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The lactoferrin receptor complex in gram negative bacteria

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Abstract Bacteria that inhabit the respiratory and genitourinary tracts of mammals encounter an iron-deficient environment on the mucosal surface where iron is complexed by the host iron-binding proteins transferrin and lactoferrin. Lactoferrin is also present in high concentrations at sites of inflammation where the cationic anti-microbial peptide lactoferricin is produced by proteolysis of lactoferrin. Several members of the Neisseriaceae and Moraxellaceae families express surface receptors, capable of specifically binding host lactoferrin and extracting the iron from lactoferrin as a source of iron for growth. The receptor is comprised of an integral outer membrane protein, lactoferrin binding protein A (LbpA), and a largely exposed surface lipoprotein, lactoferrin binding protein B (LbpB). LbpA is essential for mediating growth using lactoferrin as a sole iron source whereas LbpB only plays a facilitating role. LbpB, with the presence of a large tract of negatively charged residues, appears to protect the bacterial cell from the bactericidal effects of the lactoferricin. The lactoferrin receptors in these species appear to be essential for survival and thus may serve as potential vaccine targets.

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Distribution of lactoferrin receptors in gram-negative species

Iron serves as a cofactor for many important biological redox reactions, thus is an essential requirement for nearly all organisms (Weinberg and Weinberg 1995). Due to its solubility properties and propensity to generate toxic by-products in the presence of oxygen, most organisms have developed specialized systems for acquiring, transporting and storing iron. In the vertebrate host, the majority of iron is in the intracellular compartment, primarily in ferritin or in heme-containing proteins. Within the body, most of the extracellular iron is complexed by the serum glycoprotein, transferrin, that serves to transport iron from areas of supply to areas of need. Transferrin is an 80 kDa bi-lobed protein with two structurally equivalent lobes, each capable of binding a single ferric ion (Baker et al. 2002). Since there normally is an excess iron-binding capacity of circulating transferrin, any ferric ion that is released into the extracellular compartment is readily sequestered by transferrin.

Lactoferrin is a related glycoprotein found in mammals that is produced by mammary glands, glandular tissue on mucosal surfaces and found in secretory granules of polymorphonuclear leucocytes (Lonnerdal and Iyer 1995). It is structurally similar to transferrin and possesses similar iron binding properties, but is more effective at binding ferric ion at low pH (Baker et al. 2002). Lactoferrin is normally present in relatively small concentrations (0.18–1 μg.ml) in serum and elsewhere in the extracellular milieu within the body except at sites of inflammation but is found in high concentration in tears (1–3 mg/ml), milk (1 mg/ml), and bronchial secretions (11.5% of total protein; Kijlstra et al. 1983; Kolsto Otnaess et al. 1983; Harbitz et al. 1984). The regulation of expression of lactoferrin varies between different tissues with largely constitutive expression by glandular epithelial cells on most mucosal surfaces while mammary and uterine tissue are under hormonal regulation (Teng 2002; Ward et al. 2005). Thus while transferrin is the major iron binding protein in serum and the extracellular milieu within the body, lactoferrin is considered the major iron binding protein in bodily secretions, on the mucosal surface and at sites of inflammation.

Initially the antimicrobial properties of lactoferrin were primarily attributed to its iron sequestering abilities (Arnold et al. 1982). Subsequently, bactericidal activity was shown to be associated with an N-terminal peptide released by proteolysis of lactoferrin (Bellamy et al. 1992), suggesting that this may have a significant effect at sights of inflammation. The peptide released by acidic proteolysis, lactoferricin, has broad antimicrobial activity but relatively little effect on normal mammalian cells (Gifford et al. 2005). Although there likely would be substantial production of lactoferricin in the gastrointestinal tract from ingested lactoferrin, the extent to which this would occur on the mucosal surfaces of the respiratory and genitourinary tracts is uncertain.

The mucosal surface is the site or entry point for most bacterial infections, thus most bacterial pathogens need to be successful at colonizing the mucosal surface and competing with existing microflora. The mucosal surfaces in the gastrointestinal, respiratory and urogenital tracts represent different environmental niches that present different challenges for pathogenic bacteria. The human pathogens *Neisseria meningitidis* and *N. gonorrhoeae* that colonize the respiratory and urogenital mucosal surfaces respectively, were shown to be able to use human lactoferrin as a source of iron for growth, a presumed adaptation to the high levels of lactoferrin and low levels of free iron in these environments (Mickelsen et al. 1982). These pathogens were also able to use human transferrin as a source of iron for growth (Mickelsen and Sparling 1981), that clearly would be advantageous during invasive infection. The discovery of surface receptor proteins in these species that specifically bound host transferrin or lactoferrin (Lee and Schryvers 1988; Schryvers and Morris 1988a, b) was a critical step in the subsequent characterization of the respective iron acquisition pathways and ultimately provided avenues for exploring the prevalence of these pathways in other species and their role in survival in the host.

Studies based on binding and affinity capture assays (Gray-Owen and Schryvers 1996) and PCR-based assays (Ogunnariwo and Schryvers 1996) revealed that transferrin receptors were present in species belonging to the Neisseriaceae, Moraxellaceae and Pasteurellaceae families whereas lactoferrin receptors were only present in the first two families. The species possessing transferrin and lactoferrin receptors characteristically are highly host-adapted and host-restricted bacteria, likely a reflection of the host specificity of the receptor proteins. They are also significant pathogens for their host species. In those species that have a broad range of hosts, the receptors are found in a subset of strains that likely are host-restricted, and in the case of the receptors in *Pasteurella multocida,* represent a new type of transferrin receptor (Ogunnariwo and Schryvers 2001). These general observations have largely held up to scrutiny by bioinformatics analyses with the increasing availability of sequenced bacterial genomes.

Although the species that possess transferrin and lactoferrin receptors are important pathogens, they are primarily adapted to survival in the mucosal environment of the host and for effective means of transmission between hosts. Experiments in a human gonococcal infection model (Cornelissen et al. 1998) and in a pig model for *Actinobacillus pleuropneumoniae* infection (Baltes et al. 2002) demonstrate that the transferrin receptors are essential for survival and disease causation in the host, implying that transferrin serves as an essential iron source on the mucosal

surface. Follow up experiments in the human gonococcal model of infection revealed that optimal survival relies on the presence of both transferrin and lactoferrin receptors (Anderson et al. 2003), that either provides some ability to survive and that the transferrin receptor appears to be more important. Although several different conclusions could be drawn from this study, one interpretation is that either transferrin or lactoferrin can serve as a source of iron for growth on the human male genitourinary tract. The observation that the levels of transferrin in urine were higher than lactoferrin prior to inoculation with gonococci, infers that transferrin is readily available on the mucosal surface of the male genitourinary tract. The levels of lactoferrin in urine rose after inoculation with gonococci, but only reached levels that were comparable to those of transferrin. Although similar information is not available for the mucosal surfaces of the respiratory tract, the importance and ubiquitous presence of transferrin receptors in meningococci and species that possess this mode of iron acquisition, suggest that transferrin is readily available. The dependence upon transferrin and lactoferrin receptors for survival is clearly not shared by other related species in this environment, thus many questions remain regarding the role and selective advantage that these iron acquisition systems provide and why they result in an enhanced propensity to cause disease.

Early studies examining the ability of various species within the Neisseriaceae to use lactoferrin as a source of iron for growth clearly demonstrated that it was more prevalent in the 'pathogenic' species than in the commensal species (Mickelsen et al. 1982). However, in contrast to the ubiquitous capability in meningococcal isolates (15/15), lactoferrin utilization was only present in half of the gonococcal strains (31/59). Subsequent studies confirmed that a substantial proportion of clinical isolates were deficient in expression of the lactoferrin receptor or deficient in production of the lipoprotein component, lactoferrin binding protein B, and identified several different genetic mechanisms for the deficiencies (Anderson et al. 2003). In contrast, all the gonococcal isolates from a geographically limited, but clinically diverse, spectrum of gonococcal isolates were receptor-positive based on binding and affinity capture assays (Lee and Bryan 1989; Schryvers and Lee 1989). Thus the extent to which lactoferrin receptors are defective or absent in fresh clinical isolates of gonococci and whether this might also apply to isolates of meningococci is not fully appreciated. Preliminary studies indicate that LbpB protects against the bactericidal activity of lactoferricin (data not shown), suggesting that the selective forces for retention of the lactoferrin receptor may vary not only in different niches but also on the presence and degree of the inflammatory response.

Preliminary growth studies suggested that lactoferrin utilization was present in a number of different commensal *Neisseria* species (8/33) (Mickelsen et al. 1982) but bioinformatics analyses only confirm the presence of genes encoding lactoferrin receptors in *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica* and *N. cinerea.* These species belong to one of the three groups of *Neisseria* species defined by 16S rDNA sequence analysis (Brenner et al. 2004) that consists of the closely related asaccharolytic species *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. cinerea*, and *N. polysaccharea*. The absence of lactoferrin receptors in other commensal *Neisseria* species that reside in a similar environment begs the question of how these species deal with the forces selecting for the presence of lactoferrin receptors in *N. meningitidis*, *N. lactamica* and *N. cinerea* or whether they are adapted to different microenvironments on the mucosal surface.

Whole cell binding assays suggested the presence of host specific transferrin and lactoferrin receptors in the human pathogens *Moraxella catarrhalis* and *M. lacunata* and the bovine pathogen *M. bovis* (Gray-Owen and Schryvers 1996). The receptor proteins from *M. catarrhalis* (Bonnah et al. 1998, 1999) and *M. bovis* (Yu and Schryvers 2002) were identified and characterized by biochemical and genetic means. The distribution of lactoferrin receptors in other *Moraxella* species that reside in the respiratory or genitourinary tract of mammals is currently unknown due to the lack of experimental and sequence data. Members of the other genera within the Moraxellaceae family do not reside on mucosal surfaces of a vertebrate host thus the absence of receptor homologues in their genomic sequences was anticipated.

The ability to bind lactoferrin or use it as a source of iron for growth has been demonstrated in a variety of different bacterial species (Redhead et al. 1987; Tryon and Baseman 1987; Naidu et al. 1990, 1991,

1992; Tigyi et al. 1992; Staggs et al. 1994; Alugupalli et al. 1995; Dhaenens et al. 1997; Moshynskyy et al. 2003) but these systems are unrelated to the lactoferrin receptors in the Neisseriaceae and Moraxellaceae families and their role in iron acquisition are not well established.

Since lactoferrin receptors have only been identified in two families it begs the questions of the relatedness of LbpA and LbpB between *Neisseria* and *Moraxella* species. Although there are a limited number of published LbpA and LbpB sequences available for phylogenetic analysis, the preliminary results show that the *Neisseria* species, *M. catarrhalis* and *M. bovis* LbpAs and LbpBs are in distinct groupings (Fig. 1). This suggests that horizontal exchange between *Neisseria* and *Moraxella* species in the human host does not occur frequently. The differences between *M. bovis* and *M. catarrhalis* LbpA and LbpB is also not surprising due to their inhabiting different hosts. It is also noteworthy to mention that the *M. bovis* LbpB does not contain either of the negatively charged regions that are discussed below, which has obvious implications for the ability of *M. bovis* to bind lactoferricin.

The interaction between the surface protein, PspA, from *Streptococcus pneumoniae* and human lactoferrin has been well-characterized and its ability to protect *S. pneumoniae* from the bactericidal effects of lactoferricin suggests that this may be its primary role in vivo (Shaper et al. 2004; Senkovich et al. 2007). The lipoprotein component of the lactoferrin receptors in Gram-negative bacteria, LbpB, may perform a similar function and thus provide protection in areas of inflammation and perhaps contribute to the increased propensity to cause disease by 'pathogenic' species.

Characteristics of the lactoferrin receptor proteins

The relatively high pI of lactoferrins initially complicated the identification and characterization of the lactoferrin receptor proteins due to their propensity for nonspecific interactions. Solid phase binding assays and affinity capture experiments were routinely performed under high pH and high salt conditions to reduce nonspecific interactions and as a consequence some interactions were overlooked.

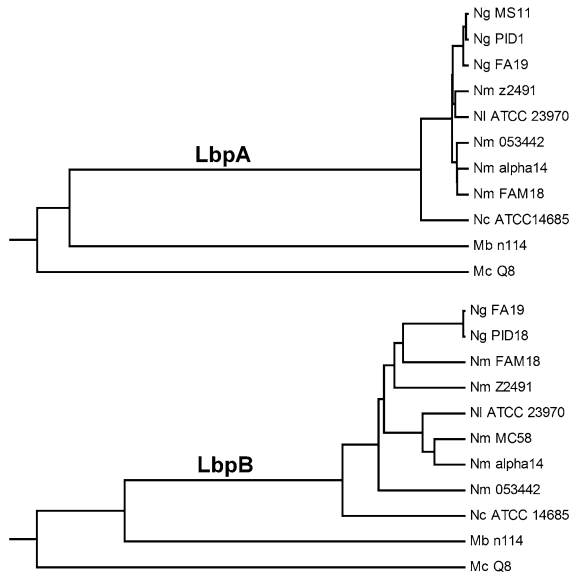

Fig. 1 Phylogenetic relationships between the proteins of the lactoferrin binding receptor complex. Published LbpA and LbpB sequences from *N. meningitidis* strains MC58, FAM18, Z2491, 053442, and α14, *N. gonorrhoeae* strains FA19, PID18, and MS11 (LbpB only as the current genome sequence contigs break in LbpA), *N. lactamica* ATCC 23970, *N. cinerea* ATCC 14685, *M. catarrhalis* Q8, and *M. bovis* N114 were aligned using ClustalW. Phylogentic trees were created using MEGA4. These results show that the LbpA is more highly conserved in *Neisseria* species than LbpB and that the *Moraxella* LbpA and LbpB proteins are quite different. That both receptor proteins are different between *M. catarrhalis* and *M. bovis* may also be a reflection of host specificity. Nm, *N. meningitidis*; Ng, *N. gonorrhoeae*; Nl, *N. lactamica*; Nc, *N. cinerea*; Mb, *M. bovis*; Mc, *M. catarrhalis*

The initial identification of the lactoferrin receptor from *N. meningitidis* described a single 100 kDa protein (Schryvers and Morris 1988a), the integral outer membrane protein now termed lactoferrin binding protein A (LbpA). Similar observations were made when isolating lactoferrin receptors from other species (Schryvers and Lee 1989). The lipoprotein component of the receptor, now termed LbpB, was only identified by affinity capture when alternate conditions for affinity isolation were explored (Bonnah et al. 1995), but resulted in an increased propensity for nonspecific background. Analogous to most transferrin receptors, genes encoding the lactoferrin receptor proteins, *lbpA* and *lbpB*, are organized in an operon with *lbpB* preceding *lbpA* (Bonnah and Schryvers 1998; Du et al. 1998; Yu and Schryvers 2002) with a third gene of unknown function included in the operon in *M. catarrhalis* (Bonnah et al. 1999).

The ability to use lactoferrin as an iron source is abolished by mutation of the gene encoding the energy transducing protein TonB (Stojiljkovic and Srinivasan 1997), the periplasmic iron binding protein FbpA (Khun et al. 1998) or the integral membrane protein LbpA but not the surface lipoprotein LbpB (Bonnah and Schryvers 1998; Bonnah et al. 1999). Thus the pathway for iron acquisition from lactoferrin, illustrated in Fig. 2, resembles other TonB-dependent pathways for acquiring iron from transferrin, iron siderophore complexes or B12 (conalbumin).

Sequence homology with TonB-dependent receptors for iron-siderophore complexes, for which structural information is available (Ferguson et al. 1998; Buchanan et al. 1999), suggests that integral outer membrane protein LbpA consists of an N-terminal plug or cork domain that inserts into a C-terminal β-barrel domain. The C-terminal β-barrel is predicted to consist of 22 β-strands and 11 extracellular loops, several of which would be substantially larger than in the siderophore receptor proteins to account for the 20 kDa difference in size. It is likely that the overall structure resembles the published structural model for TbpA although the structural features of the surface loops are unknown (Oakhill et al. 2005). The overall structure of LbpA can also be represented by a topology model (Pettersson et al. 2006) in which loops of LbpA vary in size from 4–6 residues (L1) up to 100 residues (L2). Monoclonal antibody raised against LbpA was found to recognise L2, which has also been shown to contain the most sequence diversity (Pettersson et al. 1993, 2006). This would suggest that L2 is prominent at the cell surface structure but how all 11 of the loops are arranged the extent of their exposure is unknown.

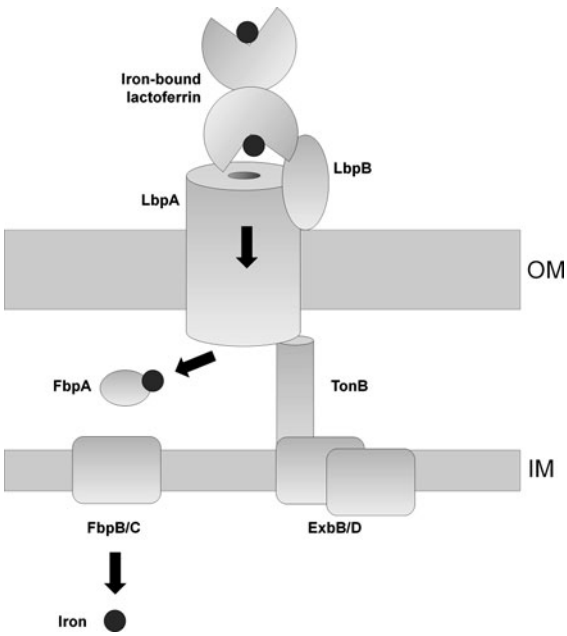

Fig. 2 Schematic diagram of the iron uptake process for the lactoferrin receptor complex. Iron-loaded lactoferrin is bound by both LbpA and LbpB. Interaction between the TonB energy transducer and LbpA results in a conformational change that enables transportation across the outer membrane. The iron is then bound by the periplasmic binding protein FbpA and transported across the inner membrane by FbpB/C

The N-terminal region of LbpA is predicted to consist of four β-strands contained within a plug domain that mediates transport of iron across the outer membrane, presumably by conformational changes or removal from the barrel. Although initial studies in *N. meningitidis* demonstrated that growth dependent upon iron from lactoferrin or transferrin was abolished by mutation of TonB (Stojiljkovic and Srinivasan 1997), a subsequent study demonstrated that this dependence could be overcome by selection for growth on hemoglobin in *N. meningitidis* or *N. gonorrhoeae* (Desai et al. 2000). Although strictly speaking this represents TonB independent growth, the most likely explanation is that mutation of an alternate system, that is somewhat homologous to the TonB, ExbB, ExbD system, enabled it to interact with the TonB box regions of TbpA, LbpA, HmbR and HpuB to energize transport. Thus this is not consistent with the presumed inability to detect a TonB box in LbpA (Pettersson et al. 2006). As illustrated in Fig. 3, the 5-residue motif of KEITV or KEVTV differs slightly from the TonB box

```
Ec FhuA   1 ----------AVEPKEDTITVTAAPAPQES 20
Ec FepA   6 ----------TPVSHIDTIVVTAA------ 19
Nm LbpA   1 AQAGGATPDAAQTQSLKEITVRAAKVG-RR 29
Nm TbpA   1 ---ENVQAGQAQEKQLDTIQVKAKKQKTRR 27
Nm HpuB   1 -----ADPAPQSAQTLNEITVTG------- 19
Nm FetA   1 -------AENNAKVVIDTITVKG------- 16
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Fig. 3 Extracted alignment of the N-terminal sequence of various TonB-dependent outer membrane proteins. *N. meningitidis* MC58 LbpB, TbpB, FetA, and HpuB protein sequences were compared with *E. coli* K12 siderophore receptors FhuA and FepA. The putative TonB-box has been framed

consensus sequence of other iron-inducible proteins (DTITV or similar) but is well conserved amongst various LbpA sequences (unpublished data).

While there is no structural information available for LbpB, internal sequence homology predicts a bi-lobed structure, and even though there is relatively little sequence identity with TbpBs, it likely shares an overall structural similarity with the recently published *A. pleuropneumoniae* TbpB structure (Moraes et al. 2009). Sequence alignments suggest that the secondary structure elements of the barrel and handle domains in the N-terminal and C-terminal lobes are broadly conserved. The structural alignment provides the opportunity to generate a structural model for LbpB (Fig. 4) and although the structure and nature of the surface exposed loops is uncertain, the placement of the loops is likely correct. An obvious difference between the LbpBs and TbpBs is in the presence of the two negatively charged regions in the C-terminal lobe of LbpB (Fig. 4, boxed). These appear co-located structurally, with the larger region I (70–90 amino acid residues) mapping to loop 2 of the C-lobe "hand" domain and region II (30–40 amino acid residues) mapping to loop 8 of the C-lobe β-barrel (Fig. 4). The structural model only shows the position of a smaller loop in place of regions I (23 amino acids) and II (18 amino acids) as any attempt to model these large negatively charged stretches of amino acids would be arbitrary. Interestingly, recent sequence analysis by this laboratory found that region I was present in all isolates tested while region II was not present in a third of isolates (unpublished data). Previous sequence analysis has shown that the sequence of these regions is highly variable and considered as possible antigenic domains recognised by the host immune systems (Biswas et al. 1999). Given the number of positively charged residues at the N-terminal of lactoferrin, it has been suggested that these regions are involved in binding lactoferrin to the receptor complex (Pettersson et al. 1999).

A characteristic feature of the lactoferrin receptors is the exquisite specificity for lactoferrin from the host (Schryvers and Morris 1988b; Yu and Schryvers 2002), despite the relatively high sequence identity and structural similarity among lactoferrins. Removal of the oligosaccharide chains from human lactoferrin did not affect binding to the meningococcal lactoferrin receptor (Alcantara et al. 1992), indicating that the interaction is mediated primarily through protein–

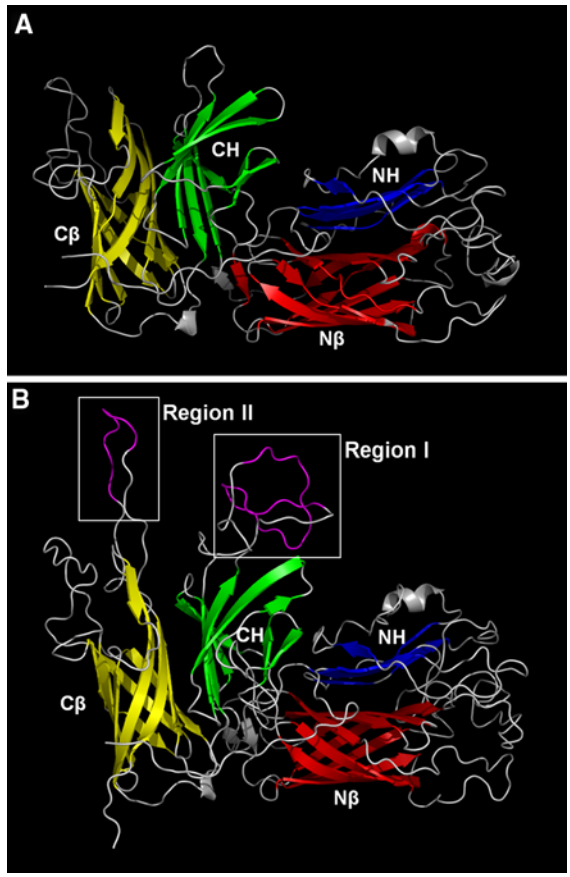

Fig. 4 Structural models of TbpB and LbpB. **a** Structural model of TbpB from *Actinobacillus pleuropneumoniae* H049 derived from crystallographic data showing the N-lobe (β-barrel labelled Nβ, "hand" domain labelled NH) and C-lobe (β-barrel labelled Cβ, "hand" domain labelled CH). **b** Structural model of *N. meningitidis* MC58 LbpB derived from the structure of TbpB, with N-lobe and C-lobe domains in the *same colours* as TbpB. The obvious differences are the two negatively charged regions (boxed), region I extending from the C-lobe hand domain (only 23 residues of approximately 80 were used in this model) and region II extending from the C-lobe β-barrel. Structures were visualised in PyMol

protein interactions. Studies with proteolytically-derived human lactoferrin subfragments demonstrated that both N-lobe and C-lobe subfragments were bound by the lactoferrin receptor from *N. meningitidis, N. gonorrhoeae* or *M. catarrhalis* and binding of all of the subfragments, with the exception of a small sub-fragment of the N-lobe, could be specifically inhibited by human lactoferrin (not bovine lactoferrin or human transferrin; Yu and Schryvers 1993a, b). A subsequent study using hybrids of human lactoferrin and bovine transferrin

demonstrated that LbpA from *N. meningitidis* or *M. catarrhalis* bound to the C-lobe of human lactoferrin. Together these two studies implicate LbpB as being responsible for binding to the N-lobe of lactoferrin, which contrasts the results from studies with TbpB that suggest the primary interaction is with the C-lobe of transferrin (Alcantara et al. 1993; Retzer et al. 1999). The study with bovine transferrin—human lactoferrin hybrid proteins demonstrated that LbpA binds to both domains of the C-lobe (Wong and Schryvers 2003), which is consistent with a model of iron removal that involves domain separation to facilitate iron removal by altering iron coordination (Schryvers and Stojiljkovic 1999).

Since LbpA is absolutely required for iron acquisition from lactoferrin, and can mediate growth on lactoferrin as an iron source in the absence of LbpB, albeit at lower levels (Bonnah and Schryvers 1998; Bonnah et al. 1999), the precise role that LbpB plays in the iron acquisition process is uncertain. The situation for TbpB in iron acquisition is similar, except that it has been shown to preferentially bind the iron-loaded form of transferrin (Yu and Schryvers 1993a, b; Retzer et al. 1998) and has been shown to be essential for survival in vivo (Baltes et al. 2002). Recent structural studies with TbpB have revealed that the relatively unstructured portion of the N-terminal 'anchor' region could extend from 60 to 100 Angstroms from the cell surface, suggesting that its primary role may be in initial capture of transferrin (Moraes et al. 2009). Structural alignments suggest that the predicted unstructured anchor region of LbpBs would be at least as long as that of the *A. pleuropneumoniae* TbpB, thus LbpB could play a similar role for iron acquisition from lactoferrin.

In addition to a potential role in iron acquisition, LbpB may provide protection against anti-bacterial cationic peptides that are part of the innate host defense system. The surface protein PspA from *Streptococcus pneumonia*e has been shown to protect against the anti-bacterial effect of lactoferricin (Shaper et al. 2004), which is released from lactoferrin by proteolysis. Preliminary experiments have shown the LbpB deficient meningococci are more susceptible to lactoferricin (data not shown), and the clusters of negatively charged residues in the C-lobe of LbpB would be the likely regions mediating this function. In view of the dual roles that the lactoferrin receptor may play in acquisition of iron from lactoferrin and protection from cationic peptides, the limited distribution of this capability amongst bacteria that colonize the respiratory mucosa is perhaps surprising. This suggests that either the species lacking lactoferrin receptors possess alternate mechanisms for dealing with lactoferrin and cationic peptides or that the availability of lactoferrin as an iron source and exposure to cationic peptides is only prevalent in certain micro-environments that the bacterial species expressing lactoferrin receptors inhabit.

Vaccine potential

Neisseria meningitidis, *N. gonorrhoeae*, and *M. catarrhalis* rely on a number of specific interactions with host proteins to inhabit the mucosa of the respiratory and genitourinary tracts in humans, the sole ecological niche that they reside in. As a consequence, animal infection models cannot adequately emulate human colonization or infection, which makes it difficult to identify potential vaccine targets that are required for disease causation. The human gonococcal infection model provided a unique opportunity to identify bacterial components required for survival and disease causation, identifying the transferrin and lactoferrin receptors as key targets (Cornelissen et al. 1998; Anderson et al. 2003). Since human infection models for *N. meningitidis* and *M. catarrhalis* are not feasible, it is reasonable to extrapolate the findings from the gonococcal experiments to these pathogens due to many similarities in their adaptation to the human host. The transferrin receptor proteins have received considerable interest as vaccine antigens (Lissolo et al. 1995; Rokbi et al. 1997; West et al. 2001; Price et al. 2007) but relatively few studies have evaluated the potential of lactoferrin receptor proteins as vaccine antigens.

A recent study evaluating the meningococcal lactoferrin receptors from *N. meningitidis* yielded mixed results (Pettersson et al. 2006). The relatively high sequence conservation of LbpA looked promising but the protein had to be produced by overexpression in *N. meningitidis* in order to achieve proper folding, which is less attractive for commercial production. In spite of the high sequence identity (93–100%, based on seven isolates) in LbpA, there

was limited cross-reactivity in the resulting antisera, suggesting that the bactericidal antibodies were primarily targeted to the variable regions in loops 2 and 5. LbpB could be produced as a recombinant protein in *E. coli*, but it also induced relatively little cross-protective antibody, which could have been anticipated due to the greater overall sequence variation in this protein. Overall these results demonstrate that the lactoferrin receptor proteins are potentially suitable vaccine targets but that effective vaccines based on native intact proteins would require a number of representative proteins in order to induce a sufficiently cross-protective response for vaccine purposes.

A more detailed understanding of the structure of the lactoferrin receptor proteins and their interactions with host lactoferrin and lactoferricin would not only provide insights into the mechanism of iron acquisition but could lead to the design of modified proteins that could serve as more suitable vaccine antigens.

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