The Importance of the Group A *Streptococcus* Capsule in the Pathogenesis of Human Infections: A Historical Perspective

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The virulence of group A streptococci (GAS) correlates closely with expression of its surface antigen, M protein, and its hyaluronic acid capsule. In studies of human GAS infection, the former has received considerable attention. For several decades, however, systematic identification of encapsulated virulent strains by the mucoid colonies they produce has been neglected in clinical studies. In part, this may be due to the capsule’s evanescent expression on artificial media, its repression during convalescent carriage, lack of expertise in recognizing its colonial morphology, and the growing tendency for clinical laboratories to eschew throat cultures in favor of rapid laboratory tests for group A polysaccharide. Older and more recent studies are reviewed here that emphasize the capsule’s basic role in infection. We believe that it is time to refocus newer clinical studies and techniques on achieving early recognition of potentially dangerous, heavily encapsulated strains of GAS for which spread may be prevented.

For >1 century since its recognition, the evanescence of the hyaluronate capsule of group A streptococci (GAS) has presented an investigative problem for assessing its role in human infections. Although much attention has focused on the surface antigen, M protein, as an essential virulence factor [1], a wealth of clinical, epidemiological, and experimental evidence indicates that the expression of the capsule shares with M protein the essential requirements for producing significant infection [2, 3].

Expression of the capsule is rapidly lost on artificial media, on fomites, and during convalescent throat carriage. Moreover, capsule recognition by clinical bacteriology laboratories requires familiarity with the “mucoid” appearance of encapsulated strains grown on blood agar. Very few clinical laboratories, however, report GAS colony morphology. In clinical practice, rapid group A antigen detection tests often have replaced blood agar throat cultures in the laboratory diagnosis of sore throat. Although rapid group A antigen detection tests are useful as negative predictors of GAS pharyngitis, they offer no clue as to the actual virulence of infecting strains. If indicated by local epidemiological circumstances, however, recognition of mucoid colony morphology on blood agar cultures may prove to be useful to flag the appearance of the highly encapsulated GAS strains that may herald an outbreak of dangerous GAS infection [4].

This review highlights early investigations of the role of the GAS capsule in human infections and relates these studies to more recent experiments that focus on emerging or genetically engineered GAS mutants of varying capsule-producing potential [3, 5]. Such studies have greatly strengthened recognition of the contribution of the hyaluronate capsule to GAS virulence.

**STUDIES OF GAS CAPSULES IN THE FIRST HALF OF THE 20TH CENTURY**

At the turn of the 19th century, Bordet [6] demonstrated that streptococcal strains isolated from blood...
cultures from septic patients were encapsulated and that this property was associated with resistance to phagocytosis. However, in broth cultures, the capsule disappeared within a few hours. By 1935, variations in GAS resistance to phagocytosis of virulent, attenuated, and avirulent strains were clearly confirmed and were summarized by Ward and Lyons [7]. They wrote, “the resistance to phagocytosis of the virulent variants appears to be associated with the presence of capsules on the organisms. . . . No capsule can be demonstrated on the avirulent variant” (p. 527). . . . “The appearance of the colony depends to some extent on the length of incubation time. The younger colonies—6 to 8 hours—are of the mucoid type, being smooth, watery, and of regular contour” (p. 520).

With Lancefield’s discovery of the GAS surface M protein as a major virulence factor and the type-specific protection afforded by its homologous M antibody [1], the capsule was relegated to a secondary level of interest, especially because it appeared to be nonantigenic [8, 9]. However, to select GAS clones likely to be most mouse virulent and to contain a large amount of M protein, Lancefield advised picking only the most highly mucoid colonies from the surface of a blood agar culture (R. C. Lancefield, personal communication).

In 1934, Seastone [10] demonstrated by Wright stains made during the first 6–8 h of GAS growth (but not thereafter) outlines of large capsules that contained within them beaded material, which he assumed to be denatured protein. These encapsulated strains were highly resistant to phagocytosis. After Kendall et al. [11] identified the capsular material as hyaluronic acid, Seastone [12] confirmed its consistent presence in the capsules of GAS strains isolated from human infections and, with Kass [13], demonstrated that hyaluronidase destroyed GAS resistance to phagocytosis and protected mice against challenge with encapsulated strains.

In 1959, Wilson [14] clearly demonstrated striking capsules formed in 3–6-h serum–enriched broth cultures of GAS strains isolated from patients with virulent human infection. On properly prepared and preserved blood agar plates, the heavily encapsulated strains formed large, domed “mucoid” colonies (figure 1A), and the size was measured by light microscopy using India ink preparations. Furthermore, the “matte” strains previously described by Ward and Lyons [7] and by Todd and Lancefield [9] were demonstrated to be initially “mucoid,” but upon drying, they developed a roughened, “matte,” collapsed (flat) surface (figure 1A). With use of phase-contrast microscopy, Wilson had also demonstrated virtually total resistance to phagocytosis of heavily encapsulated strains. In subsequent studies, resistance to “surface phagocytosis” of GAS strains by mouse and rat leukocytes was correlated with the degree of encapsulation or M protein content [15].

CLINICAL AND EPIDEMIOLOGICAL STUDIES FROM THE LATTER HALF OF THE 20TH CENTURY

In the late 1940s and early 1950s, the great epidemics of rheumatic fever among US military recruits at Warren Air Force Base in Cheyenne, Wyoming [16], and at the Naval Training Center at Great Lakes, Illinois [17], provided opportunities to identify the properties of causative GAS strains. At Warren Air Force Base, one of us (G.H.S.) obtained and preserved cultures of the extant M types (notably M5, 14, and 24) that were clearly associated with rheumatic fever. Additional similar strains were
Figure 2. The “long chain test”: Wright stain of encapsulated group A streptococci growing for 3 h in fresh human blood (A) and with added type-specific anti-M antiserum (B). Reprinted with permission from [24].

collected from the Great Lakes Naval Training Center’s Naval Medical Research Unit 4, where the site’s bacteriologist, Paul Frank, reported that the sudden appearance and spread of a highly encapsulated strain of a single M type throughout the recruit camp was regularly followed by epidemics of rheumatic fever [18]. With Alvin Coburn, who was a consultant to the unit at that time, the extreme respiratory infectivity of these strains for both men and mice was reported [19]. These rheumatic fever epidemics then became the focus of the classic studies of mass prophylaxis with injectable benzathine penicillin G [20]. The abrupt termination of an epidemic by such treatment was associated with the prompt disappearance of rheumatic fever and, simultaneously, of the highly encapsulated strains belonging to the epidemic M types 1, 3, 5, 14, 19, and 24. After mass prophylaxis in military recruits resulted in the elimination of epidemic rheumatic fever, persistent GAS carriage and sporadic pharyngitis were noted to occur relatively frequently without the reappearance of rheumatic fever [21, 22]. Definitive studies of strain virulence in these infections have not been made.

At about this time, 2 highly encapsulated strains that clearly caused rheumatic fever (an M3 and an M5) were isolated by Alan Siegel from 2 Chicago school children during their antecedent episodes of pharyngitis [23]. These virulent strains became the source of many subsequent studies of their virulence properties, and of their potential for development of M protein vaccines (see below). While studying these strains, Stollerman and Ekstedt [24] reported that encapsulation significantly shortened the chain length of GAS units, whereas, with loss of encapsulation or with growth in the presence of type-specific anti-M antibody, their chain length was greatly elongated. Wright stains made within the first 3 h after the fresh blood cultures showed striking intracapsular granules (figure 2), similar to those that had previously been reported by Seastone [10]. With use of highly encapsulated strains, the “long chain reaction” proved to be a highly sensitive test for detection of minute quantities of anti-M antibody in human serum [25, 26].

### VARIATION OF VIRULENCE OF STRAINS IN A SINGLE M TYPE

Four variants of a single M14 strain (M+ Capsule+, M+ Capsule−, M− Capsule+, and M− Capsule−) that were identified by Armine Wilson were used by one of us (G.H.S.) to study the innate host defense of germ-free mice [27] and of colostrum-deprived piglets [28]. The germ-free mice were as resistant as the conventional mice to challenge with GAS strains lacking both M protein and capsules [27] (table 1). Furthermore, the WBCs of newborn, colostrum-deprived piglets readily phagocytized M-negative, capsule-negative GAS strains and destroyed them with the same efficiency as the blood cells of normal, colostrum-fed piglets. Maximal virulence was demonstrated only in strains that were both M protein rich and heavily encapsulated [28]. During convalescence from pharyngitis, progressive loss of GAS strain virulence was clearly demonstrated by Rothbard and Watson [29, 30]. Interestingly, during epidemics of rheumatic fever at Warren Air Force Base, the GAS found in the dust of recruits’ barracks failed to infect human throats [31]. By the

<table>
<thead>
<tr>
<th>Case</th>
<th>GAS variants</th>
<th>−Log LD₅₀</th>
<th>GF</th>
<th>CM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M protein³⁺</td>
<td>Capsule²⁻</td>
<td>1.1</td>
<td>0.9</td>
<td>&gt;1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>++</td>
<td>2.8</td>
<td>2.7</td>
<td>&gt;1</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0</td>
<td>3.4</td>
<td>3.5</td>
<td>&gt;1</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>++</td>
<td>7.0</td>
<td>3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>++++</td>
<td>8.2</td>
<td>6.5</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**NOTE.** Data are from [27]. LD₅₀ is expressed as −log dilutions of the broth culture inocula.

³⁺ M protein

²⁻ Capsule size expressed as the multiples of a single coccus diameter.
In the 1980s, focal epidemics of rheumatic fever erupted in cohorts of children in several US states—notably Utah, Ohio, and Pennsylvania [41–44]—and in some US military installations [45, 46] (e.g., San Diego Naval Base in California and Fort Leonard Wood in Missouri). The M types of the strains causing these outbreaks were eventually found to be the same as the notorious rheumatogenic strains of the earlier World War II military epidemics [47]. The state of encapsulation of these strains was not reported originally, and systematic studies of their virulence were not made [48]. Later, retrospective analysis of outbreaks of invasive GAS infection that followed revealed that mucoid colonies were often identified in some isolates from affected cohorts—notably, M types 3 and 18 [49].

Finally, a retrospective study of the colonial morphology of the GAS isolates recovered from patients with pharyngitis was made during several years of recurrent waves of acute rheumatic fever in the Salt Lake City, Utah, region [50]. For strains that had been carefully preserved at −70°C, there was a clear correlation between the outbreaks of acute rheumatic fever and the prevalence of mucoid strains (figure 3) [50]. Identification of the genome of some of these strains revealed that a single virulent clone may have been responsible for the epidemics of rheumatic fever reported from the Rocky Mountain States and elsewhere [51, 52] and that a single genetically identified clone also caused an outbreak of invasive streptococcal disease among schoolchildren in Minnesota [53].

**EXPERIMENTAL STUDIES OF THE CAPSULE’S RELATION TO ADHERENCE, EPITHELIAL INTERNALIZATION, COLONIZATION, AND MUCOSAL INVASION**

By the mid-1970s, the adherence of GAS to pharyngeal cells was shown to be due to several fibronectin-binding ligands, such as lipoteichoic acid [54, 55] and the so-called F proteins [56], rather than to M protein alone. Moreover, the F proteins were shown to promote “internalization” of GAS within epithelial cells [57] and to be associated with persistent throat carriage of strains that were still M positive after administration of effective antibiotic therapy for GAS pharyngitis [58]. In contrast, the hyaluronate capsule apparently impeded GAS adherence to and internalization within Hep-2 cells and human keratinocytes [59–62]. The capsule’s role in these studies was finally clarified by the demonstration that it attaches to a hyaluronic–acid–binding protein, CD44, which is present on human epithelial cells [63–66] and which induces cytoskeletal rearrangements, resulting in disruption of intercellular junctions, thus allowing the microorganisms to remain extracellular as they penetrate the epithelium [65]. More recently, strains of M types 3 and 18 that were strongly associated with the reappearance of rheumatic fever in the United States in the 1980s were shown to avidly aggregate type IV collagen [67].

Such aggregation was found to depend on the expression of both M protein and hyaluronate and was demonstrated in vivo (mouse skin) as well as in vitro.

**THE GENETIC CONTROL OF GAS VIRULENCE**

The demonstration of the 2-component system regulating the operon that controls the hyaluronate producing genes hasA, hasB, and hasC [68–72] has helped to explain the wide variation in encapsulation and the factors affecting its expression or repression. The system consists of a membrane-integrated sensor protein (CsrS), and a cytoplasmic response negative regulator CsrR (also called CovRS). The gene (sagA) of the powerful toxin, streptolysin S, is also regulated by the same repressor [72]. The sensor protein CsrS responds to environmental signals. Contact with serum, for example, apparently derepresses
Spontaneous mutations have been found in human infections as well. For example, compared with mouse virulence of M3 strains isolated from subjects with ordinary pharyngitis, strains of M3 obtained from patients with streptococcal toxic shock exhibited greatly enhanced mouse virulence [77]. These strains were shown to contain a mutation with a deletion of the negative regulator csrR. Mouse virulence correlated with the activity or actual levels of hyaluronic acid (hasA) and streptolysin S (saga), which is also regulated by csrR. In another study, GAS gene expression by an M1 virulent strain that produced experimental pharyngitis in monkeys was measured during 3 stages of infection (incubation, acute inflammation, and convalescence). The differential expression of the 2-component regulators covR and spy0680 (M1_spy0874) was associated with all phases of disease [78]. Colony morphology of throat cultures in these monkeys in different stages of infection, however, was not noted. It was recently reported [79] that the prevalence of the M serotypes that were associated with rheumatic fever in Chicago in the 1950s and 1960s decreased significantly by the 2000s. However, encapsulation of the isolated M-typeable strains or other studies of strain virulence since those originally noted in the 1960s [34] was not reported.

ENCAPSULATION DURING CONVALESCENT-PHASE CARRIAGE

Encapsulated organisms tend to persist in experimental pharyngeal infection in mice [66,80] and in baboons [81]. In the latter case, strains expressing M protein and capsule were carried for as long as 6 weeks, whereas those that were M protein and capsule negative persisted for only 14–21 days. Noteworthy was the 1962 report from the Warren Air Force Base of GAS strains carried during convalescence from pharyngitis in an era when and in a locale where rheumatic fever and rheumatogenic strains were still prevalent [82,83]. In these studies of convalescent-phase carriage following untreated GAS pharyngitis, the infecting strain persisted as long as 2–3 months, eventually losing M protein and infectivity for monkeys. Encapsulation of the persistently carried strains was not described. To our knowledge, systematic studies of the state of encapsulation of pharyngeal “persisters” following penicillin therapy for pharyngitis have not been reported either.

In conclusion, an argument may be made for renewed surveillance of colony morphology to support clinical management of GAS pharyngitis in cohorts at risk for post-infectious or invasive GAS diseases. A 26-valent recombinant M protein vaccine that includes 80%–90% of the serotypes that cause pharyngitis and invasive disease in the United States has been tested in healthy adults and found to be immunogenic and safe [84,85]. In a recent study of invasive GAS disease in the United States [86], M serotypes included in the vaccine accounted for 79% of the total isolates; for 85% and 88% of isolates respon-

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Table 2. Limited virulence properties of group A Streptococcus (GAS) strains isolated from throat cultures of 1000 children with nonexudative pharyngitis who were not treated with antibiotics.

<table>
<thead>
<tr>
<th>Virulence property</th>
<th>No. of children (n = 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat cultures positive for β-hemolytic streptococci</td>
<td>292</td>
</tr>
<tr>
<td>GAS</td>
<td>233</td>
</tr>
<tr>
<td>M typeable</td>
<td>95</td>
</tr>
<tr>
<td>M protein&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>Low</td>
<td>9</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
</tr>
<tr>
<td>Mouse virulence&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74</td>
</tr>
<tr>
<td>None</td>
<td>16</td>
</tr>
<tr>
<td>Slight</td>
<td>16</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>94</td>
</tr>
<tr>
<td>Mucoid</td>
<td>94</td>
</tr>
<tr>
<td>Glossy</td>
<td>94</td>
</tr>
<tr>
<td>Capsules&lt;sup&gt;c&lt;/sup&gt; (India ink)</td>
<td>1</td>
</tr>
<tr>
<td>Large</td>
<td>1</td>
</tr>
<tr>
<td>Small</td>
<td>31</td>
</tr>
<tr>
<td>None</td>
<td>62</td>
</tr>
<tr>
<td>Growth in human blood&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20</td>
</tr>
<tr>
<td>Rheumatic fever</td>
<td>0</td>
</tr>
<tr>
<td>Acute glomerulonephritis</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compared with standard epidemic strain M19, high in extractable M protein.

<sup>b</sup> Mouse virulence (the lethal dose for 50%) of the 95 strains tested is expressed as −log dilutions of broth culture inocula (not virulent, <10; slightly virulent, <100; and highly virulent, >1000).

<sup>c</sup> Number of strains with the ability to increase the inoculum ≥2× after 3 h of growth in human blood.

<sup>d</sup> Pharyngeal "persisters" following penicillin therapy for pharyngitis have not been reported either.
GAS Capsule and Human Infection

Figure 3. Correlation between the outbreaks of acute rheumatic fever and the prevalence of mucoid strains in the intermountain region of Utah during 1983–2001. The number of cases of rheumatic fever encountered yearly (■) is plotted together with the percentage of mucoid strains (▲). Reprinted with permission from [50].

Sensible for necrotizing fasciitis and streptococcal toxic shock syndrome, respectively; and for 79% of isolates associated with fatal infection. The appearance of acute rheumatic fever, streptococcal toxic shock, or other invasive infections in a community should prompt close surveillance of positive culture results for the appearance of mucoid GAS colonies. Such colonies may help to detect isolates that belong to new M serotypes or others that are not presently included in vaccines [87].

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