The bactericidal/permeability-increasing protein (BPI) in the innate defence of the lower airways

Alexander Holweg*, Markus Schnare† and André Gessner*1
†Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany, and
*Institut für Immunologie, Philipps-Universität Marburg, Hans Meerweinstrasse 2, 35032 Marburg, Germany

Abstract

The human BPI (bactericidal/permeability-increasing protein), stored in primary azurophilic granula of neutrophil granulocytes and produced by mucosal epithelia, has been known for decades to bind LPS (lipopolysaccharide) with very high affinity and to efficiently kill Gram-negative bacteria. Thus BPI potentially represents a central component of the innate immune system to directly combat microbes and modulate subsequent adaptive immune responses. Especially in the lungs, which are frequently exposed to a variety of inhaled pathogens, antimicrobial innate defence molecules such as BPI, are of exceptional relevance. In the present review, we highlight possible functions of BPI during acute pneumonia and CF (cystic fibrosis)-associated chronic infections in the lung.

Introduction

In contrast with most other human vital organs, the respiratory tract is persistently exposed to a broad variety of airborne particles during breathing. Pollutants and allergens such as chemicals, pollen and dust and, more dangerously, also potentially harmful pathogens are constantly inhaled [1,2]. To fulfil its function of maximal gas exchange, the lung represents the largest epithelial surface of our body [1]. As a consequence of the constant exposure to pathogens, pneumonia and related infections of the lung are an important cause of mortality and morbidity [3]. To maintain the sterility of the lower airways, different synergistically acting mechanisms of host defence have evolved. The innate immune responses build up the first line of defence against invading pathogens by providing structural barriers, phagocytic cells such as resident alveolar macrophages and infiltrating PMNs (polymorphonuclear leucocytes) and by supplying a broad array of different antimicrobial proteins and peptides [2]. These polypeptides, produced by PMNs or epithelial cells, will either directly eliminate bacteria or destroy them indirectly by activating phospholipases or the complement system [4]. Furthermore, some of them can enhance the uptake of bacteria by phagocytes through opsonization, leading to a killing of the ingested pathogens by ROS (reactive oxygen species) or by antimicrobial proteins in the phagolysosome. When bacterial ligands are sensed by pattern recognition receptors expressed on resident cells in the lung, a downstream signalling cascade is induced leading to the secretion of cytokines and chemokines and eventually to the recruitment of the most important innate effector cell during lung infection, the PMN [5].

BPI (bactericidal/permeability-increasing protein) was first described in the late 1970s as an antibacterial protein isolated from granules of blood-derived PMNs and found to be active specifically against Gram-negative bacteria [6]. In humans it is expressed early during myelopoiesis in neutrophil precursor cells of the bone marrow and is subsequently stored in high amounts in primary granules of PMNs and to a lower extent in eosinophils [7,8]. The expression of BPI homologues is evolutionary conserved among species, including other mammals, birds, teleosts and even molluscs [9]. The molecular mass of this single-chain protein is approximately 55–60 kDa [6], and X-ray crystallography revealed a boomerang-shaped structure built up of two functional domains [9]. The cationic N-terminal domain of BPI comprises the whole bactericidal activity [10] as well as the capacity to bind the lipid A region of LPS (lipopolysaccharide) with even higher affinity than the LPS-binding protein. Thereby, BPI effectively neutralizes endotoxin-mediated pro-inflammatory responses [11–14]. More features and functions of BPI and other BPI family members during infection and inflammatory disease have been recently excellently reviewed [9,15]. The purpose of this review is to highlight the so far known functions of BPI in the context of acute and chronic human and experimental murine pulmonary diseases.

BPI in acute pneumonia

In the lungs of a healthy individual, many inhaled particles and pathogens are trapped in the thin mucus layer, produced by airway epithelial cells, coating the upper airways and are subsequently removed by the mucociliary escalator to be either swallowed or expectorated [1,4] (Figure 1A).
However, microbes that successfully pass this mechanical barrier and reach the alveoli are now confronted with a variety of antimicrobial defence molecules, among them BPI (Figure 1A). After recognition of the pathogen by pattern recognition receptors such as TLRs (Toll-like receptors) expressed on alveolar macrophages, dendritic cells and epithelial cells in the lung, the initial phase of acute pneumonia is characterized by a massive infiltration of neutrophils [5]. As BPI is stored in large amounts in primary azurophilic granules [7] and is additionally associated with the surface [16] of these main innate effector cells during acute pneumonia [5], BPI is most likely to be of central relevance for the antimicrobial defence in the lower airways. On phagocytic uptake of bacteria by neutrophils, the primary granules are rapidly degranulated into the phagosome, leading to direct killing of the pathogens through very high local concentrations of BPI and other AMPs (antimicrobial peptides/proteins) [4] (Figure 1A).

Another contribution of neutrophils to the innate immune defence is the degranulation of intracellular stores of antimicrobial contents into the surrounding inflammatory fluid. We showed that human PMNs released high amounts of BPI in a time-dependent manner into the culture supernatant after stimulation with *Pseudomonas aeruginosa* [17]. This process is completely independent of protein *de novo* synthesis and solely mediated by release from primary granules [17]. *P. aeruginosa*, among other Gram-negative bacteria, is known to be very susceptible to BPI-mediated killing [17,18]. BPI may play an important role in acute hospital-acquired or ventilator-associated infections commonly caused by *P. aeruginosa* [19]. In tracheal aspirates of mechanically ventilated newborns, who are prone to bacterial colonization and lung inflammation, extracellular BPI positively correlates with the number of infiltrating PMNs, arguing for PMNs as the most important source of BPI in the airways [20]. Besides the long known phagocytosis and degranulation processes, neutrophils were found in the last few years to employ a new third antimicrobial strategy. By expelling decondensed chromatin decorated with antibacterial proteins from their granules, they are able to trap bacteria in so-called NETs (neutrophil extracellular traps) [21] (Figure 1A). These structures actively kill captured bacteria and BPI [21] or a 25 kDa cleavage product [22] were found in NETs of human neutrophils. NETs may limit the spread of the trapped bacteria, provide a high local concentration of AMPs, and therefore facilitate synergy between these components [23]. Another important aspect highlighting the importance of BPI in innate immunity is the fact that neutrophils of newborns are selectively deficient for this protein and as a consequence have a reduced antibacterial activity against Gram-negative *Escherichia coli* [24]. This finding correlates with an increased incidence of Gram-negative sepsis among newborns [24], where the primary site of infection can be the lung. In addition, significantly elevated plasma levels of BPI have been detected in adult patients suffering from severe sepsis [25]. Despite the documented antimicrobial nature of BPI, very high plasma levels of BPI correlate with increased mortality during sepsis [26,27], probably reflecting massive bacterial encounter.
Besides the aforementioned neutrophils [6] and eosinophils [8], mucosal epithelial cells were found to be an additional source of BPI [28]. In oral, intestinal and pulmonary epithelial cell lines, BPI expression was inducible on stimulation with the stable lipoxin analogue LXA₄ and displayed antimicrobial activity against Salmonella enterica serotype Typhimurium as well as an LPS-neutralizing function [28]. Furthermore, human genital tract epithelial cells also constitutively express functional BPI contributing at least in part to bacterial killing of a commensal E. coli strain [29]. Although in sections of human oesophageal and intestinal tissue BPI was detected in the epithelial lining and, moreover, BPI was even found to be located in the cell surface of lipoxin-stimulated non-permeabilized epithelial cells [28], these epithelial cells do not secrete BPI [29]. In our own studies we could detect by flow cytometry analysis of the sputum or BALF [BAL (broncho-alveolar lavage) fluid] samples of CF (cystic fibrosis) patients a CD66b/CD14 negative cell population with intracellular BPI content besides the highly BPI positive neutrophil granulocytes [17]. Morphological analysis of these cells strongly suggested that indeed primary epithelial cells of the lower airways during Ps. aeruginosa infection express BPI too [17].

During infection of the respiratory tract, BPI might display regulatory functions in addition to direct antimicrobial activity. The cationic N-terminal domain of BPI binds the lipid A region of LPS [11–14], thereby neutralizing the potent pro-inflammatory impact of endotoxin derived from Gram-negative bacteria [9] (Figure 1A). Furthermore, BPI can deliver LPS containing outer membrane vesicles (blebs), which are shed by Gram-negative bacteria to dendritic cells [30], thereby enhancing the subsequent antigen presentation.

Since the analysis of BPI functions in vivo in the human system is very difficult, therapeutic effects of BPI have been investigated in mouse models. The administration of human BPI and BPI congeners [31] or adenoviral-mediated gene transfer of full-length [32] or N-terminal human BPI [33,34] during intraperitoneal injections with Gram-negative bacteria- or LPS-mediated negative cell population with intracellular BPI content besides the highly BPI positive neutrophil granulocytes [17]. Morphological analysis of these cells strongly suggested that indeed primary epithelial cells of the lower airways during Ps. aeruginosa infection express BPI too [17].

During infection of the respiratory tract, BPI might display regulatory functions in addition to direct antimicrobial activity. The cationic N-terminal domain of BPI binds the lipid A region of LPS [11–14], thereby neutralizing the potent pro-inflammatory impact of endotoxin derived from Gram-negative bacteria [9] (Figure 1A). Furthermore, BPI can deliver LPS containing outer membrane vesicles (blebs), which are shed by Gram-negative bacteria to dendritic cells [30], thereby enhancing the subsequent antigen presentation.

Since the analysis of BPI functions in vivo in the human system is very difficult, therapeutic effects of BPI have been investigated in mouse models. The administration of human BPI and BPI congeners [31] or adenoviral-mediated gene transfer of full-length [32] or N-terminal human BPI [33,34] during intraperitoneal injections with Gram-negative bacteria- or LPS-mediated septic shock can potently improve the survival and dampen inflammatory responses. In a model of Streptococcus pneumoniae lung infection, recombinant human BPI was shown to improve the survival of challenged mice through enhancing upper respiratory tract cell apoptosis and macrophage-bacteria interaction [35]. In contrast with the long known human orthologue, the endogenous mouse BPI has only recently been described [36,37]. We were able to demonstrate that mouse BPI is inducible on stimulation with LPS in neutrophils and dendritic cells [36] and can potently neutralize the endotoxic activity of LPS and Gram-negative bacteria [38]. We then aimed to characterize the expression of murine BPI during acute pneumonia and found 24 h post-infection a robust induction on transcriptional level in BALF derived cells (Figure 2) and in lung tissue (results not shown) of mice infected intratracheally with Ps. aeruginosa, S. pneumoniae or Streptococcus agalactiae. At this time point, the cells within the BALF are mainly infiltrating neutrophils, which up-regulate their BPI mRNA expression compared with uninfected controls or unstimulated peripheral neutrophils (Figure 2). It is therefore very likely that murine BPI will actively contribute to the defence of the lower airways during acute pneumonia, and studying experimental infections in BPI-gene-deficient mice will provide an important insight into the function of this AMP.

BPI in CF

CF is the most frequent autosomal-recessive disorder in the Caucasian population. In the majority of the patients, the disease is mediated by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene. This results in an imbalance of electrolyte transport on all epithelia including the lung. The altered electrolyte composition of the mucus leads to higher viscosity of the airway surface liquid, thereby reducing the transport velocity of the mucus on the airway epithelial cells [39] (Figure 1B). Therefore the airways of CF patients are chronically colonized with bacteria and fungi, resulting in a constant neutrophilic inflammation and degranulation. Infection with Ps. aeruginosa is the major cause of morbidity and mortality among CF-affected individuals. The infection is characterized by either acute pneumonia or chronic lung disease with periodic acute exacerbations [40–42]. Although both CF and non-CF lung epithelial cells express functional TLRs that can mediate inflammatory responses to microbes the lung epithelium of CF patients is predisposed to chronic and progressive Ps. aeruginosa infection. In the airway surface liquid of the lung multiple AMPs were identified. It was shown that the epithelium expresses the inducible β-defensins, hBD
antibodies with chronic highlighted by the correlation of the presence of these cytoplasmic autoantibodies) directed against BPI. This is course of CF is the development of ANCAs (anti-neutrophil antimicrobial activity of the lung occurring during the important complicating factor interfering with the direct applied antibiotics [51] and maybe BPI, too. Another aeruginosa in biofilms renders the bacteria highly resistant to endogenous antibacterials, phagocytosis as well as (Figure 1B). Alginate-containing biofilms protect the bacteria isolated from chronically infected CF patients display rendering antibacterial mechanisms ineffective. Ps. aeruginosa, respond to the ongoing immune response as well as to the antibiotic treatment by evasion strategies as well as to the antibiotic treatment by evasion strategies rendering antibacterial mechanisms ineffective. Ps. aeruginosa isolated from chronically infected CF patients display a mucoid phenotype by producing an alginate capsule (Figure 1B). Alginate-containing biofilms protect the bacteria from endogenous antibacterials, phagocytosis as well as complement [49,50]. In addition, the slow growth of Ps. aeruginosa in biofilms renders the bacteria highly resistant to applied antibiotics [51] and maybe BPI, too. Another important complicating factor interfering with the direct antimicrobial activity of the lung occurring during the course of CF is the development of ANCAs (anti-neutrophil cytoplasmic autoantibodies) directed against BPI. This is highlighted by the correlation of the presence of these antibodies with chronic Ps. aeruginosa lung infection [52,53] and Pseudomonas-induced lung damage [54]. In addition, the detection of BPI–ANCA resulted in a poor prognosis of the disease outcome in CF patients [55]. Most likely, the constant neutrophilic inflammation and constant degranulation of the neutrophils during CF is the cause for the appearance of these antibodies. The frequency of autoantibodies directed against BPI is up to 90% in CF patients [56,57]. In vitro, BPI–ANCAs have been shown to inhibit neutrophil-mediated killing of Ps. aeruginosa [58]. It is therefore tempting to speculate that neutralizing BPI–ANCA interfere with the bacterial killing of Ps. aeruginosa in the lungs of CF patients and thereby promote the establishment of chronic bacterial infection. It is clear that the airway surface liquids of CF patients fail to kill bacteria because of its abnormal composition [59].

Overall, the changes in the airway surface liquid in CF patients due to the defect in CFTR lead to an environment were the bacterial killing by endogenous antimicrobial mechanisms such as BPI as well as antibiotic treatment are severely altered. The reduced bacterial killing may pave the way for the chronic colonization of the lung epithelium with bacteria, and the interplay of bacterial colonization and the resulting chronic inflammatory response results in the obstructive destruction of the lung epithelium.

Future perspectives
From all existing data we can conclude that BPI is an important player of innate immunity in the lung to combat bacterial pathogens and dampen LPS-mediated, otherwise exaggerated, pro-inflammatory host responses. Since it is difficult to investigate the function of BPI during the course of infection in humans, its role and interactions with other antimicrobial or LPS-binding proteins of BPI in vivo are still ill defined. As we could recently report, we are therefore extensively investigating the properties and functions of the long neglected murine BPI [36,38]. Our group has applied gene-targeting techniques to generate BPI-deficient and human BPI-transgenic mice to further dissect its functions in different infection models to shed some light on so far unknown attributes of this intriguing innate defence protein.

Funding
This work was supported by a grant from the German Research Foundation to M.S. The work of A.G. and A.H. was funded by the German Research Foundation [grant number GZ-671-12/1-2].

References


Received 17 January 2011
doi:10.1042/BST0391045