Deficient expression of bactericidal/permeability-increasing protein in immunocompromised hosts: translational potential of replacement therapy

Christine D. Palmer*††, Eva C. Guinan*†‡ and Ofer Levy*†1

*Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115, U.S.A., †Harvard Medical School, Boston, MA 02115, U.S.A., and ‡Dana Farber Cancer Institute, Boston, MA 02115, U.S.A.

Abstract
BPI (bactericidal/permeability-increasing protein) is a 55 kDa anti-infective molecule expressed in neutrophil and eosinophil granules and on some epithelial cells. BPI's high affinity for the lipid A region of endotoxin targets its opsonizing, microbicidal and endotoxin-neutralizing activities towards Gram-negative bacteria. Several immunocompromised patient populations demonstrate BPI deficiency, including newborns, those with anti-neutrophil cytoplasmic antibodies (as in cystic fibrosis and HIV infection) and those exposed to radiochemotherapy. BPI may be replenished by administering agents that induce its expression or by administration of recombinant BPI congeners, potentially shielding BPI-deficient individuals against Gram-negative bacterial infection, endotoxemia and its toxic sequelae.

Introduction
Innate mammalian host defence against infection is based on rapid immune detection and effector mechanisms that target conserved microbial features. Activation of PRRs (pattern-recognition receptors) mobilizes both pre-formed and inducible microbicidal mechanisms. These include the oxygen-independent APPs (antimicrobial proteins and peptides) [1]. Among the APPs of human neutrophils is the BPI (bactericidal/permeability-increasing protein), BPI along with LBP (LPS (lipopolysaccharide)-binding protein) and palate, lung and nasal epithelial clone proteins is a member of the tubular lipid ('TULIP') family of lipid-binding proteins [2].

BPI structure and function
BPI is a ∼55 kDa polypeptide comprised of 456 amino acids originally identified in rabbit and human granulocyte extracts [3,4]. BPI bears structural homology with the LBP, a liver-derived plasma component that serves to deliver LPS to its cellular receptor. Unlike LBP, BPI is lysine-rich and highly cationic, features contributing to its affinity to the negatively charged LPS. In addition, hydrophobic features of BPI are also thought to contribute to its high affinity for the lipid A moiety of LPS that is common to all Gram-negative bacteria [5]. Structure–function analysis of BPI indicates that its antibacterial and LPS-neutralizing activities are localized to the N-terminal half of the protein [6], whereas the C-terminal half enhances the opsonic activity of the molecule [7]. The N- and C-terminal regions of BPI form a boomerang-like structure [8].

Anti-infective action
Consistent with the high affinity of BPI for the lipid A region of LPS, the antimicrobial action of BPI is most potently expressed against Gram-negative bacteria including Escherichia coli, Salmonella enterica serotype Typhimurium, Shigella and Enterobacter spp. [5,9]. Of note, the bactericidal activity of BPI against these bacteria is manifest at nanomolar concentrations in biological fluids such as serum, plasma and whole blood as well as inflammatory peritoneal exudates. Some Gram-negative bacteria, such as Pseudomonas aeruginosa, Klebsiella pneumoniae and Serratia spp., are relatively resistant to the bactericidal activity of BPI but remain fully susceptible to its endotoxin-neutralizing activity. At relatively high concentrations, BPI or BPI-derived peptides also have in vitro microbicidal activity against L forms of Gram-positive bacteria, fungi such as Histoplasma capsulatum and Toxoplasma gondii [9,10].

BPI may also play important roles in linking innate and adaptive immune responses. BPI increases delivery and internalization of blebs from Neisseria meningitidis to human monocyte-derived dendritic cells [11], which was associated with CD14-dependent signalling, including up-regulation of co-stimulatory molecules (e.g. CD80, CD83 and CD86), and MHC class II. Thus interactions of BPI with bacterial...
outer membrane vesicles may help link innate immune recognition of endotoxin to APC (antigen-presenting cell) delivery, presentation and adaptive immune responses.

Expression of BPI

Leucocyte expression

BPI was originally discovered as a component of rabbit granulocytes [3] and shortly thereafter identified in human neutrophils [4,12]. Human neutrophil BPI is localized to the primary or azurophilic granules, appears to be primarily membrane-associated, and is relocated after neutrophil activation, following the same route as MPO (myeloperoxidase) and CD63 [13]. BPI was subsequently also identified in eosinophils [14]. Murine BPI is expressed in granulocytes and bone-marrow-derived dendritic cells upon LPS challenge via a TLR4 (Toll-like receptor 4)-dependent pathway [15]. Inducible BPI expression in mice is reminiscent of that in the oyster, Crassostrea gigas, a marine invertebrate wherein BPI is induced in haemocytes after bacterial challenge [16].

Epithelial expression

Although BPI was first discovered as a component of leucocytes, epithelial BPI is apparently an ancient defence mechanism [17]. In the oyster C. gigas, BPI is constitutively expressed in various epithelia in contact with the external environment, including the mantle, gills, digestive tract, digestive gland diverticula and gonad follicles. In the channel catfish Ictalurus punctatus, BPI is expressed in a wide range of tissues, including head and trunk kidney, gill, skin, intestine, liver, ovary and stomach [18].

Human epithelial cells of wide origin including oral, pulmonary and GI (gastrointestinal) mucosa, express BPI [19–21]. Basal epithelial BPI expression can be further enhanced in a range of epithelial cell lines by analogues of endogenously occurring anti-inflammatory eicosanoids, namely LXs (lipoxins), including ATLα (aspirin-triggered LX analogue) [19]. LXs are products of active biochemical pathways that play key roles in resolution of inflammation [22,23]. Among their many anti-inflammatory and pro-resolving activities, LXs induce functional BPI expression on epithelial cell surfaces. Accordingly, a BPI-neutralizing antiserum blocks endotoxin-induced epithelial signalling and killing of Salmonella Typhimurium. BPI is also expressed along the murine intestine [21]. Furthermore, studies in human endo- and ecto-cervical epithelial cells showed endogenous expression of BPI mRNA and protein, as well as BPI-dependent bactericidal activity of epithelia against a commensal strain of E. coli [24], suggesting an important role for epithelial BPI expression in regulating bacterial colonization in the genital mucosa.

BPI is also expressed on rat and human skin [25], wherein it is expressed in hair follicle inner root sheath cell cytoplasm and in human scalp skin and cultured keratinocytes, as well as dermal fibroblasts [26]. It may thus play a role in defence against anaerobic bacteria in the hair follicle isthmus. Overall, epithelial BPI may represent an important agent by which the innate immune system protects epithelial surfaces against Gram-negative bacteria and their endotoxin.

BPI deficiency

A number of congenital and acquired states are associated with BPI deficiency.

Lower neutrophil BPI expression at birth

Qing et al. demonstrated that human cord blood neutrophils lack a ∼50 kDa membrane protein that binds the lipid A region of LPS [27]. Subsequently, it was demonstrated by Western blotting that neonatal cord blood neutrophils have ∼3–4-fold less intracellular BPI than adult neutrophils. This deficiency correlated with diminished neutrophil extracellular activity against E. coli K1/r [28]. Deficiency of BPI was apparently selective, as the relative levels of two other primary granule constituents MPO and the defensin peptides were indistinguishable. When stimulated with the secretagogue PMA, the neutrophils of preterm newborns release significantly less BPI per cell than adult neutrophils [29]. A recent study of plasma levels of APPs in cord blood plasma indicated a gestational age-dependent maturation of plasma BPI concentrations that remained below adult levels even at full term [30]. Taken together, these studies demonstrate an age-dependent maturation in the ability of human neutrophils to mobilize BPI to sites of infection. Although it is at present unclear as to why expression of BPI is developmentally regulated, lower BPI expression in preterm and term newborns may contribute to susceptibility to early-onset sepsis [31] and necrotizing enterocolitis [32].

Diseases associated with anti-BPI Abs (antibodies)

Some of the ANCAs (anti-neutrophil cytoplasmic Abs) that have been measured in a number of diseases are neutralizing anti-BPI Abs [33]. The frequent presence of anti-BPI Abs in diseases characterized by recurrent Gram-negative bacterial infection may reflect the role of BPI in binding and delivery of endotoxin to APCs [34]. Inhibition of the antimicrobial BPI function by BPI-ANCA demonstrates a possible mechanism of how auto-Abs may contribute to increased susceptibility for pulmonary Gram-negative bacterial infections by diminished BPI-mediated bacterial opsonization and killing [35].

TAP (transporter associated with antigen presentation) deficiency is associated with diminished HLA class I expression and recurrent Gram-negative bacterial lung infections starting in childhood [36]. Of note, BPI-ANCA occurred in five of six TAP-deficient patients. Purified IgG from BPI-ANCA-positive sera bound both C- and N-terminal portions of BPI and inhibited the antimicrobial function of BPI in vitro. These observations suggested that long-lived BPI peptide fragments may function as immunogens.
CF (cystic fibrosis) is a common genetic disease of Caucasian populations caused by a mutation in the gene for the protein CFTR (cystic fibrosis transmembrane conductance regulator) required to regulate the components of sweat, digestive juices and mucus. CF is characterized by recurrent respiratory infection, often with Gram-negative bacteria. Anti-BPI ANCA was found in the majority of CF patients [37]. Of note, high positivity of anti-BPI Abs was also noted among the youngest CF patients, before the development of clinical signs of CF, indicating that formation of ANCA might be a very early event in the disease [38]. Anti-BPI Abs recognizing the C-terminus inhibit bacterial killing [39] and correlate with disease severity [40,41].

Additional conditions are associated with anti-BPI ANCA, including HIV infection [42]. Remarkably, HIV-infected patients with AIDS suffer increased intestinal permeability associated with endotoxin translocation that could contribute to both HIV replication and cachexia [43,44].

Mucosal injury, BPI depletion and endotoxia
in patients undergoing radiochemotherapy

Patients undergoing radiochemotherapy in the context of myeloablative conditioning for HSCT (haemopoietic stem cell transplantation) become profoundly neutropenic. Observational studies suggest endotoxia and activation of an acute phase response [45] as well as depletion of plasma BPI under such circumstances [46]. This appears to be a function of the absence of neutrophils as a source of plasma BPI, as the recovering [i.e. engrafting PMN (polymorphonuclear cell)] cells have normal concentrations of BPI on a per cell basis [47]. The relative importance of plasma BPI deficiency in the susceptibility of myeloablated hosts to inflammation and frank bacteremia is unknown as is the contribution of BPI deficiency to the clinical syndrome of fever of unknown origin that occurs after aggressive chemotherapy.

Translational opportunities for the enhancement of BPI expression

A cogent approach to translational development of BPI is that it may be most helpful to patients who have relatively low BPI levels in the face of Gram-negative infection and/or endotoxia.

Passive administration of recombinant BPI congeners

The selective anti-infective actions of BPI against Gram-negative bacteria have rendered it an attractive target for biopharmaceutical development. Recombinant protein congeners based on the N-terminal half of the protein that carries the endotoxin-neutralizing and antibacterial activities have been tested in vitro, in animal models and in human clinical trials [48]. The most frequently studied congener is a 21 kDa protein fragment rBPI21 that has proven safe and non-immunogenic in over 1000 human study subjects. BPI congeners have concentration-dependent kinetics and a Vss (steady-state volume of distribution) of ∼70–140 ml/kg, probably reflecting distribution in plasma and nearby cells such as haemocytes or endothelial cells [49]. Intravenous (i.v.) loading at a dose of 2 mg/kg over 30 min, resulted in rapid mean peak rBPI21 plasma concentrations of ∼7000 ng/ml (∼300 nM) [50], while subsequent maintenance infusion (2 mg/kg over 24 h) resulted in steady-state plasma concentrations of ∼120 ng/ml (∼6 nM). At this dose, rBPI21 probably undergoes liver-mediated clearance at a rate of 25 ml/min/kg [51]. From a pharmacodynamic standpoint, rBPI congeners demonstrate rapid (<1 h) killing of susceptible Gram-negative bacteria and inhibition of bacteria (LPS)-induced TNF (tumour necrosis factor) release in whole human blood in vitro with IC50 values of ∼1–100 nM [52,53].

Administered i.v., rBPI21 blunts LPS-induced cardiorespiratory changes and plasma cytokine and clotting responses in human volunteers injected with LPS in vivo [54–56]. Phase II studies of rBPI21 suggested benefit in a number of conditions considered to feature endotoxia, including partial hepatectomy and traumatic blood loss [9]. A phase III double-blinded, placebo-controlled trial of rBPI21 in case of children with meningococcal sepsis suggested benefit in rBPI21-treated study subjects, including a significant improvement in long-term functional outcomes as reflected by a combined morbidity/mortality score [57]. However, the study did not achieve a statistically significant improvement in mortality, the primary outcome measure in this logistically challenging trial [57,58], and rBPI21 is yet to achieve FDA (Food and Drug Administration) approval.

The ability of recombinant proteins such as rBPI21 to enhance antibacterial and endotoxin-neutralizing activity of human cord blood [53] raises the possibility that supplementing the relatively low endogenous BPI stores of newborns (e.g. by i.v. administration of rBPI21) may provide clinical benefit. A population that might particularly benefit from such intervention may be very low birthweight premature infants who are at high risk for Gram-negative sepsis [59] and/or other conditions associated with endotoxia, such as necrotizing enterocolitis [32]. Of note, prolonged intubation of critically ill preterm newborns is associated with endotoxin accumulation and endotoxin-directed innate immune responses in respiratory fluid [60], raising the possibility that respiratory administration of an endotoxin antagonist such as rBPI21, that can be bioactive in vivo by the intranasal route [61], may provide benefit by reducing lung inflammation and bronchopulmonary dysplasia [62].

Potential of LX-induced BPI expression

The discovery that BPI expression by human epithelial cells can be enhanced by LXs [19] raises the possibility that these and other pro-resolving eicosanoids could be used to augment BPI expression in vivo. Indeed, LXs are endogenously expressed in gastric mucosa, and are reduced in patients with ulcerative colitis [63], a deficiency that could contribute to
Figure 1 | Approaches to the enhancement of BPI expression in BPI-deficient hosts

(A) During myeloablation, chemotherapy and radiation therapy damage the intestinal tract, allowing translocation of bacteria and bacterial products such as LPS into the systemic circulation, while simultaneously depleting neutrophils that are a major source of endogenous BPI. (B) Replenishing of BPI, either as rBPI21 to increase blood plasma BPI activity or by the addition of LX congeners to increase epithelial BPI expression may enhance killing of Gram-negative bacteria and endotoxin neutralization, and might thereby provide clinical benefit.

Conclusions

Although caution is indicated in projecting the potential clinical impact of addition of a single agent in complex inflammatory processes [67], there is at least one example of clinical benefit from addition of the APP lactoferrin as an oral agent that reduces late-onset sepsis in preterm newborns [68]. The potential clinical benefits of APPs such as BPI are likely to be greater in those who are deficient in their expression [69].

Accordingly, replenishment of BPI, an agent that has proven safe and non-immunogenic in humans, in BPI-deficient hosts, whether by infusion of rPBI21 and/or by induction of endogenous BPI expression via oral administration of LX congeners (Figure 1), holds considerable promise.

Acknowledgements

We acknowledge Peter Elsbach for helpful editorial comments before submission. O.L. acknowledges the mentorship of Dr Jerrold Weiss, Dr Peter Elsbach, Dr Charlie Serhan and Dr Michael Wessels.

Funding

Work by E.C.G. and O.L. has been supported by a Human Immunology Grant from The Dana Foundation, The DARPA (Defense Advanced Research Projects Agency), NIH R21 1R21HL089659-01A1. C.D.P. is in part supported by Greene Family Fund. O.L.’s laboratory is also supported by NIH R01 AI067353-01A1 and a pilot grant award from the Dartmouth Center for Medical Countermeasures against Radiation. The Levy and Guinan laboratories have received reagent support in the form of rPBI21 from XOMA (US) LLC.

References

Infect. Immun. 112: 1122–1130


Received 13 January 2011
doi:10.1042/BSO390994