Targeting bacterial transferrin and lactoferrin receptors for vaccines

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Abstract

A substantial disease burden in vertebrates is due to Gram-negative bacteria that exclusively inhabit the upper respiratory or genitourinary tracts of their hosts and rely on directly acquiring iron from the host iron-binding glycoproteins through surface receptor proteins. The receptors enable these bacteria to proliferate independently from their neighbors on the mucosal surface and during invasive infection of the host. The diversity in these receptors evolved over millions of years of evolution, which thus bodes well for long-lasting vaccine coverage. Experiments in food production animals provide proof of concept for the use of engineered antigens derived from the receptor proteins to prevent colonization and invasive infection in the natural host, strongly supporting development of these vaccines for use in humans.

The pathway for iron acquisition from transferrin and lactoferrin

A number of important Gram-negative pathogens of humans and food production animals that are restricted to a few bacterial lineages (Table 1) possess surface receptors for acquiring iron from the host glycoprotein transferrin (Tf) (see Glossary) and, in some lineages, lactoferrin (Lf) (Figure 1) [1]. The two-component receptors are comprised of a surface lipoprotein, Tf-binding protein B (TbpB) or Lf-binding protein B (LbpB), that captures iron-loaded Tf or Lf and delivers it to the TonB-dependent outer membrane receptors, Tf-binding protein A (TbpA) or Lf-binding protein A (LbpA). TbpB and LbpB are comprised of two lobes (structurally equivalent domains of a protein) with the N-lobe binding to the iron-loaded C-lobe of Tf or Lf. The demonstration that a portion of the N-terminal anchoring peptide is required for interacting with TbpA [2] and that portions of the anchor peptide that normally wrap around the TbpB C-lobe can assume an alpha-helical structure [3] provides insights into the mechanism by which TbpB delivers Tf to TbpA. TbpA or LbpA extract the iron from Tf or Lf and transport it across the outer membrane. As with other TonB-dependent transporters (TBDTs), TbpA and LbpA consist of an outer beta-barrel embedded in the membrane and an inner plug domain that facilitates the transport of the metal ion across the outer membrane using energy derived from an inner
membrane TonB complex. The iron is transferred to a periplasmic iron-binding protein (FbpA) that shuttles the iron across the periplasmic space to an inner membrane transport complex.

**Early adaptations to changes in iron availability**

An appreciation of the critical role that the Tf and Lf receptors play in the survival of bacteria and their proliferation on the mucosal surface is best acquired by reflecting on how Tf and Lf receptors ultimately arose and evolved as part of an ongoing adaptation to iron availability during development of life on earth [4]. Possession of these receptor proteins enable the bacteria to proliferate independently from other members of the microbial community and cause localized or systemic infections, making the receptor proteins ideal targets for vaccines.

The importance of iron for life on earth is thought to be a consequence of there being high levels of ferrous iron in primordial seas, which facilitated its use as a catalyst for biological oxidation–reduction reactions. The progressive oxygenation of the Earth’s atmosphere and oceans, with key stages at 2.3 billion and 800 million years ago [5], impacted the solubility of the ferric ion and created the need for development of systems for acquiring iron under limiting conditions. This led to production and secretion of iron-binding molecules of increasing affinity (ancestral siderophores) by microorganisms, along with specialized systems for binding and uptake, resulting in iron-containing complexes. In Gram-negative bacteria, this likely consisted of ancestral TBDTs such as siderophore receptors with a cognate periplasm to cytoplasm transport pathway (Figure 2, left panel).

There is evidence for microbial communities (stromatolites) that characteristically contain cyanobacteria (photosynthetic Gram-negative prokaryotes) being present in geological formations as early as 3.5 billion years ago [6]. This suggests that the ubiquitous presence of microbial communities (biofilms) in virtually all ecological niches today was likely established early in evolutionary history. TBDTs for other metal ions such as zinc also arose early in the evolution of Gram-negative bacteria and have subsequently expanded to include TBDTs directed against host proteins that sequester the metal ion [7]. However, zinc acquisition has not involved anything equivalent to siderophore production for iron (Figure 2, left panel). The development of an extensive spectrum of siderophores was primarily thought to be driven by competition between bacteria, but they clearly are part of the complex interactions within microbial communities [8], such as in modern day sea sediment, where there are a limited number of siderophore producers and many neighboring bacteria dependent on the production of siderophores [9].

**The single-component Tf receptors**

Single-lobed iron-binding proteins ancestral to bi-lobed Tf arose in early metazoans to acquire iron from the aqueous environment and likely to also compete with the ubiquitous microbial communities. Bi-lobed iron-binding proteins arose by gene duplication events in various metazoan lineages [10], including ancestral serum Tf, at around 670 million years ago [11]. The transition to life on land about 420 million years ago clearly impacted the
microbial community on mucosal surfaces of vertebrates and led to ancestral Tf being present on mucosal surfaces.

The evolutionary process for development of Tf receptors likely initially involved a single-component receptor (Figure 2, middle panel) arising from an ancestral TBDT that transported ferric iron and ultimately being replaced by a more efficient two-component receptor (Figure 2, right panel) that arose by around 320 million years ago [4]. Since Tf has iron preferentially bound to its C-lobe under most physiological conditions, and is the lobe bound by the bipartite receptor (Figures 1 and 2, right panel), the ancestral single-component receptor would also have recognized the C-lobe of Tf (Figure 2, middle panel, red font for Tf lobes). Since there are no remaining systems of the stages that might have been involved in progression to the ancestral bipartite receptor (i.e., a single-lobed TbpB precursor), it argues strongly for the importance of the bi-lobed TbpB in enhancing the efficiency of iron acquisition from Tf under physiological conditions in the upper respiratory tract but experimental evidence is lacking.

The TbpA2 present exclusively in modern day Gram-negative bacteria that colonize the oral or oropharyngeal mucosa of ruminants [12–14] (Figure 2, middle panel, black font for Tf lobes) likely arose in response to the unique physiological conditions on the oral mucosa in ruminants relatively recently (35 million years ago). The TbpA2 in *P. multocida* and *H. somni* binds to the N-lobe (Figure 2, middle panel, black font for Tf lobes) in contrast to the bipartite receptor present in the ruminant pathogens *Mannheimia haemolytica*, *Histophilus somni* and *Moraxella bovis* (Table 1) that binds to the C-lobe of Tf (red font for Tf lobes).

In spite of the differences in binding to bovine Tf, and the dramatic differences in the size and distribution of the extracellular loops of the beta-barrel, TbpA2 has some homology to the conserved NEVTGLGCK (QNVSGLGEV) and GAINEIEYE (SSKNDVEYE) motifs in the plug region of TbpAs and LbpAs [15] that are nearly identically positioned in structural models of TbpA2. Presumably this is a form of convergent evolution in which effective transport of an individual ferric ion by a TBDT involves a specific mechanism.

There is an FbpABC pathway for transporting iron from the outer membrane into the cytoplasm (Figure 2, middle panel) in strains of *P. multocida* from a variety of vertebrate hosts that has high sequence identity with the pathway from *M. haemolytica* (81% identity for FbpA). Since only bovine strains of *P. multocida* possess the TbpA2 receptor, there clearly are other TBDTs in strains of *P. multocida* that are capable of transporting ferric ion acquired from other sources and likely represent the type of TBDTs that were ancestors to the Tf and Lf receptors.

The evolution of the two-component Tf and Lf receptors

Analysis of the sequence changes on the surface of primate Tfs indicate that residues involved in binding to the bacterial receptor protein TbpA were preferentially mutating and ultimately responsible for the host specificity within primates over a 40-million-year time span [16]. Extension of this observation to explain the specificities of receptors in mammalian [17] and avian species [18] indicates that Tf has been available on mucosal
surfaces for at least 320 million years, when the ancestral lineages leading to birds and mammals diverged [4]. An early indication of the differences between mammalian and avian receptor proteins, that could readily be isolated with Tf affinity isolation methods, was first noticed when primers designed to the conserved NEVTGLGCK and GAINIEIYE motifs [15] were able to amplify a product from genomic DNA isolated from bacteria that inhabit mammalian hosts but not from bacteria that inhabit avian hosts. Notably there is relatively little sequence identity (<34%) between mammalian and avian TbpAs and even less sequence identity (<30%) between mammalian and avian TbpBs, likely a reflection of the long period of independent evolution from the ancestral proteins.

Lf arose in the mammalian lineage by another gene duplication event that occurred somewhere between 125 and 150 million years ago [11,19], which enabled it to acquire new functions and specialize as it was not required for the critical role in iron homeostasis. It was first recognized for its important role of limiting iron availability to microbes in milk, glandular secretions, and at sites of inflammation but the list of functions attributed to this protein has continually expanded [20]. Clearly the Lf receptor genes would have arisen from Tf receptor genes once Lf was available as a potential iron source on the mucosal surface of the upper respiratory tract, but it is interesting that it only occurred, or was acquired by horizontal transfer, in two of the bacterial lineages (Table 1, Neisseriaceae, Moraxellaceae).

The best information regarding the importance of the Tf and Lf receptors for survival on the mucosal surface of the host comes from challenge experiments with Neisseria gonorrhoeae in human male volunteers [21], demonstrating that strains lacking both receptors are not capable of causing infection and that the Tf receptor is more effective at supporting infection than the Lf receptor. Using urinary levels as a surrogate for measuring mucosal concentrations, it was shown that Tf was at much higher levels than Lf until the bacterial challenge, suggesting an inflammatory response was responsible for the increased levels.

One of the features that readily distinguishes Lf from Tf is the presence of a positively charged N-terminal region, that can result in the release of cationic antimicrobial peptides upon proteolytic cleavage [20], which likely was the driving force for the acquisition of regions rich in anionic amino acids in loop regions of the LbpB C-lobe [22]. These regions have been shown to protect against cationic antimicrobial peptides and consist of loop extensions as large as 30 kDa in the bovine Moraxella pathogens that cause pink-eye.

The Tf/Lf receptors are only present in a few bacterial lineages (Neisseriaceae, Pasteurellaceae, Moraxellaceae) [17] that likely arose in even longer time scales and characteristically have efficient natural transformation systems strongly favoring horizontal exchange within the specific bacterial lineage. This begs the question whether a gradual accumulation of receptor protein variants could provide sufficient protection to the bacteria from the host immune responses on the mucosal surfaces and, if so, is this their primary strategy? The implication for vaccine development is that if vaccines are designed to cover the known global diversity of receptor protein variants, it will provide comprehensive and long-lasting protection from infection.
Although the presence of Tf/Lf receptors on bacteria that reside on the mucosal surface of the upper respiratory tract provide a compelling argument for Tf being on the mucosal surface, there is a lack of information on the mechanisms involved. An intriguing and elegant study with *Helicobacter pylori* demonstrated that it is capable of modulating iron trafficking in the host epithelial cells by facilitating the transfer of the Tf receptor to the apical surface of the cell with the CagA effector protein to provide iron-loaded Tf [23]. It is interesting to speculate whether a similar type of process could be occurring with epithelial cells of the upper respiratory tract, whether it is done innately by the cells or whether the release of Tf is achieved by passive processes.

**Why target the Tf and Lf receptors?**

One of the primary reasons for targeting the Tf and Lf receptors is that since they are required for survival on the mucosal surface and during invasive infection, they will almost always be expressed, as iron-limiting conditions will be present under most circumstances. This means that they can not only be targeted for prevention of invasive infection but can also be targeted for prevention of colonization. Experiments with the pig pathogen *Glaesserella parasuis*, have shown that a mutant, non-Tf binding form of TbpB is capable of providing complete protection from experiment challenge [24,25] as well as prevention of natural colonization [26]. These results can serve as ‘proof of principle’ that TbpBs can be effective vaccine targets for the range of important Gram-negative pathogens of humans and food production animals that possess the Tf receptor systems.

Since the receptors represent a common adaptation to survival on the mucosal surface, insights gained from the study of these receptors from different bacteria and hosts should be used to develop a more comprehensive overall understanding of the receptors and their relationship to the immune surveillance in the host. Clearly working with bacteria from mice that inhabit the upper respiratory tract and possess Tf +/- Lf receptors would provide an ideal experimental system. The approach of specifically looking for species of *Neisseria* isolated from mice did provide the opportunity to develop a colonization model but since this species lacked Tf or Lf receptors it may not fully reflect the characteristics of the more host-adapted pathogens or pathobionts that cause human infection [27].

An alternative approach is to develop transgenic mice expressing human proteins that the bacteria interact with. Mice expressing human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) have provided significant insights into how the pathogenic *Neisseria* species (*Neisseria meningitidis* and *N. gonorrhoeae*) interact with the host and facilitate probing aspects of the immune response to pathogens on the mucosal surface [28–30]. Nonetheless, the best experimental evidence for the role of Tf on mucosal surfaces being essential for growth of bacterial pathogens comes from gonococcal challenge studies in male volunteers, in which the Tf receptor was essential for survival of the gonococcus in the male urethra [31] and the levels of Tf present in urine exceeded that of Lf until after challenge [32].
Is the sequence diversity of the Tf and Lf receptors manageable?

As highlighted in the previous sections, the bacterial Tf and Lf receptors likely arose as an adaptation for survival on the mucosal surfaces of the upper respiratory tract. The Tf and Lf receptors are present in families of bacteria that do not produce siderophores and thus are reliant on other forms of iron or xenosiderophores produced by other bacteria if they lack the Tf or Lf receptors. Since these bacteria are primarily transmitted between hosts by the ‘respiratory’ route of transmission, the selective pressures by the immune system to vary the sequence of the Tf and Lf receptor proteins would primarily occur on the mucosal surface.

The TbpB protein can extend considerable distances from cell surface due to a relatively long anchoring peptide (Figure 1). It has greater sequence diversity than the TbpA protein, which is likely a reflection of its relative accessibility to antibodies, particularly in bacteria that possess an extracellular polysaccharide capsule. The anchoring peptide could allow extension of TbpB beyond the capsule to capture iron-loaded Tf, whereas the capsule likely partially obscures TbpA from the immune system. The observation that sequence diversity of TbpB from several different porcine pathogens did not strongly correlate with bacterial species, geographic location, or time of isolation [33] suggested that the sequence diversity is not new, instead it is likely a reflection of sequence diversity being acquired over long time periods. The sequence diversity in TbpB was also largely organized into several ‘phylogenetic’ clusters with most of the sequence diversity localized to the CAP region of the TbpB N-lobe that is involved in binding Tf. There was relatively little sequence diversity within the clusters and more substantial diversity between the clusters, likely because exchanges of the loop regions that form the binding interface between TbpBs from different clusters would tend to be nonfunctional. The conclusion that, in spite of the considerable sequence diversity in TbpB, a limited number of antigens may provide a comprehensive cross-protective response against most or perhaps all strains and species, is reminiscent of previous conclusions regarding a potential vaccine targeting meningococcal meningitis [34], but will hopefully be finally tested in the native host.

A tendency for greater diversity in the N-lobe due to its binding function is obscured in LbpB due to the presence of negatively charged loop regions that are involved in binding cationic antimicrobial peptides released from the N terminus of Lf [22]. Although these loop regions have variable size and sequence, it is not clear whether this will be reflected in reduced immunological crossreactivity, or perhaps even have enhanced crossreactivity due to the common charge characteristics.

The enhanced ability of functional recombinant TbpA from N. meningitidis to provide cross-protection against infection in a mouse infection model [35] is clearly due to the lower sequence diversity, a feature that is also present in LbpA. The greater potential for cross-protection by the integral outer membrane proteins TbpA and LbpA is offset by the challenges in commercial production of integral outer membrane proteins.
Strategies for developing better antigens

The original identification of Tf receptor proteins from *N. meningitidis* [36,37] prompted interest in their use as vaccine antigens, particularly after they were shown to be effective in prevention of infection in mice [38]. Vaccine development was focused on TbpB due to its superior induction of bactericidal antibodies and infection prevention in native [39] and recombinant forms [40]. The demonstration that a limited number of recombinant TbpBs could provide broad cross-protection against a diverse collection of strains [34] led to a Phase I trial in humans [41]. Unfortunately, the commercial development of a TbpB-based meningococcal vaccine was abandoned after the trial, which clearly diminished interest and support for TbpB-based vaccines. Fortunately, ongoing and more recent efforts at developing TbpB-based vaccines are demonstrating their true potential.

One issue that may have impacted the results obtained with TbpB in humans relative to those observed in experimental animals (mice and rabbits) is the strict host specificity of binding Tf [42,43]. Solving the structure of TbpB from a porcine pathogen [44] provided the opportunity to design mutants with reduced Tf binding [3] and address the question whether binding of host Tf by TbpB during the immunization process was interfering with induction of the protective immune response (Figure 3A). Immunization and challenge experiments in pigs demonstrated that an enhanced protective immune response was seen with mutants deficient in Tf binding [24] but subsequent experiments demonstrated that not all nonbinding mutants were advantageous [25]. Due to the cost and availability of large animal experiments, it will be advantageous if mouse infection models can be used to screen for the relative efficacy of mutant proteins prior to implementing pig or cattle experiments for veterinary vaccines. The demonstrated success of mutant TbpBs in veterinary vaccines in turn can provide proof of principle for development of mutant TbpBs for human vaccines, and if mouse infection models prove to be an effective screening tool for mutant TbpBs it will provide a logical path to a commercial TbpB-based vaccine.

Solving the structure of TbpA bound to Tf [45] provided a clear indication of the junction of the extracellular loop regions and the anchoring beta-strains of the 22-strand beta-barrel and provided the opportunity for the development of the novel hybrid antigen approach for displaying TbpA loops on derivatives of TbpB [46] (Figure 3B). The utility of this approach has also been demonstrated by display of extracellular loops from other TonB-dependent proteins [47] but the parameters that influence optimal display of functional epitopes have not been established. At present it is not clear whether hybrid antigens will be the optimal way of achieving a broadly cross-protective response against the bacteria that possess the Tf or Lf receptors, but this strategy does offer the opportunity to target multiple bacterial surface proteins with a single antigen. More details on the approach and methods used for production of hybrid antigens is provided in a recently published methods chapter [48].

Concluding remarks

Although the Tf receptors along with Lf receptors may represent ideal vaccine targets due to the critical role they play in survival on the mucosal surface or during invasive infection, the path to development of effective human and veterinary vaccines has been
long and arduous. However, the available structures of TbpBs and TbpA have provided the opportunity for engineering antigens with improved immunologic properties. The presence of similar systems in pathogens of humans and food production animals has provided the opportunity to use proof of concept experiments with pig pathogens to evaluate parameters that influence potential efficacy in human pathogens and pathogens of other food production animals. The development of commercial vaccine products will provide the opportunity to determine whether they can eliminate the targeted pathogens from food production facilities and determine their impact on the microbial communities of the upper respiratory tract (see Outstanding questions). Although bacteria that reside in the upper respiratory tract of mice that possess Tf and/or Lf receptors would be an ideal experimental system to probe the host–bacteria/pathogen interaction, in their absence, development of transgenic mice can be used to study specific interactions. The successful commercialization of veterinary vaccines containing engineered Tf receptor proteins should smooth the path towards development of vaccines against human pathogens targeting similar receptors and perhaps even encourage the use of large animal experiments as surrogates to address fundamental questions relevant to vaccine development. The pathogenic species (pathobionts) in humans and food production animals appear to be more host-adapted than the commensal species, making the prospect of their replacement by commensal species unlikely in practical terms.

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Glossary

**C-lobe**
the carboxy terminal lobe of a protein.

**FbpA**
ferric binding protein A, a periplasmic binding protein responsible for transporting ferric ion across the periplasmic space and delivering it to a cytoplasmic membrane transport complex comprised of FbpB and FbpC.

**FbpB**
an integral cytoplasmic membrane transport protein for ferric ion.

**FbpC**
an ATPase that couples ATP hydrolysis to transport of ferric iron by FbpB.

**Gram-negative bacteria**
bacteria that possess a cell wall, an outer membrane, and an inner cytoplasmic membrane.

**Lactoferrin (Lf)**
a multifunctional, iron-binding host glycoprotein present in milk, glandular secretions, and neutrophils.

**LbpA**
lactoferrin-binding protein A, an integral outer membrane protein in Gram-negative bacteria that removes iron from lactoferrin and delivers it to FbpA.

**LbpB**
lactoferrin-binding protein B, a surface lipoprotein in Gram-negative bacteria that captures iron loaded lactoferrin and delivers it to LbpA.

**Lobe**
structurally equivalent domains of a protein.

**N-lobe**
the amino terminal lobe of a protein.

**Periplasm**
the space between the inner cytoplasmic membrane and the outer membrane.

**Transferrin (Tf)**
a host glycoprotein that transports iron throughout the body and is essential for iron homeostasis.

**TbpA**
transferrin-binding protein A, an integral outer membrane protein in Gram-negative bacteria that removes iron from transferrin and delivers it to FbpA.

**TbpB**
transferrin-binding protein B, a surface lipoprotein in Gram-negative bacteria that captures iron-loaded transferrin and delivers it to TbpA.

**TonB-dependent transporter (TBDT)**
an integral outer membrane protein in Gram-negative bacteria that transports essential metal ions (iron, zinc, manganese, cobalt) or metal ion complexes across the outer membrane.

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The presence of transferrin (Tf) receptors in Gram-negative bacteria that reside in the upper respiratory tract of mammals and birds indicate that they have existed for over 320 million years.

Tf/lactoferrin receptors are essential for survival of Gram-negative bacterial pathogens (pathobionts) on the mucosal surface and during invasive infection and thus are ideal targets for vaccines.

Structure-based antigen engineering can improve the immune response induced by Tf receptor proteins.

Proof of concept experiments in pigs demonstrate that antigens targeting Tf receptors can prevent infection and colonization.
Outstanding questions

How is transferrin transported onto the mucosal surface of the upper respiratory tract?

Why is transferrin transported onto the mucosal surface? What potential advantage does it confer to the host?

How can we demonstrate the importance of TbpB in acquiring iron under physiological conditions?

Will optimized immunization strategies with Tf receptor-based vaccines be able to eliminate the targeted pathogens from food production facilities?

What impact will immunization with Tf receptor-based vaccines have on the microbial communities?

Can the regions on LbpB that protect against cationic peptides serve as an effective target for vaccines?

Are bacteria that express Tf receptors present on the upper respiratory tract of all natural vertebrate species? Are they present in more bacterial lineages than currently recognized?

Can we find bacteria in mice that possess transferrin and/or lactoferrin receptors to facilitate future studies?
Figure 1. Transferrin (Tf) and lactoferrin (Lf) receptors in Gram-negative bacteria.
Receptors for Tf and Lf are comprised of a surface lipoprotein, Tf/Lf-binding protein B (TbpB or LbpB), and an integral outer membrane protein, Tf/Lf-binding protein A (TbpA or LbpA). Iron-loaded Tf/Lf are captured by TbpB/LbpB extended away from the surface tethered by their anchoring peptides, as illustrated by the Tf/TbpB complex on the right side of the figure. TbpB/LbpB then deliver Tf/Lf to TbpA/LbpA, as illustrated by LbpB/Lf on the left side of the figure. Iron is extracted from Tf/Lf and transported across the outer membrane and delivered to the periplasmic ferric-binding protein A (FbpA). The transport of iron through TbpA/LbpA derives energy from the TonB/ExbB/ExbD complex using ATP hydrolysis. The iron-loaded FbpA crosses the periplasmic space and docks onto the FbpB/C inner membrane transport complex that uses ATP hydrolysis to transport iron into the cell.
Figure 2. Evolution of systems in Gram-negative bacteria capable of iron acquisition from transferrin.

Left panel: bacteria acquire pathways for synthesis and export of iron-chelating molecules (siderophores) in order to obtain iron from various iron sources in diverse ecological niches. The resulting iron–siderophore complexes are bound by surface siderophore receptors for transport across the outer membrane (OM). The iron–siderophore complex is transferred to a siderophore-binding protein (Sbp), shuttled across the periplasmic space, and then transported into the cell by an inner membrane (IM) transport complex. Bacteria adapting to different ecological niches and microbial communities develop a diversity of additional TonB-dependent transporters (TBDTs) with similar periplasm to cytoplasm pathways for acquiring iron and other metal ions. Middle panel: an ancestral TBDT acquires the ability to bind transferrin and remove iron that is transported by an established periplasm to cytoplasm pathway. The ancestral TBDT, represented by Tf-binding protein A2 (TbpA2) (lobes in red font), was replaced by the more efficient bipartite receptor (right panel). The modern day TbpA2 (middle panel, lobes in black font) more recently evolved in the bacteria colonizing ruminants (lobes in black font) due to the unique physiological conditions. Right panel: the present day bipartite transferrin receptor has the additional surface lipoprotein, Tf-binding protein B (TbpB), that is capable of extending from the cell surface and capturing iron-loaded Tf and delivering it to TbpA for iron removal and uptake, as also illustrated in Figure 1.
Figure 3. Engineered antigen strategies.

(A) Rationale for mutant Tf-binding proteins B (TbpBs) deficient in binding transferrin (Tf). Mutation of key residues in the binding interface between TbpB and Tf can reduce or eliminate binding by Tf and thus avoid blocking of important epitopes in the native host. Immunization with native TbpB (left panel, gray Tf) in a foreign host (mice, rabbits, blue Tf) does not block key epitopes on the wild-type TbpB (gray color) as it does in the native host (i.e., pigs, humans, brown Tf) during immunization. Immunization with a mutant TbpB (right panel, yellow) in the native host (i.e., pigs, humans, brown Tf) reduces or eliminates the blocking of important epitopes during immunization.

(B) Rationale for hybrid antigen design. Tf-binding protein A (TbpA)/lactoferrin (Lf)-binding protein A (LbpA) are not soluble in aqueous solutions without detergents, in contrast to TbpB/Lf-binding protein.
B (LbpB) or their derivatives. The exposed extracellular loops on TbpA or LbpA (red and blue color) that are anchored by the antiparallel beta strands of the C-terminal beta-barrel region can be ‘transplanted’ onto the antiparallel beta-strands of the C- or N-terminal lobes of TbpB or LbpB (red or blue color). The transplanted loop (blue color, hybrid antigen) will have similar conformational constraints imposed by the anchoring beta-barrel residues as the original loop (scaffold) and thus may assume the native conformation from TbpA/LbpA on the hybrid antigen.
Table 1.
Bacterial pathogens of humans and food production animals with Tf and Lf receptors

| Host   | Pathogen                        | Disease                                                                 | TF receptor | Lf receptor | Lineage          |
|--------|---------------------------------|-------------------------------------------------------------------------|-------------|-------------|------------------|
| Human  | Neisseria meningitidis          | Meningitis, sepsis                                                      | Yes         | Yes         | Neisseriaceae    |
|        | Neisseria gonorrhoeae           | Gonorrhea                                                               | Yes         | Yes         | Neisseriaceae    |
|        | Haemophilus influenzae (type b) | Meningitis, sepsis, pneumonia                                           | Yes         | Yes         | Pasteurellaceae  |
|        | H. influenzae (non-typeable)    | Otitis media, chronic obstructive pulmonary disease (COPD), pneumonia   | Yes         | TbpA2       | Pasteurellaceae  |
|        | Moraxella catarrhalis           | Otitis media, COPD                                                      | Yes         | Yes         | Moraxellaceae    |
| Cattle | Mannheimia haemolytica          | Bovine respiratory disease (BRD)                                        | Yes         |             | Pasteurellaceae  |
|        | Pasteurella multocida           | Hemorrhagic septicemia (HS), BRD                                        |             | TbpA2       | Pasteurellaceae  |
|        | Histophilus somni               | BRD, thrombotic meningoencephalitis (TME), myocarditis                  | Yes TbpA2   |             | Pasteurellaceae  |
|        | Moraxella bovis                 | Pinkeye (infectious bovine keratoconjunctivitis) IBK                     | Yes         | Yes         | Moraxellaceae    |
| Sheep  | Bibersteinia trehalosi          | Septicemia, pneumonia                                                  | Yes         |             | Pasteurellaceae  |
| Pig    | Actinobacillus pleuropneumoniae | Pleuropneumonia                                                         | Yes         |             | Pasteurellaceae  |
|        | Haemophilus parasuis            | Glasser’s disease                                                       | Yes         |             | Pasteurellaceae  |
|        | Actinobacillus suis             | Pneumonia, septicemia                                                  | Yes         |             | Pasteurellaceae  |
| Poultry| Avibacterium sp.                | None                                                                    | Yes         |             | Pasteurellaceae  |

a Yes indicates presence of the bipartite TbpB–TbpA receptor, TbpA2 indicates the single-component receptor.