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# Hyaluronic acid capsule is a virulence factor for mucoid group A streptococci

**(bacterial polysaccharide/rheumatic fever/phagocytosis/M protein)**

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**ABSTRACT** **Mucoid strains of group A *Streptococcus* have been associated with recent outbreaks of acute rheumatic fever. The mucoid colony morphology of these strains is a result of abundant production of capsular polysaccharide, which is composed of hyaluronic acid. To study the role of the hyaluronic acid capsule in virulence, we derived an acapsular mutant from a mucoid strain of group A *Streptococcus* by transposon mutagenesis. M protein expression was not altered in the mutant strain. The mucoid wild-type strain grew in fresh human blood and was resistant to phagocytic killing *in vitro*. In contrast, the acapsular mutant failed to grow in fresh human blood and was sensitive to phagocytic killing *in vitro*. Loss of capsule was associated with a 100-fold reduction in virulence of the organisms in mice. We conclude that the hyaluronic acid capsule protects mucoid group A streptococci from phagocytosis and has an important role in virulence.**

Group A *Streptococcus* (GAS) continues to be a major cause of morbidity and mortality throughout the world, both from infections and from the postinfectious sequelae of acute rheumatic fever and poststreptococcal glomerulonephritis. Recent reports suggest that after declining in frequency in developed countries for the past half-century, the incidence of life-threatening infections due to GAS and of acute rheumatic fever is increasing (1–5). The reasons for the resurgence of serious human diseases due to GAS are not known; however, it has been suggested that the GAS strains prevalent today may have increased expression of one or more virulence factors (1, 6). One such factor may be the hyaluronic acid capsule. Occasional strains of GAS isolated from clinical sources grow as large, spreading, wet colonies on solid media; this characteristic mucoid colony morphology is due to abundant production by these strains of the GAS capsular polysaccharide, which is composed of hyaluronic acid, a high molecular weight polymer consisting of alternating residues of *N*-acetylglucosamine and glucuronic acid (7, 8). Mucoid strains of GAS have been implicated as causing unusually severe infections and frequently have been associated with individual cases or community outbreaks of rheumatic fever, including clusters of rheumatic fever cases reported recently from several regions of the U.S.A. (3, 5, 9, 10). Kaplan *et al.* (6) studied 42 GAS strains isolated from sibling contacts or patients with acute rheumatic fever during several outbreaks in the mid-1980s and found 45% to be mucoid.

Mucoid isolates are generally rich in M protein and are highly virulent in experimental animals (9, 11). M protein has been considered to be the major surface component responsible for resistance of GAS to phagocytosis (12). The fact that highly virulent, M protein-rich strains usually appear mucoid suggests that the presence of a large hyaluronic acid capsule may also confer special virulence properties on these strains. Hirst (13) attempted to define the role of the hyaluronic acid capsule in virulence by experimentally infecting mice with GAS that had been treated with leech extract (containing hyaluronidase); enzymatic decapsulation with hyaluronidase was ineffective in reducing virulence in this model. These studies were criticized by Kass and Seastone (14), who emphasized the capacity of the organisms to rapidly regenerate capsules *in vivo* and the presence of serum factors that inactivate hyaluronidase. Kass and Seastone (14) were able to show a substantial improvement in survival of mice challenged with GAS when the animals were treated with repeated doses of hyaluronidase to maintain the infecting organisms in a decapsulated state *in vivo*. Whitnack *et al.* (15) found that hyaluronidase treatment of GAS increased adherence of the organisms to peritoneal macrophages but had little effect on the ability of the bacteria to resist phagocytosis in nonimmune human blood.

It is difficult to determine from the varying conclusions of these studies what role the capsule may play in pathogenesis of GAS infection. The apparently discordant results may reflect the fact that wild-type strains studied by different investigators varied in degree of encapsulation or in expression of M protein. In addition, experimental models utilizing bacteria modified by enzyme treatment (13–15) or killed organisms (15) may not be adequate to assess the effects of encapsulation in intact, viable bacteria. Molecular genetic techniques have proved very useful in delineating the roles of potential virulence factors of a variety of bacterial pathogens by permitting the study of a single phenotypic characteristic independent of other virulence determinants. We now report the use of transposon insertional mutagenesis to derive an unencapsulated isogenic mutant strain from a mucoid wild-type strain of GAS. A single chromosomal insertion of transposon Tn*916* resulted in complete loss of hyaluronic acid capsule expression and rendered the mutant strain sensitive to phagocytic killing *in vitro* and avirulent in mice. These results indicate that the hyaluronic acid capsule of mucoid GAS protects the organism from phagocytosis and enhances virulence.

## MATERIALS AND METHODS

**Bacterial Strains.** GAS strains were 87-282, a type M18 mucoid isolate originally cultured from the throat of a child with acute rheumatic fever (provided by Edward L. Kaplan, University of Minnesota, Minneapolis); 282S1, a spontaneous streptomycin-resistant mutant of strain 87-282, described in this report; TD23, a nonmucoid transconjugant strain derived from 282S1 by Tn*916* mutagenesis, described in this

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Abbreviations: GAS, group A *Streptococcus*; cfu, colony-forming unit(s).

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report; TX4, a nonmucoid transductant of strain 87-282, and TX15, a mucoid transductant of strain 87-282, both derived from strain TD23 by bacteriophage transduction and described in this report; SS734 (2136-65), Centers for Disease Control reference strain of M protein type 18 GAS; and SS90 (Lancefield D58/40/0), Centers for Disease Control reference strain of M protein type 3 GAS (both provided by Richard R. Facklam, Centers for Disease Control, Atlanta). *Enterococcus faecalis* strain CG110, a high-frequency donor strain of transposon Tn*916*, and pAM120, a plasmid containing the entire Tn*916* sequence, were provided by Don B. Clewell, University of Michigan, Ann Arbor (16, 17). GAS strains were grown in Todd–Hewitt broth or on Todd–Hewitt agar containing 5% defibrinated sheep blood. For certain experiments, the culture medium was supplemented with tetracycline (5 μg/ml) and/or streptomycin (500 μg/ml).

**Antisera.** M protein type 18 GAS antiserum was raised in rabbits by intravenous injection of formalin-fixed organisms of GAS strain 87-282. M protein type 18 GAS reference typing serum (raised in rabbits) was obtained also from R. R. Facklam, Centers for Disease Control.

**Isolation of Mutants.** Tn*916* was transferred from *E. faecalis* donor strain CG110 to a spontaneous streptomycin-resistant mutant of wild-type mucoid GAS strain 87-282, designated 282S1, as described by O'Connor and Cleary (18). Briefly, cells of donor and recipient strains were mixed in a ratio of 1:1 and deposited on membrane filters. After overnight incubation, bacteria were removed from the filters and plated on medium containing tetracycline and streptomycin. Nonmucoid colonies were serially subcultured three times on tetracycline/streptomycin agar and then frozen at −80°C.

**Bacteriophage Transduction.** Bacteriophage transduction followed the protocol of Caparon and Scott (19). Briefly, bacteriophage A25 (provided by June R. Scott, Emory University, Atlanta) was used to infect a broth culture of the nonmucoid transconjugant TD23. A lysate of this culture was filtered free of bacterial cells and then transferred to a broth culture of wild-type strain 87-282. Transductants that had incorporated DNA from TD23 containing a Tn*916* insertion were selected on tetracycline agar.

**Southern Hybridization Analysis.** Genomic DNA was isolated from GAS strains as described by O'Connor and Cleary (18). DNA was digested with restriction endonucleases *Eco*RI or *Hin*dIII, subjected to agarose gel electrophoresis, transferred to a nylon membrane, and probed with $^{32}$P-labeled pAM120, previously labeled by nick translation, as described (20, 21). Tn*916* contains no *Eco*RI sites and a single *Hin*dIII site, so each Tn*916* insertion was identified by hybridization of the probe to a single *Eco*RI fragment and to two *Hin*dIII fragments (17).

**Measurement of Cell-Associated Hyaluronic Acid.** Bacteria were grown to early logarithmic phase in 150 ml of broth, collected by centrifugation, and washed once with water. Cells were resuspended in 1.5 ml of water and an equal volume of chloroform, mixed vigorously, and allowed to stand at room temperature for 1 hr. After centrifugation, the aqueous phase was assayed for uronic acid content by the carbazole method with testicular hyaluronic acid (Sigma) as a standard (22).

**Preparation of M Protein Extracts.** M protein extracts were prepared by acid extraction of bacterial cells as described by Lancefield (23). That equivalent quantities of bacterial cells of each strain were used for extraction was confirmed by solubilizing duplicate aliquots of the bacterial suspension in 1 M NaOH at 100°C for 5 min and then by determining total cellular protein by the method of Lowry *et al.* (24).

***In vitro* Assays of Phagocytosis.** Whole-blood phagocytic assays (the direct bactericidal test of Lancefield) were performed essentially as described (25). Opsonophagocytic killing in the presence of 10% (vol/vol) human serum was determined by using a modification of the method of Baltimore *et al.* (26). Organisms from a logarithmic-phase broth culture were mixed with leukocytes ($6 \times 10^6$) isolated from freshly drawn human blood in 0.5 ml of minimal essential medium containing 10% human serum previously absorbed with organisms of strain 282S1 to remove specific antibodies. Aliquots (25 μl) for quantitative culture were withdrawn immediately and after 1 hr of end-over-end rotation at 37°C.

**Mouse Lethality Studies.** Organisms were grown in broth to early logarithmic phase, diluted in fresh broth, and injected i.p. in female CD1 mice weighing ≈20 g. Serial 1:10 dilutions were administered to groups of six mice. Survivors were counted 3 days after challenge, and $LD_{50}$ was calculated with data from two such experiments; a total of 60 animals was used for each strain. Survival curves were fit by using the Gompertz model of the PROC PROBIT program, SAS/STAT User's Guide (SAS Institute, Cary, NC).

## RESULTS

**Derivation of a Nonmucoid Mutant Strain of GAS by Tn*916* Mutagenesis.** Tn*916* is a 16.4-kilobase (kb) transposon that carries a tetracycline-resistance marker and is capable of self-conjugation among a variety of Gram-positive species (16). Strain 87-282 is a mucoid strain of GAS (M protein type 18) originally isolated from the pharynx of a child with acute rheumatic fever. A spontaneous streptomycin-resistant mutant of strain 87-282, designated 282S1, was used as the recipient in filter mating experiments in which Tn*916* was transferred from the high-frequency donor, *Enterococcus faecalis* CG110. Transconjugants were selected on tetracycline/streptomycin agar and arose at a frequency of $10^{-4}$ to $10^{-5}$ per recipient cell recovered from the filters at the time of plating on selective media. Approximately 3000 transconjugants were screened by visual inspection for nonmucoid colony morphology. Two nonmucoid transconjugant strains were identified, designated TD23 and TD27.

Genomic DNA was isolated from selected mucoid and nonmucoid transconjugants and examined by Southern hybridization analysis. Most transconjugants were found to contain two or more Tn*916* insertions; the nonmucoid strains TD23 and TD27 had three and two transposon insertions, respectively. The nonmucoid transconjugant strain TD23 was selected for further study.

**Transduction of the Tn*916* Mutation to Wild-Type GAS Strain 87-282.** To determine which transposon insertion(s) was responsible for loss of the mucoid phenotype, we utilized bacteriophage transduction to transfer the transposon mutation from strain TD23 to the wild-type strain 87-282. We confirmed, by their sensitivity to streptomycin, that all transductants had arisen from wild-type strain 87-282. Of 14 tetracycline resistant transductants screened, 4 grew as nonmucoid colonies. Two nonmucoid transductants, TX4 and TX12, and one mucoid transductant, TX15, were examined by Southern hybridization analysis, which demonstrated a single Tn*916* insertion in the nonmucoid transductant TX4, an identical insertion in TX12, and a single insertion at a different chromosomal site in the mucoid transductant TX15 (Fig. 1). In all transductants examined, the site of transposon insertion corresponded to one of the Tn*916* insertion sites in TD23, indicating in all cases incorporation of the Tn*916*-containing chromosomal fragment of TD23 into the chromosome of the recipient cell by homologous recombination. These findings are consistent with those of Caparon and Scott (27) who also found no evidence of transposition of Tn*916* following bacteriophage transduction in GAS.

**Nonmucoid Transductant TX4 Produces No Capsular Polysaccharide.** We confirmed that the nonmucoid colony morphology of strain TX4 was due to loss of the hyaluronic acid capsule by measuring the amount of uronic acid on the

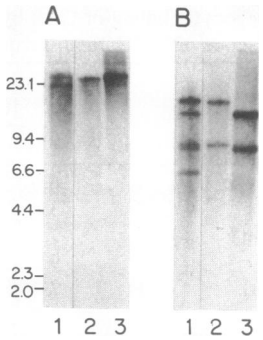


FIG. 1. Southern hybridization analysis of *Eco*RI (*A*) and *Hin*dIII (*B*) restriction digests of genomic DNA from nonmucoid transconjugant strain TD23 (lanes 1), nonmucoid transductant strain TX4 (lanes 2), and mucoid transductant strain TX15 (lanes 3), probed with $^{32}$P-labeled pAM120, which contains the Tn*916* transposon. Molecular weight standards are indicated on the left in kb. Tn*916* contains no *Eco*RI sites and a single *Hin*dIII site; so, for each Tn*916* insertion in the chromosome, digestion with *Eco*RI produces one fragment hybridizing to the probe, while digestion with *Hin*dIII results in two fragments (17). In *A*, the higher molecular weight band in the *Eco*RI digest of TD23 (lane 1) is seen to represent two incompletely resolved fragments corresponding to the distinct but similarly sized single bands in the transductants TX4 (lane 2) and TX15 (lane 3), respectively. In *B*, the higher molecular weight band in the *Hin*dIII digest of each of the two transductants (lanes 2 and 3) corresponds to a distinct band in TD23 (lane 1); the lower molecular weight bands of the two transductants correspond to a broad band in TD23 representing at least two incompletely resolved fragments. An additional anticipated *Hin*dIII fragment in TD23 is presumed to comigrate with one of the five visualized fragments.

surface of the organisms. Capsular hyaluronic acid was released from the bacterial cells with chloroform, then quantified by the carbazole method (22). The nonmucoid transductant TX4 had undetectable cell-associated hyaluronic acid, <5 fg per colony-forming unit (cfu), consistent with complete absence of capsular polysaccharide, compared with 115 fg/cfu for the parent strain 282S1 and 139 fg/cfu for the mucoid transductant TX15 (Table 1).

**M Protein Expression Is Not Altered in the Acapsular Mutant Strain TX4.** M protein expression has been shown to be correlated with resistance of GAS to phagocytosis and is considered a major virulence factor of the organism (12). In addition, strains that spontaneously lose M protein expression often convert from mucoid to glossy (nonmucoid) colonies (28). To determine whether M protein expression was altered in the nonmucoid transductant TX4, M protein extracts were prepared from strains 282S1 and TX4 by the method of Lancefield (23). Extracts both from 282S1 and from TX4 gave a reaction of identity with a Lancefield extract from a reference strain of type M18 GAS, Centers for Disease Control strain SS734 (2136-65), in Ouchterlony immunodiffusion in agarose against type M18 rabbit antiserum (Fig. 2

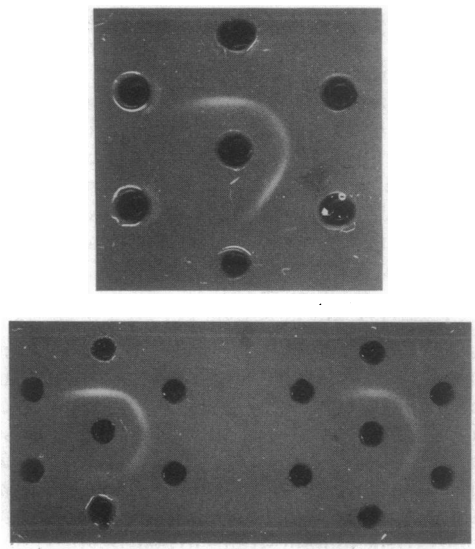

FIG. 2. Ouchterlony immunodiffusion in agarose of Lancefield extracts of GAS. The center well of each pattern received type M18 rabbit antiserum. (*Upper*) Outer wells received Lancefield extracts from the acapsular mutant strain TX4 (top well), parent strain 282S1 (upper right well), Centers for Disease Control type M18 reference strain SS734 (lower right well), and type M3 strain SS90 (bottom well). The left wells were empty. (*Lower*) Serial dilutions of Lancefield extracts prepared from strains TX4 (*Left*) or 282S1 (*Right*); outer wells of both received extract undiluted (top well), diluted 1:2 (upper right well), diluted 1:4 (lower right well), diluted 1:8 (bottom well), diluted 1:16 (lower left well). The upper left well was empty, and the center wells received type M18 rabbit antiserum.

*Upper*) (29). The same results were obtained with type M18 antiserum prepared in our laboratory or with type M18 streptococcal typing serum provided by R. R. Facklam, Centers for Disease Control. Both M18 antisera failed to react with Lancefield extracts prepared from type M3 organisms, indicating that the precipitin reaction was specific for type 18 M protein. The amount of M protein expressed on the surface of strain TX4 relative to the parent strain 282S1 was examined by preparing serial dilutions of M protein extracts from the two strains. The undiluted 282S1 extract formed a precipitin line with M18 antiserum, as did extract diluted 1:2 and 1:4; the TX4 extract gave a positive reaction when undiluted, diluted 1:2, and diluted 1:4 and a faint reaction at 1:8 (Fig. 2 *Lower*), indicating the transductant expressed a similar amount of M protein as the parent strain. Extracts were also prepared from a culture of strain 282S1 grown in the presence of hyaluronidase at 10 μg/ml to test whether the absence of the capsule influenced the efficiency of M protein extraction. The same quantity of M protein was found in extracts of strain 282S1 grown with or without hyaluronidase, indicating that decapsulation did not change significantly the yield of M

Table 1. Phenotypic characteristics of mucoid GAS strain 282S1 and of Tn*916* mutant strains

| | 282S1 | TX4 | TX15 |
|---|---|---|---|
| Colony phenotype | Mucoid | Nonmucoid | Mucoid |
| Tn*916* insertions | 0 | 1 | 1 |
| Capsule production* | 115 fg/cfu | 0 (<5 fg/cfu) | 139 fg/cfu |
| M protein expression† | 1:4 | 1:4–1:8 | Not tested |
| Growth in human blood‡ | + | − | + |
| Resistance to phagocytosis§ | + | − | + |
| $LD_{50}$ in mice, cfu × $10^{-5}$ | 1.3 | 200 | 2.6 |
| (95% confidence interval) | (0.36–4.6) | (78–530) | (0.98–14) |

*Cell-associated hyaluronic acid, quantified by the carbazole method (22).
†Highest dilution of Lancefield extract reacting in immunodiffusion with M18 antiserum.
‡Data are shown in Table 2.
§Data are shown in Table 3.

protein. No difference was found between TX4 and 282S1 in rate of growth, expression of group A carbohydrate, or hemolysis on blood agar.

**Acapsular Mutant TX4 Is Sensitive to Phagocytic Killing.** An important *in vitro* correlate of virulence is resistance to phagocytosis, defined experimentally by the ability of GAS strains to grow in fresh human blood (30). Parent strain 282S1 increased in number during incubation in fresh human blood, whereas the nonmucoid transductant TX4 showed no change or a slight decrease in cfu (Table 2). Strain TX4 grew readily in fresh plasma from which the blood cells had been removed, increasing from 115 cfu to >1000 cfu after 3 hr, confirming that the results in whole blood reflected increased sensitivity of the acapsular mutant to phagocytosis rather than inability to grow in human plasma. As a control for nonspecific effects of Tn*916* insertion, we also tested the mucoid transductant TX15, which gave results identical to those with 282S1.

Because the usual site of initial colonization and infection with GAS is the pharyngeal mucosa, we also tested the susceptibility of the mutant strains to phagocytic killing under conditions more closely resembling those in a pharyngeal exudate. For these assays, the organisms were incubated with human peripheral blood leukocytes in the presence of 10% human serum. To exclude the possibility that killing of TX4 was due to small amounts of specific antibody insufficient to opsonize the wild-type strain, the serum used in the assay as a complement source was preabsorbed with organisms of strain 282S1 to remove specific antibodies. Under these conditions the nonmucoid transductant TX4 was readily killed (77–93% decrease in cfu in 1 hr), while the number of organisms of parent strain 282S1 and the mucoid transductant TX15 remained the same or increased (Table 3). No killing was observed when serum heated at 56°C for 30 min was substituted for normal human serum, indicating that phagocytic killing was complement-dependent.

**Loss of Capsule Expression Is Associated with Loss of Virulence in Mice.** The principal mode of clearance of GAS from an infected host is phagocytic killing by fixed and circulating phagocytes. We anticipated, therefore, that loss of resistance to phagocytosis *in vitro* would be reflected in reduced virulence of the acapsular mutant TX4 *in vivo*. To determine whether the mucoid capsule was important for virulence *in vivo*, we determined the $LD_{50}$ in mice for the acapsular mutant TX4, for the parent strain 282S1, and, as a control, for the mucoid transductant TX15. The $LD_{50}$ values for the parent strain 282S1 and for the mucoid transductant TX15 were similar, $1.3 \times 10^5$ cfu and $2.6 \times 10^5$ cfu, respectively, while that for the nonmucoid transductant TX4 was $2.0 \times 10^7$ cfu (Table 1). Thus, loss of capsule expression in mutant strain TX4 was associated with an increase in $LD_{50}$ in mice of roughly 2 orders of magnitude.

Table 2. Survival in human blood of GAS

| Strain | Exp. | GAS, cfu/0.1 ml* 0 hr | 3 hr |
|---|---|---|---|
| 282S1 (mucoid wild type) | 1 | 82 | >300 |
| | 2 | 73 | >300 |
| TX4 (acapsular mutant) | 1 | 50 | 58 |
| | 2 | 155 | 99 |
| TX15 (mucoid mutant) | 1 | 41 | >300 |
| | 2 | 108 | >300 |

A logarithmic-phase broth culture of each strain was diluted in fresh broth and then 0.2 ml was mixed with 0.6 ml of freshly drawn, heparinized human blood. Aliquots (0.1 ml) were removed for quantitative culture at time 0 and after 3 hr of end-over-end rotation at 37°C.

*Colony count from a 0.1-ml aliquot of the assay mixture (mean of duplicate determinations); >300 colonies cannot be accurately counted because of "laked" or confluent mucoid colonies.

Table 3. *In vitro* phagocytic killing of GAS by peripheral blood leukocytes in the presence of 10% human serum

| Strain | Exp. | GAS, cfu × $10^{-6}$ 0 hr | 1 hr | % kill* |
|---|---|---|---|---|
| 282S1 (mucoid wild type) | 1 | 6.5 | 88 | — |
| | 2 | 3.8 | 4.0 | — |
| TX4 (acapsular mutant) | 1 | 7.8 | 0.56 | 93 |
| | 2 | 9.5 | 2.2 | 77 |
| TX15 (mucoid mutant) | 1 | 7.2 | 64 | — |
| | 2 | 3.7 | 4.6 | — |

Organisms from a logarithmic-phase broth culture were mixed with leukocytes ($6 \times 10^6$) isolated from freshly drawn human blood and 10% human serum. Aliquots (25 μl) for quantitative culture were withdrawn immediately and after 1 hr of end-over-end rotation at 37°C. Data are means of duplicate determinations.

*Percent kill = 100 − [(cfu at time 1 hr/cfu at time 0) × 100]. Dashes indicate no change or increase in cfu at 1 hr.

## DISCUSSION

The current studies show that the GAS capsular polysaccharide is an important antiphagocytic surface component of mucoid GAS that has a function distinct from the effect of M protein. M protein has been considered the surface component primarily responsible for resistance to phagocytosis (12); the ability of GAS strains to survive phagocytic killing in human blood is highly correlated with M protein expression, and this resistance can be overcome by M protein-specific antibodies. However, our results indicate that loss of encapsulation was associated with loss of resistance to phagocytic killing, even though M protein expression in the acapsular mutant was not altered. Thus, although antibodies directed against M protein are opsonic for mucoid strains, in the absence of M protein-specific antibodies (i.e., in the nonimmune host), the mucoid capsule protects these organisms against phagocytic killing. Todd and Lancefield (28) observed that certain strains of GAS give rise to spontaneous variants that have diminished or absent M protein, convert from mucoid to glossy colony morphology, and become avirulent. Loss of virulence in these strains has generally been attributed to the loss of M protein, though the change in colony type suggests that they also have diminished production of capsular hyaluronic acid (8). Our studies suggest that the change in capsule expression, rather than simply in M protein, may be critical in loss of resistance to phagocytic killing.

That loss of capsule expression dramatically attenuated the organism's virulence in mice supports the clinical observation that mucoid strains of GAS are associated with more severe infections and provides direct evidence that the GAS capsule acts as a virulence factor in these strains. While we cannot formally exclude the possibility that the diminished virulence of the acapsular mutant was due to an undetected change in another virulence factor, it is unlikely that such a factor could account for loss of resistance to phagocytosis.

The observations of Todd and Lancefield (28) that M protein and capsule expression appear to covary suggest that the level of expression of M protein, capsular hyaluronic acid, and perhaps other products may be coordinately regulated in GAS, as has been demonstrated for a variety of potential virulence factors in other bacteria (31). The existence of a virulence regulon in GAS has been proposed by Simpson *et al.* (32), who reported evidence of coregulation of expression of M protein and C5a peptidase. Whether control of capsule production is linked to expression of these or other

products remains to be determined, although our results indicate the existence of at least one genetic locus essential for production of capsule that is independent of M protein expression.

The association of mucoid strains of GAS with cases of acute rheumatic fever suggests that the presence of a mucoid capsular layer not only confers a capacity for invasive infection, but also alters the immune response of the host to the GAS organism. The pathogenesis of rheumatic fever remains poorly understood, but is thought to involve stimulation by streptococcal antigens of antibodies cross-reactive with host tissues. Hyaluronic acid itself is poorly immunogenic, presumably because of its structural identity with the ground substance of mammalian cartilage (7, 33). However, the presence of a capsular layer of hyaluronic acid may alter the antibody response to M protein or to other streptococcal products. Alternatively, the relationship between mucoid strains of GAS and rheumatic fever may reflect the capacity of such strains to persist in the host, by virtue of the antiphagocytic effect of the capsule. Prolonged pharyngeal colonization could provide a sufficient antigenic stimulus for production of potentially rheumatogenic antibodies directed against other streptococcal antigens. Our demonstration of the antiphagocytic function of the hyaluronic acid capsule provides a logical basis for the association between mucoid strains of GAS and acute rheumatic fever.

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