COMMON PROTECTIVE ANTIGENS OF GROUP A STREPTOCOCCAL M PROTEINS MASKED BY FIBRINOGEN

By ELLEN WHITNACK, JAMES B. DALE, AND EDWIN H. BEACHEY

From the Veterans Administration Medical Center and the University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38104

The surface M protein of group A streptococci is the principal virulence factor of these organisms. Streptococci lacking M protein are efficiently opsonized by the alternate complement pathway (1, 2) and in consequence are rapidly ingested and killed by host leukocytes. Streptococci possessing surface M protein are neither opsonized nor ingested unless antibody to the M protein is present (3). Two hypotheses, which are not mutually exclusive, have been advanced to explain the antiphagocytic properties of M protein: it may be toxic to the phagocyte in some way; or it may prevent opsonization of the streptococcal cells. The first possibility has not been investigated in detail. Crude, hot-acid extracts of M protein are toxic to polymorphonuclear leukocytes, but this appears to be due to the formation of antigen-antibody complexes involving non–type-specific antigens, rather than to a direct toxic effect of the M protein (4). M protein could nonetheless be toxic in the high concentrations prevailing at the surface of a streptococcal cell at its point of attachment to a phagocyte. Recently, Manjula and Fischetti (5) have speculated that M protein, which has certain structural homologies to α-tropomyosin, might disable the phagocyte by interfering with its contractile proteins.

The second possibility—that M protein interferes with opsonization—has extensive experimental support. When suspended in fresh serum, streptococci lacking M protein are opsonized by complement via the alternate pathway, which must be intact for phagocytosis to occur (2). Streptococci possessing M protein activate this pathway less efficiently (1, 6). Jacks-Weis and her colleagues (6) noted that although M-positive streptococcal cells did activate complement and bind it (C3) on their surface, the bound C3 was distributed unevenly as shown by immunofluorescence microscopy. They suggested that this patchiness prevented the smooth operation of the "zipper" mechanism of internalization proposed by Griffin et al. (7).

In a previous report, we showed that virulent M-rich streptococci, although partially resistant to opsonization when suspended in nonimmune human serum, were completely resistant when suspended in plasma (8). The opsonization-inhibiting activity of plasma was found to reside in fibrinogen: purified fibrinogen

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added to streptococci and phagocytes suspended in serum suppressed opsonization in a dose-dependent manner. These observations suggested that a reaction first reported almost 25 years ago, the precipitation of fibrinogen by extracts of M protein (9, 10), might be relevant to the known antiphagocytic functions of this molecule. We found that fibrinogen bound to the fibrillae of type 24 streptococcal cells and thereby prevented binding of complement (C3) to the cell surface (8, 11).

In the previous study, we investigated the influence of fibrinogen on nonimmune opsonization of streptococci by complement. In the present study, we investigated the influence of fibrinogen on antibody-mediated opsonization of these organisms. We present data to suggest that purified M proteins elicit antibodies directed toward both fibrinogen-binding and fibrinogen-nonbinding regions of the M protein molecule, and that certain cross-reactions between M protein serotypes involve fibrinogen binding sites. We suggest that some of the known structural and antigenic similarities among M proteins of different serotypes may be related to their ability to bind fibrinogen, which is in part the basis for their antiopsonic function.

Materials and Methods

Streptococci. Group A streptococci of types 5 (Manfredo), 6 (S43), and 24 (Vaughn) were blood-passed to achieve a high level of resistance to phagocytosis (3). Cultures were stored at −80°C and subsequently on blood agar plates at 4°C. Colonies were grown overnight at 37°C in Todd-Hewitt broth (Difco Laboratories, Detroit, MI) supplemented with 20% normal rabbit serum (Gibco Laboratories, Grand Island, NY), subcultured 1:100 in the same medium, and grown to early log phase. Organisms were harvested by centrifugation, exposed to ultraviolet light for 3 min, washed in phosphate-buffered saline (PBS)[1] (0.15 M NaCl–0.02 M phosphate, pH 7.2–7.4), and resuspended in PBS to the desired optical density.

Antisera to Streptococcal M Proteins. Large polypeptide fragments of M proteins of types 5, 6, 19, and 24—"pep M proteins"—were purified from pepsin extracts of whole organisms (12). Rabbits were immunized intracutaneously with 100 μg of pep M protein emulsified in complete Freund's adjuvant and boosted 1 wk later with an additional 100 μg dose in incomplete Freund's adjuvant as previously described (12). Antisera were inactivated by heating at 56°C for 30 min. Each antiserum was tested in an enzyme-linked immunosorbent assay (ELISA) for reaction with pep M proteins of types 5, 6, 19, and 24, using a semi-automated EIA system (PR-50, Gilford Instrument Laboratories, Inc., Oberlin, OH) (13).

Human Blood and Blood Components. Normal volunteers were selected who lacked preexisting antibody to types 5, 6, and 24 streptococci as determined by ELISA (reciprocal titers ≤200) and by opsonophagocytic tests (see below) using whole blood and blood reconstituted from serum or plasma and cells. Plasma and blood cells were obtained from whole blood to which heparin 5 U/ml (≃8 U/ml plasma) was added (heparin from hog intestine, Eli Lilly and Co., Indianapolis, IN). Erythrocytes and buffy coat were washed three times in a total of 100 ml Hanks' balanced salt solution. Serum was collected so as to minimize the formation of fibrin(ogen) degradation products and the loss of labile opsonins. Blood was drawn without stasis and allowed to clot completely during centrifugation at room temperature; serum was separated promptly and iced. The sera contained

[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; cfu, colony-forming units; ELISA, enzyme-linked immunosorbent assay; FHP, fresh heparinized (human) plasma; FHS, fresh heparinized (human) serum; pep M, purified pepsin extract of M protein; PMN, neutrophilic polymorphonuclear leukocytes.

<1 nM fibrinogen equivalent by the Thrombo-Wellcotest latex agglutination test (Burroughs Wellcome Co., Greenville, NC). Heparin was added to sera at 8 U/ml.

Opsonophagocytic Tests. In the variation of the standard opsonophagocytic test (12) employed here, 10^7 colony-forming units (cfu) of streptococci in 20 μl PBS were preincubated for 15 min at 37°C with 100 μl diluted antiserum or control plus 100 μl fibrinogen at the desired concentration, brought to room temperature, and then mixed with 200 μl fresh heparinized serum (FHS) and 150 μl washed blood cells. In some experiments, organisms were preincubated at 37°C with antiserum and either fresh serum or fresh plasma before addition of blood cells. Test mixtures were rotated at 10 rpm end-over-end at 37°C. Small samples (20 μl) were removed at 15-min intervals, smeared on glass slides, stained with Wright's stain, and examined under oil immersion. One or two hundred PMN were counted, noting the percentage with associated bacteria (percent association) without attempting to distinguish attached from ingested organisms. All values shown in the text were obtained at 30 min of incubation. The procedure differed from that previously reported (8) in that the entire buffy coat was used instead of isolated PMN, and the concentrations of PMN and streptococcal cells in the test mixtures were both reduced two- to threefold so that the baseline percent association (i.e., without antibody) was <40%, and the maximum percent association was <80%.

Bactericidal Test. The bactericidal test was performed similarly to the opsonophagocytic test, except that the inoculum was reduced by dilution to <200 cfu and the incubation period was extended to 3 h (14). 50 μl of a log-phase culture diluted in Todd-Hewitt broth was added to a mixture of 50 μl normal or immune rabbit serum and 100 μl human plasma or heparinized serum. After incubation at ambient temperature for 15 min, 200 μl washed blood cells and 150 μl additional plasma or serum were added and the mixtures rotated for 3 h at 37°C. Surviving organisms were counted by the pour plate method.

Fibrinogen. Human fibrinogen (90% clottable, Grade L, KABI Diagnostica, Stockholm, Sweden) was dissolved in PBS containing 1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) at a concentration of 10 μM, dialyzed against PBS to remove citrate, and stored at −20°C.

Affinity Chromatography and Radiolabeling of Anti-pep M5. Pep M5 (10 mg) was covalently linked by one-step carbodiimide coupling to 2.5 g CH-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as previously described (14). After the column was equilibrated with PBS, 0.5 ml of rabbit anti-pep M5 was applied and allowed to incubate for 30 min at ambient temperature. The column was then washed with PBS until the ultraviolet absorbance at 230 nm of the effluent returned to baseline. Anti-pep M5 antibodies were eluted with 0.2 M glycine–0.2 M NaCl, pH 2.8. The antibody-containing fractions were dialyzed against PBS and concentrated by membrane filtration (YM 10 membrane, Amicon Corp., Scientific Systems Division, Lexington, MA). The protein concentration of the product was 23 μg/ml as estimated by the method of Waddell (15). Radioiodination of 3 μg of protein was carried out using a commercial lactoperoxidase kit (NEZ-151, New England Nuclear, Boston, MA). Specific activity of the product was 0.2 Ci/g protein.

Binding of Radiolabeled Anti-pep M5 to Streptococcal Cells. Incubation mixtures consisted of 10^8 cfu UV-treated, log-phase streptococci, affinity-purified antibody, and fibrinogen in a total volume of 200 μl PBS containing 0.1% Tween-20. Incubations were carried out in 1.5 ml polypropylene tubes at ambient temperature for 45 min. The mixtures were centrifuged at 15,600 *g* for 2 min. The pellets were washed three times in PBS-0.1% Tween-20. Washed pellets were counted in a gamma scintillation spectrometer (Model 578, Packard Instrument Co., Downers Grove, IL).

Results

In a previous study, we demonstrated that fibrinogen in nonimmune plasma interfered with the complement-mediated opsonization of group A streptococci by binding to the M protein on the surface of the organisms. In the present study, the influence of the fibrinogen–M protein interaction on the antibody-

mediated opsonization of M-rich streptococci was investigated. For these studies, we selected types 5, 6, 19, and 24 streptococci and antisera raised in rabbits against the respective pep M proteins. Each of the antisera except anti-pep M24 showed cross-reactions with other serotypes (Table I). The use of these anti-sera in the following studies enabled an evaluation of type-specific and cross-reactive epitopes in relation to fibrinogen–M protein interactions.

Influence of Fibrinogen on Opsonization by Homologous Anti-pep M Antisera. The observation that association of type 24 streptococci with neutrophils is reduced in the presence of fibrinogen (8) was extended to the three other serotypes used in the present study. For example, type 6 streptococci were only partially resistant to uptake by leukocytes suspended in fresh serum but were completely resistant when suspended in fresh plasma (Fig. 1). Addition of rabbit antiserum raised against purified homologous pep M6 promoted uptake both in serum and in plasma in a dose-related manner, and as the antibody concentration was increased, the difference in uptake between plasma and serum was abolished (Fig. 1). Similar results were obtained with streptococci of types 5 (see Fig. 4), 19, and 24 (data not shown). These results suggested that the homologous M antibodies either competed successfully with fibrinogen for common M protein binding regions or recognized epitopes not encompassed by the fibrinogen binding site(s). To determine whether the antibodies reacted with fibrinogen binding or non-binding regions, a suboptimal concentration of homologous antiserum was added with increasing concentrations of fibrinogen to a mixture of type 24 streptococci

TABLE I

Cross-reacting Rabbit Antisera to Streptococcal Pep M Proteins

Serum	Immunizing Ag* (M-type)	Cross-reacting Ag* (M-type)	ELISA titers against:	
			Immunizing Ag*	Cross-reacting Ag*
8104	19	5	25,600	6,400
7908	5	6	102,400	51,200
7633	6	5	51,200	25,600

* Ag, pep M protein antigen.

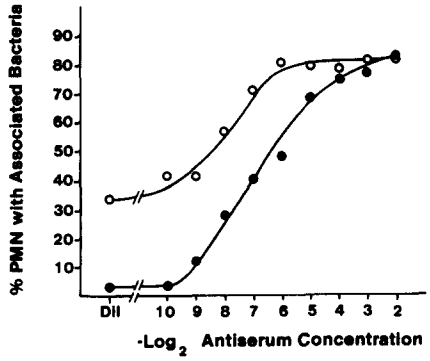

FIGURE 1. Uptake of type 6 streptococci by PMN suspended in serum (○) or plasma (●) to which anti-pep M6 in twofold increasing amounts was added. Diluent (*Dil*), 1% bovine serum albumin in phosphate-buffered saline.

and washed blood cells suspended in fresh human serum. The fibrinogen suppressed opsonization in a dose-related manner (Fig. 2). Suppression, however, was only partial, reaching a plateau at 0.12 μM of fibrinogen, the physiologic concentration of fibrinogen in plasma being $\simeq$10 μM. This finding suggested that there are antigenic sites on M protein that remain exposed and available for reaction with antibody even when fibrinogen has presumably saturated its binding sites. At the higher concentrations of antibody, however, opsonization could be partly inhibited if the organisms were exposed to fibrinogen before addition of antibody (Fig. 3), suggesting that there are other sites on M protein for which fibrinogen and antibody compete.

Influence of Fibrinogen on Opsonization by M Protein Cross-reactive Antisera. Because the preceding studies suggested a heterogeneity of opsonogenic M protein epitopes, some of which reside in fibrinogen binding regions of the molecule, we investigated the possibility that epitopes shared among different M protein serotypes may reside in the fibrinogen binding region, reasoning that fibrinogen

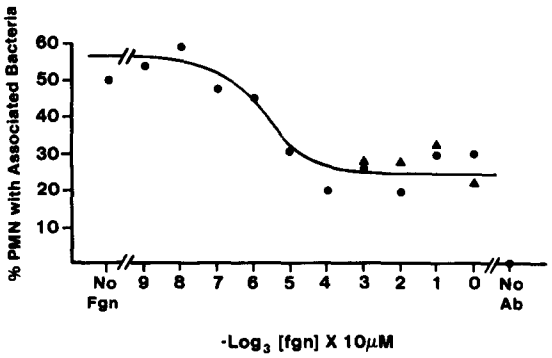

FIGURE 2. Uptake by PMN of type 24 streptococci in the presence of anti-pep M24 to which fibrinogen (*Fgn*) was added in threefold increasing concentrations up to 10 μM. Also shown are experiments in which the streptococcal cells were exposed to fibrinogen for 10 min at room temperature before adding antibody (▲). In the absence of antibody (*No Ab*), fibrinogen (10 μM) completely inhibited uptake.

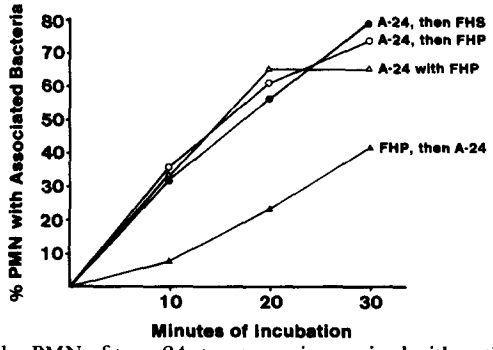

FIGURE 3. Uptake by PMN of type 24 streptococci opsonized with anti-pep M24 (*A-24*) and either fresh heparinized plasma (*FHP*) or serum (*FHS*). At this concentration of antibody, uptake was equal in serum and in plasma. However, when the organisms were exposed to plasma before addition of antibody, partial inhibition of opsonization was obtained.

binding sites on different M proteins might be structurally and therefore antigenically similar. Two cross-reactive antisera were selected because they exhibited high titers of antibody by ELISA against heterologous serotypes of M proteins, but had failed to opsonize the cross-reactive streptococci in the presence of fibrinogen in whole blood. One was an anti-pep M5 that cross-reacted with pep M6 and the other was an anti-pep M6 that cross-reacted with pep M5 (see Table I). A third cross-reactive antiserum, an anti-pep M19 that cross-reacted with pep M5 (Table I), was selected at random from the laboratory files.

First, type 5 streptococci were opsonized with serial twofold dilutions of each antiserum in the presence or absence of 5 μM fibrinogen (Fig. 4). In the homologous system (Fig. 4*A*), uptake of bacteria by PMN was suppressed by fibrinogen only at submaximal antibody concentrations, as previously noted with type 6 organisms (see Fig. 1). In contrast, uptake in the presence of cross-reacting antisera was suppressed even at antiserum concentrations that promoted maximal uptake in the absence of fibrinogen (Fig. 4, *B* and *C*); in the presence of fibrinogen, anti-pep M6 (Fig. 4*C*) failed to promote uptake at any concentration of antiserum tested. Similarly, the anti-pep M5 serum opsonized type 6 streptococci well in the absence of fibrinogen, with uptake approaching its maximum at an antiserum dilution of 1:256, whereas no opsonization could be obtained with undiluted antiserum in the presence of 5 μM fibrinogen (not shown). To confirm the ability of the type-specific and cross-reactive sera to promote killing of the streptococci, bactericidal tests corresponding to the opsonophagocytic tests shown in Fig. 4, *A* and *C* were performed (Table II). In the presence of anti-pep

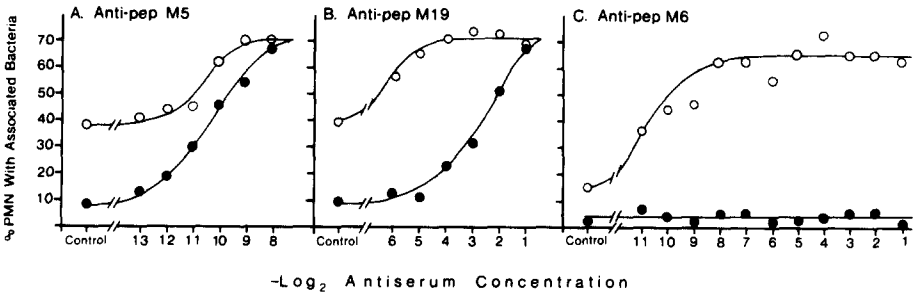

FIGURE 4. Uptake by PMN of type 5 streptococci opsonized with twofold dilutions of homologous anti-pep M5 (*A*), cross-reacting anti-pep M19 (*B*), or cross-reacting anti-pep M6 (*C*) antisera in the presence (●) or absence (○) of 5 μM fibrinogen. Control, 1% bovine serum albumin in phosphate-buffered saline (antiserum diluent).

TABLE II

Bactericidal Test of Type 5 Streptococci with Homologous and Heterologous Anti-pep M Sera

Inoculum	Colonies surviving a 3-h incubation in the presence of:					
	Normal rabbit serum and:		Anti-pep M5 (homologous) and:		Anti-pep M6 (heterologous) and:	
	Plasma	Serum	Plasma	Serum	Plasma	Serum
100	7,200	891	0	0	735	0
25	367	129	0	0	75	0

M5, leukocytes sterilized homologous type 5 organisms suspended either in serum or in plasma. In the presence of the cross-reacting anti-pep M6, type 5 organisms were sterilized only in serum, although partial reduction of growth was obtained in plasma.

Next, streptococci of types 5 and 6 were opsonized with submaximal concentrations of anti-pep M5 in the presence of increasing concentrations of fibrinogen (Fig. 5). The behavior of the homologous system was similar to that previously found for the type 24 system (see Fig. 2), in that partial inhibition was obtained that could not be increased by further addition of fibrinogen. In contrast, uptake of the heterologous type 6 organisms was completely inhibited at the lowest concentration of fibrinogen tested (Fig. 5). Similarly, uptake of type five organisms opsonized with anti-pep M6 was completely suppressed by 0.1 μM fibrinogen (not shown). These results suggested that most of the opsonic cross-reactive antibodies in these two sera were directed against epitopes lying within or closely adjacent to fibrinogen binding regions of the respective M proteins.

Inhibition of the Binding of Type-specific and Cross-reactive M Antibodies to M-rich Streptococci by Fibrinogen. Having established that fibrinogen interferes with opsonization mediated by antibodies against M protein, especially by those against cross-reactive epitopes, we next investigated the effect of fibrinogen on the binding of radiolabeled type-specific and cross-reactive antibodies to M protein on the surfaces of intact streptococci. For this purpose the anti-pep M5 serum was subjected to affinity chromatography on a column of Sepharose to which pep M5 was covalently linked (see Materials and Methods). The purified antibody preparation had ELISA titers of 1:6,400 against pep M5 and 1:800 against pep M6. The effect of fibrinogen on uptake by PMN of types 5 and 6 streptococci opsonized with the purified antibodies was compared to its effect on the binding of radioiodinated antibodies to the same organisms. The quantity of antibody was adjusted in each case so that the control values of binding and uptake for

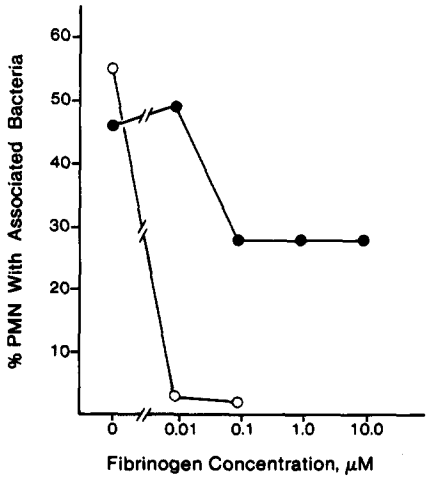

FIGURE 5. Uptake by PMN of type 5 (●) and type 6 (○) streptococci opsonized with anti-pep M5 in the presence of increasing concentrations of fibrinogen. The antiserum was diluted (~1:700) to give submaximal opsonization.

heterologous (type 6) organisms were at least as great as those for homologous (type 5) organisms. Opsonization of types 5 and 6 streptococci by the affinity-purified radioactive antibody (Fig. 6*A*) was similar to that obtained with the original serum (compare Fig. 5). Fibrinogen inhibited binding of antibody both to the homologous and to the heterologous organisms in a manner generally parallel to its inhibition of opsonization, except that the percent inhibition of uptake at any given fibrinogen concentration was greater than the percent inhibition of binding (Fig. 6*B*). This difference may be due to inhibition of fibrinogen of direct (nonimmune) complement-mediated opsonization (8) which, at the low concentrations of antibody used in these experiments, may be a significant component of the total opsonization.

Discussion

This study was undertaken with three purposes in mind: (*a*) to determine the effect of fibrinogen on opsonization of streptococci by antibody to homologous M protein; (*b*) to see whether antibodies are formed against M protein determinants masked by fibrinogen in vivo; and if so (*c*) to see whether any such determinants are shared between M protein serotypes.

Addition of sufficient quantitites of antibody to homologous M protein had much the same effect on uptake by PMN as removal of M protein from the cell surface by trypsin (8): uptake increased substantially regardless of the presence of fibrinogen in the test mixture, and the difference between uptake in serum and in plasma was eliminated. This result is not surprising since opsonization and phagocytosis of group A streptococci in the whole blood of immune persons

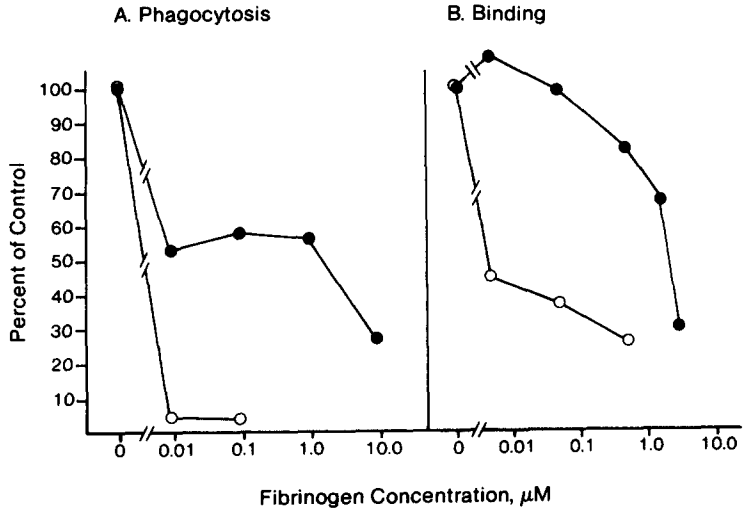

FIGURE 6. Inhibition by fibrinogen of the interaction of anti-pep M5 antibodies with type 5 (●) and type 6 (○) streptococcal cells. (*A*) Uptake by PMN of streptococci opsonized with affinity-purified anti-pep M5 antibodies diluted 1:42 for type 5, 1:11 for type 6. Control (100%) = 45% phagocytosis for type 5, 55% for type 6. (*B*) Binding of ^{125}I-labeled affinity-purified anti-pep M5 antibodies to streptococcal cells. Type 5 cells were treated with labeled antibody (1:92) mixed with unlabeled antibody (1:10). Type 6 cells were treated with labeled antibody alone (1:13). Control binding (100%) = 2,724 cpm for type 5; 5,055 cpm for type 6.

has been repeatedly documented (3). Opsonization by low levels of homologous antibody could be partly reduced in the presence of fibrinogen, but not beyond a certain point, from which we conclude that some M protein determinants remain exposed after binding of fibrinogen. Opsonization in the presence of fibrinogen may therefore be accomplished in part by antibodies against these exposed determinants.

In the absence of fibrinogen, there at least two ways that opsonization of streptococci may be accomplished: activation and binding of complement directly by cell-surface structures (1, 2, 6, 8), and binding of anti-M antibody with subsequent activation and binding of complement (3). Fibrinogen clearly inhibits the first of these (8) and therefore a reduction in bacterial uptake by PMN when fibrinogen is added to immune serum in the presence of complement would not necessarily mean that fibrinogen interferes with immune opsonization. However, we found that at the higher ranges of antiserum concentration, uptake could be partly reduced by pretreatment with fibrinogen, whereas co-treatment had no effect. Apparently certain determinants on M protein that are normally masked by fibrinogen may be occupied by antibody when present in sufficiently high concentration. This conclusion is supported by the finding that fibrinogen inhibits the binding of affinity-purified radiolabeled antibodies to streptococcal cells. Formation of antibodies against determinants normally masked by fibrinogen could occur if the pep M proteins, because they are administered intracutaneously in a mineral oil emulsion, did not form complexes with fibrinogen in vivo; or if pep M–fibrinogen complexes were degraded in the course of antigen processing so as to expose fibrinogen binding sites. Whether such antibodies are formed during a natural infection is at present unknown.

Although immunity to streptococcal infections is generally type-specific, cross-reactions between M serotypes have been encountered by many investigators beginning with the earliest studies of streptococcal immunity (16). Some of these cross-reactions are protective (opsonic), but many others have been detected only in other assays such as immunoprecipitation, hemagglutination, or complement-fixation tests using extracted M proteins (16, 17). With the development of new methods for purifying M proteins, it has been possible to show definitively that there are both opsonogenic and nonopsonogenic determinants shared by M proteins of different serotypes (18, 19). M proteins of types 6 and 14, which share antigenic determinants, yield similar tryptic peptide maps (20). Furthermore, elucidation of the primary structure of types 5, 6, and 24 M proteins has revealed significant sequence homologies among the three types (5, 21). The primary structures predict regions of α-helical coiled-coil conformation for each of these M proteins (5). This prediction has been confirmed for type 6 M protein (22). Thus, M proteins appear to have similar secondary and tertiary structures as well.

The occurrence of structural similarities and shared antigenic determinants among M proteins of different serotypes raises the possibility that some of these common features may pertain to the ability of M proteins to bind fibrinogen, and suggests an explanation for the failure of some anti-M antibodies to opsonize streptococci in vivo or in whole blood. We found that opsonization by three cross-reactive anti-pep M sera was strongly inhibited or abolished by fibrinogen

at physiologic concentration compared with opsonization by homologous antisera. Two of the sera (anti-pep M5 and anti-pep M6) are examples of apparently nonopsonic or poorly opsonic cross-reactive antibodies that become opsonic in vitro when fibrinogen is omitted from the test mixtures. The significance of such antibodies in vivo remains to be investigated.

These studies do not constitute a survey of M protein cross-reactions. There is no reason to think that all cross-reactions involve fibrinogen-binding regions of M protein, and indeed it would be surprising if this were found to be true. Nor is there reason to believe that a single fibrinogen binding epitope is shared by all M protein serotypes; none of the three cross-reactive sera reacted with type 24 M protein. These reservations notwithstanding, our data are consistent with the idea that fibrinogen binding regions of M proteins may have limited serologic specificity. Such an interpretation is attractive from the teleologic standpoint, since one might reasonably expect that in the evolution of M proteins from one serotype to another, fibrinogen binding sites would be conserved to maintain the protective function of M protein, while exposed sites would be varied to escape immune recognition. Further studies with reagents of greater specificity—monoclonal anti-M antibodies and peptide fragments of M protein and fibrinogen—may clarify these issues.

Summary

The influence of fibrinogen on the opsonization of Group A streptococci by type-specific and cross-reactive anti-M protein antisera was investigated. As previously reported for type 24 streptococci, fibrinogen inhibited the complement-mediated opsonization of types 5, 6, and 19 organisms. Rabbit antisera against large peptide fragments of purified homologous M proteins (pep M proteins) overcame the anti-opsonic effect of fibrinogen in a dose-dependent manner. In the presence of optimal amounts of antibody, bacterial uptake by PMN was equal in serum and plasma, and greater than could be obtained in serum in the absence of antibody. Polyclonal rabbit anti-pep M sera contained antibodies directed against fibrinogen-binding as well as fibrinogen-nonbinding sites or regions of the M protein molecule. Three cross-reactive anti-pep M sera included antibodies directed against fibrinogen binding sites or regions of the cross-reacting M proteins. In the two sera studied in detail, these antibodies accounted for a large part of the cross-reacting anti-M antibody present in the sera. We suggest that fibrinogen binding sites on different serotypes of M protein may be structurally and therefore antigenically similar. Conservation of fibrinogen binding sites on M proteins may be related to their protective anti-opsonic function.

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