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Immunology

Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen

(integrin/adhesion)

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ABSTRACT **Human polymorphonuclear leukocytes (PMN) have previously been shown to bind to aggregates of fibrin and to fibrinogen-coated surfaces. During their interactions with fibrinogen-coated surfaces, PMN make such close contact with the surface that a portion of the secreted elastase activity is protected from macromolecular protease inhibitors in the surrounding medium. Here we show that the receptor on PMN that mediates this interaction is complement receptor type 3 (CR3; CD11b/CD18), a molecule previously identified as a receptor for the complement protein fragment C3bi. Monoclonal antibodies against CR3 that block the binding of C3bi also block the binding of PMN to fibrinogen-coated surfaces and the formation of a protected compartment. The region of fibrinogen recognized by CR3 lies at the carboxyl terminus of the γ chain, since peptides based on this sequence effectively inhibit the binding of PMN to fibrinogen-coated surfaces. These peptides also block the binding of C3bi-coated erythrocytes to CR3, thus indicating that a single binding site is used for binding both C3bi and fibrinogen. Sequence analysis shows strong structural similarity between this region of fibrinogen and other known ligands of CR3. These studies thus indicate that CR3 functions as a receptor not only for C3bi but also for fibrinogen.**

Several observations suggest that polymorphonuclear leukocytes (PMN) bind specifically to fibrin and may be involved in dissolution of clots. PMN can be found concentrated in fibrin deposits *in vivo* (1, 2), aggregates of fibrin are phagocytosed by PMN *in vivo* (1, 2), and radioiodinated aggregates of fibrin bind avidly to PMN *in vitro* (3). Recently, we have observed that during migration across fibrinogen-coated surfaces in response to chemotactic stimuli, PMN release elastase that degrades the fibrinogen (4). Degradation of surface-bound fibrinogen by elastase is completely inhibited by the low molecular weight inhibitor MeO-Suc-Ala_2-Pro-Val-CH_2Cl (AAPVCK) but degradation cannot be completely inhibited by high molecular weight inhibitors such as soybean trypsin inhibitor (SBTI). The elastase appears to be protected from inhibitory proteins in the surrounding medium by sequestration in a compartment formed between the PMN and the substrate similar to the compartment that forms between macrophages and substrates coated with IgG or the complement protein C3 (5). This supposition is strengthened by the observation that fluoresceinated fibrinogen is lost from the substrate in patches that correspond to the margins of spread PMN (S.D.W., unpublished observations).

Here we examine the receptors on PMN that mediate adhesion to fibrinogen and the formation of the protected compartment. We find that adhesion to fibrinogen-coated surfaces is inhibited by peptides derived from the carboxyl terminus of the γ chain of fibrinogen. This is the same region that is recognized by the fibrinogen receptor of platelets, glycoprotein IIb/IIIa (gpIIb/IIIa) (6, 7). Our data, however, indicate that gpIIb/IIIa is not expressed on PMN. We show that the related molecule complement receptor type 3 (CR3; CD11b/CD18), originally recognized as a receptor for the complement protein fragment C3bi (8), functions as a receptor for fibrinogen. Monoclonal antibodies (mAbs) against CR3 block the binding of PMN to fibrinogen-coated surfaces, and peptides derived from fibrinogen competitively block the binding of C3bi to CR3.

MATERIALS AND METHODS

Reagents. Human fibrinogen depleted of plasminogen was a generous gift of L. Ossowski (Rockefeller University), and fragment D of fibrinogen was a gift of B. Kudryk (Greater New York Blood Center, New York). Fragment D exhibited a single band on SDS gels and contained no detectable C3 (<0.2%) by RIA.

Peptides. Peptide L10 (LGGAKQAGDV), based on residues 402–411 of the γ chain of human fibrinogen, was synthesized by Peninsula Laboratories (Belmont, CA). An inactive analogue of L10, AcL10, was prepared by acetylating lysine-406 as described (7). Both L10 and AcL10 were repurified by HPLC on a reverse-phase column. Peptide G15 (GQQHHLGGAKQAGDV), based on residues 397–411 of the γ chain of fibrinogen, was purchased from Sigma. Peptides C3-12 (TRYRGDQDATMS), based on residues 1390–1401 of human C3, and H12 (HHLGGAKQAGDV), based on residues 400–411 of the γ chain of fibrinogen, were generous gifts of M. Ginsberg (La Jolla, CA), and GRGDSP and GRGESP were generous gifts of M. D. Pierschbacher (La Jolla, CA).

Antibodies. mAbs OKM1 (IgG2b) and OKM10 (IgG2b), directed against the α chain (CD11b) of CR3 (8) were a gift of G. Goldstein (Ortho Pharmaceuticals, Raritan, NJ); mAb 904 (IgG1), also directed against the α chain (CD11b) of CR3 (9), was a gift of J. Griffin (Dana–Farber, Boston); mAb IB4 (IgG2a), directed against the β chain (CD18) of CR3 and the integrins LFA-1 and p150,95, was as described (8); and mAb 3G8 (IgG1), directed against the low-avidity Fc receptor of neutrophils (FcRIII; CD16), was as described (10). mAbs 10E5 and 7E3 directed against gpIIb/IIIa (11) were a gift of B. Coller (Stony Brook, NY).

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Abbreviations: PMN, polymorphonuclear leukocytes; SBTI, soybean trypsin inhibitor; gpIIb/IIIa, glycoprotein IIb/IIIa; C3bi, a complement protein fragment; mAb, monoclonal antibody; EC3b, EC3bi, and EIgG, sheep erythrocytes coated with complement protein fragment C3b, C3bi, or IgG; PBt_2, phorbol dibutyrate.

Cells. Neutrophils (PMN) were purified from fresh human blood on Ficoll/Hypaque gradients (12). Sheep erythrocytes were coated with complement protein fragment C3b (EC3b), with C3bi (EC3bi), or with IgG (EIgG) as described (13).

Adhesion of PMN to Fibrinogen-Coated Surfaces. Terasaki tissue culture plates were coated with human serum albumin (Armour Pharmaceutical, Kankakee, IL) or fibrinogen by adding the protein at 1 mg/ml in phosphate-buffered saline (PBS) and incubating at 20°C for 60 min. Plates were washed extensively and used immediately. In most experiments, the PMN were stimulated before the assay by incubating suspensions of cells with phorbol dibutyrate [PBt_2 (300 ng/ml); Sigma] for 15 min at 37°C. The cells were washed and then suspended to 2×10^6/ml in Dulbecco's PBS containing human serum albumin at 0.5 mg/ml, 3 mM glucose, and aprotinin at 0.3 unit/ml (HAP medium).

Five microliters of the PMN suspension and 5 μl of the appropriate antibody or peptide solution were added to each well of the coated Terasaki plate. Plates were incubated for 30 min at 0°C to allow settling of PMN and binding of the antibody and then warmed to 37°C for 30 min to allow cell adhesion. Unattached cells were washed away, plates were fixed with glutaraldehyde, and attachment was measured by phase-contrast microscopy. Usually triplicate samples were scored.

Attachment of Ligand-Coated Erythrocytes to PMN. Monolayers were produced by adding 5 μl of PMN (2×10^6/ml in HAP medium) to uncoated wells of Terasaki tissue culture trays and incubating at 37°C for 45 min. Where indicated, PBt_2 (300 ng/ml) was added for the last 10 min of incubation. The phorbol ester was then washed away by extensive rinsing of the plate in PBS followed by a 20-min incubation at 20°C in a large volume of HAP medium. Ligand-coated erythrocytes and the indicated peptides were then added to the monolayers. Erythrocytes were allowed to settle for 15 min at 0°C, then the plate was warmed to 37°C for 30 min. Unattached erythrocytes were removed by washing, and attachment of erythrocytes to PMN was scored by phase-contrast microscopy as described (13). Results are reported as attachment index, the number of erythrocytes per 100 PMN.

Cleavage of Fibrinogen by PMN Elastase. Fibrinogen-coated Sartorius cellulose nitrate filters were prepared and fitted into chemotactic chambers as described (4). PMN suspended in 1% human serum albumin were incubated for 60 min at 37°C in the upper well of the chemotactic chamber in the presence or absence of protease inhibitors. In some cases PMN were preincubated for 30 min at 4°C with synthetic peptides or mAb before transfer to the upper well of the chemotactic chamber. In those samples incubated with protease inhibitors, the inhibitors were present in both the upper and the lower wells. Unless otherwise stated, fMet-Leu-Phe (0.1 μM) was present in the lower well of the chemotactic chamber. At the end of an experiment, 50-μl aliquots were removed from both wells and assayed for human neutrophil elastase-derived fibrinopeptides. Release of the Aα1-21 fragment from fibrinogen-coated filters was determined by RIA as previously described (14).

RESULTS

CR3 Mediates Binding of PMN to Fibrinogen-Coated Surfaces. PMN were allowed to settle onto surfaces coated with fibrinogen or human serum albumin for 20 min at 0°C. The preparations were then warmed to 37°C for 30 min and nonadherent cells were washed away. PMN attached to and spread readily on the fibrinogen-coated surfaces. Many fewer cells attached to human serum albumin-coated surfaces, and the attached cells did not spread significantly (Fig. 1). Thus, fibrinogen-coated surfaces promote adhesion and spreading of PMN.

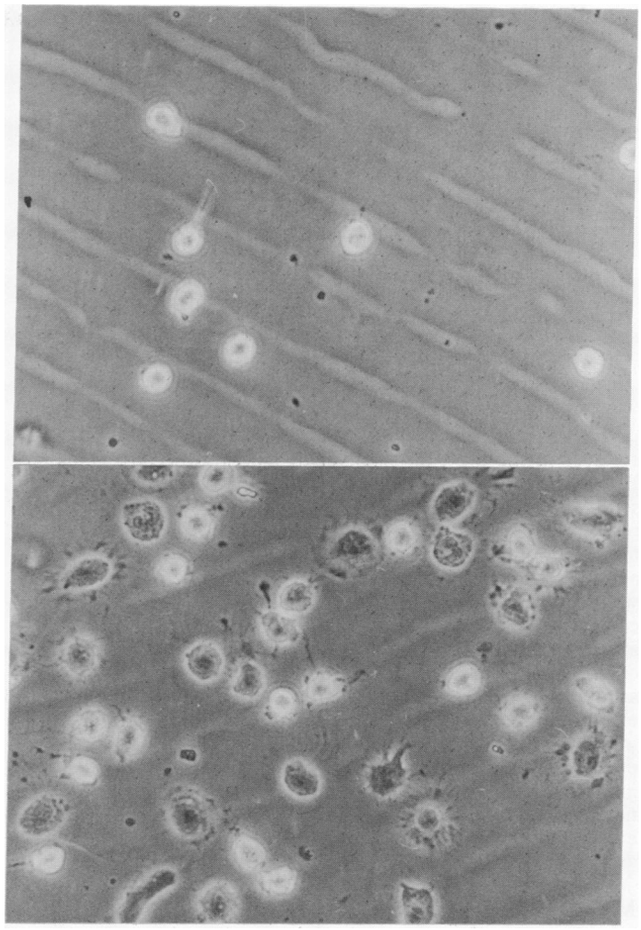

FIG. 1. Adhesion of PMN to surfaces coated with fibrinogen or human serum albumin. Unstimulated PMN were allowed to settle for 30 min at 0°C onto tissue culture plastic coated with human serum albumin (*Upper*) or fibrinogen (*Lower*). The preparations were then warmed to 37°C for 30 min, washed, fixed with glutaraldehyde, and photographed. Few cells adhered to the human serum albumin, and spreading was not observed. In contrast, PMN bound and spread on the fibrinogen-coated surface.

The "nonspecific" binding of PMN to human serum albumin-coated surfaces is known to be enhanced by agents that stimulate PMN. Bacterial lipopolysacchoride is one such agent (15) and is a common contaminant of laboratory reagents. To prevent changes in nonspecific binding from obscuring our data, PMN were intentionally stimulated with PBt_2 prior to the assay of adhesion. This treatment enhanced binding of PMN to both fibrinogen- and human serum albumin-coated surfaces (data not shown), but binding to fibrinogen was still 3-fold greater than binding to human serum albumin-coated surfaces (Fig. 2).

To determine which receptors on PMN mediate binding to fibrinogen, monoclonal antibodies against various receptors were added to the adhesion assay. Both mAb OKM10, directed against the α chain of CR3, and mAb IB4, directed against the β chain of CR3, inhibited adhesion to fibrinogen (Fig. 2). The inhibition was unlikely to be due to a steric effect since mAb 3G8, directed against the Fc receptor of PMN, had no effect on adhesion, and Fc receptors are more abundant on the cell surface than CR3 (16). More importantly, another mAb against the α chain of CR3 (OKM1) did not inhibit adhesion to fibrinogen-coated surfaces. Binding of OKM1 to CR3 does not inhibit binding of C3bi-coated erythrocytes to CR3 on monocytes (8) or on PMN (S.D.W., S.M.L.,

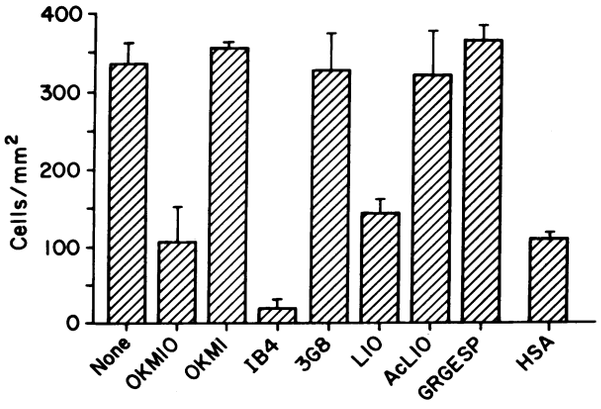

FIG. 2. Adhesion of PBt_2-stimulated PMN to fibrinogen-coated surfaces in the presence of peptides and anti-receptor antibodies. PMN were treated for 15 min with PBt_2, washed, and allowed to settle onto fibrinogen-coated surfaces for 30 min at 0°C in the presence of one of the indicated mAbs (10 μg/ml) or peptides (2 mg/ml). Preparations were then warmed to 37°C for 30 min, washed, and fixed, and cell attachment was determined. Adhesion of PMN was inhibited by mAbs OKM10 and IB4 and by peptide L10. For comparison, the nonspecific adhesion of cells to human serum albumin (HSA)-coated surfaces is also shown. Error bars show SD of triplicate measures in an experiment representative of nine separate experiments. A *t* test of paired samples showed that binding of PMN in the presence of L10 was significantly different from that in the presence of AcL10 (95% confidence) and that binding in the presence of AcL10 was not significantly different from that observed in the absence of any inhibitor. Binding to human serum albumin was not affected by any of the peptides or antibodies shown with the exception that mAb IB4 inhibited binding of PMN to both fibrinogen and human serum albumin-coated surfaces.

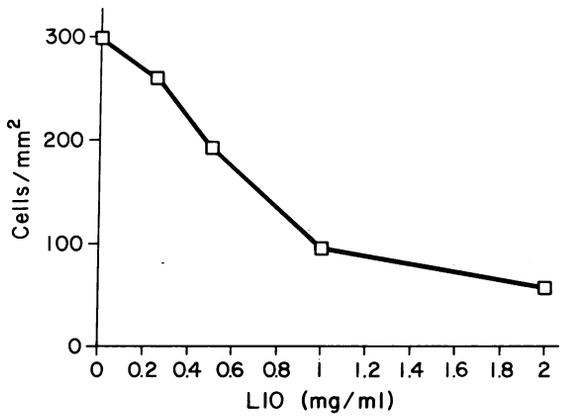

FIG. 3. Peptide L10 inhibits binding of PMN to fibrinogen-coated surfaces. Binding of PBt_2-stimulated PMN to fibrinogen-coated surfaces was measured in the presence of various concentrations of L10. Binding of stimulated PMN to human serum albumin-coated surfaces in this experiment was 51 cells/mm². This experiment is representative of three separate experiments.

M. T. C. Jong, Z. Chad, and L. G. Kabbagh, unpublished work). In contrast both OKM10 and IB4 strongly inhibit binding of C3bi to PMN (S.D.W. *et al.*, unpublished work). Thus, CR3 appears to mediate the attachment of PMN to fibrinogen, and binding to fibrinogen is inhibited by the same mAbs that inhibit the binding of C3bi.

CR3 Binds the γ Chain of Fibrinogen. To determine the portion of the fibrinogen molecule that is a ligand for CR3, synthetic peptides based on sequences in fibrinogen were used to competitively inhibit adhesion. Since platelets bind to fibrinogen by recognizing the carboxyl terminus of the γ chain, we first asked whether PMN also recognize this region. A decamer based on this sequence (LGGAK-QAGDV, referred to here as L10) blocked adhesion of PMN to fibrinogen-coated surfaces (Fig. 2). Inhibition was dose dependent with approximately 0.6 mg/ml required for half-maximal effect (Fig. 3). The specificity of the inhibition is indicated by the observation that acetylation of the lysine group abolishes the inhibitory activity of L10 in a manner similar to that observed with platelets (7). Further, the unrelated peptide GRGESP had no effect on binding (Fig. 2).

CR3 Is Necessary for the Formation of a Protected Compartment Between PMN and Fibrinogen-Coated Surfaces. During chemotaxis of PMN across a fibrinogen-coated surface, a compartment is formed between the PMN and the substrate, which sequesters secreted elastase from inhibitory proteins in the medium (4). To determine whether CR3 also mediates formation of this compartment, we measured the effect of mAbs directed against specific receptors on the elastase-mediated degradation of fibrinogen that resists inhibition by SBTI. The mAbs 7E3 and 10E5, directed against gpIIb/IIIa of platelets, had no effect on the cleavage of fibrinogen by elastase (Table 1), and binding of these mAbs to PMN was not detectable (S.D.W., unpublished observations). In contrast, mAb OKM10, directed against CR3, completely inhibited the protection of elastase from SBTI—i.e., it inhibited the formation of a protected compartment between PMN and fibrinogen. It is unlikely that the action of OKM10 is due to steric effects since other mAbs against the α chain of CR3 (904 and OKM1) had no effect on cleavage of fibrinogen by elastase. Interestingly, OKM10 effectively blocks the interaction of CR3 with C3bi, but neither OKM1 nor 904 blocks this interaction (refs. 8 and 17; S.D.W. *et al.*, unpublished work). These data suggest that CR3 is necessary not only for binding of stationary PMN to fibrinogen but also for the formation of a protected compartment between PMN and the substrate during migration across a fibrinogen-coated surface. In addition, these studies show that the site on CR3 that mediates formation of the protected compartment is the same site that mediates binding of C3bi to CR3.

Synthetic peptides based on the carboxyl terminus of the γ chain also inhibited the formation of a protected compartment between PMN and the fibrinogen-coated surface. L10 completely inhibited compartment formation while the inactive analogue, AcL10, was without effect (Table 1). In addition a dodecamer based on the carboxyl-terminal se-

Table 1. Cleavage of surface-bound fibrinogen by PMN in the presence of SBTI

Inhibitor	Release of Aα1-21, pmol/ml	
	Exp. 1	Exp. 2
None	10	8.6
3G8	ND	9.1
7E3	11	7.8
10E5	11	8.7
OKM10	0.3	0.3
OKM1	ND	9.2
904	10	8.1
H12	0.5	ND
GRGDSP	10	ND
L10	ND	0.15
AcL10	ND	7.0

PMN were incubated at 0°C for 30 min with various inhibitors. The mAbs (7E3, 10E5, 3G8, OKM1, OKM10, and 904) were used at a concentration of 20 μg/ml, and the peptides (H12, GRGDSP, L10, and AcL10) were used at 1 mg/ml. The PMN were then incubated with fibrinogen-coated filters in the presence of SBTI at 6 μg/ml and in the continued presence of inhibitors as described in *Materials and Methods*. After 60 min, supernatants were taken and assayed for Aα1-21, a fragment of fibrinogen release by elastase. ND, not determined.

quence of the γ chain of fibrinogen (HHLGGAKQAGDV, H12) that includes the L10 decamer also inhibited the sequestration of elastase from SBTI, but the unrelated peptide, GRGDSP, had no effect (Table 1). Thus, CR3 mediates the formation of a protected compartment between PMN and fibrinogen-coated surfaces by binding to the carboxyl terminus of the γ chain of fibrinogen.

Fragments of Fibrinogen Inhibit Binding of C3bi to CR3. Since the binding of both fibrinogen and C3bi by CR3 is inhibited by the same mAbs and since the sequence recognized in fibrinogen is structurally similar to that recognized in C3bi (see *Discussion*), we hypothesized that a common binding site on CR3 mediates recognition of both fibrinogen and C3bi. To test this, we measured the ability of fragments of fibrinogen to competitively inhibit the binding of C3bi-coated erythrocytes (EC3bi) to PMN. A large proteolytic fragment of fibrinogen termed fragment D, which contains the carboxyl terminus of the γ chain, effectively blocked the binding of EC3bi to PMN (Fig. 4). Similarly, both a decamer (L10) and a 15-mer (G15) from the γ chain of fibrinogen inhibited binding of EC3bi. Relatively high concentrations (≈0.4 mg/ml) of these peptides were required for half-maximal inhibition of the binding of EC3bi. These concentrations, however, were comparable to the concentrations required for inhibition of the binding of EC3bi to PMN by C3-12, a synthetic dodecamer based on the known recognition sequence in C3bi (Fig. 4). The specificity of the inhibition observed was confirmed by the observations that acetylation of the lysine of L10 eliminated its inhibitory capacity and that the unrelated peptide, GRGDSP, had no effect at the concentrations used here. In parallel experiments, none of the peptides inhibited the binding to PMN of C3b or IgG-coated erythrocytes, particles that bind to CR1 and Fc receptors, respectively (Fig. 4; data not shown). Thus, a common binding site on CR3 recognizes both C3bi and fibrinogen.

DISCUSSION

Previous studies have shown that both monocytes (3, 18) and PMN (3) bind fibrinogen and fibrin, but the receptors mediating that binding have not been identified. Platelets express a well-characterized fibrinogen receptor, gpIIb/IIIa, and several authors have suggested that the fibrinogen receptor of phagocytes is gpIIb/IIIa (19–21). In support of that hypothesis, certain mAbs against, gpIIb/IIIa such as 25E11 (19) and P140 (21) have been shown to bind to monocytes (19, 21) and PMN (19). However, the receptor on phagocytes recognized by these mAbs must be different from that on platelets because other antibodies against gpIIb/IIIa such as P4 and T10 (22), 1070 (19), P256 (21), and 7E3 (S.D.W., unpublished observations) do not bind monocytes or PMN at all, and the gel profile of protein precipitated from mononuclear cells by anti-gpIIb/IIIa antibodies differs from that of protein precipitated from platelets (18, 21). It thus appears that phagocytes express a distinct receptor that is immunologically related to gpIIb/IIIa.

CR3 is structurally homologous with gpIIb/IIIa. The β chain of CR3 is ≈40% identical to the gpIIIa of platelets (23) and, in turn, both of these proteins are related to a large family of adhesion-promoting receptors termed integrins (24). Here we report that CR3 on activated PMN can recognize surface-bound fibrinogen. PMN bind specifically to fibrinogen-coated surfaces, and that binding can be blocked by mAbs against CR3 (Fig. 1). We thus believe that CR3 constitutes the principal fibrinogen receptor of PMN. PMN do express low levels of the integrins LFA-1 and p150,95 which possess β chains identical with that of CR3. We presently cannot exclude a minor participation of these proteins in the binding of fibrinogen by PMN. Monocytes express relatively large amounts of CR3, LFA-1, and p150,95, but the interaction of monocytes with fibrinogen-coated surfaces has not yet been explored.

Both CR3 and gpIIb/IIIa recognize the same region of fibrinogen. Peptides from the carboxyl-terminus of the γ chain inhibit binding of both platelets (6, 7) and PMN (Figs. 1 and 2) to fibrinogen. Moreover, acetylation of lysine-406 destroys the ability of such peptides to inhibit the binding of both platelets (7) and PMN (Fig. 2) to fibrinogen. This portion of fibrinogen is not removed during conversion of fibrinogen to fibrin, and thus it may function in the interaction of cells with clots.

The sequence of fibrinogen recognized by CR3 shows strong structural similarity to other known ligands of CR3. It has been shown previously that CR3 recognizes a 21-residue sequence in complement protein C3 (25) and in gp63 of *Leishmania* (26), both of which contain the sequence Arg-

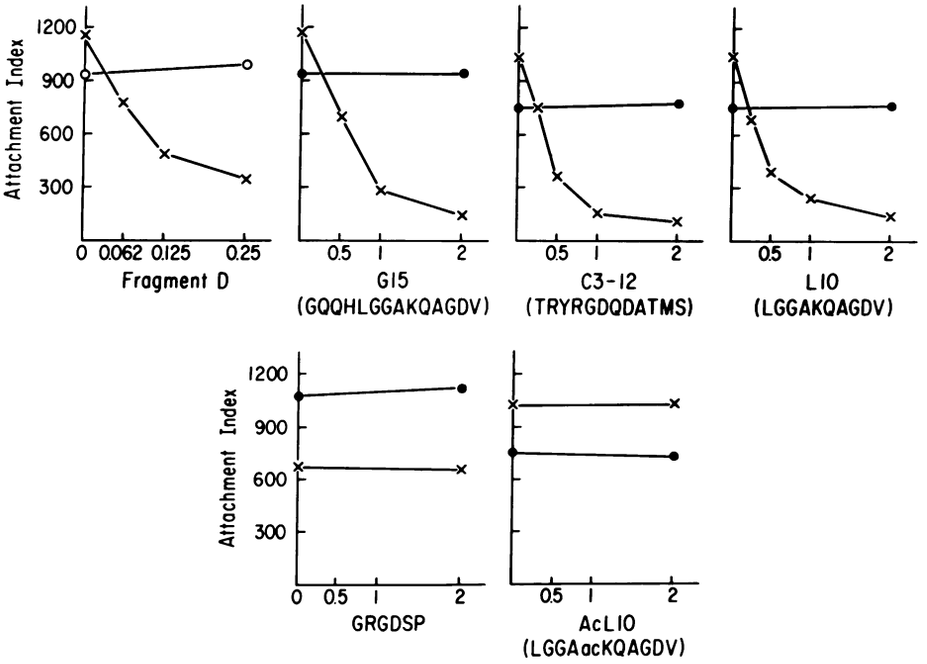

FIG. 4. Effect of peptides on the binding of EC3bi to PMN. Monolayers of PBt_2-stimulated PMN were incubated with EC3bi (×), EC3b (●), or EIgG (○) in the presence of the indicated proteins or peptides. After 30 min at 37°C, the monolayers were washed and the attachment of erythrocytes to PMN was scored by phase-contrast microscopy. Results are reported as attachment index, the number of erythrocytes bound per 100 PMN. Numbers on the abscissa refer to mg of protein or peptide per ml. Each graph is representative of at least three separate experiments. The concentration of protein or peptide required for 50% inhibition of the binding of EC3bi was as follows: fragment D, 0.08 mg/ml; G15, 0.6 mg/ml; C3-12, 0.4 mg/ml; L10, 0.4 mg/ml. No inhibition could be measured using AcL10 or GRGDSP.

Table 2. Sequences of proteins recognized by CR3

Protein	Sequence
Fibrinogen γ	His-His-Leu-Gly-Gly- -Ala-Lys-Gln-Ala-Gly-Asp-Val
Murine C3	Phe-Leu-Glu-Ile-Cys-Thr-Lys-Tyr-Leu-Gly-Asp-Val
Human C3	Ile-Leu-Gly-Ile-Cys-Thr-Arg-Tyr-Arg-Gly-Asp-Gln
Leishmania gp63	Leu-Pro-Gly-Gly-Leu-Gln-Gln-Gly-Arg-Gly-Asp-Ala

Gly-Asp. Here we narrow the site of recognition by showing that a 12-residue segment competitively inhibits binding of C3bi (Fig. 4). Comparison of this portion of human C3 with the carboxyl terminus of fibrinogen shows limited sequence similarity (Table 2). However, the comparable region of murine C3 (also a good ligand for CR3) is highly similar to the recognition site in fibrinogen, and both contain the sequence Lys-Xaa-Xaa-Gly-Asp. We hypothesize that the positive charge of the arginine in the recognition site of human C3 and gp63 is replaced by the positive charge of lysine in the recognition site of murine C3 and fibrinogen. The spectrum of sequences recognized by CR3 thus appears similar to those recognized by gpIIb/IIIa. gpIIb/IIIa binds Arg-Gly-Asp-containing sequences in fibronectin and in von Willibrand factor and a Lys-Xaa-Xaa-Gly-Asp sequence in fibrinogen (7). An important difference between CR3 and gpIIb/IIIa, however, is that CR3 does not recognize fibronectin, nor is it inhibited by peptides based on the sequence of fibronectin (refs. 23–26; Fig. 4).

The data above thus suggest that CR3 may use a single site to bind both fibrinogen and C3bi. This point is borne out by two additional observations. It has been shown previously that mAb OKM1, directed against the α chain of CR3, does not block binding of EC3bi (8), and here we show that mAb OKM1 does not block binding of PMN to fibrinogen (Fig. 1). In contrast mAb OKM10, another antibody directed against the α chain of CR3, effectively blocks binding of both C3bi (8) and fibrinogen (Fig. 1). Thus, a common epitope is involved in both recognition events. This point is confirmed by the observation that peptides from the γ chain of fibrinogen that block the binding of PMN to fibrinogen also block the binding of EC3bi to stimulated PMN (Fig. 4). Thus, CR3 binds both C3bi and fibrinogen at the same binding site.

The concentration of fragment D needed to half-maximally inhibit binding of EC3bi to PMN is ≈0.8 μM (Fig. 4). On a molar basis, fragment D is ≈300-fold more potent than the synthetic peptide L10, presumably because the structure of the protein holds the recognized region in a favorable conformation. Nevertheless, the affinity of fibrinogen for CR3 appears very low. This is not surprising since the affinity of most integrins for their ligands is also low. These receptors mediate cell adhesion events that involve interactions between surfaces on which multivalent, low-affinity interactions sum to yield the biologically important phenomenon of adhesion. We thus presume that circulating, monomeric fibrinogen will not bind CR3 to a significant extent and that clotted fibrinogen, in which the carboxyl terminus of the γ chain is multivalent, is the biologically relevant substrate for CR3.

Note Added in Proof. Altieri *et al.* (29) have recently shown that CR3 on monocytes can function as a receptor for fibrinogen. However, they observed binding to CR3 that was inhibited by monoclonal anti-CR3 antibodies that we found to be inactive, and they were unable to inhibit binding with synthetic peptides. These and other differences between the two studies suggest that the binding site for fibrinogen described by Altieri *et al.* may be different from that described here.

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