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The bactericidal/permeability-increasing protein (BPI) in infection and inflammatory disease

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Abstract

Gram-negative bacteria (GNB) and their endotoxin present a constant environmental challenge. Endotoxins can potentially signal mobilization of host defenses against invading GNB but also potentially induce severe pathophysiology, necessitating controlled initiation and resolution of endotoxin-induced inflammation to maintain host integrity. The bactericidal/permeability-increasing protein (BPI) is a pluripotent protein expressed, in humans, mainly neutrophils. BPI exhibits strong anti-microbial activity against GNB and potent endotoxin-neutralizing activity. BPI mobilized with neutrophils in response to invading GNB can promote intracellular and extracellular bacterial killing, endotoxin neutralization and clearance, and delivery of GNB outer membrane antigens to dendritic cells. Tissue expression by dermal fibroblasts and epithelia could further amplify local levels of BPI and local interaction with GNB and endotoxin, helping to constrain local tissue infection and inflammation and prevent systemic infection and systemic inflammation. This review article focuses on the structural and functional properties of BPI with respect to its contribution to host defense during GNB infections and endotoxin-induced inflammation and the genesis of auto-antibodies against BPI that can blunt BPI activity and potentially contribute to chronic inflammatory disease.

Keywords

innate immunity; inflammation; host defence; bactericidal; permeability increasing protein; autoantibodies; endotoxin

Introduction

The human host is constantly challenged by Gram-negative bacteria (GNB) and their endotoxins that are abundantly present in the surrounding environment [1]. Endotoxins (e.g., lipopolysaccharides, LPS or lipooligosaccharides LOS) are unique glycolipids found almost

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exclusively in GNB and constitute a major portion of the outer leaflet of the outer membrane that is unique to GNB (Figure 1A and B, [2]). Endotoxins consist of a highly conserved lipid A region, a core polysaccharide region and, in the case of many LPS species, a repeating tetra- or penta-saccharide that constitutes the O-antigen (Figure 1C). Endotoxins can induce potent responses in a wide range of host species including humans by rapidly inducing cellular biosynthesis and release of pro-inflammatory cytokines like tumor necrosis factor α (TNF α) and other bioactive metabolites and by rapidly activating extracellular complement, clotting, and fibrinolytic pathways [3]. Host responses to endotoxin in tissue generally promote protective inflammatory host-defense responses but similar responses systemically can lead to pathological responses ranging from fever and chills to the fulminant sepsis syndrome with multi-organ failure and high mortality [4].

Endotoxin-induced protective host responses include the recruitment and activation of inflammatory cells. Most prominent among recruited innate immune cells are the polymorphonuclear neutrophilic granulocytes (PMN) [5,6]. Neutrophils are professional phagocytic cells that produce a broad arsenal of cytotoxic products. These include antimicrobial peptide and proteins synthesized during myeloid differentiation in bone marrow and stored in cytoplasmic granules and reactive oxygen species produced *de novo* during neutrophil-microbe interaction [7]. These products can act within phagocytic vacuoles during and after phagocytosis as well as in extracellular inflammatory fluids after release from neutrophils [5,7]. Among the antimicrobial products of the neutrophil, the bactericidal/permeability-increasing protein (BPI) is unique in its antimicrobial potency and selectivity toward GNB [8]. This specificity reflects the high affinity of BPI for the conserved lipid A/ inner core region of endotoxin [9]. BPI-endotoxin interactions initiate the cytotoxic effects of BPI toward GNB and also lead to potent neutralization of endotoxin activity [10]. These properties have suggested that BPI could play an important role in arrest of GNB infection and resolution of endotoxin-driven inflammation and have stimulated pre-clinical and clinical studies of the possible protective actions of recombinant BPI in settings in which endogenous mechanisms controlling GNB infection and endotoxin-induced inflammation seem inadequate [11–15]. Studies of BPI have also led to the recognition of a variety of clinical settings in which auto-antibodies to BPI are manifest [16–18]. In many cases, the appearance of auto-antibodies to BPI follows poorly controlled GNB infection and neutrophilic inflammation. We will review current concepts of the structure and function of BPI in relation to its probable role in innate host defense against GNB infection and new data suggesting a possible link between the innate immune functions of BPI and the induction of adaptive immunity including, under unusual circumstances, that directed against BPI itself. Autoantibodies against BPI can blunt BPI activity, possibly contributing to the persistent or recurrent inflammation that is characteristic of these disorders.

BPI: expression and localization

BPI belongs to a conserved family of lipid-transfer proteins that includes as its closest relative, the LPS-binding protein (LBP). The primary structures of human LBP and BPI are ca. 45% identical [19,20]. The genes encoding BPI and LBP are located in tandem and present in a cluster on human chromosome 20 between q11.23 and q12 together with several other genes encoding products (e.g., PLUNCs) with predicted homology to BPI and LBP [21–23]. BPI has been most extensively studied in humans and New Zealand White rabbits. In both species, under resting conditions, BPI is expressed mainly in the bone marrow in myeloid precursors of neutrophils (granulocytes) and stored in primary (azurophilic) granules [24]. In contrast, the murine BPI ortholog is expressed mainly, under resting conditions, in testis and epidymis [25] and produced substantially in myeloid cells only after stimulation of mature (peripheral blood) granulocytes with inflammatory stimuli such as LPS [26,27]. Orthologs of mammalian

BPI (or LBP) have also been demonstrated in several invertebrates [27–29], suggesting evolutionary conservation of BPI-like molecules.

In humans, BPI was originally found mainly in the granules of neutrophils [8] and, to a lesser extent, in eosinophils [30]. BPI was also detected on the surface of neutrophils and monocytes [31,32], in both cases presumably originating from degranulation of neighboring activated neutrophils. In myeloid cells, BPI is expressed mainly during the promyelocytic and myelocytic stages of neutrophilic maturation, consistent with storage of BPI in azurophilic granules [33, 34]. In peripheral blood, neutrophils contain stores of BPI in the primary granules but ~no BPI mRNA or *de novo* protein expression [24]. Within the 5' upstream region of the human BPI gene are response elements for myeloid-specific transcription factors AML-1, PU.1, and Sp3, that are also involved in the expression of other granule proteins [35]. However, early studies of the human promyelocytic cell line HL-60, suggested somewhat different regulation of BPI expression vs. that of other azurophilic granule constituents [24,33]. More recent experiments in the human promyelocytic cell line NB4 have demonstrated that BPI expression accompanying neutrophilic maturation induced by all-trans retinoic acid (ATRA) correlated with binding to the BPI promoter of the transcription factors CCAAT/enhancer-binding protein (C/EBP) β and C/EBP ϵ but not C/EBP α , the latter a transcription factor involved in the expression of other azurophilic granule proteins [36]. These findings suggest a possible explanation for how the pattern of expression of BPI can be different from that of many other azurophilic granule proteins. Of note, neutrophils in human newborn blood display an apparently selective deficiency of BPI compared to other granule proteins [37] that appears to account for the reduced antibacterial activity of extracts of these cells toward BPI-sensitive GNB.

Constitutive expression of BPI on the mRNA and protein level has also been demonstrated for human gastrointestinal epithelial cells [38], dermal fibroblasts [39] as well as for basal epithelial cells of the excretory lacrimal gland ducts [40,41] and human genital tract epithelial cells [42]. A role for transcription factors of the Sp1/3 and C/EBP group in BPI expression by the human gastrointestinal epithelial tumor cell line CaCo-2 has been suggested. BPI mRNA and protein levels in these cells were low under resting conditions but could be increased ~12-fold by treatment with the stable lipoxin analog ATLa. ATLa is an anti-inflammatory lipid metabolite, deriving from short-lived arachidonic acid metabolites, that accumulate in the late (resolution) phase of tissue-based inflammatory responses [43,44]. The effects on BPI expression of ATLa are apparently specific (i.e., treatment of CaCo-2 cells with pro-inflammatory cytokines (TNF, interferon gamma, IL-4, IL-1) or Gram-negative or Gram-positive bacteria did not increase BPI expression in CaCo-2 cells) and also demonstrable in other epithelia [38,40,45]. In contrast, in dermal fibroblasts only IL-4 could raise BPI protein levels above baseline levels [39]. Similar observations were made in human endo- or ectocervical epithelial cells [42] where stimulation with increasing doses of TNF α led to an increase in IL-6 secretion but did not affect BPI expression suggesting that a more complex series of events than direct exposure of epithelial cells to microorganisms or pro-inflammatory cytokines might be needed to initiate upregulation of BPI expression in tissue.

BPI: Structure and function

BPI is a single-chain cationic protein with a molecular weight of ca. 55 kD [8]. Limited proteolysis can generate two BPI fragments comprising residues 1–199 and 200–456 in which the antibiotic and endotoxin-neutralizing functions of BPI are fully recovered with the N-terminal fragment [45]. X-ray crystallography of BPI revealed a unique boomerang-like shape which comprises two domains (residues 1–230 and 250–456, [46]) that are nearly superimposable in their 3-D organization despite marked differences in primary structure and functions. (Figure 2). Basic residues clustered at the far end of the amino-terminal domain of

BPI are thought to mediate initial electrostatic interactions with acidic sites of endotoxin located close to the lipid A region [47]. In addition, apolar pockets were demonstrated on the concave side of the molecule where host-derived phospholipids can be bound [46] that may be needed to stabilize the water soluble folded structure of BPI [46,48]. Studies using the recombinant amino-terminal domain of BPI alone or recombinant human BPI_N/LBP_C and LBP_N/BPI_C chimeric fusion proteins [48,49] have confirmed that the N-terminal domain (minimal structure of residues 13–191; [50]) is responsible for the antimicrobial cytotoxicity [45] and endotoxin-neutralizing properties of BPI [9,49] whereas the C-terminal domain of BPI is needed for BPI-dependent delivery of intact GNB and cell-free endotoxin-rich particles to specific host cells [51–53]. Reported anti-angiogenic properties have been described thus far for the BPI holoprotein and for a recombinant N-terminal fragment of BPI (residues 1–191; rBPI₂₁ [54–56]) whereas enhancement of complement activation on bacterial surfaces has been described only for the full-length BPI molecule [57].

BPI actions during host: GNB interactions

The potent and selective antibiotic and endotoxin-neutralizing properties of BPI, manifest with nM concentrations of either the full-length BPI molecule or rBPI₂₁ stimulated extensive preclinical and clinical testing of rBPI₂₁. In brief summary, in a variety of animal models, recombinant human BPI (rBPI) and its amino-terminal derivative (rBPI₂₁), were protective against lethal and sub-lethal challenges with Gram-negative bacteria and endotoxin [11,58–61]. Subsequent studies in humans, including in meningococcal sepsis, also showed a significant protective effect of BPI [12–15,61–64], although not yet sufficient to lead to an approved clinical application. However, these studies strongly suggested that BPI could act *in vivo* and help resolve GNB infection and endotoxin-induced inflammation.

In contrast to many of the clinical trials in which rBPI₂₁ was continuously infused to maintain functionally significant plasma levels and the target was systemic endotoxin and/or GNB [14,15,64], early interactions of BPI with GNB and endotoxin during natural infections are much more likely to occur in tissue, outside of the bloodstream [3]. Typically, the critical initial event in invasive GNB infections is the crossing of epithelial barriers by the microorganism, particularly those of the skin, lungs, the gastrointestinal and urogenital tracts, and the subsequent exposure of underlying host tissue to the microbe and its products (Figure 3, [3]). The outcome of an infection with GNB strongly depends on the host's ability to sense the bacteria in tissue and, if necessary, mount a timely and sufficient inflammatory response that is resolved after the bacteria have been killed and their remnants eliminated [3,65]. Sensitive and potent acute inflammatory responses followed by efficient elimination of viable bacteria and bioactive products help contain the infection and inflammation within tissue and return these sites to resting conditions after resolution of infection-induced inflammation. In many invasive GNB infections, endotoxin plays a major role in inducing acute inflammation and the clearance and detoxification of endotoxin is important in resolving host inflammatory and immune responses [66,67]. Presentation of endotoxin in these settings occurs not as aggregates of purified endotoxin, but rather, more likely, with endotoxin still an integral component of the GNB outer membrane [68–70]. This includes outer membrane vesicles (OMV or “blebs”) that are shed constitutively during bacterial growth and, to an even greater extent, under a variety of adverse conditions including sub-lethal bacterial envelope injury induced by BPI [69]. The presence of other innate immune-stimulating (e.g. porins, lipoproteins) and envelope antigenic components (e.g. outer membrane proteins) within the GNB outer membrane makes these vesicles not only primary targets for clearance and resolution of GNB/endotoxin-driven inflammation but also potentially important vehicles for delivery of antigenic material to antigen-presenting cells [69,71–73].

The activation of host cells by endotoxin requires the extraction and transfer of individual endotoxin molecules to a host cell surface receptor complex containing MD-2 and Toll-like receptor (TLR) 4 [74–76]. This transfer does not occur directly but rather requires first the interaction of LBP with the endotoxin-rich GNB outer membrane and transfer of endotoxin monomers to either soluble or membrane-bound CD14. The monomeric endotoxin:CD14 complex is the preferred substrate for MD-2 – either MD-2 alone or MD-2 pre-associated with TLR4 [74,75]. Simultaneous binding of endotoxin and TLR4 by MD-2 then triggers potent pro-inflammatory responses (Figure 4A, [74,75]).

LBP is produced constitutively in the liver and thus is constantly present in the circulation and, at lower levels, in tissue. These low levels of LBP are most favorable for extraction and transfer of endotoxin monomers to CD14 and, subsequently, to MD-2/TLR4, especially early after GNB invasion when the bacteria and endotoxin are most likely present in minute amounts. Before induction of acute inflammation, there are no neutrophils within tissue and thus no neutrophil-derived BPI. The extent to which basal expression of BPI in tissue by other cells (e.g., epithelia, connective tissue) could be functionally significant is unclear [38,39,42,77–79]. Tissue-derived BPI has been found on apical and basolateral epithelial surfaces [38,40, 42] and on dermal fibroblasts [39] derived from subcutaneous connective tissue, however, its quantitative contribution to the initial host interactions with GNB in tissue are as yet unknown. The presence of BPI at these sites could enhance the ability of local defenses to eliminate small amounts of invading bacteria and also diminish the availability of endotoxin for stimulation of sub-epithelial host cells via the LBP/CD14/MD-2/TLR4 pathway [80–82]. Under these circumstances, incidental incursions of GNB may be resolved locally without a significant inflammatory response and little or no influx of neutrophils.

Once, however, a threshold of GNB invasion and endotoxin exposure has been reached, the low levels of LBP in interstitial fluid at rest are likely sufficient to initiate delivery of LPS to mCD14/MD-2/TLR4 on endotoxin responsive resident tissue cells (e.g., macrophages) and potentially induce acute inflammation. Effects of acute inflammation include the release of bioactive substances (e.g. cytokines and chemokines) that promote the permeation of antimicrobial plasma factors into the local tissue and recruitment of neutrophils and, later, mononuclear blood cells, monocytes and lymphocytes, to the site of infection. In this setting, recruited neutrophils use much of their granule contents, including BPI, to mediate intracellular cytotoxicity after phagocytosis of the invading bacteria. In addition, recruited and activated neutrophils release some of their granule contents into the surrounding inflammatory fluid [83–86]. The released proteins may contribute to containment and killing of extracellular bacteria that have exceeded or evaded/resisted the phagocytic capacity of the neutrophils and also to elimination of bioactive bacterial remnants [87,88].

The antibiotic effects of BPI on GNB take place in two stages [89] involving: i) potentially reversible, sub-lethal alterations of the outer membrane; and ii) irreversible lesions extending to the inner cytoplasmic membrane, disrupting energy-generating and -dependent biochemical machinery and resulting in cell death [89,90]. The sub-lethal effects of BPI can be amplified by synergistic actions with other neutrophil granule proteins [91–95] and with extracellular antimicrobial proteins that are mobilized in neutrophil-rich inflammatory exudates, including group IIA phospholipase A2 (gIIA PLA2) and complement [96–98]. These combined actions can take place both within neutrophils after uptake of the PLA2 and complement with bacteria during phagocytosis [97,98] and in the extracellular inflammatory fluid [40,86,99–101]. Levels of BPI in cell-free inflammatory fluid of neutrophil-rich exudates are functionally significant (≥ 10 nM) and co-exist with other antimicrobial host factors that can act in concert with BPI [76,86,100–104]. Thus, in cooperation with other cellular and humoral components of the innate immune system, BPI can contribute to killing of invading GNB.

During and after phagocytosis, the lipid A region within bacterial endotoxin is partially deacylated by the host enzyme acyloxyacyl hydrolase [88,105]. Acyloxyacyl hydrolase converts hexa-acylated endotoxins that are potent TLR4 agonists to tetra-acylated species that are TLR4 antagonists [3,105,106]. Endotoxin associated with intact bacteria and bacterial remnants is also partially deacylated in the extracellular inflammatory fluid [88] but at a much slower rate. Thus, a more rapid detoxification mechanism may be needed in inflammatory fluids and could be mediated by locally mobilized endotoxin binding proteins, such as BPI [86]. Like LBP, BPI binds to the highly conserved lipid A region of endotoxin [9]. In contrast to the effect of binding of LBP to endotoxin, binding of BPI does not lead to extraction and transfer of endotoxin to CD14 and thus can preclude MD-2/TLR4-dependent cell activation by endotoxin [51,66]. This difference reflects differences in the structural and functional properties of the C-terminal domain of LBP and of BPI [48,51,81,107]. Additional differences in the structural and functional properties of the N-terminal domain of BPI and of LBP account for the higher affinity of BPI (vs. LBP) for purified or outer membrane-inserted endotoxin [9,52,53]. Thus, BPI can effectively compete with LBP for binding to endotoxin and thus inhibit LBP-dependent (and -independent) extraction and transfer of endotoxin to CD14/MD-2/TLR4 [81,82].

Although BPI binding to aggregates of endotoxin, cell-free outer membrane blebs or intact GNB does not engage CD14-dependent cellular interactions, BPI can promote CD14-independent interactions with specific host cells [48,51–53]. In each instance, these effects of BPI are dependent on the N-terminal domain of BPI to bind endotoxin and the C-terminal domain of BPI to somehow facilitate targeting of the BPI-coated endotoxin-rich particle to a host cell. Remarkably, which host cell is engaged seems dependent on the nature of the endotoxin-rich particle engaged by BPI. In experiments using encapsulated *Neisseria meningitidis* serogroup B and cell-free outer membrane blebs and aggregates of endotoxin derived from these bacteria, intact bacteria treated with BPI were delivered to neutrophils > monocytes, purified aggregates of endotoxin treated with BPI were delivered to monocytes > neutrophils and outer membrane blebs to monocytes and monocyte-derived dendritic cells > macrophages [51–53] (Schultz et al., manuscript submitted, Figure 4). The molecular bases of the selective association of different BPI-coated endotoxin-rich particles with specific host cells are not known.

Opsonic effects of BPI on phagocytosis by neutrophils of phagocytosis-resistant encapsulated GNB require doses of BPI sufficient to induce sub-lethal injury [52]. Uptake of the BPI-coated bacteria is accompanied by activation of the cellular respiratory burst indicating that the non-oxidative antibacterial action initiated by extracellular BPI will be followed and amplified by oxygen-dependent (and additional oxygen-independent) cytotoxic actions within the neutrophil. In contrast, BPI-dependent uptake of cell-free endotoxin by monocytes is not accompanied by apparent activation of the monocytes. In fact, low levels of endotoxin- and CD14/MD-2/TLR4-dependent activation of the monocytes that are induced by the cell-free endotoxin-rich particles are inhibited by BPI despite increased uptake of endotoxin by the monocytes [51,53]. These findings are most compatible with a role for BPI in the “silent” disposal of cell-free endotoxin that needs to be removed or detoxified by AOA to help resolve endotoxin-induced inflammation. However, the magnitude of BPI-dependent cell-free endotoxin clearance that has been observed thus far in *in vitro* studies has been quite limited ($\leq 15\%$ of that present), raising the possibility that additional co-factors, absent in the *in vitro* assays, may be needed for more efficient BPI-dependent clearance or that disposal is not a major function of BPI-dependent interactions of cell-free endotoxin with host cells.

The recent demonstration (Schultz et al., manuscript submitted) that BPI promotes delivery and uptake of purified endotoxin and, to a greater extent, outer membrane blebs to dendritic cells and not to monocyte-derived macrophages suggests a novel role for BPI in the uptake

and presentation of bacterial antigens by professional antigen presenting cells (Figure 4B). Enhanced uptake of the blebs by dendritic cells in the presence of BPI does not increase cell activation but still permits LBP/sCD14-dependent cell activation by the blebs. We speculate that a single bleb containing tens-hundreds of thousands of endotoxin molecules/particle is able to accommodate both BPI- and LBP-dependent interactions, the former increasing uptake of the particles, the latter promoting CD14/MD-2/TLR4-dependent cell activation. According to current concepts [108], initiation of TLR4 signaling from the same intracellular compartment containing internalized bacterial antigens should favor subsequent processing and MHC II-dependent presentation of fragments of the internalized protein antigens. Thus, these findings suggest that innate immune recognition of endotoxin by BPI and LBP can promote, in a complementary manner, induction of anti-bacterial adaptive immunity.

Recent studies have demonstrated that BPI has the ability to induce apoptosis in human vascular endothelial cells *in vitro* and inhibit their migration in a wound assay [54]. Moreover, the amino-terminal part of BPI (rBPI₂₁) inhibited neo-vascularization in the retina of mice [55, 56], either ischemia-induced or diabetes-induced, suggesting an antiangiogenetic effect of BPI. Its role in the context of a GNB-induced inflammation in a tissue setting remains to be elucidated. It is, however, conceivable that BPI could impair vascular access of GNB by inhibiting neo-angiogenesis in settings where chronic neutrophilic inflammation prevails, e.g. in inflammatory bowel diseases or cystic fibrosis [77,79,109,110].

Autoimmunity against BPI: BPI-ANCA

Anti-neutrophil cytoplasmic autoantibodies (ANCA), represent a heterogenous group of autoantibodies directed against neutrophil granule proteins. The presence of ANCA typically leads to a characteristic staining pattern in human neutrophils, detectable by indirect immunofluorescence microscopy [111]. Originally described in patients with rapid progressive glomerulonephritis, ANCA soon became hallmarks in the diagnostic workup and monitoring of disease activity in primary systemic vasculitides [112]. In Wegener's granulomatosis and microscopic polyangiitis, there is a strong association with ANCA against proteinase 3 (PR3) and myeloperoxidase (MPO), respectively [111]. BPI was also first described as an ANCA target antigen in vasculitides [113,114]. However, it soon became apparent that BPI is a target of ANCA in a variety of diseases of different etiologies like cystic fibrosis (CF), inflammatory bowel diseases (IBD), reactive arthritis (ReA), HIV, the recently discovered peptide transporter complex associated with antigen presentation (TAP) deficiency [16–18,113,115] and chronic obstructive pulmonary disease (COPD, Schultz, H. and Murphy, T.F., unpublished observation). Thus, BPI-ANCA could not be used as a disease-specific seromarker as PR3-ANCA is for Wegener's granulomatosis. With the exception of HIV, where the levels and function of CD4+ helper T lymphocytes are greatly disturbed [116], a common feature of BPI-ANCA associated diseases is a chronic and/or profuse exposure of host tissue to GNB and/or their endotoxin and an accompanying neutrophilic inflammation. BPI-autoantibodies have been found in the serum of up to 90% of these patients (Figure 5).

The early appearance of BPI-ANCA in some CF children, even before clinical signs of GNB infection, raised the possibility of a genetic predisposition to BPI-autoimmunity, at least in a subset of individuals with BPI-ANCA [117]. However, the apparent association in most cases of BPI-ANCA with GNB infection and neutrophilic inflammation suggest a greater circumstantial component to this auto-immune manifestation. Epitope mapping data have revealed similarities of BPI epitopes recognized by BPI-ANCA (Table 1) with specific protein sequences of infecting GNB (e.g. outer membrane proteins of *Pseudomonas aeruginosa*) suggesting that cross-reactivity followed by epitope spreading in the inflammatory setting could be a mechanism for broken tolerance against BPI [118,119]. The waning of BPI-ANCA

in CF patients after lung transplantation and a reduced pulmonary burden of *P. aeruginosa* [120,121] is consistent with this hypothesis.

An added possibility is suggested by the BPI-dependent interactions of GNB outer membrane blebs with dendritic cells briefly described above. Although the principal physiological function of these interactions may be increased bacterial antigen delivery to antigen-presenting cells leading to increased induction of adaptive immunity to bacterial outer membrane antigens, the uptake of these blebs is likely to include significant amounts of bound BPI. Thus, under conditions of pronounced and protracted GNB infection, endotoxin elaboration and extracellular BPI mobilization, uptake of BPI-coated blebs (or, perhaps, intact GNB) by dendritic cells may result in sufficient MHC II-dependent presentation of BPI by activated dendritic cells to over-ride the normal tolerance of the human host to BPI, resulting in BPI-ANCA. This mechanism would also be consistent with the diminishing levels of BPI-ANCA in CF patients after lung transplantation and fluctuations in BPI-ANCA levels with changes in clinical and infection status.

The correlation of BPI-ANCA levels in CF and IBD patients indicate that BPI-ANCA might be useful to identify subgroups of patients with higher inflammatory activity and more severe organ damage (Table 2, [16,117–122]). The ability of BPI-ANCA to interfere with the opsonic [123] and antibiotic [18,119,124] actions of BPI has been demonstrated *in vitro*. In addition, BPI-ANCA can activate TNF α -primed PMNs to produce reactive oxygen species *in vitro* [117], suggesting an additional pro-inflammatory effect these autoantibodies could have *in vivo*. The extent to which neutrophil- vs. tissue-derived BPI are targets of BPI-ANCA, distinct BPI auto-antibodies directed at different BPI epitopes and mediate different functional effects and BPI-ANCA contribute to inflammatory disease progression requires further study.

Outlook

BPI is a pluripotent protein located in neutrophils and tissue that likely plays a pivotal role in host defense against GNB and their endotoxin by means of its antibiotic and endotoxin-neutralizing and –disposing functions. Further studies are needed to understand in more detail how expression of BPI is controlled in non-myeloid tissue cells and how this may help regulate host-GNB interactions in tissue settings. A better understanding of the regulation of BPI expression in epithelial and connective tissue settings may suggest novel approaches to increasing local antibacterial and anti-inflammatory properties that could be especially useful in compromised settings such as burn patients. The ability of BPI to promote the delivery of GNB remnants to dendritic cells while still permitting LBP/CD14-dependent signaling from these same particles suggests an expansion of the roles of BPI from an innate immune effector to an inducer of antibacterial adaptive immunity. This previously unrecognized function of BPI may also create circumstances conducive to elaboration of BPI auto-antibodies. Further insight in the processing of BPI, complexed with GNB and/or OM blebs, by professional antigen presenting cells may also help clarify the genesis of BPI auto-antibodies and, by possible analogy, of other ANCA directed at other antimicrobial granule proteins. These studies may also suggest new ways to enhance host adaptive immune response to important bacterial pathogens (e.g. *N. meningitidis* serogroup B) and thus facilitate the development of new vaccine approaches.

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Figure 1. Schematic structure of outer membrane (OM) blebbing Gram-negative bacteria (GNB) (A), the envelope of Gram-negative bacteria (B) and lipo-poly (oligo)-saccharides (C)

Figure 2. Structure and function of the bactericidal/permeability-increasing protein
Ribbon diagram of the structure of the bactericidal/permeability-increasing protein including bound host phospholipids (*) (modified after [46]), major antibacterial functions are associated with the amino- and carboxy-terminal domains are indicated.

Figure 3. Interactions of BPI with Gram-negative bacteria in tissue settings

The initial interactions between Gram-negative bacteria, endotoxin and BPI normally takes place in the tissue. BPI is present on the apical and basolateral surfaces of the epithelium but if levels of BPI expression at these sites are great enough to increase local defences against invading GNB is unknown. Fibroblasts located under the epithelial lining also express BPI and could serve as a second barrier against GNB and endotoxin. However, the major source of BPI in tissue is derived from recruited and activated neutrophils. BPI can promote delivery of GNB remnants (e.g. OM blebs) to dendritic cells, facilitating induction of T-cell-dependent antibacterial immunity. BPI expression in fibroblasts and epithelia later in the immune response by IL-4 and lipoxins, respectively [38,39] may further promote elimination of GNB and resolution of endotoxin-induced inflammation.

Dendritic cells have not only the potential to ingest bacteria but can also internalize and process OM blebs and BPI delivered to them and provide a link to the adaptive immune system.

Abbreviations: *PMN* polymorphonuclear neutrophil granulocyte, *GNB* Gram-negative bacteria, *Mono* monocyte, *FB* fibroblast, *DC* dendritic cell, *BPI* bactericidal/permeability increasing protein, *OM blebs* outer membrane vesicles of GNB.

Figure 4. Roles of BPI during GNB infection

Role of BPI in the interaction of GNB and OM blebs with neutrophil granulocytes and monocytes (A). BPI can opsonize GNB and target them to be phagocytosed by PMN. It can contribute to intracellular killing of ingested GNB and also to extracellular bacterial killing when released to the extracellular space by PMN. Single endotoxin molecules from OM blebs or other endotoxin containing remnants of GNB can be extracted and processed by the LBP/CD14/MD-2/TLR-4 pathway resulting an activation of CD-14 positive monocytes. In contrast, BPI bound endotoxin is not accessible for LBP/CD14 mediated LOS/LPS extraction and does not cause an inflammatory response in monocytes [81,82,107].

Role of BPI in the interaction of OM blebs with dendritic cells (B). BPI can promote CD14-independent delivery of OM blebs to dendritic cells (DC). In parallel, LBP and sCD14 can promote MDDC activation by extraction and delivery of endotoxin to MD-2/TLR4 [108]. Subsequently, the ability of BPI and LBP to interact with the same OM bleb could allow coincidental enhancement of antigen delivery by BPI and LBP/sCD14-dependent cell activation and could thereby promote antigen-presenting functions of DC. Abbreviations: *BPI* Bactericidal/permeability-increasing protein, *GNB* Gram negative bacteria, *LBP* Lipopolysaccharides binding protein, *CD14* either membrane bound or soluble CD14, *Blebs* outer membrane vesicles of GNB, *LOS/LPS* lipooligosaccharide or lipopolysaccharide, *MD-2* Myeloid Differentiation-2, *TLR-4* TOLL-like receptor-4

Figure 5. Prevalence of BPI-autoantibodies in selected inflammatory diseases (modified after [125])

Shown is the prevalence of BPI-ANCA in selected studies of healthy persons and patients with infectious diseases, rheumatic diseases, inflammatory bowel diseases and immunodeficiency [17,18,115,118,119,126].

Abbreviations: *HIV* human immunodeficiency virus; *ReA* reactive arthritis; *TAP* transporter associated with antigen presentation

Table 1

Several BPI auto-antibodies of cystic fibrosis and inflammatory bowel disease patients react with regions on human BPI (shown in upper line, numbers correspond to amino acid residues) that are homologous to limited regions of specific proteins of GNB (shown below corresponding BPI sequence). Data are taken from references [114,118,119].

BPI Sequence Bacterial Sequence	Patients n	Bacterial protein	Origin	% Identity
50 AALQKELKRIKIPD 63 LQKELKR	5	Protein (argF- lacZ region)	<i>Escherichia coli</i>	100
397 AESDRLVGELRLDR 410 AERDRLIGGLRLDR	4	OprC	<i>Pseudomonas aeruginosa</i>	100
88 FQLPSSQISMVP 99 LHSSQISM	4	IpaD ORF	<i>Shigella dysenteriae</i>	87
137 GMSISADLKLGSNPT 151 MSIDADLK	3	Invasion Protein	<i>Salmonella enterica</i>	87
88 FQLPSSQISMVP 99 LHSSQISM	3	Invasion Protein	<i>Shigella flexneri</i>	87
99 PNVGLKFSISNAN 111 FAISNAN	2	FlgE Protein	<i>Campylobacter jejuni</i>	85

Table 2

Relation of BPI-ANCA titer to clinical status in cystic fibrosis and inflammatory bowel diseases.

Cystic Fibrosis	Inflammatory Bowel Diseases
↓ FEV ₁ and FVC [*]	↑ Aggressive course
↑ <i>P. aeruginosa</i> colonization [*]	↑ Frequency of diarrhea [*]
↑ ESR [*] and CRP [*]	↑ Neutrophil count [*]
↓ Chest X ray score [*]	↑ Crohn's disease activity index (CDAI) [*]
↓ Chest HRCT score [*]	↑ Extraintestinal manifestations [*]
	↓ Albumin [*]
	↑ ESR [*] and CRP [*]

Shown are significant differences between BPI-ANCA positive and negative patients with cystic fibrosis and inflammatory bowel diseases. Arrows heading upwards indicate a positive correlation, arrows directed downwards indicate an inverse correlation between BPI-ANCA titer and the indicated clinical parameter;

* p<0.05. Data taken from references [16,77,118–121,127–134].

Abbreviations: *FEV1* forced expiratory volume in 1 second; *FVC* functional vital capacity of the lung; *ESR* Erythrocyte sedimentation rate; *CRP* C-reactive protein; *CDAI* Crohn's disease activity index; *HRCT* high resolution computed tomography