Autoimmunity to bactericidal/permeability-increasing protein in bronchiectasis exhibits a requirement for *Pseudomonas aeruginosa* IgG response

To the Editor:

In bronchiectasis, due to diseases other than cystic fibrosis, idiopathic, genetic and environmental factors alter the airway landscape and immune responses, rendering the patients susceptible to infection, with the Gram-negative bacterium (GNB) *Pseudomonas aeruginosa* as a major contributor to mortality [1, 2]. The high prevalence of *P. aeruginosa* in bronchiectasis patients cannot be explained by a single genetic or environmental influence, and immunological permissiveness of bronchiectasis airways to this colonisation remains unexplained [2]. A similar predisposition to this pathogen is characteristic of cystic fibrosis patients, in whom a single genetic mutation (*CFTR*) shapes the abnormal lung environment.

In cystic fibrosis, where *P. aeruginosa* colonises the airways of up to 80% of patients, we and others have proposed that the inability of the innate immune system to combat *P. aeruginosa* infection is related, in part, to an autoimmune antibody response to bactericidal/permeability-increasing protein (BPI), a neutrophil antimicrobial protein [3]. Through high-affinity binding of lipopolysaccharides (LPS) on the bacterial outer envelope, BPI mediates extracellular bactericidal and LPS neutralising functions [4]. Autoantibodies to BPI were first reported in European cystic fibrosis cohorts and confirmed by us in a US cohort of adult cystic fibrosis patients [5, 6]. Notably, autoreactivity to BPI was associated with diminished lung function while *in vitro* functional studies demonstrated that anti-BPI IgG inhibits its biological activities [7–9].

In this research letter, we ask two critical questions regarding the immunological interactions that shape the bronchiectatic airway permissiveness to *P. aeruginosa* infection. 1) Do bronchiectasis patients develop autoimmunity to BPI? 2) What is the relationship between autoreactivity to BPI and chronic infection by *P. aeruginosa*? To address these questions, autoantibodies to BPI in patient sera were measured by ELISA in two bronchiectasis cohorts from the USA: one from Dartmouth Hitchcock Medical Center (DHMC) in Lebanon, NH (n=16), and the other from Oregon Health and Science University (OHSU) in Portland, OR (n=42). Immunoblotting of sera negative by ELISA yielded a low frequency of additional seropositivity (~10%). BPI autoreactivity was identified at nearly identical frequencies in the DHMC (56%) and OHSU (52%) cohorts (figure 1a and b).

We and others have reported an association between autoreactivity to BPI and the presence of *P. aeruginosa* in sputum culture of cystic fibrosis patients [5, 6]. Given these findings, we evaluated the relationship between anti-BPI autoimmunity and chronic *P. aeruginosa* infection in bronchiectasis by measuring anti-*P. aeruginosa* IgG titres in patient sera as a serological marker of chronic infection. Healthy control sera exhibited little or no reactivity against PA14 extract (figure 1b). Autoreactivity to BPI in the DHMC bronchiectasis cohort was strongly associated with the existence of an antibody response to *P. aeruginosa* (n=16, p=0.003) (figure 1c). This association was confirmed in an independent cohort of bronchiectasis patients (OHSU; n=42, p=0.002) (figure 1d). Each bronchiectasis cohort exhibited a dichotomised relationship between anti-BPI autoreactivity and the presence of anti-*P. aeruginosa* antibodies (figure 1c and d). Together, these findings represent the first report of anti-BPI autoimmunity

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Large subpopulations of patients with bronchiectasis due to diseases other than cystic fibrosis (~50% in two independent cohorts) develop autoantibodies to BPI, which arise specifically in the context of chronic *Pseudomonas aeruginosa* infection http://ow.ly/1LHZ30mtfXu

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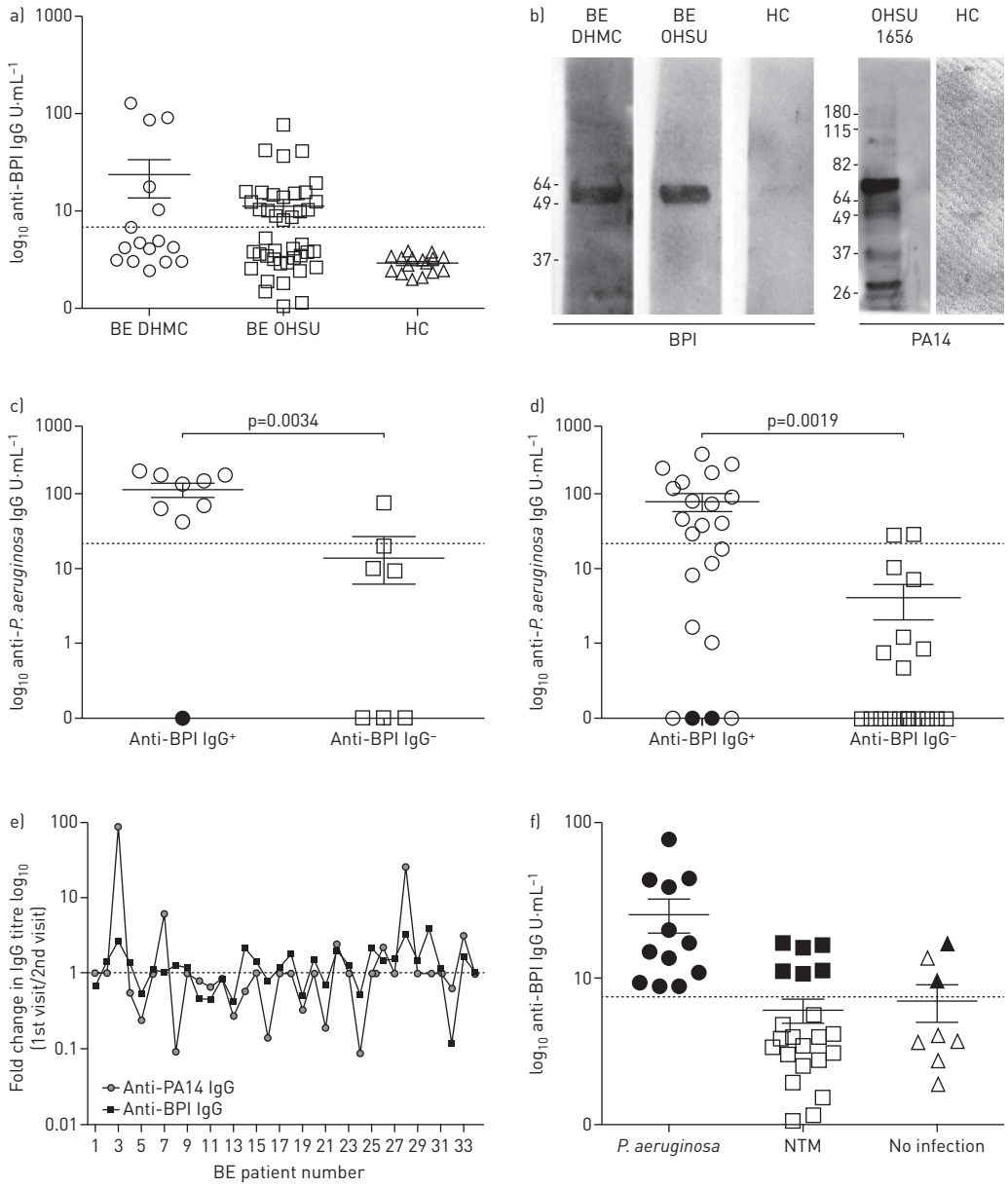

FIGURE 1 High anti-bactericidal/permeability-increasing protein (BPI) IgG titers in bronchiectasis (BE) patients associate with chronic *Pseudomonas aeruginosa* infection in a temporal and pathogen-specific manner. a) Anti-BPI IgG titers detected by ELISA in two BE cohorts: Dartmouth Hitchcock Medical Center (DHMC) (n=16, 37.5% positive) and Oregon Health and Science University (OHSU) (n=42, 45.2% positive). Anti-BPI IgG positive samples >6 U·mL^{-1}; positive cut-off determined as mean of healthy controls (HC)+2SD (n=16), represented by dashed line. b) Representative immunoblots of bronchiectasis serum reactivity to BPI (5 µg) and *P. aeruginosa* PA14 lysate (10 µg). c and d) Anti-BPI IgG positivity, determined by ELISA and immunoblot, associates with antibody reactivity to *P. aeruginosa* (PA14 lysate) in both BE cohorts: c) BE DHMC and d) BE OHSU; reactivity to *P. aeruginosa* determined by ELISA (positive cut-off of >22 U·mL^{-1} represented by dashed line); filled symbols represent positive reactivity to *P. aeruginosa* by immunoblot. e) Fold change in anti-BPI and anti-*P. aeruginosa* IgG titers over two sequential visits in a retrospective longitudinal cohort of BE patients from OHSU (n=34; r=0.623; p=8.412×10^{-5} as determined by Spearman correlation analysis). f) Anti-BPI IgG positivity in BE patients (OHSU) is associated with positive *P. aeruginosa* sputum culture. Positive sputum culture for nontuberculous mycobacterium (NTM) does not associate with anti-BPI IgG positivity in the absence of antibody response to *P. aeruginosa*. No infection: no current NTM infection. Filled symbols represent positive antibody reactivity to *P. aeruginosa*.

in bronchiectasis patients in the USA and indicate that autoreactivity to BPI develops specifically in the context of chronic *P. aeruginosa* infection, independently of a single genetic factor.

The relationship between the IgG antibody responses to *P. aeruginosa* and BPI was further examined through a retrospective longitudinal study of sera from 34 bronchiectasis patients. Serological analyses

demonstrated that levels of antibodies targeting BPI and *P. aeruginosa* changed in the same direction over two consecutive visits; *i.e.* an increase in anti-*P. aeruginosa* IgG titres was accompanied by a positive fold change in anti-BPI autoantibody titres, while a reduction in anti-*P. aeruginosa* antibody levels tracked together with a negative fold change in anti-BPI IgG titres (figure 1e). Therefore, the autoimmune response to BPI follows the same temporal pattern as the humoral response to *P. aeruginosa*.

The specificity of this interaction with *P. aeruginosa* exposure and autoantibodies to BPI was also examined in relation to nontuberculous mycobacterium (NTM). Segregation of the OHSU bronchiectasis cohort by sputum culture yielded three patient populations: history of positive *P. aeruginosa* (with some overlapping NTM positivity within 6 months), NTM positive within 6 months, or no current infection. Serological analyses of each population demonstrated that anti-BPI IgG were absent in patients colonised with NTM or in those without an infection by sputum culture, unless accompanied by a positive anti-*P. aeruginosa* IgG titre (figure 1f). A positive sputum culture for *P. aeruginosa* was reported in only 55% of patients positive for anti-*P. aeruginosa* IgG, indicating that the serological analysis captures prior, as well as current, *P. aeruginosa* infection [10].

In this study, we report that anti-BPI autoreactivity in bronchiectasis is strongly associated with chronic *P. aeruginosa* infection, characterised by the presence of anti-*P. aeruginosa* antibodies. We observed this identical association in two bronchiectasis cohorts from New England and the Pacific Northwest. The synchronised changes in antibody reactivity to *P. aeruginosa* and BPI in a longitudinal cohort of bronchiectasis patients (figure 1e) further support the model that the breaking of tolerance to BPI is mediated through an association with chronic *P. aeruginosa* infection. The remarkable conservation of the linkage between autoreactivity to BPI and chronic *P. aeruginosa* infection in bronchiectasis is heightened by: 1) the heterogeneous genetic nature of bronchiectasis [11] and 2) identical associations in cystic fibrosis [5, 6]. Together, these data argue against a human leukocyte antigen-dependent mechanism by which tolerance is broken, which is further bolstered by a similar relationship between BPI autoreactivity and immune response to *P. aeruginosa* in a bronchiectasis cohort from Japan [12]. Several potential mechanisms leading to the breach of tolerance to BPI in the context of *P. aeruginosa* infection warrant investigation: 1) molecular mimicry, 2) cross-activation of immune response due to LPS:BPI complexing and 3) cryptic epitope reveal due to differential BPI processing during inflammation. The latter model has been supported by our previous findings that *P. aeruginosa*-stimulated neutrophil extracellular trap formation leads to BPI cleavage and a possible reveal of neoepitope(s) [6].

The strength of the association between autoreactivity to BPI and chronic *P. aeruginosa* infection stands out in marked contrast to other autoantibodies against neutrophil azurophilic granule proteins (anti-neutrophil cytoplasmic antibodies (ANCA)), such as serine proteinase 3 and myeloperoxidase where no one infectious trigger has been defined [13]. In ANCA-associated vasculitis, while infectious stimuli have been implicated in the disease onset, their influence is only relevant in the context of genetic factors [14], unlike the *P. aeruginosa*–BPI interaction that argues against a genetic component of peptide restriction. Beyond the issue of immunopathogenesis of anti-BPI reactivity lie the functional implications of this autoreactivity. Three main functional roles of BPI have been proposed: 1) bactericidal, *via* LPS binding and permeabilisation of the GNB membrane; 2) inflammatory, *via* transport of GNB to dendritic cells; and 3) anti-inflammatory, *via* LPS neutralisation and down-modulation of monocyte response [15]. The temporal relationship between anti-*P. aeruginosa* and anti-BPI IgG titres suggests that autoimmune responses to BPI hinder its bactericidal and anti-inflammatory functions to facilitate colonisation/infection by *P. aeruginosa*. Thus, rather than viewing anti-BPI as a highly linked epiphenomenon of *P. aeruginosa* infection, these autoantibodies may play a causal role in the perpetuation of infection. In this model, strategies that eliminate anti-BPI reactivity may enhance clearance of *P. aeruginosa*, leading to improved airway function and clinical outcomes. Creating a model system in which the functional role of BPI and anti-BPI responses can be tested *in vivo* would seem to be a necessary first step in addressing this question.

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