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Research letter

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Letter to the Editor

Inhaled Granulocyte-Macrophage Colony Stimulating Factor for *Mycobacterium Abscessus* in Cystic Fibrosis

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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 at least 11.8% Mycobacterium abscessus complex (MABSC), a multidrug-resistant NTM, accounts for about 35%[1] and are notoriously recognized as difficult to eradicate and seriously affect 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 that is associated with poorer clinical outcome and accelerated decline in lung function. This Th2 pattern is associated with diminished IFNγ production and lesser activation of macrophages. One activator of macrophages is granulocyte macrophage-colony stimulating factor (GM-CSF) whose Toll-like receptor activation 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, though 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 persists 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 (PAP) 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: 10 y.o. delta F508 homozygous female had a 3.5 year history of persistent *M. abscessus* colonization. Nodular infiltrates and clinical decline prompted intravenous Amikacin, Cefoxitin and oral Linezolid for 2 years. Cefoxitin was discontinued, despite attempt at Cefoxitin desensitization, after 1 month due to rash. Ototoxicity due to Amikacin was noted after 4 months and was replaced with aerosolized Amikacin. Despite Linezolid (i.v.) and Amikacin (inhaled) therapy pulmonary function and body mass declined and bronchoalveolar lavage (BAL) specimens returned to smear positive with many organisms and heavy growth of *M. absessus.* The organism exhibited intermediate sensitivity to Amikacin and Cefoxitin, while sensitive to Linezolid. Aerosolized GM-CSF (Sargramostim, Genezyme, Cambridge, MA, 250 μg twice daily diluted in 2 cc saline was was added and administered on alternate weeks as previously (ref 8) via a Pari LC nebulizer, Midlothian VA. There was clinical improvement as well as a decrease in radiologic opacities within areas of extensive varicoid and cystic bronchiectasis. Clinical improvement and stability were noted. GM-CSF was continued and antibiotics were discontinued after 3 months. After remaining off antibiotics for 3 months a decision was made to recombine aerosolized GM-CSF with Linezolid (i.v.) and Amikacin (inhaled). After 4 months of combined therapy both AFB smear and cultures became negative. She remains on inhaled GM-CSF alone.

Case 2: 25 y.o. delta F508 homozygous male with CF-related diabetes mellitus had a 13 year history of persistent *M. abscessus*. New radiologic nodular infiltrates, loss of weight and fall in lung function were noted despite ongoing use of lumicaftor/ivacaftor. AFB smear showed many organisms. Aerosolized GM-CSF 250 μg BID one week on one week off was begun without

antibiotic therapy. Clinical improvement was noted without toxicity. After 6 months of GM-CSF sputum smears became negative and culture burden decreased to 1 colony per plate.

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 GM-CSF subcutaneous administration. Moser et al.[10] administered GM-CSF at 2μg/kg/day subcutaneously for approximately one year in 2 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 stabilized. However, use of aerosolized 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] Though 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 sensitized to *Aspergillous fumigatus*.[16] Human CF alveolar macrophages do not have any intrinsic dysfunction or reduced capacity to generate ROS[17] or to synthesize cytokines.[15] *Taken together, we hypothesize 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 epithelial-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 cystic fibrosis when administered by inhalation alone or in combination with antibiotics.

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Table 1.

Case (No.)	GM-CSF Duration of Therapy (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 second. BAL: bronchoalveolar lavage. AFB: acid fast bacilli.

Figure 1.

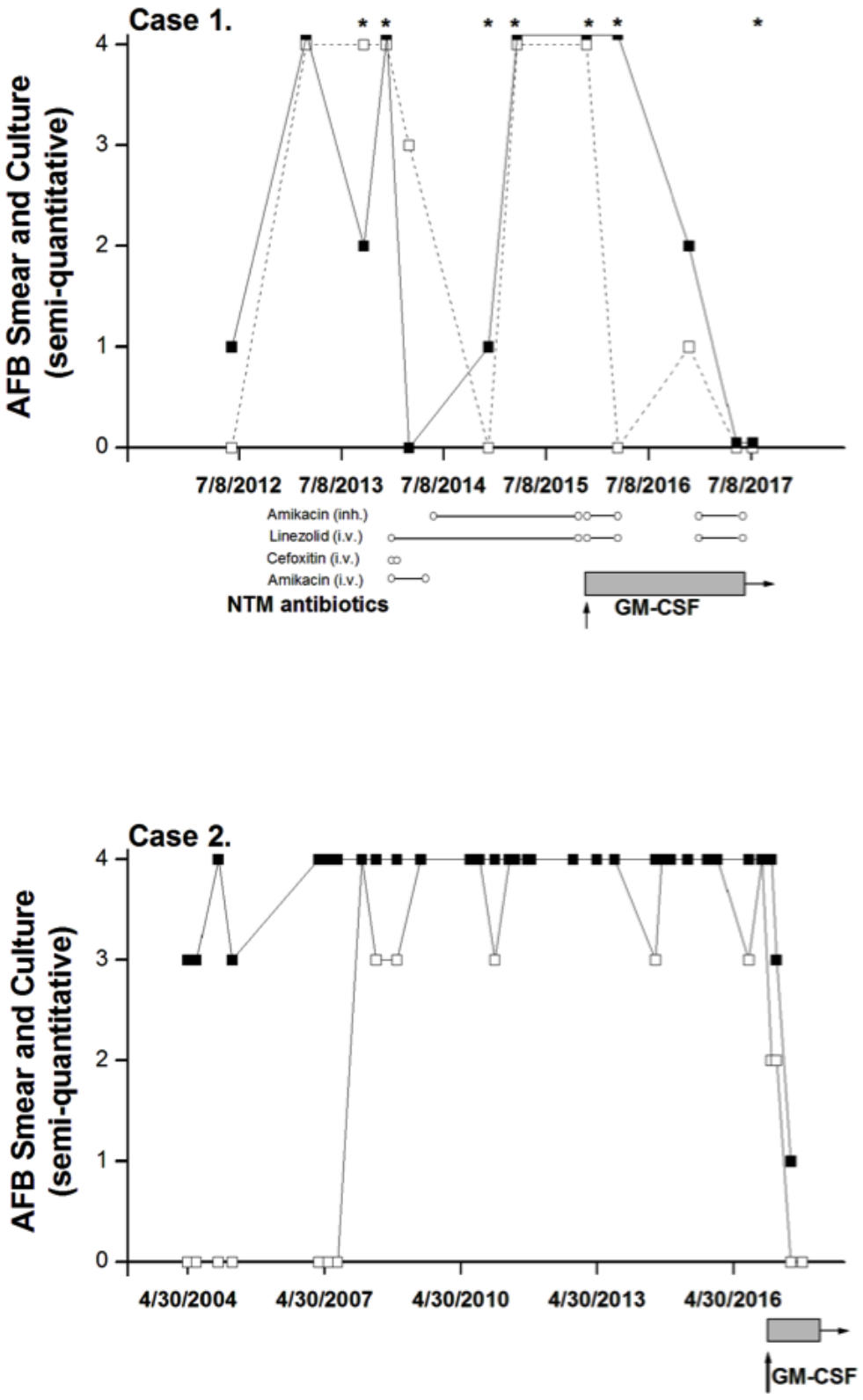
Case 1.
AFB Smear and Culture (semi-quantitative)
0
1
2
3
4
7/8/2012
7/8/2013
7/8/2014
7/8/2015
7/8/2016
7/8/2017
Amikacin (inh.)
Linezolid (i.v.)
Cefoxitin (i.v.)
Amikacin (i.v.)
NTM antibiotics
GM-CSF
Case 2.
AFB Smear and Culture (semi-quantitative)
0
1
2
3
4
4/30/2004
4/30/2007
4/30/2010
4/30/2013
4/30/2016
GM-CSF

Figure 1. AFB Smears using Auramine O Fluorescent stain and 20 X or 40 X objective (open squares) are semi-quantitative. 0 = negative, 1 = 1-2 organisms per entire smear, 2 = 3-9 organisms per entire smear, 3 = 10 or more organisms per entire smear, 4 = 1 or more organism per field. Mycobacterial cultures (closed squares) are semi-quantitative. 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. * denotes specimen obtained by bronchoalveolar lavage. Arrow indicates when GM-CSF aerosol therapy was begun.