

Fitness genes of group A streptococci in necrotizing fasciitis and myositis

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Necrotizing fasciitis and myositis caused by group A streptococci (GAS) are among the most fulminating infections, with a mortality rate of 20% to 30%. Although numerous regimens have been utilized in attempts to control these devastating infections, such as combinations of various antimicrobial agents and intravenous immunoglobulin (IVIG) as well as hyperbaric oxygen therapy, none have been the complete answer. Zhu and colleagues have utilized a transposon-directed insertion-site sequencing (TraDIS) protocol to identify 126 genes of M1 and 116 genes of M28 strains of GAS required for myositis, of which 25% encode transporters, which could be used as possible targets for future therapeutic protocols.

Group A streptococcal fasciitis and myositis

Bacteria known as *Streptococcus pyogenes*, group A, can cause a wide variety of infections that range from minor illnesses, such as strep throat and mild skin infection, to very severe and life-threatening poststreptococcal diseases, such as rheumatic fever and glomerulonephritis. Severe invasive group A streptococcal infections resulting in necrotizing fasciitis and myositis were first recognized by Pfanner (1) in 1918, who called them necrotizing erysipelas, and by Meleney (2) in 1924, who described necrotizing gangrene after infections of minor lesions due to streptococci. As a young epidemic intelligence service officer, I personally witnessed the devastating effects of group A streptococcal infection at the US Air Force Academy in Colorado Springs in 1968, during which over 1000 of the 2000 enrolled cadets became violently ill after ingesting a group A streptococcus-contaminated tuna salad in the cadet cafeteria (3). Furthermore, we then observed and reported on the large epidemic of acute rheumatic fever in 74 patients in the intermountain West in

1985–1986 (4), which persisted until 1992, during which a total of 274 cases occurred (5). Dennis Stevens and his colleagues in Boise, Idaho, then reported an outbreak of 20 cases of toxic shock-like syndrome with necrotizing fasciitis and myositis in the same intermountain region, often due predominantly to M1 and M28 group A streptococci (GAS) (6). Subsequently, a number of additional reports published on similar patients implicated streptococcal enzymes, such as streptolysin O, streptococcal pyogenic exotoxin, and streptococcal NADases (7–9).

At the University of Utah, we saw a large series of patients with invasive, toxic, necrotizing GAS infections and found that most of these individuals suffering these severe life-threatening infections had a fairly normal immune system. We and our colleagues in obstetrics and gynecology have also seen a number of patients suffering postpartum group A streptococcal infections leading to severe necrotizing tissue destruction including, in one dramatic case, the amputation of 3 of 4 limbs due to necrotizing fasciitis and myositis after pregnancy. The only abnormalities we and

our colleagues have found were related to alterations in cytokine gene expression and enhanced inflammatory cytokine production in some patients (10). Large outbreaks of group A streptococcal invasive, necrotizing infections were also reported in Canada, the United Kingdom, Sweden, etc. due to M types with apparent enhanced virulence (11). Norrby-Teglund and colleagues (12) and Barry and associates (13) as well as numerous others have employed polyclonal intravenous immunoglobulin (IVIG) and, in some cases, immunoglobulin obtained from patients who had suffered severe invasive infections to treat GAS necrotizing infections with some success. In spite of these attempts, however, there is still no conclusive proof of an effective antimicrobial or immunomodulatory therapy that is truly and verifiably effective in treating these often fatal, necrotizing infections. This underscores the need for a new approach to finding genes that contribute to the virulence of these organisms that might be targeted, in the future, for more effective therapies.

Virulence gene targeting

Although genome-wide transposon mutagenesis has been employed to investigate GAS fitness genes *ex vivo* in saliva and blood samples, there are no data from samples from human or nonhuman primates (NHP) with myositis. Since group A streptococcal infections are relatively specific in their presentation in humans and NHP compared with other animal species, such as mice, Zhu and colleagues (14) took the excellent approach of collaborating with the Animal Health Trust (Suffolk, United Kingdom) as well as with the Department of Veterinary Medicine, University of Cambridge (Cambridge, United Kingdom) to carry out these critical studies in the NHP cynomolgus macaques, which are similar to humans (Figure 1). Six animals were inoculated intramuscularly with the GAS strains M1 or M28, followed by necropsy and muscle biopsy after 28 hours.

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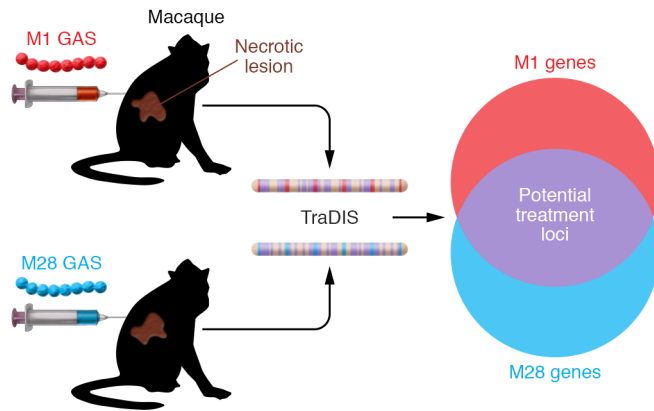


Figure 1. Using TraDIS to assess GAS myositis gene fitness in cynomolgus macaques. Cynomolgus macaques inoculated intramuscularly with the GAS strains M1 or M28 developed necrotic lesions. TraDIS was used to identify fitness genes overlapping between M1 and M28, marking these conserved regions as potential therapeutic targets in cynomolgus macaques.

Furthermore, the authors employed what is likely the newest and perhaps most sophisticated method for high-throughput genome-wide screening, termed transposon-directed insertion-site sequencing (TraDIS) (15), which has been used to analyze the gene fitness of GAS in human saliva. In these studies, the authors examined two genetically distinct and highly virulent GAS strains, M1 and M28, which frequently cause necrotizing fasciitis and myositis in humans.

TraDIS gene-fitness studies in cynomolgus macaques with GAS myositis

The authors used highly saturated transposon mutant libraries and identified 126 genes in the M1 and 116 genes in the M28 GAS strains that were crucial for fitness. Interestingly, 96% of the M1 fitness genes and 93% of the M28 fitness genes were also present in 61 of 62 additional GAS strains examined, indicating a likely critical role for these genes in most cases of GAS myositis. Several of the overlapping genes were metabolic or virulence/fitness genes, marking these conserved regions as potential therapeutic targets. The genes important in NHP myositis did not, however, often overlap with those for fitness genes in saliva (15%–21%) and revealed that, while amino acid uptake is important for GAS fitness during muscle infections, phosphate uptake is essential for growth in human saliva. Similarly, there was little overlap of these GAS fitness genes in NHP myositis with those for growth in human blood or in mouse subcutaneous infection. This suggests that the environment of the infected NHP skeletal muscle is distinct from that of human saliva or the mouse subcutaneous infection model, pointing

toward existence of unique genes involved in GAS causing other human infections, such as puerperal sepsis and pharyngitis. Of great interest is the observation that genes encoding for transporters, which promote transport of critical amino acids, ions, vitamins, and nutrients, were quite common (25%–30%) in both M1 and M28 strains. Moreover, supplementing the growth medium with these amino acids, such as methionine, restored the growth fitness of M1 and M28, indicating that blocking the uptake of these amino acids by GAS could be a potential strategy for treating GAS infection.

Future directions suggested by these results

While numerous therapies have been proposed and utilized in attempting to treat group A streptococcal necrotizing fasciitis and myositis, including poly antimicrobial therapy, polyclonal and monoclonal antibody development, and hyperbaric oxygen chamber treatment, they are yet to be adequately documented as reliably reducing mortality or lowering the profound damage due to the necrotic nature of these fulminant infections. While at present there are no data available to support the concept, this study suggests the possibility that inhibition or blocking, either locally (topically) or intravenously, of appropriate amino acids, nutrients, vitamins, or some of the GAS fitness genes in the myositis-causing strains might, in the future, lead to more successful outcomes. An attempt should be made to do this in NHP with necrotizing fasciitis or even in cultures of GAS necrotic muscle tissue *ex vivo* as the next step in possible therapeutic development.

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