CD11c/CD18 on neutrophils recognizes a domain at the N terminus of the A α chain of fibrinogen

JOHN D. LOIKE*[†], BEATE SODEIK*, LONG CAO*, SONYA LEUCONA*, JEFFREY I. WEITZ[‡],
PATRICIA A. DETMERS[§], SAMUEL D. WRIGHT[§], AND SAMUEL C. SILVERSTEIN*

*Columbia University, New York, NY 10032; [†]McMaster University, Hamilton, ON L8V 1C3, Canada; and [§]The Rockefeller University, New York, NY 10021

Communicated by S. J. Klebanoff, October 25, 1990 (received for review April 10, 1990)

ABSTRACT Fibrinogen and fibrin serve as adhesive substrates for a variety of cells including platelets, endothelial cells, and leukocytes. Previously, we identified the C terminus of the γ chain of fibrinogen as the region of the fibrinogen molecule that contains a ligand for CD11b/CD18 (complement receptor 3) on phorbol ester-stimulated polymorphonuclear leukocytes. In contrast, we report here that neutrophils stimulated with tumor necrosis factor adhere to fibrinogen-coated surfaces, but not to human serum albumin-coated surfaces, via the integrin CD11c/CD18 (p150/95). Monoclonal antibodies LeuM5 and 3.9, which are directed against the α subunit of CD11c/CD18, but not monoclonal antibodies OKM10 and OKM1, which are directed against the α subunit of CD11b/CD18, inhibit the adhesion of tumor necrosis factor-stimulated neutrophils to fibrinogen-coated surfaces. To identify the site on fibrinogen recognized by CD11c/CD18, we have examined the adhesion of tumor necrosis factor-stimulated neutrophils to surfaces coated with various fibrinogen fragments. Stimulated neutrophils adhere to surfaces coated with the N-terminal disulfide knot fragment of fibrinogen or fibrinogen fragment E. Moreover, peptides containing the sequence Gly-Pro-Arg (which corresponds to amino acids 17–19 of the N-terminal region of the A α chain of fibrinogen), and monoclonal antibody LeuM5, block tumor necrosis factor-stimulated neutrophil adhesion to fibrinogen and to the N-terminal disulfide knot fragment of fibrinogen. Thus, CD11c/CD18 on tumor necrosis factor-stimulated neutrophils functions as a fibrinogen receptor that recognizes the sequence Gly-Pro-Arg in the N-terminal domain of the A α chain of fibrinogen.

Leukocytes interact with coagulation factors in a highly specific and regulated fashion (1–5). For example, during clot formation, polymorphonuclear leukocytes (PMNs) rapidly accumulate within fibrin thrombi (6–8). Attachment of PMNs to fibrinogen/fibrin matrices is regulated by receptors located on their surfaces (1, 2, 4, 5). We (1) and others (2, 4, 5) have reported that CD11b/CD18 [complement receptor 3 (CR3)], a member of the CD11/CD18 family of leukocyte integrins, mediates PMN and monocyte adherence to surface-bound fibrinogen (Fg). Cooper *et al.* (9) reported that neutrophil binding to fibrin is blocked by an antibody directed against the 95-kDa β chain that is common to all members of the CD11/CD18 family of leukocyte integrins. Furthermore, coagulation factor X is reported (3, 10) to interact with CR3 on monocytes. Thus, CD11/CD18 mediates the interaction of PMNs and monocytes with at least three coagulation proteins, Fg, fibrin, and factor X.

We have demonstrated that the sequence Lys-Gln-Ala-Gly-Asp-Val at the C terminus of the γ chain of Fg serves as a ligand for CR3 on PMNs stimulated with phorbol esters (1). However, there is evidence that other sites on Fg also may

serve as ligands for leukocyte receptors. For example, several reports (11, 12) describe the binding of Fg fragment E or its N-terminal disulfide fragment (N-DSK) to rabbit or murine macrophages. In addition, a plasmin-generated Fg fragment has been shown to block the interaction of ¹²⁵I-labeled Fg with stimulated leukocytes (5). Since these Fg fragments all lack the Lys-Gln-Ala-Gly-Asp-Val-containing segment at the C terminus of the γ chain, other regions on Fg must serve as ligands for receptors on these cells.

In this paper, we report that adhesion of tumor necrosis factor α (TNF- α)-stimulated PMNs to Fg-coated surfaces is mediated by CD11c/CD18. LeuM5 and 3.9, monoclonal antibodies (mAbs) directed against the α subunit of the CD11c/CD18 (p150/95) integrin (13, 14), block adhesion of TNF-stimulated PMNs to surfaces coated with Fg. Using surfaces coated with well-characterized Fg fragments, we have determined that CD11c/CD18 on PMNs recognizes a Gly-Pro-Arg sequence, corresponding to residues 17–19 of the A α chain of Fg.

MATERIALS AND METHODS

Reagents. Recombinant TNF- α was generously provided by Hoffmann-La Roche. Human Fg (grade L; Kabi Vitrum, Stockholm) was depleted of plasminogen by lysine Sepharose 4B affinity chromatography in the presence of aprotinin (100 kallikrein inhibition units/ml) (15). Fg migrated as three bands of unequal intensity on reduced SDS/PAGE corresponding to the α , β , and γ chains. Fg fragments D and E were generously provided by B. Kudryk (New York Blood Center). Gel electrophoresis studies confirmed that the Fg fragment D is predominantly (>90%) the D1 form (Frag-D), in which the γ chain of this fragment extends from amino acids 86 to 411, including the C-terminal $\gamma_{395-411}$ (B. Kudryk, personal communication). The N-DSK was prepared by CnBr cleavage of Fg as described (16). Fibrinopeptide A α 1–21 (17) was synthesized by the method of Merrifield and coworker (18). Fibrinopeptides A (FPA) and B (FPB) were from Bachem. Peptide G15 (Gly-Gln-Gln-His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val) corresponding to residues 397–411 of the γ chain of Fg, Gly-His-Arg-Pro, and Gly-Pro-Gly-Gly peptides were from Sigma; Gly-Pro-Arg and Gly-Pro were from Peninsula Laboratories. Gly-Pro-Arg-Pro was obtained from Sigma, Peninsula Laboratories, and from Bachem. All peptides were dissolved in phosphate-buffered saline (PBS) and the pH of the solutions was adjusted to 7.2. Purity of Gly-Pro-Arg-Pro was established by reverse-phase HPLC with an Ultrasphere ODS C18 column (4.6 nm;

Abbreviations: CR3, complement receptor 3; Fg, fibrinogen; Frag-D, fibrinogen fragment D1; FPA, -B, fibrinopeptide A or B; mAb, monoclonal antibody; N-DSK, N-terminal disulfide knot of Fg; PMN, polymorphonuclear cell; TNF, tumor necrosis factor; HSA, human serum albumin; PDBu, phorbol dibutyrate.

[†]To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Beckman) and was found to be >98%. Endotoxin-free human serum albumin (HSA) was from Armour Pharmaceuticals.

Antibodies. Several mAbs were generously provided by colleagues. These were as follows: OKM1 and OKM10 directed against the α chain (CD11b) of CR3 (CD11b/CD18) (19), from G. Goldstein (Ortho Pharmaceuticals); LM2/1.6.11 also directed against the α chain of CR3, and TS 1/22 directed against the α chain of LFA-1 (CD11a/CD18) (20), from Timothy Springer (Dana-Farber Institute, Boston). L29 and 3.9 directed against the α chain of CD11c/CD18 (13, 14), from Lewis Lanier (Becton Dickinson) and Nancy Hogg (Imperial Cancer Research Fund, London), respectively. LeuM5 directed against the α chain of CD11c/CD18 (13) was purchased from Becton Dickinson. mAbs IB4 (19) and 60.3 (21) are directed against the common β chain of the CD11/CD18 family of integrins. mAb W6/32 is directed against HLA (22).

Cells. PMNs were isolated from fresh human blood on Ficoll/Hypaque gradients as described (1). PMNs were suspended in PBS containing 0.5 mg of HSA per ml and 5.5 mM glucose and were kept at 4°C until use.

Adhesion of PMNs. Adhesion of PMNs to protein-coated surfaces was measured as described (1). Briefly, Terasaki tissue culture plates were coated with HSA (1–10 mg/ml), Fg, or Fg fragments (1 mg/ml or 250 μ g/ml) by incubating each well with PBS containing the protein or protein fragment for 60 min at 20°C. Reducing the concentration of Fg or Fg fragments used to coat the wells from 1 mg/ml to 250 μ g/ml did not alter the number of stimulated PMNs that adhered (data not shown). Protein-coated plates were washed with PBS at 4°C and were used immediately. PMNs were suspended at 10^6 cells per ml in the absence or presence of activators, peptides, or antibodies. Five microliters of the PMN suspension was added to each protein-coated well of a Terasaki plate. Plates were incubated for 30 min at 4°C to allow PMNs to settle to the bottom of the wells and were then warmed to 37°C for 15 min to allow cell adhesion. Subsequently, unattached cells were removed by dipping the plates 10 times in PBS, inverting the plates for 20 min, and dipping the plates again 10 times in PBS, all at room temperature. The liquid in each well was removed by blotting before the cells were fixed to the plate with 2.5% glutaraldehyde. The adherent cells in each well were enumerated visually by phase-contrast microscopy. Values for PMN adherence are the means of six identical wells and are of representative experiments, each of which was repeated at least three times.

Binding of C3bi-Coated Erythrocytes. Binding of sheep erythrocytes coated with C3bi (EC3bi) to PMNs was measured as described (23, 24). Briefly, monolayers of PMNs were incubated at 37°C with TNF (1 ng/ml; 30 min) or phorbol 12-myristate 13-acetate (30 ng/ml; 15 min). The cells were then washed and incubated with mAbs (50 μ g/ml) or synthetic peptides (5 mg/ml) for 15 min at 0°C. EC3bi was then added (in the continued presence of mAb or peptides), and after a 15-min incubation at 37°C, binding of EC3bi was enumerated as "attachment index," the number of erythrocytes bound per 100 PMNs. Results are representative of three separate experiments.

RESULTS

TNF Stimulates PMN Adhesion to Protein-Coated Substrates. Attachment of unstimulated PMNs to protein-coated plastic (Figs. 1–5) varied from 30 to 90 cells per mm^2 for HSA-coated surfaces (Figs. 1, 4, 5) and from 30 to 65 cells per mm^2 for Fg-coated surfaces (Figs. 1, 2, 4, and 5). Only 10% of unstimulated PMNs that did attach to HSA-coated surfaces spread on them. About 35% of unstimulated adherent PMNs spread on Fg-coated surfaces (data not shown). Stimulation of PMNs with TNF (2 ng/ml) enhanced their

FIG. 1. Effect of anti-CD11/CD18 antibodies on adhesion of TNF-stimulated PMNs to Fg-coated surfaces. Unstimulated PMNs (5000 cells per well) or PMNs treated with TNF (2 ng/ml) were allowed to settle onto Fg-coated surfaces for 30 min at 4°C in the absence or presence of the indicated antibodies. Cells were then warmed to 37°C for 15 min, washed, fixed, and counted as described. Adhesion of untreated PMNs to surfaces coated with HSA served as a control. mAbs IB4 and 60.3 were used at 10 μ g/ml; mAbs OKM10, OKM1, and TS1/22 were used at 20 μ g/ml. mAbs LeuM5 and 3.9 were used at 5 and 25 μ g/ml, respectively.

adherence to HSA-coated surfaces 2.5-fold (Fig. 5) and to Fg-coated surfaces at least 3.5-fold (Figs. 1, 2, 4, and 5) when compared to binding of unstimulated PMNs to HSA or Fg-coated surfaces, respectively. More than 90% of TNF-treated cells were well spread on Fg-coated surfaces; in contrast, <10% of the TNF-stimulated PMNs spread on HSA-coated surfaces (data not shown). Maximal binding of TNF-treated PMNs to Fg-coated surfaces occurred within 15 min (data not shown) and was achieved with a TNF concentration of 2 ng/ml (Fig. 2). These results indicate that TNF promotes the adhesion of PMN to both Fg- and HSA-coated surfaces, but that cell spreading was markedly enhanced on Fg. The studies described below were designed to identify specific receptors and ligands that participate in the adhesion of TNF-stimulated PMNs to Fg-coated surfaces.

CD11c/CD18 Mediates Attachment of TNF-Treated PMNs to Fg. Previous work (1) showed that CR3 mediates the attachment of phorbol dibutyrate (PDBu)-stimulated PMNs to Fg-coated surfaces, and that mAb OKM10, which is directed against the α subunit of CR3 (19), blocks adhesion of PDBu-stimulated PMNs to these surfaces. However, saturating concentrations of mAb OKM10 (25) did not inhibit attachment of TNF-stimulated PMNs to Fg-coated surfaces (Fig. 1). Similarly, saturating concentrations (10–20 μ g/ml) of mAb OKM1 (Fig. 1) or LM2/1.6.11 (data not shown) directed against the α subunit of CR3 also failed to inhibit

FIG. 2. Concentration dependence of TNF on the adhesion of PMNs to Fg. PMNs stimulated with various concentrations of TNF were allowed to settle onto Fg-coated surfaces for 30 min at 4°C, warmed to 37°C for 15 min, washed, fixed, and enumerated as described.

FIG. 3. Adhesion of TNF-stimulated PMNs to fragments of Fg. Surfaces were incubated with PBS containing 250 μ g of N-DSK or Frag D per ml. Adhesion of TNF-stimulated PMNs to these surfaces was performed as described. Where indicated, the initial incubation at 4°C was performed in the presence of Gly-Pro-Arg-Pro (GPRP), FPA, A α 1-21 peptide (1 mg/ml), mAb LeuM5 (5 μ g/ml), or mAb OKM10 (20 μ g/ml).

adhesion of TNF-stimulated PMNs to Fg-coated surfaces. In contrast, mAbs IB4 and 60.3, which are directed against the β subunit of the CD11/CD18 family of glycoproteins (19, 21), inhibited the adhesion of TNF-stimulated PMNs to Fg-coated surfaces by >90% (Fig. 1). These findings suggest that CD11a/CD18 or CD11c/CD18 may mediate PMN adhesion to Fg.

mAb TS1/22, which is directed against the α chain of CD11a/CD18 [LFA-1 (20)], did not affect the adhesion of TNF-stimulated PMNs to Fg-coated surfaces (Fig. 1). In contrast, mAbs LeuM5 (13, 14) and 3.9 (14), which are directed against the α chain of CD11c/CD18, blocked adhesion of TNF-stimulated PMNs to Fg-coated surfaces (Fig. 1). LeuM5 reduced attachment of TNF-stimulated PMNs to Fg-coated surfaces by >60% at a concentration of 5 μ g/ml (Fig. 1) and by 50% at a concentration of 3–4 μ g/ml (data not shown). Control experiments showed that the presence of azide and gelatin in this antibody preparation did not significantly affect adherence of TNF-stimulated PMNs (data not shown). Similarly, mAb 3.9 (25 μ g/ml) reduced attachment of TNF-stimulated PMNs to Fg-coated surfaces by >50% (Fig. 1). mAb L29 (14), directed against an epitope on the α chain of CD11c that is different from that recognized by LeuM5, did

FIG. 4. Effects of peptides on the adherence of TNF-stimulated PMNs to Fg-coated surfaces. Attachment of PMNs treated with 2 ng of TNF per ml was assayed in the absence or presence of 1 mg of the indicated peptides per ml as described. The following peptides were used: Gly-Pro-Arg-Pro (GPRP), Gly-His-Arg-Pro (GHRP), G15 (the C terminal 15 amino acids of the γ chain of Fg), Gly-Pro (GP), Gly-Pro-Gly-Gly (GPGG). Adhesion of untreated PMNs to HSA-coated surfaces served as a control.

not inhibit adhesion of TNF-stimulated PMNs to Fg, even when used at a concentration of 25 μ g/ml (data not shown).

LeuM5 inhibited adherence of PMNs to Fg in experiments in which the cells were first incubated with TNF at 37°C for 15 min (in the absence of LeuM5), washed in PBS, and then allowed to adhere to Fg-coated wells in the presence of LeuM5. These data show that LeuM5 does not act by preventing TNF from stimulating PMNs and that CD11c/CD18-mediated adhesion of PMNs to a Fg-coated substrate is not dependent on secretory products of PMNs. Thus, the inhibitory effect of LeuM5 on PMN adherence is due to the interaction of this mAb with the cells and not to its interaction with TNF-induced secretory products.

TNF-Stimulated PMNs Bind to a Site at the N Terminus of Fg. To identify the site(s) on Fg recognized by CD11c/CD18, we examined the capacity of TNF-stimulated PMNs to adhere to surfaces coated with different fragments of Fg. A small number of unstimulated PMNs adhered (Fig. 3) but did not readily spread (data not shown) on N-DSK-coated surfaces. TNF elicited a 4-fold increase in the adherence of PMNs to N-DSK-coated surfaces, an effect blocked >50% by mAb LeuM5 but not by mAb OKM10 (Fig. 3). In contrast, approximately the same number of unstimulated and TNF-stimulated PMNs bound to surfaces coated with Frag-D (Fig. 3). These studies suggest that TNF-stimulated PMNs recognize a domain within the N- rather than the C-terminal region of the Fg molecule.

To further localize the portion of Fg that is a ligand for CD11c/CD18, we tested the capacity of several fibrinopeptides to block adhesion of TNF-stimulated PMNs to Fg-coated surfaces. FPA (corresponding to amino acids 1–16 of the A α chain) and FPB (corresponding to amino acids 1–14 of the B β chain) at concentrations ranging from 0.1 to 1 mg/ml had no effect on the adherence of TNF-stimulated PMNs to Fg-coated surfaces (Fig. 3; data not shown). In contrast, Fg fragment A α 1–21 (corresponding to amino acids 1–21 of the A α chain) reduced adherence of TNF-stimulated PMNs to Fg-coated surfaces by $\approx$ 50% (Fig. 3). These results suggest that TNF-stimulated PMNs recognize the region of the A α chain of Fg corresponding to amino acids 17–21.

Gly-Pro-Arg-Pro Blocks Adhesion of TNF-Stimulated PMNs to Fg-Coated Surfaces. Gly-Pro-Arg corresponds to amino acids 17–19 of the N terminus of the A α chain of Fg. Therefore, we examined the effects of peptides containing this sequence on adherence of TNF-stimulated PMNs to

FIG. 5. Adhesion of TNF-stimulated PMNs to HSA- and Fg-coated surfaces. Surfaces were incubated with PBS containing either 250 μ g of Fg per ml or 10 mg of HSA per ml. Adhesion of TNF-stimulated PMNs to these surfaces was performed as described. LeuM5 was used at a final concentration of 4 μ g/ml. Soluble (Sol.) Fg (final concentration, 3 mg/ml) was added to PMNs in the presence of TNF for 15 min in a gently shaking water bath at 37°C. The cell preparation then was added to protein-coated surfaces for an additional 15 min at 37°C and adhesion was monitored as described. GPRP, Gly-Pro-Arg-Pro.

Fg-coated surfaces. Gly-Pro-Arg-Pro (1 mg/ml) inhibited adhesion of TNF-stimulated PMNs to Fg-coated surfaces by >60% (Fig. 4). Inhibition of PMN adherence to Fg by this peptide was dose dependent; half-maximal inhibition occurred at ≈ 0.2 mg/ml (0.38 mM) (data not shown). Control experiments showed that Gly-His-Arg-Pro, (which corresponds to amino acids 15–18 of the β chain of Fg), peptide G15 (which corresponds to amino acids 397–411 at the C terminus of the γ chain of Fg), Gly-Pro, and Gly-Pro-Gly-Gly, all at concentrations of 1.0 mg/ml, had no significant effect on PMN adherence to Fg-coated surfaces (Fig. 4). The requirement for high concentrations of Gly-Pro-Arg-Pro to inhibit PMN adherence to Fg surfaces is consistent with our previous observations of the low efficiency with which synthetic peptides block the interaction of CR3 with C3bi-coated cells (26). This may reflect the fact that peptides act as monomeric ligands, while cell-bound C3bi and surface-bound Fg are multimeric ligands (27).

To confirm that Gly-Pro-Arg-Pro blocks PMN adhesion to ligands at the N terminus of the α chain of Fg, we examined the effects of this peptide on adherence of TNF-stimulated PMNs to surfaces coated with Fg fragments N-DSK and E. Both of these fragments lack the C terminus of the γ chain of Fg that contains the sequence Lys-Gln-Ala-Gly-Asp-Val to which CR3 binds (1). Gly-Pro-Arg-Pro blocked adhesion of TNF-stimulated PMNs to surfaces coated with either N-DSK (Fig. 3) or fragment E (data not shown). In contrast, Gly-Pro-Arg-Pro did not block adherence of TNF-stimulated PMNs to surfaces coated with Frag-D (Fig. 3). Control experiments showed that Gly-His-Arg-Pro, G15, and Gly-Pro, had no effect on PMN adherence to N-DSK-coated surfaces (data not shown). These studies indicate that CD11c/CD18 on TNF-stimulated PMNs recognizes the sequence Gly-Pro-Arg corresponding to residues 17–19 at the N-terminal region of the α chain of Fg.

Specificity of TNF-Stimulated PMN Adherence. As noted above, TNF did not promote PMN adherence to Frag-D-coated surfaces (Fig. 3), indicating that TNF does not promote PMN adhesion to all surfaces. TNF promoted adherence [but not spreading (data not shown)] of PMNs to HSA-coated surfaces (Fig. 5) by a process that was unaffected by either 4 μ g of LeuM5 per ml or 1 mg of Gly-Pro-Arg-Pro per ml (Fig. 5), whereas both compounds blocked adherence of these cells to Fg-coated surfaces (Figs. 1, 4, and 5). Thus, the inhibitory effect of LeuM5 and Gly-Pro-Arg-Pro on TNF-mediated PMN adherence is specific for Fg.

Since TNF strongly stimulates the capacity of CR3 to bind complement-coated particles (28), and since the complement binding site on CR3 also recognizes Fg (1), we determined whether LeuM5 exerts its inhibitory effect by influencing CR3. TNF-stimulated PMNs were incubated with EC3bi in the presence of mAbs (50 μ g/ml) or peptides. As expected, TNF-stimulated PMNs bound EC3bi strongly (attachment index, 754 for TNF-stimulated PMNs and 157 for untreated PMNs), and binding was inhibited >90% by mAb OKM10 (attachment index, 36). In contrast, neither LeuM5 nor an antibody against HLA (W6/32) caused any measurable inhibition of binding of EC3bi to TNF-stimulated PMNs (attachment indices, 785 and 679, respectively). Thus, OKM10 inhibits the complement-binding activity of CR3 but does not inhibit adhesion of TNF-stimulated PMNs to Fg. Conversely, LeuM5 inhibits binding of TNF-stimulated PMNs to Fg but not the complement binding activity of CR3.

To determine whether Gly-Pro-Arg-Pro affects the binding activity of CR3, we examined the effect of Gly-Pro-Arg-Pro on the binding of PDBu-stimulated PMNs to EC3bi or the adhesion of these cells to Fg-coated surfaces. Gly-Pro-Arg-Pro (5 mg/ml) had no effect on binding of EC3bi to PDBu-stimulated PMNs (attachment index, 272 for cells incubated without Gly-Pro-Arg-Pro and 346 for cells incubated with

Gly-Pro-Arg-Pro). Similarly, Gly-Pro-Arg-Pro had no effect on the attachment of PDBu-stimulated PMNs to Fg-coated surfaces. Thus, Gly-Pro-Arg-Pro does not block the ligand binding site of CR3 for either C3bi or Fg. These experiments provide complementary and concordant data. They indicate that LeuM5 and Gly-Pro-Arg-Pro block binding of TNF-stimulated PMNs to Fg-coated surfaces through their effect on CD11c/CD18 and not via an effect on CD11b/CD18.

Since plasma contains 2–4 mg of Fg per ml, we examined the effect of soluble Fg on the adhesion of TNF-stimulated PMNs to substrate-bound Fg. Adherence of TNF-stimulated PMNs to Fg-coated surfaces was reduced by only $25\% \pm 9\%$ ($n = 6$) by the presence of 3 mg of soluble Fg per ml (Fig. 5). These results indicate that conformational alterations of Fg may be required to unmask its CD11c/CD18 binding domain and/or that attachment of Fg to a surface converts it from a monomeric to a multimeric ligand, thereby enhancing its affinity for CD11c/CD18.

DISCUSSION

That the leukocyte integrin CD11c/CD18 on TNF-stimulated PMNs serves as a receptor for Fg is supported by our observations that mAbs directed against CD11c/CD18 block adherence of these cells to Fg-coated surfaces but not to HSA-coated surfaces (Figs. 1 and 5). That CD11c/CD18 recognizes a Gly-Pro-Arg containing site in the N-terminal region of the α chain of Fg is supported by our observations that Gly-Pro-Arg-Pro and α 1–21 (but FPA) block the adherence of TNF-stimulated PMNs to N-DSK-, fragment E-, and Fg-coated surfaces (Figs. 3 and 4).

We do not believe that either mAb LeuM5 or Gly-Pro-Arg-Pro induces a negative signal to reverse TNF activation of PMNs because the activation of TNF-stimulated PMNs, as measured by either the increased attachment of EC3bi to PMNs or adherence of PMNs to HSA-coated surfaces, was unaffected by LeuM5 or Gly-Pro-Arg-Pro. Nonetheless, we cannot rule out the possibility that LeuM5 or Gly-Pro-Arg-Pro may generate a negative signal that only affects TNF-mediated activation of CD11c/CD18 and not CD11b/CD18.

Other functions of CD11c/CD18 have been reported. For example, CD11c/CD18 acts as a lipopolysaccharide receptor (26, 29) and mediates the attachment of unopsonized bacteria and fungi to leukocytes (29, 30). In addition, anti-CD11c/CD18 antibodies inhibit cytolysis mediated by T-cell clones that express high levels of this integrin (31) and reduce adhesion of human monocytes and granulocytes to vascular endothelium (32).

The suggestion that CD11c/CD18 recognizes C3bi (14, 33) remains controversial. Malhotra *et al.* (33) found that solubilized CD11c/CD18 binds to C3bi-coated Sepharose; yet, an antibody against CD11c did not block the attachment of EC3bi to living phagocytes. Myones *et al.* (14) reported that mAbs LeuM5 and L29 reduced attachment of EC3bi to unstimulated PMNs, but inhibition was partial and was only observed when anti-CD11b mAbs also were present in the assay. Wright and Jong (29) observed no decrement in the binding of EC3bi upon removal of CD11c from the apical membranes of macrophages and we have shown here that LeuM5 does not block the attachment of EC3bi to TNF-stimulated PMNs. Lastly, COS cells transfected with cDNA encoding CD11c/CD18 did not bind EC3bi (34).

The ability of CD11c/CD18 to bind Fg is regulated. Unstimulated PMNs bind poorly to Fg-coated surfaces and TNF promotes CD11c/CD18-dependent attachment to these surfaces (Fig. 1). Similarly, regulation of ligand binding has been observed for other members of the CD11/CD18 family—e.g., CD11b/CD18-dependent binding of several ligands to PMNs (24, 28, 34–36) and CD11a/CD18-dependent binding of PMNs to ICAM-1 on endothelial cells (37). A curious aspect

of the regulation of Fg binding by the CD18 family of integrins is the difference in the effects of phorbol esters and TNF. Both agents cause increased expression and altered binding capacity of CR3 on PMN plasma membranes (24, 28, 36, 38). However, phorbol ester-stimulated adhesion of PMNs to Fg-coated surfaces is mediated predominantly by CD11b/CD18 (1), while TNF-stimulated PMN adhesion to Fg-coated surfaces is mediated predominantly by CD11c/CD18. The mechanisms by which these two agonists exert differential effects on the activities of CD11b and CD11c are unresolved, but they may be due to differences in the intracellular signals these agonists deliver. Our results indicate that the nature of agonist determines whether PMNs utilize CD11b or CD11c in adhering to Fg-coated surfaces.

Given that clots are formed by fibrin, one may question the physiological significance of PMN adhesion to Fg-coated surfaces. In this regard, we note that Fg binds to both soluble fibrin monomers (39) and to fibrin matrices (40). *In vivo*, where fibrin matrices are bathed in plasma rich in Fg, these matrices should be coated with Fg. We suggest that the Gly-Pro-Arg motif present in matrix bound Fg and in fibrin itself serves as a ligand to which PMNs adhere via CD11c/CD18. This hypothesis is supported by Gonda and Shainoff's report (11) that fibrin monomers readily adsorb to, and are taken up by, rabbit peritoneal macrophages and that the peptide Gly-Pro-Arg-Pro blocks this process. Their findings, coupled with those reported here, suggest that CD11c/CD18 on PMNs and macrophages is a receptor for a Gly-Pro-Arg motif in fibrin and in substrate-adherent Fg.

The authors thank Pamela Rockwell for technical assistance. This investigation was supported by grants from the National Institutes of Health (DK39110, GM40791, AI22003, AI24775, HL33210, and AI20516), the American Heart Association, and the Medical Research Council of Canada and the Ontario Heart and Stroke Foundation. J.I.W. is a scholar of the Ontario Heart and Stroke Foundation. P.A.D. is an investigator of the American Heart Association, New York City Affiliate. S.D.W. is an Established Investigator of the American Heart Association.

- Wright, S. D., Weitz, J. I., Huang, A. J., Levin, S. M., Silverstein, S. C. & Loike, J. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7734-7738.
- Altieri, D. C., Bader, R., Mannucci, P. M. & Edgington, T. S. (1988) *J. Cell Biol.* **107**, 1893-1900.
- Altieri, D. C. & Edgington, T. S. (1988) *J. Biol. Chem.* **263**, 7007-7015.
- Gustafson, E. J., Lukasiewicz, H., Wachtfogel, Y. T., Norton, K. J., Schmaier, A. H., Niewiarowski, S. & Colman, R. W. (1989) *J. Cell Biol.* **109**, 377-387.
- Altieri, D. C., Agbanyo, F. R., Plescia, J., Ginsberg, M. H., Edgington, T. S. & Plow, E. F. (1990) *J. Biol. Chem.* **265**, 12119-12122.
- Barnhart, M. I. (1965) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **24**, 846-853.
- Plow, E. F. (1986) *Blut* **53**, 1-9.
- Sherman, L. A. & Lee, J. (1977) *J. Exp. Med.* **145**, 76-85.
- Cooper, J. A., Lo, S. K. & Malik, A. B. (1988) *Circ. Res.* **63**, 735-741.
- Altieri, D. C., Morrissey, J. H. & Edgington, T. S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7462-7466.
- Gonda, S. R. & Shainoff, J. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4565-4569.
- Ritchie, D. G. & Fuller, G. M. (1983) *Ann. N.Y. Acad. Sci.* **408**, 490-500.
- Lanier, L. L., Arnaout, M. A., Schwarting, R., Warner, N. L. & Ross, G. D. (1985) *Eur. J. Immunol.* **15**, 713-718.
- Myones, B. L., Dalzell, J. G., Hogg, N. & Ross, G. D. (1988) *J. Clin. Invest.* **82**, 640-651.
- Finlayson, J. S. (1968) in *Fibrinogen*, ed. Laki, K. (Dekker, New York), pp. 39-59.
- Blomback, B., Blomback, M., Henschen, A., Hessel, B., Iwanaga, S. & Woods, K. R. (1968) *Nature (London)* **218**, 130-134.
- Weitz, J. I., Landman, S. L., Crowley, K. A., Birken, S. & Morgan, F. (1986) *J. Clin. Invest.* **78**, 155-162.
- Marglin, A. & Merrifield, R. B. (1970) *Annu. Rev. Biochem.* **39**, 841-866.
- Wright, S. D., Rao, P. E., Van Voorhis, W. C., Craigmyle, L. S., Iida, K., Talle, M. A., Goldstein, G. & Silverstein, S. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5699-5703.
- Sanchez-Madrid, F., Nagy, J., Robbins, E., Simon, P. & Springer, T. A. (1983) *J. Exp. Med.* **158**, 1785-1803.
- Beatty, P. G., Ledbetter, J. A., Martin, P. J., Price, T. H. & Hannsen, J. A. (1983) *J. Immunol.* **131**, 2913-2918.
- Barnstable, C. J., Bodner, W. F., Brown, G., Kalfre, G., Milstein, C., Williams, A. F. & Zeigler, A. (1978) *Cell* **14**, 9-20.
- Wright, S. D., Tobias, P. S., Ulevitch, R. J. & Ramos, R. A. (1989) *J. Exp. Med.* **170**, 1231-1241.
- Wright, S. D. & Meyer, B. C. (1986) *J. Immunol.* **136**, 1759-1764.
- Anderson, D. C., Miller, L. J., Schmalstieg, F. C., Rothlein, R. & Springer, T. A. (1986) *J. Immunol.* **137**, 15-27.
- Wright, S. D., Levin, S. M., Jong, M. T., Chad, Z. & Kabbash, L. G. (1989) *J. Exp. Med.* **169**, 175-183.
- Hermanowski-Vosatka, A., Detmers, P. A., Gotze, O., Silverstein, S. C. & Wright, S. D. (1988) *J. Biol. Chem.* **263**, 17822-17827.
- Detmers, P. A., Wright, S. D., Olsen-Egbert, I. & Cohn, Z. A. (1990) *FASEB J.* **4**, A2056.
- Wright, S. D. & Jong, M. T. (1986) *J. Exp. Med.* **164**, 1876-1888.
- Bullock, W. E. & Wright, S. D. (1987) *J. Exp. Med.* **165**, 195-210.
- Keizer, G. D., Borst, J., Visser, W., Schwarting, R., DeVries, J. E. & Figdor, C. G. (1987) *J. Immunol.* **138**, 3130-3136.
- Arnaout, M. A., Lanier, L. L. & Faller, D. V. (1988) *J. Cell. Physiol.* **137**, 305-309.
- Malhotra, V., Hogg, N. & Sim, R. B. (1985) *Eur. J. Immunol.* **16**, 1117-1123.
- Hogg, N. C. (1989) *Immunol. Today* **10**, 111-114.
- Lo, S. K., Detmers, P. A., Levin, S. M. & Wright, S. D. (1989) *J. Exp. Med.* **169**, 1779-1793.
- Detmers, P. A., Lo, S. K., Olsen-Egbert, I., Walz, A., Boggiolini, M. & Cohn, Z. A. (1990) *J. Exp. Med.* **171**, 1155-1162.
- Lo, S. K., Van-Seventer, G. A., Levin, S. M. & Wright, S. D. (1989) *J. Immunol.* **143**, 3325-3329.
- Berger, M., Wetzler, E. M. & Wallis, R. S. (1988) *Blood* **71**, 151-158.
- Sasaki, T., Page, I. H. & Shainoff, J. R. (1966) *Science* **152**, 1069-1071.
- Kaminski, M. & McDonagh, J. (1983) *J. Biol. Chem.* **258**, 10530-10535.