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Deficient interleukin-17 production in response to *Mycobacterium abscessus* in cystic fibrosis



To the Editor:

The respiratory tract of patients with cystic fibrosis (CF) is colonised with a high diversity of micro-organisms. Nontuberculous mycobacteria (NTM) show a high and increasing prevalence. 40% of these positive NTM cultures are caused by *Mycobacterium abscessus* [1], one of the rapidly growing NTMs present in the environment. Patients with *M. abscessus* infection are difficult to treat, due to natural and acquired antibiotic resistance [2, 3], and an infection with *M. abscessus* is controversially discussed as a contraindication for lung transplantation [4].

Immune-modulatory treatment strategies might contribute to overcome this problem. For their development, a better understanding of the defective immune response explaining the higher susceptibility of CF patients to *M. abscessus* is needed. Here we present three CF patients with *M. abscessus* infection, in whom we describe the pathogen-specific innate and adaptive cytokine production and compare this with non-CF patients with pulmonary infection caused by various NTMs: *M. abscessus* (n=1), *M. avium* (n=3), *M. kansasii* (n=2) and *M. intracellulare* (n=1).

Case 1 is a 24-year-old female patient with CF (dF508del/dF508del) with pancreatic insufficiency and *Pseudomonas* colonisation since 2003. In 2004, she presented with allergic bronchopulmonary aspergillosis (ABPA) which was successfully treated with corticosteroids. After years of infectious exacerbations she presented with an episode of haemoptysis in 2010. Shortly thereafter, *M. abscessus* was cultured from her sputum. In 2011, haemoptysis and clinical deterioration led to hospitalisation and several courses of antimycobacterial regimens (combinations of amikacin, clarithromycin, tigecycline, meropenem and clofazimine) were given without successful *M. abscessus* eradication.

Case 2 is a 23-year-old male patient with CF (dF508del/dF508del), pancreatic insufficiency and *Staphylococcus aureus* and *Aspergillus* colonisation who presented with ABPA. After a course of corticosteroids and itraconazole, he improved and serological markers for ABPA have remained at low levels ever since. After this episode, *M. abscessus* was consistently cultured and although he had no physical complaints, his pulmonary function deteriorated, and a computed tomography thorax scan showed several subpleural and intraparenchymatous nodular lesions compatible with mycobacterial disease. No clearance of *M. abscessus* was achieved, despite two courses of treatment with combination regimens of amikacin, meropenem, clarithromycin and clofazimine.

Case 3 is a 15-year-old male patient with CF (dF508del/G551D), pancreatic insufficiency and *Pseudomonas* colonisation. Since 2011 *M. abscessus* has been consistently cultured and he experienced several exacerbations in 2014, in which his pulmonary function deteriorated, despite several NTM regimens (including tigecycline,

clofazimine, linezolid and azithromycin) and treatment with ivacaftor. However, he was noncompliant with the treatment and not able to complete several regimens due to side-effects and a complex psychosocial situation. No relevant clearance of *M. abscessus* was achieved.

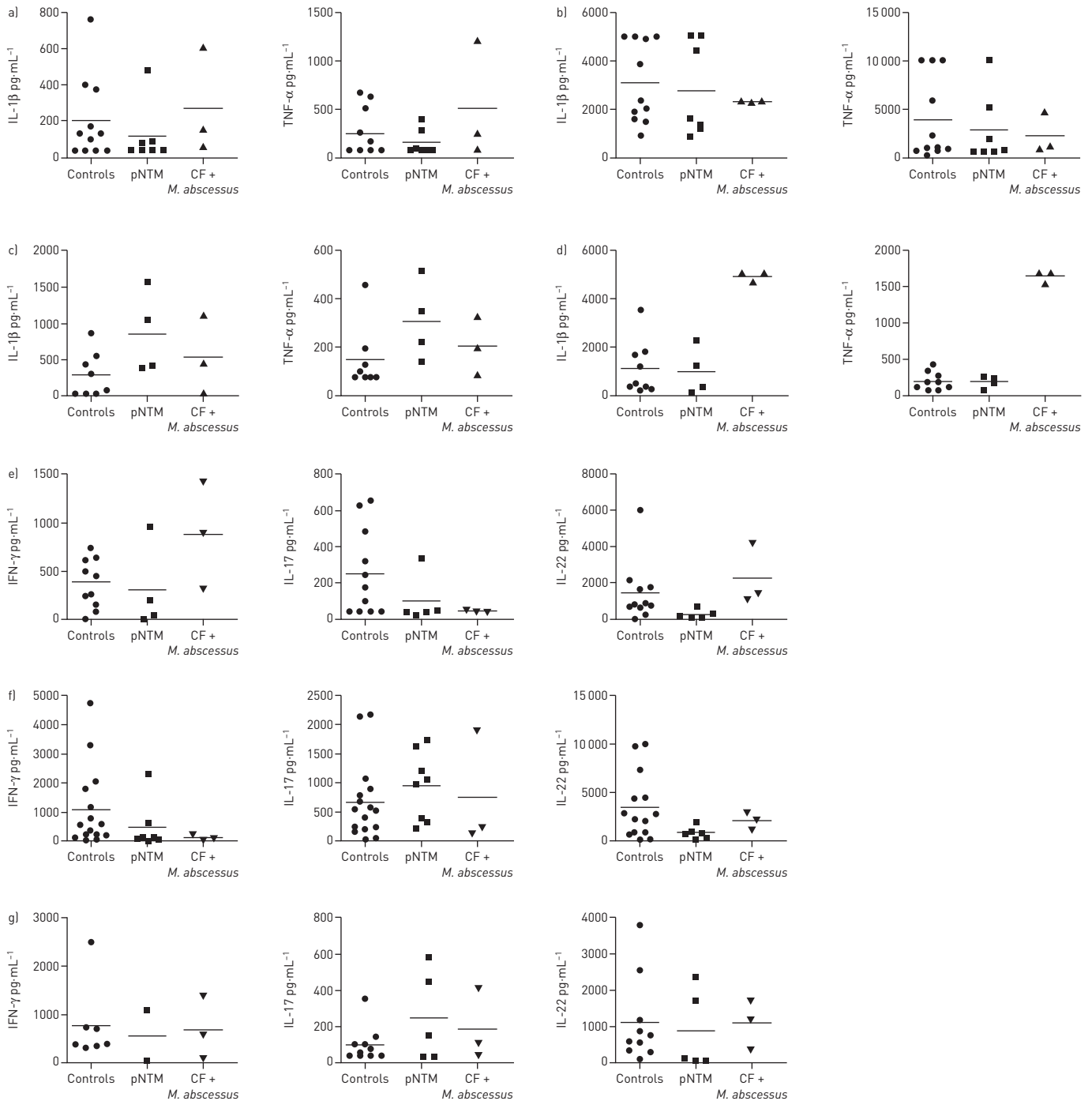


FIGURE 1 Innate and adaptive cytokine responses of three cystic fibrosis (CF) patients with *Mycobacterium abscessus* infection compared with non-CF patients with pulmonary nontuberculous mycobacteria (pNTM) infections. Peripheral blood mononuclear cells (2.5×10^6 cells·mL⁻¹) of a healthy control group (n=11), non-CF patients with pNTM infections (n=7) and three CF patients with *M. abscessus* infection were stimulated with heat-killed *M. abscessus* (clinical isolate NLA001001395) (1×10^5 cells·mL⁻¹), heat-killed *Candida albicans* [ATCC MYA-3573, UC820] (1×10^5 cells·mL⁻¹), *Escherichia coli* lipopolysaccharide [*E. coli* serotype O55:B5; Sigma-Aldrich, St. Louis, MO, USA] (1 ng·mL⁻¹) or *Aspergillus fumigatus* conidia (clinical isolate V05-27) (1×10^7 cells·mL⁻¹). The innate cytokines tumour necrosis factor (TNF)- α and interleukin (IL)-1 β were measured after 24 h and the adaptive cytokines interferon (IFN)- γ , IL-17 and IL-22 were measured using ELISA after 7 days in the cell culture supernatant. Innate cytokine response to a) *M. abscessus*; b) *C. albicans*; c) *E. coli* lipopolysaccharide; d) *A. fumigatus*. Adaptive cytokine response to e) *M. abscessus*; f) *C. albicans*; and g) *A. fumigatus*.

Peripheral blood mononuclear cells (PBMCs) isolated from the three patients described above were stimulated with *M. abscessus*, isolated and cultured from the second case, *Candida albicans*, *Escherichia coli* lipopolysaccharide (LPS) and *Aspergillus fumigatus*. CF patients and pulmonary NTM patients did not demonstrate a distinct innate cytokine profile after PBMC stimulation with *M. abscessus* or *C. albicans* (figure 1a and b). Interestingly, all NTM patients showed higher tumour necrosis factor (TNF)- α and interleukin (IL)-1 β production on LPS stimulation compared to the control group, while the *A. fumigatus*-specific innate immune response was selectively increased in the CF patients infected with *M. abscessus* (figure 1c and d).

Cystic fibrosis transmembrane conductance regulator-deficient monocytes have been described as highly reactive to LPS stimulation due to a prolonged sensing period of Toll-like receptor-4 on the cell surface [5]. Furthermore, previous studies reported a hyperresponsive innate immune system in CF patients, with elevated TNF- α , IL-1 β and IL-6 levels in the sputum [6], which is in line with the elevated innate immune profile described in the CF patients of our study. CF patients, who are often colonised with *Aspergillus*, are at high risk of developing ABPA (7.8%) [7], and two out of the three CF patients with NTM infection in this study fulfilled the criteria of ABPA. Whether and how the strongly increased innate cytokine response after *Aspergillus* stimulation, as seen in all the CF patients in this study contributes to the development of ABPA or is the result of a hypersensitivity reaction in an *Aspergillus*-colonised lung remains to be elucidated.

In contrast, the acquired immune responses revealed pathogen-specific differences. To investigate adaptive T-helper cell (Th) responses, PBMCs were stimulated with *M. abscessus*, *C. albicans* (which was used as a positive control for IL-17 production) and *Aspergillus* conidia. PBMCs from healthy subjects produced IL-17, IL-22 and interferon (IFN)- γ in response to *M. abscessus* (figure 1e). Although PBMCs isolated from both patient groups were capable of producing IL-17 in response to *C. albicans* (figure 1f), IL-17 in response to *M. abscessus* was very low in all CF patients, as well as in most of the patients with pulmonary NTM infection. In contrast, *M. abscessus*-induced IL-22 and IFN- γ was similar or even elevated in CF patients infected with *M. abscessus*, while the pulmonary NTM patients demonstrated low IFN- γ and IL-22 production upon *M. abscessus* stimulation. This suggests a selective pathogen-specific immunodeficiency in CF patients infected with *M. abscessus* shown by the total absence of *M. abscessus*-specific IL-17 production. Interestingly, the *Aspergillus*-specific Th-1 and -17 responses did not show differences between the groups.

CF is associated with a high prevalence of *M. abscessus* infections [1], but the underlying host defects leading to this increased susceptibility are not fully understood. Abnormalities in the IL-12/IFN- γ -signalling pathways are a known risk factor for mycobacterial infections [8], and decreased IL-17 production in response to *M. avium* has been described in PBMCs isolated from patients with NTM lung disease [9]. This is in line with the low Th cytokine deficiency of the pulmonary NTM patients in this study. A protective role in mucosal immunity against extracellular fungal and bacterial infections has been described for the Th-17 subpopulation, especially in the lung [10]. Although some of the healthy volunteers responded only poorly on *M. abscessus* stimulation, in most donors a robust Th-17 response was induced, as described previously [11]. The three CF patients in this study demonstrated a *M. abscessus*-specific IL-17 deficiency, while the cells were not intrinsically deficient in IL-17 production. Despite earlier reports that described significantly elevated IL-17 level in sputum and airway mucosa of CF patients, we propose that *M. abscessus*-specific IL-17 deficiency can promote the susceptibility to chronic NTM infections [12].

Treatment with biological drugs blocking innate cytokines that promote Th-17 differentiation is associated with an increased risk of NTM infections [13]. Therefore we propose the inverse correlation that patients with low IL-17-responses, such as the three CF patients in this study, might profit from an IL-17 supporting treatment regimen. Only a few studies have been performed to date. Next to treatment with IFN- γ , which had beneficial effects on the IL-17 and IL-22 production in patients with severe fungal infection [14], one might speculate about the supplementation of recombinant IL-17. Low-dose IL-17 has recently been shown to prevent diabetic nephropathy and organ fibrosis [15].

In conclusion, we describe pathogen-specific cytokine signatures in three CF patients with *M. abscessus* infection, two of them with ABPA, which might contribute to their higher susceptibility of NTM and *Aspergillus*-specific responses. The *M. abscessus*-specific IL-17 deficiency, rather than an IFN- γ defect might play a crucial role in promoting pulmonary NTM infections in CF. This might have direct implementation for different immune-modulatory treatment regimens in pulmonary NTM infection in patients, either with or without CF.



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Mycobacterium abscessus*-specific IL-17 deficiency is present in cystic fibrosis patients with *M. abscessus <http://ow.ly/U2DNL>

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