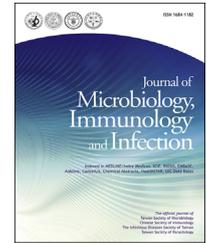




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Review Article

How *Streptococcus suis* serotype 2 attempts to avoid attack by host immune defenses



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Abstract *Streptococcus suis* (*S. suis*) type 2 (SS2) is an important zoonotic pathogen that causes swine streptococcosis, a widespread infectious disease that occurs in pig production areas worldwide and causes serious economic losses in the pork industry. Hosts recognize pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) to activate both innate and acquired immune responses. However, *S. suis* has evolved multiple mechanisms to escape host defenses. Pathogenic proteins, such as enolase, double-component regulatory systems, factor H-binding proteins and other pathogenic and virulence factors, contribute to immune escape by evading host phagocytosis, reactive oxygen species (ROS), complement-mediated immune destruction, etc. SS2 can prevent neutrophil extracellular trap (NET) formation to avoid being trapped by porcine neutrophils and disintegrate host immunoglobulins via IgA1 hydrolases and IgM proteases. Currently, the pathogenesis of arthritis and meningitis caused by SS2 infection remains unclear, and further studies are necessary to elucidate it. Understanding immune evasion mechanisms after SS2 infection is important for developing high-efficiency vaccines and targeted drugs.

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Introduction

Streptococcus suis (*S. suis*) is an important pathogen that infects humans and pigs and is distributed worldwide. *S. suis* causes meningitis, pneumonia, endocarditis, septicemia, arthritis and other symptoms in pigs, as well as meningitis, endocarditis, septicemia, permanent deafness, and even death in humans. *S. suis* can be divided into 35 serotypes based on differences in its capsule antigens.¹ In 2005, serotypes 34 and 32 were removed from the *S. suis* taxon, and serotypes 33, 26, 22 and 20 were suggested to be classified as *Streptococcus orisratti*.^{2–4} Currently, 29 verified *S. suis* serotypes exist, among which the *S. suis* type 2 (SS2) is the most widespread. The first report of human infection was in 1968, and more than 1600 SS2 infection cases have been reported since then, mainly in Southeast Asia.^{5,6} Outbreaks of human SS2 infection occurred in Jiangsu Province in 1998 and Sichuan Province in 2005 in China, causing 14 and 38 deaths, respectively.^{7,8} Therefore, SS2 is a serious danger to public health and has aroused widespread concern among researchers and the public.

SS2 is present in the respiratory tracts of normal pigs; however, the SS2 infection incidence in pigs is not directly related to the bacterial carrier rate but to the pathogenicity of the bacterial strains.⁹ Differences in SS2 strain virulence are related to virulence factors or the infectious sites.⁹ Comparative genomics, transcriptomics, proteomics, selective capture transcriptomics, suppression subtractive hybridization and other methods have been used to identify over 70 virulence factors in *S. suis*. Most are bacterial surface components, surface proteins, extracellular proteins, enzymes, or regulatory factors and are directly or indirectly involved in host cell adherence, in vivo survival, and immune escape.¹⁰ During infection, SS2 can directly affect and evade host immune responses using multiple strategies. Factor H-combining protein (Fhb) can suppress activation of alternative complement pathways by binding to complement factor H.¹¹ SS2 secretes IgM protease and IgA1 hydrolase, which can directly disintegrate host immunoglobulins. Recent studies have shown that virulent strains of SS2 can inhibit NET formation by host neutrophils.¹² SS2's capsule can suppress sphingosine-dependent immune discrimination/surveillance,¹² while its superoxide dismutase can protect against reactive oxygen species (ROS) produced by host immune cells.¹³ Therefore, studying the mechanisms behind the bacteria-host interaction contributes to understanding SS2 pathogenesis.

Main virulence factors of *S. suis* serotype 2

Surface proteins

Bacterial surface elements, especially surface proteins, are directly related to the external environment and closely related to pathogen viability and pathogenicity. Surface proteins on gram-positive bacteria such as SS2 anchor on the bacterial cell wall by covalently binding to peptidoglycan after being cut by sorting enzymes. These surface proteins are generally important virulence factors or immunogenic proteins, most of which are essential

for pathogens to infect their hosts by contributing to bacterial adhesion to cells, blood invasion, blood clotting, immune evasion, and transmembrane nutrient delivery.^{10,14,15}

Enolase

Enolase is a 45 kDa key glycolytic enzyme located in the cytoplasm and cell wall. Enolase is present in *Streptococcus* and in various serotypes and has the significant ability to combine with plasminogen. Both its C-terminal lysine residue and N-terminal region are key plasminogen binding sites. Enolase is involved in various pathological processes and autoimmune diseases after a streptococcal infection.¹⁶ Eagles found that enolase on the surface of *Streptococcus* can combine with plasminogen and fibronectin dose-dependently. *S. suis* α -enolase (SsEno) may contribute to streptococcal virulence. Huo et al. used an antibody-blocking assay in a human blood bactericidal model and found that SsEno is a potential antiphagocytic factor of *S. suis*.¹⁷ Our study found that Eno protein expression increased after *S. suis* interacted with porcine brain microvascular endothelial cells (PBMECs) and astrocytes (ACs) and promoted interleukin (IL)-8 release, thus helping *S. suis* to penetrate the blood-brain barrier.¹⁸ Furthermore, the SsEno antibody can inhibit streptococcal invasion and adhesion to brain microvascular endothelial cells.¹⁹ The SsEno protein is also an immunogenic protein. SsEno can elicit multiple IgG subtype antibody responses via subcutaneous immunization in mice.²⁰ More importantly, SS2 enolase can stimulate humoral immune responses and protect against SS2 and streptococcus type 7 in BALB/c mice.²¹

Surface antigen one (Sao)

The Sao protein is a 110 kDa protein that anchors to the bacterial surface via its C-terminus. Li discovered Sao while searching for *S. suis* functional proteins in 2006. Sao exists in most *S. suis* serotypes and has the typical characteristics of gram-positive membrane-anchoring proteins such as muramidase-released protein (MRP) and LPXTG membrane-anchoring structures. The Sao protein has 3 variations: Sao-L, Sao-M and Sao-S. Sao-M is the most common, accounting for approximately 80% of these proteins. A high concentration of anti-Sao antibodies was found in the serum of convalescent pigs, indicating that Sao is immunogenic and expressed during *S. suis* infection.²² Furthermore, ELISA detection based on Sao-M has reportedly been used to successfully monitor *S. suis* infection.^{6,25} Since Sao is a cell wall-anchoring protein, Roy et al. assumed that it could invade and adhere to epithelial cells as a first step in colonizing pathogenic hosts, but experimental results using whole blood showed that Sao was not an antiphagocytic factor.²³ Some studies have shown that Sao is a protective antigen but not an important virulence factor of SS2.²⁴ Immunization with recombinant Sao protein induces humoral reactions in pigs and mice, which weakens clinical symptoms, slows the spread of pathogens, reduces mortality, and produces cross-serotype protection.

Glutamate dehydrogenase (GDH)

The 45 kDa protein, GDH, resides on the surface of *S. suis*.^{26,27} GDH is an important functional protein in bacterial metabolism, playing important roles in bacterial carbon and nitrogen metabolism. The *gdh* gene is highly conserved and has been cloned and expressed. A fusion protein has been purified to prepare a multi-antibody that can react with proteins of the same molecular weight from other isolates, demonstrating that this protein is commonly expressed and immunogenic in different strains. The protein can also specifically react with anti-SS2 serum, indicating that it can be used as an antigen for diagnosing *S. suis* infection.^{5,28} Moreover, *S. suis* can be divided into avirulent, attenuated and virulent strains using non-denaturing gel electrophoresis on the GDH protein, indicating that GDH is a virulence factor of *S. suis*.

Evaluation of elongation factor Tu (EF-Tu)

EF-Tu is a conserved multifunctional protein involved in various cellular processes and diseases, including viral replication, apoptosis, cytoskeletal organization, signal transduction, and translation control.^{29–31} EF-Tu enables amino acid tRNAs to enter specific parts of the ribosome. Barbier showed that EF-Tu may have a role as a bacterial virulence factor and significantly affects promoting early *Pseudomonas aeruginosa* colonization.³² Cabeen and Losick reported a sudden demand for EF-Tu associated with Tse6 toxicity, which could destroy the recipient cells' core metabolism.³³ An immunoproteomics study found that EF-Tu from *S. suis* could specifically combine with human fibronectin (FN) and laminin (LN), and EF-Tu-FN or EF-Tu-LN interactions play important roles in bacterial adhesion and colonization.^{34,35} In our previous studies, we found that recombinant EF-Tu could be used to identify *S. suis* antisera. Immunizing New Zealand rabbits with EF-Tu elicited significant immune responses and protected the rabbits against lethal SS2 infective doses, indicating that EF-Tu can be used as an effective subunit vaccine candidate antigen for SS2.⁶

Sortases

Sortases are commonly expressed in gram-positive bacteria and are known as transpeptidases. Their main physiological function is to anchor the bacterial surface proteins to peptidoglycans on the cell wall, depending on the C-terminus sorting signal (CWS) domain, which recognizes LPXTG and other related motifs, thereby covalently binding those motifs containing secreted proteins to the peptidoglycan on the cell wall.^{36,37} SS2 sortases include sortases A, B, C, D, E, and F.^{38,39} Sortase A can be isolated and detected in most *S. suis* serum and is highly homologous in SS2 and *Streptococcus gordonii*.⁴⁰ Vanier found that sortase A knockout SS2 strains had significantly reduced adhesion to and invasion of cerebral microvascular endothelial cells, indicating that the C-terminus CWS contributes to *S. suis* invasion processes.⁴¹ In addition, sortase A knockout partially decreased *S. suis* from adhering to fibronectin and plasma, suggesting that other sortases may also participate in

bacterial adhesion. Although sortase A (SrtA) has long been considered an ideal drug target for treating gram-positive pathogens, the effect of sortase A on *S. suis* virulence and pathogenicity is controversial. Several studies used CD1 mice as model animals for toxicity attack tests and showed that the virulence was nearly the same between the sortase A gene knockout and wild-type strains.⁴¹ However, sortase A gene knockout in the Chinese isolate, O5ZYH33, resulted in decreased pathogenicity in pigs compared with the parent strain.³⁹

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

GAPDH in SS2 is a glycolytic enzyme that phosphorylates glyceraldehyde dehydrogenase to produce glyceral-1,3-diphosphate (DPGN), which is mainly found in bacterial cytoplasm.⁴² Madureira et al. and Zhu et al. showed that the GAPDH protein is expressed on the surfaces of *Streptococcus pyogenes*, *Erysipelothrix rhusiopathiae* and other species.^{43,44} Except the nonvirulent strain, SS2T15, 36 streptococcal strains, including 7 strains of *Streptococcus equi*, 2 strains of SS1, 24 strains of SS2, 2 strains of SS9 and 1 strain of SS7, have the *gapdh* gene. Brassard showed that *S. pyogenes* GAPDH had more than 95% amino acid sequence homogeneity with other streptococcal GAPDHs. Sichuan isolate ZY05719 and Jiangsu isolate HA9801 showed more than a 99.8% nucleotide sequence homogeneity.⁴⁷ GAPDH has many functions, including effects on membrane fusion, nuclear export, microtubule assembly, and protein phosphotransferase/kinase reactions. Eukaryotic GAPDH has been reported to regulate cell attachment and play a role in enhancing the cytoskeletal structure.⁴³ Jobin et al. studied SS2's plasminogen binding capacity and found that the GAPDH receptor could bind to and activate plasminogen, thus promoting fibronectin degradation.⁴⁵ Additionally, GAPDH deficiency in streptococcus S735 decreases bacterial binding to plasminogen by 25%.⁴⁵ Furthermore, pretreating pig tracheal ring cells with recombinant GAPDH protein increases streptococcal adhesion to cells, indicating that the streptococcal GAPDH protein is associated with bacterial adhesion to host cells.^{46,47}

Factor H-combining protein (Fhb)

Several studies have demonstrated that bacterial cell surface proteins play important roles in regulating the host's complement system, mainly by manipulating complement regulation factor H (FH). In *S. suis*, factor H-combining protein (Fhb) can specifically bind with the negative regulator H factor of the alternative pathway, inhibiting alternative pathway activation, thus helping *S. suis* to avoid complement-mediated phagocytosis and allowing its survival in the blood.¹¹ Li et al. found that Fhb (domain II) can also bind to C3b/C3d via electrostatic and hydrophobic interactions to form a large immune complex with factor H (FH), thereby enhancing bacterial antiphagocytosis by polymorphonuclear leukocytes.⁴⁸ The H-binding protein receptor binding domain (Fhb RBD) binds to the Gb2 receptor mainly via hydrogen and hydrophobic interactions.⁴⁹ Fhb is also a ligand for host cell surface proteins and

enhances the ability of *S. suis* to adhere to host cells by recognizing oligosaccharides.⁵⁰ In addition, Kong et al. confirmed for the first time that Fhb contributes to *S. suis* penetrating the human blood-brain barrier.⁵¹ Via immunoproteomic analysis, Fhb was shown to have good immunogenicity and can be used as a candidate vaccine molecule.⁵²

Metabolic and regulatory virulence elements

Pathogens produce relevant signal-regulating molecules to cope with stress from the bacterial population and the external environment while growing and infecting the host. Through these signal-regulating molecules, pathogens can quickly adjust their structure and physiological behavior to survive and proliferate in new environments. These signal-regulating molecules include energy metabolism regulators, stress-related regulators, bacterial quorum-sensing signaling molecules, bacterial units, binary regulatory systems and bacterial virulence posttranslational modification elements.⁵³ Although these factors do not directly express virulence proteins, most can regulate bacterial survival in the host, environmental tolerance and expression of virulence factors. Therefore, these molecules are important virulence-related bacterial factors and are also important targets for vaccine and novel antibacterial drug research.

Catabolite control protein A (CcpA)

A set of molecular mechanisms closely regulates the intake and energy metabolism of sugars in bacteria. For example, when a bacterium ingests and metabolizes glucose, other energy sources are inhibited. This phenomenon is called carbohydrate activation and inhibition and is very important for bacterial growth. CcpA is a key factor in inhibiting carbohydrates in a multifunctional environment or in excessive sugars by regulating related gene expression at the transcriptional level. This factor is an important bacterial virulence regulator in other streptococci.⁵⁴ Lang et al. found that the metabolic products of CcpA mutant SS2 strains differed from those of the wild strains. Furthermore, the bacterial virulence of the CcpA-deficient strains was significantly decreased,⁵⁵ possibly due to downregulated bacteriolysin, enolase, bacterial adhesion and invasion-related factors, suggesting that CcpA is also an important virulence regulator.¹³ Xiao et al. confirmed for the first time that *S. suis* meningitis strain CcpA can act as a transcription factor and regulate sRNA as an sRNA-binding protein. For example, CcpA regulates capsule expression through rSSO4, promoting bacterial adherence and invading mouse brain microvascular endothelial cells, thus causing meningitis in mice.⁵⁶

S-ribosylhomocysteine (LuxS)

To effectively resist the host immune defense system, pathogenic bacteria are pathogenic to the host only when they multiply at the infection site and reach a certain population density. Quorum-sensing controls various biological processes in bacterial populations, such as fluorescent protein production, biofilm formation, and virulence

factor expression.⁵⁷ The key factors for quorum sensing in both intraspecific and interspecific bacterial colonies are the autotrophic peptides, AI-1 (Autoinducer-1) and AI-2, and LuxS (S-ribosylhomocysteine), the key rate-limiting enzyme for AI-2 synthesis. Wang and Cao et al. reported the effects of a LuxS SS2 knockout on inducing quorum sensing and bacterial virulence, respectively. Their results showed that LuxS is an essential factor for AI-2 release in *S. suis*. LuxS knockout increases bacterial capsule thickness and H₂O₂ stress resistance but decreases adhesion, hemolytic activity and biofilm formation abilities on epithelial cells and reduces pathogenicity in piglets and zebrafish. A transcriptional analysis found that related virulence genes, such as GDH, CPS, GAPDH, Sly, FBPS and EF, were all downregulated to varying degrees.^{58,59}

Arginine deiminase system (ADS)

The ADS is conserved in different bacterial species. This system can catalyze the production of carbon dioxide, ammonia and ornithine from arginine, which produces 1 mol of ATP per 1 mol of arginine. However, activating the ADS can be affected by carbon sources, oxidative stress, temperature and changes of the arginine substrate directly or indirectly. ADS has been studied in many bacteria. ADS in bacteria such as *Listeria monocytogenes* can affect immune evasion and environmental stress responses both in vitro and in vivo, and initiating ADS metabolism in the host cells' phagolysosomes can prolong bacterial survival in cells.⁶⁰ The *S. suis* ADS contains an arcABC gene cluster, the transporter, arcD, and the system regulator, arcR.⁶¹ Gruening et al. studied the SS2 arcABC gene cluster and searched for the promoter recognition region of its regulated genes. Gruening's group also found that the arcABC function was inactivated after deleting arcA, resulting in significantly decreased cell viability under acidic stress.⁶² Marcus Fulde et al. showed that mutation of the arginine transporter, arcD, in SS2 significantly reduced arginine intake and the ability to neutralize acid and decreased the epithelial intracellular survival rate, suggesting that arcD may play an important role in adapting to the environment and regulating virulence in *S. suis*.⁶³ arcR is the only regulator of the arcABC gene cluster, and it activates the arcABC promoter by binding to the upstream transcription start site of the arcABC cluster genes.⁶¹ However, no effect of arcR has been reported on bacterial pathogenicity.

Two-component signal transduction systems (TCSTs)

Pathogenic bacteria have many signal transduction pathways, and two-component signal transduction systems (TCSTs) are the most common. The classic TCSTs consist of a histidine protein kinase located in the cell membrane that recognizes and transmits external environmental signals as well as a response element in the cytoplasm.⁵³ Five dual regulatory elements have been reported in SS2 TCSTs. Wang et al. found that the dual regulator SaK/SaR was involved in regulating the secretion of antibiotic suicin antimicrobial peptides.⁶⁴ Li et al. reported that the ciaK/ciaR dual

regulator is involved in regulating SS2 strain virulence. Knocking out this bacterial regulator weakens the bacteria's growth response, reduces the bacteria's capacity to resist antioxidant stress, and decreases bacterial growth and viability in acidic environments.⁶⁵ Han et al. discovered that the SS2 Ihk/Irr binary system was involved in bacterial adhesion, acid resistance and antioxidative stress. Further, a deletion mutant was less virulent and pathogenic, the expression of metabolically relevant proteins was down-regulated, and superoxide dismutase (SOD) was suppressed.⁶⁶ Wang and Xu et al. reported the two-component systems, VirR/VirS and NisK/nISR, respectively. A mutant strain containing a deletion of the corresponding regulatory subgenes showed weakened virulence that was easily cleared by the host, reduced adhesion and invasiveness, and a weakened ability to resist oxidative stress. The bacteria's morphological characteristics were changed, but no underlying mechanisms were analyzed.^{67,68}

Secretory virulence factor

The secretory virulence factor of *S. suis* is secreted independent of an interaction between the bacteria and the host cell but remain involved in enhancing bacterial infection, invasion and pathogenicity. *S. suis* is reported to secrete nearly 20 biologically functional protein types. Of those secretory virulence factors, suilysin (Sly) has been the most thoroughly studied. Extracellular factor (EF) is another highly studied secretory virulence factor of *S. suis*. Other *S. suis*-secreted proteins include the endonucleases, SsnA, IgG-binding proteins, phospholipase C and bacteriocin-like inhibition factors.^{10,14,69}

S. suis-secreted nuclease A (SsnA)

Bacteria interact with neutrophils to induce PMN cell degranulation to form NETs, thereby promoting the release of nucleic acid substances and granular proteins, which can form a localized high concentration of antibacterial molecules and capture and kill various pathogens in a limited area. *S. suis*-secreted nuclease A (SsnA) and other nuclease homologs, such as EndAsuis, are endonucleases that are secreted by the bacteria while *S. suis* interacts with the host cell. SsnA can hydrolyze and destroy NET particle formation by degrading NET's important component DNA, but its activity depends on a calcium or magnesium ionic environment.⁷⁰ SsnA is distributed in SS1-SS9 and can be detected in several host organs such as brain tissue and

joint cavities after infection. Moreover, SsnA is an immunogenic candidate, and its immunization together with an aluminum gel adjuvant mediates protection against the same *S. suis* infection type in mouse models.⁷¹

S. suis IgG-binding protein (SSPG)

SS2-secreted IgG-binding proteins (SSPGs) bind nonspecifically to the Fc-end of most mammalian IgG and IgA molecules and influence antibody activity. Some scholars consider that SSPGs likely act as pathogenic factors and play important roles in streptococcal infections.⁷² Experimental studies have also shown that the SSPG in group G streptococci cause bacterial aggregation, and this aggregation destroys activation of the complement pathway and complement protein-mediated opsonization.^{73,74} SSPG was listed as an important candidate in the latest review of *S. suis* virulence-related factors. Liu et al. found that SSPG induces a strong humoral immune response and provides better immune protection in animals.⁷⁵ However, reports concerning the biological function of SSPG remain limited, and SSPG's interaction with the host requires further study.

Fig. 1 summarizes the identified histories of most *S. suis* virulence factors.

Research progress on the immune evasion/escape mechanisms of pathogenic bacteria

The innate immune system includes highly integrated and networked cells and effector molecules that continuously help the host resist microbial infections in complex environments. This system includes the natural barrier of the skin and mucosal epithelium, ecological competition and antibacterial products produced by the resident flora, as well as soluble antibacterial molecules such as antimicrobial peptides, complement proteins and reactive oxygen species. The innate immune system recognizes the microbes and produces inflammatory responses after being activated by these microbes. Neutrophils and macrophages migrate to the bacterial infection sites to phagocytize and clear pathogenic microorganisms. However, pathogenic bacteria have evolved strategies to resist the innate defense mechanisms and promote spreading into the circulation and deeper into host tissues and organs. Pathogens can escape phagocytosis and elimination by neutrophils and macrophages. In conclusion, pathogenic bacteria use different mechanisms to evade the innate immune system and clearance from the host.

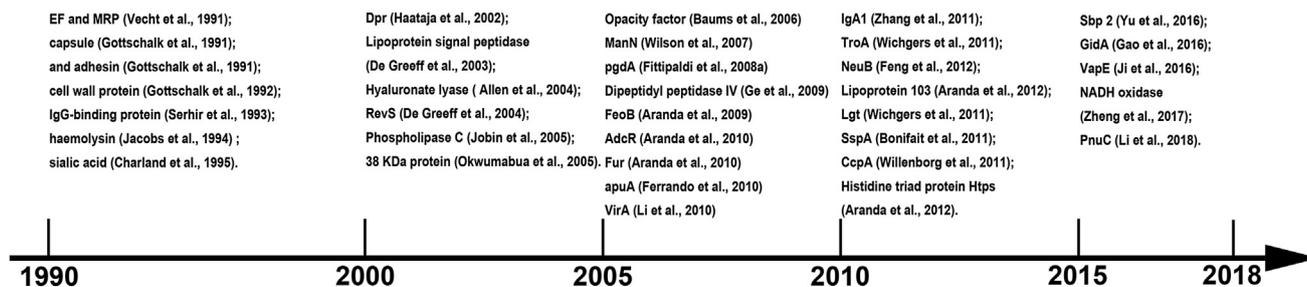


Figure 1. Timeline summary of the history of *S. suis* virulence factors.

Escape from neutrophil extracellular traps

Neutrophils are important to the host's innate immune system. Neutrophils are the first cells recruited to infection sites to clear pathogenic microorganisms by phagocytosis and degranulation. Brinkmann discovered that PMNs can kill pathogens by a third mechanism mediated by NET.⁷⁶ NETs are composed of extracellular fibrous structures formed by a DNA skeleton, antimicrobial peptides, histones and neutrophil granular proteins and are produced by activated neutrophils.⁷⁷ NET is considered broad-spectrum and highly efficient, causing little tissue damage when killing pathogenic microorganisms.^{78,79} However, pathogens have evolved several mechanisms to escape/evade the bactericidal effects of NET. Beiter found that *Streptococcus pneumoniae* was not killed after being trapped in NET. This anti-NET phenomena was largely mediated by the endolytic endoenzyme, End A, on the surface of *Streptococcus pneumoniae*, which degrades the DNA skeleton of the NET, allowing the bacteria to escape the NET.⁸⁰ Zhao et al. reported that SS2 induced NET in a mouse infection model, but nuclease treatment destroyed the NET and increased the bacterial load and TNF- α content, indicating that NET play a vital role in eliminating SS2 and regulating inflammatory responses.⁸¹ De Buhr et al. reported that virulent SS2 strains only induce a low degree of NET in porcine neutrophils or even inhibit NET formation.⁸² This may be one reason why virulent SS2 strains infect humans and pigs causing extensive bacteremia. Biofilm formation mediates SS2 escape/evasion from phagocytosis by phagocytic cells during infection but has a limited effect on NET-mediated clearance. However, biofilm formation inhibits NET formation, thus enabling SS2 to proliferate in the blood and produce extensive bacteremia.⁸³

Escape from complement deposition and activation

The complement system is the first line of defense against microbial invasion. The complement delineates innate and adaptive immunity and is a special focus of pathogenic immune escape strategies.⁸⁴ Although this enzymatic cascade of finely regulated enzymes, protein complexes, and receptors can rapidly identify and eliminate foreign objects, it also provides many intervention sites that can disrupt this balanced protein interaction network. Common strategies that pathogens use to escape the complement system include expressing proteases that cleave complement factors, producing complement inhibitory factors, and recruiting or simulating complement regulatory factors.⁸⁵ In the C3^{-/-} experimental mouse model, the complement system was demonstrated to be crucial in preventing morbidity and mortality caused by meningitis.⁸⁶ This finding indicates that escaping complement activation may be necessary for *S. suis* to survive in the host.⁸⁷ Two H-binding proteins (FHB and SUS0186) found in *S. suis* are homologous to *Streptococcus pneumoniae* pneumococcal surface protein (PspC).^{11,88} Seele et al. demonstrated IdeS_{suis} as a new complement escape factor.⁸⁷ Immunoglobulin M-degrading enzyme of *S. suis* (IdeS_{suis}) specifically cleaves porcine IgM in vivo to allow escape from

opsonization and complement-mediated killing in the blood flow, which is critical to bacterial adaption to the immune response and bacterial survival at the early phase of infection.⁸⁷ The SS2 05ZYH33 strain has 4 open reading frames that encode the proteins, NeuD, NeuC, NeuB and NeuA, which are involved in metabolizing sialic acid. Destruction of NeuB leads to capsule membrane damage, which is characteristic of decreased sialic acid content, capsule thinning, increased pH sensitivity, and increased adhesion and invasion in Hep-2 cells. Deleting NeuB leads to greater interleukin-8 secretion, increased neutrophilic chemotaxis and activation, and enhanced CD8⁺ and CD4⁺ T cell activation. Therefore, bacterial sialic acid can decrease neutrophilic recruitment by chemokines and dampen the host immune response to SS2.⁸⁹ NeuC deletion in SS2 affects capsule synthesis, rendering the bacteria more susceptible to phagocytosis.⁹⁰ How the capsular sialic acid and sialic acid modifications affect SS2 relative to host immune response evasion remains unclear, and further research is necessary.

Invasion of the central nervous system (CNS)

Two major entrances to the CNS (central nervous system) are the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF)-barrier (BCSFB). The predominant cells in the BBB are the brain microvascular cells.⁹¹ SS2 invasion and intrusion into porcine brain microvascular cells depends on adhesins and invasins and cell wall components, such as lipoteichoic acid (LTA), as well as host cell extracellular matrix proteins such as fibronectin. Cell wall-anchoring proteins and enolase may play the same roles as adhesins and invasins via fibronectin adhesion.⁹¹ We found that Eno protein expression in *S. suis* interacts with porcine brain microvascular endothelial cells (PBMEC) and astrocytes (AC), promoting the release of cytokines, such as interleukin IL-8, and thus helping *S. suis* penetrate the BBB.²¹ In addition, Eno can induce PBMEC apoptosis, which plays an important role in destroying the blood-brain barrier during SS2 infection both in vitro and in mouse experiments. Eno also promotes SS2 adhesion to the PBMEC (unpublished observations) and increases the permeability of the blood-brain barrier by inhibiting expression of the intercellular tight connexin, occludin. Hemolysin is another factor that destroys the blood-brain barrier via cytotoxic effects. The invasion and penetration of *S. suis* through the blood-cerebrospinal fluid barrier has also been demonstrated. *S. suis* can adhere to choroid plexus epithelial cells (CPEC), indicating that such adhesion is necessary to directly enter the extracellular matrix. In addition, *S. suis* can induce CPEC necrosis and may play a role in apoptosis.⁹² Capsular polysaccharides can significantly impede bacterial invasion of CPEC, suggesting that this process requires cytochrome components and surface proteins. However, the functions of these adhesins and invasins remain largely unknown. *S. suis* can also affect the blood-cerebrospinal fluid barrier, further promoting bacterial and leukocyte transport function and integrity. Other soluble factors, such as hemolysin, may also be involved in influencing the barrier function of the blood-cerebrospinal fluid.⁹³

Resistance to killing via reactive oxygen species

Phagocytosis of bacteria can induce oxidation reactions and oxygen consumption to produce ROS, which kills bacteria. This process occurs inside the cell, first via activating and operating NOX complexes to produce superoxide ($O_2^{\cdot-}$) or H_2O_2 . Both these substances can dehydrate enzymes' iron-sulfur clusters and release free iron ions (iron reaction). The iron ions can combine with H_2O_2 to produce hydroxyl radicals that kill bacteria.⁹⁴ Second, $O_2^{\cdot-}$ and H_2O_2 are converted by MPO to produce HOCl, and all HOCl produced by ROS is mainly bactericidal. Gram-positive bacteria can encode a bacterial SOD,^{95,96} which can accelerate the conversion of $O_2^{\cdot-}$ and H_2O_2 and can convert H_2O_2 into smaller molecules. Gram-negative bacteria can use catalase to convert H_2O_2 into O_2 and H_2O . However, all *S. suis* are catalase-negative, so other systems must be employed to hydrolyze H_2O_2 .⁹⁷ H_2O_2 degradation can reduce HOCl production by MPO, thus promoting bacterial resistance to ROS-mediated killing. *Streptococcus pyogenes* produces iron-binding proteins, and *Staphylococcus aureus* produces NO, both of which can inhibit the iron reaction. Certain gram-positive bacteria can enhance the antioxidant capacity to resist oxidation-mediated killing, and *Streptococcus agalactiae* produces carotenoids that hinder phagocytosis and killing.⁹⁸ Thus, bacteria resist being killed by host ROS largely by reducing the host's ROS production and increasing bacterial antioxidant production.

N-deacetylation of peptidoglycan

Peptidoglycan is the major cell wall component of gram-positive bacteria, ensuring cell wall rigidity and stability. Peptidoglycan is a comparatively conserved polysaccharide chain that helps the host to recognize the minimal peptidoglycan fragments released by lysozyme hydrolysis or the peptides released during bacterial cell wall turnover via the NOD1 and NOD2 proteins.^{99,100} However, gram-positive bacteria, including *S. suis*, have acquired various effective mechanisms to escape the host immune response by mediating peptidoglycan. SS2 pgdA expression is increased when SS2 interacts with PBMEC or neutrophils in vitro and during in vivo infection processes, indicating that peptidoglycans may undergo N-deacetylation during infection.¹⁰¹ Virulence of the SS2 PGDA-

deficient strain was reduced both in murine and swine models. Interleukin-6 and interferon- γ release was markedly impaired in PGDA-deletion mutant-infected animals compared with animals infected with the wild-type strain. This decrease may be attributed to the enhanced sensitivity of the PGDA-deletion mutant to neutrophils. Interestingly, neither the PGDA-deletion mutants nor the wild-type strain differed in antipolymyxin B, maltogenic protein II, colistin, human neutrophil peptides (HNP-1, HNP-2) or other cationic antimicrobial peptides, suggesting that PGDA may improve N-deacetylation of bacterial peptidoglycans and enhance the antineutrophilic lysozyme's effect. These results show that the PGDA transcription level is enhanced in vivo, resulting in increased N-deacetylation of peptidoglycan and greatly contributing to SS2 virulence.¹⁰²

SS2 appears to have developed several strategies to escape the host immune system, and some of these strategies are summarized in Fig. 2.^{10,103,104}

Summary and prospect

The *S. suis* immune evasion process is highly complex. This process likely occurs due to the synergistic result of several mechanisms. Many genes that contribute to SS2 immune invasion have been identified by taking advantage of the newly discovered SS2 complete genome sequence, functional genomic studies and advanced experimental techniques, thus providing important information for finding effective strategies to kill SS2 and develop new vaccines. Seeking therapeutic targets in host cells against SS2 from a host perspective has become a new research area. The latest study also found that SS2 can form biofilms to escape phagocytosis by phagocytic cells during infection and inhibit host NET formation, although biofilms cannot protect SS2 against the direct killing and clearance mediated by NET. This finding explains why SS2 survives and proliferates in the blood, causing extensive bacteremia. In murine models, the host can remove bacteria in the biofilm state via NET; eventually, the surviving mice can become immune to SS2 reinfection. This finding suggests that new perspectives and new strategies targeted at both host and bacterial aspects are needed to counter SS2 evasion mechanisms to effectively control and prevent *S. suis* diseases.

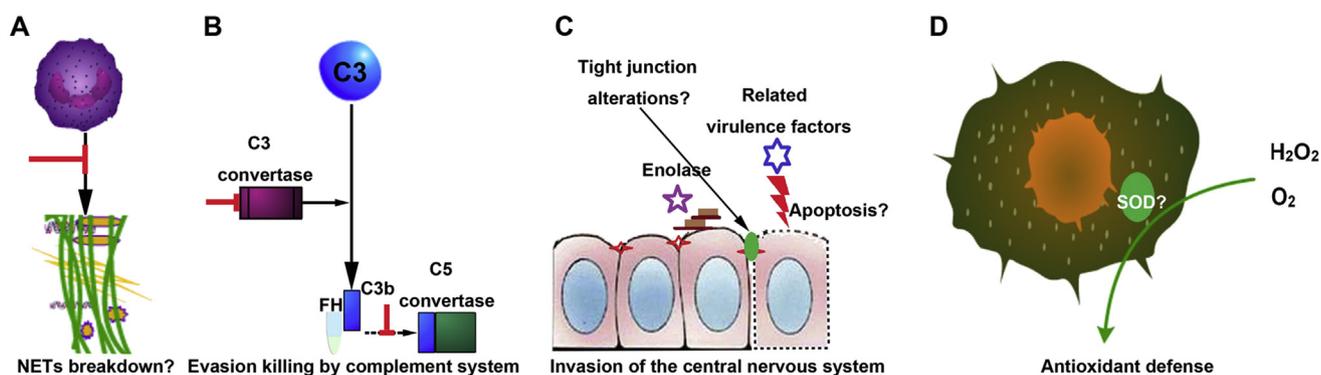


Figure 2. Evasion of host's innate immune response. (A) SS2 breaks down NET; (B) SS2 evades being killed by the complement system; (C) SS2 invades the central nervous system; (D) Antioxidant defense from SS2.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2019.03.003>.