Inhaled granulocyte–macrophage colony-stimulating factor for *Mycobacterium abscessus* in cystic fibrosis

To the Editor:

Nontuberculous mycobacteria (NTM) are an important emerging threat to cystic fibrosis (CF) patients. In North America, where the incidence of NTM in CF patients is ⩾11.8%, *Mycobacterium abscessus* complex (MABSC), a multidrug-resistant NTM, accounts for ∼35% of these [1], is notoriously recognised as difficult to eradicate, and seriously affects morbidity and mortality in CF [2] as well as lung transplantation outcomes [3, 4]. The mechanisms for the increased incidence of MABSC infection in CF patients are not known. The immune response in CF patients is directed to the Th2 response, which is associated with poorer clinical outcome and accelerated decline in lung function. This Th2 pattern is associated with diminished interferon-γ production and lesser activation of macrophages. One activator of macrophages is granulocyte–macrophage colony-stimulating factor (GM-CSF), the Toll-like receptor activation of which includes phagocytosis, bactericidal activity, oxidative burst and cell adhesion in macrophages [5]. Two experimental findings support the plausibility that reduced GM-CSF-elicited macrophage activation may contribute to NTM and MABSC infection in CF. First, alveolar macrophages from in GM-CSF$^{-/-}$ mice exhibit defective phagocytosis, bacterial killing and reduced H_2O_2 production [6]. Second, although wild-type mouse models of *M. abscessus* pulmonary infection show limited morbidity and are limited in their usefulness to study NTM therapy GM-CSF knockout models of *M. abscessus* infection, mice either succumbed to the acute infection or the infection persisted to a chronic stage in the absence of exogenous GM-CSF [7]. Previously, we [8] and others have reported the successful use of inhaled GM-CSF to treat autoimmune pulmonary alveolar proteinosis and metastatic lung metastases [9] without toxicity. Herein, we treated two CF patients with *M. abscessus* who were experiencing a decline in pulmonary function and clinical stability.

Case 1, a 10-year-old, F508del-homozygous female, had a 3.5-year history of persistent *M. abscessus* colonisation. Nodular infiltrates and clinical decline prompted intravenous amikacin, intravenous cefoxitin and oral linezolid for 2 years. Cefoxitin was discontinued after 1 month due to rash, despite an attempt at cefoxitin desensitisation. Ototoxicity due to intravenous amikacin was noted after 4 months so this was replaced with aerosolised Amikacin. Despite linezolid (intravenous) and amikacin (inhaled) therapy, pulmonary function and body mass declined, and bronchoalveolar lavage (BAL) specimens returned smear positive results for many organisms and heavy growth of *M. absessus*. The organism exhibited intermediate sensitivity to amikacin and cefoxitin, while it was sensitive to linezolid. Aerosolised GM-CSF (Sargramostim (Genezyme, Cambridge, MA, USA); 250 μg twice daily, diluted in 2 cm^3 saline) was added and administered on alternate weeks as previously described [8] *via* a Pari LC nebuliser (Pari, Midlothian, VA, USA). There was clinical improvement and a decrease in radiological opacities within areas of extensive varicoid and cystic bronchiectasis (table 1). GM-CSF was continued and antibiotics were discontinued after 3 months. After the patient remained off antibiotics for 3 months, a decision was made to recombine aerosolised GM-CSF with linezolid (intravenous) and amikacin (inhaled). After 4 months of combined therapy, both acid-fast bacillus (AFB) smear and cultures became negative (figure 1). She remains on inhaled GM-CSF alone.

Case 2, 25-year-old, F508del-homozygous male with CF-related diabetes mellitus, had a 13-year history of persistent *M. abscessus*. New radiological nodular infiltrates, loss of weight and a fall in lung function were noted despite ongoing use of lumacaftor/ivacaftor. An AFB smear showed many organisms. Aerosolised

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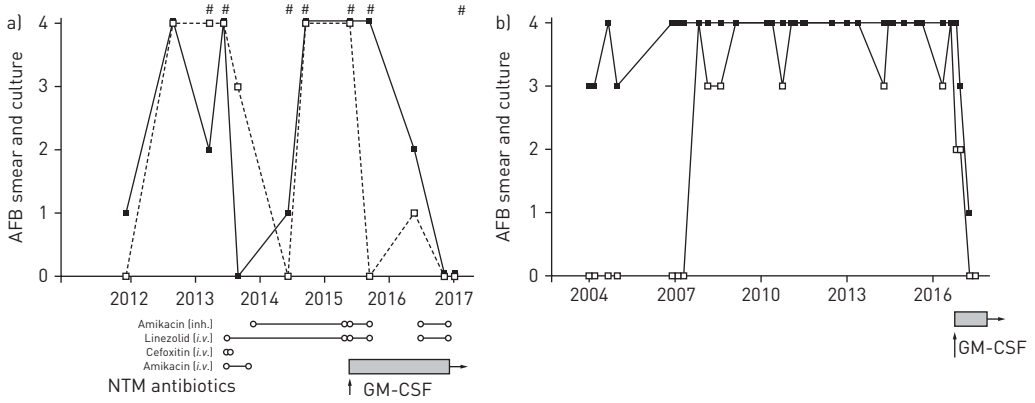

FIGURE 1 Acid-fast bacillus (AFB) smears using Auramine O fluorescent stain and 20× or 40× objective (open squares) are semiquantitative: 0, negative; 1, one or two organisms per entire smear; 2, three to nine organisms per entire smear; 3, ⩾10 organisms per entire smear; 4, one or more organisms per field. Mycobacterial cultures (closed squares) are semiquantitative: 0, negative; 1, one colony on entire plate; 2, two colonies on entire plate; 3, 3–30 colonies on entire plate; 4, >20 colonies on entire plate. #: specimen obtained by bronchoalveolar lavage. Arrow indicates when granulocyte–macrophage colony-stimulating factor (GM-CSF) aerosol therapy was begun. a) Case 1; b) case 2. Inh.: inhaled; *i.v.*: intravenous; NTM: nontuberculous mycobacterium.

TABLE 1 Pulmonary function

Case	GM-CSF therapy duration weeks	FVC % predicted	FEV_1 % predicted
1	0	62.5	64.7
	16	73.0	75.0
	90	79.0	78.0
2	0	72.5	55.2
	9	80.9	60.7
	26	81.6	63.9

GM-CSF: granulocyte–macrophage colony-stimulating factor; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s.

GM-CSF 250 µg twice daily, 1 week on 1 week off, was begun without antibiotic therapy. Clinical improvement was noted without toxicity (table 1). After 6 months of GM-CSF, sputum smears became negative and culture burden decreased to one colony per plate (figure 1).

Discrepancy between *in vitro* antibiotic susceptibility and clinical response to treatment of MABSC infection suggests that factors involving the innate immune system may play a role. There is one case report using subcutaneous administration of GM-CSF. Moser *et al.* [10] administered GM-CSF at $2\ \mu g \cdot kg^{-1} \cdot day^{-1}$ subcutaneously for ~1 year in two CF patients with *M. abscessus* infection that was resistant to antibiotic treatment. The treatment was well tolerated. One patient improved and was taken off the lung transplant list, while the other patient stabilised. However, use of aerosolised GM-CSF for the treatment of infections in humans has not been reported. Inhaled GM-CSF increases the number and function of phagocytic cells obtained from BAL [11]. GM-CSF is expressed constitutively in human airway epithelium [12]. Although human CF airway epithelial cell release of basal and stimulated GM-CSF is not different from control airway epithelial cells [13], and both *Pseudomonas aeruginosa* and *Staphylococcus aureus* increase GM-CSF expression [14] in cultured CF airway epithelial cells, BAL concentrations of GM-CSF in $CFTR^{-/-}$ mice [15] and in sputa of CF patients are significantly decreased [16]. Moreover, the airway concentrations of GM-CSF are lesser in CF patients during respiratory exacerbation and are undetectable in those sensitised to *Aspergillus fumigatus* [16]. Human CF alveolar macrophages do not have any intrinsic dysfunction or reduced capacity to generate reactive oxygen species [17] or to synthesise cytokines [15]. Taken together, we hypothesise that CF macrophages can respond to GM-CSF but that GM-CSF activity is reduced in the airway and alveolus, perhaps due to impaired diffusion of epithelially released GM-CSF in bronchiectatic airways or physical and chemical properties of abnormal CF sputum.

In summary, we report two CF patients with evidence of *M. abscessus* colonization, one with lack of response to aminoglycosides and/or linezolid, in whom aerosolized GM-CSF was well tolerated with

improved lung function. The relatively high negative impact of MABSC disease and toxicities of conventional antibiotic therapy and associated lung transplant morbidity warrant the need for future studies to investigate defects in the native immune system in CF and the potential strategies for GM-CSF treatment and prophylaxis. Augmentation of GM-CSF airway activity may improve host response to *M. abscessus* in CF when administered by inhalation alone or in combination with antibiotics.

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