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Microreview

Streptococcal toxins: role in pathogenesis and disease

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Summary

Group A *Streptococcus* (*Streptococcus pyogenes*), group B *Streptococcus* (*Streptococcus agalactiae*) and *Streptococcus pneumoniae* (pneumococcus) are host-adapted bacterial pathogens among the leading infectious causes of human morbidity and mortality. These microbes and related members of the genus *Streptococcus* produce an array of toxins that act against human cells or tissues, resulting in impaired immune responses and subversion of host physiological processes to benefit the invading microorganism. This toxin repertoire includes haemolysins, proteases, superantigens and other agents that ultimately enhance colonization and survival within the host and promote dissemination of the pathogen.

Introduction

The genus *Streptococcus* comprises several important species of human and animal pathogens, most specifically adapted to survive within a single host species. As host-adapted pathogens, streptococcal species have evolved distinctive repertoires of protein and non-protein toxins that play crucial roles in colonization, pathogenesis and dissemination. While there are examples of streptococcal toxins that are represented across species boundaries, many streptococcal toxins are species specific or even limited to certain clonal lineages within an individual species. A number of these toxins have found utility as vaccine antigens or as novel therapeutics. In this review, we divide our examination of streptococcal toxins into four groups: haemolysins, proteases, superantigens (SAgs) and miscellaneous toxins. The best-studied individual examples from each class from across the genus *Streptococcus* are used to illustrate the function of these toxin molecules and their contribution to the disease process.

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Haemolysins

The complete lysis of red blood cells by streptococci, known as β-haemolysis, was first observed in 1895 and is embodied by the characteristic zone of clearing surrounding bacterial colonies on the surface of blood agar medium (Ayers and Rupp, 1922; Molloy *et al.*, 2011). This section summarizes our current understanding of streptococcal β-haemolysins/cytolysins (β-h/c) and their key role in pathogenesis (Table 1).

Streptolysin S

The potent membrane-active haemolysin streptolysin S (SLS) is secreted by 99% of all group A *Streptococcus* (GAS) isolates at stationary phase (Yoshino *et al.*, 2010) and is related to the class I bacteriocin family of proteinaceous toxins and antimicrobial peptides (Nizet *et al.*, 2000; Cotter *et al.*, 2005). SLS belongs to the thiazole/oxazole-modified microcin class of natural products, a family of diverse ribosomally produced peptides that are post-translationally modified to contain thiazole and (methyl)oxazole heterocycles from cysteine, threonine and serine residues (Mitchell *et al.*, 2009; Melby *et al.*, 2011). The activity of SLS is both temperature and concentration dependent and is principally responsible for the characteristic zone of β-haemolysis surrounding GAS colonies cultured on blood agar plates (Betschel *et al.*, 1998; Nizet *et al.*, 2000). SLS is encoded by the chromosomal SLS-associated gene (*sag*) locus, a conserved nine-gene operon comprised of contiguous genes *sagA* to *sagI* (Nizet *et al.*, 2000). The mature SLS is a

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Table 1. Streptococcal haemolysins.

Haemolysin	Streptococcal species	Virulence role	References
Streptolysin S family haemolysins			
Streptolysin S	*Streptococcus pyogenes*	Lysis of erythrocytes, lymphocytes, neutrophils, platelets, sub-cellular organelles	(Sierig *et al*., 2003; Miyoshi-Akiyama *et al*., 2005; Lin *et al*., 2009)
	Streptococcus dysgalactiae ssp. *equisimilis*	ND	(Humar *et al*., 2002; Hashikawa *et al*., 2004)
	Streptococcus equi ssp. *equi*	ND	(Flanagan *et al*., 1998; Hashikawa *et al*., 2004)
	Streptococcus iniae	Lysis of erythrocytes, neutrophils, lymphocytes	(Locke *et al*., 2007)
	Streptococcus canis	ND	(Richards *et al*., 2012)
	Streptococcus anginosus ssp. *anginosus*	ND	(Tabata *et al*., 2013; Asam *et al*., 2015)
	Streptococcus constellatus ssp. *constellatus*	ND	(Tabata *et al*., 2014)
Cholesterol-dependent cytolysins			
Streptolysin O	*S. pyogenes*	Disrupts cytoplasmic integrity of erythrocytes, leukocytes, macrophages, platelets, epithelial cells	(Limbago *et al*., 2000; Sierig *et al*., 2003; Brosnahan *et al*., 2009; Timmer *et al*., 2009)
		Translocates NAD-glycohydrolase (*Streptococcus pyogenes* NAD-glycohydrolase) toxin into epithelial cells	(Madden *et al*., 2001)
	S. canis	ND	(Richards *et al*., 2012)
	S. dysgalactiae ssp. *equisimilis*	ND	(Tanaka *et al*., 2008)
Pneumolysin	*Streptococcus pneumoniae*	Contributes to proliferation in whole blood, and colonization of the nasopharynx and lungs	(Berry *et al*., 1989b; Kadioglu *et al*., 2000; Reiss *et al*., 2011)
Suilysin	*Streptococcus suis*	Cytotoxic to endothelial cells, epithelial cells, macrophages and neutrophils	(Allen *et al*., 2001; Lun *et al*., 2003)
β-Haemolysin/cytolysin	*Streptococcus agalactiae*	Induces proinflammatory responses by human brain microvascular endothelial cells, neuronal and hepatocyte apoptosis, promotes intrauterine colonization	(Ring *et al*., 2002; Doran *et al*., 2003; Liu *et al*., 2004; Reiss *et al*., 2011)
Intermedilysin	*Streptococcus intermedius*	Forms cytotoxic pores in CD59-positive cells, promotes adherence to and invasion of human liver cells	(Sukeno *et al*., 2005)

ND, not determined.

~2.7 kDa oxygen-stable and broad-spectrum cytolytic toxin that forms hydrophilic pores in cholesterol-containing cytoplasmic membranes to induce irreversible osmotic lysis of host cells (Todd, 1938; Bernheimer, 1967; Ginsburg, 1999; Carr *et al*., 2001). The plasma membrane damage caused by pore-forming toxins induces signalling cascades in the host cell to promote membrane repair, metal ion homeostasis and a low-energy state whereby protein synthesis is arrested (Gonzalez *et al*., 2008, 2011).

The *sagA* gene encodes pre-SLS, while the downstream genes are required for post-translational modifications, heterocycle formation, processing and export of the mature SLS exotoxin (Nizet *et al*., 2000; Nizet, 2002; Molloy *et al*., 2011). SLS enhances GAS pathogenicity by lysing a broad spectrum of host cells including erythrocytes (red blood cells), lymphocytes, neutrophils, platelets, sub-cellular organelles (e.g. lysosomes and mitochondria) and several other mammalian cell types (Ofek *et al*., 1970; Betschel *et al*., 1998), but not bacteria with intact cell walls (Bernheimer, 1966). In a murine model of cutaneous infection, GAS strains lacking SLS activity were less virulent than the wild-type (WT) isogenic parental strain (Betschel *et al*., 1998; Mitchell *et al*., 2009). Soft tissue damage in mice associated with bacterial proliferation, inflammation, vascular injury and formation of necrotic lesions also depends on the activity of SLS in GAS, group G *Streptococcus* (GGS) and *Streptococcus iniae* (Betschel *et al*., 1998; Limbago *et al*., 2000; Fuller *et al*.,

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2002; Humar *et al*., 2002; Sierig *et al*., 2003). SLS may interact synergistically with other virulence factors (e.g. M protein and streptolysin O (SLO)) and host factors (e.g. neutrophil proteases and reactive oxygen species) to induce tissue necrosis and promote the development of necrotizing fasciitis in humans (Humar *et al*., 2002).

Recently, SLS and SLO have been shown to induce endoplasmic reticulum (ER) stress and an unfolded protein response (UPR) in host cells to reduce the surplus of unfolded proteins. The activation of UPR plays a key role in cellular defences against bacterial pore-forming toxins and is an important downstream target of the p38 mitogen-activated protein kinase pathway (Bischof *et al*., 2008), a central pathway of cellular immunity. In a mechanism that is yet to be fully understood, UPR results in the transcriptional upregulation of *asns*, a gene encoding for asparagine synthetase, and the release of asparagine into the extracellular environment. The extra-cellular asparagine sensed by GAS triggers a reduction in SLS and SLO transcription and stimulates bacterial growth (Baruch *et al*., 2014). Similarly, the pore-forming toxin listeriolysin O (LLO) produced by *Listeria monocytogenes*, a facultative intracellular bacterial pathogen, induces ER stress and is required for UPR activation (Pillich *et al*., 2012). LLO is activated within the acidic phagosome, allowing the bacterium to degrade the phagosome and escape to the cytosol (Dramsi and Cossart, 2002).

Groups C and G streptococci streptolysin S homologues

Group C *Streptococcus* (GCS) and GGS comprise several species of streptococci, with the significant human pathogen *Streptococcus dysgalactiae* ssp. *equisimilis* responsible for throat, skin and soft tissue infections and invasive infections including endocarditis, bacteremia and toxic shock. Nine-gene SLS-like loci are present in GCS and GGS. The SagA peptides of GCS and GGS share 89% amino acid identity with SagA from GAS (Humar *et al*., 2002) and are responsible for the prototypical β-haemolytic phenotype on the surface of blood agar plates (Flanagan *et al*., 1998; Hashikawa *et al*., 2004). Heterologous expression of GAS SagA in a non-haemolytic *sagA* mutant in GGS restored β-haemolytic activity on blood agar (Humar *et al*., 2002).

Streptococcus iniae *streptolysin S homologue*

Streptococcus iniae is an emerging zoonotic pathogen responsible for sporadic human infections through soft tissue injuries suffered during the handling and preparation of infected fish (Weinstein *et al*., 1997; Koh *et al*., 2009). The nine-gene *S. iniae sag* operon is 73% homologous to the *sag* operon from GAS and shares the same gene order (Fuller *et al*., 2002). Encoded by the *sagA* gene, the cytolysin of *S. iniae* shares 73% identity with GAS SLS (Fuller *et al*., 2002; Locke *et al*., 2007) and lyses erythrocytes, neutrophils, lymphocytes and several tissue culture cell lines. Heterologous expression of the *S. iniae sagA* gene in a non-haemolytic Δ*sagA* mutant of serotype M49 GAS restored haemolytic activity (Fuller *et al*., 2002; Locke *et al*., 2007). *S. iniae* SLS promotes neither adherence and invasion of epithelial cells nor resistance to opsonophagocytosis (Locke *et al*., 2007). However, a SagA-deficient mutant is highly attenuated for virulence (Locke *et al*., 2007).

Intermedilysin

Streptococcus intermedius, *Streptococcus constellatus* and *Streptococcus anginosus* are members of the Anginosus group of streptococci (AGS) that colonize the oral cavity, upper respiratory, gastrointestinal and female genitourinary tracts (Whiley *et al*., 1992; Jacobs *et al*., 1995). AGS are opportunist human pathogens capable of causing liver and brain abscesses, dentoalveolar infections and endocarditis (Jacobs *et al*., 1995). The secreted intermedilysin (ILY) of *S. intermedius* binds complement receptor CD59 on human cells and forms cytotoxic pores, but only in the presence of sufficient levels of cholesterol (Nagamune *et al*., 1996; Farrand *et al*., 2008; Heuck *et al*., 2010; Johnson *et al*., 2013). ILY damages host tissues and immune cells to promote bacterial survival and dissemination (Nagamune *et al*., 1996, 2000). ILY is considered to be a major virulence factor required for adherence to and invasion of human liver cells (Sukeno *et al*., 2005).

Streptolysin O

The oxygen-sensitive, 57 kDa thiol-activated SLO exotoxin is encoded by the highly conserved *slo* gene and secreted by nearly all GAS isolates during exponential and early stationary growth phases. SLO is a cholesterol-dependent cytolysin that disrupts the cytoplasmic membrane integrity of numerous eukaryotic cell types, including erythrocytes, leukocytes, macrophages, platelets, epithelial cells and various tissue culture cell lines (Limbago *et al*., 2000; Sierig *et al*., 2003). SLO contributes to GAS β-haemolysis under the surface of blood agar medium and, in contrast to SLS, contributes negligibly to β-haemolytic activity on the surface of blood agar (Molloy *et al*., 2011). The *slo* gene is co-transcribed with the *nga* gene encoding NAD-glycohydrolase (NADase), also known as *Streptococcus pyogenes* NADase, which is actively translocated into the cytosol of human epithelial cells by SLO to deplete energy stores and promote host cell injury (Madden *et al*., 2001; Bricker *et al*., 2005; Michos *et al*., 2006). Cathelicidin antimicrobial peptide LL-37 has been shown to upregulate the expression of *slo* and hyaluronan capsule, which promotes GAS resistance to killing by human epithelial cells, neutrophils and

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macrophages (Love *et al.*, 2012). SLO blocks the clathrin-dependent pathway for GAS internalization through disruption of the keratinocyte cell surface (Logsdon *et al.*, 2011) and induces keratinocyte apoptosis through the dysregulation of calcium signalling (Cywes Bentley *et al.*, 2005). SLO expression by intracellular GAS also contributes to the inhibition of dendritic cell maturation by inducing apoptosis (Cortes and Wessels, 2009). Upon macrophage phagocytosis, SLO damages the phagolysosome membrane, preventing phagolysosome acidification and resulting in the translocation of NADase into the macrophage cytosol (Bastiat-Sempe *et al.*, 2014). SLO and NADase also inhibit the autophagic killing of GAS in pharyngeal keratinocytes (O'Seaghdha and Wessels, 2013). The toxic effects of NADase are further discussed below.

In subcutaneous, intravenous and intraperitoneal murine models of invasive disease, *slo*-deficient GAS mutants have decreased virulence compared with WT parental strains (Limbago *et al.*, 2000; Ato *et al.*, 2008; Timmer *et al.*, 2009). A recent study showed that SLO binding to A549 epithelial cells does not require cholesterol, suggesting that cholesterol is not the membrane receptor for SLO (Mozola *et al.*, 2014). In support of this hypothesis, SLO binding to a glycan on the surface of human erythrocytes is essential for pore formation (Shewell *et al.*, 2014), and the haemolytic activity of SLO can be inhibited with a specific galactose-binding lectin (Hasan *et al.*, 2014). Similarly, an exposed F-type lectin domain at the N-terminus of lectinolysin (LLY), a cholesterol-dependent cytolysin from *Streptococcus mitis*, promotes binding of LLY to fucose-rich sites on target cell membranes (Farrand *et al.*, 2008; Bouyain and Geisbrecht, 2012). SLO and an enzymatically inactive derivative are immunogenic and protective against GAS challenge in mouse vaccination models (Bensi *et al.*, 2012; Chiarot *et al.*, 2013).

Suilysin

Streptococcus suis is a pathogen of swine that is responsible for numerous diseases (meningitis, septicaemia and endocarditis) and important economic losses to the porcine industry worldwide (Fittipaldi *et al.*, 2012). This species is also an emerging zoonotic agent of meningitis and streptococcal toxic shock-like syndrome in humans. *S. suis* expresses the haemolysin suilysin (SLY), a secreted 54 kDa thiol-activated exotoxin that binds cholesterol and forms pores in eukaryotic cell membranes (Gottschalk *et al.*, 1995; Palmer, 2001). It is closely related to both GAS SLO and the pneumolysin (PLY) of *Streptococcus pneumoniae* and is cytotoxic to endothelial cells, epithelial cells, macrophages and neutrophils (Charland *et al.*, 2000; Lalonde *et al.*, 2000; Segura and Gottschalk, 2002; Chabot-Roy *et al.*, 2006). SLY allows *S. suis* to evade the innate immune response by interfering with the complement cascade and activating phagocytic cells to release proinflammatory cytokines (Lun *et al.*, 2003; Segura *et al.*, 2006). SLY-deficient mutants are attenuated for virulence in a systemic mouse infection model (Allen *et al.*, 2001), but SLY is not required for full virulence in a piglet infection model (Lun *et al.*, 2003). Recently, SLY has been shown to promote *S. suis* adherence to and invasion of human HEp-2 epithelial cells (Seitz *et al.*, 2013).

β-Haemolysin/cytolysin

Streptococcus agalactiae (group B *Streptococcus*, GBS) is the leading cause of meningitis, sepsis and pneumonia in human newborn infants and a significant agent of invasive infections among immunocompromised adults (e.g. diabetes and cancer patients) and pregnant women worldwide (Farley, 2001). The β-h/c of GBS is a non-immunogenic oxygen-stable pore-forming cytolysin and a major virulence factor expressed by most clinical isolates, with a predicted size of 78.3 kDa (Dal and Monteil, 1983; Nizet *et al.*, 1996; Spellerberg, 2000). First described in 1934 (Todd, 1934), cell surface-associated β-h/c is encoded by the *cylE* gene in the *cyl* locus, a unique 12-gene operon involved in fatty acid biosynthesis (Pritzlaff *et al.*, 2001) that is expressed by almost all strains of GBS. CylE expression is invariably associated with the production of an orange to brick-red carotenoid pigment (Tapsall, 1987; Spellerberg *et al.*, 2000) and is primarily regulated by the two-component system *covR/S* (control of virulence) (Tapsall, 1987); some have recently suggested the pigment itself may convey the haemolytic activity (Whidbey *et al.*, 2013). CylE expression is required for GBS survival in mouse and human blood *ex vivo* (Liu *et al.*, 2004). Animal studies with WT and isogenic β-h/c mutants demonstrate that haemolysin expression has proapoptotic, proinflammatory and cytotoxic effects and is necessary for full GBS virulence in multiple *in vivo* systems, including mouse models of GBS arthritis (Puliti *et al.*, 2000), meningitis (Doran *et al.*, 2003) and ascending chorioamnionitis (Randis *et al.*, 2014), a rat model of experimental GBS meningitis (Reiss *et al.*, 2011), and rabbit models of GBS septicaemia (Ring *et al.*, 2002) and pneumonia (Hensler *et al.*, 2005).

Pneumolysin

Streptococcus pneumoniae (pneumococcus) is the causative agent of pneumococcal pneumonia, meningitis, sepsis, otitis media and other less serious infections. PLY is a 53 kDa (471-amino-acid) (Walker *et al.*, 1987) cholesterol-dependent pore-forming toxin with four functional domains (Mitchell and Dalziel, 2014). In a recent study, binding of PLY domain 4 to the sialyl LewisX glycolipid cellular receptor on the surface of human

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erythrocytes was shown to be an essential step before membrane insertion and pore formation (Shewell *et al*., 2014). PLY is a cytoplasmic thiol-activated toxin with cytolytic and complement-activating properties (Lucas *et al*., 2013). PLY is localized primarily to the cell wall compartment in the absence of detectable cell lysis (Price *et al*., 2012). However, unlike other cholesterol-binding cytolysins, PLY lacks secretion signal sequences and is not actively secreted into the extracellular milieu (Cassidy and O'Riordan, 2013). The cytolytic activity of PLY and its release into the extracellular milieu are inhibited by branched stem peptides in the peptidoglycan cell wall (Greene *et al*., 2015). PLY is released into the alveolar compartment upon bacterial lysis induced by autolysis (Berry *et al*., 1989a, 1992) or by antibiotic treatment of pneumococcal pneumonia patients (Anderson *et al*., 2007). However, in the absence of cell lysis, PLY is exported from the cytoplasm and attached to the cell wall by a yet-to-be-characterized mechanism (Price *et al*., 2012). PLY is directly toxic for a wide variety of host cells and tissues and also elicits strong inflammatory responses at the site of infection, triggering signalling via TLR4 (Malley *et al*., 2003), as well as activation of the NLRP3 inflammasome (McNeela *et al*., 2010). Circulating PLY also induces myocardial injury in a mouse model of invasive pneumococcal disease and dose-dependent damage to cardiomyocytes *in vitro* (Alhamdi *et al*., 2015). In pneumococcal meningitis, the majority of the damage to the blood–brain barrier has been attributed to PLY (Zysk *et al*., 2001; Mitchell and Dalziel, 2014). Structural homology to the Fc region of immunoglobulin G (IgG) also allows PLY to activate the classical complement pathway away from intact bacteria to deplete host serum complement levels and promote survival and spread (Paton *et al*., 1984; Mitchell *et al*., 1991; Rossjohn *et al*., 1998; Alcantara *et al*., 2001). Recently, PLY was also shown to contribute to the assembly of pneumococcal biofilms (Shak *et al*., 2013). PLY activates p38 *in vitro* (Ratner *et al*., 2006) and the NLRP3 inflammasome in macrophages to stimulate the production of type I interferons following pneumococcal phagocytosis (Koppe *et al*., 2012). In murine infection models, PLY-deficient mutants have reduced proliferation in whole blood (Benton *et al*., 1995), diminished capacity to colonize the nasopharynx, induce less lung inflammation and neutrophil recruitment and are rapidly cleared from the lung, compared with WT (Berry *et al*., 1989b; Kadioglu *et al*., 2000). Genetically inactivated PLY toxoids are immunogenic and protective against lethal pneumococcal challenge in mouse vaccination models (Paton *et al*., 1991; Alexander *et al*., 1994; Kirkham *et al*., 2006). Phase I clinical trials demonstrated that PLY toxoid PlyD1 is safe and elicits functional neutralizing antibodies against the pneumococcus (Kamtchoua *et al*., 2013).

Proteases

Proteases (or peptidases) are enzymes that catalyse the hydrolysis of peptide bonds. The genus *Streptococcus* possesses a wide array of proteases that have diverse functions, including nutrient acquisition, protein maturation and quality control, and various host interactions. For the purposes of this review, however, we will focus only on those surface-exposed or secreted proteases that have direct effects on pathogenesis through their activities on host proteins and tissues.

Group A Streptococcus *cysteine protease (SpeB)*

The GAS cysteine protease SpeB is encoded in the genomes of essentially all GAS strains (Bohach *et al*., 1988; Yu and Ferretti, 1991); homologous proteins, albeit uncharacterized, are encoded in the genomes of the closely related species *Streptococcus didelphis*, *Streptococcus porcinus* and *Streptococcus pseudoporcinus*. Expression of this protein is tightly regulated at the transcriptional level, as well as post-transcriptionally through the maturation of the inactive zymogen to the active mature enzyme (for a review, see Carroll and Musser, 2011). With diverse functions in pathogenesis, including direct action on host tissues as well as roles in the maturation and surface display of other surface-exposed GAS proteins, SpeB is a veritable 'Swiss army knife' of GAS biology.

SpeB is a broad-spectrum cysteine protease structurally related to papain and has been shown to degrade numerous host proteins *in vitro*, including immunoglobulins, complement components, chemokines, cytokines, extracellular matrix proteins and numerous other host proteins (for a review, see Nelson *et al*., 2011). However, despite these studies, it has recently been shown that SpeB does not cleave immunoglobulins under physiologically relevant conditions (Persson *et al*., 2013), and thus, the true *in vivo* substrate profile might be considerably smaller. Multiple lines of evidence suggest that SpeB is important for GAS pathogenesis: passive immunization of mice with anti-SpeB antibodies or a synthetic protease inhibitor protects against infection, and low-milligram amounts of SpeB are lethal when injected into mice (Bjorck *et al*., 1989; Kapur *et al*., 1994; Nelson *et al*., 2011). Furthermore, isogenic Δ*speB* mutant GAS strains are avirulent in mice following subcutaneous (Lukomski *et al*., 1999; Cole *et al*., 2006; Terao *et al*., 2008) and intraperitoneal (Lukomski *et al*., 1997; Hollands *et al*., 2008) infection and exhibit decreased survival in human whole blood (Chiang-Ni *et al*., 2006) and serum (Honda-Ogawa *et al*., 2013).

Role in superficial infections. SpeB expression has a strong epidemiological association with isolates from superficial disease (Ikebe *et al*., 2010; Cole *et al*., 2011).

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While the exact role of this enzyme during superficial GAS disease was presumed to involve its degradation of host immune components, two recent reports have suggested alternative or complementary functions. In the first report (Barnett *et al*., 2013), SpeB conferred the ability of GAS strains to replicate in the cytosol of infected epithelial cells. SpeB was shown to degrade the host proteins that direct intracellular bacteria to the autophagy pathway, a host system for degrading cytosolic components in lysosomes that constitutes an important immune defence against intracellular bacteria. Consequently, SpeB-expressing GAS strains are able to evade this pathway and replicate in the cytosol of infected cells. In a separate study, SpeB was shown to promote the translocation of GAS across epithelial barriers by degrading occludin and E-cadherin (Sumitomo *et al*., 2013). Thus, SpeB may aid in the establishment of an epithelial replicative niche and dissemination of GAS into deeper tissues.

Role in invasive disease. Epidemiological studies have demonstrated that SpeB expression is inversely related to disease severity (Kansal *et al*., 2000; Ikebe *et al*., 2010). Loss of SpeB expression, through the accumulation of mutations in the regulatory genes *covR*/*S* and to a lesser extent *ropB*, leads to the abolishment of *speB* expression. This genetic switch has multiple consequences that lead to a hypervirulent invasive state: loss of SpeB production spares several GAS virulence factors (e.g. M protein, various SAgs and streptokinase) from proteolytic degradation, and *covR*/*S* mutations lead to the up-regulation of multiple virulence factors required for the invasive disease phenotype (for a review, see Cole *et al*., 2011).

While SpeB expression is strongly linked to GAS isolates from superficial disease, immunohistochemical analysis has shown that human tissues from necrotizing fasciitis cases are strongly positive for SpeB, suggesting that SpeB is elaborated during the establishment or progression of this disease (Johansson *et al*., 2008). Similarly, SpeB is required for full virulence in invasive infection models in mice (Lukomski *et al*., 1999; Cole *et al*., 2006; Hollands *et al*., 2008; Olsen *et al*., 2010) and nonhuman primate animal models (Olsen *et al*., 2010). While these results seemingly contradict other studies that have clearly demonstrated a genetic switch from a SpeB-positive phenotype to a SpeB-negative phenotype during invasive disease (Aziz *et al*., 2004; Cole *et al*., 2006; Sumby *et al*., 2006; Walker *et al*., 2007), it is possible that a mixed population of SpeB-positive and SpeB-negative bacteria contribute to the overall pathology of GAS invasive disease and penetration into deeper tissues (Cole *et al*., 2006).

In addition to regulation through *covR*/*S* and *ropB* mutations, it has also been recently demonstrated that SpeB activity can be inhibited by the divalent cations of zinc and copper (Chella Krishnan *et al*., 2014). While the physiological consequence of this inhibition is currently unknown, it was postulated that reversible inhibition of SpeB activity may reversibly preserve critical virulence factors required during certain stages of the infectious process.

Immune-modulating proteases

C5a peptidase. C5a peptidase (ScpA), is a serine endopeptidase that specifically cleaves and inactivates the C5a complement factor and has been implicated to play a role in inhibiting the recruitment of phagocytes to the infectious site (Ji *et al*., 1996; Collin and Olsen, 2003). While recombinant ScpA is very potent in inhibiting phagocyte chemotaxis *in vitro*, the effects of Δ*scpA* mutations in mice infections *in vivo* are much less dramatic (Ji *et al*., 1996), possibly as a result of alternative chemoattractants, such as interleukin (IL)-8, and GAS proteases that inhibit them, such as SpyCEP (below). C5a peptidase is a cell wall-anchored enzyme but can be released from the surface of GAS as a functionally active enzyme by SpeB and can thus inactivate C5a at some distance from the bacterium (Berge and Bjorck, 1995). C5a peptidase is expressed by strains of GAS (ScpA; Chen and Cleary, 1990), GBS (ScpB; Cleary *et al*., 1992) and *Streptococcus equi* ssp. *zooepidemicus* (ScpZ; Wei *et al*., 2013) and is encoded in the genomes of several other streptococcal pathogens, including *S. dysgalactiae*, *S. iniae*, *Streptococcus sanguinis*, *S. mitis* and *Streptococcus canis* (GGS). In addition to its endopeptidase activity, the C5a peptidase proteins from GAS, GBS and *S. equi* ssp. *zooepidemicus* have also been suggested to function as an invasin (Cheng *et al*., 2002b; Purushothaman *et al*., 2004; Wei *et al*., 2013). Vaccination against ScpA is protective for GAS (Park and Cleary, 2005), GBS (Cheng *et al*., 2002a; Santillan *et al*., 2008) and *S. equi* ssp. *zooepidemicus* (Wei *et al*., 2013).

SpyCEP. Group A *Streptococcus* SpyCEP is a subtilisin-like serine protease that can cleave human CXC chemokines. In its mature form, SpyCEP exists as a dimer composed of two subunits (30 and 150 kDa) generated by intramolecular autocatalytic cleavage (Zingaretti *et al*., 2010). Substrates include granulocyte chemotactic peptide-2 (CXCL6), growth-related oncogene-α, β, γ (CXCL1, 2, 3), neutrophil-activating peptide-78 (CXCL5), GRB2-related adapter protein 2 and IL-8 (CXCL8), which correspond to the murine CXC chemokines MIP-2 and KC (Hidalgo-Grass *et al*., 2004; Sumby *et al*., 2008; Chiappini *et al*., 2012). As a result of this activity, SpyCEP impairs the recruitment of neutrophils, monocytes and eosinophils to the site of infection (Zinkernagel *et al*., 2008; Chiappini *et al*., 2012). SpyCEP also promotes resistance to neutrophil killing by reducing

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the production of neutrophil extracellular traps (Zinkernagel *et al.*, 2008). SpyCEP-producing GAS strains are more virulent in murine models of invasive disease, and SpyCEP production is up-regulated in human invasive GAS isolates (Edwards *et al.*, 2005; Hidalgo-Grass *et al.*, 2006; Zinkernagel *et al.*, 2008; Turner *et al.*, 2009a). Furthermore, immunization with SpyCEP protects mice against GAS nasopharyngeal (Alam *et al.*, 2013), intramuscular and intranasal infection (Turner *et al.*, 2009b) and intramuscular infection with *S. equi* (Turner *et al.*, 2009b), demonstrating that a vaccine based on SpyCEP may provide cross-protection against multiple streptococcal species. While SpyCEP is cell wall anchored, it can be shed into the supernatants of bacterial cultures during stationary-phase growth (Chiappini *et al.*, 2012), presumably as a result of protease processing. In addition to these protease activities, SpyCEP also promotes the uptake of GAS into endothelial, but not epithelial, cells (Kaur *et al.*, 2010). Homologous proteases have also been characterized in *S. iniae* (Zinkernagel *et al.*, 2008) and *S. equi* (Turner *et al.*, 2009b) and are present in the genomes of several other *Streptococcus* species (*S. dysgalactiae*, *S. canis*, *S. didelphis*, *Streptococcus phocae*, *S. porcinus*, *S. pseudoporcinus* and *Streptococcus thermophilus*).

IdeS. IdeS (also known as Mac) is a 35 kDa secreted cysteine protease produced by strains of GAS that specifically cleaves between the two glycine residues in positions 236 and 237 in the lower hinge region of the IgG heavy chain, resulting in the separation of the Fc and Fab fragments (von Pawel-Rammingen *et al.*, 2002; Vincents *et al.*, 2004; von Pawel-Rammingen, 2012). As a homologue of CD11b, IdeS has been proposed to also inhibit phagocytosis by inhibiting Fc receptor (CD16) recognition of IgG and/or complement deposition (Lei *et al.*, 2001). This is hypothesized to prevent the recognition of antibody-opsonized bacteria by Fc receptors of immune cells and by the complement system (von Pawel-Rammingen *et al.*, 2002). Protease activity is considerably higher against soluble IgG and IgG bound in an antigen-specific manner at the Fab region than to IgG bound non-specifically to GAS M protein at the Fc region, suggesting that this enzyme has evolved to allow GAS to resist Ig-mediated phagocytosis and cytotoxicity while still allowing non-immune IgG interactions, which are believed to contribute to GAS pathogenesis (Su *et al.*, 2011). However, IdeS was recently found to not be essential for phagocyte resistance or mouse virulence, and thus, the exact role of this protein in human disease remains to be identified (Okumura *et al.*, 2013). The human protease inhibitor cystatin C can act as a cofactor for IdeS and greatly increase enzymatic activity (Vincents *et al.*, 2008). All GAS strains examined possess one of two identified variants of IdeS (IdeS and Mac-2), with considerable amino acid sequence divergence in the middle third of the proteins but with indistinguishable enzymatic activity, with the exception of the Mac-2 protein from M28 strains that displays only weak endopeptidase activity (Lei *et al.*, 2001; von Pawel-Rammingen *et al.*, 2002; Soderberg *et al.*, 2008; von Pawel-Rammingen, 2012). IdeS homologues that cleave IgG have also been identified in several other *Streptococcus* species, including *S. equi* ssp. *equi* and *S. equi* ssp. *zooepidemicus* (Lannergard and Guss, 2006; Hulting *et al.*, 2009). Additionally, an IdeS homologue that cleaves porcine IgM has recently been found in *S. suis* (Seele *et al.*, 2013).

Zinc metalloproteases

Streptococcus pneumoniae strains encode various combinations of four zinc metalloproteases: IgA1 protease, ZmpB, ZmpC and ZmpD. An unusual characteristic of all of these proteins is the presence of an LPXTG cell wall-anchoring motif near their N-terminus (Bek-Thomsen *et al.*, 2012), which in the case of IgA1 protease has been shown to be important for proper localization and enzymatic function (Bender and Weiser, 2006).

IgA1 protease specifically cleaves the IgA1 hinge region, resulting in separation of the Fc and Fab fragments (Senior and Woof, 2005a,b). This cleavage abrogates the protective effects of IgA1 in mediating complement-dependent killing of pneumococci by phagocytes (Fasching *et al.*, 2007; Janoff *et al.*, 2014) and promotes the adherence of this bacterium to respiratory epithelial cells (Weiser *et al.*, 2003). It is likely that IgA1 protease has additional roles in virulence, as mutants have reduced virulence in mice yet murine IgA1 is not a substrate for this enzyme (Chiavolini *et al.*, 2003; Janoff *et al.*, 2014). Homologous IgA1 proteases have been characterized in *S. suis*, which is important for virulence in pigs (Zhang *et al.*, 2011), and *S. sanguinis* (Gilbert *et al.*, 1988). IgA1 protease is encoded by all strains of *S. pneumoniae*, *Streptococcus pseudopneumoniae*, *Streptococcus oralis* and *S. sanguinis* and is variably present in *S. mitis* and *Streptococcus infantis* (Bek-Thomsen *et al.*, 2012).

In contrast to IgA1 protease, the ZmpC protease has multiple targets and is an important virulence factor in experimental pneumonia (Oggioni *et al.*, 2003). ZmpC has been shown to activate human matrix metalloprotease 9, a host zinc protease involved in neutrophil migration and wound repair of the respiratory epithelium (Oggioni *et al.*, 2003). ZmpC inhibits neutrophil flux by degrading the N-terminal region of P-selectin glycoprotein 1 (Surewaard *et al.*, 2013). In addition, ZmpC induces specific epithelial cell shedding of both mucin 16, a vital defence barrier of ocular epithelial cells (Govindarajan *et al.*, 2012; Menon and Govindarajan, 2013), and syndecan-1, a surface proteoglycan with diverse roles in cell–cell and cell–matrix binding, cell signalling and cytoskeletal organization

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(Chen *et al.*, 2007). Genes encoding ZmpC are variably present in *S. pneumoniae*, *S. pseudopneumoniae*, *S. mitis*, *S. oralis* and *Streptococcus gordonii* and present in all strains of *S. sanguinis* (Bek-Thomsen *et al.*, 2012).

In contrast to IgA1 protease and ZmpC, ZmpB and ZmpD have not been well characterized, and their substrates have not been identified. ZmpB induces tumour necrosis factor-α production in the respiratory tract, and a *zmpB* mutant was attenuated in pneumonia and septicaemia models of infection (Blue *et al.*, 2003; Chiavolini *et al.*, 2003). Genes encoding ZmpB are present in all *S. pneumoniae* strains as well as several other *Streptococcus* species, including *S. pseudopneumoniae*, *S. mitis*, *S. oralis*, *S. sanguinis*, *S. gordonii*, *Streptococcus vestibularis* and *Streptococcus salivarius*. Genes encoding ZmpD are sporadically distributed among *S. pneumoniae*, *S. pseudopneumoniae* and *S. mitis* (Bek-Thomsen *et al.*, 2012).

SspA

Strains of *S. suis* encode a cell wall-anchored subtilisin-like serine protease termed SspA, which is important for disease in pigs (Bonifait *et al.*, 2010; Hu *et al.*, 2010). SspA cleaves fibrinogen, preventing subsequent fibrin formation by thrombin. Purified SspA protease is toxic when added to brain microvascular endothelial cells (Bonifait *et al.*, 2011b) and induces a proinflammatory response in macrophages through a non-proteolytic mechanism (Bonifait and Grenier, 2011a). Homologous genes are present in the genomes of several other *Streptococcus* species, including *S. thermophilus*, *S. canis*, *S. agalactiae*, *S. dysgalactiae*, *S. gordonii*, *S. mitis*, *S. sanguinis* and *S. intermedius*.

Superantigens

Bacterial superantigens (SAgs) are potent microbial secreted toxins produced by GAS, group C/G *S. dysgalactiae* ssp. *equisimilis*, *Staphylococcus aureus*, *Yersinia pseudotuberculosis* and *Mycoplasma arthritidis* (Fraser and Proft, 2008). Their importance in disease pathogenesis resides in their ability to bypass the regular antigen presentation process and overstimulate immune activation. Conventional antigen presentation by antigen-presenting cells (APCs) to T cells is mediated by the interaction of the major histocompatibility complex (MHC) molecules and the T-cell receptor (TCR). TCRs are formed by five variable elements (Vβ, Dβ, Jβ, Vα and Jα), which are responsible for a highly specific immune response to a vast number of foreign antigens. All five variable elements from the TCR are needed to recognize the complex formed by the MHC and the processed antigen in order to stimulate T-cell proliferation (Fig. 1). As a result of this specificity, conventional processed antigens are only able to stimulate approximately 0.01% of the T-cell repertoire (Davis and Bjorkman, 1988). Conversely, unprocessed SAgs bind directly to the MHC II molecules on the surface of APCs and to the Vβ variable region of the TCR, which is present in up to 25% of the T-cell population (Fig. 1). When this simultaneous binding occurs, stimulated T cells and APCs secrete a vast amount of proinflammatory cytokines such as IL-2,

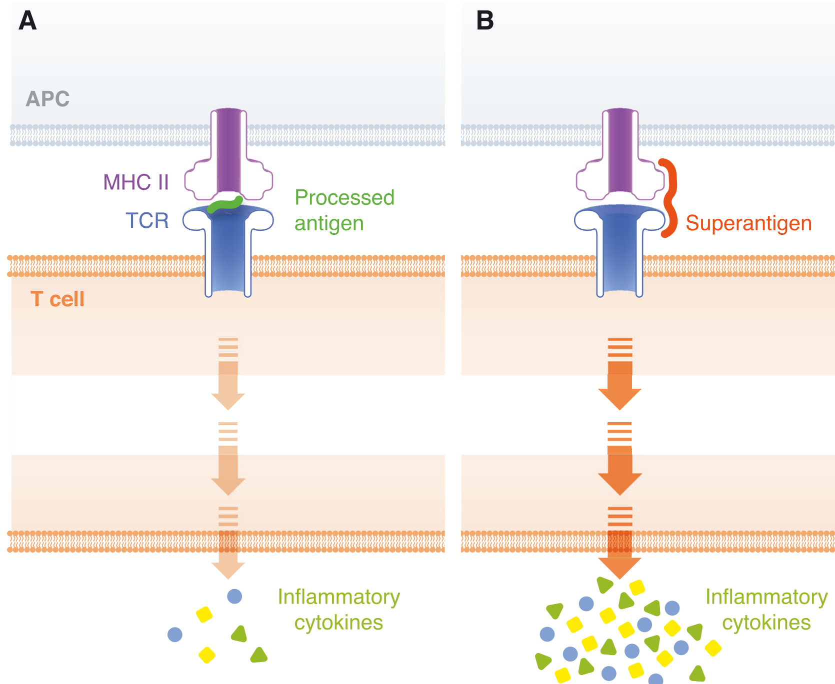

Fig. 1. A. T-cell stimulation mediated by conventional major histocompatibility complex (MHC) II antigen presentation, where large antigens are processed by antigen-presenting cells (APCs) into linear epitopes and presented by MHC II; specific recognition of the MHC–antigen complex by T-cell receptor (TCR) on the surface of T cells has to occur in order to trigger production of inflammatory cytokines.
B. Superantigen direct binding to MHC II and TCR, where binding occurs in a less specific manner, thus stimulating a significantly higher number of T cells and producing an uncontrollable cytokine release.

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interferon-γ and tumour necrosis factor-α (Jupin *et al.*, 1988; Fast *et al.*, 1989; Müller-Alouf *et al.*, 1996). This uncontrollable cytokine release is then thought to be responsible for the characteristic manifestations of streptococcal toxic shock syndrome (STSS), such as systemic vasodilatation, hypotension and multi-organ failure (Lappin and Ferguson, 2009). In addition, recent published data suggest that for some SAgs, binding to the CD28 receptor on the surface of T cells is required for the expression of Th1 cytokines (Arad *et al.*, 2011). It has been hypothesized that SAgs dysregulate the host immune response and therefore increase the chance of pathogen survival (Spaulding *et al.*, 2013). In the case of GAS, an outside–in signalling mechanism has been proposed to explain the ability of GAS to trigger STSS from mucosal surfaces, where SAgs together with SLO act synergistically to penetrate the mucosal epithelium, recruit lymphocytes to the site of infection and initiate SAg stimulation of T cells (Fig. 2) (Brosnahan, 2009; Brosnahan and Schlievert 2011). Additional new evidence suggests that SAgs provide an advantage for nasopharyngeal colonization in a humanized mouse model (Kasper *et al.*, 2014).

Structure of superantigens

Since their discovery in GAS in 1924, numerous streptococcal SAgs have been identified, particularly since the expansion of whole-genome sequencing technologies. In total, 91 unique sequences encoding complete streptococcal SAgs have been reported and a uniform nomenclature proposed (Commons *et al.*, 2014), which has been used in this review (Table 2).

Superantigens are small non-glycosylated proteins between 22 and 29 kDa. Despite low DNA sequence homology, they share a common two-domain architecture formed by the N-terminal and C-terminal domains, which are separated by a long accessible α-helix. Hydrophobic residues in solvent-exposed regions characterize the N-terminal domain, while a four-stranded β-sheet capped by a central α-helix constitutes the C-terminal domain. Most of the streptococcal SAgs contain a zinc binding site in the C-terminal domain, and the presence of zinc has been shown to be critical for MHC II binding (Proft *et al.*, 1999). In addition, the majority of streptococcal SAgs bind to the β-chain of MHC II with the exception of SpeA and SSA, which bind to the α-chain. The TCR binding site is located in a shallow cavity between the two protein domains, and different SAgs show preference for different TCR Vβ chains (Table 2) (Papageorgiou and Acharya, 1997; Proft and Fraser, 2007). Some streptococcal SAgs (SSA, SpeA, SpeI, SpeH and SmeZ) contain a CD28 binding motif situated in the SAg β-strand/hinge/α-helix domain (Arad *et al.*, 2011; Commons *et al.*, 2014).

Evolution and genetic mobility of superantigen genes

Most of the streptococcal SAgs are located on prophage regions within the genome. Only *speG*, *speJ* and *smeZ*,

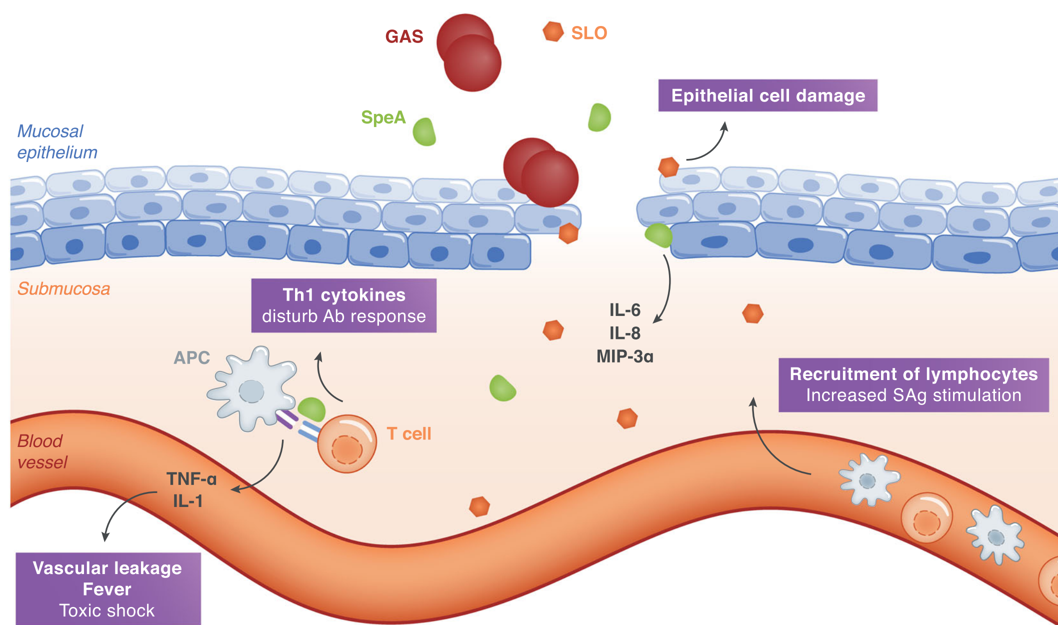

Fig. 2. Outside–in mechanism proposed by Brosnahan and Schlievert (2011), where streptolysin O (SLO) and SpeA act synergistically to cause streptococcal toxic shock syndrome (STSS) from a mucosal surface. SLO produces damage to epithelial cells allowing SpeA to access the submucosa. SpeA in turn stimulates epithelial cells to secrete chemokines that recruit lymphocytes to the site of infection where they are targets for superantigen (SAg) stimulation. Secretion of Th1 cytokines by SAg-stimulated T cells and antigen-presenting cells (APCs) skews the host's immune response, interfering with its ability to effectively combat infection. The uncontrolled release of proinflammatory cytokines can then cause vascular leakage and fever leading to STSS.

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Table 2. Streptococcal superantigens.

Superantigen	Streptococcal species	Major histocompatibility complex II binding α/β	T-cell receptor Vβ specificity	Clinical association	References
SpeA	*Streptococcus pyogenes* *Streptococcus dysgalactiae* ssp. *equisimilis*	+/−	2.1,12.2, 14.1, 15.1	Scarlet fever Streptococcal toxic shock syndrome	(Eriksson *et al*., 1999; Hartwig *et al*., 1994; Musser *et al*., 1991; Silva-Costa *et al*., 2014)
SpeC	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis* *S. dysgalactiae* ssp. *dysgalactiae*	−/+	2.1, 3.2, 12.5, 15.1	Scarlet fever Kawasaki Disease	(Li *et al*., 1997; Silva-Costa *et al*., 2014; Davies *et al*., 2015; Yoshioka *et al*., 2003)
SpeG	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis* *S. dysgalactiae* ssp. *dysgalactiae* *Streptococcus minor* *Streptococcus canis*	−/+	2.1, 4.1, 6.9, 9.1, 12.3	ND	(Proft *et al*., 1999; Sachse *et al*., 2002)
SpeH	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis* *Streptococcus equi* ssp. *equi*	−/+	2.1, 4.1, 6.9, 9.1, 12.3	ND	(Proft *et al*., 1999)
SpeI	*S. pyogenes* *S. equi* ssp. *equi*	−/+	6.9, 9.1, 18.1, 22	ND	(Proft *et al*., 2001)
SpeJ	*S. pyogenes*	−/+	2.1	ND	(Proft *et al*., 2001; McCormick *et al*., 2001)
SpeK	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis* *S. equi* ssp. *equi* *S. equi* ssp. *zooepidemicus*	−/+	1, 5.1, 23	Acute rheumatic fever	(Beres *et al*., 2002; Proft *et al*., 2003b)
SpeL	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis* *S. equi* ssp. *equi*	−/+	1, 5.1, 23	Acute rheumatic fever	(Smoot *et al*., 2002)
SpeM	*S. pyogenes* *S. dysgalactiae* ssp. *dysgalactiae*	ND	1, 23	Acute rheumatic fever	(Smoot *et al*., 2002; Miyoshi-Akiyama *et al*., 2003)
SpeN	*S. equi* ssp. *zooepidemicus*	ND	ND	ND	(Paillot *et al*., 2010)
SpeO	*S. equi* ssp. *zooepidemicus*	ND	ND	ND	(Paillot *et al*., 2010)
SpeP	*S. equi* ssp. *zooepidemicus*	ND	ND	ND	(Paillot *et al*., 2010)
SSA	*S. pyogenes* *S. dysgalactiae* ssp. *equisimilis*	+/−	1, 3, 15	Scarlet fever Streptococcal toxic shock syndrome	(Mollick *et al*., 1993; Igwe *et al*., 2003; Silva-Costa *et al*., 2014; Davies *et al*., 2015)
SmeZ	*S. pyogenes* *S. canis*	−/+	2.1, 4.1, 7.3, 8.1	Streptococcal toxic shock syndrome	(Kamezawa *et al*., 1997; Igwe *et al*., 2003; Proft *et al*., 2003a; Vlaminckx *et al*., 2003)

ND, not determined.

which are usually flanked by transposon-like elements, are located within the main chromosome external to prophages. The hypothesis that SAg genes can be horizontally transferred through phages was validated by performing *in vitro* and *in vivo* lysogenic transfer of *speC* between two different GAS isolates (Broudy and Fischetti, 2003). Moreover, lysogenic transfer of phage-carrying toxins between different streptococcal species has also been reproduced *in vitro* (Vojtek *et al*., 2008). Despite the fact that horizontal transfer of SAg genes has been demonstrated, little is known about the evolution of these genes within streptococcal species. A multi-step model for the evolution of SAgs was proposed based on genome sequence analysis of GAS, *S. dysgalactiae* ssp. equisimilis and *S. aureus*. In step 1, ancestral bacteria acquired the ancestors of streptococcal SAgs, staphylococcal enterotoxins and staphylococcal SAg-like proteins (SSLs). In step 2, ancestral *smeZ* was likely to be deleted from the *S. dysgalactiae* ssp. equisimilis genome soon after the speciation of GAS and *S. dysgalactiae* ssp. equisimilis. In step 3, ancestral SAgs and SSLs were potentially incorporated into phages that were then transferred in between

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bacterial strains. In the final step, staphylococcal SAgs (potential ancestors of streptococcal SAgs such as SSA and SpeA) were then horizontally transferred to *Streptococcus* bacteria (Okumura *et al*., 2012).

Streptococcal superantigens and disease correlation

Clinical manifestations of disease have been linked to the presence of SAgs in streptococcal pathogens. Cases of scarlet fever have been correlated with the carriage or acquisition of *ssa*, *speA* and *speC* in different studies (Silva-Costa *et al*., 2014; Davies *et al*., 2015). Additionally, *speA* and *ssa*, together with *speK*, and *smeZ* have been associated with invasive disease and STSS (Eriksson *et al*., 1999; Chatellier *et al*., 2000; Beres *et al*., 2002; Ikebe *et al*., 2002). A strong correlation has been observed between M18 isolates expressing *speL* and *speM* and acute rheumatic fever (Smoot *et al*., 2002). M89 isolates harbouring the *speK* gene have also been associated with acute rheumatic fever (Proft *et al*., 2003b).

A controversial correlation between *speC* and Kawasaki disease, a sporadic childhood inflammatory arthritis that can affect the coronary vessels (Shulman and Rowley, 2015), has been proposed (Abe *et al*., 1992; Curtis *et al*., 1995); however, contradicting epidemiological studies render a direct correlation questionable (Yoshioka *et al*., 2003). Research on the causative agents of Kawasaki disease is still ongoing. In addition, *speC* has also been correlated to psoriasis in susceptible individuals (Leung *et al*., 1993; Lewis *et al*., 1993); however, the evidence is not strong enough for a direct correlation, and the role of SAgs in disease manifestations remains unclear (Travers *et al*., 1999; Thomssen *et al*., 2000).

Other Toxins

In addition to producing toxic SAgs, haemolysins and proteases, streptococcal species produce a number of other toxins including proteins, enzymes and polysaccharides such as Christie Atkins Munch-Petersen (CAMP) factor, GBS toxin, adenosine diphosphate (ADP)-ribosyltransferase and NADase.

Christie Atkins Munch-Petersen factor

The CAMP reaction was initially described as the lysis of erythrocytes during a synergistic interaction of CAMP factor produced by GBS with the β-toxin of *S. aureus* (Christie *et al.*, 1944). Historically, this reaction has been used for the clinical identification of GBS. The CAMP factor is best characterized in GBS, where the toxin is encoded by the *cfb* gene (Podbielski *et al*., 1994). Other streptococcal species, including groups A, B, C, G, M, P, R and U, have also been reported to produce CAMP factor; and in GAS, CAMP factor is encoded by the *cfa* gene (Gase *et al.*, 1999). The CAMP factor of GBS binds glycophosphatidylinositol-anchored proteins (Lang *et al*., 2007) and subsequently functions as a pore-forming toxin, likely requiring self-oligomerization for activity (Lang and Palmer, 2003). Previously, GBS CAMP factor was called protein B, following reports that it could bind the Fc region of IgG and IgM from several mammalian species (Jurgens *et al.*, 1987). However, more recent studies could not detect a non-immune binding association between CAMP factor and human IgG, leading to the suggestion that the name protein B may be inappropriate (El-Huneidi *et al*., 2007). CAMP factor of GBS was determined to be non-pathogenic following administration to mice (Jurgens *et al*., 1987). Furthermore, CAMP factor was not essential for systemic virulence of GBS (Hensler *et al*., 2008), suggesting that CAMP factor (in the absence of *S. aureus* β-toxin) does not play a direct role in GBS pathogenesis. Recent investigations have uncovered a novel CAMP factor in GBS, designated CAMP factor II, that has been proposed to have spread to other streptococci (*Streptococcus uberis*, *S. dysgalactiae*, *S. dysgalactiae* ssp. *equisimilis* and *Streptococcus bovis*) via integrative and conjugative elements (Chuzeville *et al*., 2012).

Group B Streptococcus *toxin (CM101)*

Group B *Streptococcus* toxin, also designated CM101, is a polysaccharide exotoxin that binds to embryonic receptors expressed in the developing lung of the neonate, resulting in a strong inflammatory response (Wamil *et al*., 1997). GBS toxin also binds to tumour neovasculature in adults and for this reason has been evaluated as an anti-cancer therapeutic. In a phase I clinical trial administering GBS toxin as an anti-neovascularization agent in human cancer therapy (Harris, 1997), 5/15 patients exhibited tumour reduction or stabilization, and sera from all patients had elevated soluble E-selectin, indicative of tumour neovasculature endothelial engagement in an inflammatory process (Wamil *et al*., 1997). Historically, GBS toxin was purified from culture media of GBS isolates from neonates who had died because of GBS infection. GBS toxin has also been identified in plasma, urine and cerebrospinal fluid from infants with GBS disease and could be used as an additional tool to diagnose GBS infection in infants, which can sometimes prove difficult to diagnose (Sundell *et al*., 2000).

Adenosine diphosphate-ribosyltransferase (SpyA)

Adenosine diphosphate-ribosyltransferases covalently transfer ADP-ribose from NAD^+ to eukaryotic proteins. SpyA is a surface-exposed membrane protein of GAS that has been described as a C3-like ADP-ribosyltransferase (Korotkova *et al*., 2012). SpyA has been documented to modify cytoskeletal proteins including vimentin, tropomyosin and actin, and when expressed in HeLa cells

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following transfection, SpyA activity resulted in a loss of actin microfilaments (Coye and Collins, 2004) and inhibited vimentin polymerization, resulting in the collapse of the vimentin cytoskeleton (Icenogle *et al.*, 2012). In a mouse subcutaneous infection model, the Δ*spyA* mutant generated smaller lesions and had higher levels of mRNA encoding CXCL1 and CCL2 (both neutrophil and macrophage chemoattractants) and vimentin, compared with WT (Hoff *et al.*, 2011). Vimentin plays many roles in the cell including the organization of cellular architecture (Ivaska *et al.*, 2007), and the loss of vimentin functionality can impair wound healing (Eckes *et al.*, 2000). The findings of Hoff *et al* (2011) suggest that SpyA delays wound healing in the subcutaneous infection model. In a mouse intravenous infection model, the Δ*spyA* mutant caused higher mortality with impaired bacterial clearance, and *in vivo*, the mutant was resistant to killing by macrophages (Lin *et al.*, 2015). Lin and colleagues determined that SpyA triggers pyroptosis, a caspase-1-dependent inflammasome in macrophages, resulting in macrophage cell death and the release of proinflammatory cytokine IL-1β. This innate defence programme triggered in response to SpyA dramatically enhances clearance of GAS and restricts bacterial growth, attenuating disease progression.

NAD-glycohydrolase (Streptococcus pyogenes *NADase or NADase*)

NAD-glycohydrolase (also known as *S. pyogenes* NADase or Nga) is encoded by the *nga* gene, which is found in the same operon as *slo*. SLO and NADase act synergistically to trigger cytotoxicity. NADase is translocated through pores created by SLO and delivered into the host cell cytoplasmic compartment by a process termed cytolysin-mediated translocation (Madden *et al.*, 2001). NADase possesses both ADP-ribosyl cyclase activity and cADPR hydrolase activity (Karasawa *et al.*, 1995), and once inside the cell, NADase produces the potent second messenger cyclic ADP-ribose. Cytotoxicity mediated by the enzymatic action of NADase may be a consequence of the depletion of host cell energy stores (e.g. intracellular NAD^+) (Michos *et al.*, 2006). NADase has been shown to act within human pharyngeal keratinocytes, resulting in enhanced cell membrane injury, inhibition of bacterial internalization and induction of apoptosis (Bricker *et al.*, 2002). SLO stimulates xenophagy in these cells, and the co-expression of SLO and NADase results in prolonged intracellular survival of GAS and prevents maturation of GAS-containing autophagosomes (O'Seaghdha and Wessels, 2013). In addition, SLO and NADase mediate GAS intracellular survival and cytotoxicity within macrophages, subsequently enabling persistent infection by preventing phagolysosome acidification (Bastiat-Sempe *et al.*, 2014). Recent studies have identified NADase-inactive variants that have emerged with polymorphism at multiple residues (Chandrasekaran *et al.*, 2013). This analysis revealed an NADase-independent cytotoxic activity that was retained by the enzymatically inactive variants, suggesting another domain of NADase can mediate cytotoxicity. NADase also contributes to virulence in two mouse skin tissue infection models and a model of septicaemia, suggesting NADase plays an important role in establishing infection in the host (Bricker *et al.*, 2005; Tatsuno *et al.*, 2010).

Conclusion

While several toxins are found across streptococcal species boundaries, each of these species expresses a specific repertoire of toxic molecules that target specific aspects of host immunity and physiology. Indeed, many clones within individual species express distinct toxin profiles. These toxins play crucial roles within the host–pathogen interaction, allowing the pathogen to colonize, proliferate and disseminate. Several of these toxins have demonstrated utility as candidate vaccine antigens, in inactive forms, and in several instances, the specificity of toxin action has been experimentally utilized in the development of novel therapeutics, such as anti-cancer agents. Deepening of our knowledge of the mode of action of these toxin molecules will aid in our efforts to prevent disease caused in humans and animals by the various streptococcal pathogens.

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References

Abe, J., Kotzin, B.L., Jujo, K., Melish, M.E., Glode, M.P., Kohsaka, T., and Leung, D.Y.M. (1992) Selective expansion of T cells expressing T-cell receptor variable regions Vβ2 and Vβ8 in Kawasaki disease. *Proc Natl Acad Sci U S A* **89**: 4066–4070.

Alam, F.M., Bateman, C., Turner, C.E., Wiles, S., and Sriskandan, S. (2013) Non-invasive monitoring of *Streptococcus pyogenes* vaccine efficacy using biophotonic imaging. *PLoS One* **8**: e82123.

Alcantara, R.B., Preheim, L.C., and Gentry-Nielsen, M.J. (2001) Pneumolysin-induced complement depletion during experimental pneumococcal bacteremia. *Infect Immun* **69**: 3569–3575.

Alexander, J.E., Lock, R.A., Peeters, C.C.A.M., Poolman, J. T., Andrew, P.W., Mitchell, T.J., *et al.* (1994) Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of *Streptococcus pneumoniae*. *Infect Immun* **62**: 5683–5688.

Alhamdi, Y., Neill, D.R., Abrams, S.T., Malak, H.A., Yahya, R., Barrett-Jolley, R., *et al.* (2015) Circulating pneumolysin is a potent inducer of cardiac injury during pneumococcal infection. *PLoS Pathog* **11**: e1004836.

Allen, A.G., Bolitho, S., Lindsay, H., Khan, S., Bryant, C., Norton, P., *et al.* (2001) Generation and characterization of a defined mutant of *Streptococcus suis* lacking suilysin. *Infect Immun* **69**: 2732–2735.

Anderson, R., Steel, H.C., Cockeran, R., von Gottberg, A., de Gouveia, L., Klugman, K.P., *et al.* (2007) Comparison of the effects of macrolides, amoxicillin, ceftriaxone, doxycycline, tobramycin and fluoroquinolones, on the production of pneumolysin by *Streptococcus pneumoniae in vitro*. *J Antimicrob Chemother* **60**: 1155–1158.

Arad, G., Levy, R., Nasie, I., Hillman, D., Rotfogel, Z., Barash, U., *et al.* (2011) Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. *PLoS Biol* **9**: e1001149.

Asam, D., Mauerer, S., and Spellerberg, B. (2015) Streptolysin S of *Streptococcus anginosus* exhibits broad-range hemolytic activity. *Med Microbiol Immun* **204**: 227–237.

Ato, M., Ikebe, T., Kawabata, H., Takemori, T., and Watanabe, H. (2008) Incompetence of neutrophils to invasive group A *Streptococcus* is attributed to induction of plural virulence factors by dysfunction of a regulator. *PLoS One* **3**: e3455.

Ayers, S.H., and Rupp, P. (1922) Differentiation of hemolytic streptococci from human and bovine sources by the hydrolysis of sodium hippurate. *J Infect Dis* **30**: 388–399.

Aziz, R.K., Pabst, M.J., Jeng, A., Kansal, R., Low, D.E., Nizet, V., and Kotb, M. (2004) Invasive M1T1 group A *Streptococcus* undergoes a phase-shift *in vivo* to prevent proteolytic degradation of multiple virulence factors by SpeB. *Mol Microbiol* **51**: 123–134.

Barnett, T.C., Liebl, D., Seymour, L.M., Gillen, C.M., Lim, J.Y., Larock, C.N., *et al.* (2013) The globally disseminated M1T1 clone of group A *Streptococcus* evades autophagy for intracellular replication. *Cell Host Microbe* **14**: 675–682.

Baruch, M., Hertzog, B.B., Ravins, M., Anand, A., Cheng, C. Y., Biswas, D., *et al.* (2014) Induction of endoplasmic reticulum stress and unfolded protein response constitutes a pathogenic strategy of group A *Streptococcus*. *Front Cell Infect Microbiol* **4**: 105.

Bastiat-Sempe, B., Love, J.F., Lomayesva, N., and Wessels, M.R. (2014) Streptolysin O and NAD-glycohydrolase prevent phagolysosome acidification and promote group a *Streptococcus* survival in macrophages. *mBio* **5**: e01690–01614.

Bek-Thomsen, M., Poulsen, K., and Kilian, M. (2012) Occurrence and evolution of the paralogous zinc metalloproteases IgA1 protease, ZmpB, ZmpC, and ZmpD in *Streptococcus pneumoniae* and related commensal species. *mBio* **3**: e00303–e00312.

Bender, M.H., and Weiser, J.N. (2006) The atypical amino-terminal LPNTG-containing domain of the pneumococcal human IgA1-specific protease is required for proper enzyme localization and function. *Mol Microbiol* **61**: 526–543.

Bensi, G., Mora, M., Tuscano, G., Biagini, M., Chiarot, E., Bombaci, M., *et al.* (2012) Multi high-throughput approach for highly selective identification of vaccine candidates: the group A *Streptococcus* case. *Mol Cell Proteomics* **11**: M111.015693.

Benton, K.A., Everson, M.P., and Briles, D.E. (1995) A pneumolysin-negative mutant of *Streptococcus pneumoniae* causes chronic bacteremia rather than acute sepsis in mice. *Infect Immun* **63**: 448–455.

Beres, S.B., Sylva, G.L., Barbian, K.D., Lei, B., Hoff, J.S., Mammarella, N.D., *et al.* (2002) Genome sequence of a serotype M3 strain of group A *Streptococcus*: phage-encoded toxins, the high-virulence phenotype, and clone emergence. *Proc Natl Acad Sci U S A* **99**: 10078–10083.

Berge, A., and Bjorck, L. (1995) Streptococcal cysteine proteinase releases biologically active fragments of streptococcal surface proteins. *J Biol Chem* **270**: 9862–9867.

Bernheimer, A.W. (1966) Disruption of wall-less bacteria by streptococcal and staphylococcal toxins. *J Bacteriol* **91**: 1677–1680.

Bernheimer, A.W. (1967) Physical behavior of streptolysin S. *J Bacteriol* **93**: 2024–2025.

Berry, A.M., Lock, R.A., Hansman, D., and Paton, J.C. (1989a) Contribution of autolysin to virulence of *Streptococcus pneumoniae*. *Infect Immun* **57**: 2324–2330.

Berry, A.M., Yother, J., Briles, D.E., Hansman, D., and Paton, J.C. (1989b) Reduced virulence of a defined pneumolysin-negative mutant of *Streptococcus pneumoniae*. *Infect Immun* **57**: 2037–2042.

Berry, A.M., Paton, J.C., and Hansman, D. (1992) Effect of insertional inactivation of the genes encoding pneumolysin and autolysin on the virulence of *Streptococcus pneumoniae* type 3. *Microb Pathog* **12**: 87–93.

Betschel, S.D., Borgia, S.M., Barg, N.L., Low, D.E., and De Azavedo, J.C. (1998) Reduced virulence of group A streptococcal Tn916 mutants that do not produce streptolysin S. *Infect Immun* **66**: 1671–1679.

Bischof, L.J., Kao, C.Y., Los, F.C., Gonzalez, M.R., Shen, Z., Briggs, S.P., *et al.* (2008) Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin *in vivo*. *PLoS Pathog* **4**: e1000176.

Bjorck, L., Akesson, P., Bohus, M., Trojnar, J., Abrahamson, M., Olafsson, I., and Grubb, A. (1989) Bacterial growth blocked by a synthetic peptide based on the structure of a human proteinase inhibitor. *Nature* **337**: 385–386.

Blue, C.E., Paterson, G.K., Kerr, A.R., Berge, M., Claverys, J.P., and Mitchell, T.J. (2003) ZmpB, a novel virulence factor of *Streptococcus pneumoniae* that induces tumor necrosis factor alpha production in the respiratory tract. *Infect Immun* **71**: 4925–4935.

Bohach, G.A., Hauser, A.R., and Schlievert, P.M. (1988) Cloning of the gene, *speB*, for streptococcal pyrogenic exotoxin type B in *Escherichia coli*. *Infect Immun* **56**: 1665–1667.

Bonifait, L., and Grenier, D. (2011a) The SspA subtilisin-like protease of *Streptococcus suis* triggers a pro-inflammatory response in macrophages through a non-proteolytic mechanism. *BMC Microbiol* **11**: 47.

Bonifait, L., de la Cruz Dominguez-Punaro, M., Vaillancourt, K., Bart, C., Slater, J., Frenette, M., *et al.* (2010) The cell envelope subtilisin-like proteinase is a virulence determinant for *Streptococcus suis*. *BMC Microbiol* **10**: 42.

Bonifait, L., Vaillancourt, K., Gottschalk, M., Frenette, M., and Grenier, D. (2011b) Purification and characterization of the subtilisin-like protease of *Streptococcus suis* that contributes to its virulence. *Vet Microbiol* **148**: 333–340.

Bouyain, S., and Geisbrecht, B.V. (2012) Host glycan recognition by a pore forming toxin. *Structure* **20**: 197–198.

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Bricker, A.L., Cywes, C., Ashbaugh, C.D., and Wessels, M.R. (2002) NAD+-glycohydrolase acts as an intracellular toxin to enhance the extracellular survival of group A streptococci. *Mol Microbiol* **44**: 257–269.

Bricker, A.L., Carey, V.J., and Wessels, M.R. (2005) Role of NADase in virulence in experimental invasive group A streptococcal infection. *Infect Immun* **73**: 6562–6566.

Brosnahan, A.J., and Schlievert, P.M. (2011) Gram-positive bacterial superantigen outside–in signaling causes toxic shock syndrome. *FEBS J* **278**: 4649–4667.

Brosnahan, A.J., Mantz, M.J., Squier, C.A., Peterson, M.L., and Schlievert, P.M. (2009) Cytolysins augment superantigen penetration of stratified mucosa. *J Immunol* **182**: 2364–2373.

Broudy, T.B., and Fischetti, V.A. (2003) *In vivo* lysogenic conversion of Tox(−) *Streptococcus pyogenes* to Tox(+) with lysogenic streptococci or free phage. *Infect Immun* **71**: 3782–3786.

Carr, A., Sledjeski, D.D., Podbielski, A., Boyle, M.D., and Kreikemeyer, B. (2001) Similarities between complement-mediated and streptolysin S-mediated hemolysis. *J Biol Chem* **276**: 41790–41796.

Carroll, R.K., and Musser, J.M. (2011) From transcription to activation: how group A streptococcus, the flesh-eating pathogen, regulates SpeB cysteine protease production. *Mol Microbiol* **81**: 588–601.

Cassidy, S.K., and O'Riordan, M.X. (2013) More than a pore: the cellular response to cholesterol-dependent cytolysins. *Toxins* **5**: 618–636.

Chabot-Roy, G., Willson, P., Segura, M., Lacouture, S., and Gottschalk, M. (2006) Phagocytosis and killing of *Streptococcus suis* by porcine neutrophils. *Microb Pathog* **41**: 21–32.

Chandrasekaran, S., Ghosh, J., Port, G.C., Koh, E.I., and Caparon, M.G. (2013) Analysis of polymorphic residues reveals distinct enzymatic and cytotoxic activities of the *Streptococcus pyogenes* NAD+ glycohydrolase. *J Biol Chem* **288**: 20064–20075.

Charland, N., Nizet, V., Rubens, C.E., Kim, K.S., Lacouture, S., and Gottschalk, M. (2000) *Streptococcus suis* serotype 2 interactions with human brain microvascular endothelial cells. *Infect Immun* **68**: 637–643.

Chatellier, S., Ihendyane, N., Kansal, R.G., Khambaty, F., Basma, H., Norrby-Teglund, A., *et al.* (2000) Genetic relatedness and superantigen expression in group A *Streptococcus* serotype M1 isolates from patients with severe and nonsevere invasive diseases. *Infect Immun* **68**: 3523–3534.

Chella Krishnan, K., Mukundan, S., Landero Figueroa, J.A., Caruso, J.A., and Kotb, M. (2014) Metal-mediated modulation of streptococcal cysteine protease activity and its biological implications. *Infect Immun* **82**: 2992–3001.

Chen, C.C., and Cleary, P.P. (1990) Complete nucleotide sequence of the streptococcal C5a peptidase gene of *Streptococcus pyogenes*. *J Biol Chem* **265**: 3161–3167.

Chen, Y., Hayashida, A., Bennett, A.E., Hollingshead, S.K., and Park, P.W. (2007) *Streptococcus pneumoniae* sheds syndecan-1 ectodomains through ZmpC, a metalloproteinase virulence factor. *J Biol Chem* **282**: 159–167.

Cheng, Q., Debol, S., Lam, H., Eby, R., Edwards, L., Matsuka, Y., *et al.* (2002a) Immunization with C5a peptidase or peptidase-type III polysaccharide conjugate vaccines enhances clearance of group B streptococci from lungs of infected mice. *Infect Immun* **70**: 6409–6415.

Cheng, Q., Stafslien, D., Purushothaman, S.S., and Cleary, P. (2002b) The group B streptococcal C5a peptidase is both a specific protease and an invasin. *Infect Immun* **70**: 2408–2413.

Chiang-Ni, C., Wang, C.H., Tsai, P.J., Chuang, W.J., Lin, Y.S., Lin, M.T., *et al.* (2006) Streptococcal pyrogenic exotoxin B causes mitochondria damage to polymorphonuclear cells preventing phagocytosis of group A *Streptococcus*. *Med Microbiol Immun* **195**: 55–63.

Chiappini, N., Seubert, A., Telford, J.L., Grandi, G., Serruto, D., Margarit, I., and Janulczyk, R. (2012) *Streptococcus pyogenes* SpyCEP influences host–pathogen interactions during infection in a murine air pouch model. *PLoS One* **7**: e40411.

Chiarot, E., Faralla, C., Chiappini, N., Tuscano, G., Falugi, F., Gambellini, G., *et al.* (2013) Targeted amino acid substitutions impair streptolysin O toxicity and group A *Streptococcus* virulence. *mBio* **4**: e00387–00312.

Chiavolini, D., Memmi, G., Maggi, T., Iannelli, F., Pozzi, G., and Oggioni, M.R. (2003) The three extra-cellular zinc metalloproteinases of *Streptococcus pneumoniae* have a different impact on virulence in mice. *BMC Microbiol* **3**: 14.

Christie, R., Atkins, N.E., and Munch-Petersen, E. (1944) A note on a lytic phenomenon shown by group B streptococci. *Aust J Exp Biol* **22**: 197–200.

Chuzeville, S., Puymege, A., Madec, J.Y., Haenni, M., and Payot, S. (2012) Characterization of a new CAMP factor carried by an integrative and conjugative element in *Streptococcus agalactiae* and spreading in streptococci. *PLoS One* **7**: e48918.

Cleary, P.P., Handley, J., Suvorov, A.N., Podbielski, A., and Ferrieri, P. (1992) Similarity between the group B and A streptococcal C5a peptidase genes. *Infect Immun* **60**: 4239–4244.

Cole, J.N., McArthur, J.D., McKay, F.C., Sanderson-Smith, M.L., Cork, A.J., Ranson, M., *et al.* (2006) Trigger for group A streptococcal M1T1 invasive disease. *FASEB J* **20**: 1745–1747.

Cole, J.N., Barnett, T.C., Nizet, V., and Walker, M.J. (2011) Molecular insight into invasive group A streptococcal disease. *Nature Rev Microbiol* **9**: 724–736.

Collin, M., and Olsen, A. (2003) Extracellular enzymes with immunomodulating activities: variations on a theme in *Streptococcus pyogenes*. *Infect Immun* **71**: 2983–2992.

Commons, R.J., Smeesters, P.R., Proft, T., Fraser, J.D., Robins-Browne, R., and Curtis, N. (2014) Streptococcal superantigens: categorization and clinical associations. *Trends Mol Med* **20**: 48–62.

Cortes, G., and Wessels, M.R. (2009) Inhibition of dendritic cell maturation by group A *Streptococcus*. *J Infect Dis* **200**: 1152–1161.

Cotter, P.D., Hill, C., and Ross, R.P. (2005) Bacteriocins: developing innate immunity for food. *Nature Rev Microbiol* **3**: 777–788.

Coye, L.H., and Collins, C.M. (2004) Identification of SpyA, a novel ADP-ribosyltransferase of *Streptococcus pyogenes*. *Mol Microbiol* **54**: 89–98.

Curtis, N., Zheng, R., Lamb, J.R., and Levin, M. (1995) Evidence for a superantigen mediated process in Kawasaki disease. *Arch Dis Child* **72**: 308–311.

Cywes Bentley, C., Hakansson, A., Christianson, J., and Wessels, M.R. (2005) Extracellular group A *Streptococcus*

induces keratinocyte apoptosis by dysregulating calcium signalling. *Cell Microbiol* **7**: 945–955.

Dal, M.-C., and Monteil, H. (1983) Hemolysin produced by group B *Streptococcus agalactiae*. *FEMS Microbiol Lett* **16**: 89–94.

Davies, M.R., Holden, M.T., Coupland, P., Chen, J.H., Venturini, C., Barnett, T.C., *et al.* (2015) Emergence of scarlet fever *Streptococcus pyogenes emm12* clones in Hong Kong is associated with toxin acquisition and multidrug resistance. *Nat Genet* **47**: 84–87.

Davis, M.M., and Bjorkman, P.J. (1988) T-cell antigen receptor genes and T-cell recognition. *Nature* **334**: 395–402.

Doran, K.S., Liu, G.Y., and Nizet, V. (2003) Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. *J Clin Invest* **112**: 736–744.

Dramsi, S., and Cossart, P. (2002) Listeriolysin O: a genuine cytolysin optimized for an intracellular parasite. *J Cell Biol* **156**: 943–946.

Eckes, B., Colucci-Guyon, E., Smola, H., Nodder, S., Babinet, C., Krieg, T., and Martin, P. (2000) Impaired wound healing in embryonic and adult mice lacking vimentin. *J Cell Sci* **113**(Pt 13): 2455–2462.

Edwards, R.J., Taylor, G.W., Ferguson, M., Murray, S., Rendell, N., Wrigley, A., *et al.* (2005) Specific C-terminal cleavage and inactivation of interleukin-8 by invasive disease isolates of *Streptococcus pyogenes*. *J Infect Dis* **192**: 783–790.

El-Huneidi, W., Mui, R., Zhang, T.H., and Palmer, M. (2007) *Streptococcus agalactiae* CAMP factor/protein B does not bind to human IgG. *Med Microbiol Immun* **196**: 73–77.

Eriksson, B.K., Andersson, J., Holm, S.E., and Norgren, M. (1999) Invasive group A streptococcal infections: T1M1 isolates expressing pyrogenic exotoxins A and B in combination with selective lack of toxin-neutralizing antibodies are associated with increased risk of streptococcal toxic shock syndrome. *J Infect Dis* **180**: 410–418.

Farley, M.M. (2001) Group B streptococcal disease in nonpregnant adults. *Clin Infect Dis* **33**: 556–561.

Farrand, S., Hotze, E., Friese, P., Hollingshead, S.K., Smith, D.F., Cummings, R.D., *et al.* (2008) Characterization of a streptococcal cholesterol-dependent cytolysin with a lewis y and b specific lectin domain. *Biochemistry* **47**: 7097–7107.

Fasching, C.E., Grossman, T., Corthesy, B., Plaut, A.G., Weiser, J.N., and Janoff, E.N. (2007) Impact of the molecular form of immunoglobulin A on functional activity in defense against *Streptococcus pneumoniae*. *Infect Immun* **75**: 1801–1810.

Fast, D.J., Schlievert, P.M., and Nelson, R.D. (1989) Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. *Infect Immun* **57**: 291–294.

Fittipaldi, N., Segura, M., Grenier, D., and Gottschalk, M. (2012) Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. *Future Microbiol* **7**: 259–279.

Flanagan, J., Collin, N., Timoney, J., Mitchell, T., Mumford, J. A., and Chanter, N. (1998) Characterization of the haemolytic activity of *Streptococcus equi*. *Microb Pathog* **24**: 211–221.

Fraser, J.D., and Proft, T. (2008) The bacterial superantigen and superantigen-like proteins. *Immunol Rev* **225**: 226–243.

Fuller, J.D., Camus, A.C., Duncan, C.L., Nizet, V., Bast, D.J., Thune, R.L., *et al.* (2002) Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of *Streptococcus iniae* disease. *Infect Immun* **70**: 5730–5739.

Gase, K., Ferretti, J.J., Primeaux, C., and McShan, W.M. (1999) Identification, cloning, and expression of the CAMP factor gene (*cfa*) of group A streptococci. *Infect Immun* **67**: 4725–4731.

Gilbert, J.V., Plaut, A.G., Fishman, Y., and Wright, A. (1988) Cloning of the gene encoding streptococcal immunoglobulin A protease and its expression in *Escherichia coli*. *Infect Immun* **56**: 1961–1966.

Ginsburg, I. (1999) Is streptolysin S of group A streptococci a virulence factor? *APMIS* **107**: 1051–1059.

Gonzalez, M.R., Bischofberger, M., Pernot, L., van der Goot, F.G., and Freche, B. (2008) Bacterial pore-forming toxins: the (w)hole story? *Cell Mol Life Sci* **65**: 493–507.

Gonzalez, M.R., Bischofberger, M., Freche, B., Ho, S., Parton, R.G., and van der Goot, F.G. (2011) Pore-forming toxins induce multiple cellular responses promoting survival. *Cell Microbiol* **13**: 1026–1043.

Gottschalk, M.G., Lacouture, S., and Dubreuil, J.D. (1995) Characterization of *Streptococcus suis* capsular type 2 haemolysin. *Microbiology* **141**: 189–195.

Govindarajan, B., Menon, B.B., Spurr-Michaud, S., Rastogi, K., Gilmore, M.S., Argueso, P., and Gipson, I.K. (2012) A metalloproteinase secreted by *Streptococcus pneumoniae* removes membrane mucin MUC16 from the epithelial glycocalyx barrier. *PLoS One* **7**: e32418.

Greene, N.G., Narciso, A.R., Filipe, S.R., and Camilli, A. (2015) Peptidoglycan branched stem peptides contribute to *Streptococcus pneumoniae* virulence by inhibiting pneumolysin release. *PLoS Pathog* **11**: e1004996.

Harris, A.L. (1997) Clinical trials of anti-vascular agent group B *Streptococcus* toxin (CM101). *Angiogenesis* **1**: 36–37.

Hartwig, U.F., Gerlach, D., and Fleischer, B. (1994) Major histocompatibility complex class II binding site for streptococcal pyrogenic (erythrogenic) toxin A. *Med Microbiol Immun* **183**: 257–264.

Hasan, I., Watanabe, M., Ishizaki, N., Sugita-Konishi, Y., Kawakami, Y., Suzuki, J., *et al.* (2014) A galactose-binding lectin isolated from *Aplysia kurodai* (sea hare) eggs inhibits streptolysin-induced hemolysis. *Molecules* **19**: 13990–14003.

Hashikawa, S., Iinuma, Y., Furushita, M., Ohkura, T., Nada, T., Torii, K., *et al.* (2004) Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* **42**: 186–192.

Hensler, M.E., Liu, G.Y., Sobczak, S., Benirschke, K., Nizet, V., and Heldt, G.P. (2005) Virulence role of group B *Streptococcus* beta-hemolysin/cytolysin in a neonatal rabbit model of early-onset pulmonary infection. *J Infect Dis* **191**: 1287–1291.

Hensler, M.E., Quach, D., Hsieh, C.J., Doran, K.S., and Nizet, V. (2008) CAMP factor is not essential for systemic virulence of group B *Streptococcus*. *Microb Pathog* **44**: 84–88.

Heuck, A.P., Moe, P.C., and Johnson, B.B. (2010) The cholesterol-dependent cytolysin family of Gram-positive bacterial toxins. *Subcell Biochem* **51**: 551–577.

Hidalgo-Grass, C., Dan-Goor, M., Maly, A., Eran, Y., Kwinn, L.A., Nizet, V., *et al.* (2004) Effect of a bacterial pheromone peptide on host chemokine degradation in group A streptococcal necrotising soft-tissue infections. *Lancet* **363**: 696–703.

Hidalgo-Grass, C., Mishalian, I., Dan-Goor, M., Belotserkovsky, I., Eran, Y., Nizet, V., *et al.* (2006) A streptococcal protease that degrades CXC chemokines and impairs bacterial clearance from infected tissues. *EMBO J* **25**: 4628–4637.

Hoff, J.S., DeWald, M., Moseley, S.L., Collins, C.M., and Voyich, J.M. (2011) SpyA, a C3-like ADP-ribosyltransferase, contributes to virulence in a mouse subcutaneous model of *Streptococcus pyogenes* infection. *Infect Immun* **79**: 2404–2411.

Hollands, A., Aziz, R.K., Kansal, R., Kotb, M., Nizet, V., and Walker, M.J. (2008) A naturally occurring mutation in *ropB* suppresses SpeB expression and reduces M1T1 group A streptococcal systemic virulence. *PLoS One* **3**: e4102.

Honda-Ogawa, M., Ogawa, T., Terao, Y., Sumitomo, T., Nakata, M., Ikebe, K., *et al.* (2013) Cysteine proteinase from *Streptococcus pyogenes* enables evasion of innate immunity via degradation of complement factors. *J Biol Chem* **288**: 15854–15864.

Hu, Q., Liu, P., Yu, Z., Zhao, G., Li, J., Teng, L., *et al.* (2010) Identification of a cell wall-associated subtilisin-like serine protease involved in the pathogenesis of *Streptococcus suis* serotype 2. *Microb Pathog* **48**: 103–109.

Hulting, G., Flock, M., Frykberg, L., Lannergard, J., Flock, J.I., and Guss, B. (2009) Two novel IgG endopeptidases of *Streptococcus equi*. *FEMS Microbiol Lett* **298**: 44–50.

Humar, D., Datta, V., Bast, D.J., Beall, B., De Azavedo, J.C., and Nizet, V. (2002) Streptolysin S and necrotising infections produced by group G *Streptococcus*. *Lancet* **359**: 124–129.

Icenogle, L.M., Hengel, S.M., Coye, L.H., Streifel, A., Collins, C.M., Goodlett, D.R., and Moseley, S.L. (2012) Molecular and biological characterization of streptococcal SpyA-mediated ADP-ribosylation of intermediate filament protein vimentin. *J Biol Chem* **287**: 21481–21491.

Igwe, E.I., Shewmaker, P.L., Facklam, R.R., Farley, M.M., van Beneden, C., and Beall, B. (2003) Identification of superantigen genes *speM*, *ssa*, and *smeZ* in invasive strains of beta-hemolytic group C and G streptococci recovered from humans. *FEMS Microbiol Lett* **229**: 259–264.

Ikebe, T., Wada, A., Inagaki, Y., Sugama, K., Suzuki, R., Tanaka, D., *et al.* (2002) Dissemination of the phage-associated novel superantigen gene *speL* in recent invasive and noninvasive *Streptococcus pyogenes* M3/T3 isolates in Japan. *Infect Immun* **70**: 3227–3233.

Ikebe, T., Ato, M., Matsumura, T., Hasegawa, H., Sata, T., Kobayashi, K., and Watanabe, H. (2010) Highly frequent mutations in negative regulators of multiple virulence genes in group A streptococcal toxic shock syndrome isolates. *PLoS Pathog* **6**: e1000832.

Ivaska, J., Pallari, H.M., Nevo, J., and Eriksson, J.E. (2007) Novel functions of vimentin in cell adhesion, migration, and signaling. *Exp Cell Res* **313**: 2050–2062.

Jacobs, J.A., Pietersen, H.G., Stobberingh, E.E., and Soeters, P.B. (1995) *Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*. Clinical relevance, hemolytic and serologic characteristics. *Am J Clin Path* **104**: 547–553.

Janoff, E.N., Rubins, J.B., Fasching, C., Charboneau, D., Rahkola, J.T., Plaut, A.G., and Weiser, J.N. (2014) Pneumococcal IgA1 protease subverts specific protection by human IgA1. *Mucosal Immunol* **7**: 249–256.

Ji, Y., McLandsborough, L., Kondagunta, A., and Cleary, P.P. (1996) C5a peptidase alters clearance and trafficking of group A streptococci by infected mice. *Infect Immun* **64**: 503–510.

Johansson, L., Thulin, P., Sendi, P., Hertzen, E., Linder, A., Akesson, P., *et al.* (2008) Cathelicidin LL-37 in severe *Streptococcus pyogenes* soft tissue infections in humans. *Infect Immun* **76**: 3399–3404.

Johnson, S., Brooks, N.J., Smith, R.A., Lea, S.M., and Bubeck, D. (2013) Structural basis for recognition of the pore-forming toxin intermedilysin by human complement receptor CD59. *Cell Rep* **3**: 1369–1377.

Jupin, C., Anderson, S., Damais, C., Alouf, J.E., and Parant, M. (1988) Toxic shock syndrome toxin 1 as an inducer of human tumor necrosis factors and γ interferon. *J Exp Med* **167**: 752–761.

Jurgens, D., Sterzik, B., and Fehrenbach, F.J. (1987) Unspecific binding of group B streptococcal cocytolysin (CAMP factor) to immunoglobulins and its possible role in pathogenicity. *J Exp Med* **165**: 720–732.

Kadioglu, A., Gingles, N.A., Grattan, K., Kerr, A., Mitchell, T. J., and Andrew, P.W. (2000) Host cellular immune response to pneumococcal lung infection in mice. *Infect Immun* **68**: 492–501.

Kamezawa, Y., Nakahara, T., Nakano, S., Abe, Y., Nozaki-Renard, J., and Isono, T. (1997) Streptococcal mitogenic exotoxin Z, a novel acidic superantigenic toxin produced by a T1 strain of *Streptococcus pyogenes*. *Infect Immun* **65**: 3828–3833.

Kamtchoua, T., Bologa, M., Hopfer, R., Neveu, D., Hu, B., Sheng, X., *et al.* (2013) Safety and immunogenicity of the pneumococcal pneumolysin derivative PlyD1 in a single-antigen protein vaccine candidate in adults. *Vaccine* **31**: 327–333.

Kansal, R.G., McGeer, A., Low, D.E., Norrby-Teglund, A., and Kotb, M. (2000) Inverse relation between disease severity and expression of the streptococcal cysteine protease, SpeB, among clonal M1T1 isolates recovered from invasive group A streptococcal infection cases. *Infect Immun* **68**: 6362–6369.

Kapur, V., Maffei, J.T., Greer, R.S., Li, L.L., Adams, G.J., and Musser, J.M. (1994) Vaccination with streptococcal extracellular cysteine protease (interleukin-1 beta convertase) protects mice against challenge with heterologous group A streptococci. *Microb Pathog* **16**: 443–450.

Karasawa, T., Takasawa, S., Yamakawa, K., Yonekura, H., Okamoto, H., and Nakamura, S. (1995) NAD(+)-glycohydrolase from *Streptococcus pyogenes* shows cyclic ADP-ribose forming activity. *FEMS Microbiol Lett* **130**: 201–204.

Kasper, K.J., Zeppa, J.J., Wakabayashi, A.T., Xu, S.X., Mazzuca, D.M., Welch, I., *et al.* (2014) Bacterial superantigens promote acute nasopharyngeal infection by *Streptococcus pyogenes* in a human MHC class II-dependent manner. *PLoS Pathog* **10**: e1004155.

Kaur, S.J., Nerlich, A., Bergmann, S., Rohde, M., Fulde, M., Zahner, D., *et al.* (2010) The CXC chemokine-degrading

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protease SpyCEP of *Streptococcus pyogenes* promotes its uptake into endothelial cells. *J Biol Chem* **285**: 27798–27805.

Kirkham, L.A., Kerr, A.R., Douce, G.R., Paterson, G.K., Dilts, D.A., Liu, D.F., and Mitchell, T.J. (2006) Construction and immunological characterization of a novel nontoxic protective pneumolysin mutant for use in future pneumococcal vaccines. *Infect Immun* **74**: 586–593.

Koh, T.H., Sng, L.H., Yuen, S.M., Thomas, C.K., Tan, P.L., Tan, S.H., and Wong, N.S. (2009) Streptococcal cellulitis following preparation of fresh raw seafood. *Zoonoses Public Health* **56**: 206–208.

Koppe, U., Hogner, K., Doehn, J.M., Muller, H.C., Witzenrath, M., Gutbier, B., *et al.* (2012) *Streptococcus pneumoniae* stimulates a STING- and IFN regulatory factor 3-dependent type I IFN production in macrophages, which regulates RANTES production in macrophages, cocultured alveolar epithelial cells, and mouse lungs. *J Immunol* **188**: 811–817.

Korotkova, N., Hoff, J.S., Becker, D.M., Quinn, J.K., Icenogle, L.M., and Moseley, S.L. (2012) SpyA is a membrane-bound ADP-ribosyltransferase of *Streptococcus pyogenes* which modifies a streptococcal peptide, SpyB. *Mol Microbiol* **83**: 936–952.

Lalonde, M., Segura, M., Lacouture, S., and Gottschalk, M. (2000) Interactions between *Streptococcus suis* serotype 2 and different epithelial cell lines. *Microbiology* **146**: 1913–1921.

Lang, S., and Palmer, M. (2003) Characterization of *Streptococcus agalactiae* CAMP factor as a pore-forming toxin. *J Biol Chem* **278**: 38167–38173.

Lang, S., Xue, J., Guo, Z., and Palmer, M. (2007) *Streptococcus agalactiae* CAMP factor binds to GPI-anchored proteins. *Med Microbiol Immun* **196**: 1–10.

Lannergard, J., and Guss, B. (2006) IdeE, an IgG-endopeptidase of *Streptococcus equi* ssp. *equi*. *FEMS Microbiol Lett* **262**: 230–235.

Lappin, E., and Ferguson, A.J. (2009) Gram-positive toxic shock syndromes. *Lancet Infect Dis* **9**: 281–290.

Lei, B., DeLeo, F.R., Hoe, N.P., Graham, M.R., Mackie, S.M., Cole, R.L., *et al.* (2001) Evasion of human innate and acquired immunity by a bacterial homolog of CD11b that inhibits opsonophagocytosis. *Nat Med* **7**: 1298–1305.

Leung, D.Y.M., Walsh, P., Giorno, R., and Norris, D.A. (1993) A potential role for superantigens in the pathogenesis of psoriasis. *J Invest Dermatol* **100**: 225–228.

Lewis, H.M., Baker, B.S., Bokth, S., Powles, A.V., Garioch, J. J., Valdimarsson, H., and Fry, L. (1993) Restricted T-cell receptor Vβ gene usage in the skin of patients with guttate and chronic plaque psoriasis. *Br J Dermatol* **129**: 514–520.

Li, P.L., Tiedemann, R.E., Moffat, S.L., and Fraser, J.D. (1997) The superantigen streptococcal pyrogenic exotoxin C (SPE-C) exhibits a novel mode of action. *J Exp Med* **186**: 375–383.

Limbago, B., Penumalli, V., Weinrick, B., and Scott, J.R. (2000) Role of streptolysin O in a mouse model of invasive group A streptococcal disease. *Infect Immun* **68**: 6384–6390.

Lin, A., Loughman, J.A., Zinselmeyer, B.H., Miller, M.J., and Caparon, M.G. (2009) Streptolysin S inhibits neutrophil recruitment during the early stages of *Streptococcus pyogenes* infection. *Infect Immun* **77**: 5190–5201.

Lin, A.E., Beasley, F.C., Keller, N., Hollands, A., Urbano, R., Troemel, E.R., *et al.* (2015) A group A *Streptococcus* ADP-ribosyltransferase toxin stimulates a protective interleukin 1beta-dependent macrophage immune response. *mBio* **6**: e00133.

Liu, G.Y., Doran, K.S., Lawrence, T., Turkson, N., Puliti, M., Tissi, L., and Nizet, V. (2004) Sword and shield: linked group B streptococcal beta-hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. *Proc Natl Acad Sci U S A* **101**: 14491–14496.

Locke, J.B., Colvin, K.M., Varki, N., Vicknair, M.R., Nizet, V., and Buchanan, J.T. (2007) *Streptococcus iniae* beta-hemolysin streptolysin S is a virulence factor in fish infection. *Dis Aquat Organ* **76**: 17–26.

Logsdon, L.K., Hakansson, A.P., Cortes, G., and Wessels, M.R. (2011) Streptolysin O inhibits clathrin-dependent internalization of group A *Streptococcus*. *mBio* **2**: e00332–00310.

Love, J.F., Tran-Winkler, H.J., and Wessels, M.R. (2012) Vitamin D and the human antimicrobial peptide LL-37 enhance group A *Streptococcus* resistance to killing by human cells. *mBio* **3**: e00394-12.

Lucas, R., Czikora, I., Sridhar, S., Zemskov, E., Gorshkov, B., Siddaramappa, U., *et al.* (2013) Mini-review: novel therapeutic strategies to blunt actions of pneumolysin in the lungs. *Toxins* **5**: 1244–1260.

Lukomski, S., Sreevatsan, S., Amberg, C., Reichardt, W., Woischnik, M., Podbielski, A., and Musser, J.M. (1997) Inactivation of *Streptococcus pyogenes* extracellular cysteine protease significantly decreases mouse lethality of serotype M3 and M49 strains. *J Clin Invest* **99**: 2574–2580.

Lukomski, S., Montgomery, C.A., Rurangirwa, J., Geske, R. S., Barrish, J.P., Adams, G.J., and Musser, J.M. (1999) Extracellular cysteine protease produced by *Streptococcus pyogenes* participates in the pathogenesis of invasive skin infection and dissemination in mice. *Infect Immun* **67**: 1779–1788.

Lun, S., Perez-Casal, J., Connor, W., and Willson, P.J. (2003) Role of suilysin in pathogenesis of *Streptococcus suis* capsular serotype 2. *Microb Pathog* **34**: 27–37.

Madden, J.C., Ruiz, N., and Caparon, M. (2001) Cytolysin-mediated translocation (CMT): a functional equivalent of type III secretion in Gram-positive bacteria. *Cell* **104**: 143–152.

Malley, R., Henneke, P., Morse, S.C., Cieslewicz, M.J., Lipsitch, M., Thompson, C.M., *et al.* (2003) Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infection. *Proc Natl Acad Sci U S A* **100**: 1966–1971.

McCormick, J.K., Pragman, A.A., Stolpa, J.C., Leung, D.Y., and Schlievert, P.M. (2001) Functional characterization of streptococcal pyrogenic exotoxin J, a novel superantigen. *Infect Immun* **69**: 1381–1388.

McNeela, E.A., Burke, A., Neill, D.R., Baxter, C., Fernandes, V.E., Ferreira, D., *et al.* (2010) Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. *PLoS Pathog* **6**: e1001191.

Melby, J.O., Nard, N.J., and Mitchell, D.A. (2011) Thiazole/oxazole-modified microcins: complex natural products from ribosomal templates. *Curr Opin Chem Biol* **15**: 369–378.

Menon, B.B., and Govindarajan, B. (2013) Identification of an atypical zinc metalloproteinase, ZmpC, from an epidemic

conjunctivitis-causing strain of *Streptococcus pneumoniae*. *Microb Pathog* **56**: 40–46.

Michos, A., Gryllos, I., Hakansson, A., Srivastava, A., Kokkotou, E., and Wessels, M.R. (2006) Enhancement of streptolysin O activity and intrinsic cytotoxic effects of the group A streptococcal toxin, NAD-glycohydrolase. *J Biol Chem* **281**: 8216–8223.

Mitchell, T.J., and Dalziel, C.E. (2014) The biology of pneumolysin. *Subcell Biochem* **80**: 145–160.

Mitchell, T.J., Andrew, P.W., Saunders, F.K., Smith, A.N., and Boulnois, G.J. (1991) Complement activation and antibody binding by pneumolysin via a region of the toxin homologous to a human acute-phase protein. *Mol Microbiol* **5**: 1883–1888.

Mitchell, D.A., Lee, S.W., Pence, M.A., Markley, A.L., Limm, J.D., Nizet, V., and Dixon, J.E. (2009) Structural and functional dissection of the heterocyclic peptide cytotoxin streptolysin S. *J Biol Chem* **284**: 13004–13012.

Miyoshi-Akiyama, T., Zhao, J., Kato, H., Kikuchi, K., Totsuka, K., Kataoka, Y., *et al.* (2003) *Streptococcus dysgalactiae*-derived mitogen (SDM), a novel bacterial superantigen: characterization of its biological activity and predicted tertiary structure. *Mol Microbiol* **47**: 1589–1599.

Miyoshi-Akiyama, T., Takamatsu, D., Koyanagi, M., Zhao, J., Imanishi, K., and Uchiyama, T. (2005) Cytocidal effect of *Streptococcus pyogenes* on mouse neutrophils *in vivo* and the critical role of streptolysin S. *J Infect Dis* **192**: 107–116.

Mollick, J.A., Miller, G.G., Musser, J.M., Cook, R.G., Grossman, D., and Rich, R.R. (1993) A novel superantigen isolated from pathogenic strains of *Streptococcus pyogenes* with aminoterminal homology to staphylococcal enterotoxins B and C. *J Clin Invest* **92**: 710–719.

Molloy, E.M., Cotter, P.D., Hill, C., Mitchell, D.A., and Ross, R.P. (2011) Streptolysin S-like virulence factors: the continuing *sagA*. *Nature Rev Microbiol* **9**: 670–681.

Mozola, C.C., Magassa, N., and Caparon, M.G. (2014) A novel cholesterol-insensitive mode of membrane binding promotes cytolysin-mediated translocation by streptolysin O. *Mol Microbiol* **94**: 675–687.

Müller-Alouf, H., Alouf, J.E., Gerlach, D., Ozegowski, J.H., Fitting, C., and Cavaillon, J.M. (1996) Human pro- and anti-inflammatory cytokine patterns induced by *Streptococcus pyogenes* erythrogenic (pyrogenic) exotoxin A and C superantigens. *Infect Immun* **64**: 1450–1453.

Musser, J.M., Hauser, A.R., Kim, M.H., Schlievert, P.M., Nelson, K., and Selander, R.K. (1991) *Streptococcus pyogenes* causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. *Proc Natl Acad Sci U S A* **88**: 2668–2672.

Nagamune, H., Ohnishi, C., Katsuura, A., Fushitani, K., Whiley, R.A., Tsuji, A., and Matsuda, Y. (1996) Intermedilysin, a novel cytotoxin specific for human cells secreted by *Streptococcus intermedius* UNS46 isolated from a human liver abscess. *Infect Immun* **64**: 3093–3100.

Nagamune, H., Whiley, R.A., Goto, T., Inai, Y., Maeda, T., Hardie, J.M., and Kourai, H. (2000) Distribution of the intermedilysin gene among the anginosus group streptococci and correlation between intermedilysin production and deep-seated infection with *Streptococcus intermedius*. *J Clin Microbiol* **38**: 220–226.

Nelson, D.C., Garbe, J., and Collin, M. (2011) Cysteine proteinase SpeB from *Streptococcus pyogenes* – a potent modifier of immunologically important host and bacterial proteins. *Biol Chem* **392**: 1077–1088.

Nizet, V. (2002) Streptococcal beta-hemolysins: genetics and role in disease pathogenesis. *Trends Microbiol* **10**: 575–580.

Nizet, V., Gibson, R.L., Chi, E.Y., Framson, P.E., Hulse, M., and Rubens, C.E. (1996) Group B streptococcal beta-hemolysin expression is associated with injury of lung epithelial cells. *Infect Immun* **64**: 3818–3826.

Nizet, V., Beall, B., Bast, D.J., Datta, V., Kilburn, L., Low, D. E., and De Azavedo, J.C. (2000) Genetic locus for streptolysin S production by group A *Streptococcus*. *Infect Immun* **68**: 4245–4254.

Ofek, I., Bergner-Rabinowitz, S., and Ginsburg, I. (1970) Oxygen-stable hemolysins of group A streptococci. VII. The relation of the leukotoxic factor to streptolysin S. *J Infect Dis* **122**: 517–522.

Oggioni, M.R., Memmi, G., Maggi, T., Chiavolini, D., Iannelli, F., and Pozzi, G. (2003) Pneumococcal zinc metalloproteinase ZmpC cleaves human matrix metalloproteinase 9 and is a virulence factor in experimental pneumonia. *Mol Microbiol* **49**: 795–805.

Okumura, K., Shimomura, Y., Murayama, S.Y., Yagi, J., Ubukata, K., Kirikae, T., and Miyoshi-Akiyama, T. (2012) Evolutionary paths of streptococcal and staphylococcal superantigens. *BMC Genomics* **13**: 404.

Okumura, C.Y., Anderson, E.L., Dohrmann, S., Tran, D.N., Olson, J., von Pawel-Rammingen, U., and Nizet, V. (2013) IgG protease Mac/IdeS is not essential for phagocyte resistance or mouse virulence of M1T1 group A *Streptococcus*. *mBio* **4**: e00499–00413.

Olsen, R.J., Sitkiewicz, I., Ayeras, A.A., Gonulal, V.E., Cantu, C., Beres, S.B., *et al.* (2010) Decreased necrotizing fasciitis capacity caused by a single nucleotide mutation that alters a multiple gene virulence axis. *Proc Natl Acad Sci U S A* **107**: 888–893.

O'Seaghdha, M., and Wessels, M.R. (2013) Streptolysin O and its co-toxin NAD-glycohydrolase protect group A *Streptococcus* from xenophagic killing. *PLoS Pathog* **9**: e1003394.

Paillot, R., Darby, A.C., Robinson, C., Wright, N.L., Steward, K.F., Anderson, E., *et al.* (2010) Identification of three novel superantigen-encoding genes in *Streptococcus equi* subsp. *zooepidemicus*, *szeF*, *szeN*, and *szeP*. *Infect Immun* **78**: 4817–4827.

Palmer, M. (2001) The family of thiol-activated, cholesterol-binding cytolysins. *Toxicon* **39**: 1681–1689.

Papageorgiou, A.C., and Acharya, K.R. (1997) Superantigens as immunomodulators: recent structural insights. *Structure* **5**: 991–996.

Park, H.S., and Cleary, P.P. (2005) Active and passive intranasal immunizations with streptococcal surface protein C5a peptidase prevent infection of murine nasal mucosa-associated lymphoid tissue, a functional homologue of human tonsils. *Infect Immun* **73**: 7878–7886.

Paton, J.C., Rowan-Kelly, B., and Ferrante, A. (1984) Activation of human complement by the pneumococcal toxin pneumolysin. *Infect Immun* **43**: 1085–1087.

Paton, J.C., Lock, R.A., Lee, C-J., Li, J.P., Berry, A.M., Mitchell, T.J., *et al.* (1991) Purification and immunogenicity of genetically obtained pneumolysin toxoids and their

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conjugation to *Streptococcus pneumoniae* type 19F polysaccharide. *Infect Immun* **59**: 2297–2304.

von Pawel-Rammingen, U. (2012) Streptococcal IdeS and its impact on immune response and inflammation. *J Innate Immun* **4**: 132–140.

von Pawel-Rammingen, U., Johansson, B.P., and Bjorck, L. (2002) IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. *EMBO J* **21**: 1607–1615.

Persson, H., Vindebro, R., and von Pawel-Rammingen, U. (2013) The streptococcal cysteine protease SpeB is not a natural immunoglobulin-cleaving enzyme. *Infect Immun* **81**: 2236–2241.

Pillich, H., Loose, M., Zimmer, K.P., and Chakraborty, T. (2012) Activation of the unfolded protein response by *Listeria monocytogenes*. *Cell Microbiol* **14**: 949–964.

Podbielski, A., Blankenstein, O., and Lutticken, R. (1994) Molecular characterization of the *cfb* gene encoding group B streptococcal CAMP-factor. *Med Microbiol Immun* **183**: 239–256.

Price, K.E., Greene, N.G., and Camilli, A. (2012) Export requirements of pneumolysin in *Streptococcus pneumoniae*. *J Bacteriol* **194**: 3651–3660.

Pritzlaff, C.A., Chang, J.C., Kuo, S.P., Tamura, G.S., Rubens, C.E., and Nizet, V. (2001) Genetic basis for the beta-haemolytic/cytolytic activity of group B *Streptococcus*. *Mol Microbiol* **39**: 236–247.

Proft, T., and Fraser, J.D. (2007) Streptococcal superantigens. *Chem Immunol Allergy* **93**: 1–23.

Proft, T., Moffatt, S.L., Berkahn, C.J., and Fraser, J.D. (1999) Identification and characterization of novel superantigens from *Streptococcus pyogenes*. *J Exp Med* **189**: 89–102.

Proft, T., Arcus, V.L., Handley, V., Baker, E.N., and Fraser, J.D. (2001) Immunological and biochemical characterization of streptococcal pyrogenic exotoxins I and J (SPE-I and SPE-J) from *Streptococcus pyogenes*. *J Immunol* **166**: 6711–6719.

Proft, T., Sriskandan, S., Yang, L., and Fraser, J.D. (2003a) Superantigens and streptococcal toxic shock syndrome. *Emerg Infect Dis* **9**: 1211–1218.

Proft, T., Webb, P.D., Handley, V., and Fraser, J.D. (2003b) Two novel superantigens found in both group A and group C *Streptococcus*. *Infect Immun* **71**: 1361–1369.

Puliti, M., Nizet, V., von Hunolstein, C., Bistoni, F., Mosci, P., Orefici, G., and Tissi, L. (2000) Severity of group B streptococcal arthritis is correlated with beta-hemolysin expression. *J Infect Dis* **182**: 824–832.

Purushothaman, S.S., Park, H.S., and Cleary, P.P. (2004) Promotion of fibronectin independent invasion by C5a peptidase into epithelial cells in group A *Streptococcus*. *Indian J Med Res* **119**: S44–S47.

Randis, T.M., Gelber, S.E., Hooven, T.A., Abellar, R.G., Akabas, L.H., Lewis, E.L., *et al.* (2014) Group B *Streptococcus* beta-hemolysin/cytolysin breaches maternal-fetal barriers to cause preterm birth and intrauterine fetal demise *in vivo*. *J Infect Dis* **210**: 265–273.

Ratner, A.J., Hippe, K.R., Aguilar, J.L., Bender, M.H., Nelson, A.L., and Weiser, J.N. (2006) Epithelial cells are sensitive detectors of bacterial pore-forming toxins. *J Biol Chem* **281**: 12994–12998.

Reiss, A., Braun, J.S., Jager, K., Freyer, D., Laube, G., Buhrer, C., *et al.* (2011) Bacterial pore-forming cytolysins induce neuronal damage in a rat model of neonatal meningitis. *J Infect Dis* **203**: 393–400.

Richards, V.P., Zadoks, R.N., Pavinski Bitar, P.D., Lefebure, T., Lang, P., Werner, B., *et al.* (2012) Genome characterization and population genetic structure of the zoonotic pathogen, *Streptococcus canis*. *BMC Microbiol* **12**: 293.

Ring, A., Braun, J.S., Pohl, J., Nizet, V., Stremmel, W., and Shenep, J.L. (2002) Group B streptococcal beta-hemolysin induces mortality and liver injury in experimental sepsis. *J Infect Dis* **185**: 1745–1753.

Rossjohn, J., Gilbert, R.J., Crane, D., Morgan, P.J., Mitchell, T.J., Rowe, A.J., *et al.* (1998) The molecular mechanism of pneumolysin, a virulence factor from *Streptococcus pneumoniae*. *J Mol Biol* **284**: 449–461.

Sachse, S., Seidel, P., Gerlach, D., Gunther, E., Rodel, J., Straube, E., and Schmidt, K.H. (2002) Superantigen-like gene(s) in human pathogenic *Streptococcus dysgalactiae*, subsp *equisimilis*: genomic localisation of the gene encoding streptococcal pyrogenic exotoxin G (speG (dys)). *FEMS Immunol Med Microbiol* **34**: 159–167.

Santillan, D.A., Andracki, M.E., and Hunter, S.K. (2008) Protective immunization in mice against group B streptococci using encapsulated C5a peptidase. *Am J Obstet Gynecol* **198**: 114.e1–114.e6.

Seele, J., Singpiel, A., Spoerry, C., von Pawel-Rammingen, U., Valentin-Weigand, P., and Baums, C.G. (2013) Identification of a novel host-specific IgM protease in *Streptococcus suis*. *J Bacteriol* **195**: 930–940.

Segura, M., and Gottschalk, M. (2002) *Streptococcus suis* interactions with the murine macrophage cell line J774: adhesion and cytotoxicity. *Infect Immun* **70**: 4312–4322.

Segura, M., Vanier, G., Al-Numani, D., Lacouture, S., Olivier, M., and Gottschalk, M. (2006) Proinflammatory cytokine and chemokine modulation by *Streptococcus suis* in a whole-blood culture system. *FEMS Immunol Med Microbiol* **47**: 92–106.

Seitz, M., Baums, C.G., Neis, C., Benga, L., Fulde, M., Rohde, M., *et al.* (2013) Subcytolytic effects of suilysin on interaction of *Streptococcus suis* with epithelial cells. *Vet Microbiol* **167**: 584–591.

Senior, B.W., and Woof, J.M. (2005a) Effect of mutations in the human immunoglobulin A1 (IgA1) hinge on its susceptibility to cleavage by diverse bacterial IgA1 proteases. *Infect Immun* **73**: 1515–1522.

Senior, B.W., and Woof, J.M. (2005b) The influences of hinge length and composition on the susceptibility of human IgA to cleavage by diverse bacterial IgA1 proteases. *J Immunol* **174**: 7792–7799.

Shak, J.R., Ludewick, H.P., Howery, K.E., Sakai, F., Yi, H., Harvey, R.M., *et al.* (2013) Novel role for the *Streptococcus pneumoniae* toxin pneumolysin in the assembly of biofilms. *mBio* **4**: e00655–00613.

Shewell, L.K., Harvey, R.M., Higgins, M.A., Day, C.J., Hartley-Tassell, L.E., Chen, A.Y., *et al.* (2014) The cholesterol-dependent cytolysins pneumolysin and streptolysin O require binding to red blood cell glycans for hemolytic activity. *Proc Natl Acad Sci U S A* **111**: E5312–E5320.

Shulman, S.T., and Rowley, A.H. (2015) Kawasaki disease: insights into pathogenesis and approaches to treatment. *Nature Rev Rheumatol* **11**: 475–482.

Sierig, G., Cywes, C., Wessels, M.R., and Ashbaugh, C.D. (2003) Cytotoxic effects of streptolysin O and streptolysin S enhance the virulence of poorly encapsulated group A streptococci. *Infect Immun* **71**: 446–455.

Silva-Costa, C., Carriço, J.A., Ramirez, M., and Melo-Cristino, J. (2014) Scarlet fever is caused by a limited number of *Streptococcus pyogenes* lineages and is associated with the exotoxin genes *ssa*, *speA* and *speC*. *Pediatr Infect Dis J* **33**: 306–310.

Smoot, L.M., McCormick, J.K., Smoot, J.C., Hoe, N.P., Strickland, I., Cole, R.L., *et al.* (2002) Characterization of two novel pyrogenic toxin superantigens made by an acute rheumatic fever clone of *Streptococcus pyogenes* associated with multiple disease outbreaks. *Infect Immun* **70**: 7095–7104.

Soderberg, J.J., Engstrom, P., and von Pawel-Rammingen, U. (2008) The intrinsic immunoglobulin g endopeptidase activity of streptococcal Mac-2 proteins implies a unique role for the enzymatically impaired Mac-2 protein of M28 serotype strains. *Infect Immun* **76**: 2183–2188.

Spaulding, A.R., Salgado-Pabón, W., Kohler, P.L., Horswill, A.R., Leung, D.Y.M., and Schlievert, P.M. (2013) Staphylococcal and streptococcal superantigen exotoxins. *Clin Microbiol Rev* **26**: 422–447.

Spellerberg, B. (2000) Pathogenesis of neonatal *Streptococcus agalactiae* infections. *Microbes Infect* **2**: 1733–1742.

Spellerberg, B., Martin, S., Brandt, C., and Lutticken, R. (2000) The *cyl* genes of *Streptococcus agalactiae* are involved in the production of pigment. *FEMS Microbiol Lett* **188**: 125–128.

Su, Y.F., Chuang, W.J., Wang, S.M., Chen, W.Y., Chiang-Ni, C., Lin, Y.S., *et al.* (2011) The deficient cleavage of M protein-bound IgG by IdeS: insight into the escape of *Streptococcus pyogenes* from antibody-mediated immunity. *Mol Immunol* **49**: 134–142.

Sukeno, A., Nagamune, H., Whiley, R.A., Jafar, S.I., Aduse-Opoku, J., Ohkura, K., *et al.* (2005) Intermedilysin is essential for the invasion of hepatoma HepG2 cells by *Streptococcus intermedius*. *Microbiol Immunol* **49**: 681–694.

Sumby, P., Whitney, A.R., Graviss, E.A., DeLeo, F.R., and Musser, J.M. (2006) Genome-wide analysis of group a streptococci reveals a mutation that modulates global phenotype and disease specificity. *PLoS Pathog* **2** e5.

Sumby, P., Zhang, S., Whitney, A.R., Falugi, F., Grandi, G., Graviss, E.A., *et al.* (2008) A chemokine-degrading extracellular protease made by group A *Streptococcus* alters pathogenesis by enhancing evasion of the innate immune response. *Infect Immun* **76**: 978–985.

Sumitomo, T., Nakata, M., Higashino, M., Terao, Y., and Kawabata, S. (2013) Group A streptococcal cysteine protease cleaves epithelial junctions and contributes to bacterial translocation. *J Biol Chem* **288**: 13317–13324.

Sundell, H.W., Yan, H., Carter, C.E., Wamil, B.D., Wu, K., Gaddipati, R., *et al.* (2000) Isolation and identification of the group B streptococcal toxin CM101 from infants with sepsis. *J Pediatr* **137**: 338–344.

Surewaard, B.G., Trzcinski, K., Jacobino, S.R., Hansen, I.S., Vughs, M.M., Sanders, E.A., *et al.* (2013) Pneumococcal immune evasion: ZmpC inhibits neutrophil influx. *Cell Microbiol* **15**: 1753–1765.

Tabata, A., Nakano, K., Ohkura, K., Tomoyasu, T., Kikuchi, K., Whiley, R.A., and Nagamune, H. (2013) Novel twin streptolysin S-like peptides encoded in the *sag* operon homologue of beta-hemolytic *Streptococcus anginosus*. *J Bacteriol* **195**: 1090–1099.

Tabata, A., Sato, Y., Maya, K., Nakano, K., Kikuchi, K., Whiley, R.A., *et al.* (2014) A streptolysin S homologue is essential for beta-haemolytic *Streptococcus constellatus* subsp. *constellatus* cytotoxicity. *Microbiology* **160**: 980–991.

Tanaka, D., Isobe, J., Watahiki, M., Nagai, Y., Katsukawa, C., Kawahara, R., *et al.* (2008) Genetic features of clinical isolates of *Streptococcus dysgalactiae* subsp. *equisimilis* possessing Lancefield's group A antigen. *J Clin Microbiol* **46**: 1526–1529.

Tapsall, J.W. (1987) Relationship between pigment production and haemolysin formation by Lancefield group B streptococci. *J Med Microbiol* **24**: 83–87.

Tatsuno, I., Isaka, M., Minami, M., and Hasegawa, T. (2010) NADase as a target molecule of *in vivo* suppression of the toxicity in the invasive M-1 group A streptococcal isolates. *BMC Microbiol* **10**: 144.

Terao, Y., Mori, Y., Yamaguchi, M., Shimizu, Y., Ooe, K., Hamada, S., and Kawabata, S. (2008) Group A streptococcal cysteine protease degrades C3 (C3b) and contributes to evasion of innate immunity. *J Biol Chem* **283**: 6253–6260.

Thomssen, H., Hoffmann, B., Schank, M., Elewaut, D., Büschenfelde, K.H.M.Z., and Märker-Hermann, E. (2000) There is no disease-specific role for streptococci-responsive synovial T lymphocytes in the pathogenesis of psoriatic arthritis. *Med Microbiol Immun* **188**: 203–207.

Timmer, A.M., Timmer, J.C., Pence, M.A., Hsu, L.C., Ghochani, M., Frey, T.G., *et al.* (2009) Streptolysin O promotes group A *Streptococcus* immune evasion by accelerated macrophage apoptosis. *J Biol Chem* **284**: 862–871.

Todd, E.W. (1934) A comparative serological study of streptolysins derived from human and from animal infections, with notes on pneumococcal haemolysin, tetanolysin and staphylococcus toxin. *J Pathol Bacteriol* **39**: 299–321.

Todd, E.W. (1938) The differentiation of two distinct serological varieties of streptolysin, streptolysin O and streptolysin S. *J Pathol Bacteriol* **47**: 423–445.

Travers, J.B., Hamid, Q.A., Norris, D.A., Kuhn, C., Giorno, R. C., Schlievert, P.M., *et al.* (1999) Epidermal HLA-DR and the enhancement of cutaneous reactivity to superantigenic toxins in psoriasis. *J Clin Invest* **104**: 1181–1189.

Turner, C.E., Kurupati, P., Jones, M.D., Edwards, R.J., and Sriskandan, S. (2009a) Emerging role of the interleukin-8 cleaving enzyme SpyCEP in clinical *Streptococcus pyogenes* infection. *J Infect Dis* **200**: 555–563.

Turner, C.E., Kurupati, P., Wiles, S., Edwards, R.J., and Sriskandan, S. (2009b) Impact of immunization against SpyCEP during invasive disease with two streptococcal species: *Streptococcus pyogenes* and *Streptococcus equi*. *Vaccine* **27**: 4923–4929.

Vincents, B., von Pawel-Rammingen, U., Bjorck, L., and Abrahamson, M. (2004) Enzymatic characterization of the streptococcal endopeptidase, IdeS, reveals that it is a cysteine protease with strict specificity for IgG cleavage due to exosite binding. *Biochemistry* **43**: 15540–15549.

Vincents, B., Vindebro, R., Abrahamson, M., and von Pawel-Rammingen, U. (2008) The human protease inhibitor

cystatin C is an activating cofactor for the streptococcal cysteine protease IdeS. *Chem Biol* **15**: 960–968.

Vlaminckx, B.J., Mascini, E.M., Schellekens, J., Schouls, L. M., Paauw, A., Fluit, A.C., *et al.* (2003) Site-specific manifestations of invasive group a streptococcal disease: type distribution and corresponding patterns of virulence determinants. *J Clin Microbiol* **41**: 4941–4949.

Vojtek, I., Pirzada, Z.A., Henriques-Normark, B., Mastny, M., Janapatla, R.P., and Charpentier, E. (2008) Lysogenic transfer of group A *Streptococcus* superantigen gene among streptococci. *J Infect Dis* **197**: 225–234.

Walker, J.A., Allen, R.L., Falmagne, P., Johnson, M.K., and Boulnois, G.J. (1987) Molecular cloning, characterization, and complete nucleotide sequence of the gene for pneumolysin, the sulfhydryl-activated toxin of *Streptococcus pneumoniae*. *Infect Immun* **55**: 1184–1189.

Walker, M.J., Hollands, A., Sanderson-Smith, M.L., Cole, J. N., Kirk, J.K., Henningham, A., *et al.* (2007) DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. *Nat Med* **13**: 981–985.

Wamil, B.D., Thurman, G.B., Sundell, H.W., DeVore, R.F., Wakefield, G., Johnson, D.H., *et al.* (1997) Soluble E-selectin in cancer patients as a marker of the therapeutic efficacy of CM101, a tumor-inhibiting anti-neovascularization agent, evaluated in phase I clinical trail. *J Cancer Res Clin Oncol* **123**: 173–179.

Wei, Z., Fu, Q., Chen, Y., Li, M., Cong, P., Mo, D., and Liu, X. (2013) *Streptococcus equi* ssp. *zooepidemicus* C5a peptidase, a putative invasin, induces protective immune response in mice. *Res Vet Sci* **95**: 444–450.

Weinstein, M.R., Litt, M., Kertesz, D.A., Wyper, P., Rose, D., Coulter, M., *et al.* (1997) Invasive infections due to a fish pathogen, *Streptococcus iniae*. *S. iniae* Study Group. *New Engl J Med* **337**: 589–594.

Weiser, J.N., Bae, D., Fasching, C., Scamurra, R.W., Ratner, A.J., and Janoff, E.N. (2003) Antibody-enhanced pneumococcal adherence requires IgA1 protease. *Proc Natl Acad Sci U S A* **100**: 4215–4220.

Whidbey, C., Harrell, M.I., Burnside, K., Ngo, L., Becraft, A.K., Iyer, L.M., *et al.* (2013) A hemolytic pigment of Group B *Streptococcus* allows bacterial penetration of human placenta. *J Exp Med* **210**: 1265–1281.

Whiley, R.A., Beighton, D., Winstanley, T.G., Fraser, H.Y., and Hardie, J.M. (1992) *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J Clin Microbiol* **30**: 243–244.

Yoshino, M., Murayama, S.Y., Sunaoshi, K., Wajima, T., Takahashi, M., Masaki, J., *et al.* (2010) Nonhemolytic *Streptococcus pyogenes* isolates that lack large regions of the sag operon mediating streptolysin S production. *J Clin Microbiol* **48**: 635–638.

Yoshioka, T., Matsutani, T., Toyosaki-Maeda, T., Suzuki, H., Uemura, S., Suzuki, R., *et al.* (2003) Relation of streptococcal pyrogenic exotoxin C as a causative superantigen for Kawasaki disease. *Pediatr Res* **53**: 403–410.

Yu, C.E., and Ferretti, J.J. (1991) Frequency of the erythrogenic toxin B and C genes (*speB* and *speC*) among clinical isolates of group A streptococci. *Infect Immun* **59**: 211–215.

Zhang, A., Mu, X., Chen, B., Han, L., Chen, H., and Jin, M. (2011) IgA1 protease contributes to the virulence of *Streptococcus suis*. *Vet Microbiol* **148**: 436–439.

Zingaretti, C., Falugi, F., Nardi-Dei, V., Pietrocola, G., Mariani, M., Liberatori, S., *et al.* (2010) *Streptococcus pyogenes* SpyCEP: a chemokine-inactivating protease with unique structural and biochemical features. *FASEB J* **24**: 2839–2848.

Zinkernagel, A.S., Timmer, A.M., Pence, M.A., Locke, J.B., Buchanan, J.T., Turner, C.E., *et al.* (2008) The IL-8 protease SpyCEP/ScpC of group A *Streptococcus* promotes resistance to neutrophil killing. *Cell Host Microbe* **4**: 170–178.

Zysk, G., Schneider-Wald, B.K., Hwang, J.H., Bejo, L., Kim, K.S., Mitchell, T.J., *et al.* (2001) Pneumolysin is the main inducer of cytotoxicity to brain microvascular endothelial cells caused by *Streptococcus pneumoniae*. *Infect Immun* **69**: 845–852.

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