

FIBRONECTIN RECEPTOR OF HUMAN MACROPHAGES  
RECOGNIZES THE SEQUENCE Arg-Gly-Asp-Ser

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Fibronectin (Fn) binds to a large number of natural and artificial substrates such as collagen, fibrin, and plastic (1), and the resulting substrate-bound Fn is then recognized by cells. Upon contact, fibroblasts and many other cell types spread rapidly on Fn-coated surfaces (2). The effects of Fn on cells are not limited to the immediate motile response of spreading, since attachment to Fn alters the growth of tumor cells and the course of differentiation of embryonic cells (2). In human macrophages, interaction with Fn causes receptors for the third component of complement, C3, to avidly promote phagocytosis of both C3b- and C3bi-coated particles (3, 4). The receptors for C3b and C3bi (CR1 and CR3, respectively) mediate the binding but not the phagocytosis of C3b- and C3bi-coated particles if macrophages are spread on surfaces devoid of Fn. The "activation" of complement receptors by Fn occurs rapidly (within 45 min), is completely reversible, and is not accompanied by a change in the number of C3b or C3bi receptor molecules on the surface of the phagocyte (5).

The Fn molecule is composed of several folding domains that confer on it the ability to bind to other surface-bound molecules (1). The domain of Fn recognized by cells is comprised of 108 amino acids and occurs ~150 amino acids from the amino terminal (6, 7). Pierschbacher et al. (8, 9) have shown that the segment of the cell-binding domain of Fn recognized by normal rat kidney (NRK) cells comprises only a short sequence of amino acids: Arg-Gly-Asp-Ser (RGDS). Soluble synthetic peptides containing the RGDS sequence inhibit the attachment of NRK cells to Fn-coated surfaces, and surfaces coated with peptides containing the RGDS sequence support the attachment of NRK cells (8, 9). Peptides in which the R, G, or D residues are replaced by other amino acids are not recognized by NRK cells, though certain amino acids may be substituted for S without destroying the ability of the peptide to be recognized (9, 10). Recognition of RGDS by NRK cells is mediated by a 140,000 dalton protein that appears to be the Fn receptor (11). RGD sequences are also recognized by other receptors that promote cell adhesion, since the adhesion of *Dictyostelium* to Discoidin I (12) and of NRK cells to vitronectin (13) can be inhibited with soluble, RGD-containing peptides. Further, several ligands that promote cell attachment, such as Discoidin I, collagens, and fibrinogen, possess RGD sequences (10). Receptors that mediate cell-cell interaction may also recognize

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This work was supported by grant AI22003 from the U.S. Public Health Service and grant JRFA-103 from the American Cancer Society.

RGD since soluble, RGD-containing peptides inhibit proper movement of cells during the development of avian and amphibian embryos (14).

Here we show that the Fn receptor of human macrophages recognizes the RGDS sequence. Attachment of macrophages to surfaces coated with RGDS-containing peptides enables both the C3b and the C3bi receptors of the attached macrophages to promote phagocytosis, and soluble, RGDS-containing peptides competitively inhibit the activation of C3 receptors by surface-bound Fn. Ligation of Fn receptors by soluble, monomeric peptides, however, is insufficient to cause activation of C3 receptors. In contrast to Fn receptors, the binding of C3 receptors to their ligands is not inhibited by RGDS-containing peptides even though the ligands, C3b and C3bi, do contain an RGD sequence.

### Materials and Methods

*Reagents.* The synthetic peptides GRGDSP, GRGESP (10), peptide I, and peptide IV (8) were a generous gift of Drs. M. Pierschbacher and E. Ruoslahti. Fibronectin was purchased from the Greater New York Blood Center.

*Cells.* Human monocytes were purified on Percoll gradients and cultured in Teflon beakers as previously described (15). Such cells mature in culture, and after 5–10 d they closely resemble macrophages. These cells are referred to here as MO. Human neutrophils (PMN) were purified on Ficoll-Hypaque gradients (16). Sheep erythrocytes (E) coated with IgM (EIgM), IgG (EIgG), C3b (EC3b), or C3bi (EC3bi) were prepared as described (15).

*Substrates.* Plastic tissue culture surfaces were coated with proteins or synthetic peptides by passive adsorption. Peptides or proteins dissolved in phosphate-buffered saline (17) deficient in  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  (PD) were incubated with new, dry culture surfaces for 2 h at 20°C. The coating reagents were used at the following concentrations: Fn, 100  $\mu\text{g}/\text{ml}$ ; human serum albumin (HSA), 500  $\mu\text{g}/\text{ml}$ ; synthetic peptides, 50  $\mu\text{g}/\text{ml}$ . In some experiments, a relatively light coating of Fn was obtained by incubating the culture surfaces with a solution containing 50  $\mu\text{g}/\text{ml}$  Fn and 50  $\mu\text{g}/\text{ml}$  HSA.

*Assays of Attachment and Phagocytosis.* Monolayers of MO or PMN were obtained by incubating suspensions of phagocytes with the ligand-coated surfaces for 45 min at 37°C as described (5). The monolayers were washed, ligand-coated E were added, and the monolayers were reincubated at 37°C. Assays of attachment were read after 15 min, and assays of phagocytosis after 45 min at 37°C as previously described (15). The number of attached or ingested erythrocytes per 100 phagocytes is termed the attachment or phagocytic index, respectively.

### Results and Discussion

*RGDS-containing Peptides Do Not Bind to Complement Receptors.* The deduced sequence of human C3 contains the sequence RGDQ, 267 amino acids from the C terminus (amino acids 1393–1396) (18). This segment resides in the alpha polypeptide of C3 and is present in C3b and C3bi but not C3d. It was thus of interest to see whether RGDS-containing peptides could inhibit the binding of either C3b or C3bi to their respective receptors. Table I shows that the peptide, GRGDSP, at a concentration of 1.5 mg/ml is not capable of competitively inhibiting either the C3b or the C3bi receptors expressed on MO or PMN. The binding assay was performed using a brief incubation of phagocytes with C3-coated erythrocytes so that the binding was approximately half-maximal and a modest inhibition could have been noted. The observation that RGDS-containing peptides did not inhibit the binding of C3-coated erythrocytes to MO does not rule out the possibility that either C3b or C3bi receptors recognize the RGDQ

TABLE I  
*Interaction of C3b and C3bi with their Receptors on MO Is Not Inhibited by Soluble, RGDS-containing Peptides*

Competitor	Attachment index			
	MO		PMN	
	EC3b	EC3bi	EC3b	EC3bi
—	1,031 (100)	909 (100)	353 (94)	198 (74)
GRGDSP	1,096 (100)	823 (100)	341 (94)	230 (73)
GRGESP	1,104 (100)	886 (100)	360 (89)	209 (76)

MO (cultured 7 d in Teflon beakers) or freshly isolated PMN were allowed to spread on HSA-coated tissue culture surfaces for 45 min at 37°C. The resulting monolayers were then washed and incubated at 37°C for 15 min with the indicated C3-coated erythrocytes in the presence of 1.5 mg/ml GRGDSP, GRGESP, or without added competitor. The attachment index was then measured as described in Materials and Methods. The percent of phagocytes binding at least one erythrocyte is given in parentheses. These results are representative of four separate experiments.

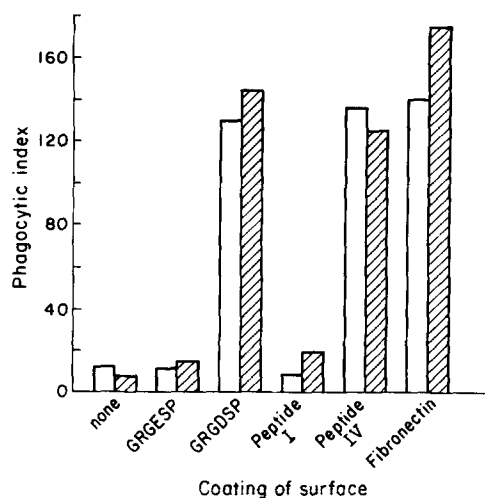


FIGURE 1. Substrate-bound, RGDS-containing peptides enhance the phagocytosis of C3b- and C3bi-coated erythrocytes. The indicated peptides were attached to plastic culture surfaces as described in Materials and Methods. MO (5-d cultures) were allowed to spread on the coated surface for 45 min at 37°C, and then the phagocytosis of EC3b (open bars) or EC3bi (hatched bars) was measured.

sequence in C3, since the conformation of that sequence may be poorly mimicked by GRGDSP. However, it is certain that neither C3b nor C3bi receptors recognize the RGDS sequence as it appears in Fn, since the concentrations of RGDS-containing peptides in Table I *did* inhibit the binding of Fn to Fn receptors (see below). Further, neither C3b nor C3bi receptors exhibited reduced activity in MO spread on Fn- or RGDS-coated surfaces (3; Fig. 1, and data not shown). A reduction in C3 receptor activity would be expected if complement receptors bound to Fn or RGDS, because such binding would cause redistribution of C3 receptors to the substrate-attached portion of the MO and subsequent diminution of receptor activity on the apical surface of the MO. Thus we believe that neither the C3b nor the C3bi receptors recognize the RGDS sequences in Fn, and neither

receptor is inhibited by concentrations of GRGDSP  $<1$  mg/ml.

*Activation of Complement Receptor Activity by Synthetic Peptides.* The capacity of peptides from the cell-binding domain of Fn to mimic the enhancement of complement-mediated phagocytosis caused by Fn was determined. Synthetic peptides were first attached to culture surfaces. MO were then allowed to spread on the peptide-coated surface, and the phagocytosis-promoting activity of C3b and C3bi receptors was measured (Fig. 1). A 30 amino acid peptide from the C-terminal region of the cell attachment domain of Fn (peptide IV) was fully as active as whole Fn in enhancing complement-mediated phagocytosis. This peptide bears the RGDS sequence previously shown to interact with Fn receptors on NRK cells (8). In contrast, a 30 amino acid peptide from the amino terminal of the cell attachment domain (peptide I) had no effect on the function of complement receptors. To further define the region of Fn recognized by MO, shorter peptides were used. The peptide GRGDSP fully activated the phagocytosis-promoting capacity of both the C3b and the C3bi receptors (Fig. 1). The high specificity for the recognition of this peptide is indicated by the observation that peptide GRGESp, in which glutamic acid is substituted for aspartic acid, is unable to activate C3-mediated phagocytosis. These experiments demonstrate that MO recognize a short RGDS-containing sequence in the cell-binding domain of Fn, and that ligation of receptors on MO by substrate-bound RGDS-containing peptides is sufficient to activate C3 receptors.

*Soluble RGDS-containing Peptides Inhibit the Action of Fn on MO.* To determine whether interaction with RGDS-containing sequences is necessary for activation of C3 receptors by Fn, MO were allowed to spread on Fn-coated surfaces in the presence of soluble RGDS-containing peptides, and phagocytosis of C3b and C3bi-coated erythrocytes was measured in the continued presence of the peptides (Fig. 2, *a* and *b*). None of the peptides tested inhibited the attachment of MO to the substrates (data not shown). This result is expected since MO do not depend on Fn for adhesion and can spread on nearly any surface except Teflon. Peptide GRGDSP did, however, cause a dose-dependent inhibition of Fn-stimulated phagocytosis of both EC3b (Fig. 2*a*) and EC3bi (Fig. 2*b*). Similar doses of

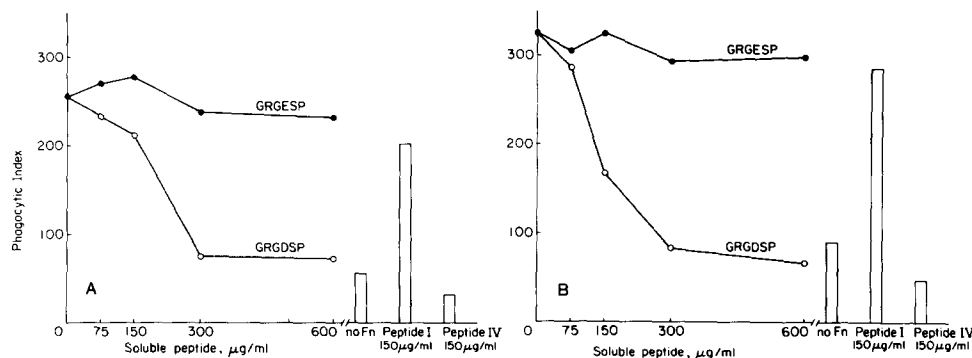


FIGURE 2. Soluble, RGDS-containing peptides inhibit the action of Fn on MO. MO (10-d cultures) were allowed to spread on Fn-coated surfaces for 45 min at 37°C in the presence of various concentrations of peptides. C3-coated erythrocytes were then added and, after an additional 45 min at 37°C, phagocytosis was scored. In these experiments, the surfaces were coated with a mixture of HSA and Fn to lessen the concentration of substrate-bound Fn. (A) Phagocytosis of EC3b. (B) Phagocytosis of EC3bi.

GRGESP did not inhibit phagocytosis of complement-coated erythrocytes (Fig. 2, *a* and *b*), suggesting that the inhibitory effect of GRGDSP is mediated by the RGDS sequence. In a parallel fashion, peptide IV, which contains an RGDS sequence, inhibited the effect of Fn but peptide I, which does not contain RGDS, did not.

Neither GRGDSP nor peptide IV inhibited the phagocytosis of EIgG (data not shown), confirming that they do not cause a general paralysis of phagocytosis but only halt the enhancement of C3-mediated phagocytosis caused by interaction of MO with Fn. Thus, interaction of MO with an RGDS sequence in Fn is necessary for Fn to augment C3-mediated phagocytosis.

GRGDSP and peptide IV inhibited the action of Fn receptors (Fig. 2), presumably because they bind the Fn receptors of MO. It is of interest that binding of such monomeric, RGDS-containing peptides does not enhance the function of complement receptors (Fig. 2, and data not shown). This result confirms our earlier finding (3) that soluble Fn does not activate complement receptors and indicates that, for Fn receptors to transduce signals to the MO, the receptors must be ligated by surface-bound ligands. The requirement that receptors be, in some way, crosslinked to transduce signals is shared by the majority of receptors for peptide hormones (19). In physiological terms, this suggests that complement receptors are not activated *in vivo* by the high levels of Fn in plasma, but are only activated at sites where Fn is deposited on a surface. Since Fn binds to fibrin, denatured collagen, and bacteria, it is likely to be deposited at sites of wounding or infection, where enhanced phagocytic activity is called for.

### Summary

When cultured human monocytes (MO) were spread on fibronectin (Fn)-coated surfaces, C3 receptors on the MO exhibited markedly enhanced capacity to promote phagocytosis. The activation of C3 receptors by Fn was mediated by a receptor that recognizes a sequence, Arg-Gly-Asp-Ser (RGDS), present in the cell-binding domain of Fn. Soluble, RGDS-containing peptides inhibited the activation of C3 receptors caused by surface-bound Fn, and surface-bound, RGDS-containing peptides themselves caused activation of the C3 receptors of attached MO. Although soluble, RGDS-containing peptides bound to Fn receptors, such monovalent ligation was insufficient to activate C3 receptors.

*Received for publication 14 May 1985.*

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