Role of Streptolysin O in a Mouse Model of Invasive Group A Streptococcal Disease

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Many of the virulence factors that have been characterized for group A streptococci (GAS) are not expressed in all clinical isolates. One putative virulence factor that is present among most is streptolysin O (Slo), a protein with well-characterized cytolytic activity for many eukaryotic cells types. In other bacterial pathogens, proteins homologous to Slo have been shown to be essential for virulence, but the role of Slo in GAS had not been previously examined. To investigate the role of Slo in GAS virulence, we examined both revertible and stable slo mutants in a mouse model of invasive disease. When the revertible slo mutant was used to infect mice, the reversion frequency of bacteria isolated from the wounds and spleens of infected animals was more than 100 times that of the inoculum, indicating that there was selective pressure in the animal for Slo+ GAS. Experiments with the stable slo mutant demonstrated that Slo was not necessary for the formation of necrotic lesions, nor was it necessary for escape from the lesion to cause disseminated infection. Bacteria were present in the spleens of 50% of the mice that survived infection with the stable slo mutant, indicating that dissemination of Slo− GAS does not always cause disease. Finally, mice infected with the stable slo mutant exhibited a significant decrease in mortality rates compared to mice infected with wild-type GAS (P < 0.05), indicating that Slo plays an important role in GAS virulence.

Streptococcus pyogenes, the group A streptococcus (GAS), is a common and important gram-positive pathogen responsible for a range of human diseases, such as the self-limiting diseases impetigo and pharyngitis and the invasive, life-threatening diseases streptococcal toxic shock syndrome and necrotizing fasciitis (7, 42). Even after bacteria are cleared from the host, sequelae, such as glomerulonephritis, rheumatic fever, and rheumatic heart disease, sometimes follow GAS infections. Reports of invasive streptococcal disease have increased in recent years, prompting greater public awareness of the dangers associated with this organism (32, 37). This disease is especially menacing because it has a rapid onset and a high mortality rate and often occurs in otherwise healthy adults.

Unlike other pathogens, for which a given strain or serotype is responsible for a specific type of disease caused by that organism, GAS bacteria do not appear to be so highly specialized with regard to the types of disease they cause. Although there are associations between GAS strains of a given M type and invasive disease (9, 20, 21, 34, 38, 40), many strains cause both life-threatening and self-limiting infections (20, 25, 27). This implies that a given strain of GAS encodes the factors necessary for the establishment of more than one kind of disease and that differential expression of these factors, presumably in response to the host environment, determines the course of infection. Definition of the virulence factors that determine the course of disease is crucial to understanding the development of invasive streptococcal disease. While some invasive infections of deep tissues are associated with a breach of the skin barrier by a puncture, cut, or burn, invasive disease also occurs after blunt trauma and in the absence of identified tissue insult (37, 43). This suggests that in addition to being opportunistic invaders, GAS bacteria are intrinsically capable of breaching host tissue barriers.

Several streptococcal proteins may be involved in the breakdown of host tissues. Among these, Plr and streptokinase have been implicated in tissue destruction through activation of the host protein plasmin (8, 28). Tissue damage specifically associated with the formation of necrotic skin lesions in mice depends on the activity of the cytolysin streptolysin S (Sls) (6). GAS also encodes a second hemolysin, streptolysin O (Slo), which has well-documented cell- and tissue-destructive activity (1). Slo is a secreted protein produced by nearly all clinical GAS isolates tested (1, 26, 36) and is the best characterized of a family of oxygen-sensitive, thiol-activated toxins that includes such established virulence factors as perfringolysin (theta toxin), pneumolysin, and listeriolysin O (2, 5, 10). Slo interacts with cholesterol in target cells to form multisubunit pores and is active on a number of eukaryotic cell types, including erythrocytes, leukocytes, macrophages, platelets, and various cell culture lines (16, 23).

Purified Slo is a very potent toxin and is lethal within minutes when injected intravenously into mice or rabbits (4, 18, 41). At sublethal doses, Slo causes dermal necrotic lesions, venous congestion, and increased vascular permeability as well as various neurological abnormalities before death (11, 15, 29). When used to infect keratinocyte monolayers, Slo mutants show an altered cytokine induction profile compared to that for a wild-type strain (30), suggesting that a subset of the host cell response to GAS infection occurs in response to Slo.

The many biological effects of Slo on the host suggest that it may act at several stages in the infectious process. However, although the effects of purified Slo are well characterized, its role in virulence for GAS infection in vivo has not been addressed directly. We investigated the role of Slo in invasive streptococcal disease using the mouse model described by Schrager et al. (33). In this model, mice are inoculated subcutaneously with a suspension of GAS. Disease progression begins with the formation of a large, raised abscess at the site of inoculation within 24 h postinfection. The abscess next develops a necrotic center, then a sunken necrotic lesion, usually within 24 to 36 h. After formation of the necrotic lesion, mice
either develop bacteremia and die or else begin to clear the infection and recover fully within 10 days. Our examination of two slo mutants of GAS in this model demonstrates that Slo is important for streptococcal virulence and suggests roles for Slo in the process of invasive disease.

MATERIALS AND METHODS

Bacterial strains and culture conditions. S. pyogenes strain AM3 (24) and its derivatives were grown in Todd-Hewitt broth supplemented with 0.2% yeast extract (ThyB) at 37°C. Plate cultures were grown on Todd-Hewitt yeast medium containing 1.5% agar (ThyA) at 37°C. Antibiotics were used at the following concentrations: streptomycin at 1,000 μg/ml, erythromycin at 0.5 μg/ml for S. pyogenes and at 500 μg/ml for Escherichia coli, and kanamycin at 300 μg/ml for S. pyogenes and at 50 μg/ml for E. coli.

Construction of slo mutant strains JRS809 and JRS821. An slo insertion mutant, JRS809, was generated in strain AM3 by insertion of the nonreplicating plasmid pSLO2 as described by Ruiz et al. (30). This plasmid contains an internal fragment of the slo gene. A single recombination event in this region integrates the plasmid within slo, resulting in two incomplete copies of the gene. Inactivation of slo was confirmed by kanamycin resistance and by a lack of Slo-specific hemolytic activity.

JRS821, an AM3 derivative containing an in-frame deletion of the central coding region of slo, was made with the plasmid pSLOD2, using the method described by Ruiz et al. (30) (Fig. 1). pSLOD2 carries the slo.300 allele (30), which is missing 303 internal nucleotides (nt) of coding sequence (nt 736 to 1029). Replacement of the wild-type allele with the allele containing the deletion was confirmed by PCR with primers 3SLO and 3SLO (30) and by the lack of Slo-specific hemolytic activity.

Hemolysis assays. Cell culture supernatants were prepared from 10-ml overnight cultures of the appropriate strains by centrifugation for 10 min at 7,500 × g and filtration of the supernatant through a 0.25-μm-pore-size filter. This cleared supernatant was then incubated for 10 min at room temperature with 10 mM dithiothreitol. Two 0.5-ml aliquots from each sample were placed in 1.5-ml sterile Eppendorf tubes, and 25 μg of cholesterol (Sigma) was added to each sample. Following a 30-min incubation at 37°C, 0.25 ml of a 2% suspension of bovine erythrocytes in phosphate-buffered saline (PBS) (31) was added to each sample, mixed by inversion, and incubated for 30 min at 37°C. Finally, 0.5 ml of PBS was added to each sample, and unlysed erythrocytes were spun out of solution for 1 min at 200 × g in a microcentrifuge. Supernatants were removed, and the release of hemoglobin was assessed by reading the absorbance at 541 nm. Erythrocytes were incubated in water as a positive control for lysis (100% lysis), and fresh ThyB was used as a negative control. Controls were treated exactly as experimental samples, except that medium rather than PBS was added to the water sample in the final step. At least three separate hemolysis assays were performed for each sample.

Reversion assays. Assays to slo + were identified by the loss of kanamycin resistance. Dilutions of overnight cultures grown in the absence of kanamycin were plated on ThyA with streptomycin (ThyA Sm) and grown overnight. These colonies were then replica plated onto ThyA with kanamycin (ThyA Km) and ThyA Sm and incubated overnight. Clones that appeared to be kanamycin sensitive were picked from the original plate and spotted onto ThyA Km and ThyA to confirm the loss of the marker. The reversion frequency (R.F.) was defined as the percent revertant bacteria divided by the number of total bacteria examined.

Inoculation of mice. Overnight cultures of the appropriate strains were used to inoculate 50 ml of ThyB in sterile Klett flasks. Cultures were incubated at 37°C until late log phase (Klett units, ~40), and bacteria were isolated by centrifugation for 10 min at 7,500 × g at 4°C. The cleared supernatant was decanted, bacterial pellets were suspended in the remaining liquid, and the cell suspensions were placed on ice. Bacteria were counted in a Petroff-Hauser chamber, and the suspension was adjusted to ~107 cells/ml with saline. Inoculation of the mice was performed by the method of Schrager et al. (33). Briefly, 5- to 6-week-old female CD1 mice (Charles River Laboratories) were anesthetized by inhalation of Metofane (Schering-Plough, Bloomfield, N.J.), and ~2 cm2 of hair was removed from the left flank with Nair (Carter products, Cranbury, N.J.). One hundred microliters of the adjusted cell suspension was injected subcutaneously with a tuberculin syringe in the center of the bare region. Mice were monitored twice daily and were euthanized by CO2 inhalation when they became hunched, lethargic, and had paralysis in the hind limbs. Mice that did not show signs of illness were euthanized at the end of the 10-day trial. Seven independent experiments involving infections with the wild-type strain AM3 were performed on 76 mice, and five separate experiments involving JRS821 infection were performed on 57 mice. Results for strain JRS809 represent a single experiment with 11 mice.

RESULTS

Construction of an slo- strain, JRS809, by insertion mutation. The slo mutation in strain AM3 was originally constructed as an insertion mutation, in which the nonreplicating plasmid pSLO2 (30) was integrated by homologous recombination into the slo locus to produce the strain JRS809. Integration of pSLO2 into slo was shown by resistance to kanamycin and by a loss of hemolytic activity. Cell culture supernatants from

FIG. 1. Construction of the slo deletion mutant JRS821 by allele replacement. (A) The wild-type allele from strain AM3 was replaced with slo.300 carried on plasmid pSLOD2 as described in Materials and Methods. The arrows indicate primers used for PCR amplification. (B) PCR amplification of slo and slo.300 with primers 3SLO and 3SLO. These primers are common to both strains, but the product generated from strain JRS821 is 303 nt smaller than that from AM3, reflecting the deletion.

Statistical analysis. Survival data were analyzed by Kaplan-Meier product-limit estimates of survival functions and were tested for significance by log rank tests and the Yates correction for the log rank test (13).
AM3 and JRS809 were analyzed for Slo-specific hemolytic activity by incubation with bovine erythrocytes in the presence or absence of cholesterol, an Slo-specific inhibitor. Hemolytic activity was detected in the supernatant of AM3 but not in that of JRS809. Hemolytic activity from the wild-type strain was reduced to background levels by the addition of cholesterol, confirming that it was due to Slo rather than to contaminating SIs (data not shown). We observed no difference between the growth rates of AM3 and JRS809 in broth cultures (data not shown).

**Disease progression and reversion of the slo mutation following infection with JRS809.** Mice were injected subcutaneously with \(-2 \times 10^7\) CFU of AM3 or JRS809. The infection progressed essentially as described by Ashbaugh et al. (3) for both strains. Within 24 h, all mice developed an abscess at the site of infection, and then a necrotic center developed, and this necrotic center finally became a sunken necrotic lesion, usually by day 3. Most of the mice died within 6 days postinfection, but mice that survived for 6 days generally survived through the end of the 10-day experiment. The surviving mice gained weight, and their lesions began to heal. There were no readily observable differences in the time it took to develop lesions or in lesion morphology between mice infected with AM3 and those infected with JRS809. Furthermore, there was no significant difference in mortality rates between the two sets of mice (data not shown).

Mice injected with wild-type or Slo\(^{-}\) bacteria lost \(-12\%\) of their initial body mass in the first day after infection. Mice injected only with sterile saline lost \(<5\%\) of their initial mass, suggesting that the stress of handling, anesthesia, and injection accounted for only a fraction of the weight loss observed when mice were infected with bacteria. Among mice that survived the infection, those infected with the slo\(^{-}\) mutant began to recover from the initial weight loss within 4 days, whereas mice infected with wild-type GAS continued to lose weight and did not begin to recover until the sixth day postinfection (Fig. 2).

This small difference in the time it took for mice to begin to recover from the initial weight loss was the only distinction observed between mice infected with the wild-type and those infected with the mutant strains.

Examination of bacteria recovered from infected mice provided some explanation of the similarities in progression of disease between infections with the wild type and those with the JRS809 strains. The JRS809 inoculum was plated for viable bacteria on ThyA\(^{+}\) plates and then replica plated onto ThyA and ThyA\(^{-}\) Km plates to screen for loss of pSLO2. The R.F. in the JRS809 inoculum was less than 0.3% (0 of 371), whereas 26% of the bacteria isolated from mice infected with JRS809 were revertants (250 of 953). Bacteria isolated from the spleens of mice that showed no signs of illness had a higher R.F. than those from the spleens of mice that became ill, whereas there was little difference in the R.F. of bacteria isolated from the wounds of the two groups of mice (Table 1).

**Construction of JRS821 by gene replacement.** To distinguish the effects of revertant bacteria from those of the slo\(^{-}\) mutant, we constructed a stable slo mutation for further examination in the mouse model. Plasmid pSLOD2 was used to replace the wild-type slo allele with slo.300, resulting in a 303-nt in-frame deletion as described in Materials and Methods (Fig. 1A). Replacement of the wild-type slo with slo.300 was confirmed by PCR. The slo allele from AM3 yielded a product that was 303 bp larger than the product from JRS821 (Fig. 1B), reflecting the internal deletion in JRS821. Hemolysis assays were performed as described for JRS809 to confirm that JRS821 lacked Slo-specific hemolytic activity. As with JRS809, there was no detectable difference in the growth rate of JRS821 from that of AM3 in broth culture (data not shown).

**Effect of Slo on virulence of GAS in murine model of skin infection.** Strain JRS821 was used to infect mice as described for the JRS809 mutant. Mice were injected subcutaneously with \(-10^7\) to \(10^9\) CFU of AM3 or JRS821 and were monitored twice daily for a period of 10 days. Mice were euthanized as they became moribund, and their wounds and spleens were cultured for the presence of streptomycin-resistant bacteria. Mice infected with the wild type and those infected with slo GAS showed no gross differences in the size and shape of necrotic lesions formed.

Mice challenged with the slo\(^{-}\) mutant strain JRS821 showed a 46% survival rate at the end of 10 days, compared to 24% survival for mice challenged with the wild-type GAS strain.
AM3 (Fig. 3). All mice that became moribund or died by day 10 had systemic disease, as confirmed by positive spleen cultures. Most of the mice (16 of 18; 89%) infected with the wild-type strain that did not show signs of systemic disease by 10 days had no detectable bacteria in their spleens (<10 CFU). In contrast, when JRS821 was used to infect mice, half of the mice that did not become ill (14 of 26; 54%) had positive spleen cultures. All mice carried viable GAS at the lesion site throughout the course of the experiment, regardless of the infecting strain.

**Hemolysis phenotype of bacteria isolated from infected mice.** Although strain JRS821 carried a gene replacement that should not have been capable of reverting to slo<sup>+</sup>, hemolysis assays were performed to confirm that bacteria isolated from mice retained the expected hemolytic phenotype. Pooled bacteria isolated from spleens of mice infected with AM3 or JRS821 were cultured overnight in ThyB<sup>+</sup>Sm, and cell culture supernatants were analyzed for Slo-specific lysis of bovine erythrocytes. As expected, bacteria isolated from mice infected with the wild-type strain showed secreted hemolytic activity, while those isolated from the JRS821-infected mice did not (Fig. 4).

**DISCUSSION**

Slo is ubiquitous among GAS strains. Slo, in contrast to many other candidate virulence factors, is expressed by nearly all GAS strains, and the amino acid sequence is conserved. Among virulence factors that appear to be present in all GAS strains, several have been found to be serologically variable. The classical example is the M protein, an important virulence factor whose antigenic diversity serves as the basis for classification of GAS strains (22, 35). More recently, sic, the streptococcal inhibitor of complement, has been found to be even more variable in sequence among GAS isolates than the M protein (17, 39). In these cases, it seems that the sequence of the protein is not essential for its pathogenic function. In contrast, the amino acid sequence of Slo appears to be conserved, since antibodies to Slo react with all strains tested and some even cross-react with the related sulfhydryl-activated toxins from other bacteria (2, 15). It seems likely that the need to preserve its cytolytic activity has served to prevent antigenic variation of Slo. This suggests that Slo activity is important for GAS in vivo and led us to expect that an slo mutant would be likely to be attenuated for virulence in an animal model of infection.

Slo is important for GAS virulence in invasive disease. We have shown Slo to be important for GAS invasive disease in a murine model of skin infection. Our results demonstrate that mice infected with a stable slo deletion mutant have a significantly higher rate of survival than mice infected with wild-type GAS. By 6 days after infection, more than 65% of mice infected with the slo mutant were alive, compared to only 30% of mice infected with wild-type GAS. Nearly half (46%) of the mice infected with the mutant strain survived to the end of the 10-day experiment, compared to only 24% of the mice infected with the wild-type strain. Half (52%) of the mice that survived infection with the slo mutant had positive spleen cultures, indicating that the infection had disseminated in these apparently healthy animals. This demonstrates that dissemination of the slo mutant does not necessarily cause disease.

When animal models were used to examine strains carrying reversible mutations in genes whose products were important for virulence, selective pressure for expression of the virulence factor within the animal led to extensive reversion of the mutation. This has been reported for mutants in the synthesis of the hyaluronic acid capsule and the M protein in mouse models of pneumonia and invasive disease (3, 19). Mutants in these factors were attenuated for virulence and were efficiently cleared by the mice, and bacteria recovered from infected animals had reverted to the wild type. We observed a similar pattern of reversion with a mutant in slo. When JRS809, an slo
GAS strain capable of reversion, was used to infect mice, 26% of the bacteria that were recovered from the animals had reverted to the wild type. This reversion frequency in mouse isolates was more than 100-fold higher than that of the inoculum. This indicates that there is strong selective pressure for expression of Slo in the mice, as there is for the M protein and the hyaluronic acid capsule, and this suggests that Slo is important for the virulence of GAS in this model.

Further support for the need for Slo in GAS pathogenesis comes from epidemiological studies. Müller-Alouf et al. (26) investigated the production of bacterial effector molecules with clinical isolates representing several types of invasive GAS disease and showed that Slo was produced in all of the strains tested. In a similar analysis of clinical isolates from invasive or superficial disease, Shiseki et al. (36) showed a correlation between invasive disease and production of high levels of Slo. While we have not examined the role of Slo for superficial disease, we have shown here that Slo is important for the full virulence of GAS in invasive disease.

Role of Slo in invasive disease. Slo has many effects on various host systems, so it is likely that Slo has more than one role for invasive disease. However, our results suggest that Slo is not important for initiating infection, because mice developed necrotic lesions at the site of the injection regardless of the infecting strain. We did not detect any differences in the time to development of a necrotic lesion, or in the morphology of the lesions formed in mice infected with the slo mutants, compared to the wild type. In dramatic contrast, mutants deficient in production of Slp, a different streptococcal hemolysin, are unable to cause necrotic lesions in mice (6). Slo and Slp have been characterized extensively in vitro and shown to have very different properties. Slo is a secreted, immunogenic, oxygen-labile protein that is specifically inhibited by free cholesterol. Slp is membrane associated, oxygen stable, nonimmunogenic, and insensitive to cholesterol. Thus, it is not surprising that the two proteins, which would be expected to be active in different host environments, would have different roles in the infection process.

Based on its ability to lyse cells and cause tissue damage, we expected Slo to be involved in the escape of GAS from the wound to underlying tissues and in the development of systemic disease. This was not the case, however, since the non-reverting slo strain JRS821 was able to disseminate from the wound. JRS821 bacteria were present in the spleens of all mice that became ill and in half (52%) of the mice that remained healthy, indicating that these mice had disseminated infection. Therefore, Slo does not appear to be necessary for the spread of GAS from the wound.

When the revertible slo insertion mutant JRS809 was used to infect mice, we observed differences in the relative reversion frequencies in the wound and spleen between mice that became ill and mice that survived the experiment. It is difficult to interpret the apparent selection for reversion in a specific host environment because Slo is a diffusible protein. One possibility is that there is a need for a threshold number of Slo+ GAS bacteria in the population to produce sufficient cytolyis for its effectiveness at a given site. There was no obvious difference in the R.F. at the wound site between healthy animals and those that became ill. However, among surviving mice that showed no signs of illness, GAS that was isolated from the spleen showed a higher frequency of Slo+ bacteria than did the wound isolates. Furthermore, the frequency of Slo+ bacteria was higher in the spleens of healthy mice than in the spleens of mice that became ill.

If the bacteria isolated from the spleens of mice were representative of all bacteria in the animal, we would expect that sick mice would show a high frequency of wild-type bacteria, whereas healthy mice would have a higher frequency of mutants. Since we saw just the opposite, a higher frequency of Slo+ bacteria in the spleens of healthy mice than in mice that became ill, it is possible that the spleen isolates represent the bacteria being cleared by the mouse rather than all bacteria present in the mouse.

If the frequencies of wild-type and slo mutant bacteria present in the blood, spleens, and wounds of infected mice could be monitored continuously in mice infected with the revertible JRS809 strain, we would expect to see equal R.F.’s in blood and wound isolates as soon as the bacteria escape from the wound to the blood. As infection progresses, the host clears bacteria from the blood, and its effectiveness in doing so ultimately determines whether or not the animal becomes ill. Therefore, we would expect to see that mice that succumbed to GAS infection were not efficiently clearing the disease-causing bacteria and so had a high frequency of Slo+ bacteria in the spleen but not in the spleen. Conversely, we would expect that by clearing the wild-type bacteria, mice would remain healthy and would show a higher frequency of Slo+ bacteria in the spleen than in the blood. Our data suggest that the frequency of Slo+ GAS bacteria which remain in the animal, rather than the frequency of Slo+ GAS bacteria which initiate systemic disease, determines the progression of disease.

Degree of attenuation of GAS Slo+ mutants. Although slo GAS bacteria were less virulent than the wild-type strain, this difference was less dramatic than that observed for some other virulence factors that have been examined using this model of GAS infection. Mutants deficient in production of the M protein or hyaluronic acid capsule were strongly attenuated (less than 10% mortality) for virulence in this model of invasive disease (3). The role of streptococcal cysteine protease has also been examined in this murine model using two different M3 strains of GAS. The results varied from no observable effect to a very clear attenuation of virulence (10 to 80% mortality) (3, 24), suggesting that cysteine protease is important for virulence in some strains of GAS.

The slo mutant JRS821 had an intermediate effect on virulence compared to strains carrying mutations in the M protein or hyaluronic acid capsule, indicating that Slo is important, but not essential, in this model of invasive disease. This may be because other cell- and tissue-destructive activities of the GAS strain partially compensate for the lack of Slo in the disease process. It is also possible that a major role of Slo occurs at an early stage of the infectious process that is bypassed by subcutaneous injection of the inoculum. For instance, Slo might be important in penetration of the GAS bacteria through the layers of skin to reach the deeper tissue. The cell and tissue destruction associated with bacterial invasion of the skin is likely to induce a cytokine profile that differs from that generated following subcutaneous injection (14, 30). If Slo has a role in counteracting the immune response specific to this type of infection, the model used here would probably not detect it.

Another consideration in evaluating the degree of virulence of the slo mutant in this model is that mice are significantly less sensitive to the toxicity of Slo than are other animals. Howard and Wallace noted that the 50% lethal dose for purified Slo was 30 times higher for mice than for rabbits or guinea pigs when adjusted for body mass (18). Thus, Slo might have a much larger role in the disease process in a different animal model and may have a larger role in human disease as well.

Conclusion. We have shown Slo to be important for full virulence in a mouse model of invasive GAS disease. Contrary to what one might expect, the cytolytic activity of Slo was not necessary for the formation of the necrotic lesion, nor was it
necessary for the spread of GAS from the lesion. However, once the bacteria had disseminated, Slo was an important factor in the ability to cause systemic disease. Thus, the important activity of Slo does not appear to be in causing localized tissue damage following subcutaneous inoculation, a process for which SlpO appears to be required.

Instead, Slo appears to be important for systemic GAS disease. The production of active Slo in the bloodstream and its activity on various cells and tissues throughout the host may elicit overstimulation of the host immune system. In support of this idea, Slo has been implicated in the host-mediated inflammation and destruction of tissues through synergy with other streptococcal proteins and host immune cells and molecules (12). If Slo is important as a general activator of the immune system, it is likely to be important for the establishment of other types of streptococcal disease in addition to invasive disease. For example, Slo could be important for streptococcal pharyngitis by eliciting inflammation of tissues near the site of infection. Arousal of such widespread activation of the immune system by Slo might also be important in the induction of streptococcal toxic shock syndrome. Likewise, immune stimulation by Slo could be important for postinfectious sequelae of GAS infections, such as acute rheumatic fever, glomerulonephritis, and the neurological diseases associated with streptococcal infection. Further analysis of the mutants constructed in this study, particularly with regard to the specific host responses to infection with wild-type or slo GAS, might lead to a better understanding of some of the complex ways that GAS interacts with the host to cause disease.

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