Vitronectin (S Protein) Augments the Functional Activity of Monocyte Receptors for IgG and Complement C3b

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An arginine-glycine-aspartic acid sequence (RGD in the single letter code for amino acids) is present in the cell attachment site of both vitronectin and fibronectin. Inasmuch as fibronectin and synthetic peptides containing RGD enhance ingestion of opsonized particles by monocytes, we investigated the effects of vitronectin on phagocytosis by monocytes of sheep erythrocytes bearing IgG (EA) or complement C3b (EC3b). Peripheral blood monocytes were isolated by countercurrent elutriation and allowed to adhere to slides that had been coated with either vitronectin or fibronectin. Next, EA or EC3b were incubated with the adherent monocytes, and phagocytosis was subsequently quantified. Vitronectin caused the same dose dependent increase in phagocytosis as fibronectin. The augmentation of phagocytosis of EA induced by vitronectin could be inhibited by the F(ab')2 fragments of anti-vitronectin IgG but not by preimmune F(ab')2. The maximum phagocytosis of EA induced by vitronectin could not be enhanced by the addition of fibronectin, suggesting that vitronectin and fibronectin act on the same population of monocytes and that the two proteins stimulate the same mechanism through which the enhanced phagocytosis is mediated. Fibronectin and vitronectin caused a tenfold increase in the attachment of EC3b to monocytes, but phagocytosis was augmented minimally. These studies demonstrate that vitronectin modulates interactions between monocytes and opsonized particles.

VITRONECTIN is a plasma protein that promotes cell spreading and adhesion.1 It has also been observed in association with the extracellular matrix of various tissue in situ, and it appears to be an important constituent of cell-substrate interactions.2 Recently, the sequence of vitronectin cDNA was determined3 and found to be virtually identical with that of S protein.4 Originally described as an inhibitor of complement-mediated cytolysis, S protein modulates the lytic activity of complement by interacting with constituents of the membrane attack complex.5,6 In addition, S protein may have a regulatory function within the coagulation system, inasmuch as it has been shown to retard heparin-accelerated inactivation of thrombin by anti-thrombin III.7,4

Vitronectin belongs to a family of adhesive proteins that includes fibronectin, fibrinogen, and von Willebrand factor (vWF). Vitronectin shares several functional similarities with fibronectin, despite the fact that except for the presence of the tripeptide, RGD, there is virtually no sequence homology between the two molecules.5 RGD is part of the cell attachment site of both vitronectin8 and fibronectin,9 although, at least on some cell types, receptors for the two proteins are discrete.10 Inasmuch as fibronectin11,12 and small synthetic peptides containing the RGD sequence13 enhance monocyte-induced phagocytosis of opsonized particles, we sought to determine if vitronectin also modulates the functional activity of the monocyte receptors for IgG (FcR) and C3b (CR1 and CR3). These studies have demonstrated that vitronectin augments FcR-mediated phagocytosis of sheep erythrocytes bearing IgG (EA) in a specific, dose-dependent fashion. In addition, vitronectin markedly enhances the attachment, but not the ingestion, by monocytes of sheep erythrocytes bearing complement C3b (EC3b).

MATERIALS AND METHODS

Buffers and reagents. The following buffers were used: veronal-buffered saline (VBS); PBS containing 0.1% gelatin (GVB); GVB containing 0.15 mmol/L CaCl2 and 1 mmol/L MgCl2 (GVB-Ni); GVB containing 0.15 mmol/L MgCl2 (GVB-Ni); GVB containing 15 mmol/L EDTA (GVB-EDTA); phosphate-buffered saline, 10 mmol/L phosphate, 150 mmol/L NaCl, pH 7.4 (PBS); Hanks balanced salt solution (HBSS); HBSS containing 0.5% human serum albumin (HBSS/A); elutriation buffer (EB), 150 mmol/L NaCl, 3 mmol/L KCl, 2 mmol/L KH2PO4, 8 mmol/L Na2HPO4, 6 mmol/L dextrose, 0.1% human serum albumin, and 1 mmol/L EDTA. The following reagents were purchased from commercial vendors: Ficoll-Hypaque, Pharmacia Fine Chemicals; Buffer for Sigma, Sigma Chemical Co.; Factor D, Sigma Chemical Co.; and cobra venom factor (CVF), Sigma Chemical Co. Antibody to vitronectin was raised in rabbits, and the IgG was isolated using 5% caprylic-acid.18 Nonimmune IgG was prepared by isolating the IgG from the sera of rabbits prior to immunization. The F(ab')2 fragments of the immune and nonimmune IgG were prepared with immobilized pepsin (Pierce Chemical, Rockford, IL). After incubation of the IgG with the immobilized pepsin according to the suggestions of the manufacturer, the reaction mixture was centrifuged, and the supernate was chromatographed on Sepharyl S-200 (Pharmacia). The fractions containing the F(ab')2 fragments [determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)] were pooled and applied to a column of...
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Protein A-Sepharose CL 4B (Sigma Chemical). The unbound protein was collected, and purity was assessed by SDS-PAGE. Affinity-purified anti-C3c\(^{18}\) was a gift of Dr Gordon D. Ross (University of North Carolina School of Medicine, Chapel Hill). Human IgG was isolated using 5% acrylamide. After dialysis, the IgG was applied to a column of Protein A-Sepharose. The column was extensively washed with PBS, and the IgG was eluted using 0.1 mol/L glycine, pH 3.0. The IgG was immediately dialyzed against PBS. Protein concentrations for C3, factor B,\(^{18}\) CVF,\(^{18}\) and IgG\(^{18}\) were determined spectrophotometrically. The concentrations of vitronectin and fibronectin were determined by Bio-Rad Protein Assay (Bio-Rad, Richmond, CA) using bovine IgG as the standard. Monoclonal anti-C3c and rabbit anti-sheep erythrocyte IgG were radiolabeled using IODO-GEN (Pierce Chemical) as previously described.\(^{17}\)

SDS-PAGE and Western blots. SDS-PAGE was performed under reducing conditions using a 6% to 12% gradient gel as previously described.\(^{20}\) A modification of the method of Towbin et al\(^{21}\) was used for the immunoblotting experiments. Peroxidase-conjugated affinity-purified goat anti-rabbit (Fab\(^{22}\)) (Tago, Burlingame, CA) was used as the second antibody, and antibody binding was detected by incubation with 50 mg diaminobenzidine (Sigma) and 50 μL 30% H\(_{2}\)O\(_{2}\).

Isolation of monocytes. Monocytes from volunteer donors were isolated by elutriation using a modification of the method of Mason and Weiner.\(^{22}\) In brief, whole blood anticoagulated with EDTA was mixed with an equal volume of 150 mmol/L NaCl containing 6% Dextran 70 and allowed to sediment for 1 hour at 1 g. The erythrocytes were removed by aspiration, and the solution was centrifuged for 5 minutes at 400 g. The supernate was aspirated, and the cell pellet was resuspended in HBSS/A. Next, the polymorphonuclear leukocytes were separated from mononuclear cells by density-gradient centrifugation using Ficoll-Hypaque.\(^{23}\) The mononuclear cells were gently aspirated, washed once in EB, and monocytes were isolated by counter-current elutriation using a modified Beckman model J-6B centrifuge (Beckman Instruments, Palo Alto, CA) equipped with an elutriation rotor (JE-6B) and a separation chamber (Beckman Instruments). After isolation, the monocytes were washed and resuspended in RPMI 1640. Non-specific esterase staining, the preparations used in these studies contained >92% monocytes.

Preparation of EA. Sheep erythrocytes were washed three times in GVB\(^{24}\) and resuspended to 1 x 10\(^{12}\)/mL. One milliliters was incubated at 37°C with 1 mL 1:4000 dilution of rabbit anti-sheep erythrocyte IgG. After 30 minutes, the cells were washed three times and resuspended to either 8 x 10\(^{12}\)/mL or 1.6 x 10\(^{13}\)/mL. Using radiolabeled rabbit anti-sheep IgG, we estimated that the EA, radiolabeled rabbit anti-sheep IgG, bore -4,800 molecules of IgG per monocyte. The EA was incubated at 37°C with I mL 1:4000 dilution of rabbit anti-sheep IgG, and the number of C3b molecules per EA was counted. After 3 minutes, the EA was incubated at 37°C with 100 μg of C3 was added to the cell pellet, and the mixture was incubated at 37°C for 20 minutes. Next, the EA was washed twice in GVB-NI and twice in GVB-EDTA, and incubated at 37°C. After 90 minutes, the cells were washed three times in GVB\(^{25}\) and 250 μL monocyte suspension (5 x 10\(^{8}\)/mL) was added to each chamber, and the supernate was placed in a 5% CO\(_{2}\) incubator at 37°C. After 30 minutes, nonadherent cells were removed by washing the chambers three times with GVB\(^{26}\). Next, 250 μL EA or EC3b (8 x 10\(^{12}\)/mL) was added to each chamber, and the slides were placed in a 5% CO\(_{2}\) incubator at 37°C. After 30 minutes, uningested EA or EC3b were lysed using hypotonic PBS (PBS diluted 1:3 with deionized water). After the supernate was aspirated, 250 μL 0.01 mol/L sodium phosphate, pH 7.4 containing 2% (vol/vol) glutaraldehyde, and 1% (wt/vol) sucrose were added to each well. After 15 minutes, the supernate was aspirated, and overlays were mounted onto the slides. Using phase microscopy (Leitz Epivert, E. Leitz, Rockleigh, NJ), phagocytosis was quantitated. The number of monocytes ingesting one or more erythrocytes (percentage of phagocytosis) and the number of erythrocytes ingested per 100 monocytes (phagocytic index) were calculated after a minimum of 200 monocytes was counted.

Effects of a combination of vitronectin and fibronectin on monoocyte-mediated phagocytosis of EA. Two hundred fifty microliters of 0.1 mol/L NaHCO\(_{3}\) or 0.1 mol/L NaHCO\(_{3}\), containing incremental concentrations of vitronectin or fibronectin were incubated in the chambers of eight-well Lab-Tek slides (Dyneact Laboratories, Alexandria, VA) at RT.\(^{24}\) After 2 hours, the slides were washed three times with PBS, and 250 μL monocyte suspension (5 x 10\(^{8}\)/mL) was added to each chamber, and the slides were placed in a 5% CO\(_{2}\) incubator at 37°C. After 30 minutes, nonadherent cells were removed by washing the chambers three times with GVB\(^{25}\). Next, 250 μL EA or EC3b (8 x 10\(^{12}\)/mL) was added to each chamber, and the slides were placed in a 5% CO\(_{2}\) incubator at 37°C. After 30 minutes, uningested EA or EC3b were lysed using hypotonic PBS (PBS diluted 1:3 with deionized water). After the supernate was aspirated, 250 μL 0.01 mol/L sodium phosphate, pH 7.4 containing 2% (vol/vol) glutaraldehyde, and 1% (wt/vol) sucrose were added to each well. After 15 minutes, the supernate was aspirated, and overlays were mounted onto the slides. Using phase microscopy (Leitz Epivert, E. Leitz, Rockleigh, NJ), phagocytosis was quantitated. The number of monocytes ingesting one or more erythrocytes (percentage of phagocytosis) and the number of erythrocytes ingested per 100 monocytes (phagocytic index) were calculated after a minimum of 200 monocytes was counted.

Effects of fluid-phase IgG on monoocyte-mediated phagocytosis. Monocytes were allowed to adhere to wells that had been previously incubated with either 250 μL vitronectin (160 μg/mL), fibronectin (160 μg/mL), or 0.01 mol/L NaHCO\(_{3}\) as described above. Nonadherent cells were removed by washing, and 250 μL of either purified human IgG (10 μg/mL) or buffer was added to the appropriate wells. After 30 minutes at 37°C, 250 μL EA (1.6 x 10\(^{12}\)) was added, and the incubation was continued for 30 minutes. Next, the wells were washed, and phagocytosis was quantitated as described above.

Effects of vitronectin and fibronectin on monoocyte-mediated phagocytosis of EC3b. Wells were incubated with 250 μL of either vitronectin (160 μg/mL), fibronectin (160 μg/mL), or buffer for 2 hours at RT. The remainder of the experiment was performed as described above except that EC3b were substituted for EA. In experiments in which attachment was quantitated, the wells were washed twice with GVB\(^{25}\), but the cells were not exposed to hypotonic buffer.\(^{29}\) The attachment index is the number of EC3b bound per 100 monocytes.
RESULTS

Analysis of vitronectin and fibronectin using SDS-PAGE and Western blotting. Intact vitronectin has an apparent mol wt of ~75 kd (Fig 1A, lane 2). Proteolysis of a 10-kd fragment from the COOH-terminal end of the native molecule creates the band whose electrophoretic mobility suggests a mol wt of ~65 kd. This 65-kd fragment contains the putative cell-attachment site (the RGD sequence is located in residues 45 through 47 from the NH2-terminus) as well as the glycosaminoglycan binding site.26 The function of the 10-kd cleavage fragment is unknown. Under reducing conditions, fibronectin has an apparent mol wt of 220 kd (Fig 1A, lane 3); however, its biologically active form is a disulphide-linked dimer (mol wt 440 kd). In the Coomassie-stained gels, we observed no contamination of either protein preparation.

To insure further that the vitronectin preparation did not contain fibronectin (and that the fibronectin preparation did not contain vitronectin), the two preparations were analyzed by Western blot. When the blots were developed using anti-vitronectin as the primary antibody, the lane containing the isolated vitronectin showed staining of both the 75 kd and 65-kd proteins (Fig 1B, lane 1), but the lane containing isolated fibronectin produced no reaction (Fig 1B, lane 2). In

![Fig 1. A: SDS-PAGE of vitronectin and fibronectin preparations. A 6% to 12% gradient gel was run under reducing conditions and stained with Coomassie blue. Lane 1, mol wt markers: lane 2, vitronectin (5 µg); lane 3, fibronectin (5 µg). Vitronectin is a single chain polypeptide whose mol wt is ~75 kd. The band whose mol wt is ~65 kd is a proteolytic fragment derived from native vitronectin. B and C: Western blots using either rabbit anti-vitronectin (B) or anti-fibronectin (C) as the primary antibody. The blots were developed following incubation with peroxidase-conjugated goat anti-rabbit IgG. B: Lane 1, vitronectin (1 µg), lane 2, fibronectin (1 µg). C: Lane 1, vitronectin (1 µg), lane 2, fibronectin (1 µg). There was no evidence of contamination of either protein preparation, and the antibodies were specific.](image)

![Fig 2. Dose-response curve of (A) vitronectin (VN)- and (B) fibronectin (FN)-induced augmentation of phagocytosis of sheep EA by freshly explanted monocytes. Data points represent the mean ± SD (n = 3). In a dose-dependent fashion, vitronectin and fibronectin enhanced both the number of monocytes ingesting EA (percentage phagocytosis) and the number of EA ingested per monocyte (phagocytic index). C: Direct comparison of the effects of vitronectin (VN) and fibronectin (FN) on monocyte-mediated phagocytosis of EA. The wells were incubated with buffer alone or with buffer containing either 40 µg vitronectin or fibronectin. Results are shown as the mean ± SD (n = 3). On a weight basis, vitronectin and fibronectin augmented phagocytosis equally.](image)
contrast, when anti-fibronectin was used as the first antibody, no reaction was observed in the lane containing the vitronectin (Fig 1C, lane 1), but staining was observed in the lane containing the isolated fibronectin (Fig 1C, lane 2). These results demonstrate both the purity of the protein preparations and the specificity of the antibodies used in these experiments.

**Effect of vitronectin and fibronectin on ingestion of EA by monocytes.** To determine if vitronectin modulates phagocytosis by monocytes of sheep erythrocytes bearing IgG, peripheral blood monocytes that had been isolated by elutriation were allowed to adhere to the wells of slides that had been previously incubated with buffer alone or with buffer containing incremental concentrations of vitronectin. After the cells were washed, EA were added to the wells, and phagocytosis was subsequently quantified. Vitronectin caused a dose-dependent increase in both the percentage of phagocytosis and the phagocytic index, and the dose–response curve developed a plateau at 10 µg/well vitronectin (Fig 2A). On a weight basis, the effects of fibronectin on monocyte-mediated phagocytosis appear to be similar to those observed for vitronectin (Fig 2B). When maximum stimulatory concentrations of vitronectin and fibronectin were used, the percentage of phagocytosis was enhanced ~2.2 times and the phagocytic index increased ~3.2 times (Fig 2C). No ingestion of unopsonized sheep erythrocytes was observed under the conditions described above. Furthermore, no enhancement of phagocytosis was observed when either BSA or gelatin was substituted for vitronectin or fibronectin (data not shown). These experiments suggest that vitronectin and fibronectin induce an otherwise quiescent subpopulation of monocytes to phagoctyze opsonized particles. In addition, the capacity of the monocytes to ingest multiple EA is also enhanced by these two proteins.

**Effects of a combination of vitronectin and fibronectin on phagocytosis of EA by monocytes.** To determine if vitronectin and fibronectin produced an additive effect on monocyte-mediated phagocytosis of EA, wells were incubated with 10 µg of either vitronectin or fibronectin. This amount was chosen because, for both proteins, it produces a near maximal enhancement of the phagocytic index. A second set of slides was incubated with buffer containing 10 µg of both vitronectin and fibronectin. A third set of slides was exposed to 20 µg of either vitronectin or fibronectin, an amount at which, individually, each protein produces its maximum stimulatory effect. If vitronectin and fibronectin were acting on different subpopulations of monocytes, or if the two proteins acted on independent stimulatory mechanisms, phagocytosis should be enhanced in an additive fashion when the two proteins are used in combination. This is not the case, however, inasmuch as the increase in phagocytosis induced by the combination of vitronectin and fibronectin is equivalent to that which occurs when the individual proteins are used in the same amount as the combination (Fig 3). These results are consistent with the concept that vitronectin and fibronectin act on the same population of monocytes and that the two proteins stimulate the same mechanism through which the enhanced phagocytosis is mediated.

**Effects of antibodies on vitronectin and fibronectin-induced enhancement of phagocytosis of EA by monocytes.** The specificity of the effects of vitronectin were investigated by determining the capacity of anti-vitronectin antibodies to inhibit the enhancement of phagocytosis induced by vitronectin. Slides were incubated with vitronectin or buffer containing no protein. After the slides were washed, the (Fab')2 fragment of anti-vitronectin, anti-fibronectin, or nonimmune IgG was added to appropriate chambers. The slides were once again washed and exposed sequentially to monocytes and EA as previously described. When anti-vitronectin (Fab')2 was added to chambers previously coated with vitronectin, the degree of phagocytosis was reduced to the level of that which occurred when no vitronectin was present (Fig 4). Nonimmune and anti-fibronectin (Fab')2 had no inhibitory activity. The effects of anti-vitronectin, anti-fibronectin, and preimmune IgG on the percentage of phagocytosis were identical to those observed for the phagocytic index (data not shown). These experiments demonstrate that the functional activity of vitronectin is inhibited by specific antibodies. Furthermore, since anti-fibronectin has no inhibitory activity, the effects on phagocytosis ascribed to vitronectin cannot be due to contamination of the vitronectin preparation with fibronectin. In a parallel set of experiments, slides were coated with fibronectin, and the capacity of the F(ab')2 fragments of anti-fibronectin, anti-vitronectin, and nonimmune IgG to inhibit fibronectin-induced enhancement of monocyte-mediated phagocytosis of EA was assessed. The results of these experiments were analogous to those described above, in that anti-fibronectin
Fig 4. (Fab')2 anti-vitronectin inhibits vitronectin (VN)-induced augmentation of phagocytosis of EA by monocytes. Wells were incubated with buffer alone or with buffer containing vitronectin (10 μg). After washing, appropriate wells were incubated with buffer alone, or with buffer containing the (Fab')2 fragment of either anti-vitronectin, anti-fibronectin, or nonimmune IgG. The wells were washed, and the phagocytic index was subsequently determined. Bars depict the mean ± SD (n = 3). Anti-vitronectin (Fab')2 completely abolished the stimulatory effects of vitronectin, whereas nonimmune and anti-fibronectin (FN) (Fab')2 had no effect. Vitronectin enhances FcR function by interacting directly with the monocyte through a recognition site that can be blocked by specific antibody.

Fig 5. Fluid-phase IgG inhibits vitronectin (VN)- and fibronectin (FN)-induced augmentation of phagocytosis of EA by monocytes. Wells were incubated with either vitronectin or fibronectin. After washing, monocytes were allowed to adhere to the slides. Next the slides were washed and either buffer alone or buffer containing 10 μg monomeric IgG were added to the appropriate wells. Without washing, EA were added, and the phagocytic index was subsequently determined. Bars depict the mean ± SD (n = 3). The enhancement of phagocytosis by vitronectin and fibronectin is mediated through the monocyte FcR, and the FcR must be unoccupied for the two proteins to exert their effects.

Effects of fluid-phase IgG on vitronectin and fibronectin-mediated enhancement of phagocytosis of EA by monocytes. Phagocytosis of EA by monocytes is mediated by the FcR. To determine if vitronectin and fibronectin influence the functional activity of monocyte receptors for C3b, sheep erythrocytes bearing ~33,000 molecules of C3b per cell were created using isolated components of the alternative pathway of complement. The attachment index of EC3b to monocytes that were adherent to slides that had been incubated with buffer containing either vitronectin or fibronectin was ~ten times greater than that observed for monocytes adherent to slides that had been incubated with buffer alone (Fig 6). In contrast, ingestion of EC3b by unstimulated monocytes was minimal, and vitronectin and fibronectin enhanced phagocytosis only modestly (Fig 6). These experiments demonstrate that both vitronectin and fibronectin modulate the functional activity of monocyte receptors for C3b. Despite the marked enhancement of attachment by both vitronectin and fibronectin, the capacity of freshly isolated peripheral blood monocytes to phagocytize EC3b appears to be extremely limited.

DISCUSSION

The studies we report here demonstrate that vitronectin interacts directly with monocytes, thereby augmenting the functional activity of both the FcR and the receptor(s) for C3b. We (in this report) and other authors have shown that fibronectin has a similar stimulatory effect on monocyte function. It was therefore essential to ensure that the vitronectin preparation used in these experiments did not contain fibronectin. The Coomassie-stained gel of the vitronectin preparation (Fig 1A, lane 2) showed only the 75-kd and 65-kd forms of vitronectin, and the Western blot of this same
Fig 6. Vitronectin (VN) and fibronectin (FN) augment the functional activity of monocyte receptors for C3b. Wells were incubated with buffer alone or with buffer containing either vitronectin (40 μg) or fibronectin (40 μg). After washing, monocytes were allowed to adhere. Next, sheep erythrocytes bearing C3bi, also known as MacI and EC3b, were added to the wells. The attachment index and the phagocytic index were subsequently determined. Results are depicted by the bars, which show the mean ± SD (n = 3). Vitronectin and fibronectin markedly enhanced attachment to EC3b to monocytes; however, phagocytosis was minimally affected.

Preparation developed using anti-fibronectin (Fig 1C, lane 1) revealed no staining. Furthermore, the F(ab')2 fragment of anti-vitronectin IgG completely inhibited the greater phagocytosis of EA induced by vitronectin, whereas anti-fibronectin F(ab')2 IgG had no effect (Fig 4). Together, these results demonstrate that the enhancement of monocyte function attributed to vitronectin is not due to the presence of fibronectin in the vitronectin preparation. Furthermore, the inhibition of vitronectin activity by antibody implies a specific receptor–ligand interaction involving the monocyte and vitronectin.

A specific receptor for vitronectin has been identified. It is a heterodimer consisting of a 125-kd α chain and a 115-kd β chain. On the basis of amino acid homology, and similarities of size and subunit structure, the vitronectin receptor belongs to a family of adhesion protein receptors that includes platelet GPIIb/IIIa, the fibronectin receptor, CR3 (the receptor for complement C3bi, also known as Mac1 and Mo 1), LFA-1, and GP 150/95. The vitronectin receptor is particularly closely related to GP IIb/IIIa, and antibodies that recognize epitopes expressed by the β subunit of the vitronectin receptor, crossreact with GP IIIa. Because of this immunologic crossreactivity, and their similarity when analyzed by SDS-PAGE, in some cases, the protein identified as GP IIb/IIIa in cells other than platelets may be the vitronectin receptor. Indeed, a preliminary report by Charo et al have suggested that this is the case for endothelial cells. By analogy, the GP IIb/IIIa-like protein reported to be present on monocytes may be the vitronectin receptor.

Fibronectin binds to GP IIb/IIIa when platelets are stimulated by thrombin, and both fibronectin and vitronectin have been reported to bind to isolated GPIIb/IIIa. Thus, in some instances, the binding of vitronectin and fibronectin to cells may not be mediated by their specific receptor but rather by a discrete membrane constituent with a recognition site for both proteins. Fibrinogen and von Willebrand factor also bind to platelet GPIIb/IIIa, and for each of these proteins, a portion of the molecule containing the RGD sequence appears to be involved in the binding interaction.

Saturable, specific binding of fibronectin to monocytes has been reported, but the monocyte receptor for fibronectin has not been isolated. Neither the characteristics of binding of vitronectin to monocytes nor identification of the binding site has been determined. Thus, whether vitronectin and fibronectin bind to their specific receptors on monocytes or to a common receptor which recognizes these, and perhaps other, cytoadhesive proteins containing RGD remains to be elucidated. The results of the experiments we report do not distinguish between these two possibilities.

Vitronectin and fibronectin cause a similar dose-dependent increase in both the percentage of phagocytosis and the phagocytic index (Fig 2A through C), and the dose–response curves develop a plateau when ~10 μg of either protein is added (Fig 2A and B). Obviously, the percentage of phagocytosis cannot exceed 100; however, that the dose–response curve for the phagocytic index develops a plateau suggests that the capacity of the monocyte to respond to the stimulus is finite. At least two explanations for this observation seem plausible. First, receptor sites for vitronectin and fibronectin may become saturated when ~10 μg of protein are added to the wells. Thus, when the receptors are fully occupied, the stimulatory signal is maximal. Second, the capacity of the FcR to respond to the stimulatory signal generated by interaction of vitronectin and fibronectin with the monocyte may be limited. According to this hypothesis, maximum enhancement of phagocytosis would not require that receptor sites for vitronectin and fibronectin be fully occupied.

We have observed that both vitronectin and fibronectin markedly augmented the attachment of EC3b to monocytes; however, these proteins caused minimal enhancement of phagocytosis of EC3b (Fig 6). The results of these experiments are in agreement with those of Wright et al but differ from those of Pommier et al, who reported that fibronectin enhances phagocytosis of EC3b by freshly explanted monocytes. The explanation for these apparent discrepancies is unclear, but differences in the methods of preparation of the monocytes or of the EC3b may be involved. Nonetheless, clearly both vitronectin and fibronectin modulate the functional activity of the monocyte receptors for C3b (Fig 6).

Monocytes bear two types of receptors, CR1 (the C3b/C4b receptor) and CR3 (the iC3b receptor) which bind C3b, and in the case of cultured monocytes, both receptors can be induced by PMA (phorbol myristate acetate), fibronectin, laminin, and serum amyloid P to mediate phagocytosis of opsonized particles. By analogy, vitronectin may also stimulate both CR1 and CR3.

The mechanism by which vitronectin and fibronectin enhance the functional activity of monocyte receptors for C3b and IgG has not been elucidated; however, in the case of...
Serum spreading factor (vitronectin) is present at the cell surface deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin suggests that receptors that mediate attachment and ingestion of opsonized particles may be a critical event in initiation and modulation of the phagocytic component of cellular immunity. In addition, fibronectin is synthesized by monocytes.

Whether monocytes also produce vitronectin constitutively remains to be determined. Recently, Chhatwal et al. have reported specific binding of vitronectin to streptococci, Staphylococcus aureus, and Escherichia coli, suggesting the possibility that under some circumstances, vitronectin may directly augment phagocytosis of opsonized bacteria by monocytes. Because of its regulatory activity in cell-substrate and cell-cell interactions, the complement system, the coagulation systems and monocyte function, vitronectin appears to be an important constituent of the processes of inflammation and repair.

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