Group A Streptococcal Virulence: New Lessons

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Group A streptococci (GAS) (*Streptococcus pyogenes*) are implicated in a wide spectrum of illnesses, perhaps the widest among human infectious agents. These illnesses include generally mild infections (eg, streptococcal pharyngitis and impetigo), severe invasive infections (eg, necrotizing fasciitis and streptococcal toxic shock syndrome [STSS]), immune-mediated nonpyogenic illnesses (eg, acute rheumatic fever, poststreptococcal acute glomerulonephritis, and poststreptococcal reactive arthritis), and perhaps the hypothesized pediatric autoimmune neuropsychiatric disorders (possibly) associated with streptococcal infection (PANDAS). It has been estimated that annually, GAS causes 600 million infections globally and approximately 500 000 deaths [1], including 10 000 to 13 000 severe invasive infections in the United States [2].

An organism with the potential to trigger a wide repertoire of illnesses might be expected to secrete and/or display on its surface a wide variety of virulence factors, and GAS does exactly that. A large group of secreted proteins and cell-associated components expressed by this organism have been proposed as virulence factors, and this theory has generally been supported by studies of isogenic mutants

in animal models and/or by pathogenic effects produced in vitro. Among others, these proteins include streptolysin O, streptolysin S, the group A-specific cell wall carbohydrate, the type-specific M protein, fibronectin-binding proteins, collagen-like proteins, laminin-binding proteins, plasminogen-binding proteins, C5a peptidase, SpyCEP, streptococcal pyogenic exotoxin B (SpeB), streptococcal inhibitor of complement (SIC), multiple pyrogenic exotoxins (Spe), and the immunologically inert hyaluronic acid (HA) capsule [3].

The capsule of GAS is a linear polymer of *N*-acetyl glucosamine and glucuronic acid that is identical to the HA capsule found in higher animals, including humans, and thus is poorly immunogenic. During the exponential phase of growth, the HA capsule is associated with the GAS cell surface, and it is shed during the stationary growth phase. The HA capsule comprises the GAS capsule, which is responsible for the mucoid appearance of GAS colonies that grow on solid medium. The role of the HA capsule in GAS virulence has been long recognized but is rather complex. The large majority of the >220 M types of GAS possess the 3 genes necessary for synthesis of the HA capsule, but the relatively common M-4 and M-22 strains in particular lack these genes and do not produce capsules, and unlike the majority of GAS strains, they produce hyaluronidase, which degrades HA. Strain-to-strain variability in the amount of capsule produced both within an M type and between M types likely reflects differences in the promoter gene region upstream of the 3 HA capsule-synthesizing genes [4].

In the 1920s and 1930s, Lancefield and Todd [5] found that the mucoid colonial morphology of GAS was correlated with increased virulence in mice. A few decades later, it was found that hyaluronidase treatment increased the susceptibility of mucoid strains to killing by human phagocytes and that hyaluronidase treatment in mice reduced GAS virulence; these studies supported a role for capsule in resistance to phagocytosis and increased virulence [6]. In addition, several epidemiologic studies found that mucoid GAS strains were associated particularly with invasive infections and with acute rheumatic fever outbreaks [7].

When genetic manipulation became possible and acapsular mutants of GAS were developed, reduced virulence in several animal models was found, and susceptibility to complement-mediated opsonophagocytic killing by leukocytes was increased. Thus, it seems that capsule enhances virulence by increasing resistance to opsonophagocytosis [8]. Results of recent studies with human pharyngeal samples suggest the possibility that downregulation of GAS capsule production is associated with chronic pharyngeal carriage [9]. In addition, CD44, which is a HA-binding protein that is expressed on multiple cell types, might be a GAS receptor in the pharynx and could facilitate GAS tissue invasion in addition to adhesion to oropharyngeal cells [10].

With this background, the 2 GAS articles in this issue of *Journal of Pediatric Infectious Diseases Society* are of interest. In the article by Flores et al [11], surveillance of GAS isolated from patients with

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different clinical syndromes over a recent 4-year period at Texas Children's Hospital yielded interesting findings. Between 2013 and 2017, the relative frequencies of infection by M-1 and M-6 (both common encapsulated types) declined, whereas the proportions of M-4, M-22, M-28, M-87, and M-89 (all nonencapsulated types) increased significantly as a cause of invasive disease and skin and soft-tissue infection. Nevertheless, the infection in 75% of the children admitted to the pediatric intensive care unit, including the 2 who died, was caused by an encapsulated strain, especially M-1. A similar significant shift toward unencapsulated types among pharyngeal strains was not found during the study period, which is curious, because the throats of children are considered the major reservoir of GAS in the community. As we found previously, there is a general ebb and flow of prevalent pediatric pharyngeal GAS strains from year to year within a given community [12], so a longer period of observation will be necessary to assess if the observed trend is sustained.

The observation in this study that unencapsulated M types of GAS were increasing in frequency in the community underscores the versatility of this organism in maintaining a virulent phenotype. As indicated above, the prominent role of the hyaluronate capsule in resistance to phagocytosis and in adhesion and invasion has historically been emphasized (reviewed in reference [13]). Although different M types of GAS have been found to rely variably on the capsule versus M protein for virulence [8], the apparent increasing frequency of genetically acapsular mutants suggests that additional compensatory virulence mechanisms are at play. Additional studies of these potentially emerging variants might uncover new and evolving

virulence determinants and potential therapeutic targets.

In the second report in this issue, by the same Houston team, 19 cases of pediatric GAS toxic shock syndrome (STSS) between 2012 and 2016 were studied retrospectively regarding clinical features in the patients, and whole-genome sequencing (WGS) was performed for all 19 GAS isolates [14]. Clinically, it was surprising that none of the patients received intravenous immunoglobulin, and only 47% of them received clindamycin despite current recommendations [15] (thought to be a result of the underrecognition by caregivers of the role of GAS in patients who present with shock).

The 19 GAS isolates were subjected to WGS to identify potential genetic signatures associated with STSS. Compared with reference sequences in the Centers for Disease Control and Prevention Active Bacterial Core Surveillance database, only the 3 M-87 isolates revealed significant differences. These 3 isolates contained a mutation in the histidine kinase component (MaeK) of the 2-component system DpiBA, which is involved in the regulation of malate metabolism. In GAS and other lactic acid bacteria, the malic acid pathway might be an alternative carbon source during stress [16]. Although the precise role of the malic enzyme pathway and its regulation in virulence are not yet clear, these findings certainly provide the substrate for additional studies using the clinical isolates of M-87 and other genetically modified strains to unravel what could be important clues in the pathogenesis of STSS. In addition, the results of this study underscore the utility of WGS as an important tool in uncovering the genetic basis of the protean clinical manifestations of GAS infections.

It is fascinating that our old acquaintance GAS continues to evolve and reveal mysteries related to its various virulence

mechanisms and its structural and secreted proteins.

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