FIBRONECTIN RECEPTORS OF PHAGOCYTES

Characterization of the Arg-Gly-Asp Binding Proteins of Human Monocytes and Polymorphonuclear Leukocytes

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The process of receptor-mediated ingestion is a regulated function of phagocytes. Phagocytosis of both complement and IgG opsonized targets can be enhanced by T cell–derived cytokines (1, 2), and complement receptor–mediated ingestion also can be markedly influenced by tumor-promoting phorbol esters (3). Work over the past several years has suggested that extracellular matrix proteins such as fibronectin (Fn),[1] laminin, and serum amyloid P component may be physiologic regulators of phagocytosis by both monocytes and polymorphonuclear leukocytes (PMN) (4–7). Exposure of monocytes to these matrix proteins will enhance phagocytosis via both IgG Fc receptors and complement receptors. For PMN, the effect of binding to the extracellular matrix on phagocytic function is more complex. Although Fn binds to unactivated PMN, it enhances complement receptor–mediated phagocytosis only after the cells have been exposed to an activating agent, such as the bacterial peptide FMLP or the complement-derived chemotaxin C5a. The reason for the different responses of the two phagocytic cell types to Fn is unknown. Previous indirect evidence suggested that the Fn receptors on the two cells were quite similar, since they recognized the same domain of Fn, and since Fn bound to both cells with similar affinity (6). Although the number of Fn receptors differed between PMN and monocytes, our previous data showed that this could not explain the difference in responsiveness of the two cell types, since FMLP and C5a did not alter Fn binding to the PMN. Binding of Fn to both monocytes and PMN was blocked by two mAbs, M1/70 and A6F10 (8), again suggesting a close relationship between the Fn receptors of the two phagocytic cells.

Recently, much progress has been made in understanding the Fn receptors on a variety of different cells (9–14). These investigations have shown that there are at least two distinct types of cellular Fn receptors, both of which recognize the amino acid sequence Arg-Gly-Asp (RGD), contained within Fn. One receptor, found initially in fibroblasts, binds to Fn but not other RGD-containing proteins.

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[1] *Abbreviations used in this paper:* CBD, chymotryptic cell binding domain of fibronectin; Fn, fibronectin; NPGB, paranitrophenyl paraguanidino benzoate; PMN, polymorphonuclear leukocytes; vWF, von Willebrand's Factor.

The second receptor, found on platelets, recognizes the RGD sequence in a variety of otherwise unrelated proteins such as fibrinogen, von Willebrand's factor (vWF), and vitronectrin (15, 16). While the fibroblast Fn receptor will bind to an enzyme-derived fragment of Fn but not to small synthetic peptides, the platelet receptor binds to the synthetic peptides with high affinity. While the two receptors both consist of two noncovalently linked polypeptide chains, the molecular weights of both chains differ between the two receptors. Despite these differences, the receptor proteins are structurally related to each other and also to the Mac-1, LFA-1, p(150,95) family of leukocyte surface molecules (17, 18).

In this work, we have studied the RGD receptors of PMN and monocytes in order to understand the functional and structural features of the Fn receptors of the two cell types. We have discovered significant differences between the RDG receptors of these cells and previously described Fn receptors. Monocytes have both fibroblastlike and plateletlike Fn receptors. In contrast, PMN have only a single type of receptor, related to the platelet RGD binding protein. However, structural and functional data suggest that the plateletlike receptors expressed by both monocytes and PMN are not identical to gp IIb/IIIa, the Fn receptor of platelets. These new receptors, unique to phagocytes, are required for the stimulatory effect of Fn and other RGD-containing proteins on ingestion of opsonized particles.

Materials and Methods

Buffers. Cell solubilization buffer (buffer A): PBS buffer containing 200 mM octylglucoside, 20 mM iodoacetamide, 2 μM pepstatin, 2 μM leupeptin, 25 μM paranitrophenyl paraguanidinobenzoate (NPGB), 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4; Column buffer (buffer B): PBS containing 10 mM Chaps, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 25 μM NPGB, pH 7.4; Elution buffer (buffer C): PBS containing 10 mM Chaps, 10 mM EDTA, and 25 μM NPGB, pH 7.4.

Proteins and Peptides. Fn and the chymotryptic cell binding domain (CBD) of 105–110 kD were prepared as described (19). Collagen type IV was the kind gift of Dr. Hynda Kleinmann, National Institute of Dental Research, Bethesda, MD. vWF was the kind gift of Dr. Sam Santoro or Dr. Evin Sadler, Washington University School of Medicine. Fibrinogen was purchased from Sigma Chemical Co., St. Louis, MO, and vitronectin was from Calbiochem-Behring Corp., La Jolla, CA. The synthetic peptides KYAVTGRGDS and CYAVTGRGDS were gifts from Dr. Steve Adams, Monsanto Corp., St. Louis, MO. The sequence YAVTGRGDS is present within the cell binding domain of human Fn (20, 21). The peptide GRGDSP was purchased from Peninsula Laboratories, Inc., Belmont, CA. The chymotryptic CBD and KYAVTGRGDS were bound to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at 3–5 mg/ml. These are called CBD-Sepharose and peptide-Sepharose, respectively. All proteins were assessed for Fn contamination by ELISA. The fibrinogen contained 3% by weight; other proteins had <0.1% Fn contamination.

Cell Labeling and Solubilization. Monocytes and PMN were isolated, and monocytes were cultured as described (4, 6, 7, 19). For cell surface labeling, $30–100 \times 10^6$ cells were washed twice in PBS and surface iodinated by chloroglycoluril (22) in the presence of 25 μM NPGB for 30 min at 0°C. After washing in PBS containing 2 mM KI, cells were washed and then incubated for 15 min in PBS containing 10 mM iodoacetamide. Cells were solubilized in buffer A. After solubilization, the suspension was centrifuged at 14,000 *g* for 10 min and the pellet was discarded.

Affinity Chromatography. The surface-labeled, solubilized cells were incubated with 2 ml of affinity-ligand Sepharose overnight at 4°C, after which the Sepharose was poured into a column, washed with buffer B, and then eluted with either one column volume of

buffer B containing 1 mg/ml GRGDSP or one column volume of buffer C. Eluted radioactivity was pooled, concentrated in a YM-30 Centricon apparatus (Amicon Corp., Danvers, MA), and analyzed by SDS-PAGE. Careful comparison of the bands seen on SDS-PAGE showed that identical molecules were visualized from both PMN and monocytes after elution by the two different methods. Therefore, most experiments were done using buffer C for elution.

Western Blots. Crude membrane fractions were prepared from PMN and monocytes by sonication, and collected by centrifugation at 100,000 *g*. These membranes were solubilized directly in SDS-PAGE sample buffer without reduction, and Western blots were performed as described (23).

Fluorescent Flow Microcytometry. Cells were stained with either mouse mAbs and fluorescent $F(ab')_2$ anti-mouse Ig (Tago, Inc., Burlingame, CA) or with rabbit polyclonal antibodies and fluorescent anti-rabbit Ig (Miles-Yeda, Rehovot, Israel), as described (24). Nonspecific fluorescence was assessed by using the monoclonal anti-human IgG HG-11 or preimmune rabbit IgG as the primary antibody. Cells were analyzed on a FACS IV; 25×10^3 cells were analyzed per point.

Phagocytosis Assays. Phagocytosis of sheep erythrocytes coated with C3b (EC3b) was done by a minor modification of our previously described method (16, 19). Proteins and peptides were adhered to wells of Labtek chambers using poly-L-lysine and glutaraldehyde, as described (25), rather than passive adherence. With this procedure, even peptides that do not usually bind to uncoated surfaces can be made to adhere to the Labtek chambers. The presence of bound ligand on the Labtek chambers was confirmed using relevant antiprotein or antipeptide antibodies and an ELISA technique.

Results

Elution of Specific Proteins from Affinity Sepharose Columns. The elution profile of radioactivity from the derivitized Sepharose columns is shown in Fig. 1. EDTA eluted specific peaks from Sepharose derivitized with either the 110-kD CBD of Fn or the synthetic peptide. When buffer A was altered to include 10 mM EGTA and 4 mM $MgCl_2$, no EDTA-elutable radioactivity bound to the column (data not shown). This suggests that phagocyte Fn receptors require Ca^{2+} for ligand recognition. When surface-labeled solubilized monocytes were chromatographed in buffer A, SDS-PAGE of the radioactivity eluted with either GRGDSP or buffer C revealed distinct proteins binding to the two different affinity columns (Fig. 2). Two bands were eluted from CBD-Sepharose with apparent molecular weights of 150×10^3 and 130×10^3. Upon reduction with DTT the two bands became one broad band at M_r 135×10^3. This is quite similar to the pattern of

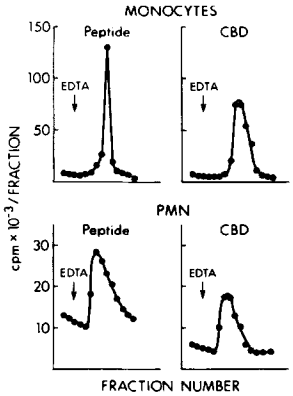

FIGURE 1. EDTA elution of ligand-affinity columns. Surface-labeled monocytes (*top panels*) or PMN (*bottom panels*) were solubilized and chromatographed on either peptide-Sepharose (*left panels*) or CBD-Sepharose (*right panels*), as described in Materials and Methods. A representative EDTA elution profile from each column is shown.

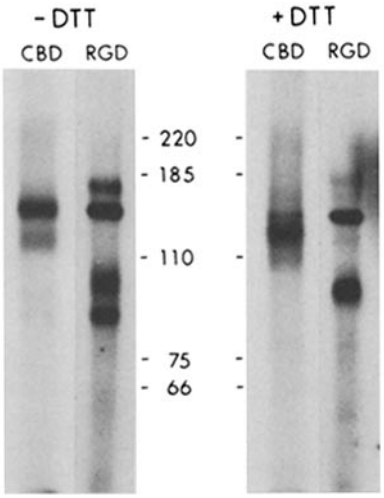

FIGURE 2. Monocyte RGD binding proteins. The eluates from CBD-Sepharose (labeled CBD) and peptide-Sepharose (labeled RGD) were pooled, concentrated, and run on 6% SDS-PAGE under nonreducing (*left*) and reducing (*right*) conditions. Two distinct profiles can be observed. The eluate from CBD-Sepharose shows two bands, which become one broad band after reduction. The eluate from peptide-Sepharose shows four bands which become two after treatment with DTT. Molecular weight markers ($\times 10^{-3}$): Fn (reduced, 220); C3b (unreduced, 185); C3b α chain (110); C3b β chain (75); BSA (66). Identical bands were obtained on elution with EDTA and with GRGDSP.

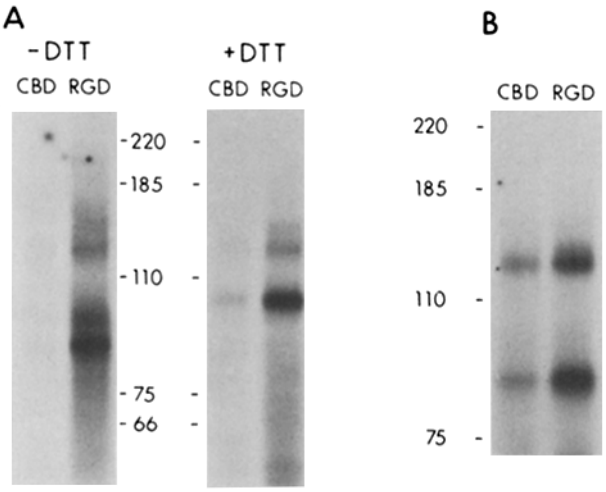

FIGURE 3. PMN RGD binding proteins. (*A*) The EDTA eluates from CBD-Sepharose (labeled CBD) and from peptide-Sepharose (labeled RGD) are compared. Prominent bands are seen in specifically bound material from the peptide-Sepharose column; only faint bands are seen in the eluate from the CBD-Sepharose column. In contrast to monocytes, however, these bands are at identical M_r to those in the peptide-Sepharose eluate. (*−DTT*) Run without reduction; (*+DTT*) eluates run with reduction. (*B*) GRGDSP eluates from CBD-Sepharose and peptide-Sepharose are compared under nonreducing conditions with longer exposure to show M_r identity between the eluates from the two affinity columns. Molecular weight markers ($\times 10^{-3}$) as in Fig. 2.

the fibroblast Fn receptor on unreduced and reduced gels (9). When solubilized monocytes were chromatographed on peptide-Sepharose, SDS-PAGE of the eluted proteins revealed four bands at 169, 150, 105, and 90 $\times 10^3$ M_r (Fig. 2). Upon reduction, only two bands were observed, at 150 and 105 $\times 10^3$ M_r. No proteins were eluted from a control BSA-Sepharose column by buffer C (not shown). Monocytes cultured in vitro for up to 14 d continued to show these differences between the proteins eluted from CBD- and peptide-Sepharose. Alveolar macrophages also had both receptors (data not shown).

In contrast, when PMN were chromatographed on the affinity matrices, the bands eluted from the CBD-Sepharose and from the peptide-Sepharose were identical (Fig. 3). SDS-PAGE revealed bands of M_r 150, 136, 100, and 90 $\times 10^3$. Upon reduction with DTT, bands were observed at 136 and 105 $\times 10^3$. Once

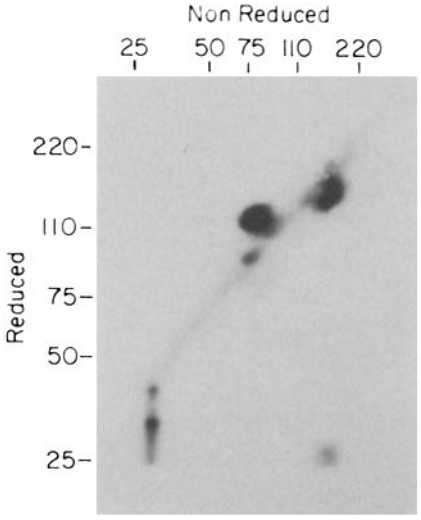

FIGURE 4. Two-dimensional SDS-PAGE of monocyte eluate from peptide-Sepharose. Peptide-Sepharose eluate was electrophoresed in two dimensions as described (38). A portion of the higher mol wt spot has moved below the diagonal, consistent with the loss of a disulfide-bonded fragment, seen faintly at the bottom of the gel. A portion of the lower mol wt spot has moved above the diagonal, consistent with the disruption of intrachain disulfide bonds.

again, no bands were eluted from BSA-Sepharose under identical conditions. In some experiments, PMN were treated with 0.5 μM FMLP before surface iodination. This treatment did not affect the amount of radioactivity eluted by EDTA or the apparent molecular weight of the eluted material from either affinity ligand column. When GRGDSP eluates from affinity columns were dialyzed to remove peptide, >70% of the eluted material from monocytes and PMN rebound to the ligand-Sepharose columns. The appearance of this rebound material on SDS-PAGE was identical to the initially bound receptors shown in Figs. 2 and 3. The disappearance of some bands upon reduction from both the monocyte and the PMN eluates from the peptide-Sepharose column may imply that in phagocytes not all α and β chain disulfide bonds are formed in surface-expressed Fn receptors. The highest M_r band from both monocytes and PMN, which disappears on reduction, probably represents α chain that was disulfide bonded to a 25×10^3 M_r chain originally synthesized as the COOH-terminus extension of the α chain (26) since this is a common feature of the integrin family (18, 27). The second band may represent α chain without covalently linked peptide, so that upon reduction its molecular weight in SDS-PAGE does not change. Thus, after reduction, both forms of the α chain run at the same M_r on SDS-PAGE. The two lower bands could represent isoforms of the Fn receptor β chain with and without intrachain disulfide bond formation. When all disulfide bonds are disrupted by DTT, both forms migrate at the same M_r, characteristic of the higher band on unreduced gels. Two dimensional SDS-PAGE, in which the first dimension was run unreduced and the second dimension reduced, is consistent with this interpretation (Fig. 4). Although the four bands are not well resolved, a portion of the α chain band has moved below the diagonal, consistent with loss of a small fragment, which is faintly visible at the bottom of the gel. A portion of the β chain band has risen above the diagonal, consistent with reduction of intrachain disulfide bands. This heterogeneity in disulfide formation in the molecules eluted from peptide-Sepharose was a frequent finding in both PMN and monocytes, but occasionally did not occur (Fig. 3*B*). The heterogeneity

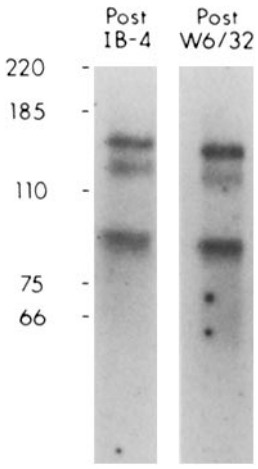

FIGURE 5. IB-4 does not recognize the PMN Fn receptor. Solubilized surface-labeled PMN were immunoprecipitated with IB-4 or the anti-HLA antibody W6/32 as a control. The supernatant from the immunoprecipitation was chromatographed on peptide-Sepharose and the GRGDSP eluates were electrophoresed. IB-4 did not remove receptor from the solubilized PMN. Molecular weight markers ($\times 10^{-3}$) as in Fig. 2.

was not influenced by omission of iodoacetamide from the washing or solubilization buffers, was unaffected by whether elution from the peptide-Sepharose was with GRGDSP or EDTA, and was never seen with platelets, with the fibroblastlike receptor from monocytes (Fig. 2 and Fig. 6, below), or with the vitronectin receptor of placenta (data not shown). These data demonstrate that monocytes have RGD receptors that differ in their affinities for synthetic peptide and the Fn CBD, while PMN show no evidence of a fibroblastlike receptor with high affinity for CBD-Sepharose. Moreover, the PMN and monocyte receptors from the synthetic peptide Sepharose may be distinct, since their α chains differ in M_r after reduction.

Phagocyte RGD Receptors Are Distinct from p(150,95) and Platelet gpIIb/IIIa. Because of the similarity in M_r of the monocyte and PMN molecules eluted from peptide-Sepharose to the known cell surface protein p(150,95) (28), we examined whether these molecules were identical or whether some of the bands eluted from peptide-Sepharose could represent p(150,95). p(150,95) is a member of a family of proteins, including Mac-1 and LFA-1, that share a common β chain. We used an mAb to this β chain, IB4, to immunoprecipitate all members of this family from solubilized surface-labeled PMN. The remaining supernatant was then chromatographed on peptide Sepharose, and radiolabeled protein eluted with GRGDSP was quantitated and run on SDS-PAGE (Fig. 5). There was no difference in quantity of receptor eluted or in its appearance on SDS-PAGE between PMN lysates precleared with IB4 and those precleared with the control anti-HLA class I antibody W6/32. The IB4 immunoprecipitation was complete as assessed by the appearance of a typical immunoprecipitation pattern on SDS-PAGE and the inability of a second incubation with IB4 to immunoprecipitate further material (data not shown).

The β chain of the platelet Fn receptor, gp IIb/IIIa, has a molecular weight of 90–95 $\times 10^3$ as well, so we next examined whether the monocyte and PMN synthetic peptide receptors were identical to gp IIb/IIIa. For this purpose, monocyte, PMN, and platelet receptors eluted from peptide-Sepharose were compared by SDS-PAGE. As shown in Fig. 6, the β chains of the three receptors

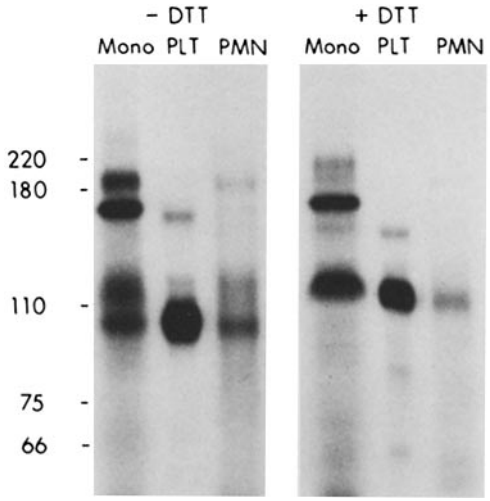

FIGURE 6. RGD receptors on monocytes, platelets and PMN. The EDTA eluates from peptide-Sepharose of surface labeled monocytes, platelets, and PMN are compared under nonreducing (*−DTT*) and reducing (*+DTT*) conditions. While the β chains of the Fn receptors of the three cell types align, the monocyte and PMN α chains do not align with gp IIb, the platelet Fn receptor α chain. Molecular weight markers ($\times 10^{-3}$) as in Fig. 2.

had similar M_r, when compared on reduced gels, although the monocytes and PMN showed heterogeneity in their β chain bands on nonreduced gels. On the other hand, the α chains of the monocyte and PMN receptors, which seem to have the same molecular weight, are quite distinct from gp IIb. The M_r of the monocyte α chain after reduction is 150×10^3, which is significantly greater than the platelet gp IIb (Fig. 6) or the α chain of the vitronectin receptor from fibroblasts, which has a β chain very similar to the platelet receptor component gp IIIa (29). To further investigate the relationship between gp IIb and the monocyte and PMN RGD receptors, we examined the binding of the gp IIb–specific mAb PMI-1 (30). PMI-1 bound minimally to monocytes and did not bind to PMN, as assessed by fluorescent flow cytometry (Fig. 7). This is further evidence that the monocyte and PMN Fn receptors are not identical to gp IIb/IIIa. To assess whether the minimal binding of PMI-1 to monocytes represented platelet contamination or crossreactivity with monocyte molecules, two experiments were performed. First we examined the binding of another antiplatelet monoclonal, 7G2, to monocytes and PMN. This monoclonal recognizes an as yet incompletely characterized antigen on platelets. It does not bind to monocytes or PMN (Fig. 8), demonstrating that there is no significant contamination of these cells by adherent platelets. Second, we examined the CBD- and peptide-Sepharose binding molecules from the monocytic cell line U937 (Fig. 9). All four monocyte cell surface bands eluted by EDTA from peptide-Sepharose were also found on U937, again showing that none of these bands arise from unrecognized platelet contamination. Moreover, PM1-1 also bound minimally to U937, as assessed by FACS, suggesting that PMI-1 may crossreact slightly with monocyte RGD receptors. When polyclonal anti-gp IIb/IIIa was used to stain monocytes and PMN for fluorescent fluorocytometry, it did bind to the phagocytes (Fig. 10). Western blots were used to further analyze the binding of this antiplatelet antibody to phagocytes. As shown in Fig. 11, the polyclonal anti-IIb/IIIa recognized both forms of the β chains of the peptide-Sepharose receptors, but only minimally crossreacted with the α chains. This polyclonal antibody

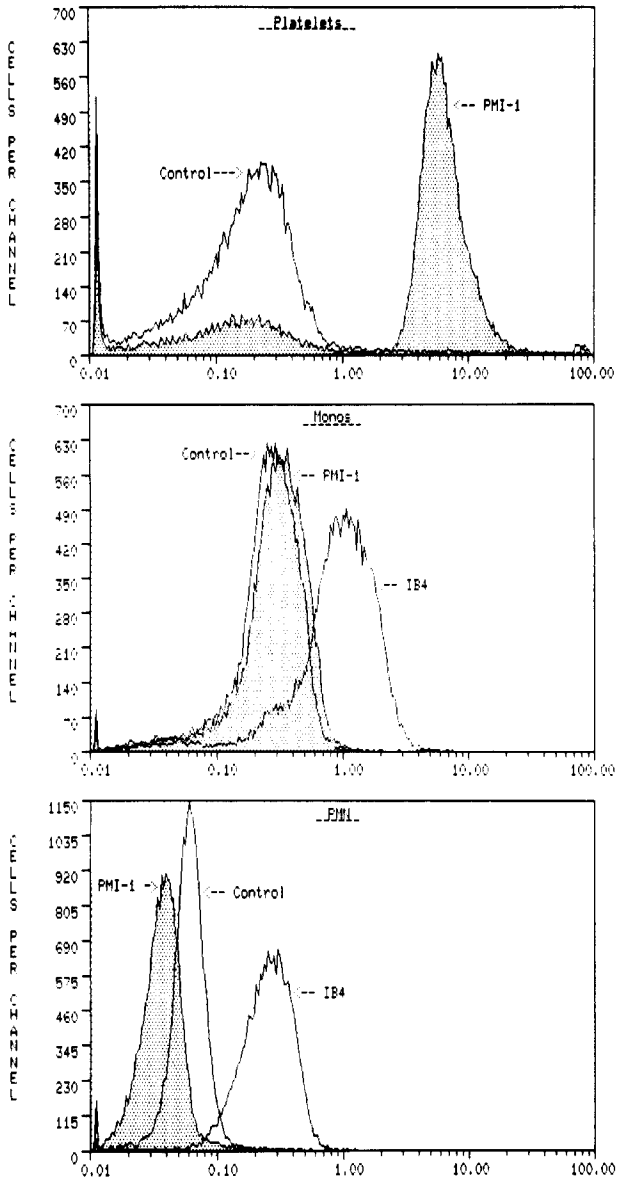

FIGURE 7. Fluorescent flow cytometric analysis of platelets, monocytes, and PMN using PMI-1. Binding of the gp IIb–specific mAb PMI-1 to platelets (*top*), monocytes (*middle*), and PMN (*bottom*) was analyzed by FACS. Cells stained with PMI-1 are shown in the shaded contours, while those stained with a negative control antibody (HG-11) and leukocytes stained with a positive control antibody (IB-4) are shown in unshaded contours. Only platelets bind PMI-1.

recognizes both α and β chains of the gpIIb/IIIa complex (31); in our laboratory, Western blot analysis of this antibody on platelets shows α chain staining to be ~80% as intense as β chain staining (data not shown). Taken together, these data suggest that both monocytes and PMN have receptors for the synthetic RGD-containing peptides that are distinct from previously described receptors for these peptides and from previously described phagocyte cell surface antigens. These phagocyte receptors do show structural similarity to the platelet gpIIb/IIIa complex, primarily in the β chain.

The Synthetic Peptide Receptor Is Involved in Enhancement of Phagocytosis.

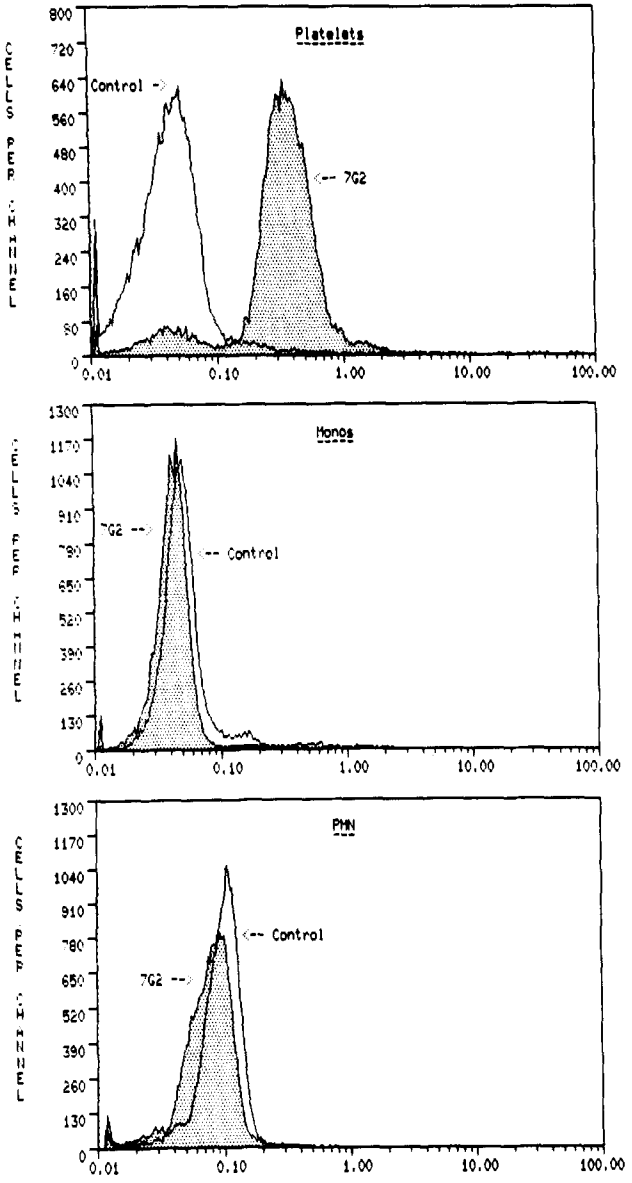

FIGURE 8. mAb 7G2 binds to platelets but not monocytes or PMN. Platelet adherence to purified monocytes and PMN was assessed with mAb 7G2. 7G2 binds strongly to platelets but not at all to monocytes or PMN, demonstrating that platelet contamination of these cell preparations is below the level of detection.

Because of the existence of two potential Fn receptors on monocytes, we sought to determine which receptor was involved in phagocytosis enhancement. To do this, we used the differences in specificity of the two receptors to design ligands that would preferentially interact with one of the receptors. When surfaces were coated with the chymotryptic cell binding fragment of Fn, which binds preferentially to the fibroblastlike receptor, no phagocytosis enhancement occurred (Fig. 12). In contrast, when surfaces were coated with the synthetic peptide, cultured monocytes efficiently ingested C3b-opsonized erythrocytes. These data confirm previous reports (19, 32) and suggest that the synthetic peptide receptor

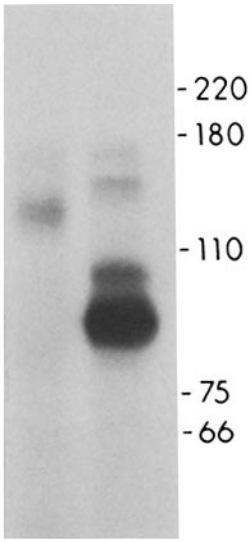

FIGURE 9. Affinity chromatography of surface-labeled U937 cells. Solubilized U937 were chromatographed as described above on CBD-Sepharose (*left lane*) or peptide-Sepharose (*right lane*). SDS-PAGE was performed under nonreducing conditions. All bands eluted from surface-labeled monocytes are also present on U937 cells.

is the more important cell surface molecule involved in phagocytosis enhancement by Fn. We also tested whether this receptor might have affinity for other RGD-containing ligands, as does the platelet Fn receptor, gp IIb/IIIa. As shown in Fig. 12, the RGD-containing molecules fibrinogen, collagen type IV, and vitronectin also enhanced phagocytosis of EC3b. In contrast, complement factor B and vWF, both of which also contain the RGD sequence, did not.

Discussion

Work over the past few years has shown that attachment to Fn can exert an important regulatory influence on the phagocytic function of human monocytes, PMN, and monocyte-derived macrophages in vitro. Thus, the biochemical and cell biologic characteristics of the receptors for Fn on these cells are of importance to the understanding of the regulation of phagocyte function. Fn receptors on a variety of cells bind to the amino acid sequence RGD, so we have characterized the RGD receptors on human phagocytes. To do this, we exploited the fact that ligand binding by RGD receptors requires Ca^{2+}. Elution of ligand affinity columns by EDTA gave identical results for phagocytes as did elution by previously described methods using synthetic peptides (9,10). The first interesting finding was that monocytes have two distinct receptors that recognize RGD, while PMN have only one. The two monocyte receptors can be distinguished structurally and functionally on the basis of their ability to bind to a chymotryptic fragment of Fn that contains the RGD sequence and to a synthetic decapeptide that is based on the Fn sequence around the RGD tripeptide. The monocyte receptor that binds to the chymotryptic Fn fragment can be eluted with either GRGDSP or EDTA and resembles the Fn receptor of fibroblasts, osteosarcoma cells, erythroid cells, and myoblasts. Because it seems less important for mediation of phagocytosis enhancement, we have not characterized it further here. However, this receptor may be important in mediating other monocyte responses to Fn, such as chemotaxis. Relatively large Fn fragments mediate the chemotaxis of

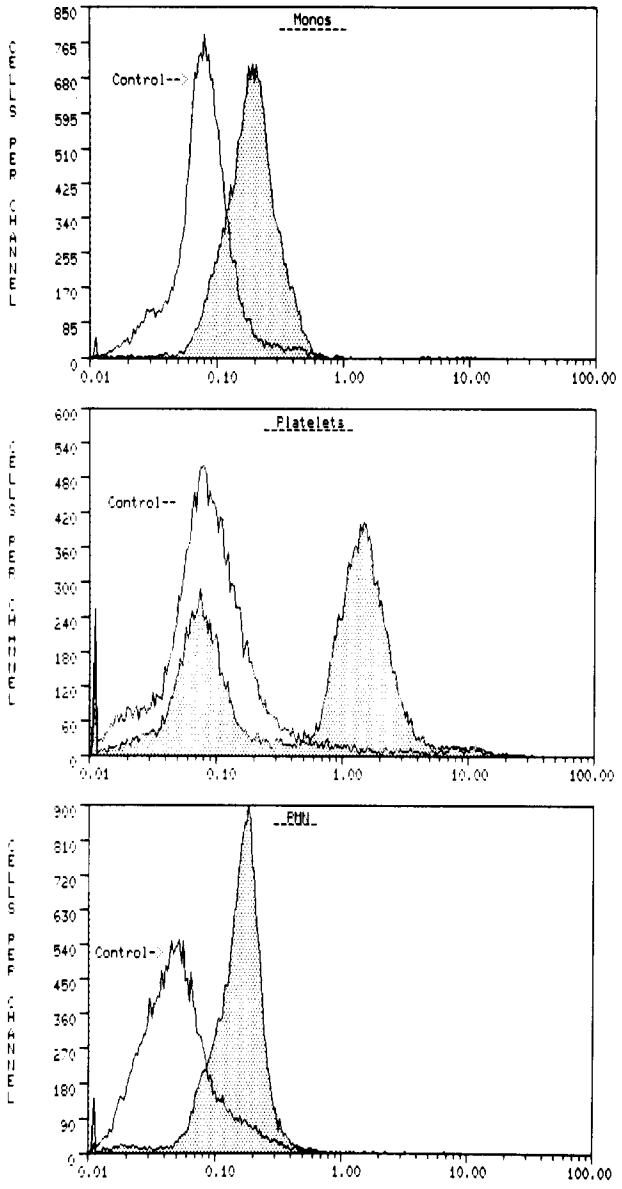

FIGURE 10. Fluorescent flow cytometric analysis of monocytes, platelets, and PMN using polyclonal anti-gp IIb/IIIa. Binding of polyclonal anti-gpIIb/IIIa to monocytes (*top*), platelets (*middle*), and PMN (*bottom*) was analyzed by FACS. Cells stained with polyclonal anti-gpIIb/IIIa are shown in shaded contours, and cells stained with preimmune rabbit serum are shown in the open contours. All three cell types stain with the polyclonal antibody.

monocytes but not PMN (33). This biological activity is consistent with both the cell distribution of the fibroblastlike receptor and its ability to bind to RGD within large enzymatic fragments of the Fn molecule.

The second RGD receptor from phagocytes differs from the fibroblastlike receptor both structurally and functionally, since it has a β chain that has a 35×10^3 M_r on unreduced SDS-PAGE and is the receptor that mediates phagocytosis enhancement. Moreover, its ligand specificity is different since it binds to the synthetic decapeptide, but binds poorly to the cell binding domain of Fn as it is expressed in the chymotryptic Fn CBD. In structure, this receptor resembles two

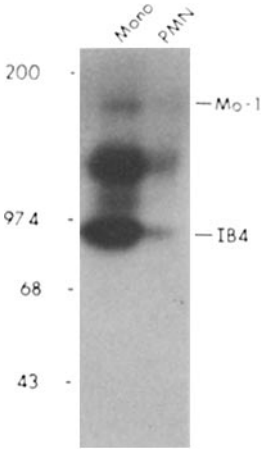

FIGURE 11. Western blot of monocyte and PMN membranes with polyclonal anti-gpIIb/IIIa. Crude monocyte and PMN membranes were electrophoresed in 6% SDS-PAGE and analyzed with polyclonal anti-gpIIb/IIIa by Western blotting. Two dark bands are seen in each lane which correspond in molecular weight to the two forms of the β chain seen in Figs. 2 and 3. The α chains of the monocyte and PMN receptors stain only minimally with the polyclonal antibody. Markers at right correspond to the molecular weights of the bands recognized by Mo-1 (Mac-1 α chain, upper mark) and by IB4 (Mac-1 β chain, lower mark). Molecular weight standards ($\times 10^{-3}$) are myosin heavy chain (200), phosphorylase B (97.4), BSA (68), and OVA (43), from a prestained molecular weight kit (Bethesda Research Laboratories, Gaithersburg, MD) electrophoresed and transferred with the membranes.

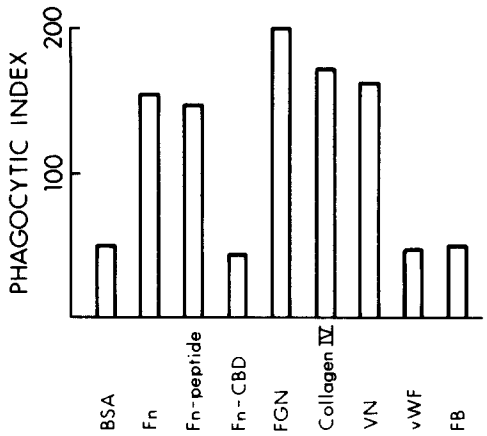

FIGURE 12. Phagocytosis enhancement by RGD-containing peptides. The ability of a variety of RGD-containing molecules to increase phagocytosis of complement-coated erythrocytes is shown. Fn, CYAVTGRGDS (*Fn-peptide*), fibrinogen (*FGN*), collagen type IV, and vitronectin (*VN*) all enhanced phagocytosis by monocytes cultured in vitro for 5 d; the chymotryptic cell-binding domain of Fn (*Fn-CBD*), non Willebrand's factor (*vWF*) and complement Factor B (*FB*) did not.

previously described cell surface molecules, the Fn receptor from platelets, gp IIb/IIIa, and the p(150,95) member of the Mac-1, LFA-1 family. However, experiments using the antibody IB4, which recognizes the β chain of p(150,95), showed that this RGD receptor was not in the Mac-1 family. The phagocyte synthetic peptide receptor seemed to have greater homology to gp IIb/IIIa, since it could be recognized by polyclonal antibodies to the platelet complex. However, further analysis showed that the homology between the phagocyte receptors and gp IIb/IIIa was almost completely limited to the β chain and that the α chains were distinct. The phagocyte peptide-Sepharose binding molecules are also structurally distinct from the vitronectin receptor of fibroblasts. Moreover, the phagocyte molecules seem to have a broader specificity for RGD-containing proteins than the vitronectin receptor. This suggests the possibility that this phagocyte receptor is a third member of the family of gp IIb/IIIa and the vitronectin receptor, which are believed to have identical β chains but distinct α chains (29).

Another interesting difference between this receptor on phagocytes and other described Fn receptors is the apparent heterogeneity of the α and β chains on

unreduced gels. Since this heterogeneity disappears on reduction, it likely reflects the fact that not all receptor molecules have complete disulfide bond formation. The evidence that this heterogeneity is not an artifact of receptor isolation is that: (*a*) elimination or inclusion of iodoacetamide made no difference to the appearance of heterogeneity; (*b*) heterogeneity was seen after elution with both EDTA and GRGDSP; (*c*) neither the fibroblastlike receptor from monocytes nor the platelet receptor isolated on peptide-Sepharose showed heterogeneity; and (*d*) heterogeneity was present in the β chain of the receptor on Western blots of monocytes and PMN without any isolation procedures. The fact that these incompletely disulfide-bonded molecules bind to peptide-Sepharose suggests that their affinity for RGD is not markedly different from those molecules with complete disulfide bond formation.

Hosein and Bianco (34) have reported a monoclonal antibody (A6F10) against the monocyte Fn receptor that recognizes a 110×10^3 M_r band on Western blots both with and without reduction. This M_r is consistent with the incompletely disulfide-bonded molecules in the β chain of the Fn receptor that binds to peptide-Sepharose. A6F10 blocks monocyte binding to Fn-coated surfaces (34), and the binding of Fn-coated microspheres to monocytes and PMNs (8), which suggests that the phagocyte receptor molecules without intrachain disulfide bonds exist and retain ligand specificity in the cell membrane as well as in detergent extracts of monocytes and PMN. An mAb that binds to both gpIIb/IIIa and to PMN and monocytes has been reported to inhibit the binding of Fn-coated particles to these cells (35). These data are also consistent with our own that there is homology between gpIIb/IIIa and the phagocyte Fn receptors. While the binding site for this monoclonal has not been localized, our data would suggest that it is in the β chain of the receptor.

Finally, our data suggest that this phagocyte-specific, synthetic peptide binding receptor generates the signal for enhanced phagocytosis. Phagocytic assays showed that the synthetic peptide will enhance phagocytosis, but the chymotryptic Fn fragment will not, in agreement with previous reports (19, 32). A6F10 also blocks phagocytosis enhancement by Fn, which is consistent with the hypothesis that it binds to the β chain of this Fn receptor. The ability of fibrinogen, vitronectin, and collagen type IV to enhance phagocytosis suggests that this receptor may recognize a number of different ligands that contain the RGD sequence. This ability to bind multiple molecules that contain RGD is also a property of gp IIb/IIIa (10). However, in our assays, vWF did not enhance phagocytosis, while it does bind to gp IIb/IIIa, which suggests that the monocyte RGD receptor is different from gp IIb/IIIa in ligand specificity as well as molecular weight.

These data leave unanswered the interesting problem of why resting monocytes will enhance phagocytosis in response to Fn, but PMN require the combined signal of Fn and an additional agent such as FMLP or C5a. The synthetic peptide receptor, which is necessary for phagocytosis enhancement, is present on both cell types, and is clearly present on the PMN plasma membrane without FMLP stimulation. One possible explanation for the difference in biological response is that the second RGD receptor on monocytes does participate in phagocytosis enhancement, perhaps by altering Fn conformation to make a more effective

ligand for the synthetic peptide receptor. We believe that this is unlikely because FMLP treatment of PMN does not induce expression of this second receptor on the plasma membrane, although it does induce phagocytic responsiveness to Fn. A second possible explanation is that the receptors of PMN and monocytes, although similar on SDS-PAGE, actually have distinct sequences. The role of FMLP might then be to bring a phagocytosis-enhancing monocytelike receptor to the PMN plasma membrane, as it does for complement receptors (36, 37). However, FMLP does not alter Fn binding to PMN (6), nor did we find increased binding to peptide-Sepharose columns after FMLP treatment of PMN in the present experiments. This makes the existence of an intracellular expressible pool of Fn receptors in PMN less likely. Finally, it is possible that the Fn receptor of the two cell types is associated with different signal transduction mechanisms and that FMLP alters the association of the PMN Fn receptor with intracellular effector systems. We currently favor this possibility to explain the difference in biological effect of Fn on PMN and monocytes.

Summary

We have defined the cell surface molecules of human monocytes and PMN that bind to the chymotryptic cell binding domain of Fn and to a synthetic peptide, KYAVTGRGDS, based on the sequence of Fn, by affinity chromatography. Monocytes express two receptors that differ in their affinity for CBD-Sepharose and peptide-Sepharose, but that both recognize the RGD sequence. Only a single receptor is purified from PMN, which resembles the monocyte surface molecule that binds to peptide-Sepharose. These receptors are not part of the Mac-1, LFA-1, p(150,95) family, but do have homology to the platelet Fn receptor, gpIIb/IIIa. Interestingly, the antigenic crossreactivity between gpIIb/IIIa and the phagocyte receptors purified on peptide-Sepharose is largely in the β chain of the receptors. The α chains appear to be distinct, based on molecular weight, antigenic analysis, and ligand specificity. This receptor also seems to be the surface molecule on monocytes that is critical for phagocytosis enhancement by Fn.

Thus, we have defined the phagocyte Fn receptor that transduces the signal for increased phagocytosis by monocytes; it may be a third member of a family of adhesion molecules that includes the gpIIb/IIIa of platelets and the vitronectin receptor of fibroblasts.

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