

The streptococcal hemoprotein receptor

A moonlighting protein or a virulence factor?

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The β -hemolytic group A streptococcus (GAS) is a major pathogen that readily uses hemoglobin to satisfy its requirements for iron. The streptococcal hemoprotein receptor in GAS plays a central role in heme utilization and binds fibronectin and laminin *in vitro*. Shr inactivation attenuates the virulent M1T1 GAS strain in two murine infection models and reduces bacterial growth in blood and binding to laminin. Shr impact on the globally disseminated M1T1 strain underscores the importance of heme uptake in GAS pathogenesis and raises the possibility of targeting heme-uptake proteins in the development of new methods to combat GAS infections.

The common group A streptococcus (GAS) is a key human pathogen that can produce severe infections as well as serious post-infection sequelae. Most GAS infections, including pharyngitis and impetigo, affect the upper respiratory track or the epidermis. Less frequently, GAS also produces invasive diseases associated with high mortality, including necrotizing fasciitis and streptococcal toxic shock syndrome. The aggressiveness of such infections gives GAS its “flesh-eating” handle. Even simple and common GAS episodes are potentially dangerous in that they may lead to glomerulonephritis (GP) or rheumatic fever (RF). These immune-based sequelae are characterized by serious renal injury (GP) or inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea) and skin (erythema marginatum).^{1,2} In developing countries and among certain disadvantaged populations, GAS-induced RF has remained prevalent and persists as a leading cause of childhood cardiovascular illnesses and mortality, despite the introduction of antibiotics. In contrast, the developed parts of the world have experienced a steady decline of GAS invasive diseases and RF episodes, until recently. In the early 80s, a worldwide resurgence of cases of GAS RF and severe invasive diseases was observed, constituting a significant change in GAS epidemiology in Western countries.^{3,4}

GAS is notorious for being highly genetically variable, with separate strains exhibiting wide differences in invasiveness, rheumatic capacity, and propensity for certain disease. Genetic recombination and horizontal gene transfer, mediated mainly by bacteriophages, are crucial vehicles in GAS genetic diversification.⁵ Serological differences observed in key surface antigens were used in early research for GAS classification.^{6,7} This serotyping was based on the anti-phagocytic M protein and the variable T antigen, which was recently found to be a pilus structure.⁸ Contemporary genotyping of the M protein (*emm* type) permitted identification of more than 200 *emm* types in GAS as listed in the *emm* database of the Center for Disease Control and Prevention⁹ (www.cdc.gov/ncidod/biotech/strep/streindex.htm). The number of T serotypes is significantly lower than that of M types in GAS populations.¹⁰ The genes encoding the pilus-associated proteins (T antigens) reside within the FCT chromosomal locus, which also encodes for fibronectin-binding and regulatory proteins. The FTC region is variable in genetic content in addition to the sequence variation seen with the T antigens.¹¹

The recent rise of severe GAS infections is neither sporadic nor localized in certain geographical areas; instead, it represents a modern-day pandemic.¹² Epidemiological studies revealed that the upsurge of

invasive infections is predominantly linked to the spread of the highly virulent M1T1 (*emm* 1) clone; although periodic increases in other clones belonging to *emm* 3, *emm* 18 and *emm* 28 types have been observed as well.^{13,14} GAS populations typically do not remain static; they rapidly evolve to form multiple subtypes. This tendency may explain the transient (4–7 y long) epidemics typically seen with certain GAS clones. Surprisingly, the M1T1 clone not only disseminated globally but has also persisted over the past 25 years.^{12,15} In light of the population-dynamic that is characteristic of GAS, the M1T1 stability appears to be unique. Today, this clone is highly prevalent in non-complicated pharyngitis and is over-represented in invasive GAS episodes. Several comprehensive molecular-genetic and epidemiological studies suggest that the M1T1 clone has separated from its ancestral M1 strain by the acquisition of a 38-kb segment from M12-type strain and sequential gaining of the DNase Sda1 and the SpeA superantigen (both encoded by separate bacteriophages).^{14,16} The acquisition of the M12 chromosomal segment enables increased expression of the streptococcal toxins Streptolysin O and NAD glycohydrolase. The enzyme Sda1 degrades the DNA traps produced by neutrophils and thus protects GAS from clearance, while SpeA activates a range of T-cell subsets and damages the

host's immune response. This acquisition of new virulence factors and the enhanced expression of others are believed to give MIT1 a significant selective advantage, which may facilitate its global spread. A noteworthy characteristic of the MIT1 clone is its ability to revert to a hyper-virulent form during infection; conversion is facilitated by mutations in the global regulatory *covRS* system.¹⁷⁻¹⁹ This event leads to comprehensive changes in GAS transcriptome, including enhanced expression of multiple virulence genes and reduction in *speB* expression. The SpeB protease degrades many of the GAS extracellular proteins that are involved in pathogenesis, including Sda1 and the M protein. A leading hypothesis asserts that Sda1 expression during infection functions as a selective force for *covRS* mutations.^{16,20}

In this issue of *Virulence*, Dahesh et al. demonstrated that the streptococcal hemoprotein receptor (Shr) is important for MIT1 virulence.²¹ This observation is intriguing in that it highlights the importance of heme uptake to pathogenesis of a highly successful GAS strain. It also raises the possibility of targeting Shr and heme-uptake mechanisms in the development of new prevention and therapeutic strategies to combat GAS. The ability to obtain and use heme during infection allows the hemolytic GAS to capitalize on the largest iron reservoir in an environment in which this essential metal is highly restricted. Significant progress in the understanding of hemoprotein utilization by GAS has been made in recent years. Shr is a key component in what appears to be a protein relay apparatus that obtains heme from hemoglobin and delivers it through the peptidoglycan layers in a cascade fashion to a dedicated ABC transporter (Sia/Hts).²²⁻²⁵ Shr, the first protein in the relay, is a 145 kDa protein that consists of a unique N-terminal region followed by two

heme-binding NEAT domains that are separated by a leucine-rich repeat segment.²⁶ Shr is anchored in the cell membrane by a short hydrophobic tail located at the receptor C-terminus. The large receptor crosses the cell wall and is exposed on the streptococcal surface for interactions with ligands and antibodies.²⁷ Interestingly, in addition to its contribution to hemoglobin utilization, Shr binds in vitro to fibronectin and laminin, and contributes to NZ131 (M49 type) attachment to epithelial cells. ECM binding was localized to one of the Shr NEAT domains (NEAT2), although the mechanism involved and the actual binding site have not been elucidated.²⁴ Therefore, like other surface proteins in GAS, Shr appears to be a moonlighting receptor involved in both iron acquisition and adherence.

Using classical genetic approach, Dahesh et al. constructed a *shr* deletion mutant and an isogenic complementation strain in the MIT1 background, and used the isogenic series to investigate Shr contributions to MIT1 biology and pathogenesis.²¹ This analysis revealed that Shr is required for the bacterial growth in blood; this growth phenotype likely resulted from a deficiency in the mutant's ability to obtain heme from the blood hemoglobin. Reduced capacity to use hemoglobin as an iron source was reported earlier for a *shr* mutant constructed in NZ131 (M49-type) background.²⁴ Surprisingly, the MIT1 *shr* mutant was also deficient in ferric chloride use during growth in iron-restricted media. In addition, this mutant exhibited reduced binding to laminin. This is an intriguing observation that highlights Shr participation in MIT1 binding to the extracellular matrix in vivo. Unlike the multiple fibronectin-binding proteins that GAS strains code for, only a few proteins in GAS interact with laminin.²⁸ Finally, loss of *shr* significantly impaired virulence in two mouse infection

models. This observation complements the finding that Shr is needed for GAS virulence in a zebrafish infection model,²⁷ and establishes the importance of Shr to pathogenesis. Further investigation is warranted to illustrate the relative contribution of heme uptake and laminin binding by Shr to GAS infection.

Like Shr, NEAT modules facilitate the capture and/or intra-molecular shuttle of heme in proteins from other Gram-positive bacteria. NEAT domains are highly diverse in primary sequence and function and can exhibit a distinctive ligand spectrum. These domains are found in one or more copies in the heme-binding proteins of the Isd systems described in *Staphylococcus*, *Bacillus* and *Listeria*.^{29,30} The multi-ligand binding that Shr NEAT2 exhibits resembles that of IsdA from *S. aureus*.³¹ In addition to its role in the staphylococcal heme relay machinery, IsdA protein is a broad-spectrum adhesin that decreases surface hydrophobicity facilitating resistance to bactericidal skin fatty acids and antimicrobial peptides.³² Despite the functional similarity observed between Shr and IsdA NEAT domains, Dahesh et al. report that Shr did not contribute to GAS surface hydrophobicity or resistance to antimicrobial peptides.²¹

The work described by Dahesh et al. establishes the importance of Shr to the GAS infection process.²¹ The *shr* gene is not limited to the MIT1 genome; in fact, this protein is highly conserved among the available GAS genomes and may need to be considered part of the GAS core virulon. Shr elicits protective immunity in both passive and active vaccination models in mice.³³ This previous observation, together with the new findings regarding its role in MIT1 pathogenesis, suggests that Shr needs to be considered in future efforts to develop a much-needed GAS vaccine.

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