

PASSIVE ACQUIRED MUCOSAL IMMUNITY TO GROUP A
STREPTOCOCCI BY SECRETORY IMMUNOGLOBULIN A

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M protein is a major surface component of group A streptococci, and more than 80 distinct serological types exist. Antigenic variability resides in the NH₂-terminal distal portion of the fibrillar M protein molecule, whereas the COOH-terminal half is highly conserved among different M serotypes (1, 2). With few exceptions, only serum IgG directed to the antigenically variable portion of M protein initiates opsonization and phagocytosis of streptococci by polymorphonuclear leukocytes (3). The presence of type-specific antibodies in serum correlates with protection against group A streptococcal pharyngitis in humans (4, 5), which involves inflammation of the nasopharyngeal mucosa and underlying tissue. Alternatively, this organism can colonize the pharyngeal mucosa leading to a carrier state without clinical signs. The carrier state can persist after the development of type-specific serum antibodies (5, 6), suggesting that opsonic IgG is protective only after streptococcal infection (i.e., pharyngitis) is established.

While humans are considered to be the primary reservoir for group A streptococci, natural infection has been reported to occur in mice with the M50 serotype (7, 8). Streptococci administered intranasally to mice can cause death by colonizing and subsequently invading the mucosal barrier and disseminating to systemic sites. Immunological protection against lethal streptococcal infection by the intranasal route might occur at either a mucosal or nonmucosal (underlying tissue, systemic) site. Secretory IgA (sIgA) can protect mucosal surfaces from colonization by pathogenic bacteria, and the effector functions of sIgA differ from those of serum-derived Igs. We investigated the role of anti-M protein-specific sIgA in protecting against streptococcal infection at the mucosa. Using a mouse model, live streptococci were mixed with affinity-purified antibodies and were administered intranasally. The data indicate that passively acquired anti-M protein sIgA given by the intranasal route protected mice against streptococcal infection, whereas opsonic serum Ig administered intranasally was without effect. The results suggest that sIgA can protect at the mucosa, and may preclude the need for opsonic IgG in preventing streptococcal infection.

Materials and Methods

Purification of Antibodies. Pooled human saliva was clarified by ultracentrifugation (50,000 g, 2 h), passed over jacalin immobilized on agarose (Pierce Chemical Co., Rockford, IL), eluted with 0.1 M melibiose, dialyzed, and concentrated. Antibodies directed to type 6 M

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protein (M6) were purified from whole serum (pooled human and hyperimmune rabbit) or from salivary IgA by passage over glutaraldehyde glass beads (Boehringer Mannheim Biochemicals, Indianapolis, IN) derivatized with M6 protein (ColiM6, the product of the M6 gene cloned in *Escherichia coli*) (9), eluted with 0.1 M glycine, pH 2.1, dialyzed, concentrated, and adjusted to 1.5% BSA/0.03 M Hepes, pH 7.4. Affinity purification on the M6 column resulted in absorption of >90% of the anti-M6 protein immunoreactivity. The opsonic mAb 3B8 (10) was added to the anti-M6 serum Ig preparation at a final concentration of 400 µg/ml to further maximize the array of immunospecificities in this fraction. The final volume of purified antibodies to M6 protein was concentrated 200-fold over unfractionated saliva for sIgA, and threefold over whole serum for serum Igs.

ELISA. Microtiter wells were coated with 0.5 (Fig. 1 A) or 1.0 µg/ml (Fig. 1 B) of ColiM6 protein. For competition ELISA (11), antibodies were preincubated (18 h, 4°C) with decreasing concentrations of ColiM6 starting at a 50-mol excess relative to the amount used to coat wells. A fixed concentration (1:200 dilution) of the two antibody preparations (anti-M6 sera Ig and sIgA) was chosen for competitive inhibition because this dilution gave a level of immunoreactivity for each component (human, rabbit, mouse) that could be measured by competition. All incubations containing antibodies were performed in 0.01 M sodium phosphate, pH 7.4/0.5 M NaCl/0.25% Brij. Secondary antibodies were conjugated with alkaline-phosphatase (Sigma Chemical Co., St. Louis, MO), and immunoreactivity was detected at A₄₀₅ using *p*-nitrophenyl phosphate substrate (to establish the endpoint titer in Fig. 1 B, the developing time was 60 min at 37°C).

Bactericidal Assay. Purified antibody or whole serum (100 µl) was added to type 6 streptococcal strain S43/192 in Todd-Hewitt broth (100 µl) and whole heparinized blood (400 µl) from a nonopsonic donor. The mixture was rotated for 3 h at 37°C, a portion was plated onto proteose peptone agar, and viable organisms were enumerated by measuring CFU (12).

Mouse Protection Assay. Type 6 streptococci (strain S43/192) were grown to mid-log phase in Todd-Hewitt broth, and each animal received (intranasally) between 5×10^4 and 5×10^5 CFU (depending on the experiment) suspended in 20 µl of control buffer or purified M6 protein-specific antibody. The time elapsed between suspension of streptococci in antibody or control buffer, and administration to mice, was no longer than 10–15 min. Intranasal inoculations were delivered to the nares of unanesthetized mice through a Hamilton syringe. Female, BALB/c *nu/+* mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were 4–6 wk old.

Ig Binding Assay. The binding of radioiodinated IgA and IgG to mid-log phase bacteria suspended in 1.5% BSA-saline was performed as previously described (13). Purified human myeloma IgA (ICN ImmunoBiologicals, Lisle, IL) and human sera IgG (CooperBiomedical, Inc., West Chester, PA) were labeled with ¹²⁵I using Iodo-beads (Pierce Chemical Company).

Results and Discussion

Pooled human salivary IgA was isolated by binding to jacalin, a lectin with specificity for human IgA1 (14). Antibodies directed to M6 protein were purified from jacalin-released sIgA or from whole serum (pooled human and rabbit) by passage over an immunoabsorbent column of the native M6 molecule, which contains both type-specific and conserved immunodeterminants. Saliva or serum was pooled from several sources to compensate for individual responses to different M protein epitopes. The M6-specific antibodies from hyperimmune rabbit serum and mAb to M6 protein were included in the serum Ig preparation to further maximize the array of immunospecificities in this fraction.

The immunoreactivity of the two antibody preparations was evaluated by ELISA. The anti-M6 sera Ig contains antibodies from three species, each of which requires a distinct secondary antibody for detection. Therefore, to evaluate the relative immunoreactivities of anti-M6 sIgA and sera Ig, a competition ELISA using a fixed antibody concentration and ColiM6 as the inhibitor was performed (Fig. 1 A). The quantity of M6 protein required for 50% inhibition of each anti-M6 serum Ig com-

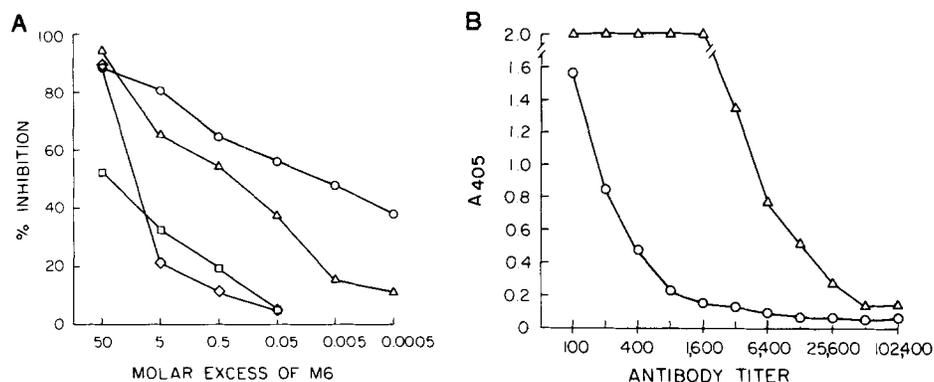


FIGURE 1. Immunoreactivity of antibody used in passive protection. (A) Competition ELISA with M6 protein. Percent inhibition of binding of purified anti-M6 sera Ig detected with secondary antibodies to human light chain (λ and κ) (Δ), rabbit IgG (\square), and mouse IgG (\diamond), and of purified anti-M6 sIgA detected with anti-human light chain (\circ). (B) ELISA measuring direct binding to M6 protein by anti-M6 sIgA (\circ) and sera Ig (Δ) detected with anti-human light chain.

ponent was at least 32-fold greater than that required to inhibit sIgA. Direct binding of sIgA to M6 protein was compared with the human component of the sera Ig preparation by using anti-light chain secondary antibody (Fig. 1 B). The endpoint titer (highest dilution reciprocal of antibody giving A_{405} equal to 1.0) of human sera Ig exceeded that of anti-M6 salivary IgA by ~ 28 -fold. The quantity of IgG present in the sIgA preparation was determined by capture ELISA and was estimated to be $<1.0\%$ (data not shown).

The bactericidal assay was used to determine the opsonic activity of the antibodies used in passive immunization (Table I). When type 6 streptococci were rotated in the blood of a donor who lacked antibodies specific for the M6 molecule, the organisms evaded phagocytosis and grew freely (control). Purified anti-M6 serum Ig displayed opsonic activity, and the organisms did not survive. In contrast, purified anti-M6 sIgA failed to promote phagocytosis; one reason for this behavior may be due to the inability of sIgA to fix complement efficiently (15). In addition, the opsonic activity of pooled sera from those individuals who served as sources for the purified sIgA and serum Ig was measured. Sera from both the serum Ig and salivary IgA sources was effective in opsonizing type 6 streptococci.

TABLE I
Bactericidal Activity of Antibody Used in Passive Immunization

Addition of:	Colony forming units*	
	Inoculum: 50	23
Sera (nonopsonic control)	8,624	4,914
Sera (from sera Ig source)	<10	<10
Sera (from sIgA source)	49	14
Purified anti-M6 sera Ig	35	<10
Purified anti-M6 sIgA	$>10,000$	3,640

* Expressed as total colony forming units.

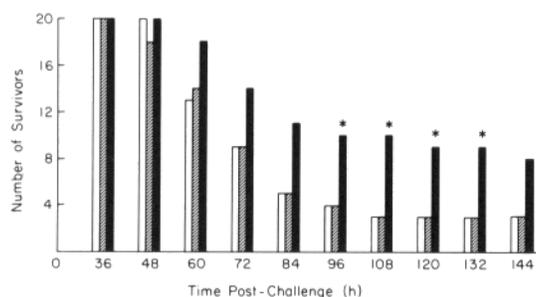


FIGURE 2. Passive protection of mice against streptococcal challenge (intranasally) by anti-M6 protein salivary IgA. Mice received live organisms suspended in 1.5% BSA/0.03 M Hepes, pH 7.4 (□, control), anti-M6 serum Ig (▨), or anti-M6 sIgA (■). Three separate experiments were performed, and a total of 20 animals are represented per group. The number of mice surviving streptococcal challenge was recorded at 12-h intervals. Significant difference (*) between sIgA and control according to χ^2 analysis ($p < 0.05$).

Fig. 2 shows that intranasal administration of anti-M6 protein salivary IgA had a significant blocking effect on mouse mortality resulting from intranasal challenge with type 6 streptococci. Beginning at 60 h post-challenge, animals given anti-M6 sIgA displayed a higher rate of survival than the control group, which had received streptococci suspended in 1.5% BSA only. Between 96 and 132 h post-challenge, the difference between these two groups was significant. Furthermore, the number of survivors in the sIgA group exceeded the control at every time point in each of three independent experiments (data not shown). However, to the contrary, mice given the anti-M6 serum Ig preparation exhibited mortality rates nearly identical to the control group.

In a separate set of two experiments, antibodies derived from a single human donor protected in a manner similar to antibodies from pooled sources. At 7 d after streptococcal challenge, 75% (15/20) of the mice given anti-M6 sIgA (intranasally) from the single donor had survived, compared with 35% (7/20) given anti-M6 sera Ig and 40% (8/20) of the 1.5% BSA control group. Thus, despite its lower immunoreactivity (Fig. 1) and lack of opsonic activity (Table I), anti-M6 salivary IgA significantly inhibited streptococcal infection at the mucosal level, whereas serum-derived anti-M6 antibodies were without effect at this site. Furthermore, antibodies derived from either pooled sources or a single donor had similar patterns of protective ability.

Many isolates of streptococci and staphylococci possess surface receptors for IgA

TABLE II
Lack of Nonimmune Binding of Ig by Type 6 Streptococci

Organism [†]	Percent bound*	
	(¹²⁵ I)-IgA	(¹²⁵ I)-IgG
S43/192	3.63 ± 0.22	1.66 ± 0.28
H36B-5	50.91 ± 2.02	1.65 ± 0.09
090R	5.30 ± 0.45	ND
Cowan I	ND	53.82 ± 2.76

* Average mean and SD of percent radioactivity bound to organisms measured in triplicate.

[†] Group A streptococci (S43/192), group B streptococci (H36B-5 and 090R), and *S. aureus* (Cowan I).

and IgG (13). To establish that strain S43/192 did not interact with Igs by a nonimmune mechanism, the binding of radioiodinated IgA and IgG to the surface of S43/192 streptococci was measured (Table II). Group B streptococci (GBS) that express an IgA binding-protein (H36B-5), and *Staphylococcus aureus* (Cowan I strain) served as positive controls for IgA and IgG binding, respectively. Strain S43/192 bound radiolabeled IgA at low levels that were comparable with that bound by a GBS strain (090R) that lacks the IgA binding protein. The data indicate that the type 6 streptococcal strain used for challenge in this study fails to bind either IgA or IgG by a nonimmune mechanism.

It does not appear that passively acquired serum Igs were used at the mucosa by a mechanism analogous to that which occurs in blood, involving complement fixation by anti-M protein IgG bound to the streptococcal surface and subsequent phagocytosis by polymorphonuclear leukocytes (16, 17). Our data is consistent with the idea that opsonic IgG is protective only after streptococcal infection is established, and suggests that sIgA alone can protect before the initiation of infection. Antibody to M protein is defined as type-specific by its ability to opsonize and phagocytize streptococci in whole blood (3). The ability to protect mucosally with sIgA, which is not opsonic, raises the possibility that antibodies directed to non-type-specific determinants may be sufficient for initial defense against streptococcal infection. Studies in progress shall compare the protective ability of sIgA directed to type-specific and conserved M protein epitopes.

Other investigators have demonstrated that total sIgA derived from immune donors can passively protect against mucosal infection by bacterial pathogens (18). Perhaps the most important finding of this passive protection study is that affinity-purified sIgA protected against infection under conditions whereby serum-derived Ig was completely ineffective. A clear distinction between sIgA and sera Ig should enable one to better understand the contribution of antigen-specific sIgA in protection against mucosal infections. This model might provide greater insight to the effector functions of sIgA and its role in blocking bacterial adherence.

Summary

We present a model in which animals are passively immunized at a mucosal site, allowing one to evaluate immunological protection at the mucosal level only. Affinity-purified, anti-M protein sIgA administered intranasally protected mice against systemic infection after intranasal challenge with group A streptococci. In contrast, anti-M protein serum Ig administered intranasally was not protective at this site, although it neutralized the antiphagocytic property of M protein and promoted phagocytosis. Protection by sIgA occurred despite the lower immunoreactivity of sIgA to purified M protein compared with serum Ig. The data suggest that sIgA can protect at the mucosa and may preclude the need for opsonic IgG in preventing streptococcal infection.

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