Journal of Translational Autoimmunity 4 (2021) 100105

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Killing three birds with one BPI: Bactericidal, opsonic, and anti-inflammatory functions

Jomkuan Theprungsirikul [a], Sladjana Skopelja-Gardner [b], William F.C. Rigby [a,b,*]

[a] Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Lebanon, NH, United States
[b] Division of Rheumatology, Department of Medicine, Geisel School of Medicine at Dartmouth, Lebanon, NH, United States

ARTICLE INFO

Keywords:
Bactericidal/permeability-increasing protein (BPI)
Gram-negative bacteria
Opsonization
Antimicrobial peptides
Anti-inflammation
Autoantibody

ABSTRACT

Bactericidal/permeability-increasing protein (BPI) is an anti-microbial protein predominantly expressed in azurophilic granules of neutrophils. BPI has been shown to mediate cytocidal and opsonic activity against Gram-negative bacteria, while also blunting inflammatory activity of lipopolysaccharide (LPS). Despite awareness of these functions *in vitro,* the magnitude of the contribution of BPI to innate immunity remains unclear, and the nature of the functional role of BPI *in vivo* has been submitted to limited investigation. Understanding this role takes on particular interest with the recognition that autoimmunity to BPI is tightly linked to a specific infectious trigger like *Pseudomonas aeruginosa* in chronic lung infection. This has led to the notion that anti-BPI autoantibodies compromise the activity of BPI in innate immunity against *P. aeruginosa*, which is primarily mediated by neutrophils. In this review, we explore the three main mechanisms in bactericidal, opsonic, and anti-inflammatory of BPI. We address the etiology and the effects of BPI autoreactivity on BPI function. We explore BPI polymorphism and its link to multiple diseases. We summarize BPI therapeutic potential in both animal models and human studies, as well as offer therapeutic approaches to designing a sustainable and promising BPI molecule.

1. Introduction

Patients with cystic fibrosis (CF), bronchiectasis, and chronic obstructive pulmonary disease (COPD) characterized by persistent airway infection by *Pseudomonas aeruginosa* exhibit increased morbidity and mortality [1–7]. Moreover, impaired lung function in these chronic lung diseases is independently associated with the production of autoantibodies against bactericidal/permeability-increasing protein (BPI) [7–10]. BPI is a protein of ~55 kDa and is stored in primary azurophilic granules of neutrophils [11,12]. Upon neutrophil encounter of Gram-negative bacteria, BPI is released to mediate bactericidal effects, phagocytosis, and uptake of bacteria by neutrophils and dendritic cells (DCs), while neutralizing the inflammatory activity of lipopolysaccharide (LPS) [11,13–19]. Given the strong association between *P. aeruginosa* chronic infection and autoreactivity to BPI [8,20–22], it is possible that autoantibodies to BPI enable bacteria to evade the immune response, contributing to worsening disease state in these patients. In this review article, we summarize and explore the functional biology of BPI in innate immunity and the possible modulation of this pathway by adaptive immune responses.

2. Structural and functional characteristics of BPI

BPI, first purified and characterized by Weiss et al., in 1978 [11,12], is a cationic antimicrobial protein, part of the BPI-fold containing (BPIF) superfamily [23,24]. Connected by a central beta pleated-sheet, the protein exhibits N- and C-terminal barrel-shaped domains that are structurally similar despite a low level of amino acid identity [23,24] (Fig. 1). A separate branch of this superfamily is the Palate Lung and Nasal epithelium Clone (PLUNC) proteins, whose expression is chiefly limited to the airway epithelial cells in vertebrates [25]. BPI is detectable at the promyelocyte stage of myeloid development [11] and subsequently found in azurophilic granules of neutrophils and to a lesser extent in eosinophils, dermal fibroblasts, macrophages, and certain mucosal epithelial cells [1,12,26–29], although dermal fibroblast and epithelial cells require stimulation for BPI expression [27,29].

The N-terminal domain of BPI binds lipopolysaccharide (LPS) with nanomolar affinity [30–32]. Neutrophil degranulation releases BPI wherein its N-terminal domain mediates binding to the negatively charged lipid A moiety of LPS expressed on the bacterial outer envelope [30,33]. This interaction destabilizes the integrity of the bacterial

* Corresponding author. Division of Rheumatology, One Medical Center Drive, Lebanon, NH, 03756, United States.
E-mail address: william.f.c.rigby@dartmouth.edu (W.F.C. Rigby).

https://doi.org/10.1016/j.jtauto.2021.100105
Received 10 April 2021; Received in revised form 10 May 2021; Accepted 15 May 2021
Available online 28 May 2021

membrane, leading to bacterial lysis and cell death in solution [8,12,13,30,33–35]. The C-terminal portion of BPI has been implicated in LPS binding and opsonization but these activities are less well understood [36,37]. BPI additionally mediates intracellular killing of Gram-negative bacteria following phagocytosis via fusion with phagolysosome containing secreted BPI [38]. The presence of BPI has also been reported in neutrophil extracellular traps, providing yet another route for bacterial killing and clearance [39,40].

The N-terminal domain of human BPI (amino acids 1–230) is linked to the C-terminal domain (amino acids 250–456) of BPI by a proline-rich hinge region (amino acids 230–250) that also contains an elastase cleavage site (amino acids 240–245) [23]. As stated above, both domains are of similar size, secondary structure, and topology which give rise to its boomerang shape. Finally, recent work suggests that BPI also binds Gram-positive *Staphylococcus aureus* lipopeptides with nanomolar affinity [32]. Binding of these lipopeptides is blocked by LPS, suggesting the N-terminal BPI domain also interacts with Gram-positive cocci [32]. Moreover, BPI was shown to enhance the immune response (elevated levels of TNFα, IL-6 and IL-8) toward Gram-positive ligands in peripheral blood mononuclear cells (PBMCs) when synthetic bacterial lipopeptides, lipoteichoic acid (major cell wall component of Gram-positive bacteria), and lysates of Gram-positive bacteria were used [32]. Interestingly, BPI was not shown to have a direct bactericidal effect on Gram-positive bacteria in previous studies [12,41].

3. Direct cytocidal activity of BPI

It has been shown that neutrophils derived from newborn umbilical cord blood express less BPI than those from adults [42,43]. Moreover, newborn neutrophils are defective in their phagocytic and bactericidal activity against *S. aureus* and *Escherichia coli* [44]. Acid extracts of newborn neutrophils also exhibit decreased antibacterial activity against serum-resistant *E. coli* [43], rendering them less effective in these infections. Chronic granulomatous disease (CGD) neutrophils exhibit defective phagocyte NADPH oxidase and hence, reduced antimicrobial hydrogen peroxide production [45]. Despite this defect against Gram-positive cocci like *S. aureus*, neutrophils from CGD patients are capable of killing *E. coli* [38], suggesting that BPI mediates bacterial clearance independent of reactive oxygen species (ROS) [38,45]. Moreover, purified antibodies to BPI from human serum has been shown to inhibit BPI (from neutrophil extracts) from killing *E. coli* [46], further validating the independent cytocidal activity of BPI.

Overall, most evidence points to the N-terminus as solely responsible for the bactericidal activity of BPI. When the cationic BPI N-terminus binds to the negatively charged LPS phosphate groups (Fig. 2), this action displaces divalent cations which disturbs the arrangement of LPS molecules and the bacterial membrane potential, causing membrane rupture [33]. This BPI-mediated outer membrane damage halts bacterial growth and allows cellular entry of other synergistic anti-microbial peptides, such as cathelicidins and defensins [13]. Additionally, BPI also acts in synergy with the complement system as BPI bactericidal activity toward *E. coli* is inhibited by C7-depleted serum but accelerated by normal serum [47,48]. Following outer membrane damage, subsequent phospholipid hydrolysis and disruption of the inner bacterial membrane ultimately lead to bacterial killing [49]. That BPI binding to LPS is essential for bacterial killing is supported by a study showing Gram-negative bacteria *Proteus mirabilis* are less susceptible to BPI killing, presumably due to steric hindrance of BPI access to the lipid A in the long polysaccharide chains with tightly packed LPS seen in this organism [50]. The targeting of LPS gives rise to bacterial mutations, such as fatty acid additions and hydroxylation or acetylation of the O-antigen, in an attempt to modify their LPS composition and structure to avoid direct target and killing by bactericidal proteins [51]. Interestingly, despite the ability of BPI to bind to lipopeptides/lipoproteins associated with Gram-positive cocci, direct cytocidal activity is not evident unless lipoteichoic acid (LTA), another major component of the cell wall of Gram-positive bacteria, is present, as LTA was shown to be an additional ligand of BPI in Gram-positive bacteria [32]. Moreover, there has been evidence showing that L-forms (i.e. lacking cell wall) of the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* are susceptible to BPI inhibition and killing due to lack of protection of the cytoplasmic membrane [52].

4. Opsonophagocytic activity of BPI

While the bactericidal activity of BPI has been the main focus of the *in vitro* functional studies, the opsonophagocytic function of BPI has not been well understood. Though the N-terminal domain mediates the BPI: LPS binding, the structurally similar carboxy-terminal domain is thought to mediate bacterial opsonization [8,36,37]. The C-terminal BPI fragment was also shown to inhibit inflammation triggered by endotoxin, this activity required 5–10 fold higher molar protein concentrations than the N-terminal domain [31]. Gram negative bacteria, *E. coli* K1/r, pre-incubated with native human BPI are ingested by neutrophils and monocytes [36]. This activity is missing with the recombinant N-terminal domain ($rBPI_{21}$), suggesting the C-terminus promotes

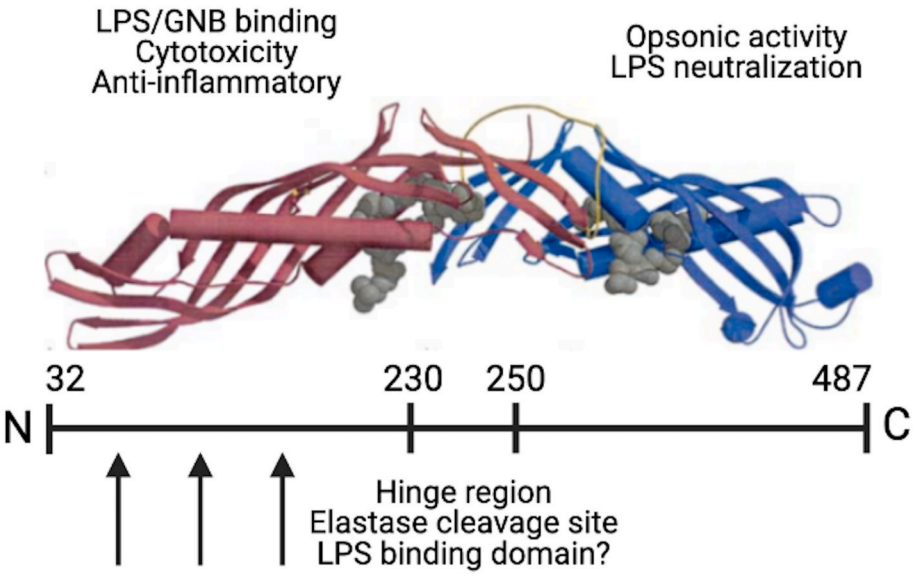

Fig. 1. Structural and functional characteristics of BPI. BPI (amino acids 32–487 after signal sequence cleavage) mediates at least three innate immune activities. Separate functional activities exist in the N-terminus and C-terminus. LPS binding enables direct cytocidal activity against gram-negative bacteria (GNB) and blocks LPS-induced TNF. The N-terminus (aa 32–230) has three LPS binding sites (black arrows). The C-terminus (aa 250–487) opsonizes *P. aeruginosa* bound to the N-terminus and is necessary for clearance of *P. aeruginosa in vivo*. The C-terminus and hinge (aa 231–249) region exhibits LPS binding activity *in vitro* suggesting their possible contribution to *P. aeruginosa* clearance. Structure of BPI shown here was reported previously [24].

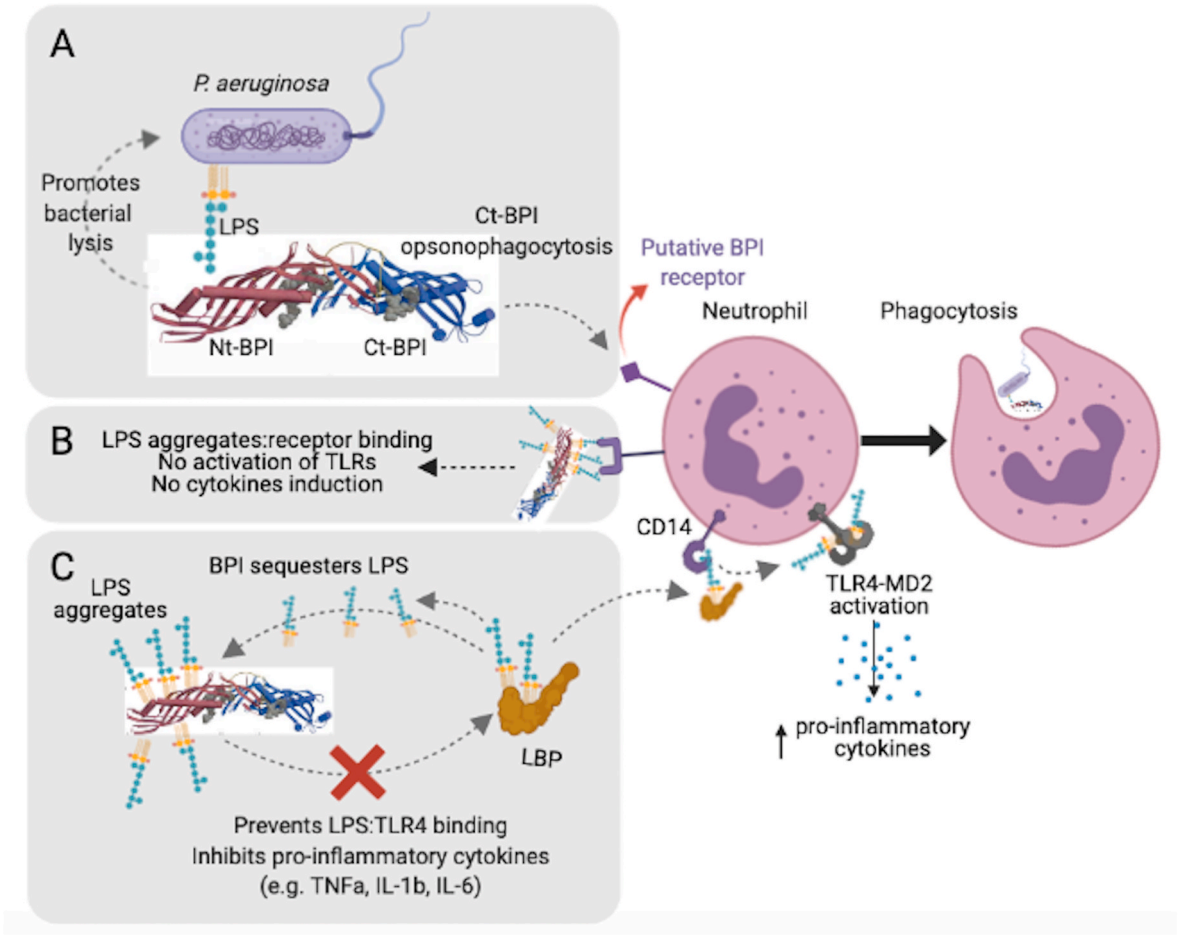

Fig. 2. Proposed model of BPI functions in bactericidal, anti-inflammatory, and opsonophagocytosis of *Pseudomonas aeruginosa*. (A) The cationic N-terminal portion of BPI binds to the negatively-charged LPS contained in the outer membrane of *P. aeruginosa,* and promotes bacterial lysis by destabilizing integrity of bacterial membrane. By binding to LPS, BPI also acts to inhibit pro-inflammatory cytokines released by the host through LPS neutralization. The C-terminal portion of BPI opsonizes *P. aeruginosa* through a putative BPI receptor on neutrophil, resulting in opsonophagocytosis of the bacteria. Presence of BPI is critical due to its anti-inflammatory and phagocytosis induction properties. **(B)** LPS aggregates bind to a receptor on neutrophils but do not activate TLR and therefore, no pro-inflammatory cytokines are released. **(C)** LPS-binding protein (LBP) catalyzes and disperses LPS aggregates and delivers the monomers to CD14/TLR-4 receptor complexes, triggering the release of pro-inflammatory cytokines. Due to its high affinity for LPS, BPI increases the size of LPS aggregates, thereby sequestering LPS from interacting with LBP and blunting inflammatory activities of monocytes by CD14-independent and dependent mechanisms. Structure of BPI shown here was reported previously [24].

bacterial phagocytosis, possibly via direct binding (Fig. 2). This model was supported by the observation that the uptake of Gram-negative bacteria by DCs is promoted by the C-terminal domain [8]. However, addition of the recombinant BPI (full length) to serum promotes phagocytosis of *E. coli* but not *S. aureus* by promoting complement activation (deposition of C3b/iC3b fragments) on the bacterial surface, possibly indicating indirect effects of BPI on phagocytosis [53]. The importance of the opsonophagocytic role of BPI has recently been highlighted by the *in vivo* studies using BPI-deficient mice. The absence of BPI impaired neutrophil phagocytosis and clearance of *P. aeruginosa* in acute infection. The ability of BPI-deficient mice to clear *P. aeruginosa* was corrected with the administration of neutrophil-purified human BPI [129]. Intracellular uptake of exogenous BPI and *P. aeruginosa* complex was observed, reinforcing the notion that BPI mediates phagocytosis *in vivo* [129]. Recent study has shown that CD18, a β2 integrin expression facilitates the uptake of both motile and nonmotile *P. aeruginosa* strains by phagocytes [54]. BPI-enhanced phagocytosis and *P. aeruginosa* clearance were inhibited by CD18 blockade *in vivo*. These data further supports evidence of phagocytosis rather than bactericidal activity of BPI [129]. Therefore, BPI not only facilitates leukocyte clearance of Gram-negative bacteria, but it also promotes antigen uptake and presentation, which can serve as a necessary link between innate anti-bacterial defenses and induction of adaptive immune responses [55].

5. Anti-inflammatory activity of BPI

In addition to facilitating the clearance of Gram-negative bacteria, BPI has been reported to exhibit anti-inflammatory effects by regulating LPS-triggered cytokine responses (Fig. 2). BPI belongs to the family of lipid-transfer proteins mentioned earlier including LPS binding protein (LBP) that is present in normal serum [56,57]. However, unlike LBP, which facilitates pro-inflammatory activation of monocytes by LPS, BPI binding to LPS blunts its ability to trigger endotoxin activation [58]. LBP catalyzes and disperses LPS aggregates and delivers the monomers to CD14/TLR-4 receptor complexes, triggering the release of pro-inflammatory cytokines [58]. Due to its high affinity for LPS, BPI increases the size of LPS aggregates, thereby sequestering LPS from interacting with LBP and blunting inflammatory activities (i.e. pro-inflammatory cytokine production and release) of monocytes by CD14-independent and dependent mechanisms [37,58–60] (Fig. 2). This anti-inflammatory effect is seen with the N-terminal domain [31,61] and in one study the C-terminal domain alone [31]. Interestingly, despite the ability of BPI to bind to lipopeptides and lipoproteins on *S. aureus,* no

inhibition of cytokine release is seen, suggesting specificity of BPI effects on LPS-mediated activation and cytokine release [32]. In fact, there was a dose-dependent increase in TNFa, IL-6, and IL-8 secretion with increasing concentration of BPI in the presence of lipopeptides and lipoproteins, suggesting that BPI enhances the immune response toward Gram-positive ligands [32].

Recent *in vivo* studies of acute *P. aeruginosa* infection have demonstrated enhanced neutrophil recruitment and inflammatory cytokine production in the absence of BPI [129]. Administration of exogenous human BPI reduced cellular inflammation and cytokine production (TNF, IL-6, IL-1b) at the site of infection [129]. Therefore, besides mediating specific and direct killing of Gram-negative bacteria and facilitating bacterial opsonization, BPI also mediates endotoxin neutralization via mechanisms that simultaneously work to eradicate bacterial infection and to dampen excessive inflammation.

6. Autoantibodies to BPI and their impact on BPI-dependent immunity

6.1. Etiology

Mysteriously, BPI and other contents of the azurophilic granules have been recognized as frequent targets of humoral autoimmunity [62]. Anti-neutrophil cytoplasmic autoantibodies (ANCA) are found in primary vasculitic syndromes, granulomatosis and polyangiitis (GPA) and microscopic polyangiitis (MPA) exhibit specificity for azurophilic granular proteins proteinase 3 (PR3) and myeloperoxidase (MPO) [63–66]. These relationships prompted the discovery of BPI-ANCA in a subset of vasculitis patients in the early 1990s [67,68]. Despite their shared origin in neutrophil granules, the etiology and the specificity of these responses are not understood [69]. Defects in the progression of apoptosis or in the removal of apoptotic cells [69–71] have been proposed for the production of ANCA *in vivo*. In this regard, the presence of these autoantigens on neutrophil extracellular traps (NETs) is proposed to lead to the breaking of tolerance to self-protein [72]. ANCA targeting of different NET-associated bactericidal proteins rarely track together despite the fact that those proteins are localized to the same neutrophil azurophilic granules [73,74], suggesting the disease- or infection-specific nature of ANCA. Autoantibodies to BPI have a remarkable restriction to *P. aeruginosa* infection, and presence of BPI antibodies is associated with worse disease outcome [6,10,14].

Anti-BPI autoantibodies have been peculiar for a number of reasons, most notably their strong linkage with patients suffering from various diseases, particularly cystic fibrosis (CF) [10,75,76] and bronchiectasis [6,67], but also including inflammatory bowel diseases (IBD) [63,77,78], vasculitis [63,67,68,79], reactive arthritis [80], necrotizing and crescentic glomerulonephritis [81], and primary sclerosing cholangitis [82,83]. The etiopathogenesis of BPI autoantibodies is unknown. Three possible models for breaking of immune tolerance to BPI have been proposed: i) molecular mimicry, ii) cross-activation of immune response to BPI:bacterial complex, and iii) immune response to BPI cryptic epitope generated from interaction with bacteria [6,10] (Fig. 3A–C).

i) The relationship of autoimmunity to specific infection remains obscure except for *P. aeruginosa* particularly in the lung. Our recent studies have indicated molecular mimicry as the less likely mechanism. In a cohort of bacteremic patients, we showed that BPI autoantibodies were present in patients with Gram-positive as well as Gram-negative sepsis [84]. Anti-BPI antibodies in bacteremic patients (acute infections) were of low-avidity [84], compared to those in CF or bronchiectasis (chronic infections) patients [6,10], suggesting the breaking of tolerance to BPI arises through affinity maturation rather than cross-reactivity to *P. aeruginosa* [10] (Fig. 3B and C). Thus, high avidity anti-BPI antibodies are restricted to patients with chronic lung infection by *P. aeruginosa.* In contrast, IBD patient sera frequently exhibits anti-BPI reactivity, these autoantibodies are of low-avidity in contrast to that seen in the lung infection by *P. aeruginosa* (unpublished observation).

ii) Apart from the lung infection and its relationship to ANCA, colonic mucosal levels of BPI are increased in IBD patients [85] and are associated with anti-BPI antibodies in ulcerative colitis patients [77]. However, while higher BPI protein levels are reported in serum of bacteremia patients, there was no correlation between serum BPI protein levels and anti-BPI IgG responses [84]. This evidence suggests a requirement of both presence of BPI and chronic infection/inflammation conditions for the BPI autoantibodies to be generated.

iii) There has been evidence suggesting that exposure of BPI cryptic epitope generated from *P. aeruginosa* interactions (i.e. *P. aeruginosa* elastase) [39] leads to generation of autoantibodies to BPI. We showed that presence of cleaved BPI protein in bronchoalveolar lavage (BAL) samples of CF patients is strongly associated with IgA antibodies to *P. aeruginosa* and BPI [10]. This evidence is consistent with a model by which cleaved BPI antigen formed in the BAL arises in the presence of chronic airway infection by *P. aeruginosa*, and contributes to the breaking of tolerance to BPI in the lungs. This model has been described in Fig. 3C. Additionally, the presence of *P. aeruginosa* infection in the airways could lead to increased neutrophil recruitment to the infection site. Since neutrophil elastase is also contained within neutrophil azurophilic granules alongside BPI [86,87], the concomitant release of both BPI and neutrophil elastase from the activated neutrophils could play a role in cleaving BPI at its elastase sensitive site [23], exposing its cryptic epitope.

6.2. Effects on BPI function

In the presence of BPI autoantibodies, studies in both European and United States CF cohorts have proposed that the innate immune system fails to combat airway *P. aeruginosa* infection [19,20,39,88]. Anti-BPI autoantibodies strongly associate with severity of disease, including poor lung function in CF, bronchiectasis, and COPD [6,7,10]. The strong association between high-avidity anti-BPI autoantibodies with chronic *P. aeruginosa* airway infection [8,20–22], suggests that the efficiency of neutrophils to clear Gram-negative bacteria may be compromised by the autoantibodies to BPI (Fig. 4).

Several *in vitro* studies have attempted to delineate the functional effects of BPI autoantibodies. Goat anti-BPI antibodies neutralize the antibacterial activity of BPI against *E. coli* [89]. Anti-BPI autoantibodies purified from CF or IBD sera prevent BPI-mediated phagocytosis and inhibit neutrophil-mediated killing of Gram-negative bacteria [19,78,90–92]. Anti-BPI antibodies against both the N- and the C-terminal domains isolated from IBD patient sera were able to inhibit bactericidal activity of BPI, and were associated with a more aggressive disease in IBD [78]. For CF, the majority (72%) of anti-BPI antibodies were specific to the C-terminus [19]. While these *in vitro* studies and strong clinical correlations suggest autoantibody-mediated inhibition of BPI function, they do not establish clear functional consequences of autoreactivity to BPI, let alone the phenotype of anti-BPI antibodies in neutralizing BPI function and facilitating bacterial persistence *in vivo*. Having demonstrated functional non-redundancy of BPI in combating *P. aeruginosa* infection *in vivo* [129], we can now interrogate the role for BPI autoantibodies in both acute and chronic murine infection models. These approaches will also allow us to investigate the etiology of anti-BPI autoantibodies *in vivo*.

7. BPI gene polymorphisms and their link to disease

IBD such as Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing inflammatory disorders of the intestine in which the underlying pathophysiology is unknown. Other than in neutrophils, BPI

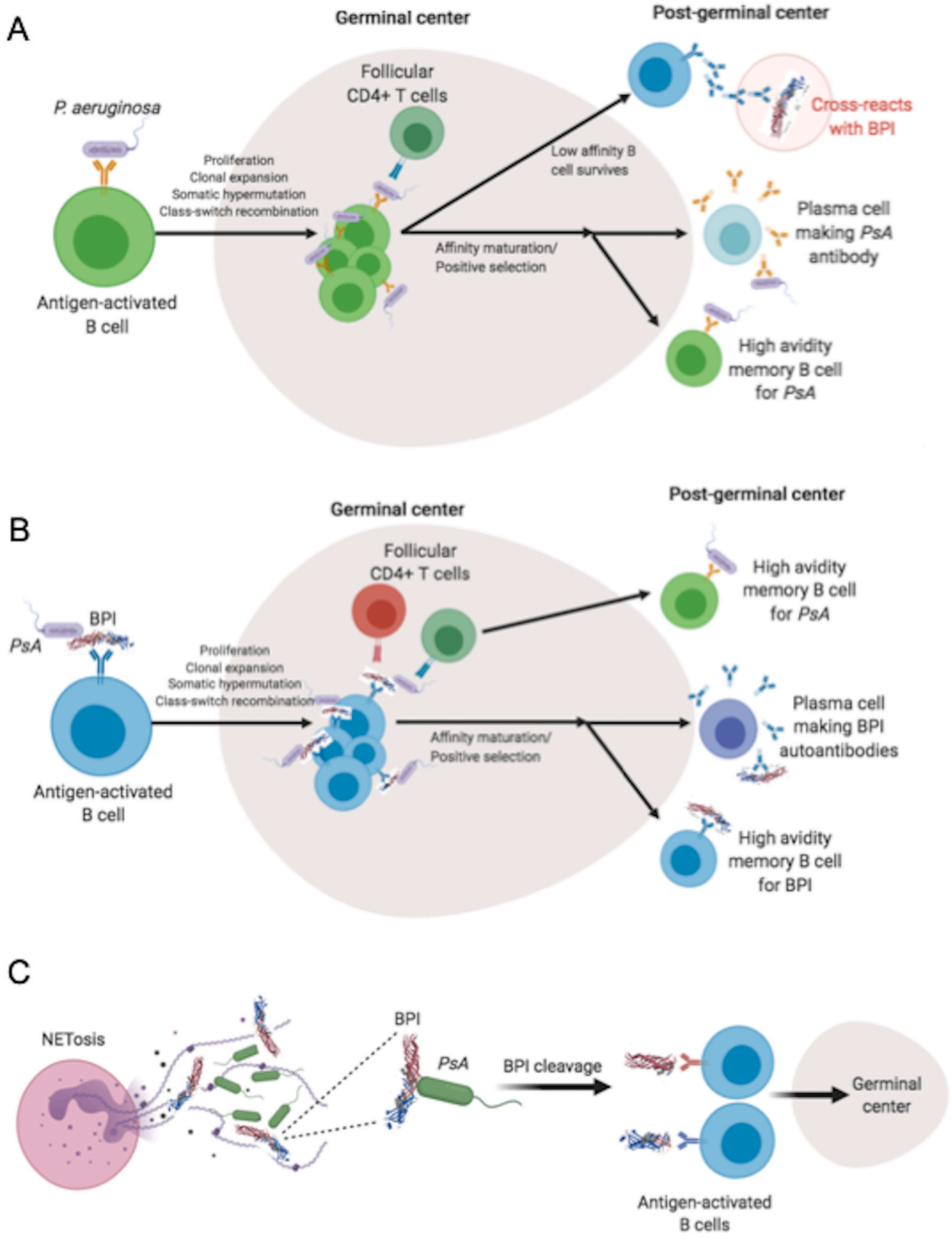

Fig. 3. Proposed models of the etiology of anti-BPI autoantibodies. (A) *Molecular mimicry*: Antigen-activated B cell captures *P. aeruginosa*, undergoes proliferation, clonal expansion, somatic hypermutation, and class-switching in the germinal center before affinity maturation into high avidity plasma cell and memory B cell targeting *P. aeruginosa*, which can cross-react with BPI antigen. **(B)** BPI:*P. aeruginosa* complex enhances uptake of BPI into the germinal center. Antigen-activated B cell captures the complex, undergoes proliferation, clonal expansion, somatic hypermutation, and class-switching in the germinal center. The BPI-*P. aeruginosa* antigens are presented to the T cells, going through class switching and affinity maturation to make high avidity memory B cells and plasma cells targeting either BPI and *P. aeruginosa* antigens. **(C)** *Generation of cryptic epitopes* of cleaved BPI through interaction of *P. aeruginosa* elastase and BPI elastase-sensitive region (amino acids 240–245). Newly generated cryptic BPI epitopes then get picked up by antigen-activated B cells before going through proliferation in the germinal center. Structure of BPI shown was reported previously [24]. *PsA* represents *P. aeruginosa*.

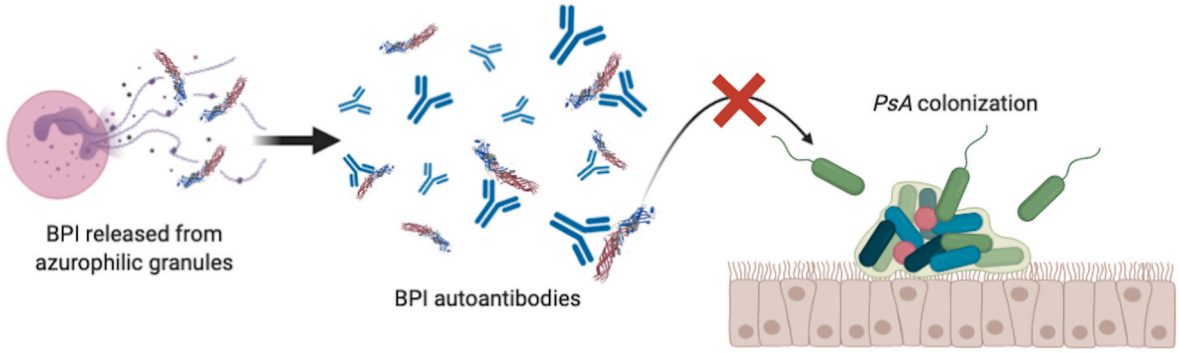

Fig. 4. Presence of BPI autoantibodies compromises the bactericidal effect of BPI. Anti-BPI autoantibodies neutralize the ability of BPI to bind to LPS and kill *P. aeruginosa*, allowing the persistence of *P. aeruginosa* infection in cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease. Structure of BPI shown here was reported previously [24]. *PsA* represents *P. aeruginosa.*

is also produced on the apical and apicobasal surfaces of the human gastrointestinal epithelium [91]. Interestingly, BPI protein concentrations are elevated in gut tissue of UC patients [85,93,94], and BPI-ANCA are elevated with increasing severity of clinical status in IBD [77]. Previously, a gene variant polymorphism of BPI (Lys216Glu) was described in a case control study of sepsis patients [95]. Subsequently the Lys216Glu polymorphism was shown to be associated with both CD and UC [96–98]. It has been speculated that any impairment in the functions of BPI protein through BPI gene polymorphism may alter pathogen recognition by the innate immune system, eventually giving rise to undesirable outcomes in IBD [97], potentially due to gut dysbiosis.

Additional SNPs in the same superfamily of proteins have been associated with increased infection. Bactericidal/permeability-increasing protein fold–containing family member A1 (BPIFA1), formerly known as SPLUNC1, is one of the most abundant proteins produced by epithelial cells of the upper and proximal lower respiratory tract [99,100]. BPIFA1 single-nucleotide polymorphisms (G allele of rs1078761) is reported to be associated with decreased expression of BPIFA1 and with reduced lung function and more severe disease in CF [101,102]. BPI mutation PstI (T→C) polymorphism in intron 5 was associated with an increased risk of developing COPD [103].

Some efforts have been made in investigating the contribution of BPI polymorphisms in other diseases. While a genetic polymorphism in alpha 1-antitrypsin (A1AT) is linked to PR3-ANCA occurrence with a pathogenic role in systemic vasculitis [104], BPI polymorphisms do not appear to contribute to genetic predispositions for granulomatosis with polyangiitis disease [105]. Whether other BPI gene polymorphisms predispose to other diseases harboring chronic infections is still an area under investigation.

8. BPI therapeutic potential

Studies examining the possible therapeutic benefit of BPI often utilize recombinant N-terminal fragments of human BPI: $rBPI_{21}$ or $rBPI_{23}$. The only differences lie in the number of amino acids ($rBPI_{21}$: 1–193, $rBPI_{23}$: 1–199), and the mutation of amino acid at position 132 (cysteine 132 is changed to alanine for $rBPI_{21}$) to reduced dimer formation [106]. This mutation reduces $rBPI_{21}$ heterogeneity and loss of activity is observed in $rBPI_{23}$ while retaining the N-terminal bioactivities of BPI [107], leading to its entry into several clinical trials [108–111]. Experimental animal models and preclinical and clinical studies in humans have demonstrated that exogenously administered recombinant BPI peptides intravenously can exert protective effects in the bloodstream [61,112,113]. Table 1 summarizes the role of BPI in different diseases.

8.1. Animal models

Intravenous administration of a recombinant $rBPI_{21}$ in animal models of sepsis [114], pneumonia [115], and endotoxemia contributed

Table 1
Bactericidal/permeability-increasing protein (BPI) and its role in different diseases. (References: CF: [19,20,39,75,76,88,101,102,124]; BE:[6,67]; COPD: [7,103,125]; Vasculitis: [63,66–68,79,105,126,127]; IBD Crohn's: [77,78,82,96–98]; IBD UC: [77,78,94,96–98]; Sepsis/bacteremia: [84,89,95,114,122]; Pneumonia: [116,128]; Endotoxemia: [107]; Hemorrhage (trauma): [108]; Meningococcal disease: [12,109–111,120].

Disease	Location of action	Function of BPI in these diseases	BPI autoantibody association	Prevalence of BPI autoantibody in selected patient cohorts	BPI gene polymorphism predisposition
Cystic fibrosis	Airways, lungs	Anti-inflammatory, anti-microbial, bacterial clearance	Yes	17.9–83% (49.45% pooled)	Yes
Bronchiectasis	Airways, lungs	Anti-inflammatory, anti-microbial, bacterial clearance	Yes	52–56%	Not reported
Chronic obstructive pulmonary disease	Airways, lungs	Anti-inflammatory, anti-microbial, bacterial clearance	Yes	48.15%	Yes
Vasculitis	Airways, kidneys	BPI-ANCA binding activates neutrophils, enhances vascular injury	Yes	45%	No
Inflammatory Bowel Disease: Crohn's	Intestinal tracts	Anti-inflammatory	Yes	14–75%	Yes
Inflammatory Bowel Disease: Ulcerative colitis	Intestinal tracts	Anti-inflammatory	Yes	29–75%	Yes
Sepsis/bacteremia	Systemic	Anti-inflammatory	Yes	46.7–64.7%	Yes
Pneumonia	Lungs	Anti-inflammatory, bacterial apoptosis	Not reported	17–38%	Not reported
Endotoxemia	Systemic	Anti-inflammatory	Not reported	Not reported	Not reported
Hemorrhage (trauma)	Site specific	Anti-inflammatory, anti-microbial	Not reported	Not reported	Not reported
Meningococcal disease	Systemic	Endotoxin clearance, bacterial inhibition	Not reported	Not reported	Not reported

to a significant reduction (>95% survival rate compared to <40% in control group) in mortality, associated with a reduction in serum LPS and TNF [114]. The combination of antibiotics with $rBPI_{21}$ in an animal model of radiation-induced bone marrow aplasia was associated with survival rates of 65–80%, significantly greater than the 0–25% observed with control/antibiotics [113]. Intravenous infusion of $rBPI_{23}$ has also been shown to reduce acute lung injury in endotoxemic pigs by ameliorating LPS-induced hypoxemia, functional upregulation of opsonin receptors on circulating phagocytes, and alveolitis [107]. Intraperitoneal injection of BPI has been shown to enhance *P. aeruginosa* uptake into the neutrophils, facilitates bacterial clearance from the peritoneal cavity, and reduce inflammation in mouse model deficient in BPI harboring acute peritoneal infection [129].

Promising data exist in other model systems. The therapeutic effects of BPI may not be limited to Gram-negative bacteria, as intranasal administration of $rBPI_{21}$ in TLR-4 deficient mice infected with Gram-positive pathogen *Streptococcus pneumoniae*, led to enhanced upper respiratory tract bacterial apoptosis and prolonged survival [116].

Besides this possible therapeutic value in acute bacterial infections, BPI has shown utility in the treatment of burn wounds. Post-burn administration of $rBPI_{23}$ reduced the incidence of bacterial translocation in mice [117]. Moreover, $rBPI_{23}$ reduced neutrophil deposition in lungs and skin in rats after burn injury [118]. Additionally, BPI was shown to inhibit the infectivity of Influenza A virus strain H1N1, H3N2, and H5N1 due to its ability to modify the structure of virus particles leading to the breakdown of virus capsid, its ability to inhibit the replication of the virus, and its ability to inhibit the activation of human PBMCs by the virus shown in lower titers of IFNa and IL-6 [119]. These effects were seen only with human but not murine BPI [119]. This evidence further expands the scope of BPI therapeutic use.

8.2. Human studies

In patients with acute hemorrhagic trauma and meningococcal disease, clinical trials using $rBPI_{21}$ have shown beneficial but limited effects of the recombinant N-terminus BPI fragment on the outcome of the disease [108–111]. In a clinical trial study involving endotoxin challenge of human volunteers, $rBPI_{23}$ neutralized endotoxin, suggesting $rBPI_{23}$ is capable of attenuating the potentially deleterious effects of blood endotoxin in humans [61,112]. Moreover, $rBPI_{23}$ also reduced the activation of the fibrinolytic and coagulation cascades after low-dose endotoxin infusion in human volunteers [113]. In children with severe meningococcal sepsis, $rBPI_{21}$ was administered and proven to be effective in meningococci inhibition and bacterial endotoxin clearance, reducing clinically significant morbidities and improving the functional outcome of children with severe meningococcemia [110,120].

8.3. Therapeutic approaches

Due to rapid clearance from the circulation and short half-life of BPI *in vivo*, there are major limitations to the therapeutic utility of BPI and recombinant BPI fragments in the clinical settings [46,121]. This could possibly be the reason to why therapeutic usage of BPI did not go through late-stage clinical trials and into the market. Due to the functional nature of the amino terminus of BPI, $rBPI_{21}$ and $rBPI_{23}$ lacked the opsonic activity conferred by the C-terminus. Given our findings that human BPI in its full form containing both N- and C-terminus is essential for bacterial phagocytosis *in vivo* [129], with a limitation of short BPI half-life, other formulations of BPI that prolong its turnover time in circulation may tremendously benefit the functionality and practicality of BPI therapy. A chimeric protein consisting of N-terminal domain of lipopolysaccharide-binding protein (LBP) and the C-terminal domain of BPI demonstrated expanded duration of activity in circulation, as well as survival benefit and endotoxin reduction in neutropenic rats with *P. aeruginosa* sepsis [122]. Adeno-associated virus 2 (AAV2)-BPI_{700}--fragment crystallizable gamma one 700 ($Fc\gamma1_{700}$) chimeric gene transferred mice has shown a prolonged half-life of BPI *in vivo* and protection against minimal lethal dose of *E. coli* infection through $BPI_{1\text{-}199}$-Fcγ1 protein expression [123]. This pharmacokinetic property of chimeric protein is beneficial for clinical dosing and administration. By increasing the time BPI can remain functionally active in circulation, this would allow broader utilization of the protein for therapeutic usage in patients.

9. Conclusion

Unlike antibiotic therapies that lack LPS neutralization properties and are prone to bacterial-resistance, BPI effects in killing bacteria, neutralizing bacterial endotoxins, while avoiding generation of antibiotic-resistant bacterial strains due to its membrane-targeting nature are proven to be useful for a new class of anti-bacterial therapy. With its nontoxic properties, bactericidal and opsonic activity, anti-inflammatory effects, and potential to exhibit synergistic interaction with conventional antibiotics, BPI remains a promising therapeutic molecule in mediating infection and inflammation in different diseases. Together with the fact that BPI is derived from the host itself, this makes it a safe and promising therapeutic molecule to be used against other Gram-negative bacterial infection in other diseases that may not be mentioned in this review. Clinical trials have indicated N-terminal domain of BPI to be effective for its bactericidal properties. There are still many more avenues to explore the C-terminal domain of BPI for its opsonophagocytosis properties. There is a possible risk for patients with anti-BPI antibodies for the BPI therapy to not be as effective, or that the introduction of BPI treatment could lead to anti-BPI antibodies generation. Presence of BPI autoantibodies and their linkage to worse disease outcome warrants further investigation into the mechanism leading to the induction of those antibodies as well as their roles in diseases.

Credit author statement

Jomkuan Theprungsirikul: Conceptualization, Writing – original draft preparation. Sladjana Skopelja-Gardner: Writing- Reviewing and editing. William F. C. Rigby: Supervision, Funding acquisition, Writing-Reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Cystic Fibrosis Foundation (CFF) [grant number: RIGBY 20G0]. All diagrams were created with Biorender .com.

References

[1] D. Aichele, M. Schnare, M. Saake, M. Röllinghoff, A. Gessner, Expression and antimicrobial function of bactericidal permeability- increasing protein in cystic fibrosis patients, Infect. Immun. 74 (2006).
[2] E. Kerem, M. Corey, R. Gold, H. Levison, Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with Pseudomonas aeruginosa, J. Pediatr. 116 (1990).
[3] A. Pamukcu, A. Bush, R. Buchdahl, Effects of Pseudomonas aeruginosa colonization on lung function and anthropometric variables in children with cystic fibrosis, Pediatr. Pulmonol. 19 (1995).
[4] M.R. Kosorok, et al., Acceleration of lung disease in children with cystic fibrosis after Pseudomonas aeruginosa acquisition, Pediatr. Pulmonol. 32 (2001).
[5] R.L. Henry, C.M. Mellis, L. Petrovic, Mucoid Pseudomonas aeruginosa is a marker of poor survival in cystic fibrosis, Pediatr. Pulmonol. 12 (1992).
[6] S. Skopelja-Gardner, et al., Autoimmunity to bactericidal/permeability-increasing protein in bronchiectasis exhibits a requirement for Pseudomonas aeruginosa IgG response, Eur. Respir. J. 53 (2019).

[7] Y. Tian, et al., BPI-ANCA in chronic obstructive pulmonary disease with pulmonary Pseudomonas aeruginosa colonisation: a novel indicator of poor prognosis, Br. J. Biomed. Sci. 75 (2018).
[8] H. Schultz, J. Hume, D.S. Zhang, T.L. Gioannini, J.P. Weiss, A novel role for the bactericidal/permeability increasing protein in interactions of gram-negative bacterial outer membrane blebs with dendritic cells, J. Immunol. 179 (2007).
[9] J.C. Jennette, R.J. Falk, Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease, Nat. Rev. Rheumatol. 10 (2014).
[10] J. Theprungsirikul, et al., Dissociation of systemic and mucosal autoimmunity in cystic fibrosis, J. Cyst. Fibros. 19 (2020) 196–202.
[11] J. Weiss, I. Olsson, Cellular and subcellular localization of the bactericidal/permeability-increasing protein of neutrophils, Blood 69 (1987).
[12] J. Weiss, P. Elsbach, I. Olsson, H. Odeberg, Purification and characterization of a potent bactericidal and membrane active protein from the granules of human polymorphonuclear leukocytes, J. Biol. Chem. 253 (1978).
[13] O. Levy, A neutrophil-derived anti-infective molecule: bactericidal/permeability-increasing protein, Antimicrob. Agents Chemother. 44 (2000).
[14] M. Carlsson, S. Shukla, A.C. Petersson, M. Segelmark, T. Hellmark, Pseudomonas aeruginosa in cystic fibrosis: pyocyanin negative strains are associated with BPI-ANCA and progressive lung disease, J. Cyst. Fibros. 10 (2011).
[15] R.J. Ulevitch, P.S. Tobias, Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin, Annu. Rev. Immunol. 13 (1995).
[16] E.T. Rietschel, H. Brade, Bacterial endotoxins, Sci. Am. 267 (1992).
[17] I. Wittmann, et al., Murine bactericidal/permeability-increasing protein inhibits the endotoxic activity of lipopolysaccharide and gram-negative bacteria, J. Immunol. 180 (2008).
[18] P. Elsbach, The bactericidal/permeability-increasing protein (BPI) in antibacterial host defense, J. Leukoc. Biol. 64 (1998).
[19] R. Mahadeva, et al., Anti-neutrophil cytoplasmic antibodies (ANCA) against bactericidal/permeability-increasing protein (BPI) and cystic fibrosis lung disease, Clin. Exp. Immunol. 117 (1999).
[20] U. Lindberg, M. Carlsson, T. Hellmark, M. Segelmark, BPI-anca provides additional clinical information to anti- pseudomonas serology: results from a cohort of 117 Swedish cystic fibrosis patients, J. Immunol. Res. 2015 (2015).
[21] H. Kobayashi, O. Kobayashi, S. Kawai, Pathogenesis and clinical manifestations of chronic colonization by pseudomonas aeruginosa and its biofilms in the airway tract, J. Infect. Chemother. 15 (2009).
[22] C. Aebi, F. Theiler, C.C. Aebischer, M.H. Schoeni, Autoantibodies directed against bactericidal/permeability-increasing protein in patients with cystic fibrosis: association with microbial respiratory tract colonization, Pediatr. Infect. Dis. J. 19 (2000).
[23] L.J. Beamer, S.F. Carroll, D. Eisenberg, Crystal structure of human BPI and two bound phospholipids at 2.4 Angstrom resolution, Science 80– (1997) 276.
[24] L.J. Beamer, Structure of human BPI (bactericidal/permeability-increasing protein) and implications for related proteins, Biochem. Soc. Trans. 31 (2003).
[25] C.D. Bingle, L. Bingle, Characterisation of the human plunc gene, a gene product with an upper airways and nasopharyngeal restricted expression pattern, Biochim. Biophys. Acta Gene Struct. Expr. 1493 (2000).
[26] J. Calafat, et al., The bactericidal/permeability-increasing protein (BPI) is present in specific granules of human eosinophils, Blood 91 (1998).
[27] P.H. Reichel, et al., Bactericidal/permeability-increasing protein is expressed by human dermal fibroblasts and upregulated by interleukin 4, Clin. Diagn. Lab. Immunol. 10 (2003).
[28] A. Balakrishnan, M. Schnare, D. Chakravortty, Of men not mice: bactericidal/permeability-increasing protein expressed in human macrophages acts as a phagocytic receptor and modulates entry and replication of gram-negative bacteria, Front. Immunol. 7 (2016).
[29] G. Canny, et al., Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia, Proc. Natl. Acad. Sci. U.S.A. 99 (2002).
[30] H. Gazzano-Santoro, et al., High-affinity binding of the bactericidal/permeability-increasing protein and a recombinant amino-terminal fragment to the lipid A region of lipopolysaccharide, Infect. Immun. 60 (1992).
[31] C. Eng Ooi, J. Weiss, M.E. Doerfler, P. Elsbach, Endotoxin-neutralizing properties of the 25 kD N-terminal fragment and a newly isolated 30 kD C-Terminal fragment of the 55-60 kD bactericidal/permeability-increasing protein of human neutrophils, J. Exp. Med. 174 (1991).
[32] S. Bülow, et al., Bactericidal/permeability-increasing protein is an enhancer of bacterial lipoprotein recognition, Front. Immunol. 9 (2018).
[33] A. Wiese, K. Brandenburg, S.F. Carroll, E.T. Rietschel, U. Seydel, Mechanisms of action of bactericidal/permeability-increasing protein BPI on reconstituted outer membranes of gram-negative bacteria, Biochemistry 36 (1997).
[34] B. Beutler, E.T. Rietschel, Innate immune sensing and its roots: the story of endotoxin, Nat. Rev. Immunol. 3 (2003).
[35] X. Wang, P.J. Quinn, Endotoxins: lipopolysaccharides of gram-negative bacteria, Subcell. Biochem. 53 (2010).
[36] N.M. Iovine, P. Elsbach, J. Weiss, An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N- and C-terminal domains, Proc. Natl. Acad. Sci. U.S.A. 94 (1997).
[37] N. Iovine, J. Eastvold, P. Elsbach, J.P. Weiss, T.L. Gioannini, The carboxyl-terminal domain of closely related endotoxin-binding proteins determines the target of protein-lipopolysaccharide complexes, J. Biol. Chem. 277 (2002).
[38] J. Weiss, L. Kao, M. Victor, P. Elsbach, Oxygen-independent intracellular and oxygen-dependent extracellular killing of Escherichia coli S15 by human polymorphonuclear leukocytes, J. Clin. Invest. 76 (1985).
[39] S. Skopelja, et al., The role for neutrophil extracellular traps in cystic fibrosis autoimmunity, JCI Insight 1 (2016).
[40] S. Skopelja-Gardner, et al., Regulation of Pseudomonas aeruginosa-mediated neutrophil extracellular traps, Front. Immunol. 10 (2019).
[41] P. Elsbach, et al., Separation and purification of a potent bactericidal/permeability-increasing protein and a closely associated phospholipase A 2 from rabbit polymorphonuclear leukocytes: observations on their relationship, J. Biol. Chem. 254 (1979).
[42] G. Qing, S. Howlett, R. Bortolussi, Lipopolysaccharide binding proteins on polymorphonuclear leukocytes: comparison of adult and neonatal cells, Infect. Immun. 64 (1996).
[43] O. Levy, et al., Impaired innate immunity in the newborn: newborn neutrophils are deficient in bactericidal/permeability-increasing protein, Pediatrics 104 (1999).
[44] W.C. Wright, B.J. Ank, J. Herbert, E.R. Stiehm, Decreased bactericidal activity of leukocytes of stressed newborn infants, Pediatrics 56 (1975).
[45] Di Roos, Chronic granulomatous disease, Br. Med. Bull. 118 (2016).
[46] P. Elsbach, Weiss, J. Role of the bactericidal/permeability-increasing protein in host defence, Curr. Opin. Immunol. 10 (1998).
[47] J. Weiss, et al., Human bactericidal/permeability-increasing protein and a recombinant NH2-terminal fragment cause killing of serum-resistant gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria, J. Clin. Invest. 90 (1992).
[48] L.M. Madsen, M. Inada, J. Weiss, Determinants of activation by complement of group II phospholipase A2 acting against Escherichia coli, Infect. Immun. 64 (1996).
[49] B.A. Mannion, J. Weiss, P. Elsbach, Separation of sublethal and lethal effects of the bactericidal/permeability increasing protein on Escherichia coli, J. Clin. Invest. 85 (1990).
[50] C. Capodici, S. Chen, Z. Sidorczyk, P. Elsbach, J. Weiss, Effect of lipopolysaccharide (LPS) chain length on interactions of bactericidal/Permeability-increasing protein and its bioactive 23-kilodalton NH2-terminal fragment with isolated LPS and intact Proteus mirabilis and Escherichia coli, Infect. Immun. 62 (1994).
[51] J.S. Gunn, Bacterial modification of LPS and resistance to antimicrobial peptides, J. Endotoxin Res. 7 (2001).
[52] A.H. Horwitz, R.E. Williams, P.S. Liu, R. Nadell, Bactericidal/permeability-increasing protein inhibits growth of a strain of Acholeplasma laidlawii and L forms of the gram-positive bacteria Staphylococcus aureus and Streptococcus pyogenes, Antimicrob. Agents Chemother. 43 (1999).
[53] H. Nishimura, et al., Bactericidal/permeability-increasing protein promotes complement activation for neutrophil-mediated phagocytosis on bacterial surface, Immunology 103 (2001).
[54] S. Demirdjian, D. Hopkins, N. Cumbal, C.T. Lefort, B. Berwin, Distinct contributions of CD18 integrins for binding and phagocytic internalization of Pseudomonas aeruginosa, Infect. Immun. 88 (2020).
[55] H. Schultz, J.P. Weiss, The bactericidal/permeability-increasing protein (BPI) in infection and inflammatory disease, Clin. Chim. Acta 384 (2007).
[56] R.R. Schumann, et al., Structure and function of lipopolysaccharide binding protein, Science 249 (80-) (1990).
[57] C.J. Kirschning, et al., Similar organization of the lipopolysaccharide-binding protein (LBP) and phospholipid transfer protein (PLTP) genes suggests a common gene family of lipid-binding proteins, Genomics 46 (1997).
[58] P.S. Tobias, K. Soldau, N.M. Iovine, P. Elsbach, Weiss, J. Lipopolysaccharide (LPS)-binding proteins BPI and LBP form different types of complexes with LPS, J. Biol. Chem. 272 (1997).
[59] P. Elsbach, J. Weiss, The bactericidal/permeability-increasing protein (BPI), a potent element in host-defense against gram-negative bacteria and lipopolysaccharide, Immunobiology 187 (1993).
[60] M.A. Dentener, E.J.U. Von Asmuth, G.J.M. Francot, M.N. Marra, W.A. Buurman, Antagonistic effects of lipopolysaccharide binding protein and bactericidal/permeability-increasing protein on lipopolysaccharide-induced cytokine release by mononuclear phagocytes: competition for binding to lipopolysaccharide, J. Immunol. 151 (1993).
[61] M.A.M. Von der Möhlen, et al., Inhibition of endotoxin-induced cytokine release and neutrophil activation in humans by use of recombinant bactericidal/permeability-increasing protein, J. Infect. Dis. 172 (1995).
[62] S. Skopelja-Gardner, J.D. Jones, W.F.C. Rigby, NETtling" the host: breaking of tolerance in chronic inflammation and chronic infection, J. Autoimmun. 88 (2018).
[63] S. Hauschild, et al., ANCA in systemic vasculitides, collagen vascular diseases, rheumatic disorders and inflammatory bowel diseases, Adv. Exp. Med. Biol. 336 (1993).
[64] F.J. Van Der Woude, et al., Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker OF disease activity IN WEGENER'S granulomatosis, Lancet 325 (1985).
[65] J.C. Jennette, J.R. Hoidal, R.J. Falk, Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3 (I), Blood 75 (1990).
[66] R.J. Falk, J.C. Jennette, Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis, N. Engl. J. Med. 318 (1988).
[67] R. Mahadeva, et al., Vasculitis and bronchiectasis in a patient with antibodies to bactericidal/permeability increasing protein and α1-antitrypsin deficiency, Chest 112 (1997).

[68] M.H. Zhao, S.J. Jones, C.M. Lockwood, Bactericidal/permeability-increasing protein (BPI) is an important antigen for anti-neutrophil cytoplasmic autoantibodies (ANCA) in vasculitis, Clin. Exp. Immunol. 99 (1995).
[69] D. Reumaux, P. Duthilleul, D. Roos, Pathogenesis of diseases associated with antineutrophil cytoplasm autoantibodies, Hum. Immunol. 65 (2004).
[70] D. Söderberg, M. Segelmark, Neutrophil extracellular traps in ANCA-associated vasculitis, Front. Immunol. 7 (2016).
[71] Y. Kusunoki, et al., Peptidylarginine deiminase inhibitor suppresses neutrophil extracellular trap formation and MPO-ANCA production, Front. Immunol. 7 (2016).
[72] S. Sangaletti, et al., Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity, Blood 120 (2012).
[73] C. Roozendaal, C.G.M. Kallenberg, Are anti-neutrophil cytoplasmic antibodies (ANCA) clinically useful in inflammatory bowel disease (IBD)? Clin. Exp. Immunol. 116 (1999).
[74] H.K. Choi, S. Liu, P.A. Merkel, G.A. Colditz, J.L. Niles, Diagnostic performance of antineutrophil cytoplasmic antibody tests for idiopathic vasculitides: metaanalysis with a focus on antimyeloperoxidase antibodies, J. Rheumatol. 28 (2001).
[75] M.H. Zhao, et al., Autoantibodies against bactericidal/permeability-increasing protein in patients with cystic fibrosis, QJM - Mon. J. Assoc. Physicians 89 (1996).
[76] L. Dorlöchter, et al., Anti-neutrophil cytoplasmatic antibodies and lung disease in cystic fibrosis, J. Cyst. Fibros. 3 (2004).
[77] R.S. Walmsley, et al., Antineutrophil cytoplasm autoantibodies against bactericidal/permeability-increasing protein in inflammatory bowel disease, Gut 40 (1997).
[78] S. Schinke, et al., Autoantibodies against the bactericidal/permeability-increasing protein from inflammatory bowel disease patients can impair the antibiotic activity of bactericidal/permeability-increasing protein, Inflamm. Bowel Dis. 10 (2004).
[79] A. Fukuhara, et al., Systemic vasculitis associated with anti-neutrophil cytoplasmic antibodies against bactericidal/permeability increasing protein, Intern. Med. 52 (2013).
[80] H. Schultz, et al., BPI-ANCA is found in reactive arthritis caused by Yersinia and Salmonella infection and recognise exclusively the C-terminal part of the BPI molecule, Scand. J. Rheumatol. 29 (2000).
[81] J.J. Yang, R. Tuttle, R.J. Falk, J.C. Jennette, Frequency of anti-bactericidal/permeability-increasing protein (BPI) and anti-azurocidin in patients with renal disease, Clin. Exp. Immunol. 105 (1996).
[82] M.P. Stoffel, et al., Anti-neutrophil cytoplasmic antibodies (ANCA) directed against bactericidal/permeability increasing protein (BPI): a new seromarker for inflammatory bowel disease and associated disorders, Clin. Exp. Immunol. 104 (1996).
[83] C. Roozendaal, et al., Antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis: defined specificities may be associated with distinct clinical features, Am. J. Med. 105 (1998).
[84] J. Theprungsirikul, et al., Low-avidity autoantibodies against bactericidal/permeability-increasing protein occur in gram-negative and gram-positive bacteremia, Infect. Immun. 88 (2020).
[85] H. Monajemi, et al., Inflammatory bowel disease is associated with increased mucosal levels of bactericidal/permeability-increasing protein, Gastroenterology 110 (1996).
[86] B. Korkmaz, M.S. Horwitz, D.E. Jenne, F. Gauthier, Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases, Pharmacol. Rev. 62 (2010).
[87] Z. Fu, M. Thorpe, S. Akula, G. Chahal, L.T. Hellman, Extended cleavage specificity of human neutrophil elastase, human proteinase 3, and their distant ortholog clawed frog PR3-three elastases with similar primary but different extended specificities and stability, Front. Immunol. 9 (2018).
[88] H. Schultz, et al., Anti-neutrophil cytoplasmic antibodies directed against the bactericidal/permeability-increasing protein (BPI) in pediatric cystic fibrosis patients do not recognize N-terminal regions important for the anti-microbial and lipopolysaccharide-binding activity of BPI, Pediatr. Allergy Immunol. 11 (2000).
[89] Y. Weinrauch, et al., Extracellular accumulation of potently microbicidal bactericidal/permeability-increasing protein and p15s in an evolving sterile rabbit peritoneal inflammatory exudate, J. Clin. Invest. 95 (1995).
[90] A. Šedivá, et al., Antineutrophil cytoplasmic antibodies directed against bactericidal/permeability-increasing protein detected in children with cystic fibrosis inhibit neutrophil-mediated killing of Pseudomonas aeruginosa, Microb. Infect. 5 (2003).
[91] H. Schultz, et al., ANCA against the bactericidal/permeability increasing protein (BPI-ANCA) can compromise the antibiotic function of BPI in a Wegener's granulomatosis patient, Clin. Exp. Rheumatol. 21 (2003).
[92] H. Schultz, et al., BPI-ANCA of pediatric cystic fibrosis patients can impair BPI-mediated killing of E. coli DH5α in vitro, Pediatr. Pulmonol. 37 (2004).
[93] O. Levy, G. Canny, C.N. Serhan, C S, Expression of BPI (bactericidal/permeability-increasing protein) in human mucosal epithelia, Biochem. Soc. Trans. 31 (Pt4) (2003) 795–800.
[94] M.M. Haapamäki, et al., Bactericidal/permeability-increasing protein in colonic mucosa in ulcerative colitis, Hepato-Gastroenterology 46 (1999).
[95] J.A. Hubacek, et al., Gene variants of the bactericidal/permeability increasing protein and lipopolysaccharide binding protein in sepsis patients: gender-specific genetic predisposition to sepsis, Crit. Care Med. 29 (2001).
[96] H. Akidotlessn, et al., Association between bactericidal/permeability increasing protein (BPI) gene polymorphism (Lys216Glu) and inflammatory bowel disease, J. Crohn's Colitis 5 (2011).
[97] W. Klein, et al., A polymorphism of the bactericidal/permeability increasing protein (BPI) gene is associated with Chrohn's disease, J. Clin. Gastroenterol. 39 (2005).
[98] G. Can, et al., Bactericidal permeability increasing protein gene polymorphism is associated with inflammatory bowel diseases in the Turkish population, Saudi J. Gastroenterol. 21 (2015).
[99] C.D. Bingle, L. Bingle, C.J. Craven, Distant cousins: genomic and sequence diversity within the BPI fold-containing (BPIF)/PLUNC protein family, Biochem. Soc. Trans. 39 (2011).
[100] C.J. Britto, L. Cohn, Bactericidal/permeability-increasing protein fold-containing family member A1 in airway host protection and respiratory disease, Am. J. Respir. Cell Mol. Biol. 52 (2015).
[101] A. Saferali, et al., Polymorphisms associated with expression of bpifa1/bpifb1 and lung disease severity in cystic fibrosis, Am. J. Respir. Cell Mol. Biol. 53 (2015).
[102] A. Saferali, et al., Immunomodulatory function of the cystic fibrosis modifier gene BPIFA1, PloS One 15 (2020).
[103] C.Z. Chen, et al., The role of bactericidal/permeability-increasing protein in men with chronic obstructive pulmonary disease, COPD 9 (2012).
[104] V.L.M. Esnault, et al., Alphal-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis, Kidney Int. 43 (1993).
[105] P. Jagiello, et al., Association study of Wegener granulomatosis and the functionally relevant A645G polymorphism in the bactericidal/permeability increasing protein (BPI) gene, Int. J. Immunogenet. 32 (2005).
[106] A.H. Horwitz, et al., Expression and characterization of cysteine-modified variants of an amino-terminal fragment of bactericidal/permeability-increasing protein, Protein Expr. Purif. 8 (1996).
[107] T.J. Vandermeer, et al., Bactericidal/permeability-increasing protein ameliorates acute lung injury in porcine endotoxemia, J. Appl. Physiol. 76 (1994).
[108] D. Demetriades, et al., Bactericidal/permeability-increasing protein (rBPI21) in patients with hemorrhage due to trauma: results of a multicenter phase II clinical trial, J. Trauma Inj. Infect. Crit. Care 46 (1999).
[109] M. Levin, et al., Recombinant bactericidal/permeability-increasing protein (rBPI21) as adjunctive treatment for children with severe meningococcal sepsis: a randomised trial, Lancet 356 (2000).
[110] B.P. Giroir, et al., Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in children with severe meningococcal sepsis, Lancet 350 (1997).
[111] O. Levy, P. Elsbach, Bactericidal/permeability-increasing protein in host defense and its efficacy in the treatment of bacterial sepsis, Curr. Infect. Dis. Rep. 3 (2007).
[112] R.J. De Winter, et al., Recombinant endotoxin-binding protein (rBPI23) attenuates endotoxin- induced circulatory changes in humans, J. Inflamm. 45 (1995).
[113] M.A.M. Von der Mohlen, et al., Inhibition of endotoxin-induced activation of the coagulation and fibrinolytic pathways using a recombinant endotoxin-binding protein (rBPI23), Blood 85 (1995).
[114] T.J. Evans, A. Carpenter, D. Moyes, R. Martin, J. Cohen, Protective effects of a recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in an animal model of gram-negative sepsis, J. Infect. Dis. 171 (1995).
[115] C.J. Kelly, et al., Role of bactericidal permeability-increasing protein in the treatment of gram-negative pneumonia, Surgery 114 (1993).
[116] A. Srivastava, H. Casey, N. Johnson, O. Levy, R. Malley, Recombinant bactericidal/permeability-increasing protein rBPI21 protects against pneumococcal disease, Infect. Immun. 75 (2007).
[117] Z.M. Earley, et al., Burn injury alters the intestinal microbiome and increases gut permeability and bacterial translocation, PloS One 10 (2015).
[118] J. Hansbrough, et al., Effects of recombinant bactericidal/permeability-increasing protein (rBPI23) on neutrophil activity in burned rats, J. Trauma Inj. Infect. Crit. Care 40 (1996).
[119] O. Pinkenburg, et al., The human antimicrobial protein bactericidal/permeability-increasing protein (bpi) inhibits the infectivity of influenza a virus, PloS One 11 (2016).
[120] B.P. Giroir, P.J. Scannon, M. Levin, Bactericidal/permeability-increasing protein - lessons learned from the phase III, randomized, clinical trial of rBPI21 for adjunctive treatment of children with severe meningococcemia, in: Critical Care Medicine, vol. 29, 2001.
[121] C.J. Fisher, et al., Human neutrophil bactericidal/permeability-increasing protein reduces mortality rate from endotoxin challenge: a placebo-controlled study, Crit. Care Med. 22 (1994).
[122] S.M. Opal, et al., Activity of lipopolysaccharide-binding protein-bactericidal/permeability- increasing protein fusion peptide in an experimental model of Pseudomonas sepsis, Antimicrob. Agents Chemother. 39 (1995).
[123] Q.L. Kong, et al., BPI700-Fcγy1700 chimeric gene expression and its protective effect in a mice model of the lethal E. coli infection, Chin. Med. J. 119 (2006).
[124] K. Iwuji, et al., Prevalence of bactericidal/permeability-increasing protein autoantibodies in cystic fibrosis patients: systematic review and meta-analysis, Pediatric, Allergy, Immunology, and Pulmonology 32 (2019).
[125] Y. Tian, et al., Clinical significance of BPI-ANCA detecting in COPD patients with Pseudomonas aeruginosa colonization, J. Clin. Lab. Anal. 33 (2019).
[126] S. Takeda, et al., The pathogenicity of BPI-ANCA in a patient with systemic vasculitis, Front. Immunol. 10 (2019).

[127] C.A. Langford, G.S. Hoffman, Rare diseases bullet 3: wegener's granulomatosis, Thorax 54 (1999).
[128] P. Steiner, M. Otth, C. Casaulta, C. Aebi, Autoantibodies against bactericidal/permeability-increasing protein (BPI) in children with acute pneumonia, FEMS Immunol. Med. Microbiol. 57 (2009).
[129] J. Theprungsirikul, S. Skopelja-Gardner, A.S. Burns, R.M. Wierzbicki, W.F. C. Rigby, Bactericidal/permeability-increasing protein (BPI) preeminently mediates clearance of *Pseudomonas aeruginosa in vivo* via CD18-dependent phagocytosis, Front. Immunol. 12 (2021) 659523, https://doi.org/10.3389/fimmu.2021.659523.