

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

Elizabeth A. Wayner* and William G. Carter*‡; and Randolph S. Piotrowicz§ and Thomas J. Kunicki§

*Department of Biochemical Oncology, Fred Hutchinson Cancer Research Center and Pacific Northwest Research Foundation, Seattle, Washington 98104; ‡Department of Pathobiology, University of Washington, Seattle, Washington 98104; and

§The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin 53233

Abstract. We have identified monoclonal antibodies that inhibit human cell adhesion to collagen (PIH5), fibronectin (PIF8 or PID6), and collagen and fibronectin (PIB5) that react with a family of structurally similar glycoproteins referred to as extracellular matrix receptors (ECMRs) II, VI, and I, respectively. Each member of this family contains a unique α subunit, recognized by the antibodies, and a common β subunit, each of ~ 140 kD. We show here that ECMR VI is identical to the fibronectin receptor (FNR), very late antigen (VLA) 5, and platelet glycoproteins Ic-IIa and shall be referred to as FNR. Monoclonal antibodies to FNR inhibit lymphocyte, fibroblast, and platelet adhesion to fibronectin-coated surfaces. ECMRs I, II, and FNR were differentially expressed in platelets, resting or activated lymphocytes, and myeloid, epithelial, endothelial, and fibroblast cell populations, sug-

gesting a functional role for the receptors in vascular emigration and selective tissue localization. Tissue staining of human fetal skin localized ECMRs I and II to the basal epidermis primarily, while monoclonal antibodies to the FNR stained both the dermis and epidermis. Experiments carried out to investigate the functional roles of these receptors in mediating cell adhesion to complex extracellular matrix (ECM) produced by cells in culture revealed that complete inhibition of cell adhesion to ECM required antibodies to both the FNR and ECMR II, the collagen adhesion receptor. These results show that multiple ECMRs function in combination to mediate cell adhesion to complex EMC templates and predicts that variation in ECM composition and ECMR expression may direct cell localization to specific tissue domains.

RECENTLY, we identified a family of cell surface glycoproteins that mediate fibroblast adhesion to specific components of the extracellular matrix (ECM)¹ and are referred to as extracellular matrix receptors (ECMRs) I and II (Wayner and Carter, 1987). Monoclonal antibody to ECMR II (PIH5) specifically inhibited fibroblast (Wayner and Carter, 1987) and nonactivated platelet (Kunicki et al., 1988) adhesion to collagen, while monoclonal antibody against ECMR I (PIB5) inhibited fibroblast adhesion to fibronectin (FN), collagen, and laminin (Wayner and Carter,

1987). Both members of this ECMR family contained a common β and a unique α subunit, each with a molecular mass of ~ 140 kD. The β subunit was identified as the β_1 subunit of the Integrin (Hynes, 1987) family of receptors (Wayner and Carter, 1987). Subsequent studies (Takata et al., 1988) indicated that the functionally defined PIB5 and PIH5 monoclonal antibodies reacted with the α_3 and α_2 subunits of the very late antigens (VLA), respectively. Since the monoclonal antibodies previously used to describe the VLA 3 and VLA 2 antigens (J143 [Fradet et al. 1984] and 12F1 [Pischel et al. 1987], respectively) had no apparent functional activity, it is now possible to describe the VLA 3 and VLA 2 antigens as ECMRs for collagen and possibly other ligands.

Despite promiscuity in binding capability, ECMR I was remarkably similar to the fibronectin receptor (FNR) described by Pytela et al., (1985) and others (Damsky et al., 1985; Brown and Juliano, 1985, 1986). Furthermore, monoclonal antibodies to ECMR I partially inhibited cell adhesion to FN

T. J. Kunicki is an established investigator (83-186) of the American Heart Association.

1. *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; ECM, extracellular matrix; ECMR, extracellular matrix receptor; FN, fibronectin; FNR, fibronectin receptor; LAK, lymphokine activated killer; PBMC, peripheral blood mononuclear cells; VLA, very late antigens.

(Wayner and Carter, 1987). Since neither the anti-ECMR I nor II monoclonal antibodies reacted with the FNR isolated by RGDS peptide elution from FN-Sepharose, we concluded that ECMR I and II represented two new members of the family of ECMRs that included the FNR. However, without a specific probe for the FNR it was not possible to evaluate the cooperative roles of multiple ECMRs in mediating cell localization in tissue. For example, previously described polyclonal and monoclonal antibodies that react with the Integrin β_1 subunit inhibit cell adhesion to multiple ECM components (Horwitz et al., 1985) by reacting with multiple ECMRs. Similarly, RGD-containing peptides that inhibit cell adhesion to ECM components are also not specific enough for evaluating the cooperative role of multiple ECMRs. This is due to the functional expression of the RGD sequence in many adhesive ECM components, including FN, thrombospondin, vitronectin, osteopontin, vWF, and tenasin (Ruoslahti and Pierschbacher, 1987).

In the present studies: (a) we describe monoclonal antibodies PIF8 and PID6 that specifically inhibit the adhesion of human cells to FN, but not collagen or laminin. These antibodies react with a 140-kD surface receptor, termed ECMR VI; (b) we show that ECMR VI is identical to the FNR, to platelet glycoproteins Ic-IIa, and VLA 5, and that PIF8 and PID6 specifically inhibit fibroblast, lymphocyte, and platelet adhesion to fibronectin-coated surfaces; (c) we examine the differential expression of the ECMRs on multiple human cell populations both in culture and in tissue. The results suggest their widespread distribution and possible function as mediators of cell adhesion to the ECM in tissue; (d) the availability of multiple monoclonal antibodies that inhibit the function of specific ECMRs allowed us to evaluate their combined roles in mediating cell adhesion to ECM produced by WI-38 human embryonic lung fibroblasts. Our results indicate that multiple ECMRs function to mediate cell adhesion to ECM and predicts the possible combined action of multiple ECM and ECMR components in localizing cells to specific tissue domains.

Materials and Methods

Materials

Phenylmethylsulfonyl fluoride (PMSF), N-ethylmaleimide, diisopropyl fluorophosphate, 2-mercaptoethanol, BSA, Triton X-100, EDTA, protein A-Agarose, and V8 protease (from *Staphylococcus aureus*, strain V8, protease type XVII) were purchased from Sigma Chemical Co. (St. Louis, MO). Lactoperoxidase and glucose oxidase were from Calbiochem-Behring Corp. (San Diego, CA). Fluorescein-conjugated goat anti-mouse IgG and IgM (H and L chains) or rhodamine-conjugated goat anti-rabbit IgG and IgM (H and L chains) were obtained from Tago Inc. (Burlingame, CA). Rabbit anti-mouse IgG (H + L) antiserum was obtained from Cappel Laboratories (Malvern, PA). ^{51}Cr -sodium chromate was from New England Nuclear (Boston, MA). ^{125}I was from Amersham Corp. (Arlington Heights, IL). The gly-arg-gly-asp-ser (GRGDS) peptide was obtained from Peninsula Laboratories, Inc. (Belmont, CA). Human recombinant interleukin-2 was a generous gift from Dr. D. Urdal (Immunex Corp., Seattle, WA).

Cells and Cell Culture

Normal diploid human embryonic lung fibroblasts, WI-38 cells, and SV-40 virus transformants of WI-38 cells, WI-38 VA13 cells, and a human fibrosarcoma (HT1080) were obtained from the American Type Culture Collection (Rockville, MD). Primary cultures of human fetal skin fibroblasts were prepared by collagenase digestion of tissue. Human fetal skin was obtained from Dr. Thomas H. Shepard (Central Lab for Human Em-

bryology, University of Washington, Seattle, WA). The human carcinoma cell lines: MKN 45 (poorly differentiated gastric adenocarcinoma), MKN 28 (well differentiated gastric adenocarcinoma), HuTu 80 (carcinoma of the duodenum), MCF-7 (breast adenocarcinoma), A431 (epidermoid carcinoma), and QG-56 (squamous cell carcinoma of the lung) were obtained from Dr. Sen-iroh Hakomori (Fred Hutchinson Cancer Center, Seattle, WA). Human Tera-2 cells (human embryonic carcinoma) were obtained from Dr. Bruce Fenderson (Biomembrane Institute, Seattle, WA). Fibroblast and carcinoma cell lines were grown as monolayer cultures in RPMI 1640 supplemented with 10% FBS. OC-1 cells were obtained from sterile ascites removed from a patient with metastatic carcinoma of the ovary and were used fresh in suspension for flow cytometry. Primary cultures of normal human neonatal foreskin keratinocytes were obtained from Dr. Pritinder Kaur at the Fred Hutchinson Cancer Research Center.

Peripheral blood mononuclear cells (PBMC) from normal human donors were purified on ficoll/hypaque ($d = 1.077$) according to a standard protocol (Mishell and Shiigi, 1980). Adherent monocytes were obtained by allowing unfractionated PBMC to adhere on tissue culture dishes in the presence of 2% FBS for 30 min at 37°C. The plastic nonadherent peripheral blood lymphocytes were collected and consisted of 80-90% T lymphocytes by cell surface phenotype analysis (CD2+, CD3+). The adherent cells consisted of 80-90% monocytes by morphological criterion. Granulocytes were purified from ficoll/hypaque pellets by ammonium chloride lysis of red blood cells and contained >95% neutrophils and <5% eosinophils, basophils, and lymphocytes. Human lymphokine activated killer (LAK) cells (Grimm et al., 1982) were prepared by incubating plastic nonadherent peripheral blood lymphocytes at 2×10^6 cells/ml in Click's medium supplemented with 10% FBS and 500 U/ml human recombinant interleukin-2 for 7-14 d. The monoclonal HLA B7 specific human cytotoxic T lymphocyte (CTL) cell line was established using a standard protocol (Glasebrook and Fitch, 1980; Brooks, 1983). CTL and LAK cells demonstrated the surface phenotype of classical cytotoxic T lymphocytes (CD2+, CD3+, CD8+, CD4-, Leu 11b-, OKM1-). All other lymphoid cell lines, Molt 4 (T cell leukemia), HL-60 (promonocyte cell line), and BLCL (Epstein Barr virus transformed B lymphocyte cell line) were maintained in suspension in RPMI 1640 supplemented with 10% FBS. Unactivated human platelets were obtained from the peripheral blood of normal donors as described (Kunicki et al., 1985).

Antibodies

Rabbit polyclonal antibodies prepared against the FN receptor as described by Pytela et al. (1985) were a generous gift from Dr. M. D. Pierschbacher (La Jolla Cancer Research Federation, La Jolla, CA). Monoclonal antibody AIA5 against the common Integrin (Hynes, 1987) β_1 subunit of the VLA family of receptors (Hemler, 1988) was obtained from Dr. Martin Hemler (Dana-Farber Cancer Inst., Boston, MA). Monoclonal anti-ECMR I and II (Wayner and Carter, 1987) and their relation to the VLAs (Takada et al., 1988) have been described. Rabbit polyclonal antibodies to type VI collagen and fibronectin were prepared and characterized as previously described (Carter, 1982). Rabbit polyclonal antibodies to laminin were prepared and found to be specific for laminin by immunoblotting and solid phase radioimmunoassay.

Monoclonal anti-ECMR VI (anti-FNR) was produced by the methods of Oi and Herzenberg (1980) and Taggart and Samloff (1983), as described in detail (Wayner and Carter, 1987). Immune spleens from RBF/Dn mice (Jackson Labs, Bar Harbor, ME) immunized with 100 μl of packed LAK cells were removed and fused with NS-1/FOX-NY myeloma cells. Viable heterokaryons were selected in RPMI 1640 supplemented with adenine/aminopterin/thymidine, AAT, (Taggart and Samloff, 1983). Heterokaryons producing antibody directed to the fibronectin receptor were screened by specific inhibition of cell adhesion to FN. Selected hybrids were cloned by limiting dilution with thioglycolate-induced, irradiated BALB/c peritoneal macrophages or thymocytes as feeder cells.

Inhibition of Cell Adhesion

Antibodies that would alter cell adhesion to either or both FN or collagen Types I and III were selected as previously described (Wayner and Carter, 1987). Briefly, 48-well virgin styrene plates were coated with pepsin-generated type I and III collagen or human plasma FN (5 $\mu\text{g}/\text{ml}$). The plates were blocked with PBS (137 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl_2 , 10 mM Na_2HPO_4 , 1.5 mM K_2HPO_4) supplemented with 10 mg/ml heat denatured BSA. HT1080 cells were labeled with $\text{Na}_2^{51}\text{CrO}_4$ (50 $\mu\text{Ci}/\text{ml}$ for 2-4 h), washed, and 5×10^4 cells per well were incubated with the hybridoma culture supernatants (1:2 dilution in PBS supplemented with 1 mg/ml heat dena-

tured BSA) or control myeloma cell culture supernatant for 15 min at room temperature. The cells were allowed to adhere to the protein-coated surfaces in the presence of the hybridoma supernatants for 15–30 min at 37°C. Nonadherent cells were removed by washing with PBS, and the adherent cells were dissolved in SDS/NaOH, and bound ⁵¹Cr-cpm were quantitated in a gamma counter.

Platelet adhesion to FN and collagen-coated surfaces was as previously described (Kunicki et al., 1988).

Preparation of Adhesive Proteins

FN: human plasma FN was purified from human plasma by affinity chromatography on gelatin-Sepharose according to the method of Engvall and Ruoslahti (1977). Pepsinized collagen types I and III: all collagens were isolated from fresh human placenta after digestion with pepsin as described by Miller and Rhodes (1982). Laminin: mouse laminin was isolated from Englebreth-Holmswam sarcoma grown in BALB/c mice as described by Timpl et al. (1979).

Preparation of WI-38 ECM

WI-38 cells were grown to confluence in 24-well tissue culture plates in the presence of 10 µg/ml ascorbate. The adherent cells and ECM were extracted basically as previously described to prepare an adherent ECM composed primarily of FN and type VI collagen, previously referred to as GP140 (Carter, 1982, 1984): (a) emipgen BB zwitterionic detergent to remove membrane, and cytoplasmic components; (b) 2 M urea in 1 M NaCl to solubilize cytoskeletal and nuclear components; (c) digestion with DNase I (10 µg enzyme/ml PBS for 30 min at 37°C) to remove residual DNA present in the ECM layer. The stable ECM was then used in adhesion and inhibition studies as described in Fig. 9.

Immune Precipitation and Sequential Immune Precipitation

HT1080, LAK cells, and platelets were surface labeled with radioactive iodine using the lactoperoxidase/glucose oxidase method (Hynes, 1973), followed by extraction with 1% vol/vol Triton X-100 detergent in 25 mM Tris-HCl buffer, pH 7.5, (Triton/Tris) containing 1 mM diisopropyl fluorophosphate, or 1 mM PMSF plus 2 mM N-ethylmaleimide as protease inhibitors. Immune precipitation and sequential immune precipitation were performed as previously described (Wayner and Carter, 1987).

V8 Protease Peptide Analysis

Peptide analysis came after the basic procedure of Cleveland et al. (1977) with modifications as previously described (Wayner and Carter, 1987).

Isolation of RGDS-eluted FNR: Reactivity of FNR with Class I, II, and VI Monoclonal Antibodies

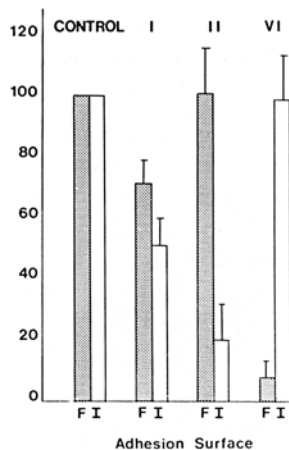
FN receptor was isolated from octylglucoside extracts basically as described by Pytela et al. (1985) and as follows. Labeled HT1080 cells were extracted in 100 mM octylglucoside detergent in PBS at 4°C containing 1 mM PMSF as a protease inhibitor. The octylglucoside extracts were chromatographed on insoluble FN at 4°C and washed with octylglucoside (25 mM) in PBS. A 140-kD bound receptor was specifically eluted with the peptide gly-arg-gly-asp-ser (1 mg/ml). FN-Sepharose was prepared as previously described (Wayner and Carter, 1987).

PAGE

Polyacrylamide slab gels containing SDS (SDS-PAGE gels) were prepared after the basic stacking gel system of Laemmli (1970) and as previously described (Wayner and Carter, 1987). Prestained protein standards for relative molecular mass estimation were obtained from Bethesda Research Laboratories (Gaithersburg, MD) and were as follows: lysozyme, 14,300 kD; β-lactoglobulin, 18,400 kD; α-chymotrypsin, 25,700 kD; ovalbumin, 43,000 kD; BSA, 68,000 kD; phosphorylase B, 97,400 kD; and myosin (H chain) 200,000 kD.

Two-dimensional SDS gels that were run under reducing and nonreducing conditions were performed as described (Kunicki et al., 1988).

Figure 1. Isolation of ECMR VI-specific monoclonal antibodies. Effect of anti-ECMR I, II, and VI monoclonal antibodies on adhesion of HT1080 cells to fibronectin- or collagen-coated surfaces. ⁵¹Cr-labeled HT1080 cells were incubated in the presence of hybridoma culture supernatants anti-ECMR I (PIB5), anti-ECMR II (PIH5), and anti-ECMR VI (PIF8), and allowed to adhere to fibronectin- (F) or type I collagen- (I) coated plastic surfaces. After nonadherent cells were removed, adherent cells were solubilized and radioactivity quantitated in a gamma counter. Results are expressed as a percent of control (myeloma culture supernatant) adhesion (mean of three experiments ± standard deviation).



Fluorescence Analysis of Receptor Expression

Expression of ECMR classes I, II, and VI on cells in suspension was analyzed by flow cytometry on a FACS II cell sorter (Becton Dickinson & Co., Sunnyvale, CA). Expression of ECMRs on adherent cells was determined by immunofluorescence microscopy as described (Carter and Wayner, 1988).

Tissue Staining

The distribution of ECMRs I, II, and VI in tissue was determined by fluorescence microscopy of cryostat sections. Cryostat sections (6 µ) were prepared from human fetal skin, and lung or adult skin and tonsil samples embedded in OCT medium after snap freezing in isopentane/liquid nitrogen. All sections were fixed in 4% paraformaldehyde in PBS before incubation in primary antibodies and secondary fluorescent antibodies (Carter and Wayner, 1988). The samples were examined with a Zeiss fluorescence microscope using a 40× oil immersion objective and photographed with Kodak T-Max film shot at ASA 800. In control experiments, no fluorescence of rhodamine was detected using the fluorescein filters or vice versa.

Results

Isolation of Monoclonal Antibodies that Specifically Inhibit Cell Attachment to FN

Monoclonal antibodies that would inhibit cell adhesion to FN were obtained by screening hybridoma culture supernatants for the specific inhibition of fibroblast adhesion to FN-coated surfaces. The antibodies that were obtained using this protocol, PIF8 and PID6, reacted with a cell surface receptor referred to as ECMR VI and specifically inhibited HT1080 cell adhesion to FN but not collagen (Fig. 1) or laminin-coated surfaces (data not shown). Specific inhibition of HT1080 cell adhesion with the anti-ECMR II (PIH5) and anti-ECMR VI (PIF8) monoclonal antibodies was observed on collagen or FN-coated surfaces, respectively (Fig. 1). As previously reported (Wayner and Carter, 1987), anti-ECMR I (PIB5) monoclonal antibody partially inhibited fibroblast attachment to both FN and collagen (Fig. 1).

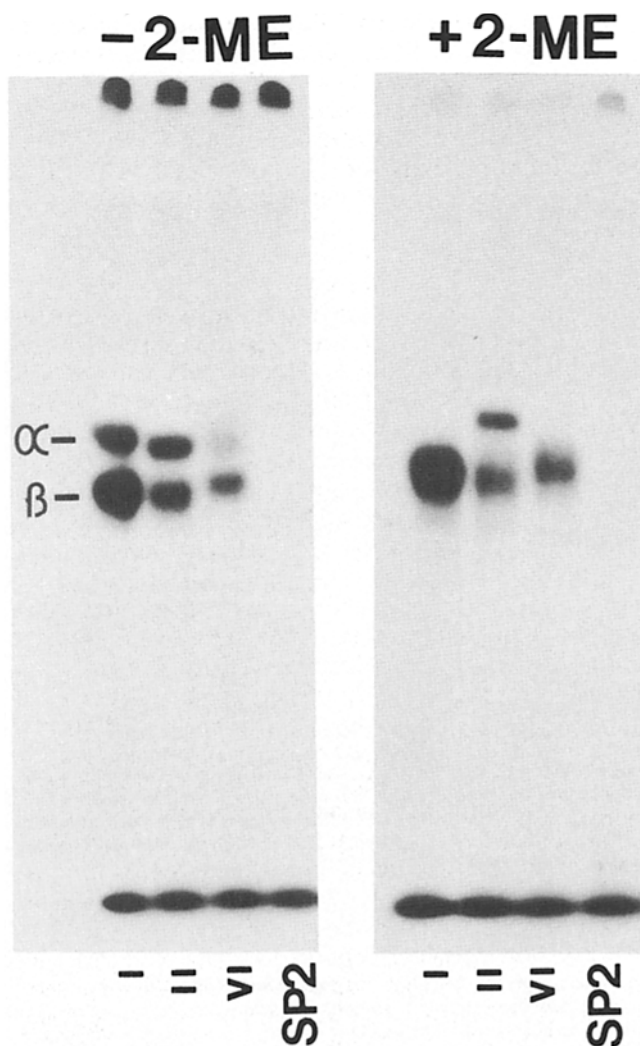


Figure 2. The effect of 2-mercaptoethanol on the migration of the α and β subunits of ECMRs I, II, and VI. HT1080 cells were surface labeled with radioactive iodine, then extracted with Triton X-100 detergent. Aliquots of the extracts were then immune precipitated with SP2 culture supernatant or anti-ECMR I (PIB5), II (PIH5), and VI (PIF8) monoclonal antibodies. The immune precipitates were run on 7% SDS-PAGE gels in the presence (+2-ME) or absence (-2-ME) of 2-mercaptoethanol and visualized by autoradiography. Migration of the α and β subunits of the receptors in the absence of reducing agent is indicated.

Biochemical Characteristics of ECMR VI and Comparison with ECMRs I and II

The PIF8 (Fig. 2) or PID6 monoclonal antibodies both immune precipitated a single cell surface protein containing two noncovalently linked subunits of 147 and 125 kD, termed α and β , respectively, from Triton X-100 detergent extracts of ^{125}I surface-labeled HT1080 cells when run on SDS-PAGE gels in the absence of 2-mercaptoethanol (Fig. 2, see -2-ME column). In the presence of 2-ME, ECMR VI migrated as a single band of ~ 135 – 140 kD (Fig. 2, see +2-ME column). HT1080 ECMR VI appeared to be indistinguishable from ECMR I by single dimension SDS-PAGE analysis under both reducing and nonreducing conditions. However, ECMR VI was easily distinguishable from ECMR II due to the characteristic migration of ECMR II under

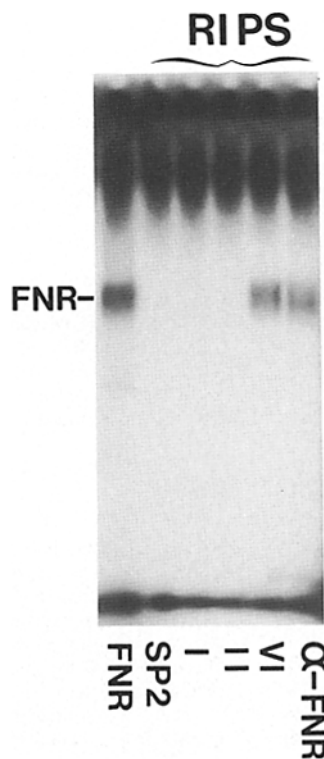


Figure 3. Isolation of the HT1080 FNR by elution with RGDS-containing peptide from FN-Sepharose and immune precipitation of purified FNR with anti-ECMR VI monoclonal antibody. Surface-labeled HT1080 cells were extracted with octylglucoside detergent (100 mM) in PBS at 4°C. The octylglucoside extracts were chromatographed on FN-Sepharose at 4°C. The FNR was eluted with the peptide gly-arg-gly-asg-ser (1 mg/ml), and immune precipitated with SP2 culture supernatant, anti-ECMR I (PIB5), II (PIH5), or VI (PIF8) monoclonal antibodies, or polyclonal antibodies specific for the FNR (α -FNR).

reducing conditions (Fig. 2, +2-ME) as resolvable α and β subunits.

In a series of control experiments (results not shown) using previously described techniques (Wayner and Carter, 1987), we have characterized ECMR VI in relation to ECMRs I and II as follows. (a) Metabolic pulse-chase studies revealed that ECMR VI was not a precursor to any other ECMR and suggested that the functionally defined monoclonal antibodies, PIF8 and PID6, recognized epitopes on the α or α - β subunit combinations. (b) Sequential immune precipitation of the ECMR I, II, and VI antigens with the appropriate antibodies from Triton X-100 detergent extracts of ^{125}I -labeled HT1080 cells indicated that neither antibody class recognized antigen from the other ECMR classes. However, sequential immune precipitation with PIF8 quantitatively precipitated all the PID6 antigen present in the extracts, confirming the inhibition of cell adhesion results (Fig. 1) that indicated that the PIF8 and PID6 monoclonal antibodies were reacting with epitopes present on the same receptor. (c) Peptide mapping of the α subunits of ECMRs I and II produced two distinct peptide maps (Wayner and Carter, 1987). Similarly, the peptide map of the α subunit of ECMR VI was also distinct from either ECMR I or II. The peptide maps of the HT1080 ECMR I, II, and VI β subunits were virtually identical, suggesting a high degree of amino acid sequence homology and clearly identifying all three receptors as members of the same ECMR family. In addition, a polyclonal antibody that reacts with the β subunit of the FNR and a monoclonal antibody, A1A5 that reacts with the Integrin β_1 subunit of the VLA family of receptors, both reacted with the β subunit from all three ECMRs, confirming that the β subunits of the ECMRs were identical to the β_1 subunit of the Integrin super family of receptors.

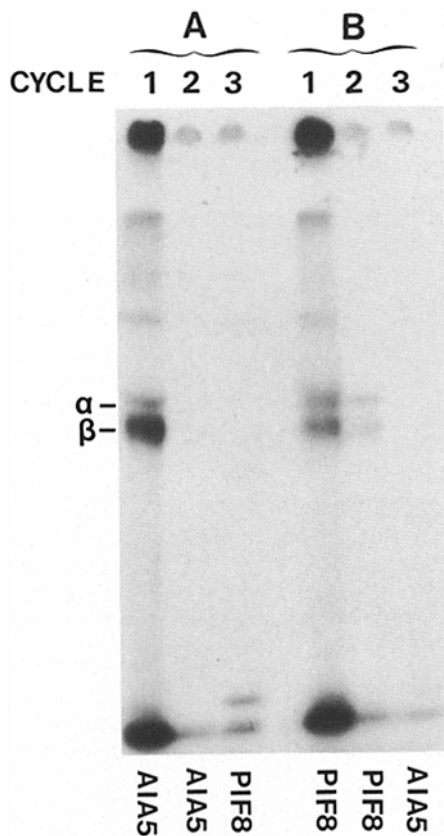


Figure 4. Sequential immune precipitation of FNR (ECMR VI) from K562 cells: ECMR VI is identical to VLA 5. K562 cells were surface labeled with ^{125}I and extracted with Triton X-100 detergent. Hemler et al. (1987) has previously published that K562 cells express primarily VLA 5. Aliquots of labeled extract were subjected to three cycles of immune precipitation as follows. (A, cycle 1) Monoclonal antibody AIA5 was used to precipitate all VLA antigen containing the common β_1 subunit that is recognized by the AIA5 monoclonal antibody. (cycle 2) The extract was reprecipitated with AIA5 to ensure that all VLA antigen was quantitatively removed. (cycle 3) The VLA-free extract was then immune precipitated with anti-FNR (PIF8) monoclonal antibodies. (B) The experiment was also performed in the reverse direction preclearing first with PIF8 then with AIA5 to examine whether monoclonal anti-FNR could preclear VLA 5. The antigens brought down in the three immune precipitation steps were analyzed on SDS-PAGE gels (7.5%) in the absence of 2-mercaptoethanol followed by autoradiography.

ECMR VI Is Identical to the FNR, VLA 5, and Platelet Glycoprotein Ic-IIa

The relationship of ECMR VI to the FNR described by Pytela et al. (1985) was examined by immune precipitation of affinity-purified and RGDS-eluted FNR (Fig. 3). FNR was purified by affinity chromatography of octylglucoside detergent extracts of ^{125}I surface-labeled HT1080 cells on FN-Sepharose. The bound FNR was specifically eluted with the GRGDS peptide but not the control peptide, Tuftsin. Monoclonal antibodies prepared against the ECMR VI immune precipitated the purified FNR (Fig. 3). Affinity-purified FNR also reacted with rabbit polyclonal antibodies prepared against the FNR (Pytela et al., 1985). This polyclonal anti-

body preparation precleared ECMR VI antigen, and cross reacted with ECMR VI antigen affinity purified with monoclonal antibody (data not shown). Neither ECMR I nor II specific monoclonal antibodies reacted with the GRGDS-eluted FNR (Fig. 3). These data show that the ECMR VI, recognized by the monoclonal antibodies PIF8 and PID6, is identical to the FNR and will therefore be referred to as FNR.

Takada et al. (1987) have recently suggested that VLA 5 is identical to the FNR and is the major VLA expressed in K562 cells. However, no monoclonal antibody specific for the α subunit of VLA 5 has been described. As seen in Fig. 4, PIF8 monoclonal antibody quantitatively immune precipitated all the antigen detected by the common β_1 subunit-specific monoclonal antibody, AIA5, from K562 cells. Both PIF8 and PID6 also completely abrogated K562 cell adhesion to FN-coated surfaces (results not shown). Therefore, PIF8 and PID6 clearly react with the VLA 5 antigen and importantly, completely inhibit K562 cell adhesion to the FN ligand.

Piotrowicz et al. (1988) have recently reported that adhesion of nonactivated platelets to fibronectin is mediated by platelet glycoprotein Ic-IIa. As seen in Fig. 5, monoclonal antibodies PIF8 and PIH5 specifically inhibit platelet adhesion to fibronectin and collagen, respectively. In contrast to results obtained with HT1080 cells, platelet adhesion to FN was only partially inhibitable with PIF8. This was in contrast to the virtual quantitative nature of the inhibition of platelet adhesion to collagen with PIH5 monoclonal antibody. Conceivably, platelet adhesion to FN may be mediated by more than one receptor, only one of which is recognized and inhibited by the PIF8 monoclonal antibody.

The antigen immune precipitated with the PIF8 antibody from ^{125}I -labeled platelet extracts was identified as platelet glycoproteins Ic and IIa by two-dimensional gel analysis and corresponded to the α and β subunits of ECMR VI (FNR), respectively (Fig. 6).

Expression of ECMRs I, II, and FNR on Multiple Human Cell Populations

Analysis of the expression of ECMRs I, II, and FNR on a wide variety of human cell populations revealed the univer-

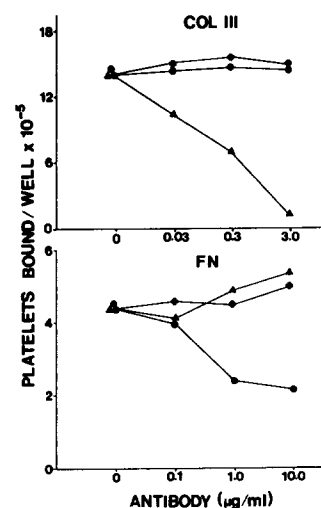


Figure 5. Inhibition of platelet adhesion to FN and collagen with PIF8 and PIH5 monoclonal antibodies, respectively. The effect of monoclonal IgG on the adhesion of ^{51}Cr -labeled platelets to microtiter trays coated with fibronectin (FN) or type III collagen (COL III) was assessed. APl is an IgG1 murine monoclonal antibody specific for human platelet glycoprotein Ib (Okita et al., 1985). The number of platelets adherent to wells coated with BSA was routinely <1% of that observed in fibronectin-coated wells. ●, PIF8; ▲, PIH5; ♣, APl.

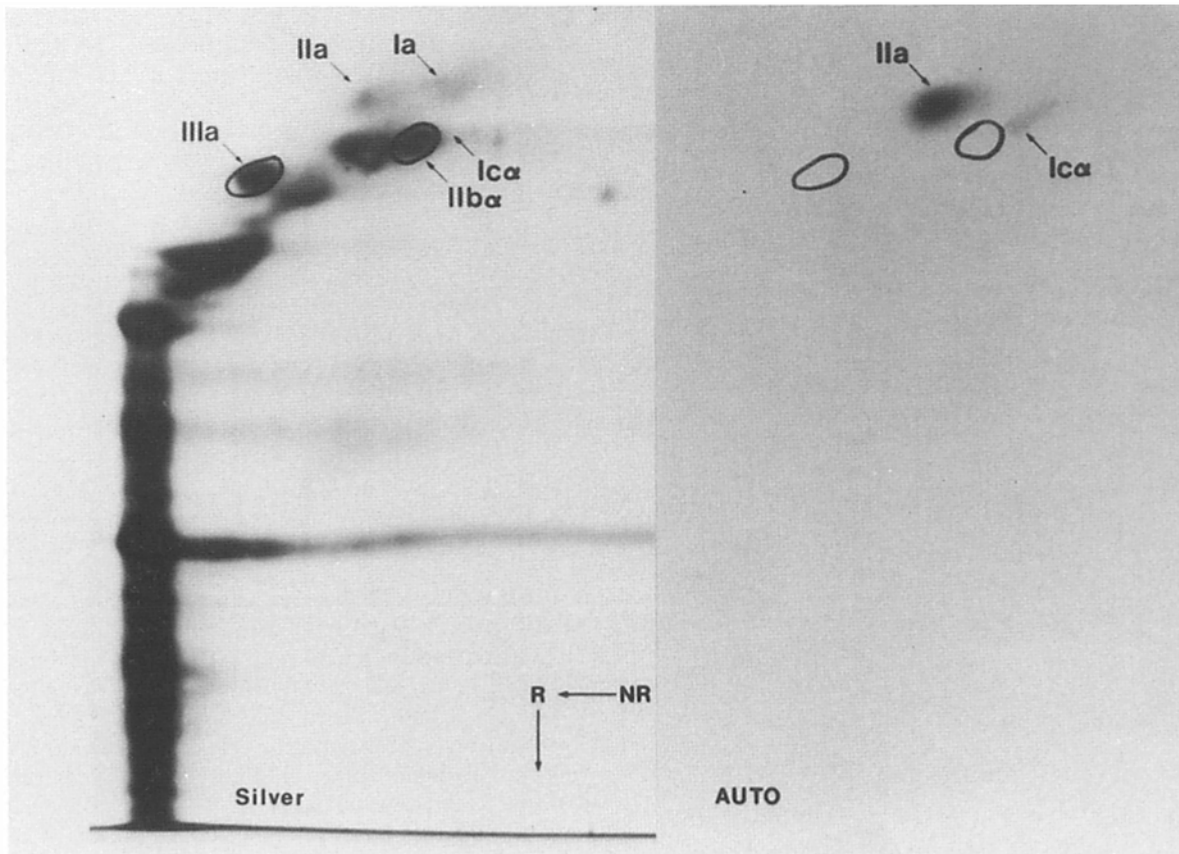


Figure 6. Identification of the α and β subunits of ECMR VI in platelets as platelet glycoprotein Ic and IIa, respectively. Radioiodinated antigens precipitated by PIF8 were mixed with 100 μ g of nonlabeled platelet protein and subjected to electrophoresis under nonreducing (right to left) and then reducing (top to bottom) conditions. Gel stained with silver stain (*Silver*). The positions of known nonlabeled platelet proteins are indicated. (*AUTO*) Autoradiograph of gel containing the PIF8 antigen. The positions of radioiodinated proteins that comigrate with glycoproteins Ic and IIa are indicated. The migration of IIb and IIIa are also indicated as loops.

sal expression of these receptors (Table I). What is also immediately apparent from these data is that different cell populations express different quantities of receptors. Peripheral blood mononuclear cells, platelets, and fibroblasts were positive for FNR, while epithelial cells expressed little FNR. Alternatively, epithelial cells were positive for ECMR I, while expression of this receptor on mesodermally derived cells was restricted primarily to activated lymphocytes (CTL and LAK), monocytes, or transformed fibroblasts. Umbilical vein endothelial cells, resting small lymphocytes, and platelets were highly positive for the collagen receptor, ECMR II, and expressed little or no ECMR I. As we have previously reported expression of ECMR I by cytotoxic T lymphocytes requires activation with specific antigen (CTL) or interleukin-2 (LAK). This is in agreement with our previous finding that activated CD8⁺ T lymphocytes adhere to collagen and laminin-coated surfaces (Wayner and Carter, 1987; Wayner and Carter, manuscript submitted for publication).

Expression of the ECMRs in Human Fetal Skin

We expected that localization of the ECMRs in tissue would correspond to regions that contained the appropriate ECM ligand. As seen in Fig. 7, type VI collagen and FN were localized throughout the dermis of human fetal skin. Anti-

bodies specific for types I or VI collagen and fibronectin did not stain epidermis. In contrast, laminin was localized to the basement membrane zone and did not extend into the dermis except in association with hair follicles as in the field shown (Fig. 7) or in glandular epithelium, not shown. The PIB5 monoclonal antibody to the promiscuous ECMR I localized to the basal cells of the epidermis that lie in contact with laminin located in the basement membrane zone. In contrast, ECMR II (PIH5), the collagen specific receptor, localized throughout the epidermis but more intensely in the more differentiated cells than the basal cells that are in contact with the basement membrane. FNR (PIF8), in contrast to ECMRs I and II, had a much wider distribution and was detected in abundance in both the dermis and epidermis. We observed similar patterns for the expression of ECMRs I, II, and FNR in epithelial and stromal cell populations in various human tissue including adult skin, tonsil, fetal lung, and intestine.

Collagen-binding Site on ECMR II Is Blocked in the Dermis

Interestingly, antibody to the ECMR II (PIH5) collagen receptor failed to localize in the collagen-rich dermis (Fig. 7). This was even more surprising since we routinely detected ECMRs I, II, and FNR on fibroblasts derived from

Table I. Expression of ECMRs I, II, and VI (FNR) in Human Cells

Cells	Fluorescence intensity [‡]		
	I (PIB5)	II (PIH5)	VI (PIF8)
Lymphocytes			
Nonadherent peripheral blood lymphocytes	-(*)	+++(*)	+(*)
B lymphocyte (EBV transformed)	-	-	+
Molt 4 (T cell leukaemia)	-(*)	-(*)	++(*)
JU KAT (T cell leukaemia)	-(*)	++(*)	+(*)
Cytotoxic T lymphocyte (CTL)	+(*)	+++(*)	+++(*)
Lymphokine activated killer (LAK)	+++(*)	+++(*)	+++(*)
Fibroblasts			
Primary fetal skin fibroblasts	+	++	+
WI-38 (embryonic lung)	+(*)	++(*)	+(*)
WI-38 VA13 (SV-40 transformed)	+++(*)	++++(*)	+++(*)
HT-1080 (fibrosarcoma)	+++(*)	++++(*)	++(*)
Epithelial cells (carcinomas)			
Primary keratinocytes	++	++	+/-
MKN 28 (gastric, adeno)	++	++	+/-
MKN 45 (gastric, adeno)	++	++	+/-
QG 56 (lung, squamous)	+/-(*)	+++(*)	-(*)
A431 (epidermoid)	+++	+	+/-
MCF-7 (breast, adeno)	+++	+	+/-
Hutu-80 (duodenum)	+	+	+/-
Tera-2 (teratocarcinoma)	+	-	-
OC-1 (ovarian)	+++	-	+/-
Others			
Adherent monocytes	+	+++	++
Granulocytes	-	+/-	-
HL-60 (promonocyte)	-	-	+
K562 (erythroleukemia)	-	-	++
Endothelial cells (umbilical)	+/-	+++	+
Platelets (unactivated)	-(*)	+++(*)	+(*)

[‡] Fluorescence intensity was determined by flow cytometry on nonadherent cells and by fluorescence microscopy on adherent cells. The asterik indicates those results that have been confirmed by immune precipitation with the indicated antibodies and SDS-PAGE gel analysis. The minus sign indicates no detectable fluorescence or band formation on SDS-PAGE gels.

primary cultures of the same skin used for tissue staining (Table I). In control experiments other membrane glycoproteins, including the ECMR III 90-kD intrinsic membrane glycoprotein (Wayner and Carter, 1987; Carter and Wayner, 1988) or the FNR, were readily detected in the dermis and epidermis with monoclonal antibodies (Fig. 7). Thus, our failure to detect staining in the dermis with ECMR II (PIH5) monoclonal antibodies was not due to inaccessibility of the plasma membranes of the stromal cells. The PIH5 monoclonal antibody used in the above studies was originally selected for its ability to inhibit cell adhesion to collagen and therefore may react with an epitope in or near the collagen-binding site on the ECMR II α subunit (Figs. 1 and 2). Because the dermis is rich in its content of collagen types that have been shown to mediate fibroblast adhesion via ECMR II, we reasoned that the epitope recognized by PIH5 on cells in the dermis might be blocked with ligand. Alternatively, the epitope recognized by the PIH5 monoclonal antibody may be cryptic or conformation dependent. To test these possibilities, we rescreened our battery of hybridomas for a monoclonal antibody that reacted with an epitope on the ECMR II α subunit that was distal from the collagen-binding site and would therefore not inhibit cell adhesion. Monoclonal antibody P4B4 was selected on the basis of the above criteria and was characterized as follows (results not shown).

(a) P4B4 precleared ECMR II, but not ECMR I, from detergent extracts of HT1080 cells. (b) pH-induced subunit dissociation experiments indicated that, like PIH5, P4B4 reacted with the α subunit of ECMR II. (c) In contrast to the PIH5 monoclonal antibody, P4B4 had no effect on cell adhesion to collagen indicating that it reacted with an epitope on the II α subunit that was distal from the collagen binding site (Fig. 8). (d) Both the PIH5 and P4B4 monoclonal antibodies were of the IgG_{2b} subclass and therefore had similar access capabilities in the tissue.

Using P4B4, ECMR II was found to distribute in both the dermis and epidermis in contrast to the results obtained with PIH5 (Fig. 7). Two conclusions can be made from these data: (a) the ECMR II codistributes in dermis with interstitial collagen as well as in epidermis, and (b) virtually all of the epitope recognized by the PIH5 monoclonal antibody is blocked or cryptic in the dermis, possibly because it is bound to collagen. In contrast, in the epidermis, where collagen deposition is minimal (Fig. 7), the epitopes recognized by both the PIH5 and P4B4 antibodies are both detected.

Role of Multiple ECMRs in Mediating Cell Adhesion to a Complex ECM Containing FN and Type VI Collagen

The availability of monoclonal antibodies to ECMRs I, II, and FNR that selectively inhibit the function of these recep-

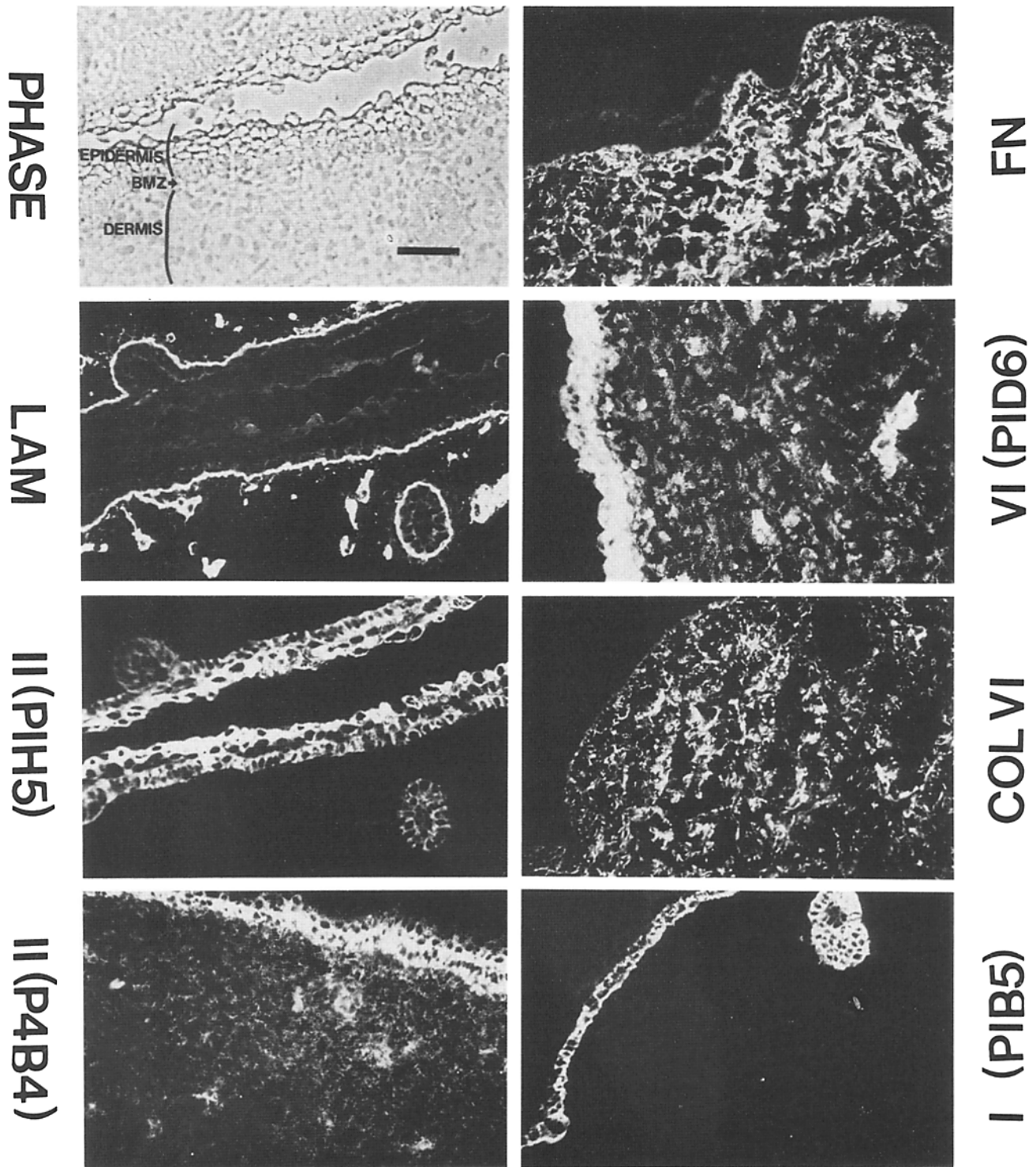


Figure 7. Localization of ECMRs I, II, and FNR (ECMR VI) in human fetal skin sections by immunofluorescence microscopy. Cryostat sections of human fetal skin were incubated with the indicated mouse monoclonal antibodies to ECMRs I, II, and VI. Where indicated, rabbit polyclonal antibodies were used to localize type VI collagen (*COL VI*), fibronectin (*FN*), and laminin (*LAM*). Bound antibodies were detected with rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-mouse IgG. In the section marked *PHASE*, the tissue layers corresponding to epidermis, basement membrane zone (*BMZ*), and dermis are indicated. *PHASE*, *LAM*, and *II (PIH5)*: same field. *COL VI*, and *I (PIB5)*: same field. *FN* and *VI (PID6)*: different fields. Monoclonal antibodies *PIH5* and *P4B4* both react with the α subunits of ECMR II, the collagen receptor. *P4B4* does not inhibit cell adhesion to collagen, while *PIH5* does. *P4B4* stains both the dermis and epidermis and *PIH5* stains only the epidermis. Bar, 50 μ M.

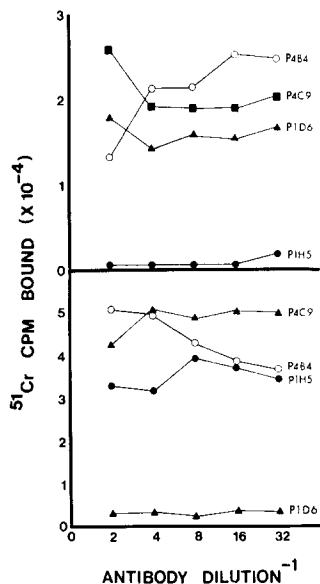


Figure 8. Inhibition of HT-1080 cell adhesion to collagen and FN using monoclonal antibodies P4B4 and PIH5 directed to different epitopes of ECMR II. Surfaces coated with collagen (type I, *top*) and fibronectin (*bottom*) were incubated with ⁵¹Cr-labeled HT-1080 cells in the presence of serial twofold dilutions of the indicated monoclonal antibodies in culture medium. *PID6*, anti-ECMR VI. *P4B4*, anti-ECMR II α . *PIH5*, anti-ECMR II α . *P4C9*, anti-CD18 (LFA, β_3 subunit). Anti-CD18 is a negative control since the β_3 subunit is not expressed on HT1080 cells.

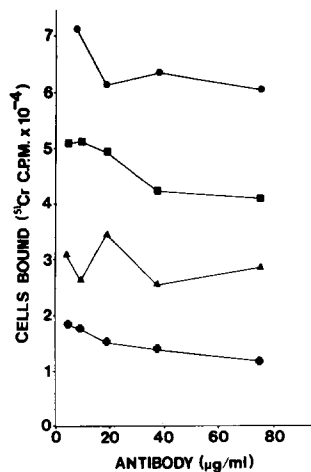


Figure 9. The function of ECMRs II and VI in mediating WI-38 VA13 cell adhesion to WI-38 ECM. WI-38 cells were grown to confluence in 24-well culture plates then extracted to prepare confluent, cell-free layers of ECM as described in Materials and Methods. This ECM has been previously characterized and contains primarily polymeric FN and type VI collagen. ⁵¹Cr-Labeled WI-38 VA13 cells were added to the ECM layers in the presence of the indicated purified monoclonal antibodies incubated for 60 min, then nonadherent cells

were removed by washing and the adherent cells dissolved and counted in a gamma counter. Where indicated, equal quantities of PIH5 and PID6 were added together to obtain the indicated final concentration of antibody. ●, CONT; ■, PIH5; ▲, PID6; and ◆, PIH5 + PID6.

tors allows us to evaluate their individual or combined roles in cell adhesion to complex ECM. The effect of monoclonal antibodies PIH5 and PIF8 on adhesion of WI-38 VA13 cells to WI-38 ECM is shown in Fig. 9. WI-38 ECM is composed primarily of polymeric fibronectin and type VI collagen (Carter, 1982; 1984). Each antibody alone only partially inhibits cell adhesion to ECM even at saturating levels. However, PIF8 and PIH5 in combination completely inhibited the WI-38 VA13 cell adhesion to WI-38 ECM. These data suggest that ECMR II and FNR function together in mediating adhesion to complex ECM composed of multiple ligands.

Discussion

A Model for the Role of Multiple ECMRs in Mediating Cell Adhesion to the ECM

A summary of our conclusions concerning the role of ECMRs I, II, and FNR (ECMR VI) in mediating cell adhesion to a complex ECM are presented in Fig. 10. What is im-

mediately evident from this model is that two classes of receptors exist: those like ECMR II and FNR that specifically interact with collagen and fibronectin, respectively, and those that are promiscuous, like ECMR I, that can interact with multiple ECM components. We have previously suggested that the extracellular disulfide-dependent assembly of type VI collagen (Carter, 1982) is dependent on cell surface contact with the ECM (Carter, 1984). It is conceivable that ECMRs like II and FNR that are involved in cell adhesion might also be involved in assembly or polymerization of collagen and FN in the ECM (Carter, 1982, 1984). We have reported that other membrane components also interact with collagen, such as the 90-kD phosphorylated, transmembrane glycoprotein, termed ECMR III (Carter and Wayner, 1988; Wayner and Carter, 1987). These components have no

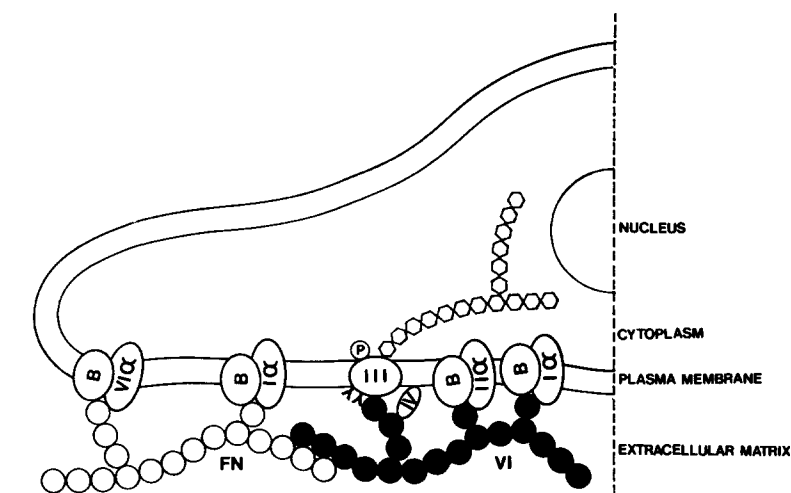


Figure 10. Model showing fibroblast adhesion to a complex ECM: interaction of ECMRs I, II, and FNR (ECMR VI) with their ECM ligands, FN, and type VI collagen. Polymeric FN and type VI collagen interact with each other and both induce cell adhesion (Carter, 1982; 1984). FN and type VI collagen specifically interact with the FNR (ECMR VI) and ECMR II, respectively, to mediate cell adhesion to the ECM. In addition, both FN and type VI collagen interact with the promiscuous ECMR I. ECMR III, a 90-kD transmembrane, phosphorylated, glycoprotein interacts with the cytoskeleton and binds to collagen but has no defined role in cell adhesion to the ECM (Carter and Wayner, 1988). ECMR III is homologous to lymphoid homing receptors (Gallatin et al., 1986) and this intercellular adhesion may be modulated by interactions with the ECM (Carter, Wayner, and Gallatin, unpublished data). ECMR IV, a peripheral membrane protein, binds to collagen but also has no observed role in cell adhesion to the ECM.

Table II. Cell Substratum Adhesion Mediated by Extracellular Matrix Receptors (ECMRs) Containing Unique α and Common Integrin* β_1 Subunits

ECMR [§]	ECM ligand	α Subunit MW [†] (α - β_1)	Identifying monoclonal antibodies [§]	VLA nomenclature	Related receptors
—	—	$\alpha_0 = 160$	(CSAT, JG22)	—	Avian Integrin I [¶]
—	—	$\alpha_1 = 200$	(TS 2/7, SR84)	VLA-1	—
II	Collagen Types I-VI	$\alpha_c = 150$	<u>PIH5</u> , (PIH6, P4B4, 12F1)	VLA-2	Ia/IIa
I	Fn, Lam, Col (Promiscuous?)	$\alpha_3 = 150$	<u>PIB5</u> , (J143)	VLA-3	Avian Integrin 2
—	—	$\alpha_4 = 140$	(B-5G10, HP2/1)	VLA-4	—
VI	Fn	$\alpha_f = 150$	<u>PIF8</u> , <u>PID6</u>	VLA-5	FNR ^{**} , Ic/IIa ^{¶¶}
—	—	$\alpha_6 = 150$	(GoH3)	VLA-6	Ic (?) ^{¶¶}

* The β_1 subunit of the Integrin family (Hynes, 1987).

† α Subunit designation based on the ligand, or the subscript used by the original discoverer. Relative molecular weight (MW) estimation under nonreducing conditions.

§ Underlined monoclonal antibodies react with the α subunits or α - β complexes and inhibit cell adhesion to the indicated ECM ligand. Antibodies in parenthesis either do not inhibit activity or are of unknown inhibitory activity.

|| VLA of M. E. Hemler (1988) and references therein. The relation of ECMRs to VLAs is described in Takata et al. (1988).

¶ Buck and Horwitz (1987) and references therein.

** Pytela et al. (1985).

¶¶ Hemler et al. (1988) and Sonnenberg et al. (1987).

§§ Wayner and Carter (1987).

||| Kunicki et al. (1988).

¶¶ Piotrowicz et al. (1988).

defined role in cell adhesion to the ECM. However, the recent observation that ECMR III is homologous to the lymphoid cell homing receptor (Carter, Wayner, and Gallatin, unpublished data) that mediates lymphoid cell interactions with high endothelium (Gallatin et al., 1986) suggests that ECMR III may be involved in intercellular adhesion.

Relation of ECMRs I, II, and VI (FNR) to Receptors Described by Others

As described by Hynes (1987), the Integrin super family contains a number of families of receptors. Each member of a particular family expresses a unique α and a common β subunit, termed β_1 - β_3 . The relationship of ECMRs I, II, and VI (FNR) to other receptors is summarized in Table II. Human ECMRs I, II, and VI, described here, all share the common Integrin β_1 subunit. We have shown that the functionally defined ECMRs I and II are homologous to the VLA 3 (monoclonal antibody J143) and VLA 2 (monoclonal antibody 12F1) differentiation antigens (Takada et al., 1988). Fradet et al. (1984) have also examined the tissue distribution of antigens recognized by the J143 monoclonal antibody (VLA 3/ECMR I). Our findings, in general, do agree with their results. However, the differential staining results we obtained with monoclonal antibodies P4B4 and PIH5 to ECMR II (Fig. 7) suggest that interactions of the ECMRs with ligands can dramatically affect the observed tissue distribution results. Alternatively, monoclonal antibodies to the same antigen that recognize distinct epitopes may also affect tissue distribution results.

ECMR VI is homologous to the prototype FNR (Pytela et al., 1985) and to the recently described VLA 5 antigen (Takada et al., 1987). In the present study we have shown that platelet glycoprotein Ic-IIa, involved in the adhesion of nonactivated platelets to FN (Piotrowicz et al., 1988), is identical to the fibroblast FNR. Furthermore, nonactivated platelet adhesion, like fibroblast and lymphoid adhesion to

FN, can be inhibited with the PIF8 and PID6 monoclonal antibodies. In recent work (Kunicki et al., 1988), we have determined that the α and β subunits of the collagen receptor, ECMR II (PIH5), correspond to platelet glycoproteins Ia and IIa, respectively.

In Table II, we have used the ECMR nomenclature (Wayner and Carter, 1987) when referring to these receptors because they were identified as components involved in interacting with ECM components. The VLA terminology does not reflect the relationship of the receptors to the ECM nor even to the differentiation state of cells, since as we have shown here and as pointed out by Hemler, the VLAs are often not very late appearing antigens (Hemler, 1988). Similarly, the nomenclature used for platelet membrane glycoproteins does not reflect the function of the receptors. As suggested by Buck and Horowitz (1987), the α subunit of the ECMR II has been designated α_c on the basis of its affinity for collagen (Table II).

Function and Expression of Multiple ECMRs in Mediating Cell Distribution in Tissue

ECMRs were differentially expressed by various cell populations (Table I and Fig. 7). In general, epithelial cells expressed elevated levels of ECMRs I and II while circulating mononuclear cells and platelets expressed relatively high levels of the ECMR II and VI. Characteristically, expression of ECMR I was present in only monocytes and activated or transformed cell populations. Interestingly, only a subpopulation of PBMC and platelets expressed the FNR. Cell adhesion to a complex WI-38 ECM, composed primarily of FN and type VI collagen, was mediated by at least two receptors, ECMR II and FNR. Conceivably, ECMR I may also play a role in this adhesion process since monoclonal antibody PIB5 against ECMR I, also partially inhibited (30%) the adhesion of WI-38 VA13 cells to the WI-38 ECM. It is clear from these data that multiple receptors contribute to cell

adhesion to complex WI-38 ECM. One interpretation of these results suggests that a cell expressing different ECMRs can adhere to one or more different ligands in complex ECM in an additive manner. In this interpretation, each ligand-receptor complex would function independently. Alternatively, different ligand-receptor complexes may function cooperatively, with each complex altering, (positively or negatively) the function of other ligand-receptor complexes. The availability of multiple monoclonal antibodies that inhibit the adhesive function of unique ECMRs will be useful in further evaluating these possible mechanisms and the function of these receptors in mediating morphogenic and cell infiltration processes in tissue. We propose that the differential expression of various ECMRs for multiple ECM components (Table I) combined with the cooperative function of multiple ECMRs may provide a template for localizing cells to specific tissue domains such as the dermis or epidermis of skin.

We would like to acknowledge the excellent technical assistance of Mr. Todd Bouchard. We would also like to thank Dr. M. Pierschbacher for the gift of rabbit polyclonal antibody to the fibronectin receptor and Dr. Martin Hemler for monoclonal antibody A1A5 to the common β subunit of the VLA family.

This work was supported by grant BC-419 from the American Cancer Society and grants ROI-CA38801, HL3279, and HL28444 from the National Institutes of Health.

Received for publication 23 November 1987, and in revised form 11 July 1988.

References

- Brooks, C. G. 1983. Reversible induction of natural killer cell activity in cloned murine cytotoxic T lymphocytes. *Nature (Lond.)* 305:155-158.
- Brown, P. J., and R. L. Juliano. 1985. Selective inhibition of fibronectin-mediated cell adhesion by monoclonal antibodies to a cell-surface glycoprotein. *Science (Wash. DC)* 228:1448-1451.
- Brown, P. J., and R. L. Juliano. 1986. Expression and function of a putative cell surface receptor for fibronectin in hamster and human cell lines. *J. Cell Biol.* 103:1595-1603.
- Buck, C. A., and A. F. Horwitz. 1987. Cell surface receptors for extracellular matrix molecules. *Annu. Rev. Cell Biol.* 3:179-205.
- Carter, W. G. 1982. Transformation-dependent alterations in glycoproteins of the extracellular matrix of human fibroblasts: characterization of GP250 and the collagen-like GP140. *J. Biol. Chem.* 257:13805-13815.
- Carter, W. G. 1984. The role of intermolecular disulfide bonding in deposition of GP140 in the extracellular matrix. *J. Cell Biol.* 99:105-114.
- Carter, W. G., and E. A. Wayner. 1988. Characterization of the class III collagen receptor, a phosphorylated transmembrane glycoprotein expressed in nucleated human cells. *J. Biol. Chem.* 263:4193-4201.
- Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* 252:1102-1106.
- Damsky, C. H., K. A. Knudsen, D. Bradley, C. A. Buck, and A. F. Horwitz. 1985. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. *J. Cell Biol.* 100:1528-1539.
- Engvall, E., and E. Ruoslahti. 1977. Binding of soluble forms of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer.* 20:1-5.
- Fradet, Y., C. Condo-Cardo, T. Thomson, M. E. Daly, W. F. Whitmore, Jr., K. O. Lloyd, M. R. Melamed, and L. J. Old. 1984. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA.* 81:224-229.
- Gallatin, W. M., T. P. St. John, M. Siegelman, R. Reichert, E. C. Butcher, and I. L. Weissman. 1986. Lymphocyte Homing Receptors. *Cell.* 44:673-680.
- Glasebrook, A. L., and F. W. Fitch. 1980. Alloreactive cloned T cell lines. *J. Exp. Med.* 151:876-895.
- Grimm, E. A., A. Mazumder, H. Z. Zhang, and S. A., Rosenberg. 1982. Lymphokine activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.* 155:1923-1941.
- 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: characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight β subunit. *J. Biol. Chem.* 262:3300-3309.
- Horwitz, A., K. Duggan, R. Greggs, C. Decker, and C. Buck. 1985. The cell substratum attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. *J. Cell Biol.* 101:2134-2144.
- Hynes, R. O. 1973. Alteration of cell-surface proteins by viral transformation and by proteolysis. *Proc. Natl. Acad. Sci. USA.* 70:3170-3174.
- Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549-554.
- Kunicki, T. J., P. J. Newman, D. L. Amrani, and M. W. Mosesson. 1985. Human platelet fibrinogen: Purification and hemostatic properties. *Blood.* 66:808-815.
- Kunicki, T. J., D. J. Nugent, S. J. Staats, R. P. Orzechowski, E. A. Wayner, and W. G. Carter. 1988. The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. *J. Biol. Chem.* 263:4516-4519.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
- Miller, E., and R. Rhodes. 1982. Preparation and characterization of the different types of collagen. *Methods Enzymol.* 82:33-64.
- Mishell, B. B., and S. M. Shiigi. 1980. Selected Methods in Cellular Immunology. W. H. Freeman & Co. Publishers, San Francisco, CA. 205-208.
- Oi, V. T., and L. A. Herzenberg. 1980. Immunoglobulin producing hybrid cell lines. In Selected Methods in Cellular Immunology. B. B. Mishell and S. M. Shiigi, editors. W. H. Freeman & Co. Publishers, San Francisco, CA. 351-373.
- Okita, J. R., D. Pidard, P. J. Newman, R. R. Montgomery, and T. J. Kunicki. 1985. On the association of glycoprotein Ib and actin-binding protein in human platelets. *J. Cell Biol.* 100:317-321.
- Piotrowicz, R. S., R. P. Orzechowski, D. J. Nugent, K. M. Yamada, and T. J. Kunicki. 1988. Glycoprotein Ic-IIa functions as an activation-independent fibronectin receptor of human platelets. *J. Cell Biol.* 106:1359-1364.
- Pischel, K. D., M. E. Hemler, C. Huang, H. G. Bluestein, and V. L. Wood. 1987. Use of the monoclonal antibody 12F1 to characterize the differentiation antigen VLA-2. *J. Immunology.* 138:226-233.
- Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1985. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell.* 40:191-198.
- Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC)* 238:491-497.
- Sonnenberg, A., H. Janssen, F. Hogervorst, J. Calafat, and J. Hilgers. 1987. A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J. Biol. Chem.* 262:10376-10383.
- Taggart, R. T., and I. M. Samloff. 1983. Stable antibody-producing murine hybridomas. *Science (Wash. DC)* 219:1228-1230.
- Takada, Y., C. Huang, and M. E. Hemler. 1987. Fibronectin receptor structures within the VLA family of heterodimers. *Nature (Lond.)* 326:607-609.
- Takada, Y., E. A. Wayner, W. G. Carter, and M. E. Hemler. 1988. Extracellular matrix receptors, ECMR II and ECMR I, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. *J. Cell. Biochem.* 37:385-393.
- Timpl, R., H. Rohde, P. G. Robey, S. I. Rennard, J. M. Foidart, and G. R. Martin. 1979. Laminin—a glycoprotein from basement membranes. *J. Biol. Chem.* 254:9933-9937.
- Wayner, E. A., and W. G. Carter. 1987. Identification of multiple cell adhesion receptors for type VI collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β subunits. *J. Cell Biol.* 105:1873-1884.