HUMAN NEUTROPHIL ADHERENCE TO LAMININ IN VITRO

Evidence for a Distinct Neutrophil Integrin Receptor for Laminin

By JOHN F. BOHNSACK,* STEVEN K. AKIYAMA,¶** CAROLINE H. DAMSKY,‡‡ WILLIAM A. KNAPE,‡ AND GUY A. ZIMMERMAN§‖

*From the Departments of *Pediatrics, ‡Pathology, and §Medicine, the ‖Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah College of Medicine, Salt Lake City, Utah 84132; the ¶Departments of Biochemistry and Oncology, Howard University College of Medicine, Washington, DC 20060; the **Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; and the ‡‡Departments of Anatomy and Stomatology, University of California, San Francisco, California 94143*

Neutrophils (polymorphonuclear leukocytes [PMNs][1]) migrate through endothelium and subendothelial basement membrane when they leave the circulation and enter the extravascular compartment. PMNs migrate by a process of regulated, reversible adherence to tissues, rather than by swimming (1, 2). The molecular mechanisms involved in PMN adherence endothelial cells (EC), the first cell that PMNs encounter during migration from the blood to the extravascular space, are under active investigation, and it is clear that molecules expressed by both the PMN and the EC are important in regulating this adhesive interaction (3–8). Less is known about the molecular mechanisms involved in PMN adhesion to connective tissue proteins in the subendothelial basement membrane and interstitial space, the next structures encountered by the migrating leukocyte.

A compelling body of experimental evidence has demonstrated that PMN receptors, called the CD11/CD18 glycoprotein complex, are involved when stimulated PMNs adhere to biologic surfaces (3–6, 9–14). The CD11/CD18 glycoprotein complex consists of three heterodimeric proteins, each with a separate α chain, and a common β chain (15). The β chain is one of three β chains identified in a larger family of related cell surface heterodimers called the integrins, which are all involved in cell-cell and cell-protein interactions (16). The clinical course of patients congenitally

This work was supported in part by a Fellowship from the Arthritis Foundation and by a grant from the Thrasher Research Fund (J. F. Bohnsack); by U. S. Public Health Service grants CA-14178, CA-45515 (S. K. Akiyama), and CA-42032 (C. H. Damsky); by a Grant-in-Aid from the American Heart Association (H711417) (G. A. Zimmerman); and by the Nora Eccles Treadwell Foundation (G. A. Zimmerman). G. A. Zimmerman is an Established Investigator of the American Heart Association.

Address correspondence to John Bohnsack, Department of Pediatrics, Division of Clinical Immunology and Allergy, University of Utah School of Medicine, Salt Lake City, UT 84132.

[1] *Abbreviations used in this paper:* EC, endothelial cell; HSA, human serum albumin; PAF, platelet-activating factor; PMN, polymorphonuclear leukocytes.

lacking the CD11/CD18 integrins is marked by poor or absent accumulation of PMNs at sites of bacterial infections. Stimulated PMNs from such patients fail to adhere to unactivated EC and to certain matrix proteins in vitro (3, 17–20). mAbs directed against the common β chain of the CD11/CD18 completely inhibit adherence of stimulated PMNs from normal donors to unactivated EC monolayers and to certain matrix proteins in vitro (3, 4, 6), and systemic administration of anti-CD11/CD18 antibodies to experimental animals inhibits tissue accumulation of PMNs in response to chemoattractants (21, 22). These observations have demonstrated the importance of CD11/CD18-dependent PMN-adhesive interactions in the directed migration of PMNs into sites of inflammation. However, PMNs can also adhere in vitro to certain surfaces, notably thrombin, LPS, or cytokine-stimulated EC monolayers, by mechanisms that do not require CD11/CD18 integrins (4, 5, 23). The contribution of such CD11/CD18-independent PMN-adhesive interactions to PMN recruitment into inflammatory sites is still being characterized.

In the experiments reported here, we have investigated the receptors involved in stimulated PMN adherence to several extracellular matrix molecules in vitro. We have found that the adherence of stimulated PMNs to the majority of protein-coated surfaces tested can be totally inhibited by the anti-CD18 mAb, IB4. In contrast, stimulated PMN adherence to laminin, the major glycoprotein of mammalian basement membrane, was only partially inhibited by mAb IB4. This CD11/CD18-independent adherence appears to involve a distinct PMN adherence receptor for laminin. These observations are important because interaction of phagocytes with laminin and other interstitial proteins may alter their functional state (24–26), in addition to influencing directed migration.

Materials and Methods

Buffers and Reagents. Human serum albumin for infusion (HSA; Cutter Biologicals, Berkeley, CA), recombinant human C5a (C5a), FMLP, PMA (Sigma Chemical Co., St. Louis, MO), platelet activating factor (PAF; Avanti Polar Lipids, Inc., Birmingham, AL), gelatin (Type II from swine skin; Fisher Scientific Co., Pittsburgh, PA), and purified human plasma fibronectin (Collaborative Research, Lexington, MA) were purchased. Recombinant human TNF-α was a gift from Cetus Corp. (Emeryville, CA). Murine laminin and collagen IV purified from the EHS sarcoma, and DPGYIGSR peptide, were gifts of Dr. Hynda Kleinman (National Institute of Dental Research, Bethesda, MD). Synthetic GRGDSP peptide was purchased (Telios Pharmaceuticals, La Jolla, CA). HBSS and HBSS containing 1.2 mM Ca^{2+} and 0.8 mM Mg^{2+} ($HBSS^{2+}$) were purchased from M.A. Bioproducts (Walkersville, MD).

Antibodies. Murine mAb IB4 (IgG2a), which binds to the β chain (CD18, β_2 of the integrin family) of the CD11/CD18 (27), was a gift from Dr. Samuel Wright (Rockefeller University, NY). Murine mAb B6H12 (IgG1), which recognizes the RGDS-binding leukocyte response integrin (25) was a gift from Dr. Eric Brown (Washington University, St. Louis, MO). Murine mAb W6/32 (IgG2a), which recognizes a common determinant on the HLA class I molecule (28); rat mAb M1/70 (IgG2b), which recognizes the α_2 chain of the CD11/CD18 (CD11b, Mac-1, or CR3; 15); and rat mAb R4-6A2 (IgG1), directed against murine IFN-γ (29), were obtained from the American Type Culture Collection (Rockville, MD). AIIB2 and BIVF2 are rat mAbs raised against the JAR choriocarcinoma cell line (30). AIIB2 (IgG1) and mAb 13 (rat IgG2a; 31) bind to the β subunit (β_1) of the VLA integrins. BIVF2 is a control mAb (IgG2a) that binds to the JAR cell but does not recognize an integrin. The various mAbs used in these experiments are summarized in Table I.

IB4 was purified from ascites using protein A-Sepharose. W6/32 and B6H12 were purified from ascites by octanoic acid precipitation (32). M1/70, AIIB2, and BIVF2 were used as tissue culture supernatants. R4-6A2 was partially purified by $(NH_4)_2SO_4$ precipitation from

tissue culture supernatant, while mAb 13 was purified from tissue culture supernatant by $(NH_4)_2SO_4$ precipitation and ion exchange chromatography.

Subclass determination of AIIB2 and BIVF2 was performed with a commercially available kit (The Binding Site, San Diego, CA). The concentrations of mAbs in tissue culture supernatant were determined by ELISA, using rat IgG (Calbiochem-Behring Corp., La Jolla, CA) as a standard.

Rabbit polyclonal antiserum against the 67-kD laminin receptor was a gift from Dr. Tomeo Sequi-Real (National Institute of Dental Research). Rabbit antibody against laminin, and $F(ab')_2$ fragments of the IgG fraction, were prepared as previously described (24).

EC Monolayers and Subendothelial Matrix. Confluent primary monolayers of human umbilical vein EC were prepared as previously described in 24-mm tissue culture plastic wells (4, 33, 34). Subendothelial matrices were prepared by lysis of the overlying endothelial monolayers with 0.5% Triton X-100 for 30 min at 37°C, followed by treatment with 0.1 M NH_4OH for 10 min at room temperature (34). The subendothelial matrices were then washed copiously with HBSS before use in the adherence assay.

PMN Adherence Assay. The method for determining PMN adherence to protein-coated plastic wells and to endothelial cell monolayers has been previously described in detail (4, 33, 34). PMNs were obtained from healthy adult donors, or from a patient with leukocyte adhesion deficiency (CD11/CD18 deficiency). The clinical and laboratory characteristics of the patient with CD11/CD18 deficiency will be the subject of a more complete report (Shigeoka, A. O., and J. F. Bohnsack, manuscript in preparation). Briefly, this female patient's clinical course has been characterized by recurrent cutaneous infections with no pus formation and a peripheral blood leukocytosis since her first several months of life. PMNs from this patient completely lack detectable CD11/CD18 as determined by using flow cytometry or by immunoprecipitation, using mAb IB4 to detect the β chain of the CD11/CD18 complex. PMNs were isolated from heparinized blood by dextran sedimentation, hypotonic lysis, and Ficoll-Hypaque density gradient centrifugation, as previously described (4, 33, 34). Purified PMNs were suspended in $HBSS^{2+}$ containing 0.5% HSA ($HBSS^{2+}$/HSA) and labeled with ^{111}Indium oxine (^{111}In) by incubation with the ^{111}In for 5 min at 37°C, followed by washing to remove free ^{111}In. ^{111}In-labeled PMNs were suspended to 5.5×10^6/ml in $HBSS^{2+}$/HSA. For adherence to protein-coated substrates, 16-mm plastic tissue culture wells (Nunc, Roskilde, Denmark) were coated with purified protein by incubation of 500 μl of protein diluted in HBSS for at least 2 h at 37°C. The wells were then aspirated and washed once with HBSS just before the adherence assay. Preliminary studies revealed that PMNs adhere readily to uncoated tissue culture plastic wells, likely by a charge-induced mechanism. This high basal adherence was inhibited in a concentration-dependent manner by precoating the wells with a variety of proteins. Maximal inhibition of basal adherence occurred when wells were precoated with proteins at 50 μg/ml. All proteins were therefore used at 50 μg/ml to coat the wells, and basal adherence was found to be approximately equal to all protein-coated wells used in this report (see Results). Adherence to endothelial cell monolayers and subendothelial matrices was determined in 24-mm diameter wells prepared as described above.

PMNs were preincubated with mAb for 10 min at room temperature before addition to the wells. mAbs were present throughout the course of the incubation. ^{111}In-PMNs (225 μl) were added to 16-mm wells and 450 μl of ^{111}In-PMNs to 24-mm wells, followed by addition of 25 or 50 μl, respectively, of the different agonists. The PMNs were then allowed to adhere for various lengths of time at 37°C. At the end of the incubation interval, nonadherent cells were removed by gentle aspiration and one wash with 0.5 ml HBSS. Nonadherent cells were saved and counted. Counts associated with adherent cells were recovered by two cycles of lysis with 1 M NH_4OH and vigorous scraping with a cotton tip applicator. The percentage of adherent cells was calculated as a percentage of the total number of counts added to each well.

In experiments to determine the requirements for divalent cations for PMN adherence, PMNs prepared in $HBSS^{2+}$/HSA were pelleted by centrifugation and resuspended in HBSS/HSA containing either EGTA, $CaCl_2$, or $MgCl_2$ at a final concentration of 1 mM.

In experiments to determine the receptor responsible for CD11/CD18-independent adherence, ^{111}In-PMNs isolated from normals were incubated at 1.1×10^6/ml, with mAb IB4 at 50 μg/ml to maximally inhibit CD11/CD18-dependent adherence. PMA-stimulated adherence to laminin in the presence of IB4 was compared in paired experiments to PMA-stimulated

adherence to laminin in the presence of IB4 and various mAbs, and expressed as the percent maximal adherence, calculated as follows: percent maximal adherence = 100× [(PMA + IB4) − (PMA + IB4 + mAb)]/[(PMA + IB4) − (BASAL)]; where (PMA + IB4), (PMA + IB4 + mAb), and (BASAL) represent the percent adherence under each of these conditions.

Immunoprecipitation of ^{125}I Surface-labeled PMNs. Purified human PMNs were washed four times in PBS, pH 7.4. PMNs (1–2 × 10^8) were labeled with 2 mCi of $Na^{125}I$ for 15 min using 250 μg of chloroglycoluril (35; Sigma Chemical Co.), washed four times with PBS containing 2 mM KI, and solubilized in 0.01 M Tris, 0.145 M NaCl, pH 7.4, containing 0.5 mM EDTA, 25 μM nitroparaguanidinobenzoate, 2 μg/ml aprotonin, and 2 μg/ml leupeptin (lysis buffer) for 30 min at 37°C. After the solubilization, the lysates were centrifuged for 10 min in a microfuge to remove insoluble material, and precleared successively with Sepharose 4BCL and protein A-Sepharose. Aliquots of precleared cell lysates were mixed with AIIB2 (1 μg), mAb 13 (5 μg), or BIVF2 (1 μg) for 1 h at 4°C. Immune complexes were precipitated with 50 μl of goat anti-rat IgG agarose (Sigma Chemical Co.). After mixing for 2 h at 4°C, the precipitates were washed exhaustively with lysis buffer, and the pellets boiled in sample buffer for SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli (36) using an 8% resolving gel and 4% stacking gel. Reduced samples were treated with 2% 2-ME.

Flow Cytometry. Purified PMNs (10^6 in 0.5 ml of HBSS/HSA) were incubated with mAbs for 30 min on ice, washed twice, and then incubated with fluorescein-labeled goat anti-rat antibody for an additional 30 min on ice. The cells were then washed, fixed with 2% paraformaldehyde in 0.01 M PO_4, 0.145 M NaCl, pH 7.4, and analyzed on a Cytofluorograph II (Ortho Diagnostic Systems Inc., Westwood, MA).

Statistical Analysis. Significance was determined using student's *t*-test for paired and unpaired samples.

Results

Phorbol-stimulated PMNs Adhere to Matrix Proteins. We previously demonstrated that stimulated human PMNs adhere in an enhanced fashion to complex subendothelial matrices (14, 34). To examine the role of specific proteins in this interaction, we assayed the adhesion of radiolabeled PMNs to matrices of laminin, collagen IV, fibronectin, and gelatin, as well as to complex subendothelial matrices and EC monolayers (Fig. 1). There was very low adhesion of unstimulated PMNs to each surface ("basal adhesion"). This demonstrates that unstimulated PMNs from the peripheral circulation do not avidly adhere to surfaces, and that exposure of unstimulated PMNs to these proteins or to unstimulated EC does not alone significantly increase PMN adherence. When PMNs were stimulated with PMA, there was a dramatic (4–10-fold) increase in adhesion; enhanced adhesion occurred when laminin, collagen IV,

TABLE I
mAbs Used in this Study

mAb	Species	Subclass	Antigen
IB4	Mouse	IgG2a	β_2 (CD18)
W6/32	Mouse	IgG2a	HLA class I
B6H12	Mouse	IgG1	PMN LRI
AIIB2	Rat	IgG1	β_1 of VLA
mAb 13	Rat	IgG2a	β_1 of VLA
BIVF2	Rat	IgG2a	JAR cells
R4-6A2	Rat	IgG1	Murine IFN-γ
M1/70	Rat	IgG2b	CD11b

All target antigens are human unless otherwise specified.

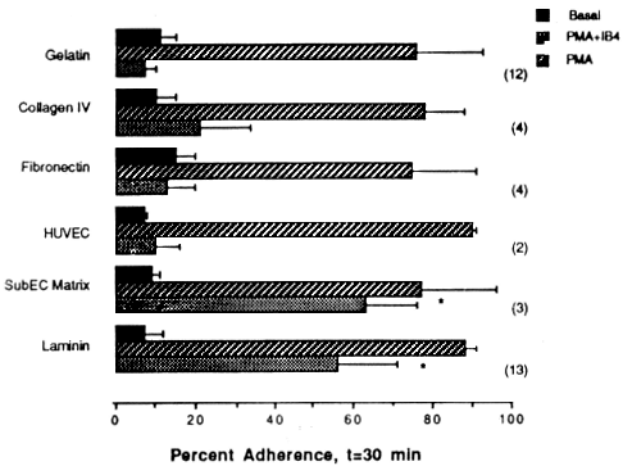

FIGURE 1. Inhibition of adherence of PMA-stimulated PMNs to various substrates by the anti-CD18 mAb, IB4. Tissue culture wells were coated with the various purified proteins at 50 μg/ml. Monolayers of human umbilical vein endothelial cells (HUVEC) and subendothelial matrix (SubEC matrix) were prepared as described in Materials and Methods. PMA (10 ng/ml) stimulated adherence to all substrates to approximately the same extent after a 30-min incubation. mAb IB4 completely inhibited adherence of PMNs to all substrates except SubEC matrix and laminin. (*) $p < 0.01$ compared with basal adherence to the same substrate. Percent adherence is the percentage of PMNs adherent after a 30-min incubation relative to the total number added per well. Data for each substrate are the means ± SD. The number of determinations for each substrate is indicated in parentheses.

or fibronectin was the substrate, as well as with complex subendothelial matrices. This result suggests that one or more of these proteins, which are major components of the subendothelium (37), is a "target" for adhesive molecules on the surface of stimulated PMNs. To examine the mechanism of recognition of the proteins by activated PMNs, we determined the effect of the anti-CD18 mAb, IB4, on stimulated adherence of PMNs to these surfaces, since we previously showed that PMNs stimulated with chemotactic factors adhere to a variety of proteins by a CD11/CD18-dependent mechanism (4, 14). IB4 prevented enhanced PMA-stimulated PMN adherence to fibronectin, gelatin, and collagen IV, as well as to EC (Fig. 1). An isotype-matched control mAb, W6/32, had no effect on stimulated PMN adherence (not shown). This result supports previous observations that indicate that CD11/CD18 glycoproteins can interact with a variety of soluble and cell surface–associated ligands (3, 4, 38–42). In contrast, the percentage of PMA-stimulated PMNs adhering to subendothelial matrices was reduced by only 35% by IB4 (Fig. 1). Furthermore, there was IB4-resistant adherence of PMA-stimulated PMNs to laminin (Fig. 1), suggesting that laminin confers this property on the subendothelial matrix. Therefore, we further characterized the mechanisms of stimulated PMN adherence to laminin.

Laminin Is Recognized by Stimulated PMNs. A trivial explanation for the IB4-resistant binding was that laminin did not coat the plastic surface evenly, allowing PMNs to bind to exposed plastic (see Materials and Methods). Phase-contrast microscopy demonstrated, however, that stimulated PMNs were uniformly distributed over the laminin-coated wells, arguing against this possibility. Furthermore, $F(ab')_2$ fragments of polyclonal IgG antibody directed against laminin completely abolished the IB4-resistant adherence of PMA-stimulated PMNs to wells coated with laminin, whereas $F(ab')_2$ fragments of preimmune IgG did not affect the IB4-resistant adherence (not shown). In a second strategy, we prepared composite matrices that con-

tained both laminin and collagen IV. In these experiments, tissue culture wells were first coated with collagen IV, washed, and then incubated with either laminin or buffer. After an additional incubation, the wells were again washed and the adherence of PMA-stimulated PMNs was examined in the presence or absence of IB4. Laminin binds specifically to collagen IV (43), and laminin bound to the collagen IV–coated wells supported IB4-resistant adherence of PMA-stimulated PMNs (Fig. 2). These data demonstrate that the IB4-resistant adherence of the stimulated PMNs resulted from an interaction of the PMNs with the laminin molecule itself.

Stimulated PMNs Adhere to Laminin by CD11/CD18-dependent and CD11/CD18-independent Mechanisms. PMA-stimulated PMN adherence to laminin was time dependent, with an onset within 5 min and maximal adherence at 30–60 min (Fig. 3). IB4-resistant adherence was present at 5 min and increased with time (Fig. 3). Increasing the concentration of IB4 from 10 to 50 μg/ml did not abolish PMA-stimulated PMN adherence to laminin (from 56 ± 8 to 46 ± 15% with 10 and 50 μg/ml of IB4, respectively). Preincubating the PMNs with IB4 at 30 μg/ml at 37°C with constant agitation for 90 min also did not lead to a further reduction in PMA-stimulated PMN adherence to laminin when compared with PMA-stimulated adherence of PMNs not preincubated with IB4 (Table II). In the latter experiments, we followed the protocol that Nathan et al. (44) used to demonstrate that the massive respiratory burst produced by TNF-stimulated PMNs adherent to extracellular matrix proteins is CD11/CD18 dependent. These data suggest that the IB4-resistant PMN adherence to laminin did not merely reflect a higher affinity of laminin for the CD11/CD18.

To examine this issue in a different way, we measured the stimulated adherence of PMNs from a patient with leukocyte adhesion deficiency. These PMNs, which completely lack CD11/CD18 on their surface either in the basal state or after treatment with PMA (as measured by analysis with flow cytometry using anti-CD11b and anti-CD18 mAb), were less adherent to laminin than were control PMNs (Table III). This observation further demonstrates that the CD11/CD18 contributes to PMN

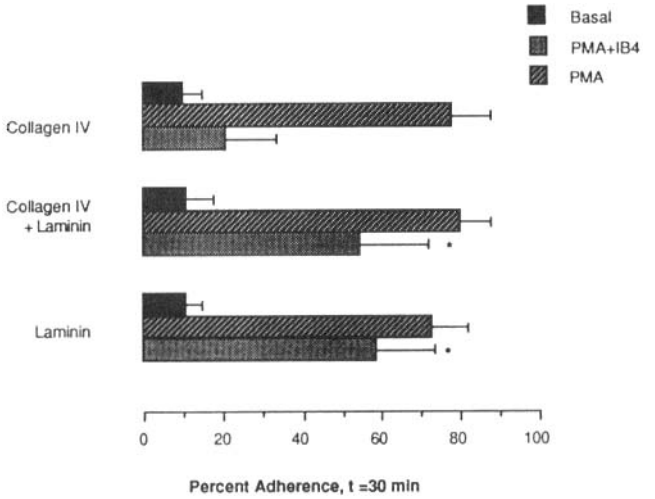

FIGURE 2. Laminin bound to collagen IV supports IB4-resistant adherence. Tissue culture wells were coated with collagen IV (50 μg/ml), then washed, and either laminin or buffer was added. After an additional incubation, the plates were washed and adherence tested in the presence or absence of PMA (10 ng/ml) and mAb IB4 (10 μg/ml). Data are the means ± SD from four separate experiments. (*) $p < 0.01$, compared with basal adherence to the same substrate.

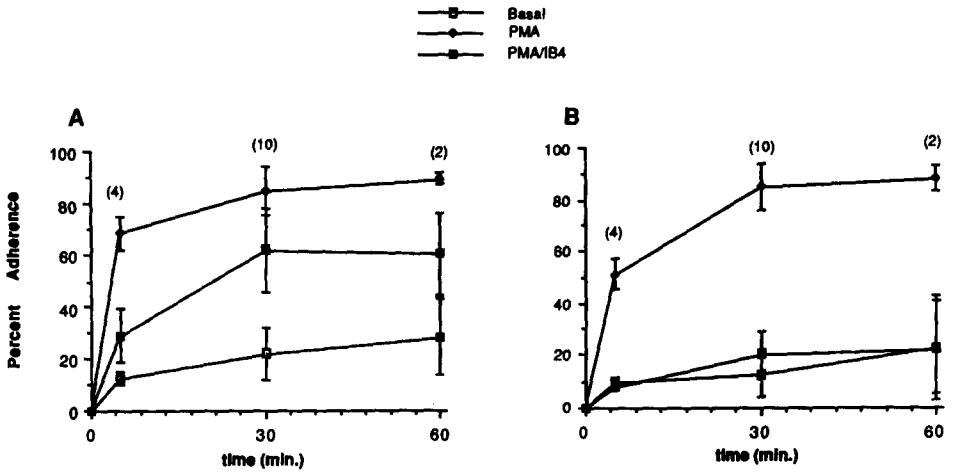

FIGURE 3. The expression of IB4-resistant adherence to laminin is time dependent. PMNs were tested for adherence to (*A*) laminin or (*B*) gelatin at different times after addition of PMA (10 ng/ml), in the presence or absence of IB4 at 10 μg/ml. IB4-resistant adherence was detectable as early as 5 min after addition of PMA in laminin-coated wells and persisted for at least 60 min. PMA-stimulated adherence to gelatin at each time point was always completely inhibited by IB4. Data are means ± SD. The number of separate experiments for each time point is indicated in parentheses.

adherence to laminin. However, there was a three to fourfold increase in adhesion of PMA-stimulated PMNs from the CD11/CD18-deficient patient to laminin, whereas there was no increased adhesion to gelatin or EC (Table III). These data demonstrate that PMA-stimulated PMNs adhere to laminin by a mechanism that does not require expression or upregulation of the CD11/CD18.

The Expression of CD11/CD18-independent PMN Adhesion to Laminin Is Agonist Specific and Dependent on the Presence of Divalent Cations. Several other agonists were examined for their ability to stimulate CD11/CD18-independent adherence to laminin. As shown in Fig. 4, FMLP, PAF, and recombinant human C5a all stimulated PMN adherence to laminin. This adherence was completely CD11/CD18 dependent when assayed after a 30-min incubation. In contrast, calcium ionophore A23187 and TNF-α

TABLE II
Preincubation of PMNs with IB4 Does not Abolish PMN Adherence to Laminin

Agonist	No preincubation	90-min preincubation
Basal	9 ± 2	6 ± 1
PMA (10 ng/ml)	88 ± 1	77 ± 1
PMA + IB4	64 ± 1	56 ± 3

PMNs were preincubated alone or with IB4 at 30 μg/ml for 0 or 90 min at 37°C with constant agitation, before determining adherence to laminin in the absence (basal) or presence of PMA at 10 ng/ml. Data are the means and SDs of one experiment performed in triplicate.

TABLE III
Stimulated PMNs from a CD11/CD18-deficient Patient Adhere to Laminin

Subject	Percent adherence		
	Laminin	Gelatin	EC monolayers
Patient			
Basal	9 ± 4 (3)	8 ± 1 (3)	7.8 (1)
Stimulated	28 ± 2 (3)	6 ± 1 (3)	3.0 (1)
Normal			
Basal	21 ± 3 (3)	12 ± 0 (3)	9.4 (1)
Stimulated	94 ± 1 (3)	92 ± 0 (3)	74.1 (1)

Adherence of PMNs from a patient with CD11/CD18 deficiency or from a normal control subject to laminin, gelatin, or EC monolayers was compared. PMNs were either allowed to adhere in the absence of any stimulus (basal) for 30 min or in the presence of PMA (laminin and gelatin), or phorbol dibutyrate (EC monolayers) at 10 ng/ml. Data are the means and SDs from one experiment. The numbers in parentheses are the numbers of replicates in this experiment. Data for adherence to laminin and gelatin are representative of three separate experiments. Adherence to EC monolayers was performed once.

both stimulated PMN adherence to laminin in a CD11/CD18-independent manner, but not to the same extent as did PMA (Fig. 4). Thus, an agonist important in inflammatory tissue injury, TNF-α (45), as well as pharmacologic agonists (PMA, calcium ionophore), induced CD11/CD18-independent PMN adhesion to laminin, whereas all the chemotactic factors induced adhesion that was completely mediated by the CD11/CD18.

Resuspending the PMNs in buffer without added divalent cations completely inhibited PMA-stimulated adherence to gelatin but not to laminin (Fig. 5). This adherence to laminin was completely CD11/CD18 dependent (inhibited by IB4), and was probably supported by the small amount of divalent cation remaining in the buffer since chelation of divalent cations with EGTA completely abolished the ad-

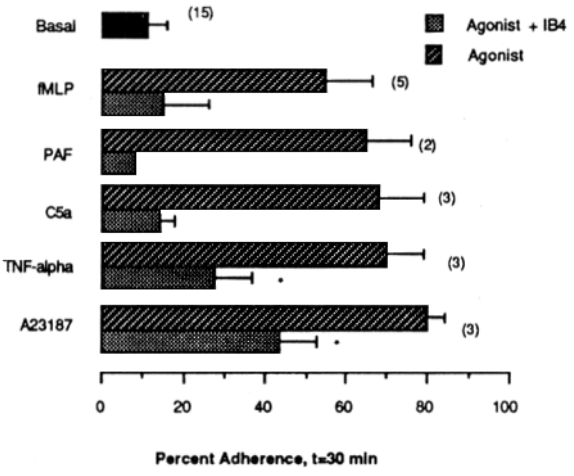

FIGURE 4. The expression of IB4-resistant PMN adherence to laminin is agonist dependent. The ability of IB4 (10 μg/ml) to inhibit adherence of PMNs stimulated with various agonists was tested on laminin substrates. IB4 completely inhibited adherence of PMNs to laminin stimulated with FMLP at 10^{-6} M, PAF at 10^{-6} M, or recombinant human C5a at 100 ng/ml, but did not completely inhibit adherence stimulated by recombinant TNF-α at 10^{-9} M, or calcium ionophore A23187 at 500 ng/ml. IB4 completely inhibited PMN adherence to gelatin for every agonist tested. The number of experiments performed for each separate experiment is indicated in parentheses. (*) $p < 0.01$ compared with basal adherence to laminin.

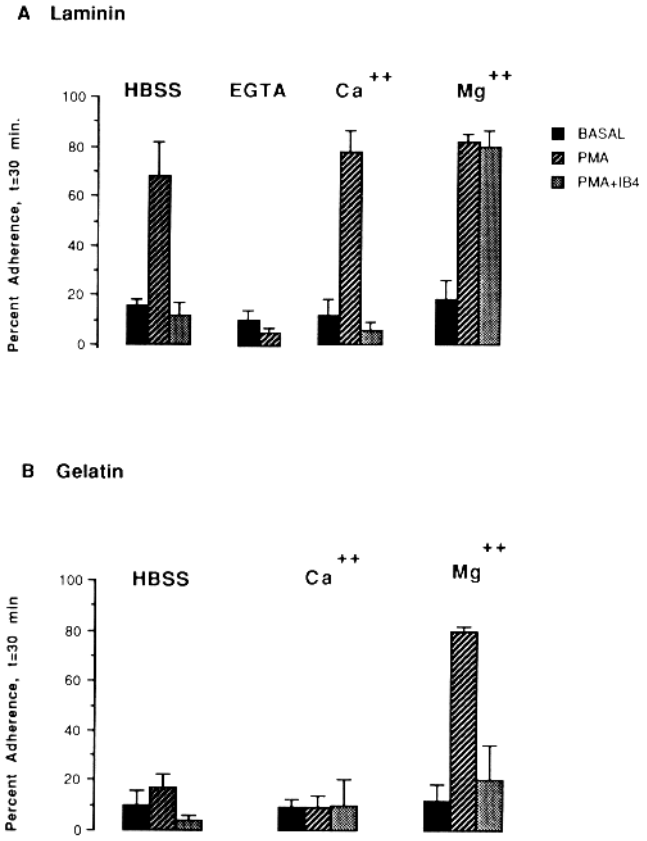

FIGURE 5. CD11/CD18-independent adherence to laminin is divalent cation dependent. ^{111}In PMNs were pelleted and resuspended in HBSS/HSA (HBSS without added divalent cations) or in HBSS/HSA containing 1 mM EGTA, 1 mM Ca^{2+}, or 1 mM Mg^{2+}. Adherence of PMNs to laminin or to gelatin was determined after 30 min in the presence or absence of PMA at 10 ng/ml.

herence of PMA-stimulated PMNs to laminin (Fig. 5). The addition of 1 mM Mg^{2+}, but not 1 mM Ca^{2+}, restored PMN adherence to gelatin. This concentration of Mg^{2+} also supported CD11/CD18-dependent adherence of PMNs to laminin, whereas CD11/CD18-independent adherence did not occur in the presence of 1 mM Ca^{2+} alone (Fig. 5). These data demonstrate that CD11/CD18-independent adherence to laminin is Mg^{2+} dependent.

CD11/CD18-independent PMN Adherence to Laminin Is Inhibited by Anti-VLA mAbs. We next attempted to determine what alternative adherence receptor mediates CD11/CD18-independent PMN adherence to laminin. We found that neither polyclonal antibody directed against the 67-kD laminin receptor present in PMN granules (46), nor DPGYIGSR peptide, a sequence in laminin that binds to the 67-kD receptor (47), inhibited CD11/CD18-independent adherence (not shown). We then considered members of the β-1 integrin family, since at least three of these,VLA-2, VLA-3, and VLA-6, have been shown to mediate cell adhesion to laminin (48–50). mAb AIIB2 and mAb 13, which bind to the common β_1 subunit of the VLA family of integrins, significantly inhibited adherence of normal PMNs to laminin when the mAb IB4 was used to block CD11/CD18-dependent adherence (Fig. 6). In experiments similar to those shown in Fig. 6, mAb 13 inhibited CD11/CD18-independent

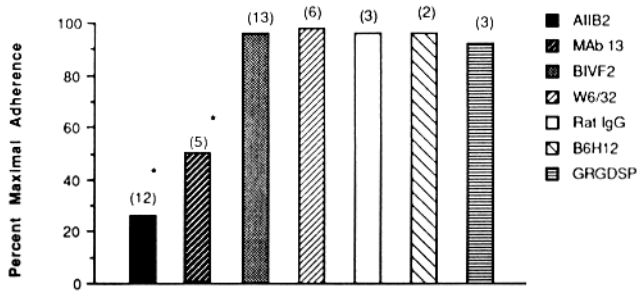

FIGURE 6. Anti-β_1 mAbs significantly inhibit CD11/CD18-independent adherence to laminin. Adherence of PMNs to laminin after 30 min in the presence of PMA at 10 ng/ml and IB4 at 50 μg/ml was compared with adherence of PMA-stimulated PMNs to laminin in the presence of IB4 and various mAbs, rat IgG, or peptide GRGDSP. Final concentrations of AIIB2 and BIVF2 were 2 μg/ml, while mAb 13, W6/32, B6H12, and rat IgG were at 50 μg/ml. GRGDSP was used at a final concentration of 300 μg/ml. Numbers in parentheses indicate the number of separate experiments. (*) $p < 0.01$ compared with paired experiments with PMA + IB4 only.

adherence at concentrations as low as 2 μg/ml, but inhibition was most consistent at concentrations $\geq$10 μg/ml. In the absence of IB4, neither AIIB2 nor mAb 13 inhibited PMA-stimulated adherence of PMNs from normal donors to laminin (not shown). Polyclonal rat IgG, synthetic peptide GRGDSP, and mAbs BIVF2, W6/32, and B6H12 did not inhibit CD11/CD18-independent adherence (Fig. 6).

The effect of the anti-β_1 mAbs was most clearly observed when adherence of PMNs from the CD11/CD18-deficient patient was examined. PMA-stimulated adherence of PMNs to laminin in this non-CD11/CD18-mediated system was completely blocked by mAb AIIB2 (Table IV) and by mAb 13 (Fig. 7). Inhibition by mAb 13 was concentration dependent, with complete inhibition occurring at concentrations of mAb 13 $\geq$10 μg/ml (Fig. 7). The control mAbs BIVF2 and W6/32 did not inhibit adherence of CD11/CD18-deficient PMNs to laminin (Table IV and Fig. 7).

mAb AIIB2 Recognizes a Receptor on the Surface of PMNs. We determined whether AIIB2 and mAb 13 recognize an antigen on PMNs, since it has previously been reported that PMNs do not possess VLA receptors on their surface (51). Both mAb AIIB2 and mAb 13 immunoprecipitated a protein from ^{125}I surface-labeled PMNs. Under both reducing and nonreducing conditions, the immunoprecipitate migrated on SDS-PAGE as a broad band with an M_r of ~125,000–135,000 daltons (Fig. 8).

TABLE IV

AIIB2 Inhibits Stimulated Adherence of CD11/CD18-deficient PMNs to Laminin

	Percent adherence		
Agonist	No mAb	AIIB2	BIVF2
Basal	6 ± 2	ND	ND
PMA	25 ± 1	5 ± 1	23 ± 4

Adherence of PMNs from a CD11/CD18-deficient patient to laminin in the presence or absence of PMA at 10 ng/ml was determined after a 30-min incubation as described in Materials and Methods. PMNs were preincubated with tissue culture supernatant containing mAb AIIB2 or BIVF2 for 10 min before and throughout the course of the adherence assay at a final concentration of 2 μg/ml. Data are the means ± SDs from one experiment performed in quadruplicate, representative of two experiments.

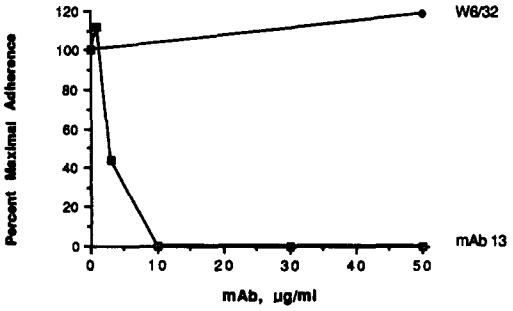

FIGURE 7. Anti-β_1 mAb 13 inhibits adherence of PMA-stimulated CD11/CD18-deficient PMNs to laminin. mAb 13 or W6/32 was incubated with PMNs from a CD11/CD18-deficient patient at the indicated concentrations, and adherence of the PMNs to laminin was determined after 30 min in the presence of PMA at 10 ng/ml.

This band was clearly distinguishable from the VLA antigens (VLA-2, VLA-5, and VLA-6; 52) present on platelets, which appeared as two bands at 135,000–140,000 and 115,000–120,000 daltons (Fig. 8). In addition, experiments with flow cytometry indicate that both mAbs 13 and AIIB2 specifically bind to PMNs (Fig. 9). These data demonstrate that the anti-β_1 mAbs both identify a surface protein on PMNs and block PMN adhesion to laminin, indicating that human PMNs possess a surface receptor, distinct from the CD11/CD18, that mediates adherence to this basement membrane protein. This distinct PMN adherence receptor appears to be a member of the β_1 family of integrins.

Discussion

These experiments demonstrate that adherence of stimulated human PMNs to laminin involves the CD11/CD18 glycoprotein complex, since the anti-CD18 mAb, IB4, decreased agonist-stimulated PMN adherence to laminin. However, laminin possesses a unique property as an adherence substrate for stimulated PMNs, since

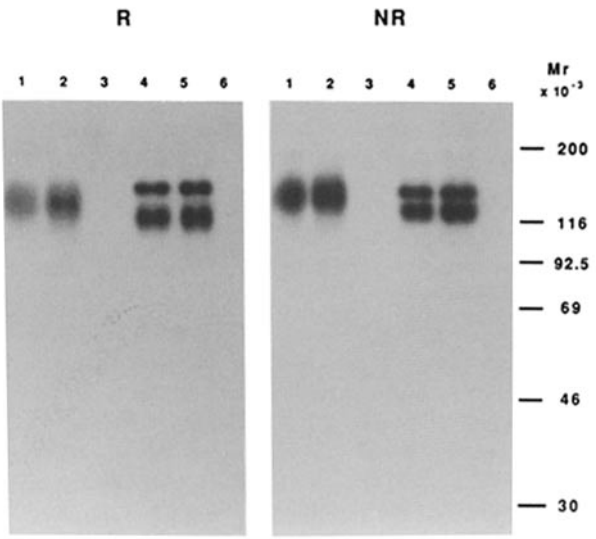

FIGURE 8. Autoradiography of 8% SDS-PAGE analysis of immunoprecipitated ^{125}I surface-labeled PMNs and platelets. (Lanes *1–3*) PMNs; (lanes *4–6*) platelets. (Lanes *1* and *4*) AIIB2; (lanes *2* and *5*) mAb 13; (lanes *3* and *6*) BIVF2. R, samples reduced with 2% 2-ME; NR, nonreduced samples. PMNs were not stimulated before being labeled and solubilized with detergent.

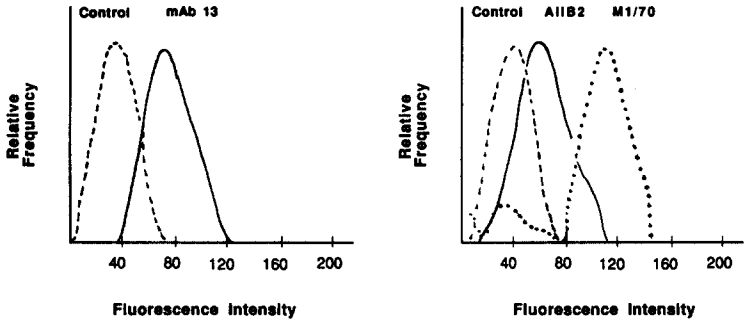

FIGURE 9. Flow cytometry of PMNs from normal donors stained with anti-β_1 mAb 13 (3 μg) or AIIB2 (1 μg), anti-CD11b mAb M1/70 (12 μg), or the control mAbs BIVF2 (3 μg; *left*) or R4-6A2 (1 μg; *right*). See Materials and Methods for details of experimental procedures. Fluorescence intensity is expressed on a three-decade log scale.

adherence of PMA-stimulated PMNs to laminin, and laminin-containing subendothelial matrix, was only partially inhibited by mAb IB4, whereas PMA-stimulated PMN adherence to all other surfaces tested was completely inhibited by IB4.

There were at least two possible explanations for the difference between PMA-stimulated PMN adherence to laminin and adherence to the other substrates examined. One possibility was that CD11/CD18 mediates both the IB4-sensitive and IB4-resistant adherence to laminin, but that the affinity of CD11/CD18 for laminin was greater than for the other ligands, such as gelatin or fibronectin. This explanation seems unlikely since even very high concentrations of IB4 and prolonged preincubation of PMNs with IB4 at 37°C failed to inhibit the PMA-stimulated PMN adherence to laminin. In addition, PMA induced PMNs from a CD11/CD18-deficient patient to adhere to laminin-coated substrates, but not to gelatin or endothelial cell monolayers, documenting that adhesion to laminin occurs in the absence of leukocyte β_2 integrins.

The alternative explanation, therefore, is that there is a separate PMN receptor for laminin. A member of the VLA integrin receptor family would be a good candidate to be such a PMN laminin receptor since several members of the VLA family, VLA-2, VLA-3, and VLA-6, have been reported to mediate adherence of connective tissue cells and platelets to laminin substrates (48–50). Furthermore, integrins require divalent cations for ligand binding (16), and CD11/CD18-independent adherence to laminin requires the presence of the divalent cation Mg^{2+} for expression (Fig. 5). Indeed, we found that mAbs 13 and AIIB2, which recognize the common β_1 subunit of the VLA integrins, significantly inhibited the CD11/CD18-independent adherence of PMA-stimulated PMNs to laminin.

It has previously been reported that VLA receptors are not present on mature PMNs, since flow cytometry experiments did not detect binding of the anti-β_1 mAb A1A5 to PMNs (51). By contrast, our flow cytometry experiments indicate that mAbs 13 and AIIB2 do bind to PMNs, although the observed fluorescence intensity indicates that the antigen recognized by mAbs 13 and AIIB2 is present on the surface of unstimulated PMNs at low density. It is interesting to note that both mAbs 13 and AIIB2 differ from A1A5 in that both mAb AIIB2 and mAb 13 block adherence

of cells to several extracellular matrix molecules (31, 53), suggesting that these mAbs have a different affinity for the VLA β chain, or that they bind to a separate epitope on the β_1 chain than mAb A1A5. We also found that these anti-β_1 mAbs immunoprecipitate a protein from surface-labeled PMNs. Interestingly, this precipitate does not contain a band that migrates like the β_1 chain immunoprecipitated from surface-labeled platelets. There are several possible explanations for the discrepancy. One is that the mAbs crossreact with a non-VLA molecule on PMN cell surface membranes. If so, our functional data indicate that this molecule mediates cell binding to laminin, identifying it as a novel receptor for matrix proteins. Another possibility is that the β chain is poorly labeled on PMNs by the method we have used, and only the α chain is recognizable on the autoradiograms. Finally, the VLA β chain from PMNs may have a different M_r from that in platelets, due, for example, to differences in glycosylation. If so, the broad band seen in Fig. 8 may be the β chain, or a closely spaced dimer of α and β chains. These possibilities are currently under investigation.

These observations clearly demonstrate that PMNs have at least two separate mechanisms by which they adhere to laminin, one that is CD11/CD18 dependent and one that is mediated by an independent laminin receptor that has similarity to a VLA β_1 integrin. These findings confirm that PMNs can adhere by CD11/CD18-independent mechanisms (4, 5, 23). It is not clear what contribution such CD11/CD18-independent adherence makes to PMN recruitment into sites of inflammation. The inability of PMNs from CD11/CD18-deficient patients to accumulate at sites of infection suggests that the CD11/CD18 is a dominant and critical receptor involved in PMN adherence and migration in vivo. However, PMNs may utilize regulated expression of different surface receptors to allow controlled adhesion to different structures (endothelial cells, connective tissue proteins, epithelial cells of the airway, peritoneum, etc.) that are encountered as they migrate to extravascular sites. The surface molecule that we describe may be such a receptor. In addition, distinct PMN receptors for extracellular matrix proteins, such as the PMN receptor for laminin described here or the recently described PMN RGDS integrin (25), may allow the PMN to discriminately recognize its extravascular environment, and alter its functional state accordingly (24–26).

Summary

We used mAbs against polymorphonuclear leukocyte (PMN) surface proteins to investigate the mechanisms by which stimulated human neutrophils (PMNs) adhere in vitro to laminin, the major glycoprotein of mammalian basement membrane. mAb IB4, which is directed against the common β_2 chain of the CD11/CD18, only partially inhibited the adherence of PMA-stimulated PMNs to both laminin and to subendothelial matrices. In contrast, IB4 completely inhibited PMA-stimulated PMN adherence to gelatin, fibronectin, collagen IV, and endothelial cell monolayers. PMA-stimulated PMNs from a patient with severe congenital CD11/CD18 deficiency also adhered to laminin, but not to gelatin or endothelial cell monolayers. Therefore, PMA-stimulated PMNs adhere to laminin by both CD11/CD18-dependent and CD11/CD18-independent mechanisms. Expression of CD11/CD18-independent adherence to laminin was agonist dependent, occurring after stimulation with the cal-

cium ionophore A23187 and recombinant TNF-α, but not with the chemotactic factors FMLP, platelet activating factor, or recombinant human C5a. Expression of CD11/CD18-independent adherence was also divalent cation dependent, occurring in the presence of Mg^{2+} but not Ca^{2+} as the sole added divalent cation. The mAbs AIIB2 and 13, which are directed against the β_1 subunit of the VLA integrins, significantly inhibited the CD11/CD18-independent adherence of normal PMNs to laminin, and completely abolished the adherence of CD11/CD18-deficient PMNs to laminin. Both anti-β_1 mAbs bound to PMNs, as demonstrated by flow cytometry, and immunoprecipitated a membrane molecule of M_r 130,000 daltons from ^{125}I-labeled, detergent-solubilized PMNs. These data suggest that human PMNs possess β_1 and β_2 classes of integrins, and that both mediate PMN adherence.

We thank the following who generously supplied reagents used in these studies: Dr. Samuel Wright for mAb IB4; Dr. Hynda Kleinman, for laminin, collagen IV, and DPGYIGSR peptide; Dr. Eric Brown for mAb B6H12; and Dr. Tomeo Sequi-Real for antibody against the 67-kD receptor. We also thank Drs. Thomas McIntyre, Charles Parker, and Eric Brown for many helpful comments. We express our appreciation to Holly Nichols, Becky Robertson, and Margaret Hawley for excellent technical assistance, and to Dr. Ann Shigeoka for assistance in obtaining PMNs from the CD11/CD18-deficient patient.

Received for publication 7 September 1989 and in revised form 18 December 1989.

References

1. Snyderman, R. and E. J. Goetzl. 1981. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science (Wash. DC).* 2131:830.
2. Gallin, J. I. 1980. The cell biology of leukocyte chemotaxis. *In* Cell Biology of Inflammation. G. Weissman, editor. Elsevier Science Publishers B. V., Amsterdam, The Netherlands. 299–335.
3. Harlan, J. M., P. D. Killen, F. M. Senecal, B. R. Schwartz, E. K. Yee, R. F. Taylor, P. G. Beatty, T. H. Price, and H. D. Ochs. 1985. The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium in vitro. *Blood.* 66:167.
4. Zimmerman, G. A., and T. M. McIntyre. 1988. Neutrophil adherence to human endothelium in vitro occurs by CDw18 (Mol, MAC-1/LFA-1/GP150,95) glycoprotein-dependent and -independent mechanisms. *J. Clin. Invest.* 81:531.
5. Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan. 1986. An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin-1, and tumor necrosis factor-alpha increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.* 136:4548.
6. Tonnesen, M. G., D. C. Anderson, T. A. Springer, A. Knedler, N. Avdi, and P. M. Henson. 1989. Adherence of neutrophils to cultured human microvascular endothelial cells. Stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. *J. Clin. Invest.* 83:637.
7. Bevilacqua, M. P., J. S. Pober, D. L. Mendrick, R. S. Cotran, and M. A. Gimbrone, Jr. 1987. Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 184:9238.
8. Lewinsohn, D. M., R. F. Bargatze, and E. C. Butcher. 1987. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes and other leukocytes. *J. Immunol.* 138:4313.
9. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. The lymphocyte function associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of

the immune system. *Annu. Rev. Immunol.* 5:223.
10. Dana, N., B. Styrt, J. D. Griffin, R. F. Todd III, M. S. Klempner, and M. A. Arnaout. 1986. Two functional domains in the phagocyte membrane glycoprotein Mo-1 identified with monoclonal antibodies. *J. Immunol.* 137:3259.
12. Wright, S. D., J. I. Weitz, A. J. Huang, S. M. Levin, S. C. Silverstein, and J. D. Loike. 1988. Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. *Proc. Natl. Acad. Sci. USA.* 85:7734.
13. Anderson, D. C., L. J. Miller, F. C. Schmalstieg, R. Rothlein, and T. A. Springer. 1986. Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessments employing subunit-specific monoclonal antibodies. *J. Immunol.* 137:15.
14. Carveth, H. J., J. F. Bohnsack, T. M. McIntyre, M. Baggiolini, S. M. Prescott, and G. A. Zimmerman. 1989. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cell and to subendothelial matrix proteins. *Biochem. Biophys. Res. Commun.* 162:387.
15. Sanchez-Madrid, F., J. A. Nagy, E. Robbins, P. Simon, and T. A. Springer. 1983. A human leukocyte differentiation antigen family with distinct α subunits and a common β subunit. The lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med.* 158:1785.
16. Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549.
17. Anderson, D. C., F. C. Schmalsteig, M. A. Arnaout, S. Kohl, M. F. Tosi, N. Dana, G. J. Buffone, B. J. Hughes, B. R. Brinkley, W. D. Dickey, J. S. Abramson, T. Springer, L. A. Boxer, J. M. Hollers, and C. W. Smith. 1984. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (gp138): a common relationship to diminished cell adherence. *J. Clin. Invest.* 74:536.
18. Buchanan, M. R., C. A. Crowley, M. A. Rosin, M. A. Gimbrone, Jr., and B. B. Babior. 1982. Studies on the interaction between gp-180-deficient neutrophils and vascular endothelium. *Blood.* 60:160.
19. Buescher, E. S., T. Gaither, J. Nath, and J. I. Gallin. 1985. Abnormal adherence-related functions of neutrophils, monocytes, and Epstein-Barr virus-transformed B cells in a patient with C3bi receptor deficiency. *Blood.* 65:1382.
20. Anderson, D. C., and T. A. Springer. 1987. Leukocyte adhesion deficiency: An inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu. Rev. Med.* 38:175.
21. Arfors, K. E., C. Lundberg, L. Lindblom, K. Lundberg, P. G. Beatty, and J. M. Harlan. 1987. A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. *Blood.* 60:338.
22. Price, T. H., P. G. Beatty, and S. R. Corpuz. 1987. In vivo inhibition of neutrophil function in the rabbit using monoclonal antibody to CD18. *J. Immunol.* 139:4174.
23. Luscinkas, F. W., A. F. Brock, M. A. Arnaout, and M. A. Gimbrone, Jr. 1988. Endothelial-leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J. Immunol.* 142:2257.
24. Bohnsack, J. F., H. K. Kleinman, T. Takahashi, J. J. O'Shea, and E. J. Brown. 1985. Connective tissue proteins and phagocytic cell function: laminin enhances complement and Fc-mediated phagocytosis by cultured human macrophages. *J. Exp. Med.* 161:912.
25. Gresham, H. D., J. L. Goodwin, P. M. Allen, D. C. Anderson, and E. J. Brown. 1989. A novel member of the integrin receptor family mediates Arg-Gly-Asp-stimulated neutrophil phagocytosis. *J. Cell Biol.* 108:1935.
26. Nathan, C. F. 1987. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J. Clin. Invest.* 80:1550.

27. Wright, S. D., P. E. Rao, W. C. VanVoorhis, L. S. Craigmyle, K. Iida, M. A. Talle, E. F. Westberg, G. Goldstein, and S. C. Silverstein. 1983. Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. *Proc. Natl. Acad. Sci. USA.* 80:5699.
28. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA, and other human cell surface antigens. *Cell.* 14:9.
29. Spitalny, G. L. and Havell, E. A. 1984. Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activities. *J. Exp. Med.* 159:1560.
30. Werb, Z., P. M. Tremble, O. Behrendtsen, E. Crowley, and C. H. Damsky. 1989. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J. Cell Biol.* 109:877.
31. Akiyama, S. K., S. S. Yamada, W. T. Chen, and K. M. Yamada. 1989. Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly and cytoskeletal organization. *J. Cell Biol.* 109:863.
32. Steinbuch, M., and R. Audran. 1969. The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch. Biochem. Biophys.* 134:279.
33. Zimmerman, G. A., T. M. McIntyre, and S. M. Prescott. 1985. Thrombin stimulates the adherence of neutrophils to human endothelial cells in vitro. *J. Clin. Invest.* 76:2235.
34. Zimmerman, G. A., T. M. McIntyre, and S. M. Prescott. 1986. Thrombin stimulates neutrophil adherence by an endothelial cell-dependent mechanism: characterization of the response and relationship to platelet-activating factor synthesis. *Ann. NY Acad. Sci.* 485:349.
35. Markwell, M. A K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril. *Biochemistry.* 17:4807.
36. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
37. Hay, E. D. Cell Biology of Extracellular Matrix. 1982. Plenum Publishing Corporation, NY. 417 pp.
38. Beller, D. I., T. A. Springer, and R. D. Schreiber. 1982. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med.* 156:1000.
39. Ross, G. D., J. A. Cain, and P. J. Lachmann. 1985. Membrane complement receptor type three (CR3), has lectin-like properties analogous to bovine conglutinin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. *J. Immunol.* 134:3307.
40. Wright, S. D., and M. T. C. Jong. 1986. Adhesion-promoting receptors on human macrophages recognize *Escherichia coli* by binding to lipopolysaccharide. *J. Exp. Med.* 164:1876.
41. Altieri, D. C., and T. S. Edgington. 1988. The saturable high affinity association of factor X to ADP-stimulated monocytes defines a novel function of the Mac-1 receptor. *J. Biol. Chem.* 263:7007.
42. Marlin, S. D., and T. A. Springer. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1) *Cell.* 51:813.
43. Woodley, D. T., C. N. Rao, J. R. Hassell, L. A. Liotta, and G. R. Martin. 1983. Interactions of basement membrane components. *Biochim. Biophys. Acta.* 761:278.

44. Nathan, C., S. Srimal, C. Farber, E. Sanchez, L. Kabbash, A. Asch, J. Gailit, and S. D. Wright. 1989. Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD11/CD18 integrins. *J. Cell Biol.* 109:1341.
45. Sherry, B., and A. Cerami. 1988. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. *J. Cell Biol.* 107:1269.
46. Yoon, P. S., L. A. Boxer, L. A. Mayo, A. Y. Yang, and M. S. Wicha. 1987. Human neutrophil laminin receptors: activation-dependent receptor expression. *J. Immunol.* 138:259.
47. Graf, J., R. C. Ogle, F. A. Robey, M. Sasaki, G. R. Martin, Y. Yamada, and H. K. Kleinman. 1987. A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67000 laminin receptor. *Biochemistry.* 26:6896.
48. Languino, L. R., K. R. Gehlsen, E. Wayner, W. G. Carter, E. Engvall, and E. Ruoslahti. 1989. Endothelial cells use alpha$_2$beta$_1$ integrin as a laminin receptor. *J. Cell Biol.* 109:2455.
49. Horwitz, A., K. Duggan, R. Greggs, C. Decker, and C. Buck. 1985. The cell substrate attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. *J. Cell Biol.* 101:2134.
50. Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.).* 336:487.
51. Hemler, M. E., C. Huang, and L. Schwartz. 1987. The VLA protein family: characterization of five distinct cell surface heterodimers each with a common 130,000 Mr subunit. *J. Biol. Chem.* 262:3300.
52. Hemmler, M. E., C. Crouse, Y. Takada, and A. Sonnenberg. 1988. Multiple Very Late Antigens (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor) and VLA-6 structures. *J. Biol. Chem.* 263:7660.
53. Hall, D. E., E. Crowley, L. F. Reichardt, and C. Damsky. 1988. Beta$_1$ integrins adhesion receptors mediate cell attachment to two major attachment promoting domains of laminin. *J. Cell Biol.* 107:800A.