

INHIBITION OF COMPLEMENT-MEDIATED OPSONIZATION
AND PHAGOCYTOSIS OF *STREPTOCOCCUS PYOGENES* BY D
FRAGMENTS OF FIBRINOGEN AND FIBRIN BOUND TO
CELL SURFACE M PROTEIN

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The surface M protein of group A streptococci is responsible for resistance of the organism to complement-mediated opsonization and phagocytosis (1). We have shown that M protein on intact streptococcal cells binds fibrinogen (2, 3) and that the bound fibrinogen prevents opsonization by complement in nonimmune human blood (2). Bound fibrinogen also inhibits opsonization by low concentrations of anti-M antibody of the homologous serotype and can, in some cases, prevent opsonization by heterologous crossreactive anti-M sera (4). The resistance to phagocytosis of M-positive streptococci suspended in serum is intermediate between that of organisms suspended in plasma and organisms from which the M protein has been removed by trypsin (2). Accordingly, we have concluded that binding of fibrinogen contributes to the antiopsonic activity of M protein in whole blood.

The present investigation was undertaken to see if digested fibrinogen and fibrin bind to M protein and, if so, whether they retain the antiopsonic properties of intact fibrinogen. This question arises because most streptococcal infections occur in tissue, not blood; consequently, the organisms might be exposed to degraded fibrinogen and fibrin rather than, or in addition to, intact fibrinogen, particularly since they elaborate a plasminogen activator, streptokinase (5). We show that a highly purified peptide fragment of type 24 streptococcal M protein (pep M24) binds to the D region of the fibrinogen molecule (see scheme of fibrinogen degradation, Fig. 1), including a 38 kD subfragment of fragment D, and that binding of D fragments to intact streptococcal cells is inhibited by pep M24. The binding affinity of the various D fragments is on the average ~30-fold lower than that of native fibrinogen; nonetheless, at physiologic concentrations, digested fibrinogen retains the ability to block complement-mediated opsonization and phagocytosis of streptococcal cells. Similarly, digested fibrin clot, in which the D fragments exist as crosslinked dimers, is also antiopsonic. We conclude that digested fibrinogen and fibrin bind to M protein on strepto-

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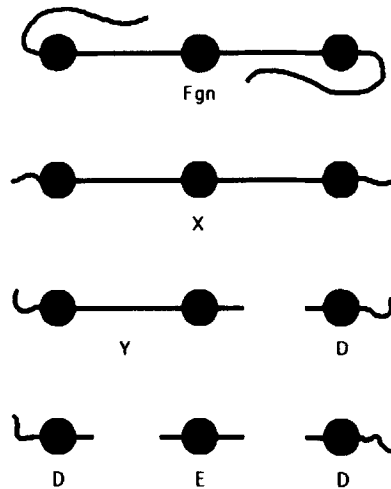


FIGURE 1. Plasmic digestion of fibrinogen (*Fgn*), after reference 6. Fibrinogen is a dimer composed of three pairs of polypeptide chains that join near their amino termini via extensive disulfide bridging, creating the central nodular domain. Nearer the carboxy termini, the three chains on each side are extensively interconnected by disulfide bonds, creating the outer two nodular domains. These disulfide knot regions resist plasmic digestion. Plasmin attacks the extended chains, creating fragments X, Y, D, and E. All are heterogeneous, owing to the numerous points of plasmic attack on the molecule.

coccal cells, and that this binding prevents effective opsonization and phagocytosis of the organisms.

Materials and Methods

Streptococci. A group A strain of M serotype 24 (Vaughn) was blood-passed to a high level of resistance to phagocytosis. Cultures were stored at -80°C . Organisms were subcultured to early log phase in Todd-Hewitt broth (Difco Laboratories, Detroit, MI) supplemented with 20% normal rabbit serum (Gibco Laboratories, Grand Island, NY), 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 7.4), and resuspended in PBS at 10^{10} colony-forming units (CFU)/ml.

M Protein. A polypeptide fragment of type 24 M protein was extracted by limited peptic digestion of whole organisms and purified by ion exchange and gel filtration chromatography. The product, designated pep M24, was judged to be pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7).

³H-Labeled and Unlabeled Fibrinogen (3). Human fibrinogen (grade L, 90% clottable; KABI Diagnostica, Stockholm, Sweden) was dissolved in PBS at a concentration of 3.4 or 13.6 mg clottable protein/ml (10 or 40 μM), dialyzed against PBS to remove salts, and stored at -20°C . For tritium labeling, fibrinogen was dissolved in 0.2 M sodium borate, pH 9.2, at a concentration of 10 μM , dialyzed against the same buffer, and subjected to reductive N-methylation at 4°C with 0.1 vol of a 1% aqueous solution of [³H]formaldehyde (85 mCi/mmol; New England Nuclear, Boston, MA), followed by 0.3 vol of sodium borohydride (12.8 mg/ml), according to the method of Rice and Means (8) as modified by Grinnell (9). The product was dialyzed against PBS and stored at -20°C . Specific activity was 1.8 Ci/mmol. We have previously shown that ³H-fibrinogen retains the binding characteristics and antiopsonic properties of the unlabeled molecule (3).

Plasmic Digests of Fibrinogen. Fibrinogen, 10 or 40 μM in PBS, was treated with 0.1

¹ Abbreviations used in this paper: CFU, colony-forming units; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

U/ml plasmin (P-4895; Sigma Chemical Co., St. Louis, MO) at 37°C for the desired time interval, at which time digestion was stopped by addition of 0.8 mg/ml soybean trypsin inhibitor (T-9003; Sigma Chemical Co.). For overnight digests, trypsin inhibitor was added after 12–16 h incubation. Controls included pre-mixed plasmin and trypsin inhibitor added to fibrinogen or PBS. Plasmic digestion was carried out in the absence of calcium to allow more extensive degradation of fragment D (see Discussion).

Binding of M Protein to Fibrinogen Degradation Products. Fibrinogen digests were heated for 2 min at 100°C in an equal volume of nonreducing sample buffer (0.6 M Tris/12% glycerol/1.2% SDS/0.001% brom phenol blue, pH 6.8). Quadruplicate sets of samples were subjected to SDS-PAGE on four 7% acrylamide gels (10). The separated proteins on each gel were transferred to nitrocellulose paper (0.2 μ m, 15 \times 15 cm²; Schleicher and Schuell, Inc., Keene, NH) by the method of Towbin et al. (11). The resulting transblots were treated with the desired reagents, each treatment consisting of a 30 min soak in blocking buffer in the appropriate pH (0.01 M Tris/0.9% NaCl/0.05% Tween-20/3% bovine serum albumin), followed by addition of reagent to the buffer, further incubation at ambient temperature for 2 h, and finally, nine 5-min washes in 100 ml of 0.01 M Tris/0.9% NaCl/0.05% Tween-20, pH 7.5. Reagents included antisera diluted 1:1000 at pH 7.5 and 0.2 μ M pep M24 at pH 5.0. Treatments of the four transblots were (a) peroxidase-labeled goat anti-human fibrinogen (Cappel Laboratories, Cooper Biomedical, Malvern, PA); (b) rabbit anti-fragment D (Diagnostica Stago, Asnieres, France), then peroxidase-labeled anti-rabbit Ig (Cappel Laboratories); (c) rabbit anti-fragment E (Diagnostica Stago), then peroxidase-labeled anti-rabbit Ig (Cappel Laboratories); and (d) pep M24, then rabbit anti-pep M24 (7), then peroxidase-labeled anti-rabbit Ig (Cappel Laboratories). The treated transblots were developed by incubation in 100 ml of 0.02 M Tris/2.9% NaCl, pH 7.5, to which were added 200 μ l of 30% hydrogen peroxide and 120 mg of horseradish peroxidase color development reagent (Bio-Rad Laboratories, Richmond, CA) dissolved in 40 ml cold (-20°C) methanol.

Binding of Fibrinogen and Fibrin(ogen) Degradation Products to Streptococcal Cells. In experiments in which digests of fibrin or fibrinogen were used to inhibit the binding of ³H-fibrinogen to streptococcal cells, assay mixtures consisted of 20 nM ³H-fibrinogen, serial dilutions of inhibitor, 0.1% Tween-20, and 3 \times 10⁸ CFU streptococci (added last) in a total volume of 200 μ l PBS. After 45 min incubation at ambient temperature, binding was measured by counting 80 μ l of each assay mixture and 80 μ l of each supernatant to determine percentage of counts bound (3). For experiments in which pep M24 was used to inhibit the binding of digested ³H-fibrinogen, washed pellets were counted rather than mixtures and supernatants, because of the lower binding affinity of the fibrinogen fragments. Assay mixtures consisted of an overnight digest of 0.7 μ M ³H-fibrinogen, 0.1% Tween-20, and serial dilutions of pep M24 in a total volume of 170 μ l PBS. After 45 min preincubation at ambient temperature, 3 \times 10⁸ CFU streptococci in 30 μ l PBS were added and the mixture further incubated for 45 min. Pellets were washed three times in 1.25 ml PBS/0.1% Tween-20. For counting, samples were placed in vials containing 0.5 ml PBS/0.1% Tween-20 and 1 mg pronase (Protease Type XIV; Sigma Chemical Co.), incubated at 37°C for 1 h, mixed with 10 ml Hydrofluor (National Diagnostics, Inc., Somerville, NJ) and counted in a Packard Tricarb 460C liquid scintillation counter (3).

To determine the effect of pH on the binding of 20 nM ³H-fibrinogen to streptococcal cells, assays were performed as described above, except that the following buffers were substituted for PBS: 0.08 M citrate, pH 2.82–5.34; 0.8 M citrate and 0.16 M phosphate in varying ratios to yield pH 5.46–6.56; 0.16 M phosphate, pH 5.91–7.57; 0.16 M Tris, pH 7.11–8.97; and 0.16 M glycine, pH 8.85–9.91. Phosphate buffer, 0.2 M at pH 7.0–7.6, was used in the counting vials in place of PBS to neutralize the samples before pronase digestion.

Electron Microscopy. Streptococci (3 \times 10⁸ CFU) were treated with 10 μ M fibrinogen, digested 10 μ M fibrinogen, or control buffer for 20 min at ambient temperature, washed three times in PBS/0.1% Tween-20, fixed in 2.5% glutaraldehyde/0.1% sodium cacodylate at pH 7.2, postfixated in osmium tetroxide, dehydrated in alcohol, and embedded in Spurr's medium for transmission electron microscopy (12).

Detection of Bound Complement (C3) by Immunofluorescence Microscopy. Streptococci (3×10^8 CFU) were treated for 30 min at 37°C with 35% fresh human serum and either digested 4.4 μ M fibrinogen, serial 10-fold dilutions of 4.4 μ M undigested fibrinogen, or blank containing plasmin and trypsin inhibitor. The cells were washed three times in PBS and incubated for 30 min at ambient temperature in 100 μ l of a 1:160 dilution of fluorescein-labeled anti-human C3 (Cappel Laboratories). The treated cells were again washed three times, smeared on glass slides, embedded in 20% polyvinyl alcohol (Gelvatol; Monsanto Co., St. Louis, MO) in 0.01 M phosphate at pH 7.2, and examined under oil immersion with a fluorescence microscope (2).

Opsonophagocytic and Bactericidal Tests. Normal volunteers were selected who lacked antibody to type 24 M protein as determined by enzyme-linked immunosorbent assay (reciprocal titers, <200) and by opsonophagocytic tests using whole blood and blood reconstituted from serum and washed cells. Serum, plasma, and blood cells were obtained as described previously (4). Opsonophagocytic test mixtures (470 μ l) consisted of 200 μ l serum or plasma, 1.6 U heparin, 150 μ l washed blood cells (erythrocytes and buffy coat), 100 μ l test fluid, and, finally, 20 μ l of streptococcal suspension containing 10^7 CFU. Mixtures were incubated with rotation at 37°C. Small samples (20 μ l) were removed at 45 min, smeared on glass slides, stained with Wright's stain, and examined under oil immersion. 100 neutrophils were counted, and the percentage with associated bacteria was noted. Bactericidal tests were performed similarly, except that live cultures were used, the inoculum was reduced by dilution to <200 CFU, and incubation was carried out for 3 h. Inocula and surviving organisms were counted by pour plates.

Degradation Products of Crosslinked Fibrin. Crosslinked fibrin degradation products were prepared by a standard clinical method for euglobulin clot lysis time (13) in which plasma is anticoagulated by decalcification with 0.01 M sodium citrate, then precipitated in 19 vol of 0.014% acetic acid to remove plasmin inhibitors, redissolved in 1 vol of 0.01 M borate/0.9% NaCl at pH 9.0, and recalcified by addition of 1 vol 0.025 M CaCl₂. Upon recalcification, the fraction clots within a few minutes. The clot is spontaneously digested by the plasmin in the fraction upon further incubation at 37°C for 1–3 h. In the present study, crosslinking of the clot was confirmed by insolubility in 5 M urea at 37°C for 24 h (14) and by SDS-PAGE (see Results). Digestion was allowed to proceed for 12 h after disappearance of the clot, at which time 1 mg/ml soybean trypsin inhibitor was added. Controls included unrecalcified plasma, citrated serum, and unrecalcified plasma to which soybean trypsin inhibitor was added at the outset to prevent digestion. All fractions were dialyzed against PBS before use.

Results

In our previous studies (3, 4), we presented evidence that the surface M protein of the streptococcal cell is a high-affinity binding site for fibrinogen. This evidence includes (a) precipitation of fibrinogen by a highly purified 33.5 kD fragment of type 24 M protein (pep M24); (b) dose-dependent inhibition of binding of fibrinogen to streptococcal cells by this peptide; (c) inhibition of binding of anti-M antibody to streptococci by fibrinogen and vice versa; and (d) prevention of fibrinogen binding by a gentle proteolytic treatment of the streptococcal cells that removes the M protein but leaves the surface fibrillae partially intact. Accordingly, we wished to see if pep M24 reacted with fibrinogen degradation products.

Reaction of Fibrinogen Degradation Products with a Polypeptide Fragment of Type 24 M Protein (Pep M24). Timed fibrinogen digests were subjected to SDS-PAGE and transferred to nitrocellulose paper by electroblotting. One such transblot was developed with peroxidase-labeled antifibrinogen. To determine the origin of the fragments within the intact molecule, two other transblots were treated

with anti-D and anti-E sera, these two regions of the fibrinogen molecule being immunologically distinct (15). These transblots were compared with a fourth transblot treated with pep M24. The results (Fig. 2) show that pep M24 reacts with all species that bind anti-D antibody, including several closely spaced bands at 38 kD designated D", but not with fragments reacting only with anti-E. Pep M24 failed to bind to individual polypeptide chains of fibrinogen and its derivatives when reacted with transblots of the same-timed digests reduced with mercaptoethanol before electrophoresis.

Binding of Fibrinogen Degradation Products to Intact Streptococcal Cells. We next sought to determine whether fibrinogen degradation products would bind to M protein on intact streptococcal cells. Type 24 cells were treated with an overnight plasmic digest of fibrinogen containing D and E fragments. The cells absorbed the same D-related fragments as had reacted with pep M24 (Fig. 3;

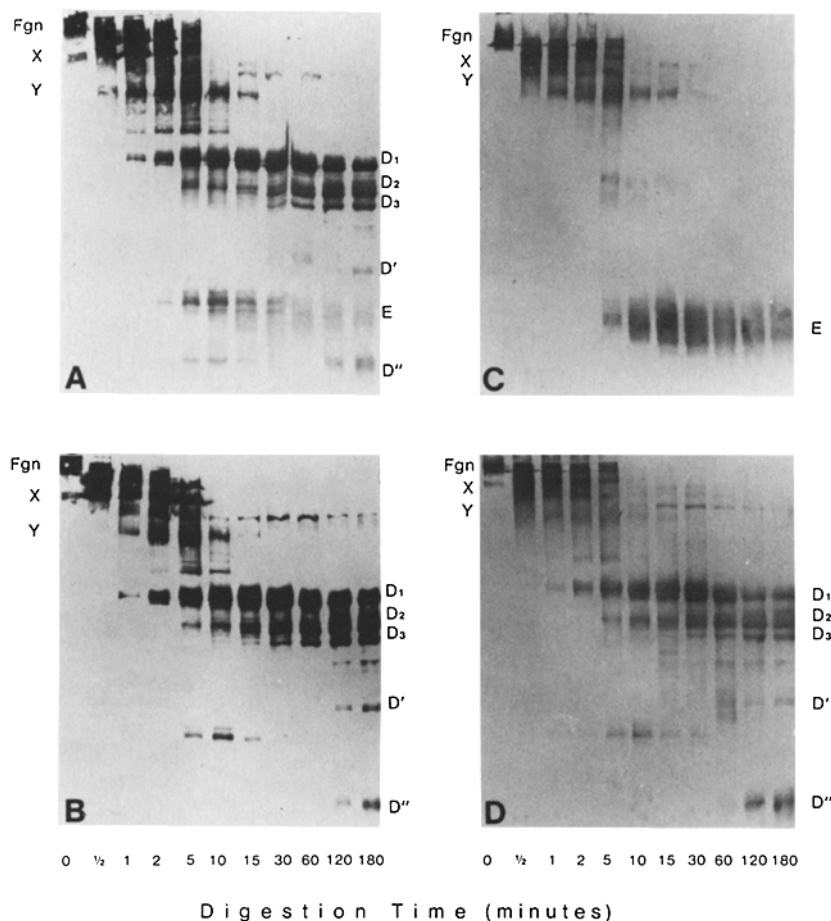


FIGURE 2. Transblots of timed digests of fibrinogen (*Fgn*), reacted with (A) antifibrinogen; (B) a polypeptide fragment of type 24 streptococcal M protein (pep M24); (C) anti-fragment E, which reacts with species containing the central nodular domain; and (D) anti-fragment D, which reacts with species containing one or both outer nodular domains. Pep M24 reacts with the same species as anti-D, but not with species reacting only with anti-E.

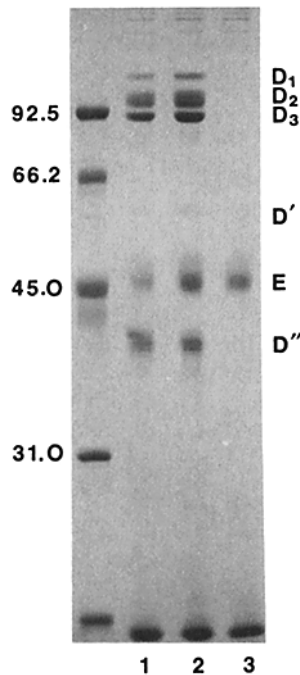


FIGURE 3. SDS-PAGE of an overnight plasmic digest of fibrinogen. (1) Unabsorbed digest; (2) digest absorbed with type 24 streptococci that have been treated with trypsin (1 mg/ml in PBS for 30 min at 37°C) to remove the M protein-bearing surface fibrillae; (3) digest absorbed with untreated type 24 streptococci.

compare Fig. 2). Similarly, plasmin treatment of fibrinogen-coated streptococci released fragment E but not the D fragments. In transmission electron micrographs, the cells treated with digested fibrinogen showed the same increased density of the M protein-bearing fibrillar layer as cells treated with undigested fibrinogen (Fig. 4).

To confirm that the D fragments bind to the same site on the bacterial cells as does intact fibrinogen, an overnight plasmic digest was used to inhibit the binding of ^3H -fibrinogen to type 24 streptococci. Complete inhibition was obtained (Fig. 5). When a single concentration of digested fibrinogen was used to inhibit the binding of ^3H -fibrinogen at a range of concentrations, a parallel shift in the binding isotherm was obtained. Assuming two inhibitory fragments per fibrinogen molecule, the data (Fig. 5) indicate that the average affinity of the fragments (inhibitory constant, 100 nM) is ~30-fold lower than that of intact fibrinogen (dissociation constant, 3 nM). To further confirm the role of M protein in the binding of D fragments to intact cells, pep M24 was used to inhibit the binding of digested ^3H -fibrinogen. Concentration-dependent inhibition was obtained (Fig. 6).

Inhibition of Opsonization and Phagocytosis of Streptococci by Fibrinogen Degradation Products. Having found that D fragments, like fibrinogen, bind to M protein on streptococcal cells, we next wanted to see if the bound fragments retained the ability to prevent opsonization by complement and, therefore, phagocytosis

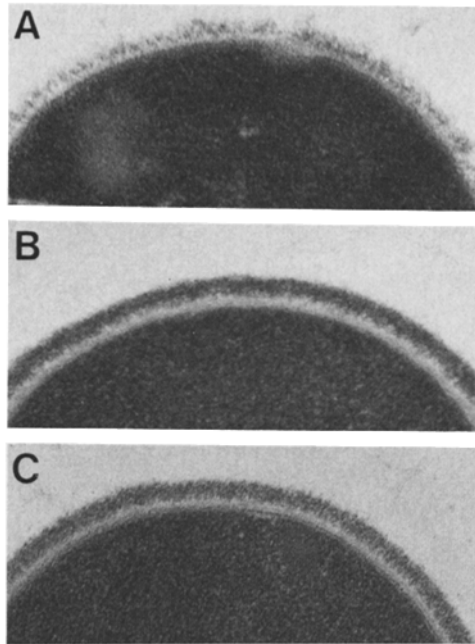


FIGURE 4. Electron micrographs of type 24 streptococci treated with (A) control buffer; (B) undigested fibrinogen; (C) an overnight plasmic digest of fibrinogen as shown in Fig. 3. Original magnification: $\times 60,000$.

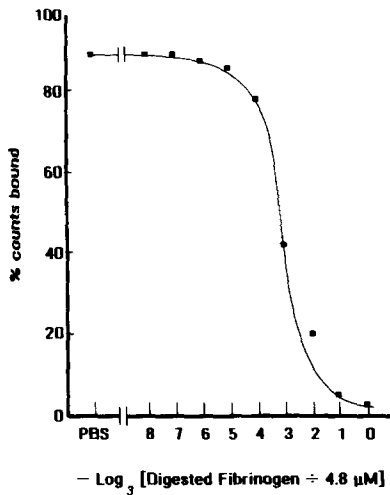


FIGURE 5. Concentration-dependent inhibition of the binding of 20 nM ^3H -fibrinogen to type 24 streptococci by an overnight plasmic digest of 4.8 μM unlabeled fibrinogen serially diluted as shown.

by neutrophils. Streptococci were treated with fresh nonimmune human serum containing an overnight plasmic digest of 4.4 μM fibrinogen, undigested 4.4 μM fibrinogen diluted 10^0 to 10^{-2} , or control buffer. All test mixtures contained equal quantities of plasmin and soybean trypsin inhibitor. Treated cells were

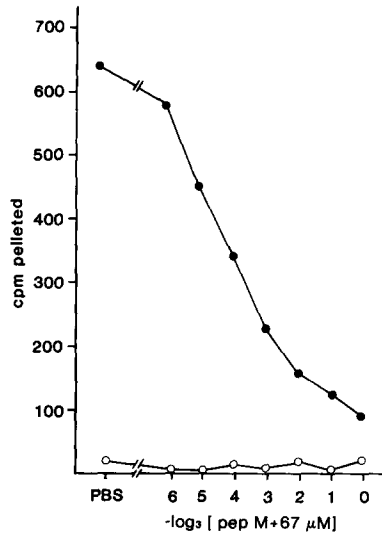


FIGURE 6. Concentration-dependent inhibition of the binding of digested $0.7 \mu\text{M}$ ^3H -fibrinogen to type 24 streptococci by pep M24 (●). In a control series, streptococci were omitted to rule out precipitation of the labeled fragments by the pep M24 (○).

washed and stained with fluorescein-labeled anti-C3. As in previous studies (2), control cells stained brightly and evenly, whereas cells treated with 4.4 or $0.44 \mu\text{M}$ fibrinogen did not fluoresce. Cells treated with digested fibrinogen fluoresced dimly and unevenly (Fig. 7). A similar uneven fluorescence was obtained with fibrinogen diluted 100-fold (not shown).

To determine the extent to which this inhibition of complement deposition impairs phagocytosis by neutrophils, streptococci were incubated with fresh serum, washed blood cells, and threefold dilutions of digested or undigested fibrinogen. Neutrophils were scored for associated bacteria. Digested fibrinogen proved to be ~ 50 times less potent as an inhibitor (assuming two active fragments per fibrinogen molecule), but at concentrations $>1 \mu\text{M}$ before digestion, the fragments were fully effective in preventing recognition of the bacteria by neutrophils (Fig. 8). This result was confirmed with a bactericidal test (Table I).

Degradation Products of Crosslinked Fibrin. In the formation of a fibrin clot, fibrin monomer (which differs from fibrinogen in the absence of two pairs of small peptides from the central E region) first polymerizes via noncovalent (or very weak covalent) interactions between the D and E regions of adjacent molecules, after which covalent crosslinks are formed enzymatically in the presence of calcium between the gamma and alpha chains of the D domains (16). The gamma-gamma crosslinks resist plasminic degradation; consequently, dimers of fragment D are formed in the process of digestion. These dimers are noncovalently complexed with fragment E as in the intact fibrin polymer (17). We prepared digests of crosslinked fibrin from plasma using a standard technique for euglobulin clot lysis (see Materials and Methods). Controls included unrecalcified plasma (to prevent crosslinking), unrecalcified plasma treated with soybean trypsin inhibitor to prevent digestion, and serum. The presence of

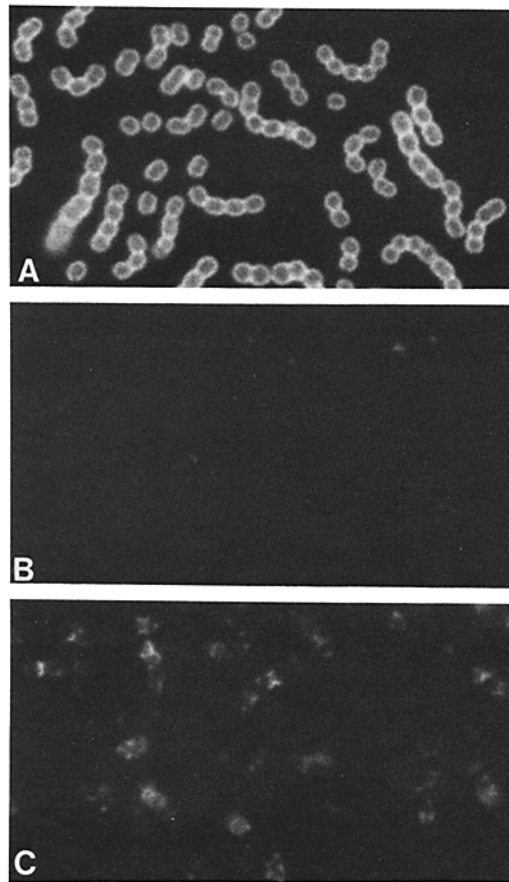


FIGURE 7. Staining of streptococcal cells with fluorescein-labeled anti-C3 after treatment with fresh human serum and (A) control buffer; (B) undigested fibrinogen; or (C) an overnight plasmic digest of fibrinogen.

dimeric D, monomeric D, and undigested fibrin(ogen) in the three plasma fractions was confirmed by SDS-PAGE (Fig. 9) and by immunoblotting with anti-D serum.

In each case, the D-containing species present could be absorbed by intact streptococcal cells and eluted with SDS (Fig. 9), and each plasma fraction inhibited binding of ^3H -fibrinogen to streptococcal cells (Fig. 10). In these experiments the ^3H -fibrinogen, the inhibitor, and the cells were incubated together (see Materials and Methods). Inhibition by the crosslinked digest was virtually identical when tested by pretreating the cells with serial dilutions of digest, washing them, and then exposing them to 20 nM ^3H -fibrinogen. All the plasma fractions inhibited opsonization (Fig. 11), but the crosslinked digest was consistently less effective than the noncrosslinked digest or the undigested plasma fraction. The reason for this was unclear, but was not due to any intrinsic opsonic activity in the preparation, since no uptake of bacteria could be obtained when heat-inactivated serum was substituted for fresh serum in the phagocytosis test

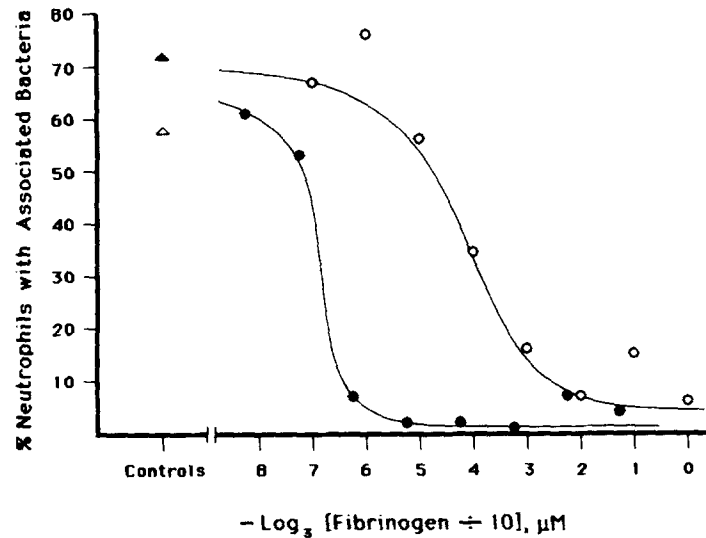


FIGURE 8. Uptake of type 24 streptococci by neutrophils suspended in fresh serum and dilutions of 10 μ M fibrinogen (●) or an overnight plasmic digest of 10 μ M fibrinogen (○). Controls included PBS (Δ) and a blank containing plasmin and soybean trypsin inhibitor (\blacktriangle) as in the undiluted fibrinogen and digest samples.

TABLE I
Effect of Plasmin-digested Fibrinogen on Killing of Type 24
Streptococci by Leukocytes and Serum

Inoculum*	Number of colonies after 3 h incubation with blood cells and serum plus:		
	Blank [‡]	Digest of 10 μ M fibrinogen	10 μ M fibrinogen
8	0	3,548	875
20	77	5,489	2,640
63	143	19,140	15,180
157	630	42,570	28,710

* Pour-plate counts of 100 μ l of each culture dilution.

[‡] PBS containing plasmin and soybean trypsin inhibitor as in the digested and undigested fibrinogen preparations.

mixtures; furthermore, the crosslinked digest inhibited binding of C3 slightly less than did the noncrosslinked digest in immunofluorescence studies (not shown). In control experiments, none of the fractions had any effect on the uptake by neutrophils of an avirulent, M-negative strain (D58X).

Effect of pH on Binding of ³H-Fibrinogen to Streptococcal Cells. The pH of inflammatory exudates may be lower than that of blood; values as low as 5.6 have been reported (see reference 18). In a final experiment, we measured the binding of ³H-fibrinogen to type 24 streptococci in overlapping buffer systems over the pH range 2.8–10. Binding was maximal between pH 4 and 6 (Fig. 12), falling off substantially below pH 3.8. Binding would, therefore, be expected to occur at the pH levels encountered in vivo.

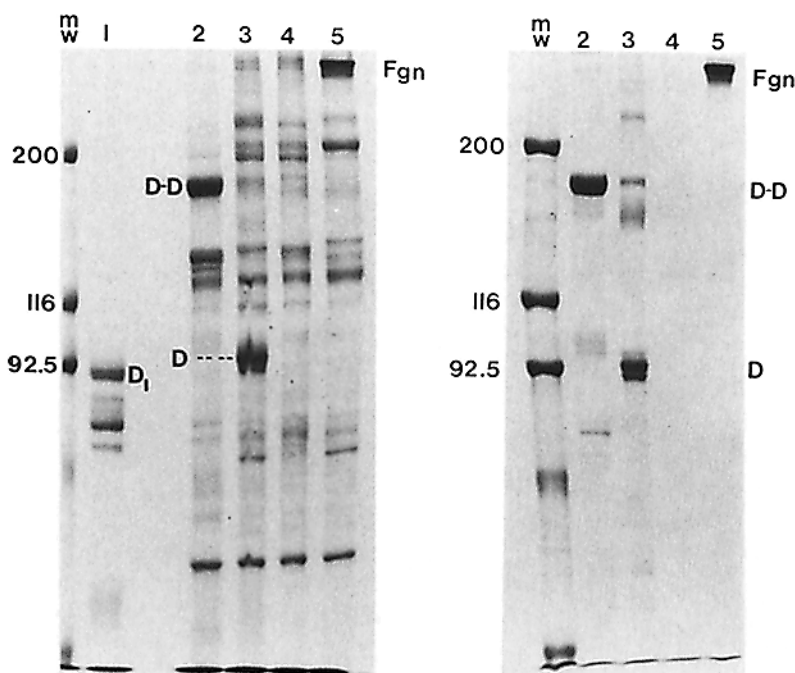


FIGURE 9. SDS-PAGE on 6% polyacrylamide gels under nonreducing conditions. (Left, lane 1) An overnight plasimic digest of fibrinogen (*Fgn*); (2) autodigest of recalcified plasma euglobulin (crosslinked fibrin clot) containing D dimers; (3) autodigest of nonrecalcified plasma euglobulin (noncrosslinked fibrin or fibrinogen) containing D monomers; (4) serum euglobulin fraction; (5) nonrecalcified plasma euglobulin to which soybean trypsin inhibitor was added to prevent digestion. (Right) The same fractions after elution from type 24 streptococcal cells with SDS (corresponding lane numbers).

Discussion

In this study, we have shown that group A streptococci bind the D family of fibrinogen and fibrin degradation products, i.e., the fragments containing the outer nodular domain of the fibrinogen molecule. This finding is in accord with the previously reported observation of Runehagen et al. (19) who found that ^{125}I -labeled X, Y, and D fragments bound to various group A strains. We have previously shown that the binding site for intact fibrinogen is M protein (3). This appears to hold true for the degradation products also, since (a) purified M protein (pep M24) reacts with the same fragments as bind to the intact streptococcal cells; (b) pep M24 inhibit the binding of digested fibrinogen to the cells; and (c) digested fibrinogen competitively inhibit the binding of intact fibrinogen.

The binding affinity of D fragments averages ~ 30 -fold lower than that of fibrinogen, and digested fibrinogen digest is ~ 50 times less potent in blocking uptake of streptococci by neutrophils. Despite their lower binding affinity and proportionately lower antiopsonic potency, fibrinogen degradation products completely inhibit phagocytosis at concentrations $>1 \mu\text{M}$. The concentration of fibrinogen in blood is normally $\sim 10 \mu\text{M}$ and may increase two- to threefold in inflammatory states (20). Provided that vascular permeability increased sufficiently at the site of inflammation to allow free passage of fibrinogen into the

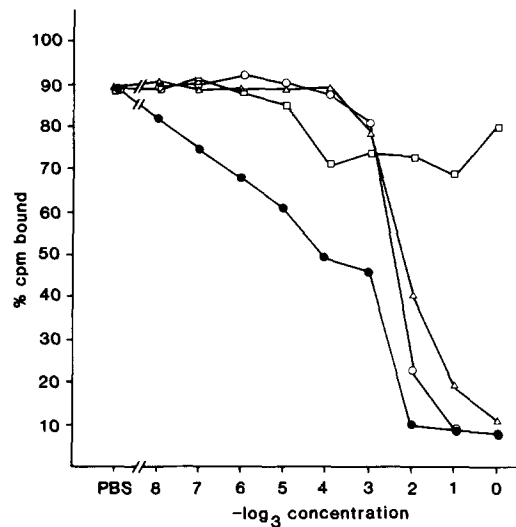


FIGURE 10. Inhibition of binding of 20 nM ³H-fibrinogen to type 24 streptococcal cells by the fractions shown in Fig. 9: digested fibrin clot (●); digested noncrosslinked fibrin(ogen) (○); undigested noncrosslinked fibrin(ogen) (△); and serum (□). The concentration of the undiluted inhibitors was nominally one-fourth that of whole plasma.

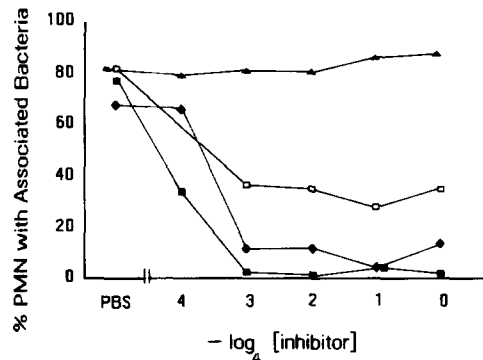


FIGURE 11. Inhibition of uptake by neutrophils (PMN) of type 24 streptococci by the fractions shown in Fig. 9: digested crosslinked fibrin clot (□); digested noncrosslinked fibrin(ogen) (◆); undigested noncrosslinked fibrin(ogen) (■); and serum (▲).

extracellular space, the concentration of fibrinogen and its degradation products would be sufficient to block opsonization even if degradation of fibrinogen were complete.

We prepared fibrinogen degradation products in the absence of calcium so that D fragments smaller than D1 would be generated (21), permitting certain preliminary conclusions about the M protein-binding site on fibrinogen. Fragment D3 differs from D1 in the loss of a segment of the carboxy terminus of the gamma chain. This segment contains several functional sites, including a fibrin polymerization site, two sites for covalent gamma-gamma crosslinking, and binding sites for platelet fibrinogen receptor, staphylococcal clumping factor, and ionized calcium (see reference 22). The M protein-binding site is retained

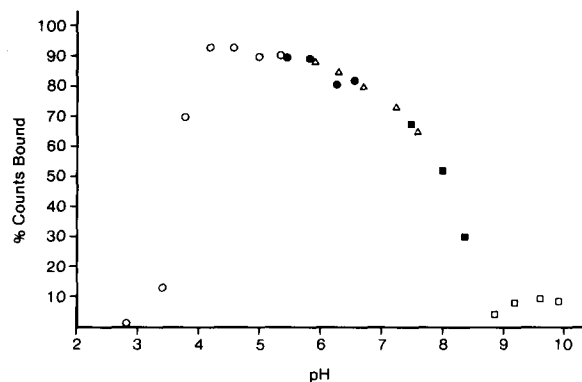


FIGURE 12. Binding of 20 nM ^3H -fibrinogen to type 24 streptococci as a function of pH. Buffer systems were citrate (○), citrate-phosphate (●), phosphate (△), Tris (■), and glycine (□).

on fragment D3 and would, therefore, appear to differ from these sites. We have investigated the effect of calcium (≤ 10 mM), EDTA (≤ 0.6 mM), and EGTA (10 mM) on binding of ^3H -fibrinogen to type 24 streptococci; all were without effect (unpublished observation).

An incidental observation of this study reproduces an earlier finding of Jacks-Weis and colleagues (23), namely that complement deposited in the uneven pattern shown in Fig. 7 does not promote phagocytosis by neutrophils. In that study, the pattern was obtained using serum without added fibrinogen or fibrinogen degradation products. These investigators proposed that the uneven distribution of the bound complement prevented efficient enclosure of the bacterial cells in a phagosome by the "zipper" mechanism of Griffin et al. (24). We have noticed (2) that even when complement is deposited evenly over the entire surface of M-positive streptococci, the bacteria remain somewhat more resistant to phagocytosis than M-negative cells. We suspect that complement deposited on the cell wall is partially inaccessible to receptors on the leukocyte because of hindrance by the projecting surface fibrillae.

In conclusion, fibrin and fibrinogen degradation products of the D family bind to M protein on group A streptococci, and, like fibrinogen, block complement-mediated opsonization and phagocytosis in the absence of anti-M antibody. This process may be relevant to circumstances in which fibrin(ogen)olysis is occurring, such as inflammatory exudation in tissue infections.

Summary

The biological effects of the binding of fibrin(ogen) degradation products to M protein-bearing group A streptococci were investigated. Type 24 group A streptococci bind fibrinogen degradation products of the D family, but not fragment E. Binding appears to be mediated by M protein, since a large peptide of this molecule (pep M24) bound to fragments containing the terminal domains of the fibrinogen molecule (D, X, and Y), but not fragment E, and pep M24 inhibited the binding of digested fibrinogen to streptococcal cells. An M protein-binding site occurs on fragment D3 and, therefore, differs from several functional sites present on D1 but not D3, including the fibrin polymerization site, the two

γ chain crosslink sites, and the binding sites for platelet fibrinogen receptor, staphylococcal clumping factor, and ionized calcium. Bound fibrinogen degradation products prevented deposition of C3 on the streptococcal cell surface, and, in consequence, prevented phagocytosis by neutrophils in nonimmune blood. The average affinity of D fragments for the streptococcal cell surface was ~ 30 times lower than that of native fibrinogen, and a terminal plasminic digest was ~ 50 times less potent in inhibiting opsonization by C3. However, physiologic concentrations of digested fibrinogen sufficed to inhibit opsonization and phagocytosis completely. Digests of crosslinked fibrin clot also inhibited opsonization, although slightly less effectively than did fibrinogen digests. The antiopsonic effect of fibrin(o)gen degradation products may be relevant to circumstances in which fibrin(o)genolysis is occurring, e.g., exudation and suppuration.

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