reduced long-term survival [43], impaired macrophage function [44], and a propensity to develop lung and other infections; with no detectable disturbance in their steady-state haematopoiesis.

In the current study, there was no haematopoietic responsiveness and this was consistent with the other aerosolised GM-CSF study by ANDERSON *et al*. [23]. However, it must be kept in mind that patients with PAP have attenuated haematopoietic responsiveness to GM-CSF [20, 21, 43], possibly due to the presence of GM-CSF neutralising antibodies [16, 20, 31]. It is possible that anti-GM-CSF titre predicts response to subcutaneous GM-CSF therapy in idiopathic PAP [31]. These antibodies were even suggested to be used in the diagnosis of idiopathic PAP using a latex agglutination test with a sensitivity and specificity of 100 and 98%, respectively [16, 31]. However, this needs to be validated while keeping in mind that these antibodies can be seen in healthy persons [45], or in persons treated with GM-CSF [46].

Four of the study patients have had sustained recovery following cessation of treatment with GM-CSF. Reports of ''spontaneous resolution'' occurring in idiopathic PAP vary between ~8% of 303 cases [2], to 13 out of 21 patients following lung lavage [7]. Although the auto-antibody to GM-CSF may be related to the development of PAP, other factors controlling, or influencing, macrophage clearance of surfactant, as well as the permanence of auto-antibody to GM-CSF may influence the rate of spontaneous remission. The rate of improvement in the study patients makes it unlikely that initial improvement following use of aerosolised GM-CSF was due to do to ''spontaneous resolution''. For example, smoking may be casual to some cases of PAP, but in several of the current cases the treatment response was achieved without smoking cessation despite smoking cessation counselling, a finding previously reported by KAVURU *et al*. [21]. Although ~ 72% of patients with idiopathic PAP have a history of smoking [2], effects of smoking are likely to be concerted with other influences of alveolar GM-CSF activity.

Bearing in mind the small number of patients and the retrospective nature of the study, many important questions remain unanswered, including the optimal dose of GM-CSF, the optimal duration of therapy, the long-term outcome, response rates compared to subcutaneous GM-CSF, possible late toxicities, and individual characterisations that predict treatment response. As the disease is rare, a national registry is required to answer these questions.

In conclusion, the present study is the first to report the successful treatment of human pulmonary alveolar proteinosis with aerosolised granulocyte-macrophage colony-stimulating factor. The results appear to be better than when granulocyte-macrophage colony-stimulating factor is administered systemically. No pulmonary toxicity could be determined during prolonged use. When coupled to the current authors previous study in patients with lung metastases, granulocyte-macrophage colony-stimulating factor is a well tolerated biological aerosol. The role of adjunctive immunotherapy with granulocyte-macrophage colony-stimulating factor aerosols on dendritic cell function in bronchogenic cancer [47, 48], central airway tumours, macrophage function in pulmonary infections, such as tuberculosis [49, 50], and atypical mycobacterium may extend the role of this biological aerosol. In idiopathic pulmonary alveolar proteinosis, larger prospective studies with a long-term follow-up are needed to confirm the present findings and to establish the long-term safety of this novel treatment.

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