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Role of M Protein in Pharyngeal Colonization by Group A Streptococci in Rats

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As the initial step in infection, group A streptococci (GAS) colonize either the nasopharyngeal mucosa or the skin of humans. A number of virulence factors have been implicated in the colonization phase of pathogenesis based upon their in vitro activities, but the in vivo data supporting their role in colonization of the host tissues is lacking. In this investigation, the potential requirement for M protein in pharyngeal colonization by GAS was explored by using near-isogenic strains in experimental animal studies. Fischer rats were infected by intranasal and oral inoculation with both M-positive and M-negative *Streptococcus pyogenes* strains. Colonization of the pharyngeal area by the streptococci was monitored at various time intervals. Both M-positive and M-negative strains colonized during the first week following infection, indicating that M protein was not necessary for this initial colonization. Two M-positive strains of *S. pyogenes* were recovered from the rats up to 23 weeks following inoculation, while the colonization levels for M-negative strains decreased rapidly in the second and third weeks, becoming negligible by the fourth week. This indicates a potential role for M protein in the persistence of colonization at this mucosal surface. Colonization of rats with either M-positive strain of *S. pyogenes* also resulted in the appearance of salivary and serum antibody responses. This in vivo model should allow further investigation into factors required for GAS disease, including the examination of the potential role of the host immune response both in modulation of the pharyngeal surface and in modulation of antigenic changes in M protein or other surface factors.

Group A streptococcal (GAS) colonization of the nasopharynx in humans is frequently associated with an inflammatory response, characterized by edema and lymphoid hyperplasia of the posterior pharynx, accompanied by fever, and commonly referred to as strep throat. In some individuals, however, colonization of the pharynx is asymptomatic. A substantial number of humans (over 10% in some studies) are considered to be carriers of GAS (19). In these individuals, beta-hemolytic streptococci appear to persist in the nasopharyngeal region, frequently in the absence of either clinical symptoms or host immune response (22). Other individuals have shown prolonged carriage of GAS in the presence of a host response (25, 26), a condition which potentially could contribute to the development of sequelae.

Early studies with monkeys suggested that M protein on the surface of streptococcal strains was required for the development of a streptococcal infection (44). In both humans and monkeys, protection against streptococcal infection was shown to be linked to the development of type-specific antibodies elicited to M protein (28). Over the last several decades, a number of possible roles for M protein in streptococcal infection have been suggested: protection from host defenses because of M protein's antiphagocytic effect (reviewed in references 13, 34, and 45), adherence to a pharyngeal specific substance as a prerequisite for colonization (12, 18, 41, 42), a scaffold for extending another adhesive molecule away from the surface of the bacterial cell (1), or aggregation of M^+ bacteria after an initial adhesion step (9). Although M protein is best known for its antiphagocytic effect, it remains uncertain as to when and how this activity influences pathogenesis. Protection against phagocytosis would seem to be most critical at invasive steps after the bacterium had crossed the epithelial surface. Substantial evidence suggests that M protein may play a role in the earlier steps in colonization as well. For example, intranasally administered vaccines consisting of M protein non-type-specific epitopes have given protection against colonization and death in a mouse model (2, 3, 6, 7).

The potential role of the M protein or other streptococcal surface proteins in colonization may now be explored with strains exhibiting genetically defined differences in surface components because of recent advances in the ability to make stable defined mutations in GAS strains (8). Surface components can be tested in appropriate systems to define their molecular roles in initial adhesion to eukaryotic cell tissue, in tissue tropism, in affinity for or persistence in a particular colonization site, or in protection from or impairment of host defenses.

Rats have frequently proven to be useful models for studies of both antigenic variation and bacterial adhesion of various indigenous streptococci inhabiting the oral cavity (5, 16, 21, 41). They have also been a useful model for several infections native to humans, including dental caries resulting from *Streptococcus mutans* infection (30), respiratory disease caused by members of the genus *Mycoplasma* (10), and arthritis induced by streptococcal cell wall antigens (43). An infant rat model has been of enormous value in exploring pathogenesis of *Haemophilus influenzae* and group B streptococci (24, 31, 36, 38, 39).

In order to clarify the role, if any, of M protein in GAS colonization of the pharyngeal mucosa, we examined the abilities of GAS strains with defined mutations to colonize the upper respiratory tract of the Fischer rat. The significance of colonization differences was examined with respect to two phenotypic distinctions between the near-isogenic strains used for the study: the presence or absence of M protein and/or C5a peptidase, an inhibitor of chemotaxis.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Strain JRS4 is a streptomycin-resistant derivative of strain D471, a human isolate of *Streptococcus pyogenes* (37). Strain S43-29R is a streptomycin-resistant derivative of *S. pyogenes* (2) which was made virulent for mice by 29 intraperitoneal passages through mice (28). Both strains are of the M6 serotype. Strains JRS75 and JRS145 are M-negative derivatives of strain JRS4 which were obtained from June Scott (Emory University, Atlanta, Ga.) and Michael Caparon (Washington University, St. Louis, Mo.). These two strains have a deletion of the gene encoding the M6.1 protein in strain JRS4 with the concomitant insertion of an antibiotic resistance gene replacing the *emm*6.1 allele, which encodes the M6 protein. In strains JRS75 and JRS145, the deletion in *emm*6.1 extends from bp 5 to about 2550 or from bp 1480 to 2500, respectively (numbers in *emm*6.1 as per reference 20). JRS75 is kanamycin resistant because of the insertion of the *aph*A3 gene in place of the *emm*6.1 allele (32, 32a), and JRS145 is chloramphenicol resistant because of the insertion of the *cat*86 gene in place of the *emm*6.1 allele (17). Both of these strains are considered isogenic to the parental strain, JRS4, and are M negative.

Bacteria were grown in standing cultures of Todd-Hewitt broth with added 2% yeast extract (THY) at 37°C for 16 to 18 h. Viability counts were performed by serial dilution plating on THY plates. Viability counts for the overnight cultures ranged from 1.8×10^9 CFU/ml to 2.0×10^9 CFU/ml. To prepare the inoculum for colonization experiments, cultures of GAS were grown overnight, harvested by centrifugation, washed once in fresh THY broth, and then resuspended to 10^{10} CFU/ml. The number of CFU was determined from optical density readings at 550 nm with a curve derived which correlated with numbers of CFU on the THY agar plates. The presence or absence of M6 protein was monitored with a sample of each inoculum by extracting surface proteins by using a phage-encoded lysin (33) and visualizing M6 protein by immunoblotting with M6-specific antisera.

Animal procedures. Fischer CDF344 rats (originally obtained from Charles River Laboratories, Wilmington, Mass.) used in these studies were derived from a breeding colony of germfree animals maintained under sterile conditions in Trexler plastic isolators in the University of Alabama at Birmingham Gnotobiotic Rat Facility. Pups were removed from the isolator at 20 to 30 days of age and were then maintained in a laminar flow hood in sterile covered cages and provided autoclaved food and water ad libitum.

At the time of infection, 8- to 12-week-old rats were placed on water containing streptomycin (1,000 μg/ml) to lower their natural flora and facilitate colonization by the test streptococcal strains. We have, however, not determined if streptomycin treatment of the rats was a necessary requirement for the colonization results obtained with the test strains of *S. pyogenes*. A proportion of the natural flora in the experimental rats was resistant to streptomycin. These organisms were observed in animals on water with antibiotic after 48 h, indicating that competition for colonization with the natural flora is required in this model after day 2 postinfection. In this study, rats were maintained on streptomycin-containing water throughout all experiments. No additional antibiotics were used during colonization experiments so as not to influence the results.

Rat colonization. Except as noted, groups of four to six female Fischer rats per strain were used and each experiment was performed at least three times. Prior to infection, throat swabs were taken to determine if any beta-hemolytic organisms were present. No beta-hemolytic organisms were found in any rat prior to infection or in any of the uninfected control rats throughout the experiment. At the time of infection, 20 μl of inoculum was introduced intranasally and 50 μl was introduced orally; the inoculum contained 10^9 CFU in 100 μl.

After infection, throat swabs were taken from the pharyngeal region of each rat biweekly for a period of 8 weeks in most experiments (noted if otherwise) by using a Calgiswab (Spectrum Laboratories, Los Angeles, Calif.). Throat swabs were streaked onto blood agar plates (Difco blood agar base no. 2 plus 5% defibrinated sheep blood) containing 1,000 μg of streptomycin per ml. Plates were incubated at 37°C for 18 h in a candle jar. On the following day, the number of beta-hemolytic colonies isolated from each infected rat was recorded (2), sample beta-hemolytic colonies were streak purified, tested for bacitracin sensitivity, and grown in pure culture, and a frozen isolate was stored. A positive throat swab was one in which one or more beta-hemolytic colonies were present. In most experiments, samples were taken at 16 time points spanning 8 weeks. Two experiments were extended to 20 and 23 weeks, respectively.

Comparison of colonization using isogenic M-positive and M-negative strains. In one series of experiments, three groups of six rats were infected with strain JRS4, JRS75, or JRS145 and assessment of pharyngeal colonization was monitored as described above. The results of three identical complete experiments were summarized and are presented. GAS strains isolated from the rats at each time point were stored as freezer cultures and tested for bacitracin sensitivity (to verify that they were GAS) and kanamycin (for JRS75) or chloramphenicol (for JRS145) resistance.

Strains were analyzed for colonization probability during four consecutive time periods postinfection: 0 to 1 week, 2 to 3 weeks, 4 to 5 weeks, and 6 to 8 weeks. Clearance of GAS or colonization failure was indicated by three consecutive negative throat swabs from an individual animal within one of these time periods. This format allowed the examination of both relative initial colonization rates and of persistence of colonization in the groups infected with the three strains.

Analysis of secretions. Salivary secretions were induced by injection of pilocarpine (0.75 mg/100 g of body weight) into anesthetized rats. The salivary secretions and sera collected at the time of sacrifice were stored frozen at −80°C. For an experiment employing two M6-positive strains, an enzyme-linked immunosorbent assay (ELISA) was used to assay for antibodies directed against M6 protein present in serum and saliva at times of sacrifice ranging between 65 and 135 days postinfection. Wells were coated with 5 μg of purified recombinant M6 protein per ml, prepared from *Escherichia coli* (14), suspended in carbonate buffer. Salivary immunoglobulin A (IgA) directed against M6 was detected with biotinylated monoclonal anti-rat IgA antibody (1/500; Zymed Laboratories, Inc., San Francisco, Calif.). Serum Ig levels of anti-M6 antisera were detected with a biotinylated goat anti-rat Ig reagent (1/2,000; Southern Biotechnology Associates, Birmingham, Ala.). The biotinylated antibodies were detected after binding to streptavidin-alkaline phosphatase, the addition of substrate (Sigma 104) in diethanolamine buffer, and reading of the optical density at 405 nm in a Vmax ELISA plate reader (Molecular Devices, Menlo Park, Calif.).

Statistical and data analysis. Data for each experiment were analyzed with the program Systat 5.2 and survival module of the statistical analysis computer software by

Systat, Inc. (40). The Kaplan-Meier estimation for nonparametric analysis was used to analyze probabilities of colonization, and the log rank test was used to compare colonization probabilities for different streptococcal strains (23, 29). An analysis of variance and Tukey's test were also performed for the 1st week, 2nd and 3rd weeks, 4th and 5th weeks, and 6th to 8th weeks. P values of <0.05 were considered significant.

Variation in GAS isolates. The potential variation occurring in the bacterial strain as it was harbored in the pharynx of Fischer rats was examined as follows. Single-colony isolates from each throat culture were grown as individual pure cultures and then stored initially as frozen cultures. M-protein extracts from individual isolates were made, using phage lysin from a volume of cells equivalent to 1.0 ml of a broth culture with an optical density of 1.0 at 600 nm (15). Ten microliters of each lysin extract was then separated on sodium dodecyl sulfate gels (27), electrophoretically transferred to nitrocellulose, and visualized with antibody in a Western blot (4). Five duplicate Western blots were produced from each set of 15 extracts, and these were developed separately with four different monoclonal antibodies and a polyclonal rabbit antiserum elicited against strain JRS4. The M6 protein on the blots was examined for any differences in quantity, size, or antibody reactivity among distinct colony isolates from an individual throat culture. Fifteen separate colonies from the same throat isolate gave identical results when examined in this manner.

RESULTS

M protein was not required for initial colonization of the pharyngeal region of Fischer rats. Strain JRS4 is an M6.1-positive GAS strain (37) which was the parent strain for the construction of two mutant GAS strains, JRS75 and JRS145, that are lacking M6.1 protein (17, 32). On three separate occasions, three groups of Fischer rats were infected, one with each strain, as described in Materials and Methods. Pharyngeal colonization was monitored for a period ranging from 2 to 3 months. It was observed that many of the rats infected with the parent strain sustained colonization throughout the experiment. In contrast, many of the rats infected with either mutant strain reached a colonization endpoint during the course of the experiment. This endpoint was defined as three consecutive negative throat swabs from a single rat.

To analyze these results, all the data collected were broken into four time quadrants: 0 to 1 week postinfection, 2 to 3 weeks postinfection, 4 to 5 weeks postinfection, and 6 to 8 weeks postinfection. The Kaplan-Meier estimation was used to determine the probability of colonization during each of the four time quadrants postinfection, and the differences in both initial and sustained colonization were examined (Fig. 1).

Initial colonization probabilities for the wild-type group (JRS4) and the mutant groups (either JRS75 or JRS145) fell between 88 and 76%, with no significant difference between the groups ($P = 0.559$, data not shown). These high initial colonization rates during the first 3 days of the experiment indicate that the experimental protocol was sufficient for an infection leading to sustained colonization of the pharynx in Fischer rats. Because no M protein is present for the mutant strains, these results also show that the presence of M protein on the surface is not required during the most initial stage of colonization of the nasopharyngeal surface (37).

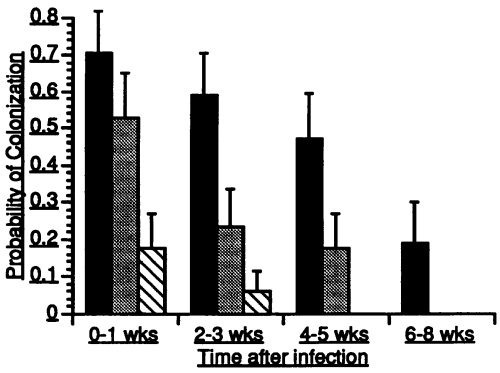

FIG. 1. Summary of results from three colonization experiments with strains JRS4 (M^+) (■), JRS75 (M^-) ▨, and JRS145 (M^-) (▧). The y axis shows the probability of positive colonization ± the standard deviation as estimated by the Kaplan-Meier estimator; the x axis shows the time range during which samples were taken. P values for significance of results for isogenic strains as compared with those for the parent are 0.05 and 0.007 for JRS75 and JRS145, respectively.

M protein may be required for persistence of colonization of the pharyngeal region. By the end of the first week, the colonization probability had dropped to 70, 53, and 18% for groups infected by JRS4, JRS75, and JRS145, respectively (Fig. 1). For the first week, there was no significant difference between JRS4 and JRS75 ($P = 0.436$), while the difference between JRS4 and JRS145 was significant ($P = 0.041$).

When the colonization probabilities for JRS4 (M^+), JRS75 (M^-), and JRS145 (M^-) streptococcal strains were analyzed for the complete 8-week time period, the differences between the wild-type and mutant strains were highly significant ($P = 0.001$) (Fig. 1). This result indicates a difference in the persistence of colonization among these strains. The M-positive strain (JRS4) persisted longer in the pharyngeal cavity of rats than either M-negative strain. Colonization by the JRS145 M-negative strain persisted for the least amount of time after infection, with colonization lasting less than 6 weeks in all animals. There was also a significantly higher percentage of culture-positive swabs from animals infected with the M-positive strain than with either of the M-negative strains, JRS75 and JRS145 ($P = 0.05$ and $P = 0.007$, respectively).

In all instances, isolates taken from the rats were observed to maintain the phenotypes of the infecting strain: JRS4, Sm^r $M_{6.1}{}^+$; JRS75, M^- Sm^r Km^r; JRS145, M^- Sm^r Cm^r. There also appeared to be a difference in persistence of colonization between the two mutant M-negative strains ($P = 0.025$); these strains differ from each other in the size of the deletion present at the *emm*6.1 locus (14, 29). Other than their constructed allelic variation at the *emm*6.1 locus, few, if any, genetic differences should exist between these three strains. Thus, the phenotypic distinctions between the isogenic strains caused by the mutated *emm*6.1 allele are likely to be associated with this difference in persistence.

Two unrelated M6-positive strains of *S. pyogenes* colonized equally well. To add support to the assertion that the phenotypic distinctions between genetically related strains were responsible for colonization differences, two unrelated, but phenotypically similar, GAS strains were studied. JRS4 is the parent strain for the isogenic strain comparison; S43-29R is a mouse-virulent strain that was passaged intraperitoneally in mice (28). The abilities of these two

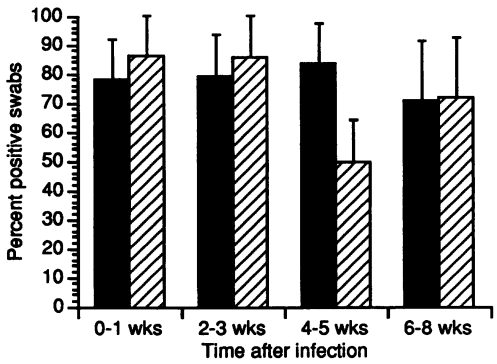

FIG. 2. Summary of results from three colonization experiments comparing strains JRS4 (■) and S43/29R (▨). The y axis shows percent positive throat swabs ± the standard deviation; the x axis shows the time range during which samples were taken.

unrelated M6-positive GAS strains to colonize the nasopharynx were compared in an experiment repeated three times (Fig. 2; one example in Table 1). Strains of the M6 serotype were used because of the availability of M6-specific antisera in order to study the interaction of the host immune response to this antigen at a mucosal surface and the potential variability of the M6 antigen in relation to that host immune response.

TABLE 1. Colonization of the pharynx of Fischer rats by *S. pyogenes* JRS4 and S43/29R[a]

Day post-infection	No. of beta-hemolytic colonies/throat swab						
	JRS4				S43/29R		
	Rat 1[b]	Rat 2[b]	Rat 3[c]	Rat 4[c]	Rat 1[d]	Rat 2[d]	Rat 3[d]
2	1	1	2	3	0	0	0
3					1	12	1
4	2	3	4	11	0	3	3
6					3	1	16
8					2	2	1
9	30	40	100	100			
10					2	33	7
11	200	100	21	35			
14	100	200	100	12			
16					2	0	2
24	200	7	100	100			
27					0	0	3
34					0	3	0
36	41	0	3	13			
43	20	2	0	3			
54					0	4	36
63	32	7	1	1			
71	3	4					
84	8	20					
87	0	10					
91	4	8					
99	5	0					
105	38	2					
112	2	1					
114	3	11					
122	4	1					
162	3	4					

[a] Rats were infected on day 1 and were placed on water containing streptomycin (1,000 μg/ml) at the time of infection.
[b] Sacrificed on day 162.
[c] Sacrificed on day 65.
[d] Sacrificed on day 84.

TABLE 2. M6-specific serum and salivary antibody measured after colonization of the rat nasopharynx by GAS[a]

Day post-infection	Total serum anti-M6 activity[b]		Salivary IgA anti-M6 activity[b]	
	Strain JRS4	Strain S43/29R	Strain JRS4	Strain S43/29R
65	458 ± 60		1.5 ± 0.5	
69		4,000 ± 400		79 ± 30
84		4,188 ± 700		10 ± 3
134	506 ± 40		19 ± 10	
162	500 ± 50		1.5 ± 1	

[a] Antibody levels were determined by ELISA (see Materials and Methods for details).
[b] Values represent the means of duplicate assays of two or three rat serum samples and are the reciprocal dilutions giving the 50% endpoints of antibody titration ± ranges for different sera.

When adult Fischer rats were infected intranasally and orally with 10^9 CFU of either one of these two strains, each strain was capable of colonizing the pharyngeal mucosa for the entire time course of the experiments (4 weeks in three instances; Fig. 2). No significant differences in colonization of the pharyngeal cavities of rats between the two M6-positive strains were found. The average percentages of positive throat swabs through week 8 were 78 and 74% for JRS4 and S43/29R, respectively (Fig. 2).

Neither was there any significant difference in the persistence of these two strains in colonization of the rat pharyngeal cavities. Colonization by either strain persisted for at least 8 weeks, as shown by over 50% positive throat swabs taken (Fig. 2). Rats colonized with either strain showed no signs of illness throughout the course of the experiments. Although the number of beta-hemolytic colonies per throat swab varied extensively from swab to swab, a decline in this number was seen over the course of an experiment (example in Table 1) for both M6-positive strains. In contrast, the percentage of throat swabs that detected colonization (one or more GAS colonies) remained about the same throughout the course of the experiment. One experiment was extended for a 3-month period with no change in the percentage of positive throat swabs detected (data not shown).

Antibodies directed against M6 protein that were generated in response to colonization were detected in both serum and saliva by ELISA in samples taken at several different times postinfection (Table 2). Although present, salivary IgA anti-M6 antibody activity was quite low (Table 2). Serum anti-M6 antibody activities were about 10-fold higher after colonization with strain S43-29R than with JRS4. This difference in antibody levels was not reflected in any significant difference in colonization between these two strains. Control rats not challenged with the test streptococcal strain showed no antibody activity to M6 protein (data not shown).

Variability of surface antigen M protein during colonization. The potential modulation in the expression of M6 protein during colonization was addressed in these studies by examining the M protein present in sequential GAS isolates from a single rat taken at different time points postinfection. Lysin-extracted proteins from isolates taken at days 10, 43, 105, and 162 postinfection with the M6.1-positive strain JRS4 are shown in Fig. 3. No isolate-specific differences in the size or quantity of M6 protein were found. Lysin-extracted proteins from different isolates were also indistinguishable from those in the infecting strain in their detection with three separate monoclonal antibodies that

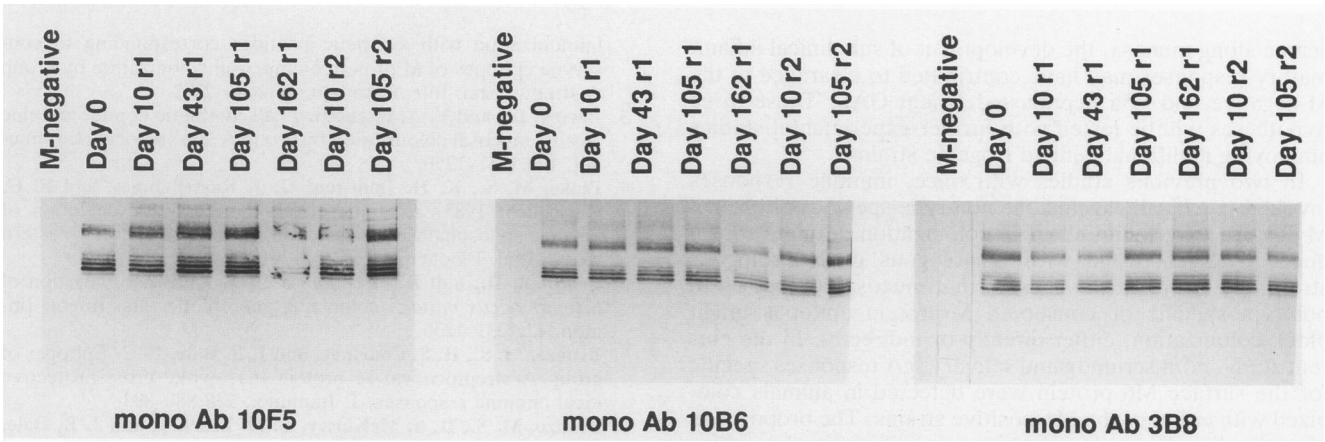

FIG. 3. Sample Western blots of M protein extracted from isolates taken from rats at different times postinfection with strain JRS4. Lane 1, lysin extract from the M-negative strain JRS75; lane 2, lysin extract from the inoculum used for infection; lanes 3 through 6, separate isolates taken from rat 1; lanes 7 and 8, isolates from a second rat (lane numbers are from left to right). Immunoblots were developed with the monoclonal antibodies indicated at the bottom of each gel.

recognize M6 protein; results for two of these isolates are shown in Fig. 3. Additional isolates from time points over 30 days postinfection confirmed that there was also no significant decline in the amount of M protein expressed on those isolates taken late in infection as compared with those taken early in infection.

DISCUSSION

All four *S. pyogenes* strains tested, including S43-29R (M6 positive), JRS4 (M6.1 positive), JRS75 (M negative), and JRS145 (M negative), colonized the nasopharynx of streptomycin-treated Fischer rats when the rats were infected intranasally and orally. This observation suggests that each strain can survive and replicate in the nasopharynx in the presence of reduced numbers of indigenous oral flora. Indeed, all strains were maintained at this site even after the development of an indigenous oral flora resistant to streptomycin at about 48 h (data not shown).

M protein did not appear to be associated with the initial ability of strains to colonize the pharyngeal region of the rat in this model. Two near-isogenic M-negative strains which had specific deletions in the gene encoding M protein yielded numbers of positive throat swabs equivalent to those for their parental M6-positive strain in the initial week following infection. However, the M6-positive parental strain was significantly more persistent in remaining in a pharyngeal location. Indeed, the M6-positive strains can be recovered up to 5 months after infection (data not shown). The difference in persistence between GAS strains suggests that either M protein or another factor varying between these near-isogenic strains is associated with persistence of GAS pharyngeal colonization.

There are several possible explanations for the significant differences in colonization observed with these isogenic strains. Two possibilities are based upon roles in pathogenesis that have previously been attributed to M protein. First, the antiphagocytic activity of M protein could serve as some protection from phagocytosis for the GAS during colonization and thus allow persistence at the nasopharynx. It is unclear whether this protective role would be invoked at the mucosal surface because of the relative scarcity of polymorphonuclear leukocytes at this anatomical site. Secondly, M protein could assist in persistent colonization because of its binding capabilities. M protein has been reported to contribute to pharyngeal specific binding capacity of bacterial cells (12, 41, 42). The binding of M protein to a specific epithelial cellular receptor could occur upon initial interaction with the epithelial surface or as a second step in adhesion after initial contacts have been made (18). Alternatively, M protein may serve to assist in the aggregation of bacteria already bound to cell surfaces (9). Recent observations suggest that the binding activities of M protein are not required for initial interactions with epithelial tissue (9, 17), and the present rat colonization studies show that M protein is apparently also not required for initial colonization. Previously detected binding activities of M protein could still play a role in the persistence of colonization observed in this experimental system.

There was a difference in the persistence of colonization between the two M-negative streptococcal strains, suggesting that factors in addition to M protein contribute to the survival of the organism on the epithelial surface of the pharynx. However, the M-positive strain not only persisted longer than the M-negative strains after infection of the rats but throat swabs were more frequently positive than those from rats infected with either M-negative strain. In addition, the numbers of beta-hemolytic colonies recovered from M-positive animals, as compared with those recovered from animals infected with the mutant organisms, were often higher (data not shown). Thus, infection of rats with the streptococcal strain expressing M protein appeared to result in heavier, as well as more persistent, colonization of pharyngeal tissues.

An additional possibility is that the results observed are not attributable to M protein but to a second difference between the near-isogenic strains used in the study. In the construction of the deletion of the *emm*6.1 gene in both JRS75 and JRS145, a small segment of DNA extending approximately 500 bp downstream of the *emm*6.1 allele was also deleted (17, 32). This 500-bp region includes the promoter and first 100 codons of the *scpA* gene (8, 11, 20a). Therefore, it is likely that the isogenic M-negative strains are also lacking the C5a peptidase activity expressed by GAS strains. Conceivably, this activity could also play a role in the persistence of GAS at a colonization site; strains lacking the activity might be more likely to recruit inflammatory cells via the complement component C5a. Although polymorphonuclear leukocytes and complement are normally

scarce along mucosa, the development of subclinical inflammatory responses may have contributed to clearance of the M-negative and C5a peptidase-deficient GAS. These three hypotheses will be tested with further experimental studies employing additional defined isogenic strains.

In two previous studies with mice, immune responses invoked specifically against the non-type-specific epitopes of M protein were found to lower colonization during a subsequent GAS challenge with homologous or heterologous strains (3, 6, 7). This suggested that mucosal antibodies to non-type-specific or conserved M-protein epitopes might block colonization, either directly or indirectly. In the current study, both serum Ig and salivary IgA responses specific for the surface M6 protein were detected in animals colonized with either of the M6-positive strains. The proportions of antibodies directed against type-specific epitopes versus non-type-specific epitopes were not determined. However, the development of specific antibody responses did not result in the clearance of GAS bacteria from the pharyngeal region. The mechanism of persistent colonization, despite the presence of specific antibody seen in this study and in the human carrier state, is unknown; perhaps antibodies directed against the non-type-specific region are explicitly required for the abrogation of colonization as seen in the mouse studies (3, 7).

The possibility that variation of the M6 protein occurred in response to antibody during colonization was explored by examining GAS isolates taken at different time points throughout the experiment. No variation in either the apparent amount or size of M6 protein was found. However, it is unclear if the antibody responses at the mucosal surfaces were of sufficient levels to effect clearance of the organisms, as the levels of salivary IgA were low. Additional studies are needed to characterize the interaction of specific antibody, as well as other host responses, with chronic infection by GAS.

These experiments indicate that the rat model described in this study may be useful for exploring various aspects of initial colonization at a pharyngeal site by GAS. The rat studies encompass several facets of GAS infection in humans, in which prolonged asymptomatic colonization by GAS has been noted in the presence of immune responses against the infecting bacteria (35). Thus, this experimental system may prove to be useful for understanding factors contributing to this type of streptococcal carrier state.

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