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MicroReview

The role of zinc in the interplay between pathogenic streptococci and their hosts

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Summary

Recent studies on pathogenic streptococci have revealed that zinc is a pivotal metal ion in their interaction with the host. In these streptococci, systems exist that ensure optimal use of zinc from the surrounding milieu, as well as export of zinc when concentrations exceed tolerance levels. Zinc uptake is of crucial importance for the virulence of streptococci, whereas elevated zinc levels induced in the host during infection are detrimental for these pathogens. The expression or activity of a number of putative surface proteins and virulence factors depends on zinc. Moreover, several metal sensor proteins that mediate the transcriptional response to zinc in streptococci have recently been characterized. A number of components of zinc- and other metal ion-acquisition systems are suitable as protective antigens and may be future targets for the development of new vaccines, thus providing opportunities for the development of novel therapies. This review will discuss the recent advancements in the important field of metal ion biology in relation to the virulence of pathogenic streptococci, with a central focus on zinc homeostasis in *Streptococcus pneumoniae*.

Introduction

In order for bacterial pathogens to survive inside their hosts, they need to be able to efficiently exploit and use the available nutrients from their surrounding milieu. Several studies have shown that in pathogenic bacteria there is a

complex intertwinement of metabolic (regulatory) networks and the functioning and expression of virulence factors (Brown *et al.*, 2008; Gorke and Stulke, 2008; Hendriksen *et al.*, 2008; Poncet *et al.*, 2009; Somerville and Proctor, 2009; Amon *et al.*, 2010; Eisenreich *et al.*, 2010; Carvalho *et al.*, 2011; Alteri and Mobley, 2012; Cole, 2012). One of the most highlighted nutritional factors at the intersection of nutrient metabolism and virulence are trace metal ions (Schaible and Kaufmann, 2004; Papp-Wallace and Maguire, 2006; Riccardi *et al.*, 2008; Jakubovics and Valentine, 2009; Dupont *et al.*, 2011; Klein and Lewinson, 2011; Osman and Cavet, 2011; Cassat and Skaar, 2012; Wakeman and Skaar, 2012). All forms of life require trace metal ions for proper functioning and it is estimated that approximately 30% of all proteins and almost 50% of all enzymes contain trace metal ions as cofactors for their faithful activity and/or structure (Tainer *et al.*, 1991; Andreini *et al.*, 2008; Waldron *et al.*, 2009).

In this review, we have discussed the role of trace metal ions, with an emphasis on zinc, in the lifestyle of an important class of pathogenic bacteria, comprised of the important human pathogen *Streptococcus pneumoniae* and other related streptococci.

Why zinc?

Many studies have focused on the importance of iron in the host–pathogen interplay (Brown and Holden, 2002; Schaible and Kaufmann, 2004; Nairz *et al.*, 2010; Hammer and Skaar, 2011). However, other trace metal ions like copper, zinc and manganese have been shown to contribute to the virulence of streptococci as well and recently significant insights into the pivotal role of zinc have been obtained (Fig. 1).

Zinc can function as a structural and/or as a catalytic component of proteins (Berg and Shi, 1996). Furthermore, it is (like copper) a highly competitive metal ion [high in the Irving-Williams series of the stability of metal complexes: $Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II)$] that can replace the cognate metal ion of certain metal sensory proteins (Reyes-Caballero *et al.*, 2010; Guerra *et al.*, 2011). Due to a high cellular zinc-chelating capacity, free

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Fig. 1. General overview of the possible roles and effects of zinc in pathogenic streptococci and their hosts. The figure was mostly based on research performed in *S. pneumoniae*, although also elements from other pathogenic streptococci were incorporated. See text for details. Big one-sided arrows arising from the central 'zinc' indicate the effect of zinc on different processes (boxed) in the cell. Although not indicated, the boxed processes are interrelated as well. Small one-sided arrows indicate transcriptional activation/de-repression, and lollypop structures transcriptional repression. $Zn = Zn^{2+}$, $Mn = Mn^{2+}$ and $Cu = Cu^{2+}$.

zinc concentrations are kept very low in the cell (Outten and O'Halloran, 2001). Despite the low free zinc concentration in the cell, the total cellular concentration of zinc is in the order of magnitude of 0.1 mM (Finney and O'Halloran, 2003; Jacobsen *et al.*, 2011). Zinc is less redox-sensitive than copper and iron (Outten and O'Halloran, 2001) and might therefore, like manganese, play a role in protection against oxidative stress as for example caused by ROS (reactive oxygen species) produced via Fenton reaction (Gaballa and Helmann, 2002; Faulkner and Helmann, 2011; Ortiz de Orue Lucana *et al.*, 2012).

In bacteria, zinc is present in approximately 5% of all proteins (Andreini *et al.*, 2006b; 2008; Decaria *et al.*, 2010). A recent study suggests that more than 200 copper/zinc-containing proteins (9% of the proteome) are encoded by the genome of *S. pneumoniae* (Sun *et al.*, 2011). Indeed, several important enzymes in streptococci require zinc for their proper activity. Notable examples include pneumococcal phosphorylcholine esterase that affects decoration of the cell surface with choline, thereby influencing the complement of surface-exposed choline-binding proteins, which in turn might be important virulence factors (Lagartera *et al.*, 2005; Hammerschmidt,

2009), and also peptidoglycan deacetylase, which protects against host lysozymes and is a virulence factor (Blair *et al.*, 2005). Other groups of proteins that (likely) bind zinc are zinc-containing alcohol dehydrogenases (Lanie *et al.*, 2007; Stroehrer *et al.*, 2007; Potter *et al.*, 2010), Pht (Polyhistidine triad) family proteins (Panina *et al.*, 2003; Rioux *et al.*, 2010; Loisel *et al.*, 2011; Plumptre *et al.*, 2012), ribosomal proteins (Panina *et al.*, 2003), several zinc metalloproteases (Zmp) that are implicated in virulence (Blue *et al.*, 2003; Chiavolini *et al.*, 2003; Oggioni *et al.*, 2003; Camilli *et al.*, 2006; Bek-Thomsen *et al.*, 2012), laminin-binding protein LmB/AdcAll (Loisel *et al.*, 2008; Linke *et al.*, 2009), and the major antigen and exotoxin SpeA1 of *Streptococcus pyogenes* (Baker *et al.*, 2001; 2004; Galeotti *et al.*, 2012). Thus, it is evident that in streptococci zinc plays a vital role in protein structure and function.

Zinc in the human host

In the human body, zinc is estimated to interact with up to 10% of all proteins and after iron, it is the second most abundant trace element (Whittaker, 1998; Zalewski *et al.*,

2005). Appropriate levels of zinc are important for the functioning of the immune system (Rink and Gabriel, 2000; Andreini *et al.*, 2006a,b). The importance of zinc for immunity is further illustrated by observations of an elevated incidence of several infectious diseases in developing countries that are associated with a poor zinc status, like acute respiratory infections, otitis media and diarrhoea. Dietary supplementation with zinc may have a positive impact on the prevalence of these infectious diseases (Sazawal *et al.*, 1998; Black and Sazawal, 2001; Fischer and Black, 2004; Coles *et al.*, 2008); however in case of pneumonia there is conflicting data available (Basnet *et al.*, 2012).

The zinc concentration in the human body varies among different tissues. For example, plasma zinc concentrations are between 12 and 16 μM (Rink and Gabriel, 2000; Ibs and Rink, 2003; Jayaram *et al.*, 2011), while lung alveolar lavages contain as low as 1–2 μM zinc (Harlyk *et al.*, 1997) and sputum contains around 1 μM (50 $\mu\text{g l}^{-1}$) zinc (Jayaram *et al.*, 2011). The concentration of freely accessible zinc in the airway epithelium and other mucosal surfaces is thought to be relatively high (Zalewski *et al.*, 2005), whereas in plasma, virtually all zinc is bound to plasma proteins, primarily albumin (Magneson *et al.*, 1987; Wang *et al.*, 2003; Jayaram *et al.*, 2011).

During inflammation and infections, zinc levels are elevated in several body compartments (Milanino *et al.*, 1993), although levels in the plasma may drop due to redistribution to the liver, which produces elevated levels of the zinc-binding metallothioneins and displays upregulation of zinc transporters (Peretz *et al.*, 1991; Milanino *et al.*, 1993; Sturniolo *et al.*, 1998; Liuzzi *et al.*, 2005; King, 2011). In addition, upon bacterial infection, zinc levels may be depleted locally by the action of human calprotectin released from neutrophils (Kehl-Fie and Skaar, 2010), which chelates these metal ions and potentially inhibits bacterial growth at the sites of inflammation, as shown for abscesses caused by *Staphylococcus aureus* (Sohnle and Hahn, 2000; Lusitani *et al.*, 2003; Corbin *et al.*, 2008; Kehl-Fie *et al.*, 2011). However, this might be counteracted from the side of pathogenic bacteria by upregulation/engagement of high-affinity zinc uptake systems, as has been shown in an animal model for gut infection by *Salmonella typhimurium* (Liu *et al.*, 2012) and lung infection by the nosocomial pathogen *Acinetobacter baumannii* (Hood *et al.*, 2012). Moreover, comparison of zinc concentrations in infected and uninfected tissues in mice indicated that the zinc to manganese ratio in infected tissues has increased 2.4-fold in lungs, 2.8-fold in the brain, 5.3-fold in the nasopharynx and 24.4-fold in the blood serum (McDevitt *et al.*, 2011).

Thus, it seems plausible that human pathogens like *S. pneumoniae* and its relatives have to cope with both conditions of zinc limitation and zinc excess in their hosts.

Systems involved in zinc homeostasis in streptococci

In pathogenic streptococci, several systems involved in zinc homeostasis have been studied. In *S. pneumoniae*, a high-affinity zinc transport unit is present that consists of an ABC transporter AdcBC with two extracellular zinc-binding proteins, AdcA and AdcAll (also called laminin-binding protein LmB). These are partially redundant in binding zinc on the pneumococcal surface (Loisel *et al.*, 2008; Bayle *et al.*, 2011) and are homologous to the *Escherichia coli* major zinc-binding protein ZnuA (Li and Jogl, 2007). In addition, *S. pneumoniae* contains a manganese ABC transporter, the PsaBCA complex, which can bind zinc as well, although it is not involved in high-affinity zinc transport (Lawrence *et al.*, 1998; McDevitt *et al.*, 2011). Similar systems have been studied in other streptococci like *Streptococcus suis* (Aranda *et al.*, 2009; Zheng *et al.*, 2011), *S. pyogenes* (Janulczyk *et al.*, 2003; Brenot *et al.*, 2007), *Streptococcus gordonii* (Jakubovics *et al.*, 2000; Loo *et al.*, 2003) and *Streptococcus agalactiae* (Bray *et al.*, 2009). The homologues of AdcAll in *S. agalactiae* and *S. pyogenes*, which both also have been shown to bind zinc, are involved in adhesion to the extracellular matrix protein laminin, in invasion of HMBE (human brain microvascular endothelial) cells and adhesion to epithelial cells (Sommer *et al.*, 1992; Spellerberg *et al.*, 1999; Elsner *et al.*, 2002; Terao *et al.*, 2002; Tenenbaum *et al.*, 2007; Linke *et al.*, 2009; Ragunathan *et al.*, 2009). The *adcAll* (*lmB*) gene in all of these streptococci is in an operon with a gene encoding a histidine triad protein PhtD that also binds zinc (Panina *et al.*, 2003; Kunitomo *et al.*, 2008; Loisel *et al.*, 2011). Streptococci contain several more proteins belonging to the zinc-binding Pht protein family, in which they are unique compared with non-streptococcal species (Adamou *et al.*, 2001; Plumtre *et al.*, 2012). These Pht family proteins are thought to scavenge manganese and zinc on the bacterial cell surface, and may serve as a reservoir for these metal ions during their starvation (Rioux *et al.*, 2010).

A zinc export system is encoded by *czcD* in *S. pneumoniae*, belonging to the cation diffusion facilitator (CDF) family, which is present in other streptococci as well (Kloosterman *et al.*, 2007). This gene provides resistance against zinc stress, and to a lesser extent cobalt stress (Kloosterman *et al.*, 2007). In addition to this system, in *S. pyogenes* a gene called *pmtA* has been implicated in zinc export (Brenot *et al.*, 2007). This gene is repressed by PerR, but gets expressed upon peroxide stress, leading to an intracellular zinc starvation response (Brenot *et al.*, 2007).

Another protein that might play a role in zinc homeostasis is Dpr, which is a ferritin-like protein that can bind and oxidize iron with the concomitant consumption of hydrogen peroxide, thereby preventing the Fenton reaction and thus

free radical stress. This protein has been shown to bind zinc in *S. pyogenes*, offering protection against zinc stress (Haikarainen and Papageorgiou, 2010; Tsou *et al.*, 2010; Haikarainen *et al.*, 2011). In *S. pneumoniae*, *dpr* is regulated by the RitR orphan response regulator that regulates iron metabolic genes (Ulijasz *et al.*, 2004), and its expression is iron-dependent (Gupta *et al.*, 2009).

Zinc-dependent transcriptional regulators in streptococci

In many bacteria, like *E. coli* and *Bacillus subtilis*, Zur (Zinc uptake regulator) regulates zinc uptake genes (Panina *et al.*, 2003). However, in streptococci the MarR family regulator AdcR is involved in regulation of zinc uptake genes (Panina *et al.*, 2003). During zinc starvation, AdcR binding to its target promoters is relieved, thereby activating the expression of zinc acquisition genes and ensuring the maintenance of intracellular zinc levels (Aranda *et al.*, 2008; Ogunniyi *et al.*, 2009; Shafeeq *et al.*, 2011a). Interestingly, the AdcR regulon includes genes encoding the Pht family of zinc-binding proteins (Shafeeq *et al.*, 2011a). In addition, in some streptococci AdcR regulates alternative ribosomal genes, which might be induced upon zinc starvation leading to lower consumption of zinc by ribosomes (Panina *et al.*, 2003; Brenot *et al.*, 2007; Aranda *et al.*, 2009).

A recently identified novel zinc-stress regulator, SczA, which is conserved in streptococci, activates the *czcD* zinc-efflux gene upon encountering high levels of zinc (Kloosterman *et al.*, 2007). This occurs via an interesting mechanism involving both a repressor site that functions during low zinc levels and an activator site needed for induction of transcription during high zinc levels (Kloosterman *et al.*, 2007). Besides *czcD*, SczA also induces expression of two downstream genes, *nmlR* and *adhC*. NmlR activates *adhC* expression, which has been proposed to encode a GSNO (S-nitrosoglutathione) or GS (glutathione)-aldehyde reductase, providing resistance against NO (Stroeher *et al.*, 2007; Potter *et al.*, 2010). Induction of expression of this gene would be consistent with the prediction that AdhC contains zinc, and moreover with a model wherein AdhC is necessary to prevent accumulation of nitrosylated zinc-binding cysteine-containing sites in proteins.

Microarray experiments suggest that AdcR tunes expression of zinc-acquisition systems encoded by the *adc* and *pht* genes when zinc in the medium is scarce (lower than a few μM) (Shafeeq *et al.*, 2011a), and SczA takes over control at higher levels of zinc in the growth medium ($> 10\text{--}20\ \mu\text{M}$; Jacobsen *et al.*, 2011) by proper adjustment of expression of the CzcD zinc-efflux pump (Kloosterman *et al.*, 2007; Jacobsen *et al.*, 2011). This plausible mechanism of the regulation of zinc homeostasis whereby AdcR and SczA act at distinct cytoplasmic

concentrations of zinc is consistent with the metal-binding affinities of both zinc sensors (Reyes-Caballero *et al.*, 2010; Jacobsen *et al.*, 2011).

Streptococci also contain a manganese-responsive regulator. In *S. pneumoniae*, this regulator, PsaR, has been shown to be responsible for manganese-dependent repression of the manganese uptake system PsaBCA, as well as the choline-binding protein PcpA and the surface-associated protein PrtA (Kloosterman *et al.*, 2008). In *S. pyogenes*, *S. agalactiae* and *S. mutans*, this regulator is also involved in the response to iron and seems to have an impact on other important physiological processes as well, such as biofilm formation, genetic competence, oxidative stress tolerance and adherence (Berry and Paton, 1996; Dintilhac *et al.*, 1997; Novak *et al.*, 1998; 2000; Marra *et al.*, 2002; Tseng *et al.*, 2002; McAllister *et al.*, 2004; Johnston *et al.*, 2006; Anderton *et al.*, 2007; Bray *et al.*, 2009). Interestingly, the regulon of PsaR was upregulated under conditions with high zinc concentrations (Kloosterman *et al.*, 2008; Jacobsen *et al.*, 2011), likely by preventing manganese-induced interaction of PsaR with its cognate promoters (Kloosterman *et al.*, 2007), and by competing with manganese uptake through PsaA (McDevitt *et al.*, 2011).

Zinc also interferes with the regulation of copper homeostasis genes in *S. pneumoniae* via the copper-responsive regulator CopY, as, like in *Enterococcus hirae*, zinc is necessary for CopY-dependent repression (Solioz and Stoyanov, 2003; Solioz *et al.*, 2010; Shafeeq *et al.*, 2011b). The physiological relevance of this is unknown, but it could be a way for the cell to ensure a proper balance between copper and zinc.

The regulators and their regulons discussed above are all highly conserved in different strains of *S. pneumoniae* (Table S1). Especially the *adcCBA* and *psaBCA* genes are also conserved in other streptococcal species, underscoring their important functions in the lifestyle of these bacterial species. However, some of the *pht* genes are specific to *S. pneumoniae* and also the *sczA* regulon is absent in several streptococci, implying differences in zinc homeostasis.

Zinc in the interplay of the pathogen and the host

The importance of zinc for pathogenic streptococci as well as for their hosts implies a role for zinc during colonization and infection. AdcB was found to be important for full virulence of *S. pneumoniae* in a STM lung infection study (Hava and Camilli, 2002). Combined mutation of the genes encoding the zinc-binding proteins AdcA and AdcAll completely abolished virulence in models of nasopharyngeal carriage, pneumonia and sepsis, suggesting that efficient zinc uptake is needed to survive in all of the corresponding host compartments, and that zinc is preva-

lently limiting there (Bayle *et al.*, 2011). Despite the abolished virulence, the *adcA/adcAII* double mutant was still able to grow, albeit worse than wild-type, in diluted BALF (bronchoalveolar lavage fluid) and human serum. It could be that specific and local sequestration of zinc, by for example, metallothioneins or calprotectin renders zinc concentrations in certain microniches (see also above) in the host tissues low enough to prevent growth of the *adcA/adcAII* mutant. AdcA and AdcAII could as well have other roles during infection in the host. Indeed, the *adcA-adcAII* double mutant showed a lag phase even in zinc-replete conditions, suggesting a role besides zinc acquisition. In line with a function that is seemingly unrelated to zinc, the permease component AdcB, was found to be important for adhesion to human epithelial cells (Song *et al.*, 2008). Moreover, in *S. gordonii* the *adc* operon contributed to biofilm formation (Loo *et al.*, 2003) and zinc uptake by the proteins encoded in the *adc* operon in *S. pneumoniae* is important for competence (Dintilhac *et al.*, 1997). The individual contributions of AdcA and AdcAII to *in vivo* fitness remain unclear and it is tempting to speculate that each protein has a unique way of contributing to the infection processes. In *S. pyogenes*, deletion of *adcAII* alone already confers a strongly debilitated virulence and a defective growth phenotype during zinc depletion (Weston *et al.*, 2009).

Given the fact that there is a strict zinc-dependent control of expression of genes involved in zinc homeostasis by AdcR, it is to be expected that at least at some stages during infection there is no need for these systems, namely when zinc levels are high. Indeed, a recent study shows that during infection, *S. pneumoniae* might encounter elevated concentrations of zinc and that upon infection of mice the ratio of zinc to manganese increases dramatically, especially in the plasma and in the nasopharynx, leading to cellular deprivation of manganese via competition of zinc on the manganese transporter PsaA (McDevitt *et al.*, 2011). This would lead to expression of the PsaR regulon (Kloosterman *et al.*, 2008; Jacobsen *et al.*, 2011), causing a different set (compared with zinc limitation) of virulence genes to be expressed, namely (i) *psaA*, involved in virulence and other important processes, like oxidative stress resistance, penicillin stress, competence and adhesion via interaction with human E-cadherin (Berry and Paton, 1996; Dintilhac *et al.*, 1997; Novak *et al.*, 1998; 2000; Marra *et al.*, 2002; Johnston *et al.*, 2004; McAllister *et al.*, 2004; Obert *et al.*, 2006; Anderton *et al.*, 2007), (ii) the gene encoding the choline-binding protein PcpA, involved in adhesion (Hendriksen *et al.*, 2009), biofilm formation (Moscoso *et al.*, 2009) and virulence (Hava and Camilli, 2002), and (iii) *prtA*, which contributes to systemic infection and to cleavage of the iron binding and transporting compound apolactoferrin (Bethe *et al.*, 2001; Mirza *et al.*, 2011). In

addition to elevated zinc levels, it is likely that local zinc scarcity exists, explaining the *in vivo* phenotype of the *adcA/adcAII* mutant.

Supporting the hypothesis that bacteria are exposed to both high and low concentrations of zinc during infection, zinc-efflux systems have been reported to play a role in virulence as well. In *Helicobacter pylori*, a pathogen of the human gastric mucosa, the *cznABC* operon is required for metal ion homeostasis by providing resistance to zinc, cadmium and nickel. The *cznABC* operon was required for gastric colonization, showing a significant importance of metal ion homeostasis for survival of *H. pylori* in the gastric environment (Stahler *et al.*, 2006). Although the role of the zinc efflux gene *czcD* in streptococcal virulence has not been studied so far, given the fact that increases in tissue zinc levels have been observed during pneumococcal infection (McDevitt *et al.*, 2011), this system might contribute significantly. Another example of a link between zinc-resistance and virulence is *S. pyogenes* PmtA, which likely functions as a zinc exporter and enhances oxidative stress resistance (Brenot *et al.*, 2007). Dereglulation of metal homeostasis as a result of *perR* deletion is thought to render *S. pyogenes* avirulent. So, while general effects of zinc homeostasis on virulence genes are evident in several pathogenic bacteria including *S. pneumoniae*, it remains to be resolved at which stages of the infection process the different zinc-regulated and zinc transport genes are required. Streptococci could as well encode hitherto unknown zinc transporters that help in fine-tuning of zinc homeostasis. Moreover, certain proteins having a role in the infection process require zinc. For example, in *S. pyogenes*, Spel interacts strongly with AdcAII/LmB and AdcA. Since Spel, a potent virulence factor in this bacterium, needs zinc for activity, this interaction makes sense (Galeotti *et al.*, 2012). Also, the Pht family proteins, which have zinc-binding capacity, may play roles in protection against the immune system, for example by neutralizing complement, and they might also contribute to adherence (Rioux *et al.*, 2010; Plumtre *et al.*, 2012). In addition, the functionality of numerous other proteins could be affected by zinc during colonization and infection, such as the zinc metalloproteinases, which are involved in virulence as well, and zinc-containing alcohol dehydrogenases (Potter *et al.*, 2010; Bek-Thomsen *et al.*, 2012).

Availability of micronutrients such as zinc could also be influenced the presence of other bacterial species, as may the case in the nasopharynx, colonization of which by *S. pneumoniae* is a prerequisite for subsequent infection. So far, this is an unexplored area of study.

Use in therapies

Since the colonization and virulence of pathogenic streptococci displays a clear dependence on trace metal ion

homeostasis systems (Brown and Holden, 2002; Schaible and Kaufmann, 2004; Riccardi *et al.*, 2008; Jakubovics and Valentine, 2009; Klein and Lewinson, 2011; Cassat and Skaar, 2012) there could be possibilities to use these systems as targets for anti-infective therapies. Either it could be possible to generate compounds that inhibit such systems, or vaccines based on these systems could be developed (Klein and Lewinson, 2011). One class of vaccine targets could be the Pht family proteins, which are highly conserved in pneumococcal strains (see Table S1), especially PhtD which shows very little sequence variability (Rioux *et al.*, 2010; Plumptre *et al.*, 2012). All Pht family proteins were able to elicit a high level of protection in virulence settings, although in a study by Ogunniyi *et al.* (2007) the level of protection observed for PhtE and PhtB was not very strong. PhtD and E, as well as PcpA were found to raise antibody responses in humans (Khan *et al.*, 2012; Pichichero *et al.*, 2012), which might compromise pneumococcal adhesion to epithelial cells as this is mediated by these proteins (Khan *et al.*, 2002; Hendriksen *et al.*, 2009). Moreover, PhtE, PhtD and ZmpB were found in an antigen screen using human and mice sera (Beghetto *et al.*, 2006). PcpA also protects against sepsis and lung infection, but despite the fact that it has been reported as an adhesion factor, not against colonization of the nose (Glover *et al.*, 2008). Lmb, which binds zinc and presumably has a similar role as AdcAll in *S. pneumoniae*, is conserved in all strains of *S. agalactiae* and might be a good vaccine candidate as well (Ragunathan *et al.*, 2009). *S. pneumoniae* contains up to four zinc metalloproteases: IgA1, ZmpB, C and D (Bek-Thomsen *et al.*, 2012). Especially ZmpB, highly conserved among serotypes and involved in pneumococcal virulence, is an interesting vaccine candidate, which offers protection against pneumococcal invasive disease in mice, in particular when combined with other antigens (Gong *et al.*, 2011).

Adequate levels of zinc in the host might reduce the incidence of infectious diseases, including those caused by *S. pneumoniae* (Sazawal *et al.*, 1998; Black and Sazawal, 2001; Strand *et al.*, 2001; 2003; Fischer and Black, 2004; Coles *et al.*, 2008; Basnet *et al.*, 2012). Zinc also reduces the toxicity of pneumolysin to cochlear hair cells *in vitro* due to an inhibition of the toxin's incorporation and aggregation in(to) the plasma membrane, a phenomenon which could be a basis for treatment of pneumococcal otitis media (Franco-Vidal *et al.*, 2008).

So far, inhibitory molecules have not been generated based on metal ion homeostasis systems, but such compounds could be well targeted at one of the zinc uptake or efflux systems as described above (Klein and Lewinson, 2011). Thus, a proper understanding of zinc homeostasis in relation to the colonization by and virulence of streptococci might open up new avenues for protection against infections.

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