

Cytokine-induced Respiratory Burst of Human Neutrophils: Dependence on Extracellular Matrix Proteins and CD11/CD18 Integrins

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Abstract. Human polymorphonuclear leukocytes (PMN) released large quantities of hydrogen peroxide in response to tumor necrosis factor, but only when the cells were adherent to surfaces coated with extracellular matrix proteins. The PMN did not respond when exposed to cytokines and matrix proteins in suspension, or when exposed to cytokines while adherent to surfaces coated with stearic acid. PMN from children with genetic deficiency of the CD11/CD18 integrins underwent a normal respiratory burst upon adherence to uncoated polystyrene, but not in response to tumor necrosis factor when tested on polystyrene that was coated with serum, fibronectin, vitronectin, fibrinogen, thrombospondin, or laminin. Anti-CD18 antibodies, alone of sixteen antibodies tested, induced

a similar defect in PMN from normal donors, when the PMN were tested on surfaces coated with serum, fibrinogen, thrombospondin, or laminin; no defect was induced by the anti-CD18 monoclonal antibody IB4 in normal PMN tested on surfaces coated with fibronectin or vitronectin. Thus, for cytokines to induce a respiratory burst in PMN, the cells must be able to use CD11/CD18 integrins and must interact with matrix proteins in the solid phase. CD11/CD18, which is already known to serve as a receptor for fibrinogen, may also be a receptor for thrombospondin and laminin. Finally, receptor(s) exist on PMN for fibronectin and vitronectin which are not blocked by the anti-CD18 antibody IB4 but which are nonetheless CD11/CD18 dependent.

HUMAN polymorphonuclear leukocytes (PMN)¹ can undergo a large respiratory burst in response to subnanomolar concentrations of tumor necrosis factor- α (TNF α), TNF β , colony stimulating factor for granulocytes and macrophages (CSF-GM), or colony stimulating factor for granulocytes (CSF-G), but only if the PMN are adherent to surfaces coated with plasma, serum, or proteins of extracellular matrix (40, 41). There are few if any other instances in which adherence of PMN capacitates them to respond to stimuli that are otherwise ineffective. The goal of the present study was to determine if capacitation by adherence requires ligation of specific adhesion receptors.

Integrins are a superfamily of heterodimeric, transmembrane glycoproteins which act as receptors for various glycoproteins of extracellular matrix or cell surface (33). Interaction of integrins with surface-bound ligands supports adhesion

1. *Abbreviations used in this paper:* CSF-G, colony stimulating factor for granulocytes; CSF-GM, colony stimulating factor for granulocytes/macrophages; FBG, fibrinogen; FN, fibronectin; KRPG, Krebs-Ringer phosphate buffer with glucose; LAD, leukocyte adhesion deficiency; LAM, laminin; PMA, phorbol myristate acetate; PMN, neutrophilic polymorphonuclear leukocytes; r, recombinant; TNF, tumor necrosis factor; TSP, thrombospondin; VN, vitronectin.

of phagocytes and their spreading, diapedesis, and phagocytosis. Engagement of phagocyte integrins apparently does not trigger secretion, for example of hydrogen peroxide (23, 66) or arachidonate (1), although exposure of macrophages to an anti-integrin antibody for 1-3 d did increase the amount of hydrogen peroxide released subsequently in response to a secretagogue (23).

A family of integrins apparently restricted to leukocytes is comprised of CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1, complement receptor type 3), and CD11c/CD18 (p150,95) (34, 54). Leukocyte adhesion deficiency (LAD) is a genetic disease resulting from markedly decreased cell surface expression of all three of these heterodimers, a consequence of deficiency or abnormality in mRNA for CD18 (34). The function of CD11/CD18 molecules in cell adhesion has been inferred from the defects displayed by PMN of children with LAD or by normal PMN treated with anti-CD11 or anti-CD18 antibodies (5, 11, 19, 26, 47, 59, 69). PMN in LAD patients migrate poorly through tissues to sites of inflammation, and, like normal PMN treated with anti-CD11 or anti-CD18 antibodies, attach and spread poorly in vitro. The in vitro studies have dealt with adherence of PMN to surfaces

of nonphysiologic or complex composition: uncoated glass, plastic, or nylon wool; glass or plastic coated with serum; or cultured endothelial cells. Four specific protein ligands for CD11/CD18 have been identified: C3bi (65), ICAM-1 (39), coagulation factor X (4), and fibrinogen (FBG) (2, 68). Of these, only FBG can be considered a matrix protein. Thus, while it is likely that CD11/CD18 molecules interact with matrix components, there is little information on the specificity of CD11/CD18 integrins for individual proteins of extracellular matrix.

Using PMN from children with LAD, normal PMN treated with anti-integrin antibodies, and surfaces coated with purified matrix proteins, the present work shows that solid-phase FBG, thrombospondin, laminin, fibronectin, and vitronectin capacitate PMN to secrete hydrogen peroxide in response to cytokines. Each of these interactions requires expression of CD11/CD18 molecules on the PMN.

Materials and Methods

PMN

Donors of venous blood (collected with heparin) were three children with LAD whose clinical histories have been described (Detmers, P., S. D. Wright, E. Olsen-Egbert, R. Adamowski, Z. Chad, L. B. Kabbash, and Z. A. Cohn, manuscript in preparation), the parents of one of them, three other children referred for evaluation of leukocyte function defects, and seven healthy adults. Blood from the LAD patients and a normal donor was collected in Montreal, brought to New York by courier, and processed within ~5 h of collection, together with blood that was processed immediately after collection from an additional normal donor in New York. Results were comparable for either source of normal donors' cells. For assays of H₂O₂ release, PMN were isolated on a one-step Ficoll-Hypaque gradient (Neutrophil Isolation Medium; Los Alamos Diagnostics, Los Alamos, NM) as described (40), with the following modification. For patients with marked leukocytosis, the ratio of leukocytes to surface area of the interface was adjusted by layering 0.25 ml blood on 4 ml of isolation medium in a 15-ml tube, or <1.4 ml blood on 10 ml of isolation medium in a 50-ml tube. PMN (>98% PMN, <2% lymphocytes) were washed and resuspended at 7.5×10^5 /ml in Krebs-Ringer phosphate buffer with glucose (KRPG) (40). For determination of CD11/CD18 levels, PMN were purified on a two-step Ficoll-Hypaque gradient as described (24) and resuspended in RPMI containing human serum albumin and aprotinin. Levels of CD11a, CD11b, CD11c, and CD18 on the surface of unstimulated PMN were measured by flow cytometry using mAbs TSI/22, OKM10, Leu M5, and IB4, respectively. In addition, phorbol myristate acetate (PMA) was added to mobilize possible intracellular reserves of CD11/CD18 molecules to the cell surface (7, 10, 53), as monitored by rosetting of erythrocytes coated with complement component C3bi. By both assays, CD11/CD18 molecules were >96% deficient on cells from each of the three LAD patients, were not measured but presumed normal on cells from two of the healthy adults, and were documented to be normal on PMN of all other donors.

Preparation of Surfaces

Initial studies used tissue culture grade polystyrene plates with 6-mm-diam flat-bottomed wells (No. 25860; Costar Data Packaging Corp., Cambridge, MA); later experiments used the modified Primaria plates (No. 3872; Falcon Labware, Oxnard, CA) (discussed in reference 41). The agents used, their sources, the amounts plated per well, and the diluent (25 μ l/well) were as follows. FBS (HyClone Laboratories, Logan, UT); undiluted (in some experiments with FBS-coated wells, the reaction solution [see below] was also made to contain 0.1% fresh autologous human plasma). Pure human plasma FBG (Sigma Chemical Co., St. Louis, MO); 100 μ g in RPMI 1640 medium (HyClone Systems, Lenexa, KS). Pure human plasma FN (New York Blood Center); 3.4 μ g in KRPG. Laminin (LAM) (purified >95% from basement membrane of the Engelbreth-Holm-Swarm tumor) (Collaborative Biomedical Products, Waltham, MA); 0.5–10.0 μ g in RPMI 1640. Human thrombospondin (TSP); purified from the calcium ionophore-stimulated releasate of human platelets by sequential heparin Sepharose and anion exchange chromatography (16, 37); 3.5–10.0 μ g in 0.15 M NaCl.

Vitronectin (VN); purified from human plasma by mAb and heparin affinity chromatography (27); 1.5–4.0 μ g in KRPG. The FBG, FN, LAM, TSP, and VN each gave only the expected bands on polyacrylamide gel electrophoresis. In other wells, 250 μ g fatty acid/50 μ l methanol was added and the plates placed in a vacuum dessicator until the solvent evaporated. All plates were then incubated in humidified 5% CO₂/95% air for 1–2 h. Wells containing protein were washed three times with 0.9% NaCl. Each well was then given 100 μ l of KRPG containing 2.4–3.5 nmol scopoletin, 0.5 μ g of horseradish peroxidase, and 1 mM Na₂S₂O₈ (reaction solution). Triggering agents and antibodies were added in 10 μ l. Plates were incubated at 37°C in air for 30 min before addition of cells (1.5×10^4 /20 μ l per well).

Treatment of PMN with Antibodies

PMN were exposed to the antibodies listed in Table I while in agitated suspension in FBS-coated tubes as follows. Polypropylene microfuge tubes (Brinkmann Instruments Co., Westbury, NY) were coated with FBS by end-over-end rotation at 37°C for 1 h. The tubes were washed extensively with 0.9% NaCl. Antibodies were added (where indicated, addition of antibodies was delayed), followed by PMN to a final concentration of 7.5×10^5 /ml in KRPG in a volume of 0.5–1.2 ml per tube of 1.5 ml capacity, or 0.24–0.38 ml per tube of 0.5 ml capacity. The tubes were shaken mechanically at 150 horizontal cycles/min at 37°C for 90 min. 20 μ l of cell suspension was then transferred to each well. Wells contained sufficient additional antibody to maintain the stated concentration. In some experiments with antiserum, PMN were washed after preincubation, and antiserum was not added to the wells, to avoid coating the wells with serum proteins. Bubbles in the wells were popped with a 26-gauge needle to avoid fluorescence artefacts. PMN preincubated as described above could release as much H₂O₂ in response to PMA as PMN from the same preparations tested without preincubation. However, results were markedly inferior if the cell density during preincubation was fivefold lower, the volume of cell suspension per tube threefold lower, the agitation 1.4-fold faster, the tube made of polystyrene, the coating with FBS omitted, or the suspension allowed to remain stationary at any time before the cells were transferred to the plates. In each experiment it was demonstrated that antibody treatment itself elicited no peroxide release.

H₂O₂ Release

Triggering agents included pure, recombinant (r) TNF α and rTNF β (Genentech, Inc., South San Francisco, CA), rCSF-GM and rCSF-G (Amgen, Inc., Thousand Oaks, CA, and Genetics Institute, Cambridge, MA), and PMA. The cytokines have been described (40, 41). Except in dose-response studies, all triggering agents were used at 100 ng/ml, which affords a maximal response (40, 41). H₂O₂ release was measured at 15-min intervals during a 2-h incubation in air at 37°C by the decrease in fluorescence of scopoletin as measured by a plate-reading fluorimeter (Flow Laboratories, Inc., McLean, VA). Results were calculated as described (21). Each condition was tested in triplicate. When most of the SEM within one experiment were smaller than the symbols used in the figure, SEM have not been displayed. Where pooled data are reported, they represent means \pm SEM from the indicated number of separate experiments, each datum being the mean of triplicates from one experiment.

TNF Receptors

Specific binding of ¹²⁵I-rTNF α to PMN was measured as reported (48). In brief, 2×10^5 PMN in 0.2 ml KRPG containing 5 mM Na₂S₂O₈ and 10% FBS were incubated with ¹²⁵I-rTNF α (4×10^5 cpm) on a rocking platform for 4 h at 4°C with or without 100-fold excess of unlabeled rTNF α , washed four times by centrifugation, and counted in a gamma spectrometer.

Reagents

Where not otherwise stated, reagents were from Sigma Chemical Co. Bacterial lipopolysaccharide contamination of reagents was monitored by the chromogenic limulus amoebocyte lysate test (Whittaker M. A. Bioproducts, Walkersville, MD) and found not to exceed 20 pg/ml at the concentrations used.

Results

Previous studies (40, 41; Nathan, C., unpublished observations) established that uncoated polystyrene or glass surfaces

Table I. Antibodies Used in This Study

Antibody	Type and class	Specificity	Reference No.
IB4	Mouse mAb IgG _{2a}	CD18	63
60.3	Mouse mAb IgG _{2a}	CD18	9
TS1/22	Mouse mAb IgG ₁	CD11a	50
OKM1	Mouse mAb IgG _{2b}	CD11b	12
904	Mouse mAb IgG ₁	CD11b	20
Leu M5	Mouse mAb IgG _{2b}	CD11c	36
BH2C6	Mouse IgM	p157 of human PMN (not CD11)	46
Anti-VLA-β	Rabbit serum	FN, LAM, collagen receptors	30
B6H12	Mouse mAb IgG ₁	PMN FN receptor	13
Anti-VNR	Rabbit IgG	VN receptor	51
OKM5	Mouse mAb IgG ₁	CD36; platelet gp IV; TSP receptor	6,35,52
8A6-D11	Mouse mAb IgG ₁	CD36: see OKM5	*
7E3	Mouse mAb IgG ₁	Platelet gp IIb/IIIa; receptor for FN, FBG, VN, von Willebrand factor	17
9.3F10	Mouse mAb IgG _{2a}	HLA class II	55
3C10	Mouse mAb IgG _{2b}	p55 of mononuclear phagocytes	55
R24	Mouse mAb IgG ₃	Ganglioside GD3	32

* Barnwell, J., A. Asch, M. Maya, and M. Aikawa, manuscript submitted for publication.

triggered a respiratory burst from PMN without additional stimulation. Polystyrene or glass coated with plasma, FBS, FBG, FN, TSP, VN, or LAM no longer triggered a respiratory burst, but allowed PMN to mount a respiratory burst in response to cytokines. In agitated suspension, PMN did not respond to cytokines, even when matrix proteins were added in solution. These observations indicated that responsiveness to cytokines required adherence of PMN to a surface coated with certain proteins, but did not prove that these proteins had a role more specific than preventing polystyrene or glass from triggering the respiratory burst.

Cytokine-stimulated H₂O₂ Secretion Specifically Requires Adherence to Surfaces Coated with Certain Proteins

To test the specificity of adherence to matrix proteins for enabling a response, we compared rTNFα-induced H₂O₂ secretion from PMN on plastic surfaces coated with FBS or FN to their response on surfaces coated with lipids. The

most suitable lipid of those screened was stearic acid, by three criteria: (a) PMN adhered as well to plastic coated with stearic acid as to plastic coated with matrix proteins (22), in that the PMN resisted dislodgment through three cycles of flicking the plates dry and flooding them with saline (not shown); (b) stearic acid-coated surface neither triggered much H₂O₂ release by itself, nor (c) interfered with the abil-

Table II. Hydrogen Peroxide Release from CD11/CD18-deficient and CD11/CD18-replete PMN: Triggering by Contact with Polystyrene or by Phorbol Ester

Donor*	Uncoated surface (no other stimulus)‡	Coated surfaces with PMA§
H ₂ O ₂ , nmol/1.5 × 10 ⁴ PMN¶		
LAD-1	0.80 ± 0.18 (4)	1.08 ± 0.07 (17)
LAD-2	1.38 ± 0.10 (3)	1.44 ± 0.08 (12)
LAD-3	ND	1.44 ± 0.06 (4)
Mother	ND	1.03 (1)
Father	ND	1.14 (1)
Ped-1	ND	0.99 (1)
Ped-2	ND	1.42 (1)
Ped-3	ND	1.20 (1)
Normals	1.35 ± 0.13 (8)	1.78 ± 0.06 (24)

* LAD-1, 2-yr-old boy with leukocyte adhesion deficiency. LAD-2, 2-yr-old boy with LAD. LAD-3, 4-yr-old girl with LAD. Mother and Father, mother and father of LAD-1. Ped-1, 3-d-old premature girl with unexplained neutrophilic leukocytosis and negative microbiologic cultures. Ped-2, 5-yr-old girl with unexplained recurrent abscesses. Ped-3, 6-yr-old boy with unexplained recurrent abscesses. Normals, seven healthy adult men. CD11/CD18 expression was documented to be deficient on PMN from LAD-1-3 and normal for all other donors, except two of the normals, on whose PMN CD11/CD18 were not tested.

‡ Respiratory burst triggered by contact of PMN with tissue culture grade polystyrene (conventional or Primaria types).

§ Respiratory burst triggered by phorbol myristate acetate (100 ng/ml) in PMN adherent to polystyrene surfaces coated with FBS, human plasma, FN, FBG, TSP, VN, or LAM; such coated surfaces induced little or no respiratory burst by themselves.

¶ Mean ± SEM for number of experiments in parentheses, each in triplicate, at the termination of the response (90-120 min).

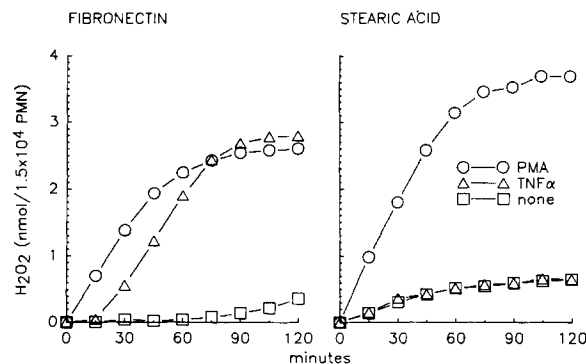


Figure 1. A polystyrene surface coated with FN but not stearic acid capacitated PMN to release H₂O₂ in response to rTNFα (100 ng/ml). The response to PMA (100 ng/ml) was not restricted by either surface.

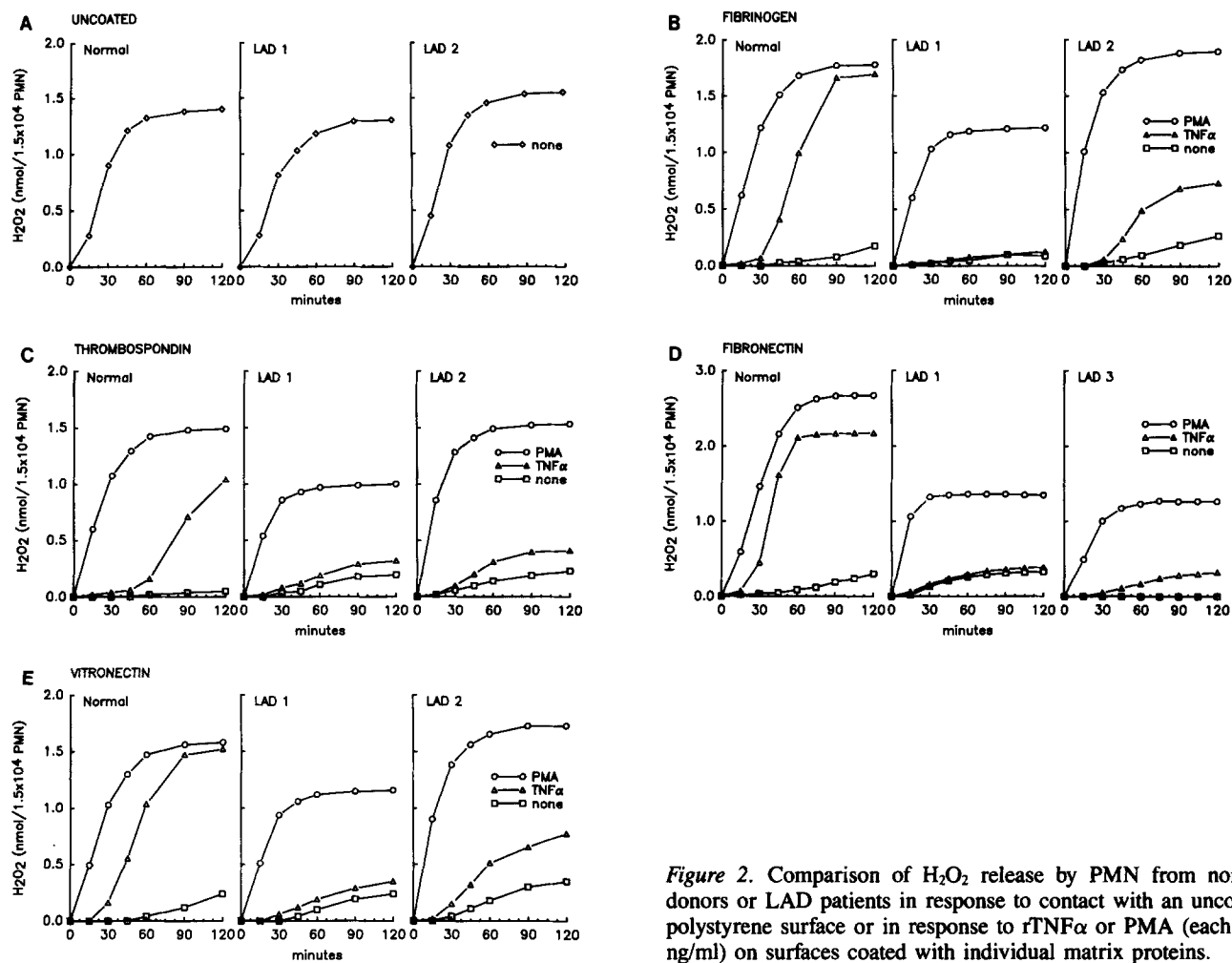


Figure 2. Comparison of H_2O_2 release by PMN from normal donors or LAD patients in response to contact with an uncoated polystyrene surface or in response to $rTNF\alpha$ or PMA (each 100 ng/ml) on surfaces coated with individual matrix proteins.

ity of PMA to do so. Yet, as shown in Fig. 1, adherence to a stearic acid-coated surface did not capacitate PMN to secrete H_2O_2 in response to $rTNF\alpha$. Results were similar in six such experiments. Thus, adherence as such was not sufficient for a response to $rTNF\alpha$, but rather, adherence to surfaces coated with particular substances, such as FN. This raised the possibility that adhesion receptors might be involved.

Deficient Cytokine Response by PMN from Patients with LAD

PMN from each of three patients with LAD released large amounts of H_2O_2 over 90–120 min in response to PMA (Table II). These amounts averaged 74% as much as from PMN of the adults used as controls in most of the same experiments, and equalled or exceeded the amounts released by

Table III. Hydrogen Peroxide Release from CD11/CD18-deficient and CD11/CD18-replete PMN: Triggering by $rTNF\alpha$ *

Donor	Percent of PMA-triggered response [†]					
	FBS	FN	FBG	TSP	VN	LAM
LAD-1	12 ± 2 (5)	-6 ± 8 (3)	4 ± 2 (3)	8 ± 3 (4)	7 (2)	8 (1)
LAD-2	15 ± 9 (3)	12 (2)	15 (2)	8 (2)	13 (2)	19 (1)
LAD-3	8 (1)	15 (1)	0 (2)	0 (1)	ND	ND
Mother	138 (1)	ND	ND	—	—	—
Father	153 (1)	ND	ND	—	—	—
Ped-1	128 (1)	ND	ND	—	—	—
Ped-2	70 (1)	ND	ND	—	—	—
Ped-3	72 (1)	ND	ND	—	—	—
Normals	75 ± 5 (9)	95 ± 7 (3)	78 ± 7 (3)	76 ± 10 (4)	92 (2)	103 (1)

* Tissue culture polystyrene was coated with FBS, FN, FBG, TSP, VN, or LAM before adding PMN from the donors described in Table II.

[†] Calculated as: 100 (nmol H_2O_2 released in response to 100 ng/ml $rTNF\alpha$ minus that released without a triggering agent)/(nmol released in response to PMA minus that released without a triggering agent) at the same time point. Time points selected for comparison were the latest at which the H_2O_2 released without a triggering agent remained $\leq 10\%$ of the response triggered by PMA, and ranged from 45 to 120 min. Presentation of results from late time points minimizes the apparent deficiency in TNF-induced responses by LAD cells relative to normal cells, because responses by normal cells tended to end earlier than responses by LAD cells. Results are means ± SEM for the number of experiments in parentheses, each in triplicate.

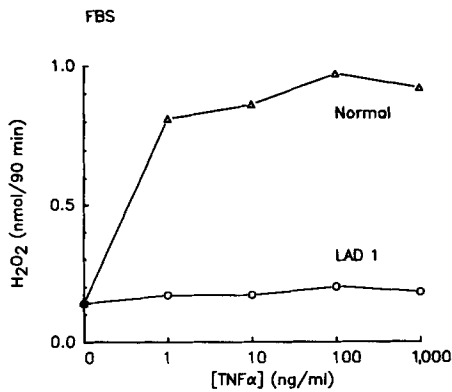


Figure 3. Inability of high concentrations of rTNF α to overcome the unresponsiveness of PMN from LAD patients. The surface was coated with FBS.

PMN from three other children and from the healthy parents of one of the subjects. The normality or near normality of the PMA-induced respiratory burst of adherent LAD PMN was consistent with previous comparisons of normal and LAD PMN in suspension, under conditions where smaller amounts of respiratory burst products were measured during much shorter periods, usually ≤ 5 min (5, 11, 19, 42). A finding of special importance for the present analysis was that uncoated polystyrene triggered H₂O₂ release from the PMN of LAD patients as well as or nearly as well as from normal PMN (Table II and Fig. 2). Thus, in a setting where matrix proteins were not involved, PMN from children with LAD had no defect in mounting a large respiratory burst as a consequence of adherence.

In contrast, LAD cells were grossly deficient in their ability to respond to rTNF α , when assayed in plates coated with FBG, FN, TSP, VN, LAM, or FBS. Considering each combination of day, donor, and surface as a separate test, the deficient rTNF α response of LAD cells was demonstrated in each of 35 trials (Table III). Fig. 2 shows such responses on each surface on which the responses of normal PMN have not been illustrated previously (40, 41). The interpatient variability evident in Fig. 2 did not correspond to differences in surface expression of CD11/CD18 (see Materials and Methods), and remains unexplained.

The deficient response of PMN from LAD patients was not a consequence of quantitative insensitivity to rTNF α , since the defect was manifest over a 1,000-fold range of rTNF α concentrations on the plateau of the dose-response curve for normal PMN (Fig. 3). In five experiments with normal PMN, multipoint binding curves indicated the presence of $1,400 \pm 500$ specific binding sites for TNF per cell with K_d of $3.9 (\pm 1.8) \times 10^{-9} M^{-1}$ (mean \pm SEM), comparable to previous reports (e.g., reference 48). In two experiments involving 10 measurements with cells from two LAD patients at three concentrations of TNF, specific binding was 1.6- (± 0.14) fold as much as binding to normal PMN. Thus, there was no evidence for a deficiency of TNF receptors on LAD PMN. Finally, LAD PMN were also deficient in their secretion of H₂O₂ in response to rCSF-G and rCSF-GM (not shown).

The deficient response of PMN from LAD patients could not be attributed to the patients' age, nor to effects of marked leukocytosis or recurrent infection, because no deficiency was evident using CD11/CD18-replete PMN from pediatric

patients ranging in age from 3 days to 6 years. One of these children had marked leukocytosis and two had recurrent abscesses (Table III).

Effect of Anti-CD18 or Anti-CD11 mAbs on the Response of Normal PMN

The results with LAD patients raised the possibility that CD11a, CD11b, CD11c, and/or CD18 might serve as adhesion receptors for each of five matrix proteins. To test this hypothesis further and to try to determine which of the CD11/CD18 molecules was critical, we used mAbs specific to each.

In a previous study (40), 15 min treatment at 4°C with 2 $\mu g/ml$ of the anti-CD18 mAb IB4 had no impact on the response of PMN on FBS-coated plates. We therefore exposed PMN to higher concentrations of mAb for longer times at a higher temperature. As shown in Fig. 4 *a*, PMN in suspension at 37°C required ≥ 68 min pre-exposure to 30 $\mu g/ml$ IB4 to block completely their subsequent response to rTNF α . Exposure for the same time at 4°C did not suffice, nor was pre-exposure fully effective unless followed by continued exposure to IB4 during the assay. With a 90-min pre-exposure at 37°C, $\geq 90\%$ suppression required $\geq 10 \mu g/ml$ IB4 (Fig. 4 *b*).

Using conditions optimal for inhibition by IB4 (30 $\mu g/ml$

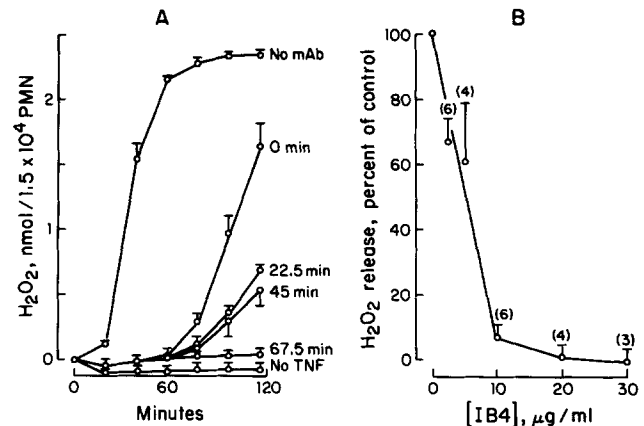


Figure 4. Ability of anti-CD18 IB4 to inhibit H₂O₂ release from normal PMN in response to rTNF α on FBS-coated surfaces. (*A*) Dependence on duration of earlier exposure of PMN to 0 or 30 $\mu g/ml$ mAb in agitated suspension at 37°C. PMN were preincubated for 90 min with or without mAb before the start of the assay (time 0). The response of adherent cells was then followed over 120 min, as indicated on the abscissa. For the topmost curve, no mAb was added at any time. For the other curves, 30 $\mu g/ml$ mAb was present in the assay stage. The numbers to the right of each curve specify that mAb was not also present during the preincubation (0 min) or was added at 22.5, 45.0, or 67.5 min before time 0. In the absence of TNF (100 ng/ml), no H₂O₂ was released by PMN from any of these groups; results from one rTNF α -free control are displayed. Data are means \pm SEM for triplicates. (*B*) Dependence of inhibition on the concentration of IB4. PMN were exposed to the indicated concentration of mAb in agitated suspension at 37°C for 90 min before the assay, as well as throughout the measurement of the response to rTNF α (100 ng/ml). Data are means \pm SEM for the number of experiments shown in parentheses, each in triplicate. H₂O₂ release was measured at time points selected by the criterion given in the legend of Table III (mean \pm SEM, 105 \pm 17 min), and averaged 1.68 \pm 0.19 nmol/1.5 $\times 10^4$ PMN in the absence of IB4. The concentration of IB4 giving 50% inhibition averaged 4.9 \pm 1.0 $\mu g/ml$.

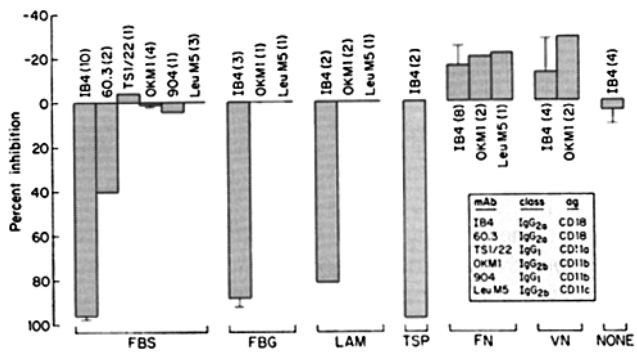


Figure 5. Effect of anti-CD11/CD18 mAbs on the ability of normal PMN to release H_2O_2 in response to $rTNF\alpha$ (100 ng/ml) during incubation on surfaces coated with the indicated proteins. PMN were exposed to 30 $\mu g/ml$ of each mAb at 37°C for 90 min in advance of and during the assay. Results are means \pm SEM from the number of experiments in parentheses, each in triplicate. Time points were selected for comparison as in Table III. Percent inhibition was calculated in comparison to the no-antibody control after subtracting H_2O_2 released without a triggering agent.

for 90 min at 37°C before and during the assay), up to five other anti-CD11/CD18 mAbs were tested on each of the six protein-coated surfaces. As shown in Fig. 5, IB4 inhibited the response completely on surfaces coated with FBS, FBG, LAM, and TSP, but not at all on FN, VN, or uncoated plastic. The latter results ruled out nonspecific effects of IB4 on the cells or the assay. On FBS-coated plates, an independently derived anti-CD18 mAb (60.3) was effective. However, anti-CD11a, CD11b, or CD11c mAbs were noninhibitory on any surface tested. The anti-CD11b panel included mAbs reacting with either of the known binding sites on CD11b (3, 20, 62, 65).

Lack of Effect of Other Anti-Integrin and Control Antibodies

The foregoing results suggested that while CD18 might be critical to PMN interaction with surfaces coated with FBS, FBG, LAM, and TSP, a different integrin might be more important in the interaction with FN and VN. However, as shown in Fig. 6, there was no inhibition by anti-VLA- β (which binds to FN and laminin receptors on other cell types [29]), 7E3 (which binds gp IIb/IIIa [17], a receptor on platelets for FN, VN, FBG, and von Willebrand factor [45]), B6H12 (which binds to a FN receptor on PMN [13]), an antibody that binds VN receptors on a variety of cells (51), or two mAbs (OKM5, 8A6-D11) against CD36, an 88-kD glycoprotein that serves as a TSP receptor on several cell types but which has not been detected on PMN (6, 35, 52). A variety of other mAbs that do or do not bind PMN served as further controls, emphasizing the specificity of effects seen with IB4. The tetrapeptide RGDS, a known inhibitor of many integrins (33), interfered nonspecifically with the peroxide assay at concentrations $\geq 70 \mu g/ml$ and thus was not informative in this system (not shown).

Discussion

Substrate-bound matrix proteins and CD11/CD18 integrins are required for PMN to initiate a respiratory burst in re-

sponse to soluble cytokines. The ability to secrete H_2O_2 in response to cytokines is associated with cell spreading and blocked by dihydrocytochalasin B (40, 41), but not by colchicine or nocodazole (Nathan, C., unpublished observations). Thus, microfilaments may be involved in transmission of a capacitating signal from CD11/CD18. This would be consistent with the association of some integrins with the actin-based cytoskeleton (33).

We do not know how adherence capacitates secretion. With T lymphocytes, antibodies that cross-link CD11a or CD18 enhance synergistically the increase in intracellular Ca^{++} , release of interleukin-2, and proliferation induced by antibodies that cross-link the antigen receptor (15, 57). With PMN, binding of CD11/CD18 to extracellular matrix proteins (but not binding by soluble mAbs) may likewise capacitate responses triggered by cytokine receptors. PMN adhesion receptors and cytokine receptors may even capacitate each other's functions reciprocally, generating positive feedback during the lag period that precedes secretion of H_2O_2 . Previous observations are compatible with the occurrence of reciprocal interactions between integrins and secretagogue receptors. For example, binding of FBG to the polyspecific platelet integrin gpIIb/IIIa is essential for intracellular alkalization induced by the binding of epinephrine to the α_2 adrenergic receptor (8), while activation of platelets with thrombin promotes association of gpIIb/IIIa with the actin-based cytoskeleton (44).

CD18 and/or CD11 may function as receptor(s) for FBG, TSP, and LAM, in that surfaces coated with purified FBG, TSP, or LAM enabled PMN to respond to $rTNF\alpha$; PMN deficient in CD11/CD18 failed to exhibit this response; and the response of normal PMN on surfaces coated with FBG, TSP, or LAM was blocked specifically by the anti-CD18 mAb, IB4. All three of these matrix proteins may bind to CD11/CD18 at site(s) bound by IB4 or obstructed by the binding of IB4. Alternatively, CD11/CD18 may be required for the function of receptors for these proteins. CD11/CD18 molecules have previously been shown to bind FBG (2, 68). In contrast, although two distinct TSP receptors have been described on cells other than PMN (6, 38), an interaction of PMN with TSP, and the possibility that CD11/CD18 may serve as a TSP receptor, have not previously been noted. In future studies, the ability of CD11/CD18 to bind TSP should

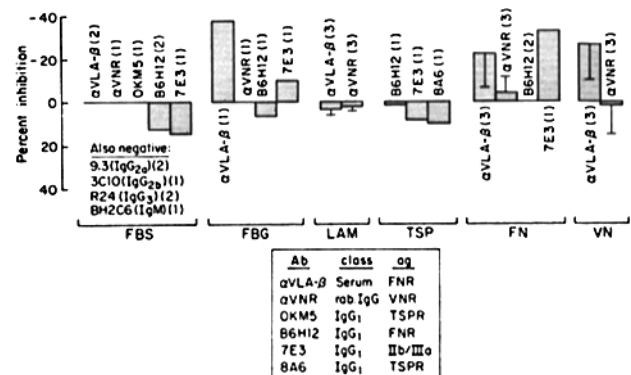


Figure 6. Lack of inhibition of the cytokine response by antibodies against integrins other than CD11/CD18, mAbs against other surface antigens of PMN, or isotype controls of irrelevant antigenic specificity. Anti-VLA- β antiserum was used at 1:300. Other details are as in Fig. 5.

be tested directly. PMN do bind laminin (14). Laminin receptors have been characterized biochemically on cells other than PMN, some of the receptors having structural features of integrins (25, 49). To our knowledge, no previous reports have raised the possibility that CD11/CD18 may serve as a laminin receptor or regulate function of laminin receptors.

PMN bind FN (13). However, an interaction of PMN with VN has apparently not been described. The present work indicates that PMN can interact with FN and VN via receptor(s) that are probably distinct from the receptors for FBG, TSP, and LAM, and from the IB4 epitope of CD18. This interpretation hinges on results that are seemingly paradoxical. On the one hand, PMN could not respond to rTNF α on surfaces coated with FN or VN if the PMN were genetically deficient in CD11/CD18. On the other hand, treatment of normal PMN with mAb IB4 did not interfere with the ability of FN or VN to support a cytokine response. Of several possible explanations, we will discuss two for which there is precedent.

First, there may be FN and VN receptor(s) that are structurally distinct from CD11/CD18, but whose signals must be transmitted via a CD11- or CD18-dependent pathway. It is known that information can be exchanged between integrin molecules: adherence of macrophages to a FN-coated surface capacitates CD11b/CD18 molecules on the nonadherent surface of the cell to mediate the ingestion of C3bi-coated erythrocytes (63). Alternatively, CD18 may be part of the FN and VN receptor(s) of PMN, but FN and VN may bind to site(s) distinct from those recognized by or obstructed by mAb IB4. A noncross-reactive pattern of inhibition by different mAbs supports the inference that CD11b has two to three different binding sites for the ligands C3bi, FBG (2), and phosphorylated microbial carbohydrates (62).

Two observations suggest that CD11/CD18 molecules can capacitiate PMN to secrete H₂O₂ in response to rTNF α by serving as adhesion receptors in their own right, rather than solely by mediating or regulating the function of other adhesion receptors. First, PMN required CD11/CD18 to respond on surfaces coated with FBG; CD11/CD18 molecules seem to comprise all demonstrable PMN binding sites for FBG (2, 68). Second, surfaces coated with complement component C3bi also capacitated PMN to respond to rTNF α (Nathan, C., and S. Wright, unpublished observations); CD11/CD18 molecules comprise all detectable binding sites on PMN for C3bi (62, 64).

The present study did not find evidence for the involvement of integrins other than CD11/CD18 in the FN and VN receptor activity of PMN. Three families of integrins with distinct β chains are presently recognized: β 1 or VLA, which includes at least two FN receptors, termed VLA-5 (ECMR VI) and ECMR I (28, 60); β 2, consisting of the CD11/CD18 molecules; and β 3, which includes a VN receptor and gp IIb/IIIa, which binds both FN and VN. The β 1 family is unlikely to participate in the response of PMN to matrix components, since these molecules have been considered absent from PMN (28, 60). In a recent study, flow cytometry detected weak binding of an anti-VLA- β mAb to PMN, but without any difference between PMN from normals and PMN from the LAD patients studied here (Wright, S. D., P. A. Detmers, R. Adamowski, Z. Chad, L. G. Kabbash, and M. J. Pabst, manuscript in preparation). In the present work, a polyclonal antibody to the β 1 chain did not inhibit the re-

sponse of PMN on surfaces coated with FN or VN. Similarly, with regard to β 3 integrins, PMN are not thought to express gp IIb/IIIa, and neither a polyclonal anti-VN receptor antibody nor a mAb to gp IIb/IIIa was inhibitory. A FN-binding protein has been purified from PMN, which reacts with a polyclonal anti- β 3 antibody (13); a mAb against this protein had no effect on the response of PMN on FN. Of course, some of these reagents, particularly the mAbs, could have bound to a relevant receptor and failed to inhibit its function. Thus, these studies do not rule out the participation of known integrins. Likewise, the possibility cannot be excluded that the underlying defects in LAD PMN may affect not just CD18 mRNA (34) but also other molecules involved in adhesion.

Inhibition of the cytokine response required prolonged preexposure to anti-CD18 mAb at 37°C and the continued presence of high concentrations of antibody throughout the assay. This might reflect that PMN contain a large intracellular store of CD11/CD18 and can mobilize it to the cell surface (7, 10, 53). Engagement of a small fraction of CD11/CD18 molecules may be sufficient for a maximal response, and CD11/CD18 molecules exteriorized at the site of contact with the substrate may be partly protected from antibodies (61, 67). Prolonged preincubation at 37°C may permit endocytosed mAb to complex with intracellular CD18 before CD11/CD18 molecules are mobilized to the protected sites where PMN make contact with the matrix protein-coated surface.

It now appears that a secretory response of PMN can be controlled by ligation of three classes of receptors at the same time on the same cell. The ability of PMN to secrete large amounts of H₂O₂ in response to TNF α / β , CSF-G, and CSF-GM is presumably controlled by the known receptors for these cytokines (43, 48, 58). This response is suppressed by adenosine at levels found in plasma (22), an effect most likely mediated through the A₂ receptor (18). The present results indicate that a third type of receptor, CD11/CD18, is required to capacitiate this secretory response. The respiratory burst, which serves to kill microbes, can also cause or promote damage to host tissue (31). For PMN in circulation, lack of a positive signal from adhesion receptors may lessen the likelihood of secretion in response to cytokines that have spilled into the blood from inflammatory sites. The secretory response to cytokines may normally be confined instead to PMN that have aggregated, adhered, or emigrated in a manner dependent in large part on CD11/CD18. Consistent with this speculation, experimental animals have been protected from inflammatory tissue damage by administration of anti-CD18 mAb (56).

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References

- Aderem, A., S. D. Wright, S. C. Silverstein, and Z. A. Cohn. 1985. Ligated complement receptors do not activate the arachidonic acid cascade in resident peritoneal macrophages. *J. Exp. Med.* 161:617-622.
- Altieri, D. C., R. Bader, P. M. Mannucci, and T. S. Edgington. 1988. Oligospecificity of the cellular adhesion receptor MAC-1 encompasses an inducible recognition specificity for fibrinogen. *J. Cell Biol.* 107:1893-1900.
- Altieri, D. C., and T. S. Edgington. 1988. A monoclonal antibody reacting with distinct adhesion molecules defines a transition in the functional state of the receptor CD11b/CD18 (Mac-1). *J. Immunol.* 141:2656-2660.
- Altieri, D. C., J. H. Morrissey, and T. S. Edgington. 1988. Adhesive receptor Mac-1 coordinates the activation of factor X on stimulated cells on monocytic and myeloid differentiation: an alternative initiation of the coagulation protease cascade. *Proc. Natl. Acad. Sci. USA.* 85:7462-7466.
- Anderson, D. C., F. C. Schmalsteig, M. A. Arnaut, 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): common relationship to diminished cell adherence. *J. Clin. Invest.* 74:536-551.
- Asch, A. S., J. Barnwell, R. L. Silverstein, and R. L. Nachman. 1987. Isolation of the thrombospondin membrane receptor. *J. Clin. Invest.* 79:1054-1061.
- Bainton, D. F., L. J. Miller, T. K. Kishimoto, and T. A. Springer. 1987. Leukocyte adhesion receptors are stored in peroxidase-negative granules of human neutrophils. *J. Exp. Med.* 166:1641-1653.
- Banga, H. S., E. R. Simons, L. F. Brass, and S. E. Rittenhouse. 1986. Activation of phospholipases A and C in human platelets exposed to epinephrine: role of glycoproteins IIb/IIIa and dual role of epinephrine. *Proc. Natl. Acad. Sci. USA.* 83:9197-9201.
- Beatty, P. G., J. A. Ledbetter, P. J. Martin, T. H. Price, and J. A. Hansen. 1983. Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. *J. Immunol.* 131:2913-2918.
- Berger, M., J. O'Shea, A. S. Cross, T. M. Folks, T. M. Chused, E. J. Brown, and M. M. Frank. 1984. Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J. Clin. Invest.* 74:1566-1571.
- Bowen, T. J., H. D. Ochs, L. C. Altman, T. H. Price, D. E. Van Epps, D. L. Brautigan, R. E. Rosin, W. D. Perkins, B. M. Babior, S. J. Klebanoff, and R. J. Wedgwood. 1982. Severe recurrent bacterial infections associated with defective adherence and chemotaxis in two patients with neutrophils deficient in a cell-associated glycoprotein. *J. Pediatr.* 101:932-940.
- Breard, J., E. L. Reinherz, P. C. Kung, G. Goldstein, and S. F. Schlossman. 1980. A monoclonal antibody reactive with human peripheral blood monocytes. *J. Immunol.* 124:1943-1948.
- Brown, E. J., and J. L. Goodwin. 1988. Fibronectin receptors of phagocytes: characterization of the Arg-Gly-Asp binding proteins of human monocytes and polymorphonuclear leukocytes. *J. Exp. Med.* 167:777-793.
- Bryant, G., C. N. Rao, M. Brentani, W. Martins, J. D. Lopes, S. E. Martin, L. A. Liotta, and E. Schiffmann. 1987. A role for the laminin receptor in leukocyte chemotaxis. *J. Leukocyte Biol.* 41:220-227.
- Carrera, A. C., M. Rincon, F. Sanchez-Madrid, M. Lopez-Botet, and M. O. de Landazuri. 1988. Triggering of co-mitogenic signals in T cell proliferation by anti-LFA-1 (CD18, CD11a), LFA-3, and CD7 monoclonal antibodies. *J. Immunol.* 141:1919-1924.
- Clezardin, P., J. L. McGregor, M. Manach, F. Robert, M. Dechavanne, and K. J. Clemetson. 1984. Isolation of thrombospondin released from thrombin-stimulated human platelets by fast protein liquid chromatography on an anion-exchange Mono Q column. *J. Chromatog.* 296:249-256.
- Coller, B. S. 1985. A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. *J. Clin. Invest.* 76:101-108.
- Cronstein, B. N., E. D. Rosenstein, S. B. Kramer, G. Weissmann, and R. Hirschhorn. 1985. Adenosine: a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A₂ receptor on human neutrophils. *J. Immunol.* 135:1366-1371.
- Crowley, C. A., J. T. Curmutte, R. E. Rosin, J. Andre-Schwartz, J. I. Galin, M. Klempner, R. Snyderman, F. S. Southwick, T. P. Stossel, and B. M. Babior. 1980. An inherited abnormality of neutrophil adhesion. Its genetic transmission and its association with a missing protein. *N. Engl. J. Med.* 302:1163-1168.
- Dana, N., B. Styrt, J. D. Griffin, R. F. Todd, III, M. S. Klempner, and M. A. Arnaut. 1986. Two functional domains in the phagocyte membrane glycoprotein Mo1 identified with monoclonal antibodies. *J. Immunol.* 137:3259-3263.
- De la Harpe, J., and C. F. Nathan. 1985. A semi-automated micro-assay for H₂O₂ release by human blood monocytes and mouse peritoneal macrophages. *J. Immunol. Methods.* 78:323-336.
- De la Harpe, J., and C. Nathan. 1989. Adenosine regulates the respiratory burst of cytokine-triggered neutrophils adherent to biological surfaces. *J. Immunol.* In press.
- Ding, A., S. D. Wright, and C. Nathan. 1987. Activation of mouse peritoneal macrophages by monoclonal antibodies to Mac-1 (complement receptor type 3). *J. Exp. Med.* 165:733-749.
- English, D., and B. R. Andersen. 1974. Single-step separation of red blood cells, granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. *J. Immunol. Methods.* 5:249-252.
- Gehlsen, K. R., L. Dillner, E. Engvall, and E. Ruoslahti. 1988. The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science (Wash. DC).* 241:1228-1229.
- Harlan, J. M., P. D. Kilen, 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.* 66:167-178.
- Hayman, E. G., M. D. Pierschbacher, Y. Ohgren, and E. Ruoslahti. 1983. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. *Proc. Natl. Acad. Sci. USA.* 80:4003-4007.
- Hemler, M. E. 1988. Adhesive protein receptors on hematopoietic cells. *Immunol. Today.* 9:109-113.
- Hemler, M. E., C. Crouse, Y. Takada, and A. Sonnenberg. 1988. Multiple very late antigen (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor), and VLA-6 structures. *J. Biol. Chem.* 263:7660-7665.
- Hemler, M. E., C. Huang, and L. Schwarz. 1987. The VLA protein family. *J. Biol. Chem.* 262:3300-3309.
- Henson, P. M., and R. B. Johnston, Jr. 1987. Tissue injury in inflammation. Oxidases, proteinases, and cationic proteins. *J. Clin. Invest.* 79:669-674.
- Houghton, A. N., D. Mintzer, C. Cordon-Cardo, W. Welt, B. Fliegel, S. Vadhan, E. Carswell, M. R. Melamed, H. F. Oetgen, and L. J. Old. 1985. Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. *Proc. Natl. Acad. Sci. USA.* 82:1242-1246.
- Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549-554.
- Kishimoto, T. K., N. Hollander, T. M. Roberts, D. C. Anderson, and T. A. Springer. 1987. Heterogeneous mutations in the β subunit common to the LFA-1, Mac-1 and p150,95 glycoproteins cause leukocyte adhesion deficiency. *Cell.* 50:193-202.
- Knowles, D. M., II, B. Tolidjian, C. Marboe, V. D'Agati, M. Grimes, and L. Chess. 1984. Monoclonal anti-human monocyte antibodies OKM1 and OKM5 possess distinctive tissue distribution, including differential reactivity with vascular endothelium. *J. Immunol.* 132:2170-2174.
- Lanier, L. L., M. A. Arnaut, R. Schwarting, N. L. Warner, and G. D. Ross. 1985. p150/95, third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur. J. Immunol.* 15:713-718.
- Lawler, J. W., H. S. Slayter, and J. E. Coligan. 1978. Isolation and characterization of a high molecular weight glycoprotein from human blood platelets. *J. Biol. Chem.* 253:8609-8616.
- Lawler, J., R. Weinstein, and R. O. Hynes. 1988. Cell attachment to thrombospondin: the role of ARG-GLY-ASP, calcium, and integrin receptors. *J. Cell Biol.* 107:2351-2361.
- 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.* 51:813-819.
- 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.* 80:1550-1560.
- Nathan, C. F. 1989. Respiratory burst in adherent human neutrophils: triggering by colony stimulating factors CSF-GM and CSF-G. *Blood.* 73:301-306.
- Nauseef, W. M., P. de Alarcon, J. F. Bale, and R. A. Clark. 1986. Aberrant activation and regulation of the oxidative burst in neutrophils with Mo1 glycoprotein deficiency. *J. Immunol.* 137:636-642.
- Nicola, N. A., and L. Peterson. 1986. Identification of distinct receptors for two hemopoietic growth factors (granulocyte colony-stimulating factor and multipotential colony-stimulating factor) by chemical cross-linking. *J. Biol. Chem.* 261:12384-12389.
- Phillips, D. R., L. K. Jennings, and H. H. Edwards. 1980. Identification of membrane proteins mediating the interaction of human platelets. *J. Cell Biol.* 86:77-86.
- Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Ruoslahti. 1986. Platelet membrane glycoprotein IIb/IIIa: member of a family of arg-gly-asp specific adhesion receptors. *Science (Wash. DC).* 231:1559-1562.
- Pytowski, B., T. G. Easton, J. E. Valinsky, T. Calderon, T. Sun, J. K. Christman, S. D. Wright, and J. Michl. 1988. A monoclonal antibody to a human neutrophil-specific plasma membrane antigen. Effect of the antibody on the C3bi-mediated adherence by neutrophils and expression of the antigen during myelopoiesis. *J. Exp. Med.* 167:421-439.
- Rosen, H., and S. Gordon. 1987. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro

- and inflammatory cell recruitment in vivo. *J. Exp. Med.* 166:1685-1701.
48. Shalaby, M. R., M. A. Palladino, Jr., S. E. Hirabayashi, T. E. Eessalu, G. D. Lewis, H. M. Shepard, and B. B. Aggarwal. 1987. Receptor binding and activation of polymorphonuclear leukocytes by tumor necrosis factor- α . *J. Leukocyte Biol.* 41:196-204.
 49. Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.)*. 336:487-489.
 50. Springer, T. A., D. Davignon, M.-K. Ho, K. Kurzinger, E. Martz, and F. Sanchez-Madrid. 1982. LFA-1 and Lyt-2,3, molecules associated with T-lymphocyte-mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. *Immunol. Rev.* 68:171-195.
 51. Suzuki, S., W. S. Argraves, R. Pytela, H. Arai, T. Krusius, M. D. Pierschbacher, and E. Ruoslahti. 1986. cDNA and amino acid sequences of the cell adhesion protein receptor recognizing vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. *Proc. Natl. Acad. Sci. USA.* 83:8614-8618.
 52. Talle, M. A., P. E. Rao, E. Westberg, N. Allegar, M. Makowski, R. S. Mittler, and G. Goldstein. 1983. Patterns of antigenic expression on human monocytes as defined by monoclonal antibodies. *Cell. Immunol.* 78:83-99.
 53. Todd, R. F., III, M. A. Arnaout, R. E. Rosin, C. A. Crowley, W. A. Peters, and B. M. Babior. 1984. Subcellular localization of the large subunit of Mo1 (Mo1a; formerly gp 110), a surface glycoprotein associated with neutrophil adhesion. *J. Clin. Invest.* 74:1280-1290.
 54. Todd, R. F., III, and D. R. Freyer. 1988. The CD11/CD18 leukocyte glycoprotein deficiency. In *Hematology/Oncology Clinics of America*. Vol. 2. J. Curnutte, editor. W. B. Saunders Company, Philadelphia, PA. 13-31
 55. Van Voorhis, W. C., R. M. Steinman, L. S. Hair, J. Luban, M. D. Witmer, S. Koide, and Z. A. Cohn. 1983. Specific antimononuclear phagocyte monoclonal antibodies. Application to the purification of dendritic cells and the tissue localization of macrophages. *J. Exp. Med.* 158:126-145.
 56. Vedder, N. B., R. K. Winn, C. L. Rice, E. Y. Chi, K.-E. Arfors, and J. M. Harlan. 1988. A monoclonal antibody to the adherence-promoting leukocyte glycoprotein, CD18, reduces organ injury and improves survival from hemorrhagic shock and resuscitation in rabbits. *J. Clin. Invest.* 81:939-944.
 57. Wacholtz, M. C., S. S. Patel, and P. Lipsky. 1989. LFA-1 is an activation antigen for human T lymphocytes. *J. Exp. Med.* In press.
 58. Walker, F., and A. W. Burgess. 1985. Specific binding of radioiodinated granulocyte-macrophage colony-stimulating factor to hemopoietic cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:933-939.
 59. Wallis, W. J., D. D. Hickstein, B. R. Schwartz, C. H. June, H. D. Ochs, P. G. Beatty, S. J. Klebanoff, and J. M. Harlan. 1986. Monoclonal antibody-defined functional epitopes on the adhesion-promoting glycoprotein complex (CDw18) of human neutrophils. *Blood.* 67:1007-1013.
 60. Wayner, E. A., W. G. Carter, R. S. Piotrowicz, and T. J. Kunicki. 1988. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.* 107:1881-1891.
 61. Weitz, J. I., A. J. Huang, S. L. Landman, S. C. Nicholson, and S. C. Silverstein. 1987. Elastase-mediated fibrinogenolysis by chemoattractant-stimulated neutrophils occurs in the presence of physiologic concentrations of antiproteases. *J. Exp. Med.* 166:1836-1850.
 62. Wright, S. D., S. M. Levin, M. T. C. Jong, Z. Chad, and L. G. Kabbash. 1989. CR3 (CD11a/CD18) expresses one binding site for Arg-Gly-Asp-containing peptides, and a second site for bacterial lipopolysaccharide. *J. Exp. Med.* 169:175-184.
 63. Wright, S. D., M. R. Licht, L. S. Craigmyle, and S. C. Silverstein. 1984. Communication between receptors for different ligands on a single cell: ligation of fibronectin receptors induces a reversible alteration in the function of complement receptors on cultured human monocytes. *J. Cell Biol.* 99:336-339.
 64. Wright, S. D., P. E. Rao, W. C. Van Voorhis, 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.* 80:5699-5703.
 65. Wright, S. D., P. A. Reddy, M. T. C. Jong, and B. W. Erickson. 1987. C3bi receptor (complement receptor type 3) recognizes a region of complement protein C3 containing the sequence Arg-Gly-Asp. *Proc. Natl. Acad. Sci. USA.* 84:1965-1968.
 66. Wright, S. D., and S. C. Silverstein. 1983. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J. Exp. Med.* 158:2016-2023.
 67. Wright, S. D., and S. C. Silverstein. 1984. Phagocytosing macrophages exclude proteins from the zones of contact with opsonized targets. *Nature (Lond.)*. 309:359-361.
 68. 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.* 85:7734-7738.
 69. Zimmerman, G. A., and T. M. McIntyre. 1988. Neutrophil adherence to human endothelium in vitro occurs by CDw18 (Mo1, MAC-1/LFA-1/GP 150,95) glycoprotein-dependent and -independent mechanisms. *J. Clin. Invest.* 81:531-537.