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Tissue tropisms in group A *Streptococcus*: what virulence factors distinguish pharyngitis from impetigo strains?

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Abstract

Purpose of review—Group A streptococci (GAS) are a common cause of pharyngitis and impetigo, and distinct throat strains and skin strains have been long recognized. This review aims to describe recent advances in molecular differences between throat and skin strains, and the pathogenic mechanisms used by virulence factors that may distinguish between these two groups.

Recent findings—Recent findings include a new typing scheme for GAS strains based on sequence clusters of genes encoding the entire surface-exposed portion of M protein; correlations between *emm*-based typing schemes, clinical disease and surface adhesins; covalent bond formation mediated by GAS pili and other adhesins in binding to host ligands; a key role for superantigens in oropharyngeal infection via binding major histocompatibility complex class II antigen; and migration of GAS-specific Th17 cells from the upper respiratory tract to the brain, which may be relevant to autoimmune sequelae.

Summary—The gap between molecular markers of disease (correlation) and virulence mechanisms (causation) in the establishment of tissue tropisms for GAS infection currently remains wide, but the gap also continues to narrow. Whole genome sequencing combined with mutant construction and improvements in animal models for oropharyngeal infection by GAS may help pave the way for new discoveries.

Keywords

group A *Streptococcus*; impetigo; M protein; pharyngitis; pili

INTRODUCTION

Group A *Streptococcus* (GAS) is one of the most prevalent bacterial pathogens causing human disease worldwide, ranging from the fairly mild to highly severe. Organisms of this species are remarkable for the extensive diversity in their spectrum of virulence factors and surface antigens. Importantly, those variant features can be used to organize GAS strains into subgroups that, in turn, correlate with distinct clinical features.

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Conflicts of interest

There are no conflicts of interest.

The primary ecological niche of GAS is the superficial epithelium of the oropharynx or skin. It is at these tissues that GAS can cause mild infections (>700 million/year) or persist in an asymptomatic carrier state (at the throat) [1,2]. Impetigo predominates in tropical and subtropical regions (transmission via skin contact), whereas pharyngitis spikes when the weather drives people indoors (transmission via respiratory droplets) and thus, geospatial distances arise because of environmental factors that favor one mode of transmission over the other. It has been long recognized that there exist discrete ‘throat strains’ and ‘skin strains’ and in more recent years the molecular basis for this distinction has been delineated to some extent.

The molecular underpinnings for the tendency of a given GAS strain to preferentially infect either the throat or skin is not completely understood. However, recent discoveries advance our knowledge of the molecular markers and virulence factors which define the clinically distinct subgroups of GAS strains causing pharyngitis vs. impetigo.

MOLECULAR CORRELATES OF GROUP A STREPTOCOCCUS DISEASE

Classical throat and skin strains [3] were recognized as such based on their M protein serotype, defined by the N-terminal amino acid sequence at the tips of the M protein cell surface fibril [4]. Some 20 years ago a nucleotide-based typing scheme arose based on the extreme 5′ end of the *emm* gene, with more than 230 *emm* types characterized to date [5,6]. Around that same time, three major *emm* pattern groups were defined based on sequence differences in the more highly conserved 3′ end of *emm* and flanking *emm-like* genes (i.e., *mip* and *enn*), encoding the cell wall-spanning domain of M and M-like proteins (Fig. 1a) [7–9,10]. *emm* type is highly predictive of *emm* pattern group based on the vast majority (97%) of GAS strains measured for both features [13] and thus, reasonably good inferences can be made for *emm* pattern based solely on knowledge of *emm* type.

The *emm* pattern group – known as *emm* patterns A–C, D, and E – displays highly significant associations with pharyngitis and impetigo [10,13,14]. A meta-analysis of 23 pharyngitis and six impetigo population-based surveys conducted throughout the world [10, 11] shows that pattern A–C strains represent ~47% of pharyngitis isolates but only 8% of impetigo isolates (Fig. 1b); *emm* pattern A–C strains represent the classical throat strains and are designated ‘throat specialists’. In sharp contrast, pattern D strains represent ~50% of impetigo isolates, but only ~2% of pharyngitis isolates; they are classical skin strains and designated ‘skin specialists.’ In contrast, *emm* pattern E isolates account for almost equal fractions of throat and skin infections and as a group, they are designated ‘generalists.’

The M proteins discussed in this review belong to *emm* pattern groups A–C (M1, M3, M12, M18) and E (M89). A new molecular typing scheme for GAS developed in late 2014 is based on the phylogeny of the sequence of the entire surface-exposed portion of mature M protein, which contains the determinants of M serotype plus functional binding domains. The scheme is known as ‘*emm* cluster’ typing whereby 97% of *emm* types fall into a major clade (X or Y), and 82% of *emm* types are assigned to one of 16 well supported sequence clusters [12]. Since its introduction, *emm* cluster analysis has been widely adopted for molecular epidemiology studies [15–20].

The *emm* cluster typing scheme can be applied to the *emm* typing and *emm* pattern data for the 29 population-based surveillance studies on GAS pharyngitis and impetigo infections, as reported in [10■,11]. Figure 1c shows that more than 99% of *emm* pattern A–C isolates (so-called throat specialists) have an *emm* type belonging to clade Y, largely within clusters AC1–5. Similarly, the vast majority of *emm* pattern D isolates (so-called skin specialists) also belong to clade Y, but largely in *emm* clusters D1–5, with D4 being most dominant. In contrast, the *emm* pattern E isolates (generalists) are restricted to clade X, although cluster E6 contains ~14% of the total pattern D isolates. The fractional ratio of pharyngitis to impetigo isolates is highly skewed for clusters AC1–5 (high) and D1–5 (low) (Fig. 1d). Overall, the *emm* cluster scheme, based on the entire surface-exposed portion of M protein, is highly consistent with the *emm* pattern scheme that is based on the C-terminal peptidoglycan-spanning domain of *emm* and *emm-like* flanking genes.

MULTIFUNCTIONAL M PROTEIN

The strong correlations between *emm* genotypes and clinical phenotypes provide a molecular handle for elucidating the basis of tissue site preferences for infection at either the throat or skin. Several functional domains within M proteins, and their predicted binding motifs, strongly correlate with *emm* clusters, including plasminogen-binding in *emm* cluster D4 and fibrinogen-binding in most A–C clusters within clade Y [12■■].

The differential binding activities for human host proteins are potential determinants of tissue tropism. In particular, the plasminogen-binding M protein of *emm* cluster D4 (known as PAM) is associated with a very strong preference for impetigo over pharyngitis (Fig. 1e). Many recent studies have enriched our knowledge of the molecular specificity of PAM and its interaction with streptokinase [21–25]. Despite advances on M protein-based GAS–host interactions, precisely how differential modulation of the coagulation–fibrinolysis pathways [26] may translate into bacterial tissue tropisms remains elusive.

FCT-REGION PRODUCTS AND ADHERENCE

Ten years ago the discovery was made that T-antigens, which form the basis for a second major serotyping scheme for GAS, are comprised of thin, elongated pili [27]. This finding had a profound impact on our recent understanding of GAS epidemiology and pathogenesis [28–40]. The fibronectin- and collagen-binding proteins and T-antigen loci, known as the FCT-region, harbors the T-antigen genes and lies ~300 kb from *emm* on the GAS chromosome. It encodes pilus proteins and additional adhesins and has at least nine major forms [41–44]. There are significant correlations between several genes within the FCT-region and *emm* pattern and/or *emm* type [10■,45–48] (Fig. 2a–c).

A signature feature of GAS pili is that the backbone and accessory pilin proteins spontaneously form covalent intramolecular isopeptide bonds promoting mechanical stabilization to counteract the shearing forces that occur during bacterial adherence to the host epithelium [49–55]. A more recent, major advance lies in the finding that internal thioester bond formation leads to covalent linkage of GAS adhesins to host ligands, whereby GAS adhesins act as ‘chemical harpoons’ [53,56,57■,58■,59]. Motifs for thioester domains

(TEDs) are present in the pilin tip adhesin (Cpa) and adhesins encoded by other FCT-region genes (PrtF1/SfbI and the FbaB form of PrtF2) (Fig. 2a).

The highly reactive thioester bonds of PrtF1/SfbI and PrtF2/FbaB (but not Cpa) bind human fibrinogen present in purified form and plasma, and fibrinogen-associated with epithelial cell surfaces mimicking an inflammatory state [58■]. Furthermore, thioester binding to fibrinogen involves only a single Lys residue, highlighting the specificity of the binding reaction. If indeed GAS pili and other FCT-region products are determinants of tissue tropism, as supported by strong epidemiological correlates, how might TEDs confer tissue-specific binding? Do there exist other physiologically relevant host ligands for GAS-mediated thioester bond formation, in addition to fibrinogen?

Sequence heterogeneity is particularly high among pilin backbone proteins (e.g., FctA), pilin tip proteins (e.g., Cpa) and the N-terminal half of PrtF1/SfbI [44,45,60]. Some structural forms of Cpa have two distinct TEDs, whereas other Cpa forms are devoid of putative TEDs [56,57■]. Is thioester bond formation and host target ligand specificity influenced by the adjacent polypeptide sequence and conformation of FCT proteins? Insofar as the fibrinogen ligand, fibrinogen binding by M1 protein blocks phagocytosis of GAS by neutrophils, but also inhibits GAS adherence to epithelial cells [61■]. Do M1 protein–fibrinogen interactions counteract processes mediated by TEDs and FCT-region proteins? The PrtF1/SfbI and PrtF2/FbaB proteins that bind fibrinogen via TEDs in the N-terminal region are multifunctional and also act as adhesins by binding fibronectin through C-terminal repeat domains [58■]. PrtF1 and PrtF2 promote cellular invasion by GAS into epithelial and/or endothelial cells, whereby the mechanism of cellular uptake is dictated by the specific PrtF1/PrtF2 molecular form [62,63]. Differential transcription and posttranslational modifications of FCT-region proteins, often influenced by the microenvironment of the infection site within the host, likely add further levels of complexity to the possible mechanisms by which thioester bond formation might confer host tissue specificity for throat (pharyngitis) vs. skin (impetigo) infections caused by GAS.

NEW INSIGHTS ON GROUP A STREPTOCOCCUS PATHOGENESIS AT THE UPPER RESPIRATORY TRACT

One of the challenges to understanding the pathogenesis of GAS on a molecular level derives from the fact that GAS is strictly a human pathogen. Mice engrafted with human skin provide a highly valid model for impetigo, whereby low physiological inoculum doses of GAS yield histopathological alterations that capture many of the key features of human disease (reviewed in [64]). In contrast, animal models for GAS infection at the upper respiratory tract (URT) have, historically, had many shortcomings, partly because of the very high inoculum doses of GAS required.

GAS can cause acute pharyngitis, but far more often GAS persists at the URT in a semidormant carrier state wherein the human host has no disease symptoms and transmission rates are probably quite low. The strong association between *emm* genotypes and the throat (Fig. 1) is limited to pharyngitis, and does not include asymptomatic carriage.

One major challenge has been to experimentally distinguish between these two forms of GAS–host interactions (carriage vs. pharyngitis) at the URT.

M3 GAS recovered from the URT of humans lacking signs of infection underwent whole genome sequencing in an effort to identify nucleotide polymorphisms that are unique to carriage strains, via comparison to invasive disease isolates [32–35]. Phenotypes favorable to carriage (as measured by adherence and/or invasion of epithelial cells), and less favorable for invasive disease (e.g., survival in blood, soft-tissue infection), include decreased hyaluronic acid capsule production, decreased transcription of the gene encoding Mga (an activator of *emm* gene transcription), increased cell surface expression of the collagen-like protein SclA, and pleiotropic effects resulting from an amino acid change in the sensor kinase LiaS [32–35]. However, nasopharyngeal colonization by GAS in the mouse does not strictly parallel these particular carriage vs. invasive genotypes [65–67], perhaps indicative of complex relationships involving multiple factors.

It appears that a high bacterial burden during nasopharyngeal GAS colonization of mice better represents pharyngitis as opposed to carriage. However in humans, acute pharyngitis is often accompanied by a purulent exudate that is typically not observed in mice. Also in humans, a specific immune response acts to clear a GAS URT infection by ~7–10 days, whereas asymptomatic carriage can linger for weeks or even months [2,68,69]. The duration of GAS colonization in mice can extend well beyond what is typical for acute pharyngitis in humans and it is not known whether long-term mouse colonization is a better mimic of pharyngitis or throat carriage in this particular regard.

Consistent with the findings of a classical study [70], several recent reports show that hyaluronic acid capsule production enhances nasopharyngeal colonization in the mouse. Truncation of the multifunctional regulator RocA, which modulates the CovRS two-component system, leads to hyperencapsulation of GAS of several M-types [71–73,74■]. In M18 strains, truncated *rocA* leads to increased longevity of nasopharyngeal carriage of GAS in mice and enhanced GAS transmission by a respiratory route, as evidenced by bacterial shedding [71]. Similarly, the truncated RocA gene confers hyperencapsulation in M3 strains [74■,75,76]; its effect on GAS behavior in URT models has not yet been reported. The hyaluronic acid capsule facilitates GAS adherence to the oropharyngeal epithelium by binding human CD44 [77]. Recent mouse studies show that capsular hyaluronic acid is also essential for GAS dissemination from the URT to locally draining lymph nodes, via interaction with lymphatic vessel endothelial receptor-1 [78■■]. The authors speculate that hyperencapsulated M18 GAS get trapped in the lymphoid tissue, thereby limiting GAS spread to the bloodstream and perhaps offering an explanation for why M18 strains are rarely recovered from invasive disease but are often associated with acute rheumatic fever.

A recently emerged M89 clade of GAS associated with a high incidence of invasive disease lacks hyaluronic acid capsule but displays enhanced production of the exotoxins nicotinamide adenine dinucleotide (NAD)-glycohydrolase (NGA, which is also known as SPN) and streptolysin O (SLO) [79■,80■■]. The *nga-slo* locus of the new M89 clade is most similar in sequence to that of M12 and modern M1 strains, suggesting acquisition from a recent common ancestor [79■,80■■,81■■,82]. In a mouse model for soft-tissue infection, the

capsule is dispensable if NGA/SPN and SLO toxin levels are high [80■]. The precise evolutionary history of the epidemic M89 clade remains somewhat controversial for European vs. North American clones [83–85]. The modern M1 strain, which is frequently recovered from cases of pharyngitis [86], yields a higher bacterial burden and longevity in URT colonization in a nonhuman primate model as a direct consequence of its newly acquired *nga-slo* locus [81■]. An accessory gene region (AGR-3) that lies immediately downstream of *nga-slo* is differentially distributed among GAS strains in accordance with the *emm* pattern group, as are isoforms of the *spn/nga-ifs* (*ifs*, gene encoding immunity factor for SPN) gene products [10■,48,87].

A hallmark feature of GAS is the production of superantigens (SAGs), which act as virulence factors in severe invasive disease [88,89]. Since invasive disease is rare, the ecological viewpoint begs the question: What purpose do SAGs serve the organism within its primary niche? A recent study examined the role of SAGs in GAS–nasopharyngeal interactions using transgenic mice expressing human leukocyte antigen (HLA) class II antigens [90■]. Using an M18 strain, a 10000-fold increase in bacterial burden in nasal tissue was evident for HLA-humanized mice as compared with unmodified mice (although GAS inoculum doses were quite high in these experiments). Importantly, deletion of select SAG genes abrogated this effect. Furthermore, vaccination with a toxoid form of SAG protected mice from nasopharyngeal challenge with GAS, paving a novel path toward GAS disease control [90■]. There exists more than 10 variant SAG genes that have a differential presence/absence among GAS strains [91■,92,93■], and correlations between SAG alleles and *emm* pattern group have been noted [94]. The degree to which recent acquisition of SAG genes leads to emergence of new clones as a consequence of enhanced URT colonization and/or transmission is a topic for future inquiries.

GAS induces a robust Th17 response in nasal-associated lymphoid tissue (NALT) in mice, which is the functional equivalent of human palatine tonsils [95–97]. In a recent advance, intranasal challenge of mice with live GAS promoted migration of GAS-specific Th17 cells from NALT into the brain, with breakdown of the blood–brain barrier, extravasation of IgG, and loss of excitatory synaptic proteins within the central nervous system [98■]. This animal model holds great promise for better understanding the induction of autoimmune disease triggered by GAS infection at the URT, such as acute rheumatic fever (specifically, Sydenham’s chorea) and possibly other neuropsychiatric disorders [99]. The M-types of the classical rheumatogenic GAS [100,101] are recognized as a subset of the throat specialist strains, although that concept continues to be challenged [20].

CONCLUSION

A gap in our understanding remains between the molecular correlates of GAS disease and the pathogenic mechanisms of key virulence factors mediating superficial throat vs. skin infection. More precise characterization of the genes and nucleotide sequences that distinguish the populations of throat and skin strains, combined with improved animal models for GAS disease, are likely to strengthen the connections between epidemiology correlates and tissue tropism mechanisms.

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KEY POINTS

- A new M protein-based cluster scheme identifies GAS having strong tissue site preferences for superficial infection at either the skin (impetigo) or throat (pharyngitis).
- Pili and adhesins of the FCT-region correlate with clinical disease and are strong candidates for establishing tissue tropisms; they can function as chemical harpoons by forming covalent thioester bonds with host ligands, such as fibrinogen.
- Virulence factors that enhance GAS colonization at the URT in mice include SAgS and hyaluronic acid capsule; however, the importance of the latter in disease can depend on the remaining spectrum of virulence factors produced by the bacterium.

FIGURE 1.

emm region and clinical correlates. (a) Genetic structure of the *emm* chromosomal region depicting *emm* genes, flanking *emm*-like genes, and *emm* pattern groupings A-C, D, and E. Note that only the *emm* genes harbor *emm* type-specific determinants. Based on [7-9,10]. (b) Distribution of 4674 pharyngitis isolates and 765 impetigo isolates, in accordance with *emm* pattern groups A-C, D, and E. (c) Distribution of *emm* pattern groups across *emm* clades. Plot depicts the percentage of GAS isolates [as described in (b)] within each *emm* pattern group that are associated with either clade X or clade Y [10,11,12]. (d)

Distribution of 4674 pharyngitis isolates and 765 impetigo isolates, in accordance with *emm* clades and *emm* clusters (excluding 29 isolates that do fall into any of the four *emm* clade/cluster groups shown) [10■,11,12■]. The fractional ratio of pharyngitis to impetigo isolates for each *emm* cluster group is indicated. (e) Distribution of pharyngitis ($N=1852$) and impetigo ($N=277$) isolates among select *emm* clusters. The *emm* clusters associated with fibrinogen-binding include A-C1, A-C3, A-C4, A-C5, and D5 (plus *emm* types that are not assigned a cluster and are not shown) [12■]. GAS, group A streptococci.

FIGURE 2.

FCT-region genes and *emm* pattern group correlates. (a) The nine known FCT-region forms, depicting genes encoding pilus structural proteins (blue), nonpilus adhesins (red), transcriptional regulators (light green), and enzymes involved in pilus assembly (gray). FCT-8 differs from FCT-4 in a transcriptional regulatory gene form (not shown). Because FCT-region assignment is often oligonucleotide-based, some FCT-7 forms may have divergent *cpa* alleles that yield false-negatives and otherwise would be assigned FCT-4. The *prtF2* locus corresponds to a combination of both the *fbaB* and *pfbp* genotype forms. Based on [10,41,42,44,45]. (b) Distribution of *prtF1*, *cpa*, and *prtF2* loci among *emm* pattern groups. Based on 113 GAS strains of 111 distinct *emm* types, as reported in [45]. Fisher's exact test (two-tailed) for comparisons between *emm* pattern A–C and D strains (**, $P < 0.01$). (c) Distribution of FCT-region forms (FCT-1 through FCT-8) among *emm* pattern groups [based on same strains as in (b)] GAS, group A streptococci.