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Early View

Research letter

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Research Letter

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

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In non-cystic fibrosis bronchiectasis (BE), 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 BE patients cannot be explained by a single genetic or environmental influence, and immunologic permissiveness of BE airways to this colonization remains unexplained (2). A similar predisposition to this pathogen is characteristic of CF patients, in whom a single genetic mutation (*Cftr*) shapes the abnormal lung environment.

In CF, where *P. aeruginosa* colonizes the airways of up to 80% of patient, 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 anti-microbial protein (3). Through high-affinity binding of lipopolysaccharides (LPS) on the bacterial outer envelope, BPI mediates extracellular bactericidal and LPS neutralizing functions (4). Autoantibodies to BPI were first reported in European CF cohorts and confirmed by us in a United States cohort of adult CF patients (5, 6). Notably, autoreactivity to BPI was associated with diminished lung function while *in vitro* functional studies demonstrated that anti-BPI IgG inhibit its biological activities (7-9).

In this research letter we ask two critical questions regarding the immunologic interactions that shape the BE airway permissiveness to *P. aeruginosa* infection: i) Do BE patients develop autoimmunity to BPI? and ii) 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 BE cohorts from the United States: one from

Dartmouth Hitchcock Medical Center (DHMC) in Lebanon, New Hampshire (n=16) and the other from Oregon Health & Science University (OHSU) in Portland, Oregon (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-B).

We and others have reported an association between autoreactivity to BPI and presence of *P. aeruginosa* in sputum culture of CF patients (5, 6). Given these findings, we evaluated the relationship between anti-BPI autoimmunity and chronic *P. aeruginosa* infection in BE by measuring anti-*P. aeruginosa* IgG titers in patient sera as a serologic marker of chronic infection. Healthy control sera exhibited little or no reactivity against PA14 extract (Figure 1B). Autoreactivity to BPI in the DHMC BE 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 BE patients (OHSU, $n= 42$, $p = 0.002$) (Figure 1D). Each BE cohort exhibited a dichotomized relationship between anti-BPI autoreactivity and the presence of anti-*P. aeruginosa* antibodies (Figure 1C-D). Together, these findings represent the first report of anti-BPI autoimmunity in BE patients in the United States 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 BE patients. Serologic 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 titers was accompanied by a positive fold change in anti-BPI autoantibody titers, while a reduction in anti-*P. aeruginosa* antibody levels tracked together with a negative fold change in anti-BPI IgG titers (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 BE 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. Serologic analyses of each population demonstrated that anti-BPI IgG were absent in patients colonized with NTM or in those without an infection by sputum culture, unless accompanied by a positive anti-*P. aeruginosa* IgG titer (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 serologic analysis captures prior, as well as current, *P. aeruginosa* infection (10).

In this study, we report that anti-BPI autoreactivity in BE is strongly associated with chronic *Pseudomonas aeruginosa* infection, characterized by the presence of anti-*P. aeruginosa* antibodies. We observed this identical association in two BE cohorts from New England and the Pacific Northwest. The synchronized changes in antibody reactivity to *P. aeruginosa* and BPI in a longitudinal cohort of BE 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 BE is heightened by: i) the heterogeneous genetic nature of BE (11) and ii) identical associations in CF (5,6). Together, these data argue against an HLA-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 BE cohort from Japan (12). Several potential mechanisms leading to the breach of tolerance to BPI in the context of *P. aeruginosa* infection warrant investigation: i) molecular mimicry, ii) cross-activation of immune response due to LPS:BPI complexing, and iii) 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 revel 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 permeabilization of GNB membrane, 2) inflammatory, via transport of GNB to DCs and 3) anti-inflammatory, via LPS neutralization and down modulation of monocyte response (15). The temporal relationship between anti-*P. aeruginosa* and anti-BPI IgG titers suggests that

autoimmune responses to BPI hinder its bactericidal and anti-inflammatory functions to facilitate colonization/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 which 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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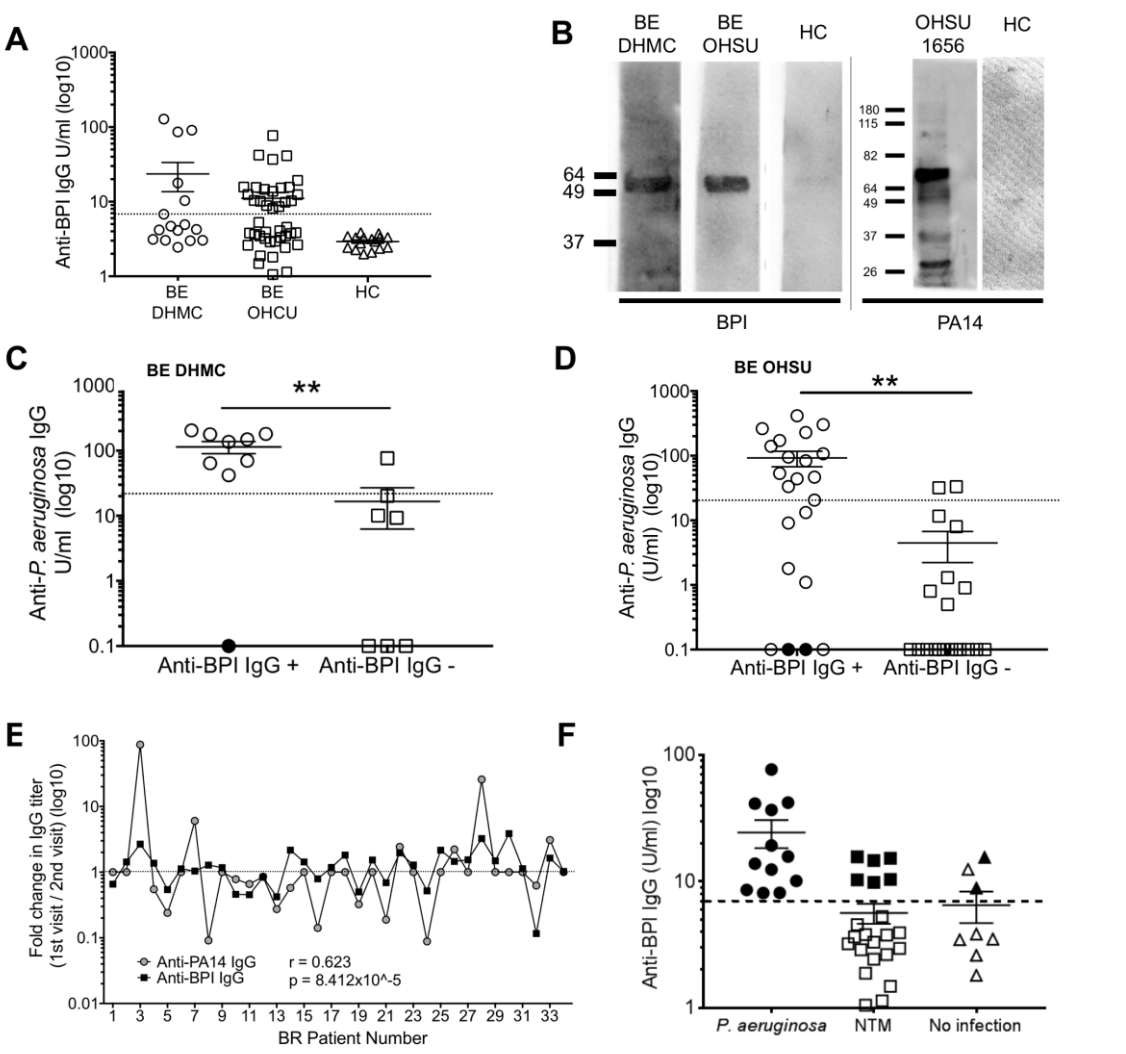

Figure 1: High anti-BPI IgG titers in BE patients associate with chronic *P. aeruginosa* infection, in a temporal and pathogen-specific manner. (A) Anti-BPI IgG titers detected by ELISA in two non-CF bronchiectasis cohorts: BE DHMC (n=16, 37.5% positive) and BE OHSU cohort (n=42, 45.2% positive). Anti-BPI IgG positive samples > 6 U/ml; positive cutoff determined as mean of healthy controls + 2SD (n=16), represented by dashed line. **(B)** Representative immunoblots of bronchiectasis serum reactivity to BPI (5µg) and *P. aeruginosa* PA14 lysate (10µg). **(C-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 **p=0.0034 and (D) BE OHSU **p=0.0019; reactivity to *P. aeruginosa* determined by ELISA (positive cutoff of >22 U/ml 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 Oregon Health and Science University (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 non-tuberculous 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*.