

Meet the relatives: a family of BPI- and LBP-related proteins

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Until recently, two key members of the innate immune response to Gram negative bacteria, bactericidal permeability-increasing protein (BPI) and lipopolysaccharide (LPS)-binding protein (LBP), have been considered to be members of a small family of lipid-binding proteins that also contains cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). A recent paper has characterised three related proteins that are expressed in the mouth, nose and upper airways. Taken together with other recent data, it is clear that a large family of such proteins exists and these additional members might also function in the innate immune response.

Epithelial surfaces are constantly exposed to potential pathogens and support large, complex, commensal, microbial populations, which elicit no significant response despite intimate contact with host tissues. The innate immune system, which acts as the first line of host defence and without prior exposure to pathogen, is of central importance in maintaining such host–microbe homeostasis. Many of the molecules associated with the innate immune response specifically interact with and respond to the bacterial surface. Two of the proteins crucial to the mediation of signals from the surface of Gram-negative bacteria are lipopolysaccharide (LPS)-binding protein (LBP) and bactericidal permeability-increasing protein (BPI) [1,2]. LBP and BPI, which are predicted to be structurally similar [3], both bind the Lipid A component of LPS from the outer envelope of Gram-negative bacteria, although they are generally considered to have antagonistic functions. LBP alarms the host to the presence of minute amounts of LPS and can therefore be considered to be proinflammatory, whereas BPI renders LPS non-inflammatory [1,2]. The predominance of these pro- and anti-inflammatory pathways might ultimately determine the host response to bacteria [2,4].

As well as being predicted to be structurally similar, LBP and BPI are encoded by related genes, which are themselves products of a gene duplication event, and which have remained adjacent to each other at chromosomal location 20q11.23 [5]. Two further related proteins have also been characterised, phospholipid transfer protein (PLTP) (also located on chromosome 20) and

cholesteryl ester transfer protein (CETP) (located on chromosome 16). PLTP and CETP function as lipid transfer proteins (but not specifically in host defence) [6] and thus the BPI–LBP–PLTP–CETP family of proteins appear to share the ability to bind to and transfer lipid molecules.

Defining a new family of BPI- and LBP-related proteins

Based on a combination of sequence similarity, strengthened by structure predictions and conserved gene structures, there is now evidence for a new family of at least nine (human) or twelve (mouse) genes that encode proteins homologous to the BPI–LBP–PLTP–CETP family. The recent paper by Andraut *et al.* [7] characterises the cDNAs and expression patterns of human and mouse orthologues of three of these genes, which they term *RYA3*, *RY2G5* and *RYSR* [7]. This study complements the recent papers of Mulero *et al.* [8], England *et al.* [9] and ourselves [10,11], which together establish the genomic locus on chromosome 20 q11.21, depicted in Figure 1. This locus is close to the *BPI* and *LBP* genes at 20q11.23 [5] and supports the contention that these genes evolved from a common ancestor. This paper also helps to establish a pattern of expression that is predominantly targeted to the mouth, nose and the conducting airways of the lung. The evidence to date is that this family is restricted to air-breathing vertebrates [11]; conserved loci being present in mice, rats and cows [10–12]. It appears appropriate to consider these proteins as members of a distinct subfamily because they are clustered in the genome at a site distinct from the other genes, they have overlapping expression patterns and exhibit predicted structural variability, which contrasts with the high degree of predicted structural conservation in the BPI–LBP–PLTP–CETP family [10,11].

Pointers towards a function in innate immunity

The function of the proteins in the PLUNC (palate lung and nasal epithelial clone)–RY–BPIL (BPI-like) family is not known. Indeed, given the diversity of function of the BPI–LBP–PLTP–CETP proteins [1,2,6], it might be that members of the family have distinct functions. The main evidence supporting related functions is that the proteins have similar, although not identical, sites of expression in the oral, nasopharyngeal and respiratory epithelium [7–11], which contrasts with the variety of sites of expression of the BPI–LBP–PLTP–CETP proteins. The

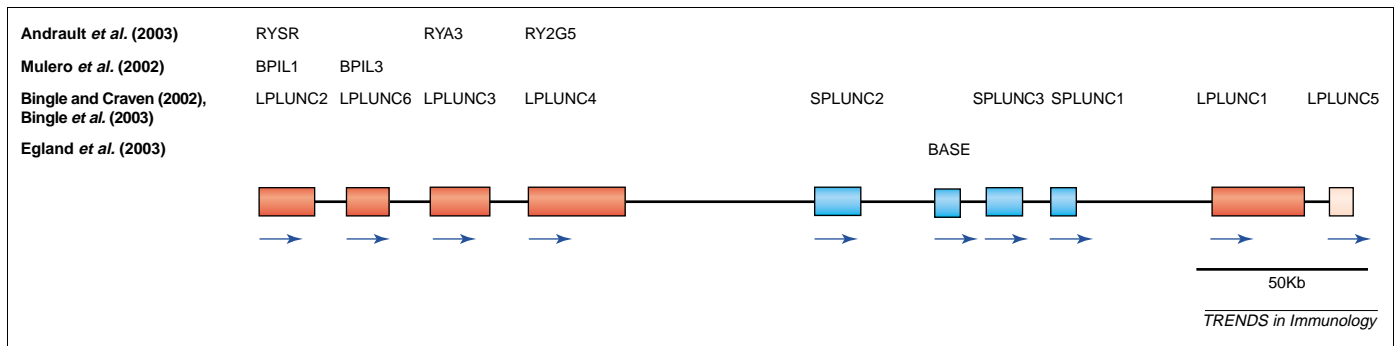


Figure 1. The human *PLUNC-RY-BPIL* gene cluster on chromosome 20q11.21. The locus (and nomenclature) is derived from Andraut *et al.* [7], Mulero *et al.* [8], Bingle and Craven [10], Bingle *et al.* [11] and Egland *et al.* [9], as indicated. The differentiation between the LPLUNC proteins (red boxes) and the SPLUNC proteins (light blue boxes) is that LPLUNC proteins are homologous to both domains of BPI-LBP-CETP-PLTP, whereas SPLUNC proteins have homology to the N-terminal domain [10]. The pale red box indicates the position of a pseudogene for the human orthologue of rodent LPLUNC5 [11]. The blue arrows indicate the direction of transcription. An analysis of all of the members of this family that have been identified across a wide range of species can be found in Ref. [11]. Abbreviations: BPIL, BPI-like; PLUNC, palate lung and nasal epithelial clone; PSP, parotid secretory protein; SPLUNC, short PLUNC.

genomic clustering of the genes might indicate the conservation of common expression control elements but is more likely to simply reflect the relatively recent duplication events that have given rise to this family. The definition of 'short' and 'long' proteins (Figure 1) corresponding to either one or both domains of BPI-LBP-PLTP-CETP [3,10] might also reflect a fundamental distinction in function.

The two main functions that have been proposed for the PLUNC-RY-BPIL proteins are in the innate immune response and in olfaction. However, to date no direct evidence has been presented for either function. The argument for a role in olfaction arises primarily from the original identification of expression of rat *RY2G5* and *RYA3* in the rat olfactory mucosa [13]. Andraut *et al.* also point out that some odorant binding proteins are also expressed in the genital sphere, which is similar to the pattern observed for the protein that they term RYSR [7]. Countering these arguments is the question of why some of the proteins are also found much further down the airways than one would normally expect to find molecules involved in olfaction.

The suggestion of a role for this family of proteins in innate immunity stems from the predicted structural homology to BPI and LBP [3,10], which suggests that these proteins might interact with LPS. Indeed Ghafouri *et al.* have recently demonstrated binding of PLUNC proteins to an LPS-coated surface [14]. However, Andraut *et al.* also point out that PLTP is able to interact with LPS and PLTP is not normally considered to be part of the innate immune system [6,7]. A possible mode of binding of LPS to BPI or LBP has been inferred from the crystal structure of BPI, which strongly implicates a role for the hydrophobic pocket in each domain [3], although peptide studies have also implicated the positively charged residues in the tip of the N-terminal domain, which are not well conserved in the PLUNC-RY-BPIL proteins [2,3]. Because similar hydrophobic pockets are also predicted to exist in PLTP and to be involved in its lipid transfer functions [6], and hydrophobic interactions are rather non-specific, it is not entirely surprising that the hydrophobic pocket should interact with LPS. By the same token, the observation of LPS binding to PLUNC proteins is not compelling evidence of physiological relevance. There are a few additional

observations, however, that add weight to the suggestion that members of this family might have a role in host defence against bacteria. The rat SPLUNC (short PLUNC) protein, PSP (parotid secretory protein), interacts with bacterial membranes [15] and human SPLUNC1 is present in the antimicrobial fraction of human nasal secretions [16]. Human SPLUNC1 is increased in the sputum of patients with chronic obstructive pulmonary disease [17].

The upper airways, nose and mouth are clearly sites of frequent exposure to inhaled or ingested bacteria, which is also part of the argument in favour of a role in innate immunity. However, the role might not be directly bactericidal; instead it might be part of the pathogen-sensing mechanisms analogous to the functions of LBP or conversely it might be to damp-down an excessive response to such bacteria or to provide a bacteriostatic function that allows the presence of a commensal bacterial flora.

Why so many members in the family?

A key question is why are there so many different PLUNC-RY-BPIL proteins? It is certainly true that many host defence proteins, for example defensins and Toll-Like receptors, are members of large gene families, which enables the host to mount complex and specific responses to multiple pathogens. One feature that emerged from structure predictions of the PLUNC-RY-BPIL family of proteins is that although the BPI-LBP-PLTP-CETP proteins are predicted to have similar 3D structures, there is significantly more variability among the proteins of the PLUNC-RY-BPIL family [10]. The variability appears to reside primarily in the apical tip of the N-terminal domain [10]. In BPI, this structure has been associated with its bactericidal properties [2]. Elucidating the functional role of this variability in the PLUNC-RY-BPIL family may also shed light on the mechanisms of action of the BPI-LBP-PLTP-CETP family.

As is not uncommon in the genomic era, much more is currently known about the existence of the PLUNC-RY-BPIL family than is known about their function. The challenge to the immunological community now is to devise

the experiments that can test their putative role in host defence.

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