Identification and Characterization of the T Lymphocyte Adhesion Receptor for an Alternative Cell Attachment Domain (CS-1) in Plasma Fibronectin

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Abstract. Using mAb technology (Wayner, E. A., W. G. Carter, R. Piotrowicz, and T. J. Kunicki. 1988. J. Cell Biol. 107:1881-1891), we have identified a new fibronectin receptor that is identical to the integrin receptor α4β1. mAbs P3E3, P4C2, and P4G9 recognized epitopes on the α4 subunit and completely inhibited the adhesion of peripheral blood and cultured T lymphocytes to a 38-kD tryptic fragment of plasma fibronectin containing the carboxy-terminal Heparin II domain and part of the type III connecting segment (IIICS). The ligand in IIICS for α4β1 was the CS-1 region previously defined as an adhesion site for melanoma cells. The functionally defined mAbs to α4 partially inhibited T lymphocyte adhesion to intact plasma fibronectin and had no effect on their attachment to an 80-kD tryptic fragment containing the RGD (arg-gly-asp) adhesion sequence. mAbs (P1D6 and P1F8) to the previously described fibronectin receptor, α5β1, completely inhibited T lymphocyte adhesion to the 80-kD fragment but had no effect on their attachment to the 38-kD fragment or to CS-1. Both α4β1 and α5β1 localized to focal adhesions when fibroblasts that express these receptors were grown on fibronectin-coated surfaces. These findings demonstrated a specific interaction of both receptors with fibronectin at focal contacts.

In conclusion, these findings show clearly that cultured T lymphocytes use two independent receptors during attachment to fibronectin and that (a) α5β1 is the receptor for the RGD containing cell adhesion domain, and (b) α4β1 is the receptor for a carboxy-terminal cell adhesion region containing the Heparin II and IIICS domains. Furthermore, these data also show that T lymphocytes express a clear preference for a region of molecular heterogeneity in IIICS (CS-1) generated by alternative splicing of fibronectin pre-mRNA and that α4β1 is the receptor for this adhesion site.

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eg and others (reviewed by Hynes, 1987; Hemler, 1988) have described specific cell surface receptors for the extracellular matrix (ECM) components collagen, fibronectin, and laminin. The functions of the extracellular matrix receptors (ECMRs I, II, and VI) we described were defined by affinity chromatography (Wayner and Carter, 1987; Staatz et al., 1989) and by preparing mAbs that specifically inhibited the interaction of cells with purified ligands (Wayner and Carter, 1987) or ECM (Wayner et al., 1988). The ECMRs are members of the integrin (Hynes, 1987) family of cell adhesion molecules and possess unique α subunits complexed to the integrin β1 subunit (Wayner and Carter, 1987; Wayner et al., 1988). ECMR VI is identical to the prototype fibronectin receptor (Pytel et al., 1985), α5β1, platelet glycoprotein (gp) Ic/IIa, and VLA 5; ECMR II is identical to α2β1, platelet gp Ia/IIa and VLA 2 (Hemler et al., 1987b); and ECMR I is identical to α3β1 and VLA 3 (Kunicki et al., 1988; Takada et al., 1988; Wayner et al., 1988). Monoclonal antibodies to α2β1, α3β1 and α5β1 (P1H5, P1D6, and P1B5) inhibit fibroblast or platelet adhesion to collagen, fibronectin and laminin-coated surfaces (Kunicki et al., 1988; Wayner et al., 1988).

The β1 integrins are differentially expressed in cultured cells and tissue, and demonstrate clear differences in activation dependent expression. For example, expression of α5β1 in hematopoietic cells is restricted to subpopulations of thymocytes and peripheral blood lymphocytes, monocytes, acute
lymphocytic or myelogenous leukemias, activated T cells, migrating hematopoietic precursor cells, and some cultured T, B, or erythroleukemia cell lines (Bernardi et al., 1987; Cardarelli et al., 1988; Garcia-Pardo et al., 1989; Giancotti et al., 1986; Liao et al., 1987; Savagner et al., 1986; Wayner et al., 1988).

In experiments designed to examine the function of α5β1 in lymphocytes, we observed that resting peripheral blood and cultured T lymphocytes (Molt 4 or Jurkat) expressed an affinity for fibronectin independent of the prototype fibronectin receptor, α5β1. Although these cells attached to fibronectin-coated surfaces (unpublished), they expressed low or undetectable levels of α5β1 recognized by our functionally defined mAb, PID6 (Wayner et al., 1988). Furthermore, T lymphocyte adhesion to fibronectin could only be partially inhibited by PID6- or RGD-containing peptides, suggesting the involvement of other receptors for fibronectin in the adhesion process. Alternatively, adhesion of other cells to fibronectin such as malignant or transformed fibroblasts and activated T lymphocytes (lymphokine-activated killer [LAK] cells) could be completely inhibited by PID6. This suggested that resting peripheral blood T lymphocytes and cultured T cell leukemias express multiple independent and functional fibronectin receptors.

Therefore, we identified an alternative fibronectin receptor by preparing mAbs that specifically inhibited the adhesion of T lymphocytes to fibronectin. This receptor was identical to the integrin receptor α4β1 and mediated the attachment of peripheral blood lymphocytes, cultured T cell lines, and RD cells to plasma fibronectin. Furthermore, as we have shown (Garcia-Pardo, A., and O. C. Ferreira, manuscript submitted for publication) T lymphocytes expressed a clear preference for a 38-kD tryptic fragment of plasma fibronectin (Garcia-Pardo et al., 1987) containing the Heparin II domain and 67 amino acid residues of the type III connecting segment (IIICS) spanning the CS-1, CS-2, and CS-3 regions defined by Humphries et al. (1986, 1987). T lymphocytes attached only to CS-1 and mAbs to α4β1 (P3E3, P4C2, P4G9) completely inhibited T lymphocyte adhesion to the 38-kD fragment and to CS-1. T lymphocytes also attached (with much lower affinity) to a site present in the heparin II domain and mAbs to α4β1 also inhibited this interaction. The functionally defined mAbs to α4β1 did not inhibit T lymphocyte adhesion to an 80-kD tryptic fragment of plasma fibronectin containing the RGD sequence, whereas antibodies to α5β1 completely inhibited this interaction. These data show that T lymphocytes bear at least two receptors for fibronectin and clearly identify α4β1 as the receptor for adhesion site(s) located in the carboxy-terminal region of plasma fibronectin.

Materials and Methods

Materials

PMSE, N-ethylmaleimide, leupeptin, diisopropyl fluorophosphate, 2-mercaptoethanol, BSA, Triton X-100, Protein A-agarose, soybean trypsin inhibitor, 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. (La Jolla, CA). TPCK-trypsin was from Cooper Biomedicals (Malvern, PA). Fluorescein-conjugated goat anti-mouse IgG and IgM (heavy [H] and light [L] chains) or rhodamine-conjugated goat anti-rabbit IgG and IgM (H and L chains) were obtained from Tago, Inc. (Burlingame, CA). R-phycocerythrin-conjugated streapavidin was from BioMeda (Foster City, CA). Rabbit anti-mouse IgG (H and L) antiserum was obtained from Cappel Laboratories (Malvern, PA). [51Cr] Sodium chromate was from New England Nuclear (Boston, MA). [125I] was from Amersham Corp. (Arlington Heights, IL). Human recombinant IL 2 was a generous gift from Dr. D. Urdal (Immunex Corp., Seattle, WA). Laminin was purchased from Collaborative Research, Inc. (Bedford, MA) and purified plasma fibronectin and collagen types I and III were prepared as previously described (Wayner and Carter, 1987; Wayner et al., 1988).

Cells and Cell Culture

RD (human rhabdomyosarcoma) and HT1080 (human fibrosarcoma) cells were obtained from the American Type Culture Collection (Rockville, MD). PBMC, platelet, and granulocyte populations from normal human donors were prepared as described (Kunicld et al., 1988; Wayner et al., 1988). Peripheral blood cells from patients with acute lymphocytic, large granular lymphocyte (LGL), or myelogenous leukemia were obtained from Dr. I. Bernstein and Dr. T. Loughran (Fred Hutchinson Cancer Research Center). Human LAK cells (500 U/ml IL 2) and the monoclonal HLA B7-specific human cytotoxic T lymphocyte (CTL) cell line, CIC4, were prepared according to standard protocols (Grimm et al., 1982; Glasebrook and Pich, 1983; Brooks, 1983; Wayner and Brooks, 1984; Wayner and Brooks, 1985). The EBV-transformed B lymphocyte cell line (BLCL) ST-1, was derived from the donor spleen used in the production of the CIC4 CTL line. All other cell lines and cell culture conditions were as previously described (Wayner and Carter, 1987; Wayner et al., 1988).

Antibodies

A rabbit polyclonal antibody, AB33, prepared against the cytoplasmic domain of the fibronectin receptor, α5β1, (Roman et al., 1987) was used to detect α5β1 in focal adhesions. mAbs A1A5, against the common integrin (Hynes, 1987) β1 subunit of the VLA family of receptors (Hemler, 1988) and BS-G10 to the VLA 4 α subunit (Hemler et al., 1987) were obtained from Dr. Martin Hemler (Dana-Farber Cancer Institute, Boston, MA). mAbs to the integrin receptors α5β1 (PIBS), α2β1 (PIH5), and α5β1 (PID6) have been described. PIH5 and PID6 inhibit fibroblast and platelet adhesion to collagen and fibronectin-coated substrates, respectively (Wayner and Carter, 1987; Kunicld et al., 1988; Wayner et al., 1988).

mAbs to lymphocyte adhesion receptors were produced by the methods of Oh and Herzenberg (1980) and Taggart and Samloff (1983) as described (Wayner and Carter, 1987; Wayner et al., 1988). Spleens from RBF/Om mice immunized with 100 μl of packed T lymphocytes were removed and fused with NS-1/FIOX-NY myeloma cells. Viable heterokaryons were selected in RPMI 1640 medium supplemented with adenosine/aminopterin/thymidine (Taggart and Samloff, 1983). Hybridomas producing antibody directed to lymphocyte adhesion receptors were screened by specific inhibition of lymphocyte adhesion to fibronectin-coated surfaces and cloned by limiting dilution.

Inhibition of Cell Adhesion to Intact Fibronectin and Fibronectin Fragments

Antibodies that would alter cell adhesion to purified plasma fibronectin, tryptic fragments and CS peptides were identified as previously described (Wayner and Carter, 1987). Briefly, 48-well virgin styrene plates were coated with 5 μg/ml human plasma fibronectin. The plates were blocked with PBS supplemented with 10 mg/ml heat-denatured BSA (HBSA). T lymphocyte or HT1080 cells were labeled with Na251CrO4 (50 μCi/ml) for 2-4 h) and washed, and 5 × 104 HT1080 or cultured T cells or 5 × 104 PBL/well were incubated with hybridoma culture supernatants (1:2 dilution in PBS supplemented with 1 mg/ml HBSA) 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 supernatant for 15-30 min (HT1080) or 2-4 h (lymphocytes) at 37°C. Nonadherent cells were removed by washing with PBS, and the adherent cells were dissociated in SDS/NaOH and bound 51Cr counts per minute were quantitated in a gamma counter.

Immune Precipitation, Sequential Immune Precipitation, V8 Protease Peptide Mapping, and PAGE

Viable cells were surface labeled with 125I as described (Wayner and Carter, 1987) followed by extraction with 1% vol/vol Triton X-100 deter-
gent or 0.3% [3-cholamidopropyl]dimethylammonio]-l-propanesulfo-
ate (CHAPS) detergent in 50 mM PBS, pH 7.2. In some cases, 1 mM CaCl2 was added to the lysis buffer. 1 mM diisopropyl fluorophosphate, 1 mM N-ethylmaleimide, 1 μg/ml leupeptin, and 1 μg/ml soy-
bean trypsin inhibitor were used as protease inhibitors. Immune precipita-
tion and sequential immune precipitations were performed exactly as previ-
ously described. Peptide analysis followed the basic procedure of Cleveland et al. (1977) with modifications as described (Wayner and Carter, 1987). Polyacrylamide slab gels containing SDS (SDS-PAGE) gels were prepared following the basic stacking gel system of Laemmli (1970).

Preparation of Tryptic Fragments from Human Plasma Fibronec
tin and Synthesis of CS Peptides

Human plasma fibrinectin was a generous gift from Dr. Horowitz and Dr. R. Schulman (New York Blood Center, New York). Fibrinectin was dig-
gested with TPCK-trypsin for 90 min at 37°C, and the digest was fraction-
ated by affinity and ion-exchange chromatography as previously described (Garcia-Pardo et al., 1987, 1989). Two overlapping peptides spanning the initial 48 residues of the type III connecting segment (IIICS) region of hu-
mam fibrinectin (CS-1 and CS-2) were synthesized and coupled to rabbit IgG as described (Humphries et al., 1986, 1987).

Fluorescence Analysis of Receptor Expression

Expression of ECNRs on cells in suspension was analyzed by one- or two-
color flow cytometry on a dual laser cell sorter (EPICS 750; Coulter Elec-
tronics, Hialeah, FL). Positive fluorescence was determined on a three-
decade log scale and fluorescence intensity (log FI) was expressed as mean 
channel number (0-255). Background fluorescence for a nonimmune mouse IgG negative control was determined for each cell population and sub-
tracted. Adherent cells were trypsinized and allowed to recover for 15 min at 37°C in the presence of serum before use for flow cytometry. For one- or two-color fluorescence measurements, 106 cells in suspension were incu-
bated for 30 min first with protein G-Sepharose-purified goat IgG (20 g/ml) and then with first-stage antibodies at 4°C for 60 min, washed in HBSS containing 10 mg/ml HBSA and 0.02% sodium azide (HBBSA/SA), and incubated with FITC-conjugated rabbit anti-mouse IgG for 40°C at 4°C in HBBSA/SA. They were washed and fixed in cold 2% parafor-
maldehyde (prepared fresh) in PBS. For two-color fluorescence, the purified and biotinylated mAb was added to the FITC-stained and fixed cells to a final concentration of 1 μg/ml in HBBSA/SA and incubated at 4°C for 60 min. Prior fixation with 2% paraformaldehyde had little effect on expres-
sion of lymphocyte integrin receptors. The fixed cells were washed and in-
cubated in 0.5 ml HBBSA/SA containing phycoerythrin-conjugated strepavidin (Bionetics Laboratory Products, Charleston, SC) at 1/50 for 30 min at 4°C. Finally, the stained cells were washed and fixed again in 2% paraformaldehyde in PBS and held at 4°C in the dark for analysis on the flow cytometer.

Localization of Receptors in Focal Adhesions

Adherent cells were trypsinized, washed in RPMI 1640 supplemented with 1 mg/ml BSA plus 100 μg/ml soybean trypsin inhibitor, and allowed to ad-
here to acid-washed and silanized glass cover slips coated with fibrinectin, laminin, or collagen (20 μg/ml) in the absence of serum for 1-4 h as de-
scribed (Carter, W. G., and E. A. Wayner, manuscript in preparation). At the end of the incubation, nonadherent cells were removed and adherent cells were fixed in 100 mM sodium cacodylate, 100 mM sucrose, 4.5 mM CaCl2, 2% formaldehyde for 20 min. They were permeabilized with 0.5% Triton X-100 for 5 min, washed, and blocked with 25% goat serum in PBS. The permeabilized cells were stained with antibodies to specific receptors (60 min at room temperature), washed, incubated with either FITC-con-
jugated goat anti–mouse or rhodamine-conjugated goat anti–rabbit IgG (45 min at room temperature), and washed again. The cover slips were inverted onto glass slides for fluorescence and interference reflection microscopy as described (Lizard and Lochner, 1976).

Tissue Staining

The distribution of the integrin receptors in tissue was determined by fluorescence microscopy of cryostat sections. Cryostat sections (6 μm) were prepared from human skin, tonsil, or tumor 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 antibo-
dies and secondary fluorescent antibodies as described (Carter and Wayner, 1988). In control experiments, no fluorescence of rhodamine was detected using the fluorescein filters or vice versa.

Results

Identification of an Alternative Fibronec
tin Receptor

Cultured T lymphocytes (Molt 4), K562, RD (rhabdomyo-
sarcoma), and HT1080 (fibrosarcoma) cells, and freshly de-
rivived PBL (not shown) adhered to fibronec
tin-coated sur-
faces (Fig. 1, open bars). However, Molt 4 and RD cells expressed low or undetectable levels of the prototype fibronec
ntin receptor (integrin α5β1) recognized by monoclonal antibody P1D6 (Fig. 1, striped bars). Consistent with this, adhesion of Molt 4 and RD cells to fibronectin could not be completely inhibited by P1D6 (Fig. 1, solid bars). Alternati-
vely, adhesion of cells to fibronec
tin that expressed abundant α5β1 (HT1080 and K562) could be effectively inhibited by P1D6. Furthermore, the synthetic peptide RGDs did not completely inhibit T lymphocyte adhesion to plasma fibronec
tin (50-70% for Molt 4 or Jurkat cells vs. 80-90% for fibroblasts and 100% for K562-1 cells). Together, these data suggested that some cells, such as T lymphocytes, express fibronec
ntin adhesion receptors other than α5β1.

We attempted to identify other putative fibronec
tinin recep-
tors by preparing mAbs to cultured T lymphocytes and screening them for their ability to specifically inhibit lym-
phocyte but not fibroblast adhesion to fibronec
tin-coated sur-
faces. Using this protocol several mAbs (P4C2, P3E3, P4G9) were identified that inhibited cultured T lymphocyte but not HT1080 cell adhesion to fibronec
tinin (Table I). Immune pre-
cipitation from Triton X-100 detergent lysates prepared with 125I-surface–labeled PBL (not shown), Molt 4 or HT1080 (Fig. 2) cells showed that the inhibitory mAbs (data shown for P3E3) reacted with a single protein present in lympho-
cyte extracts that migrated at M, 150,000 (p150) in the pres-
ence (not shown) or absence (Fig. 2) of reducing agent. Under these immune precipitation conditions p150 lacked an apparent α-β subunit structure and did not co-migrate with either the α or β subunit of the integrin receptors α2β1 or α3β1 (Fig. 2). The antigen immune precipitated from Triton X-100 detergent extracts prepared with chronically activated CD8+ LAK cells or CTL (not shown) contained, in addition to p150, relatively large quantities of two smaller proteins that migrated at M, 80,000 and 70,000 in the presence (not shown) or absence of reducing agent. V8 protease peptide mapping revealed that p80 and p70 were proteolytic fragments of p150 (not shown). These lower molecular weight forms could be immune precipitated from chronically acti-
vated T cells even when detergent extracts were prepared in the presence of multiple protease inhibitors (legend to Fig. 2). p80 and p70 were virtually absent from extracts prepared with resting PBL, cultured T (Molt 4, Jurkat), or B cell leukemias and RD cells (not shown).

The biochemical characteristics of p150 suggested that it might be related to the VLA 4 antigen described by Hemler (Hemler et al., 1987a). This was confirmed by sequential immu-
nee precipitation (not shown) with a VLA 4-specific mAb, B5-G10. p150 was established as an α subunit of the integrin super family by its association with E1 when immune pre-
cipitations were carried out after CHAPS detergent (0.3%) solubilization of 125I-surface–labeled T lymphocytes in the
presence of 1 mM Ca\(^{2+}\) (Fig. 3). Under these conditions \(\alpha_4\) was precipitated as a heterodimer with \(\beta_1\). The identity of \(\beta_1\) was confirmed by V8 protease peptide mapping (not shown). The \(\alpha_4\beta_1\) heterodimer immune precipitated from T lymphocytes with the inhibitory mAbs (P3E3, P4C2, and P4G9) was shown to be distinct from the prototype fibronectin receptor, \(\alpha_5\beta_1\), immune precipitated with P1D6 by three criteria. (a) The relative quantities of \(\alpha_4\beta_1\) and \(\alpha_5\beta_1\) present in detergent extracts of T lymphocytes were distinct with higher levels of \(\alpha_4\beta_1\) being present (Fig. 3). This was in agreement with the data we obtained using flow cytometry (Fig. 1). (b) In sequential immune precipitation experiments, mAbs to \(\alpha_4\beta_1\) did not preclear \(\alpha_5\beta_1\) (not shown). (c) The V8 protease peptide maps derived from the \(\alpha_4\) and \(\alpha_5\) subunits precipitated with mAbs P3E3 and P1D6 were clearly distinguishable (not shown). Furthermore, under the conditions (0.3\% CHAPS and 1 mM CaCl\(_2\)) used to solubilize the conjugate of \(\alpha_4\beta_1\) from Jurkat cells (Fig. 3) another protein of higher molecular weight (p180) also reacted with the mAbs or coprecipitated with \(\alpha_4\beta_1\). p180 was absent from extracts prepared with P1D6 mAb (Fig. 3), nonlymphoid cells or Triton X-100 detergent extracts prepared in the absence of Ca\(^{2+}\). The relationship of p180 to other integrins is not known. Because \(\alpha_4\) could be immune precipitated without \(\beta_1\) after solubilization of T cells with Triton X-100 in the absence of Ca\(^{2+}\) this revealed that the inhibitory mAbs recognized epitopes present on the \(\alpha_4\) subunit (Fig. 2).

### Table I. Specific Inhibition of Lymphocyte Adhesion to Plasma Fibronectin by mAbs P3E3, P4C2, and P4G9

| Cells     | SP2 | P1D6 (\(\alpha_5\beta_1\)) | P3E3 | P4C2 | P4G9 |
|-----------|-----|--------------------------|------|------|------|
| % of control         | 100 | 43                        | 38   | 10   | 52   |
| Jurkat    | 100 | 22                        | 33   | 12   | 48   |
| Molt 4    | 100 | 18                        | 12   | 8    | 39   |
| HT1080    | 100 | 5                         | 98   | 93   | 104  |

Cells were labeled with \(^{51}Cr\) and allowed to adhere to plasma fibronectin-coated (20 \(\mu\)g/ml) plastic surfaces (5 \(\times\) 10\(^5\) for PBL or 105 for HT1080 or Molt 4 and Jurkat) for 30 min (HT1080 cells) or 2-4 h (Molt 4 or Jurkat) at 37°C in the presence of SP2 myeloma or hybridoma culture supernatants diluted 1:2 with fresh medium. Results are expressed as a percent of the \(^{51}Cr\) counts per minute bound to the SP2-positive control. Data shown are from a single experiment. In general, the inhibition observed for the new inhibitory mAbs ranged from 50 to 80\%, with P4C2 always being the most efficient inhibitor of lymphocyte adhesion to plasma fibronectin. The inhibition obtained for P1D6 ranged from 10 to 70\% for PBL and 50 to 80\% for cultured T lymphocyte cell lines. This variability correlated with cell surface expression of \(\alpha_5\beta_1\).

**Figure 1.** Adhesion of T lymphocytes (Molt 4), K562-1, RD, or HT1080 cells to plasma fibronectin, inhibition with P1D6 mAb, and cell surface expression of \(\alpha_5\beta_1\). \(^{51}Cr\)-labeled cells (10\(^5\) cells/ml) were incubated with P1D6 mAb (50 \(\mu\)g/ml) or mouse IgG (50 \(\mu\)g/ml) for 60 min at 4°C and allowed to attach to fibronectin-coated (20 \(\mu\)g/ml) plastic surfaces in the presence of P1D6 (solid bars) or mouse IgG (open bars) for 30 min (HT1080 or RD) or 4 h (Molt 4 or K562) at 37°C. Adhesion to plasma fibronectin (pFN) is expressed as \(^{51}Cr\) bound to the plastic surfaces. Cell surface expression of \(\alpha_5\beta_1\) was determined by flow cytometry by staining of cells in suspension with mAb P1D6. Log P1D6 fluorescence (striped bars) is expressed as mean channel number (0-255) above background.

**Figure 2.** Immune precipitation of lymphocyte fibronectin receptor from HT1080, Molt 4, or chronically activated CD8+ T (LAK) cell detergent extracts. \(^{125}I\)-labeled Molt 4, LAK, or HT1080 cells were extracted with 1\% Triton X-100 in the presence of 1 mM PMSF, 1 mM N-ethylmaleimide, 1 \(\mu\)g/ml leupeptin, and 1 mM di-isopropyl fluorophosphate as protease inhibitors. Aliquots of these extracts were immune precipitated with mAbs directed to \(\alpha_3\beta_1\) (PIB5), \(\alpha_2\beta_1\) (PIH5) and \(\alpha_4\beta_1\) (P3E3). The immune precipitated antigens were run on 7.5\% SDS-PAGE gels in the absence of 2-mercaptoethanol and visualized by autoradiography. The three bands immune precipitated with P3E3 from T lymphocytes are indicated (arrows).

### Distribution of \(\alpha_4\beta_1\) and \(\alpha_5\beta_1\) in Cultured Cells and Tissues

As has been previously reported (Hemler et al., 1987a), \(\alpha_4\beta_1\) and \(\alpha_5\beta_1\) are expressed in a variety of cultured cells and tissues. In human fibroblasts, \(\alpha_4\beta_1\) is present on the cell surface, and its expression is upregulated by treatment with PDGF. In contrast, \(\alpha_5\beta_1\) is absent from normal fibroblasts but is induced on activated fibroblasts. In malignant cells, \(\alpha_5\beta_1\) is often overexpressed, while \(\alpha_4\beta_1\) is typically downregulated. This differential expression suggests that these integrins play distinct roles in mediating cell adhesion and migration.

**Table I. Specific Inhibition of Lymphocyte Adhesion to Plasma Fibronectin by mAbs P3E3, P4C2, and P4G9**

| Cells     | SP2 | P1D6 (\(\alpha_5\beta_1\)) | P3E3 | P4C2 | P4G9 |
|-----------|-----|--------------------------|------|------|------|
| % of control         | 100 | 43                        | 38   | 10   | 52   |
| Jurkat    | 100 | 22                        | 33   | 12   | 48   |
| Molt 4    | 100 | 18                        | 12   | 8    | 39   |
| HT1080    | 100 | 5                         | 98   | 93   | 104  |

Cells were labeled with \(^{51}Cr\) and allowed to adhere to plasma fibronectin-coated (20 \(\mu\)g/ml) plastic surfaces (5 \(\times\) 10\(^5\) for PBL or 10\(^5\) for HT1080 or Molt 4 and Jurkat) for 30 min (HT1080 cells) or 2-4 h (Molt 4 or Jurkat) at 37°C in the presence of SP2 myeloma or hybridoma culture supernatants diluted 1:2 with fresh medium. Results are expressed as a percent of the \(^{51}Cr\) counts per minute bound to the SP2-positive control. Data shown are from a single experiment. In general, the inhibition observed for the new inhibitory mAbs ranged from 50 to 80\%, with P4C2 always being the most efficient inhibitor of lymphocyte adhesion to plasma fibronectin. The inhibition obtained for P1D6 ranged from 10 to 70\% for PBL and 50 to 80\% for cultured T lymphocyte cell lines. This variability correlated with cell surface expression of \(\alpha_5\beta_1\).
was widely distributed on nucleated hematopoietic cells (Table II). Two-color flow cytometry revealed that all lymphocyte subpopulations derived from spleen, tonsil, and peripheral blood expressed abundant \( \alpha_4 \beta_1 \). In addition, peripheral blood monocytes, freshly derived acute lymphocytic (T or B) leukemias, all LGL and myelogenous leukemias, and cultured T and B lymphocyte cell lines we examined expressed abundant \( \alpha_4 \beta_1 \) (Table II). Normal human blood platelets and granulocytes were negative for \( \alpha_4 \beta_1 \). In contrast, the only hematopoietic cell populations that expressed \( \alpha_5 \beta_1 \) were activated T cells, platelets, monocytes, and granulocytes; acute lymphocytic (T or B) and myelogenous leukemias; and cultured K562, HL-60, and U937 cells. Some cultured T (Molt 4 or Jurkat) and B (ST-1) cell lines expressed low levels of \( \alpha_5 \beta_1 \) as detected by P1D6 mAb. In some normal individuals, a subpopulation of PBL were positive for P1D6 fluorescence detected by flow cytometry. We are investigating the nature of this subpopulation of PBL that expresses \( \alpha_5 \beta_1 \). YT cells, a CD3- T cell lymphoma, were completely negative for P1D6 by flow cytometry. These results show that the major fibronectin receptor constitutively expressed by T lymphocytes is \( \alpha_4 \beta_1 \) and as we have previously reported (Wayner...)

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**Figure 3.** Identification of lymphocyte specific fibronectin receptor as integrin \( \alpha_4 \beta_1 \). \( ^{125} \)I-surface labeled Jurkat cells were extracted with 0.3% CHAPS in the presence of 1 mM CaCl\(_2\), 1 mM diisopropyl-fluorophosphatase, 1 mM PMSF, 1 mM N-ethylmaleimide, 1 \( \mu \)g/ml leupeptin, and 2 \( \mu \)g/ml soybean trypsin inhibitor. Aliquots of the extracts were then immune precipitated with myeloma (SP2) culture supernatant or with mAbs P3E3, P4C2, P4G9, or with P1D6 (anti-\( \alpha_5 \beta_1 \)). The immune precipitates were run on 8% SDS-PAGE gels in the absence of reducing agent and visualized by autoradiography. (left) Molecular weight markers. The \( \alpha_5 \) and \( \beta_1 \) subunits are indicated as are the bands present in immune precipitates prepared with P3E3, P4C2, and P4G9 (arrows).

**Table II. Distribution of the Fibronectin Receptors \( \alpha_4 \delta_1 \) and \( \alpha_5 \beta_1 \) on Human Cells**

| Cells                      | \( \alpha_4 \delta_1 \) (P4G9) | \( \alpha_5 \beta_1 \) (P1D6) |
|----------------------------|-------------------------------|-------------------------------|
| Hematopoietic cells*       |                               |                               |
| PBL                        | +++                           | +/−                           |
| LGL (CD3−, CD16+)          | +++                           | +/−                           |
| Monocytes (CD16+)          | +/−                           | +/−                           |
| Granulocytes               | −                             | +                             |
| Platelets                  | −                             | +                             |
| Spleen                     | +/−                           | +                             |
| Tonsil                     | +/−                           | +                             |
| ALL (T or B)               | +                             | +                             |
| LGL leukemia (CD3+, CD16+) | +/−                           | +/−                           |
| AML                        | +/−                           | +/−                           |
| BLCL                       | +                             | +                             |
| Molt 4 (CD3+, CD4+)        | +/−                           | +/−                           |
| Jurkat (CD3+, CD4+)        | +/−                           | +/−                           |
| YT (CD3−)                  | +                             | +                             |
| PHA blasts (CD4+)          | +++                           | +                             |
| CTL (CD3+, CD8+)           | +++                           | +/−                           |
| LAK (CD3+, CD8+)           | +/−                           | +++                           |
| HL-60                      | +                             | +/−                           |
| U937                       | +/−                           | +/−                           |
| K562-1                     | −                             | +                             |
| Fibroblasts†               |                               |                               |
| HFF (p5)                   | +                             | +                             |
| HT1080                     | +/−                           | +                             |
| RD                         | +/−                           | +/−                           |
| VA13                       | +                             | +/−                           |
| Epithelial cells           |                               |                               |
| OC-11                      | −                             | −                             |
| OVCA-41                    | −                             | −                             |
| T47D                       | −                             | −                             |
| QG56                       | −                             | −                             |
| HUVEs (p1)†                | −                             | −/−                           |

Relative fluorescence intensity was determined as described in Materials and Methods by flow cytometry. Each plus corresponds to 50 channels on a three-decade log scale from 0 to 255, −, no detectable fluorescence above background. +/−, a positive shift in fluorescence above background. mAbs used in all flow cytometry experiments were P4G9 and P1D6 (10 \( \mu \)g/ml). Fc receptors were blocked by preincubation with Protein G-Sepharose-purified goat IgG (20 \( \mu \)g/ml). Protein G-Sepharose-purified mouse IgG was used a negative control for background staining and detection of possible residual Fc receptor interaction. In general, data obtained with Fc receptor- (CD16) positive cell populations has to be interpreted with caution. In addition, the phenotype of cells in culture can vary from experiment to experiment. The reason for this is unknown. The data presented here are representative of the phenotype expressed most often by cultured cell lines.

* PBL, tonsil or splenic lymphocytes were prepared by standard protocols without enzymatic degradation and stained in suspension for flow cytometry. Peripheral blood subpopulations were distinguished either by two-color flow cytometry analysis or by the use of forward light and high angle (90°) scatter. PBL, resting peripheral blood lymphocytes from normal individuals. CTL, CD8+ long-term, antigen-specific cytotoxic T lymphocyte cell line. LAK, CD8+ non-antigen-dependent lymphokine-activated killer cells.
† Fibroblasts were trypanized and analyzed for cell surface expression of receptors by flow cytometry. Trypsin was inactivated with soybean trypsin inhibitor and the cells were allowed to recover at 37° C for 10 min before use. HFF, human neonatal foreskin fibroblasts, passage 5 (p5).
‡ OC-1 cells were derived from the ascites fluid of a woman with ovarian carcinoma. They were fresh frozen and thawed just before staining. These cells are nonadherent in culture.
§ OVCA-4 cells are a well-established adherent cell line derived from an ovarian carcinoma.
¶ HUVEs, large vessel endothelial cells obtained from Cell Systems (Seattle, WA) and were used at passage 1. They were derived from human umbilical cords and grown to confluence in serum-free media (Cell Systems). They exhibited typical characteristics of HUVEs.
Localization of α4β1 and α5β1 in focal adhesions on fibronectin-coated surfaces. RD cells were trypsinized and allowed to adhere to silanized and fibronectin-coated (20 μg/ml) glass cover slips in the absence of serum for 1 h at 37°C. At the end of this time, the cells were prepared for localization of receptors in focal adhesions as described (Materials and Methods). (A and C) Focal adhesions (arrows) visualized by interference reflexion microscopy when RD cells are adhered to fibronectin. (B) Reorganization of the prototype fibronectin receptor α5β1 stained with antibody AB33 to the focal adhesions (arrows). (D) Reorganization of α4β1 stained with P4G9 (FITC) also to the focal adhesions when RD cells are adhered to fibronectin (arrows). A and B are the same field and C and D are the same field.

et al., 1988), expression of α5β1 in T lymphocytes is restricted to leukemic or activated cultured cells. Interestingly, most fibroblast cell lines expressed low levels of α4β1, whereas large-vessel endothelial cells and cultured epithelial cells were negative for α4β1 by flow cytometry.

In tissue, α4β1 was present in adult spleen, lymph node, and tonsil and essentially absent from all other tissues we examined (not shown). In addition, the relative quantities of the fibronectin adhesion receptors expressed by cells in specific tissue domains varied dramatically. For example, PBL and lymphocytes in tonsil cortex and germinal center areas expressed large quantities of α4β1 but virtually no α5β1. α4β1 was also found in epithelial regions in adult lymphatic tissue, but whether this was the result of lymphocyte infiltration of these areas or expression of α4β1 by lymphatic epithelial cells was unclear.

α4β1 Localizes in Fibronectin-dependent Focal Adhesions

There is a specific reorganization of cell surface adhesion receptors to the focal adhesions when cells are grown on the appropriate ligands in the absence of serum (reviewed by Burridge et al., 1988). As some fibroblasts express α4β1, we investigated whether this receptor would distribute into focal adhesions when fibronectin was used as the adhesion substrate. As can be seen in Fig. 4, A and C, the primary focal contact sites or focal adhesions could be visualized by interference reflexion microscopy (Izzard and Lochner, 1976) when RD cells were grown on fibronectin. As we and others have reported (Roman et al., 1989), in the absence of serum, α5β1 was concentrated at the focal adhesions when RD cells were grown on fibronectin (Fig. 4 B, arrows) but not laminin-coated surfaces (not shown). Likewise, staining with mAb P4G9 (Fig. 4 D, arrows) revealed that α4β1 was also concentrated in focal adhesions when cells were grown on fibronectin- but not laminin-coated surfaces (not shown). These results demonstrate a specific interaction of α4β1 with fibronectin present in focal adhesions, the primary adhesion structure of cultured cells.

The presence of both receptors in focal contacts suggested the possibility that α4β1 and α5β1 bind to distinct adhesion sequences in fibronectin. Evidence for this was obtained when P4C2 and PID6 were used simultaneously to inhibit cell adhesion to intact plasma fibronectin. PID6 and P4C2 when used together completely inhibited adhesion of T lymphocytes and partially inhibited adhesion of RD cells to intact plasma fibronectin (Table III). Interestingly, unlike T lymphocytes, neither PID6 nor P4C2 alone were good inhibitors of RD cell adhesion to intact plasma fibronectin. RD cell adhesion to fibronectin could be efficiently inhibited by PID6 and P4C2 only when used together.

α4β1 Functions as the Receptor for an RGD-independent Alternative Attachment Site in Fibronectin

The preceding results (Tables I and III, Figs. 1 and 4) clearly indicated that attachment of some cells to plasma fibronectin was mediated by two independent cell surface receptors, α4β1 and α5β1. It has been well documented that the ligand for α5β1 in fibronectin is the 80-kD cell-binding domain that...
Table III. Combined Effect of mAbs P1D6 and P4C2 on T Lymphocyte and RD Cell Adhesion to Fibronectin

| Cells | Antibody | Specificity | Adhesion % of control ± SD |
|-------|----------|-------------|---------------------------|
| RD    | IGG      | -           | 100                       |
|       | P1D6     | α5β1        | 81 ± 11                   |
|       | P4C2     | α4β1        | 99 ± 7                    |
|       | P1D6 + P4C2 |       | 36 ± 8                   |
| Jurkat| IGG      | -           | 100                       |
|       | P1D6     | α5β1        | 26 ± 9                    |
|       | P4C2     | α4β1        | 38 ± 14                   |
|       | P1D6 + P4C2 |       | 0                         |

Cells were labeled with 51Cr and incubated in the presence of the indicated mAbs (50 μg/ml) or purified mouse IgG (50 μg/ml) for 1 h at 4°C. They were then applied to plasma fibronectin-coated (20 μg/ml) surfaces in RPM11640/1 mg/ml HBSA and incubated at 37°C for 30 min (RD cells) or 2 h (Jurkat). At the end of this incubation, nonadherent cells were washed off with warm PBS and the bound counts per minute were solubilized and quantitated in a gamma counter. Results from several experiments were pooled and are expressed as mean percent (relative to control) ± SD.

contains the RGD sequence (Pierschbacher and Ruoslahti, 1984; Pytela et al., 1985). To determine the region of fibronectin that interacts with α4β1, we examined the adhesion of cultured T lymphocytes to various proteolytic fragments of plasma fibronectin (see Fig. 5, A and B), as well as the effect of mAbs P1D6 and P4C2 on lymphocyte adhesion to these fragments. As shown in Fig. 6, Jurkat, YT, and Molt 4 (not shown) cells attach to a 38-kD fragment containing the Heparin (Hep) II domain much more efficiently than to an RGD-containing fragment (80 kD). Jurkat and Molt 4 cells also attach in a dose-dependent manner to another Hep II domain-containing fragment of 58 kD (not shown). Maximum cell attachment to the 58-kD fragment, however, reached only 30% of that achieved by the 38-kD fibronectin fragment. This suggests that the 38-kD fragment contains a high-affinity attachment site for T lymphocytes. T lymphocytes did not adhere to the NH2-terminal 29-kD fragment containing the Hep I domain of plasma fibronectin (not shown). In general, freshly derived PBL showed a similar pattern of attachment as Jurkat or Molt 4 cells and the ability of freshly derived PBL to bind to the 80-kD fragment correlated with expression of α5β1 (not shown). Other hematopoietic cell lines, such as K562 cells (Fig. 6) exhibited a clear preference for the 80-kD fragment of plasma fibronectin, whereas RD cells expressed promiscuous adhesion to all the fragments of plasma fibronectin tested, except the NH2-terminal 29-kD fragment (not shown). RGDS (1 mg/ml) partially inhibited (50%) Jurkat cell adhesion to intact fibronectin and completely (100%) inhibited their adhesion to the 80-kD fragment. Jurkat cell adhesion to the 38-kD fragment was unaffected by RGDS (up to 1 mg/ml).

As we have previously shown (Table I and Fig. 1), mAbs to α4β1 and α5β1 partially inhibited T lymphocyte adhesion to intact plasma fibronectin (Fig. 7, top). As expected, P1D6 completely inhibited adhesion of T cells to the 80-kD fragment, which contains the RGD adhesion sequence (Fig. 7, middle). P1D6 did not inhibit T lymphocyte adhesion to the 38- (Fig. 7, bottom) or 58-kD (not shown) fragments. In contrast, P4C2 completely inhibited T lymphocyte adhesion to the 38-kD fragment and had no effect on adhesion to the 80-kD fragment (Fig. 7). Furthermore, adhesion of T lymphocytes to the 58-kD fragment which also contains Hep II could be inhibited by P4C2. In every case other T lymphocyte cell lines which express both α4β1 and α5β1 (such as Jurkat cells) behave exactly as Molt 4 cells (Fig. 7). As seen in Table II, K562 cells express only α5β1. Adhesion of K562 cells to the 38- (Fig. 6) and 58-kD fragments (not shown) was greatly inhibited by P4C2.

Figure 5. (A) Domain structure of human plasma fibronectin (pFN) showing the origin of the fragments used in this study. (B) SDS-PAGE gel analysis (10% acrylamide) demonstrating the purity of the fragments. The 80-kD fragment had the NH2-terminal amino acid sequence SD(YVSPR)(LQF, and therefore begins at position 874 of the fibronectin molecule (Kornblith et al., 1985). This fragment contains the cell binding domain (Cell) and the RGD sequence of fibronectin (⁎). The 58- and 38-kD fragments had the NH2-terminal amino acid sequence TAGPDQ-TEMTIEGLQ. Both fragments contain the COOH-terminal heparin binding domain (Hep II) and result from a different cleavage of the two fibronectin chains by trypsin. The 38-kD fragment comprises the first 67 amino acid residues of the alternatively spliced connecting segment of fibronectin (IIICS) (Garcia-Pardo et al., 1987) and it is therefore derived from the A chain. The 38-kD fragment does not contain the REDV adhesion site recognized by B16-F10 melanoma cells (Humphries et al., 1986, 1987). The 58-kD fragment is derived from the B chain of fibronectin and lacks the IIICS region (Garcia-Pardo, unpublished). The 58-kD fragment also contains the COOH-terminal fibrin binding domain of fibronectin (Fib II), and is similar to previously reported fragments from this region of plasma fibronectin (Click and Balian, 1985; Rogers et al., 1985). The bands are visualized by staining with Coomassie blue.
cocytes would recognize and bind to CS-1 and if α4β1 was the affinity adhesion site for melanoma cells (Humphries et al., 1986, 1987). We have shown here that the 38-kD fragment contains a high-affinity adhesion site recognized by T lymphocytes and that α4β1 is the receptor that mediates T lymphocyte adhesion to 38 kD. This fragment does not contain the CS-5 site but it does contain the entire CS-1 region (Fig. 5) contains at least two sites responsible for mediating cell adhesion to fibronectin. This fragment does not contain the CS-5 site but it does contain the entire CS-1 region (Fig. 5) contains at least two sites responsible for mediating cell adhesion to fibronectin.

**α4β1 Is the T Lymphocyte Receptor for CS-1**

The IIICS region present on the A chain of plasma fibronectin (Fig. 5) contains at least two sites responsible for mediating cell adhesion to fibronectin (Humphries et al., 1986, 1987, 1988). Using a series of overlapping synthetic peptides spanning the entire IIICS region (CS peptides), Humphries and co-workers showed that the CS-1 (NH2-terminal) and CS-5 (COOH-terminal) peptides contained adhesion sequences recognized by mouse melanoma cells (Humphries et al., 1986, 1987). We have shown here that the 38-kD fragment contains a high-affinity adhesion site recognized by T lymphocytes and that α4β1 is the receptor that mediates T lymphocyte adhesion to 38 kD. This fragment does not contain the CS-5 site but it does contain the entire CS-1 region (Garcia-Pardo et al., 1987), which was defined as a high-affinity adhesion site for melanoma cells (Humphries et al., 1987). Therefore, it was of interest to determine if T lymphocytes would recognize and bind to CS-1 and if α4β1 was the receptor involved in this interaction.

T lymphocytes (Jurkat or Molt 4 cells) recognize and attach to CS-1 (rabbit IgG conjugate) -coated plastic surfaces (Table IV). Jurkat cells do not attach to CS-2 (rabbit IgG conjugate) -coated surfaces or to plastic surfaces coated with rabbit IgG alone (not shown). Furthermore, mAbs to α4β1 (P4C2) completely inhibited T lymphocyte adhesion to CS-1, whereas antibodies to α5β1 (P1D6) had absolutely no effect (Table IV). As we have previously shown, antibodies to α4β1 completely and specifically inhibited T lymphocyte adhesion to the 38-kD fragment (Table IV), whereas antibodies to α5β1 specifically inhibited adhesion to the RGD containing 80-kD fragment.

**Discussion**

In this study, we have described a new fibronectin receptor, identical to the integrin receptor α5β1 (Hemler et al., 1987a), preferentially expressed by nucleated hematopoietic cells. Identification of α4β1 as a specific fibronectin receptor was based on (a) inhibition of cell adhesion to fibronectin by mAbs P4C2, P3E3, and P4G9, and (b) specific reorganization and concentration of α4β1 into fibronectin-dependent focal adhesions. These findings suggest that α4β1 and α5β1 function together as primary mediators of cell adhesion to fibronectin.

mAbs to either α5β1 or α4β1 partially inhibited T lymphocyte adhesion to intact fibronectin. However, inhibition of T lymphocyte or RD cell adhesion to fibronectin was most efficient when the inhibitory antibodies to α4β1 and α5β1 were used together. This suggested that these receptors recognized independent sites on intact fibronectin. It has been well documented that the ligand for α5β1 is the cell binding domain containing the RGD sequence (Pytel et al., 1985). Analysis of the region of fibronectin recognized by α4β1 was accomplished by examining the ability of the functionally defined mAbs to inhibit lymphocyte adhesion to purified tryptic fragments of plasma fibronectin. Although T lymphocytes attached to the 80-kD cell binding domain (Fig. 5 A) they demonstrated a clear preference for a non-RGD-
jugates (1:100). After a 2-h incubation at 37°C, nonadherent cells were washed containing region located on a 38-kD tryptic fragment de-
Jurkat cells were labeled with 5~Cr and incubated in the presence of the indi-
the A and B chains of plasma fibronectin, respectively. They
38-kD fragment was three times more efficient than the 58-
was located on the 38-kD fragment. On a molar basis, the
ment. However, the high-affinity lymphocyte binding site
the present study we have shown that T lymphocytes also
mAbs (10/~g/ml) or purified mouse IgG (50 t~g/mi) for 1 h at 4°C. They were
fibronectin fragments (10/~g/ml) or the CS-1 and CS-2 rabbit IgG con-
Table IV. Inhibition of T Lymphocyte Adhesion
to CS-1 Peptide with mAbs to a4B1

| Ligand | IgG | Antibody P4C2 | P1D6 |
|--------|-----|--------------|------|
| 80 kD  | 8,580 ± 214 | 7,154 ± 398 | 202 ± 105 |
| 38 kD  | 22,680 ± 1,014 | 114 ± 78 | 24,917 ± 352 |
| CS-1   | 44,339 ± 513 | 841 ± 555 | 42,897 ± 728 |
| CS-2   | 2,576 ± 214 | 553 ± 258 | 435 ± 168 |

Jurkat cells were labeled with 3Cr and incubated in the presence of the indi-
cated mAbs (50/~g/ml) or purified mouse IgG (50/~g/ml) for 1 h at 4°C. They were then applied to plastic surfaces coated with the 80- and 38-kD tryptic plas-
ma fibronectin fragments (10/~g/ml) or the CS-1 and CS-2 rabbit IgG con-
jugates (1:100). After a 2-h incubation at 37°C, nonadherent cells were washed
for warm PBS and the bound counts per minute were solubilized and quanti-
titated in a gamma counter. Results from several experiments were pooled and
are expressed as 3Cr counts per minute bound to the adhesion surface ± SD.

Table IV. Inhibition of T Lymphocyte Adhesion
to CS-1 Peptide with mAbs to a4B1

Recently, Bernardi et al. (1987) and Liao et al. (1987, 1989) reported that some B lymphocyte cell lines bind to a region of plasma fibronectin located within the carboxy-
terminal Hep II domain. Liao et al. (1987) identified an integrin-like receptor on B cells. However, it is not clear whether the protein they described was a4B1 or a5B1. Ber-
nardi et al. (1987) also identified fibronectin receptors expressed by B lymphocytes. Interestingly, in this study, B cells that attached to fragments containing Hep II expressed a receptor similar to a4B1, whereas cells that attached to the RGD-containing cell adhesion domain expressed a receptor similar to a5B1. Together, the results of these previous reports and the present findings provide clear evidence in sup-
port of (a) the existence of an alternative adhesion domain present in the carboxy-terminal region of plasma fibronectin and (b) a role for a4B1 as the receptor for this alternative adhesion site. It will be interesting to determine the precise amino acid sequences responsible for a4B1 interaction with fibronectin. As neither of the 38- or 58-kD fragments nor CS-1 contains an RGD sequence (Kornblith et al., 1985; Garcia-Pardo et al., 1988), it is clear that characterization of the ligand for a4B1 will identify a new amino acid sequence important for cell adhe-
sion to fibronectin. Because the 38-kD fragment does not contain CS-5 (Garcia-Pardo et al., 1987), the minimal amino acid sequence responsible for T lymphocyte adhesion to 38 kD and therefore the ligand for a4B1 in these cells is not arg-
glu-asp-val or REDV (Humphries et al., 1986).

Like a2B1, the a4 subunit is weakly associated with the B1 subunit. The data presented here (Fig. 2) and our previous findings (Wayner and Carter, 1987; Wayner et al., 1988) show that the functionally defined mAbs to a2B1 and a4B1 selectively interact with epitopes present on the a subunits, based on immune precipitation of a2 or a4 without B1 after subunit dissociation. These results suggest that the unique a subunit is responsible for determining the ligand-binding specificity of each a-B complex. This concept is now further supported by the observations presented here that a5 and a4, which are both complexed with B1, mediate adhesion to dist-
tinct sites on fibronectin. This is not to suggest that the B subunit is not important in binding, but that the specificity of receptor-ligand interactions is determined by a or a unique a-B complex.

It is interesting that although LAK cells expressed abundant cell surface a4B1, it did not appear to be a functional receptor; P1D6 completely inhibited LAK cell adhesion to fibronectin (not shown). The reason for this could be that LAK cells express a degraded form of a4 (see Fig. 2). In ad-
bation, because they are activated, LAK cells over express a5B1 when compared with resting peripheral blood or leukemic T cells (Table II). In other cells that express larger quantities of a5B1 relative to a4B1 (K562-1 and HT1080) adhesion to the 80-kD RGD-containing domain via a5B1 is dominant (see K562-1 cells, Fig. 6). This implies that regulation of receptor expression determines the ability of a cell to recognize and bind to different sites on fibronectin. Furthermore, it is also possible that coexpression of the two recep-
tors for fibronectin could increase the avidity of cell binding, for example Jurkat and RD cells, which express relatively promiscuous adhesion to fibronectin when compared with YT cells, which express only a4B1.

The regulation of cell adhesion to fibronectin is potentially
complex even under the simplest possible conditions, which assume that α5β1 and α4β1 function independently of each other and do not overlap during interaction with the two binding sites on fibronectin. Variation from this simple state provides opportunities for exquisite sensitive regulation of cell adhesion. At the least complex level, this regulation can be roughly categorized as (a) processes that control the synthesis and/or exposure of the binding sites on the ligand and (b) regulation of functional expression of the receptors. Experiments at both levels are currently available and include, the observation that lymphocytes and specific antigen induce α5β1 expression on T lymphocytes followed by increased cell adhesion to fibronectin (Wayner et al., 1988, and unpublished). In addition, the control of mRNA splicing in the IIICS region of fibronectin (Kornbluth et al., 1985) during wound healing or inflammation may dictate the specificity of receptor-ligand binding in resting or activated T cells. Variations from the simple state are intriguing but require additional experimentation to even begin to identify the multitude of potential mechanisms.

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Note added in proof. We have identified a minimal peptide derived from the carboxy terminal portion of CS-1, LHGPEDLYPST, which inhibits T lymphocyte adhesion to plasma fibronectin, 38 kD, and CS-1.

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