Integrin α8β1 Promotes Attachment, Cell Spreading, and Neurite Outgrowth on Fibronectin

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The integrin α8 subunit, isolated by low stringency hybridization, is a novel integrin subunit that associates with β1. To identify ligands, we have prepared a function-blocking antiserum to the extracellular domain of α8, and we have established by transfection K562 cell lines that stably express α8β1 heterodimers on the cell surface. We demonstrate here by cell adhesion and neurite outgrowth assays that α8β1 is a fibronectin receptor. Studies on fibronectin fragments using RGD peptides as inhibitors show that α8β1 binds to the RGD site of fibronectin. In contrast to the endogenous α5β1 fibronectin receptor in K562 cells, α8β1 not only promotes cell attachment but also extensive cell spreading, suggesting functional differences between the two receptors. In chick embryo fibroblasts, α8β1 is localized to focal adhesions. We conclude that α8β1 is a receptor for fibronectin and can promote attachment, cell spreading, and neurite outgrowth on fibronectin.

INTRODUCTION

The integrins are a large family of heterodimeric cell surface molecules that serve as receptors for extracellular matrix molecules (ECM)1, for members of the Ig family, and for complement and blood clotting proteins (reviewed in Hynes, 1992). Integrins play an important role in development, wound healing, and immune responses (reviewed in Adams and Watt, 1993). Certain bacteria and viruses use integrins as receptors or coreceptors for entry into the host cell (reviewed in Isberg and Van Nhieu, 1994). Each member of the integrin family is a heterodimer composed of one α and one β subunit. To date, 15 different α subunits and eight different β subunits have been characterized in vertebrate species. Most α subunits appear to associate with only one β subunit but a few form heterodimers with more than one β subunit. Some heterodimers have multiple identified ligands; some have only one; for others, including α8β1, ligands are not well characterized (reviewed in Sonnenberg, 1993). In this paper, we demonstrate that α8β1 is a functionally important receptor for fibronectin (FN).

FN is a major component of the ECM and different alternatively spliced forms are expressed in developmentally regulated patterns (ffrench-Constant and Hynes, 1989). Several different integrins serve as receptors for FN and interact with two principal domains within FN. One of these, the central cell-binding domain, contains the RGD sequence within the 10th FNIII repeat. The second domain is located close to the carboxy terminus of FN and contains the alternatively spliced IIICS domain and an adjacent heparin binding sequence. The integrins α5β1, αvβ1, αvβ3, αvβ6, and perhaps α3β1 and α7β1 bind to the RGD site (Pytel et al., 1985a, b; Cheresh and Spiro, 1987; Charo et al., 1990; Dedhar and Gray, 1990; Vogel et al., 1990; Elices et al., 1991; Busk et al., 1992; Gu et al., 1994; Weinacker et al., 1994). The integrin α1β1β3 interacts with both the RGD site and a second site within the 9th FNIII repeat (Pytel et al., 1986; Bowditch et al., 1991, 1994). Sequences located within the 8th and 9th FNIII repeats synergize with the RGD site in promoting α5β1 inte-
grin-dependent functions such as cell adhesion and cell spreading (Obara et al., 1988; Aota et al., 1991; Kimizuka et al., 1991; Nagai et al., 1991). The integrin α4β1 binds to IIICS and heparin binding domains of FN whereas the integrin α4β7 binds to the IIICS region (Wayner et al., 1989; Elices et al., 1990; Garcia et al., 1990; Guan and Hynes, 1990; Mould et al., 1990; Ruegg et al., 1992). Within IIICS, two sequences named CS-1 and CS-5 have been identified, each promoting αβ1-dependent adhesion. CS-1 appears to be at least two orders of magnitude more active than CS-5 and is therefore the dominant cell binding site (Humphries et al., 1986, 1987; Mould et al., 1990, 1991). The minimal active sequences within CS-1 and CS-5 has been mapped to the tripeptide LDV and tetrapeptide REDV, respectively (Humphries et al., 1986). Interaction of α4β1 has also been observed with an IDAPS pentapeptide sequence located within the heparin binding domain of FN (Mould and Humphries, 1991).

Recently it has been shown that the ligand specificity of integrin receptors may be regulated. For example, α4β1 interacts under certain conditions with the RGD site within FN (Sanchez-Aparicio et al., 1994). Interaction of α4β1 with RGD was only observed after activation of α4β1 with an antibody directed against the β1 subunit (Sanchez-Aparicio et al., 1994). In addition, divalent cations are known to influence ligand specificity of numerous integrins including α4β1 (Matsui and Hemler, 1993a,b; Shimizu and Mobley, 1993). Ligand specificity may also be influenced by the lipid environment (Conforti et al., 1990; Smyth et al., 1992). Finally, it may be regulated in a cell type–specific manner. For example, integrin α2β1 serves as a collagen receptor on some cells and as a collagen and laminin receptor on others (Elices and Hemler, 1989; Kirchhofer et al., 1990; Chan and Hemler, 1993).

The integrin α8 is expressed as a heterodimer with β1 in both primary chicken embryo fibroblasts and primary sensory neurons (Bossy et al., 1991; Varnum-Finney et al., 1995). Immunohistochemical localization of α8 in the developing chicken embryo revealed a striking expression pattern. During embryonic development α8 is abundantly expressed on several classes of projection axons in the central and peripheral nervous systems, suggesting a role in establishment of axonal projections in the developing embryo. In addition α8 is expressed at moderate levels in several epithelia adjacent to basal laminae (Bossy et al., 1991).

To investigate the function of α8β1 heterodimers, it is essential to identify its ligand(s). For this purpose, we established K562 cell lines expressing α8β1 heterodimers and generated antibodies to α8’s extracellular domain. We used these cells and antibodies to examine possible interactions of α8β1 with purified ECM and Ig-superfamily members known to be expressed on fasciculating axons in projection tracts. We present evidence here that α8β1 is a FN receptor that mediates attachment of K562 cells and neurite outgrowth by embryonic sensory neurons. Within FN, α8β1 recognizes the RGD site. Interestingly, even though both α5β1 and α8β1 receptors expressed in K562 cells bind to FN, only the latter promotes extensive cell spreading of these cells, suggesting functional differences between the two receptors.

**MATERIALS AND METHODS**

**Materials**

Different preparations of human FN and FN fragments were purchased from Calbiochem (San Diego, CA) and Life Technologies (Gaithersburg, MD). Human vitronectin was purchased from Calbiochem and human thrombospondin-1 from Life Technologies. Rat collagen I and IV were purchased from Collaborative Research Inc (Bedford, MA). EHS laminin was purified as described (Timp et al., 1979). Ig molecules and restrictin were a kind gift from Dr. T. Brümmendorf, MPI Tübingen, Tübingen, Germany. They were immunofluorescence purified from embryonic chicken brain as described (Brümmendorf et al., 1993). Axiom-1–expressing cell lines were kindly provided by Dr. P. Sonderegger, University of Zürich, Zürich, Switzerland (Rader et al., 1993). The RGD and RGE peptides were purchased from Life Technologies. Monoclonal antibodies (Mabs) to α5 (B1E5 and BIIG2) and β1 (AILB2) have been described elsewhere (Werb et al., 1989; Hall et al., 1990) and were a kind gift from Dr. Carolyn Damsky, University of California, San Francisco, CA. The monoclonal antibody Mab16 to α5 (Akiyama et al., 1989) was a kind gift from Dr. Kenneth Yamada, National Cancer Institute, Bethesda, MD. The β1-specific–activating Mab TS2/16 was a kind gift from Dr. M. Hemler (Dana-Farber Cancer Institute, Boston, MA). Antibodies to αvβ5 (P1F6) and vinculin were purchased from Life Technologies and Sigma Chemical (St. Louis, MO), respectively. The αecyto and αβcyto antiserum raised to the cytoplasmic domain of α5 and α8, respectively, and CHAV-1, the Mab to chicken αv, have been described earlier (Tardiff et al., 1988; Bossy et al., 1991; Neugebauer and Reichardt, 1991). Secondary antibodies were purchased from Jackson Immunoresearch (West Grove, PA). NHS sulfo-biotin was purchased from Pierce Chemical (Rockford, IL). Other reagents for immunological techniques were purchased from Pharmacia (Upsala, Sweden) or Pierce Chemical. BSA was purchased from Serva (Heidelberg, Germany).

**Generation of Polyclonal Antiserum against the Extracellular Domain of α8**

The α8εx antibody was raised by immunizing rabbits with a sodium docetyl sulfate-polycyclamide gel electrophoresis (SDS-PAGE)-purified glutathione-S-transferase α8 fusion protein of 93 kDa. This fusion protein was obtained by expressing the plasmid αMFpGEX2T in Escherichia coli. The αMFpGEX2T plasmid contains the Nael-EcoRI fragment (nucleotide 379-2329) of the chick α8 cDNA G4 (Bossy et al., 1991) cloned into the SmaI and EcoRI sites of the pGEX2T vector (Pharmacia). The nucleotide sequence of the G4 cDNA indicates that this clone has in fact a deletion from nucleotide 654 to 752 of the chick α8 sequence (Bossy et al., 1991). Therefore, the fusion protein corresponds to 26 kDa of glutathione-S-transferase fused with 67 kDa of the chick α8 subunit. The chick α8 part encodes amino acids 62 to 710 of the α8 subunit except for amino acids 150 to 185. Immunizations were performed at Caltag Laboratories (South San Francisco, CA).
Transfections and Selection of a8-Expressing Cell Lines

K562 cells were maintained as suspension cultures in RPMI 1061 supplemented with 10% fetal bovine serum. To generate a8-expressing sublines, the chicken a8 cDNA (Bossh et al., 1991) was cloned into pAuwneo3', a eukaryotic expression vector containing the Friend spleen focus-forming virusLTR as a promoter and a neomycin resistance gene (kindly provided by Dr. Arthur Weiss, University of California, San Francisco, CA). A full length chicken a8 cDNA was first constructed by ligating the EcoRI-Aval fragment (nucleotide 1 to 569) of the G4 cDNA, the Aval-BglII fragment (nucleotide 570-1256) of the C4 cDNA, and the BglII-BamHI fragment (nucleotide 1257-3420) of the G1.6 cDNA (Bossh et al., 1991) into the EcoRI site and a blunt HindIII site of the blue script KS' vector (Stratagene, La Jolla, CA). A 3.4-kb Small fragment of this subclone containing the full length a8 cDNA was recloned into pAuwneo3'. K562 cells were transfected with the Lipofectin reagent (Life Technologies) and neomycin-resistant pools were expanded and screened by DNA blot. Positive pools were single cell cloned by limited dilution. Individual single cell-derived subclones were expanded and further screened by Western blot analysis with the a8cyt antibody. Some of the positive clones were analyzed for a8β1 cell surface expression by immunoprecipitation of cell surface-labeled cells.

Cell Surface Labeling

Cell surface iodination of K562 and K88 cells was carried out as described previously (Tomasselli et al., 1988). For cell surface biotinylation, 106 cells were harvested, washed twice with PBS, and resuspended in 1 ml PBS supplemented with 20 mM Na-N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5. NHS sulfo-biotin was added to a final concentration of 200 μg/ml and cells were incubated for 30 min at room temperature. The cells were washed twice with PBS and lysed in 2 ml lysis-buffer (50 mM Tris-HCl, pH 7.5, 50 mM N-octyl-p-D-glucopyranoside, 15 mM NaCl, 1 mM phenylmethylsulfonyl fluoride). Cell debris was removed by centrifugation and extracts were stored at −80°C.

Affinity Purification of a8ex Antiserum

Inclusion bodies from bacteria expressing a GST-a8 fusion protein containing almost the complete extracellular domain of a8 (amino acid 62 to 710 without an internal deletion) were purified according to Harlow and Lane (1988). The proteins were separated by SDS-PAGE, transferred to nitrocellulose, and the area containing a8-GST (as identified by Western blot analysis with GST antibodies and by staining with Ponceau S) was cut out from the filters. The filters were incubated for 1 h in PBS supplemented with 3% BSA to block nonspecific binding sites. Filters were pre-eluted for 15 min at room temperature with 100 mM sodium-glycine, pH 2.5, followed by multiple washes in PBS. The protein A-purified IgG fraction from a8ex sera was incubated for 2 h at room temperature with the filters containing the a8-GST fusion protein. The filters were washed multiple times with PBS and bound IgG molecules were eluted by incubation for 15 min at room temperature in 100 mM Na-glycine, pH 2.5. The eluate was immediately adjusted to neutral pH with 1 M Tris-HCl, pH 8.3. The eluate was dialyzed against Tris-buffered saline (TBS; 24 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl) and stored in aliquots at −20°C. Typically <1% of the input IgG fraction was recovered as affinity-purified a8-specific antiserum.

Affinity Purification of Integrin a8β1

Integrin a8β1 was purified from K88 cells by affinity chromatography over a8cyt antiserum coupled to protein A beads. Before coupling of the antiserum to protein A beads, the antiserum itself was first affinity purified against the peptide that was used to generate the antiserum. All procedures were carried out as described by Harlow and Lane (1988), with minor modifications. Briefly, 10 mg of peptide containing most of the a8 cytoplasmic domain (Bosssh et al., 1991) was coupled to 1.2 ml CNBr-activated Sepharose beads. Between 3 and 5 mg of protein A-purified IgG preparations of a8cyt were applied to the peptide column, eluted, dialyzed, and coupled with dimethylpimelimidate (Fierce Chemi-cal) to protein A beads. K88 cells (4 × 106 cells) were lysed, washed, and cell surface biotinylated and extracts were prepared in lysis buffer as described above. Antibody affinity columns were equilibrated in lysis buffer; the extracts were passed three times through the column by gravity flow, the column was washed extensively with column buffer, and bound proteins were eluted with lysis buffer supplemented with 2 mg/ml a8 cyto-peptide. Eluate fractions were stored for 3 to 4 days at 4°C or for extended periods at −70°C.

Immunoprecipitations, Gel Electrophoresis, and Western Blots

Protein gel electrophoresis, Western blot analysis, and immunoprecipitation experiments were carried out as described (Miller et al., 1992) with the following modifications for cell surface biotinylated molecules. Antibodies for immunoprecipitation of cell surface biotinylated molecules were covalently coupled to protein A or G beads with dimethylpimelimidate (Harlow and Lane, 1988). After immunoprecipitation, gel electrophoresis, and transfer of the proteins to nitrocellulose membranes, the membranes were blocked for 1 h with solution I (5% BSA, 5% nonfat dry milk, 0.1% Tween 20 in PBS). Filters were incubated for 1 h with streptavidin-horse radish per-oxidase (Zymed, South San Francisco, CA) in solution I, followed by three 10-min washes with solution II (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20). Incubation with enhanced chemiluminescence reagents and autoradiography followed the instructions of the manufacturer (Amersham, Arlington Heights, IL).

Immunofluorescence

Breast muscle chicken embryo fibroblasts were prepared as described (Rein and Rubin, 1968) and cultured in DME-H21 supplemented with 10% fetal bovine serum. Multilayer glass slides (Nunc, Naperville, IL) were precoated for at least 1 h at room temperature with 1 mg/ml poly-L-lysine and washed multiple times with PBS before use. Where indicated, the poly-D-lysine precoated slides were further coated for 3 h at 37°C with 5 μg/ml FN, followed by a 1-h incubation at 37°C with 10 mg/ml BSA. Chicken embryo fibroblasts were trypsinized, washed once in growth medium, and plated either overnight in growth medium on poly-L-lysine–coated slides or plated for 2–4 h in the absence of serum onto FN-coated slides. Cells were incubated at 37°C in a 10% CO2 incubator. Cells were fixed for 10 min at 0°C with 4% paraformaldehyde, blocked for 1 h in solution A (10% normal goat serum, 0.1% Tween 20, 1% glycine, 2% BSA in PBS), incubated for 1 h at room temperature with primary antibody in solution A, washed five times with solution A, incubated for 1 h at room temperature with secondary antibody, washed five times with solution A, embedded in FITC-Guard (TESTOG, Chicago, IL), and analyzed by immunofluorescence microscopy.

Cell Adhesion and Cell Spreading Assays

Cell adhesion assays were essentially carried out as described elsewhere (Hall et al., 1987; Neugebauer and Reichardt, 1991). Linbro titrater 96-well plastic dishes (Flow Laboratories, McLean, VA) were coated for 3 h at 37°C or O/N at 4°C with the substrate molecules and diluted with PBS to the indicated concentration. A total vol of 100 μl substrate solution was applied to each well. Nonspecific binding sites were blocked by incubating coated wells for 1 h at 37°C with 10 mg/ml BSA in PBS. Cells were counted, harvested, washed once with PBS supplemented with 1 mM EDTA, washed once with TBS (24 mM Tris, pH7.4, 137 mM NaCl, 2.7 mM

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KCl), and resuspended in TBS containing 0.1% BSA, 2 mM glucose, and divalent cations at varying concentrations. Cells (10^6) were plated out per well. Cells were incubated for 1.5 h at 37°C, washed three to five times with TBS containing the appropriate divalent cation, and fixed for 15 min with 2% paraformaldehyde. Cells were stained for 5 min with 2.5% crystal violet in 20% ethanol, washed three times with water, and retained dye was solubilized in 1% SDS and quantitated by A_560 in a microtiter plate reader (Flow Laboratories). Values were normalized against BSA-coated wells. Usually, determinations were carried out in duplicates, triplicates, or quadruplicates and most experiments were carried out more than three times. Error bars indicate the standard error of the mean.

For antibody inhibition experiments, cells were preincubated for 15 min on ice with the antibody. Antibody was present throughout the duration of the adhesion assay. All antibodies with the exception of the commercial anti-a5 MAb P1F6 were purified over protein A or G columns before use in adhesion assays. The β1-specific ALLB2 antibody was either used as an IgG preparation or as ascites supernatant with identical results. The a8ɛx antibody was affinity purified against the a8 fusion protein before use (see above). Antibody concentrations for inhibitions were as follows: ALLB2 (anti-β1) 0.1–1.0 µg/ml, E105 (anti-α5) 1–25 µg/ml, a8ɛx (anti-a8) 500 µg/ml, and unrelated polyclonal IgG (C5) 150 µg/ml. Antibody P1F6 (anti-a8ɛx) was supplied as ascites. It effectively blocked a8ɛx-mediated vitronectin adhesion of K562 cells at a 1:200 dilution and was used at 1:100 or 1:200 dilution for function inhibition studies on FN.

Neurite Outgrowth Assays

For neurite outgrowth assays, embryonic day 7–8 chick dorsal root ganglia were dissected and dissociated by incubation for 10 min at 37°C in 0.05% trypsin/FBS followed by trituration. The cells were washed in F12 medium, resuspended in F12 medium supplemented with 10% fetal bovine serum, and plated for 1–3 h onto tissue culture dishes. Nonneuronal cells tend to attach to these dishes whereas neuronal cells could be removed from the dishes with the medium. Neuronal cells were harvested by centrifugation and resuspended in F12 medium supplemented with 1% BSA and 100 ng/ml nerve growth factor. The cells were plated onto 96-well plates (10^4 cells/well) that had been precoated with nitrocellulose (Lagenaur and Lemmon, 1987) followed by coating with FN or a fragment of FN. The cells were gently centrifuged onto the dish and incubated for 4–7 h at 37°C in a 5% CO₂ atmosphere. Where indicated, antibodies were added to the wells simultaneously with the cells. To assay effects of various antibodies, the fraction of cells with neurites extending at least two cell diameters was quantified.

RESULTS

Characterization of α8β1-Expressing K562 Cell Lines

K562 cells are suitable for the identification of novel integrin ligands because they appear to express only two endogenous integrin receptors: moderate levels of α5β1, which binds FN (Arroyo et al., 1992; Chan and Hemler, 1993; Delwel et al., 1993), and low levels of αvβ5, which binds vitronectin (VN) and possibly FN (Pasqualini et al., 1993). Function-inhibiting antibodies to each of these receptors exist, making it possible to express additional integrins and examine binding to any ECM adhesive glycoprotein, including FN or VN. The K562 cell lines employed here express very low levels of αvβ5 and exhibit αvβ5-dependent adhesion to VN, but not to FN (see below). To identify ligands for α8β1, we transfected K562 cells with an α8 cDNA in an expression vector and established clones derived from single cells. Multiple α8-expressing K562 cell lines were identified by antigen blot analysis employing a polyclonal antiserum raised against the cytoplasmic domain of α8. In each cell line, the antiserum recognized a 160-k protein, the reported Mᵢ for α8 (Bossy et al., 1991), which was not present in parental K562 cells (our unpublished observations).

To analyze cell surface expression of α8 and α8β1 heterodimers, integrins were immunoprecipitated from membrane extracts of cell surface biotinylated K562 cells and α8 transfectants. Figure 1 shows the data for one representative clone, KA8, but similar results were obtained with additional clones. Either β1-specific or an α5-specific Mab precipitated α5β1 heterodimers from parental K562 cells and KA8 cells. The α5 subunit was resolved into three bands presumably representing differentially glycosylated forms of α5 (Figure 1A, lane 1). Anti-β1, but not anti-α5 precipitated an additional protein of Mᵢ 160 k from KA8, but not K562 membrane extracts (Figure 1A, lane 4). This protein must be the α8 subunit for several reasons:

1) The Mᵢ 160-k protein was only observed in transfectants, but not in parental cell lines. Its size is consistent with the reported size for α8 (Bossy et al., 1991).
2) A protein of the same Mᵢ, was specifically immunoprecipitated from KA8 but not K562 cells by two antisera raised against separate domains of α8 (Figure 1B). An antibody to the cytoplasmic domain (α8cyto) coprecipitated bands of Mᵢ 160 k and 115 k, the expected sizes of α8 and β1. An antibody to the extracellular domain of α8 (α8ex) precipitated the Mᵢ 160-k, but not the Mᵢ 115-k band, suggesting that it disrupted the association between α8 and β1. Other anti-integrin antibodies have previously been shown to dissociate integrin heterodimers (Horwitz et al., 1985).
3) The β1-associated Mᵢ 160-k protein was completely removed by an α8cyto antibody column (Figure 1C, lane 6). Before coupling, the α8cyto antibody was affinity purified against the peptide used to generate the antibody to ensure absolute specificity. Although all of the α8β1 heterodimer was retained on the column, all the α5β1 heterodimer was in the flow-through (Figure 1C, lanes 5 and 6).
4) The electrophoretic mobility of the Mᵢ 160-k protein decreased in the presence of the reducing agent dithiothreitol, consistent with the previously reported behavior of chick α8, which suggests that the mature α8 subunit is cleaved into two fragments linked by disulfide bonds (Bossy et al., 1991; our unpublished observations).

We conclude that KA8 cells express both α5β1 and α8β1 integrin heterodimers on the cell surface. Both heterodimers appear to be expressed at similar levels.
Figure 1. Integrin expression in K562 cells and α8 transfectants. (A) Extracts from cell surface biotinylated K562 and KA8 cells were immunoprecipitated with the indicated antibodies (anti-β1 [AIIB2] and anti-α8 [α8cyto]; anti-α5 [B1E5]) separated on 6% polyacrylamide gels, transferred by blotting to nitrocellulose, reacted with streptavidin-horseradish peroxidase conjugates, and visualized by chemiluminescence. (B) KA8 cells were cell surface iodinated and integrins were precipitated with the indicated antibodies. After electrophoretic separation, proteins were visualized by autoradiography. The * marks potential breakdown products. (C) Extracts from cell surface biotinylated KA8 cells were passed over an α8cyto antibody column. The flow through fraction (FT) and the eluate (E) were analyzed by immunoprecipitation with β1 antibody (lanes 4 and 6) or the eluate was loaded directly onto the gel without immunoprecipitation (lane 5). As a control, input material (IP) was also analyzed by immunoprecipitation with the indicated antibodies (lanes 1–3). After electrophoresis and transfer to nitrocellulose, filters were reacted with streptavidine-horseradish peroxidase conjugates and proteins were visualized by chemiluminescence.

(Figure 1A). No other β1 class integrins were detected on the cell surface. Antigen blot analysis with antibodies specific for β1, α5, αv, and β5 indicated that each subunit is expressed at similar levels in K562 and KA8 cells (our unpublished observations). In agreement with earlier findings (Arroyo et al., 1992; Chan and Hemler, 1993; Delwel et al., 1993) we did not detect any expression of α3 or α4 in K562 cells.

Screen for α8β1 Ligands

The expression pattern of α8 within epithelia suggested that it may interact with components of basement membranes. In addition, its expression within major axonal tracts on fasciculating axons raises the possibility that α8 may interact with counter receptors on axonal surfaces. To identify possible α8β1 ligands, several purified ECM molecules and immunofinity–purified Ig-superfamily members known to be expressed on axons were assayed in cell adhesion assays (Table 1). As previously reported, endogenous K562 integrins show low ligand binding efficacy in Mg2+ but ligand binding can be activated by Mn2+ or by the β1-specific–activating Mab TS2/16 (Arroyo et al., 1992; Chan and Hemler, 1993). Therefore, cell adhesion assays were carried out in the presence of different concentrations (1 to 10 mM) of either Mg2+, Ca2+, Mn 2+ or a combination of these ions. Similar experiments were performed in the presence of Mg2+ or Ca2+ and the β1-activating antibody TS2/16.

Parental K562 cells and α8 transfectants adhered to several preparations of FN, VN, F11, DM-GRASP, and restrictin. Adhesion to F11, DM-GRASP, and restrictin appeared to be mediated by receptors other than α8β1 because it was not inhibited by antibodies to either the α8 or β1 subunit (our unpublished observations). These interactions were therefore not further analyzed. α8β1-mediated interactions were observed with substrates coated with FN (see below). In some assays interactions were also observed with VN; however, these interactions showed great variability depending on the VN preparation. Clearly, the VN preparations were not contaminated with FN as determined by Western blot analysis with FN-specific antibodies (our unpublished observations). The reason for this incon-
sistency is at present unclear, but raises the possibility that αβ1 is a receptor for VN in addition to FN.

**Adhesion to FN**

In the presence of Mg$^{2+}$, both K562 and KA8 cells adhered weakly but significantly to FN. In the presence of Ca$^{2+}$, adhesion was close to background levels (Figure 2A). In the presence of either cation, KA8 cells often showed slightly stronger adherence. In the presence of Mn$^{2+}$, both cell lines adhered strongly to FN. Levels of adhesion to FN in the presence of Mn$^{2+}$ were defined as 100% because they were indistinguishable from adhesion to the control substrate poly-D-lysine (our unpublished observations). In dose response curves, half maximal binding was observed for both cell lines at 5 μg/ml or <1 μg/ml coating concentration in the presence of Mg$^{2+}$ or Mn$^{2+}$, respectively (Figure 2B). The curves did not differ significantly between the two cell lines.

To identify which integrins mediate interactions of these cells with FN, effects of subunit-specific antibodies were examined. Results in Figure 3 show that MAbs directed either against β1 (MAb AIIB2) or α5 (MAb B1E5) almost completely inhibit binding of K562 cells to FN. Anti-αβ5 (MAb P1F6) and several other unrelated polyclonal antisera (our unpublished observations) did not interfere with adhesion. Adhesion of KA8 cells to FN was also eliminated by the anti-β1 MAb. In contrast, however, anti-α5 MAbs were not effective in blocking KA8 cell adhesion. Three different anti-α5 MAbs (B1E5, BIIG2, and MAb16) failed to block adhesion of KA8 and two additional α8-expressing cell lines (our unpublished observations).

To analyze interactions mediated by αβ1, the effect of anti-αβ1 antibody was tested in the same assay (Figure 3). The antibody was affinity purified using the αβ fusion protein to ensure specificity. Incubation with affinity-purified αβ1 antibody alone did not inhibit binding of K562 or KA8 cells to FN; however, the antibodies did inhibit attachment of KA8 cells to FN significantly when used in combination with an α5 MAb (B1E5). As controls, other antibody combinations were also
tested. Simultaneous addition of anti-\(\alpha 5\) (MAb B1E5) and anti-\(\alpha \beta 5\) (MAb P1F6) did not significantly reduce adhesion of KA8 cells to FN (Figure 3). Adhesion was also not inhibited by a combination of anti-\(\alpha 5\) (MAB B1E5) and IgG preparations of several unrelated polyclonal antisera (our unpublished observations). Thus, these data provide strong evidence that two different integrin heterodimers, \(\alpha 5\beta 1\) and \(\alpha 8\beta 1\), mediate KA8 cell adhesion to FN. The integrin \(\alpha \beta 5\) does not appear to have contributed to FN adhesion in these assays. The \(\alpha \beta 5\) MAb employed here was clearly active, because in parallel assays it efficiently inhibited adhesion of K562 cells to VN (our unpublished observations).

**Anti-\(\beta 1\) MAb TS2/16 Activated Adhesion to FN**

\(\beta 1\)-integrin-dependent adhesion of K562 cells to ECM molecules in the presence of \(\text{Mg}^{2+}\) can be enhanced by TS2/16, an anti-\(\beta 1\) MAb (Arroyo et al., 1992; Chan and Hemler, 1993). Adhesion of either K562 or KA8 cells to FN was strongly up-regulated by TS2/16 (Figure 4). TS2/16-promoted adhesion of K562 cells to FN was completely inhibited by a MAb against the \(\alpha 5\) subunit (B1E5). In contrast, KA8 adhesion to FN was partially, but not completely inhibited by the \(\alpha 5\) MAb. Although anti-\(\alpha 8\) alone had only weak inhibitory affects, in combination with anti-\(\alpha 5\) it virtually eliminated adhesion of KA8 cells to FN. A MAb to \(\alpha \beta 5\) (P1F6) did not reduce adhesion significantly. These data also demonstrate that KA8 cells use both \(\alpha 5\beta 1\) and \(\alpha 8\beta 1\) heterodimers as receptors for FN.

In the presence of \(\text{Mg}^{2+}\) and TS2/16, anti-\(\alpha 5\) was partially effective alone in blocking FN adhesion of KA8 cells; however, in the presence of \(\text{Mn}^{2+}\), anti-\(\alpha 5\) alone was ineffective (compare Figures 3 and 4). This suggests that adhesion of KA8 cells to FN in the presence of TS2/16 is more strongly dependent on \(\alpha 5\beta 1\) than adhesion in the presence of \(\text{Mn}^{2+}\). As one possible interpretation, \(\text{Mn}^{2+}\) may activate \(\alpha 5\beta 1\) and \(\alpha 8\beta 1\) heterodimers equally well, whereas MAb TS2/16 may be much more effective at activating the \(\alpha 5\beta 1\) heterodimer.

**Cell Spreading on FN**

To analyze cell spreading and other possible morphological responses, the parental K562 cells and three different \(\alpha 8\) transfectants were plated at low density on FN; representative results are shown for subclone KA8 (Figure 5). In the presence of \(\text{Mg}^{2+}\), both K562 and KA8 cells adhered weakly to FN and the cells maintained a rounded morphology (Figure 5, panels a and b). In the presence of \(\text{Mn}^{2+}\), K562 cells spread slightly on FN (Figure 5, panel c). In contrast, KA8 cells spread strongly on FN and elaborated numerous lamellopodia-like protrusions (Figure 5, panel d). Spreading was evident within 30 min after plating. Spreading of KA8 cells was inhibited by anti-\(\beta 1\) (Figure 5, panel e) or by affinity-purified \(\alpha 8\) antibody (Figure 5, panel f), but not by anti-\(\alpha 5\) (Figure 5, panel g) or an unrelated polyclonal antibody (Figure 5, panel h). Extensive cell spreading of KA8 cells but not K562 cells was also induced in the presence of \(\text{Mg}^{2+}\) and the \(\beta 1\)-activating antibody TS2/16 (Figure 6, a and b). Again, spreading was inhibited by anti-\(\alpha 8\) (Figure 6, panel d) but not anti-\(\alpha 5\) antibody (Figure 6, panel c).

We conclude that integrin \(\alpha 8\beta 1\) but not \(\alpha 5\beta 1\) can promote extensive cell spreading of K562 cells on FN. Importantly, both heterodimers were expressed at similar levels on the cell surface of KA8 cells (Figure 1). Thus, the results suggest that the two integrins \(\alpha 5\beta 1\) and \(\alpha 8\beta 1\) are functionally different.

**Focal Adhesion Localization**

We were unable to detect \(\alpha 8\beta 1\) or \(\alpha 5\beta 1\) in focal adhesions in K562 cells because very few, if any such structures are present, as assessed by staining with

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**Figure 4.** Inhibition of TS2/16-stimulated adhesion to FN. Adhesion to FN (5 \(\mu g/ml\) coating concentration) was measured in the presence of 1 mM \(\text{Mg}^{2+}\) and the integrin-activating antibody TS2/16. The blocking antibodies were as follows: anti-\(\beta 1\) (AIIB2), anti-\(\alpha 5\) (B1E5), anti-\(\alpha 8\) (P1), and anti-\(\alpha \beta 5\) (P1F6). The values were normalized as indicated in Figure 3. For antibody concentrations see MATERIALS AND METHODS.
Figure 5. Spreading assay. K562 cells (a and b) or KA8 cells (c-h) were plated onto FN (5 μg/ml coating concentration) in the presence of either 1 mM Mg²⁺ (a and c) or 1 mM Mn²⁺ (b and d-h); in the absence of antibody (a-d); or in the presence of an anti-β1 antibody A1B2 (e), anti-α8 antibody α8ex (f), anti-α5 antibody B1E5 (g), unrelated polyclonal IgG (h). Cells were photographed 30–45 min after plating. For antibody concentrations see MATERIALS AND METHODS. Scale bar, 100 μm.
Integrin α8β1 Promotes Attachment, Spreading

Figure 6. Spreading assay. K562 cells (a) or KA8 cells (b-d) were plated onto FN (5 μg/ml coating concentration) in the presence of 1 mM Mg²⁺ and the β1-activating antibody TS2/16; in the absence of function-blocking antibodies (a and b); in the presence of anti-α5 antibody B1E5 (c); in the presence of anti-α8 antibody a8ex (d). Cells were photographed 30–45 min after plating. For antibody concentrations see MATERIALS AND METHODS. Scale bar, 100 μm.

vinculin antibodies (our unpublished observations). We therefore analyzed the localization of α8 in primary chick embryo fibroblasts, which have previously been shown to express α8β1 heterodimers (Bossy et al., 1991) and assemble numerous focal adhesions (reviewed in Burridge et al. 1990). Accordingly, chick embryo fibroblasts were allowed to attach and spread on FN for 2–4 h. By immunofluorescence α8 and vinculin were colocalized in focal adhesions (Figure 7, a and b). Interestingly, α8 was predominately observed within focal adhesions at the edges of cells but rarely in more central regions of the cells. Localization of α8 in focal adhesions was also observed when the fibroblasts were plated overnight on poly-D-lysine-coated cover slips (Figure 7, c and d). Under these conditions, these fibroblasts synthesized high quantities of FN and assembled it into an extensive matrix (our unpublished observations). Thus it seems likely that endogenously synthesized FN accounts for the formation of focal adhesions containing α8 in this experimental paradigm.

Integrin α8β1 Recognizes the RGD Site within FN
To identify the binding site(s) for α8β1 within FN, adhesion assays were carried out with various chymotryptic FN fragments (40 kDa, 45 kDa, 120 kDa) in the presence of Mn²⁺. The FN120 fragment contains the RGD sequence recognized by many different integrins including α5β1. The C-terminal FN40 fragment contains binding sites for integrin α4β1 and α4β7. The N-terminal FN45 fragment contains sites that mediate FN matrix assembly and collagen binding. No integrin has been shown to bind within this region of FN (for a review see Sonnenberg, 1993).

In the presence of Mn²⁺ (Figure 8) or the β1-activating antibody TS2/16 (our unpublished observations) both K562 and KA8 cells adhered strongly to FN120 but not significantly to FN40 or FN45 (our unpublished observations). Antibody inhibition experiments confirmed that the adhesion to FN120 was dependent on both α5β1 and α8β1 (Figure 8).
Figure 7. Focal adhesion localization. Chicken embryo fibroblasts were plated for 4 h on FN-coated surfaces (a and b) or for 16 h on poly-β-lysine-coated surfaces (c and d). Cells were fixed and stained for either vinculin (a and c) or integrin α8 (b and d) and analyzed by immunofluorescence microscopy. Vinculin was visualized with a fluorescein-coupled secondary antibody and α8 with a texas-red coupled secondary antibody. Scale bar, 100 μm.

These results were virtually identical to those obtained with full length FN (Figure 3). RGD peptide competition experiments revealed that adhesion of both cell lines to FN120 was completely inhibited by an RGD peptide, but not by a control RGE peptide (Figure 9). This data suggests that α8β1 recognizes the same RGD sequence within FN as is recognized by α5β1.

Figure 8. Antibody inhibition of adhesion to FN120. Adhesion of K562 and KA8 cells to FN120 (5 μg/ml coating concentration) was measured in the presence of 1 mM Mn²⁺ and the indicated antibodies: anti-β1 (AIIB2), anti-α5 (B1E5), anti-α8 (α8ex), and anti-αv (P1F6). The values were standardized as indicated in Figure 3. For antibody concentrations see MATERIALS AND METHODS.
Integrin $\alpha\beta 1$-Dependent Neurite Outgrowth of Sensory Neurons

Integrin $\alpha\beta 1$ is highly expressed on axons of embryonic sensory neurons within the developing nervous system (Bossey et al., 1991). To analyze a possible function of $\alpha 8$ in neuronal differentiation, we performed neurite outgrowth assays of embryonic day 7 and 8 dorsal root ganglion neurons in culture on full length FN and FN fragments (Table 2). It has previously been demonstrated that dorsal root ganglion neurons respond with robust neurite outgrowth when plated onto different ECM substrates such as laminin or FN (Rogers et al., 1983; Tomaselli et al., 1986; Humphries et al., 1988). These cells express integrin $\alpha\beta 1$ heterodimers (Varnum-Finney et al., 1995). When plated in the presence of nerve growth factor, dorsal root ganglion neurons extended neurites on FN, FN120, or FN40 within 4 h. Neurite outgrowth on FN and FN120 was partially inhibited by affinity-purified $\alpha 8e X$ antibody, suggesting that $\alpha\beta 1$ is involved in neurite outgrowth on FN. Neurite outgrowth on FN40 was not inhibited by $\alpha 8e X$ consistent with the observation that $\alpha\beta 1$ expressing KA8 cells do not adhere to FN40 (our unpublished observations).

Neurite outgrowth assays on FN120 were also carried out in the presence of antibodies to $\alpha v$ and $\beta 1$. Both antibodies partially inhibited neurite outgrowth. When added together, the $\alpha v$ and $\beta 1$ antibodies completely inhibited neurite outgrowth. This suggests that $\beta 1$ and $\alpha v$ integrins account for most and perhaps all dorsal root ganglion neurite outgrowth on FN120. The data also indicate that $\alpha v$ must associate with a $\beta$ subunit distinct from $\beta 1$ on these cells. Antibodies specific for $\alpha 8$ and $\alpha v$ together also had an additive but partial inhibitory effect on neurite outgrowth. This suggests that $\alpha\beta 1$ and at least one additional $\beta 1$ integrin is involved in neurite outgrowth on FN120. A likely candidate would be $\alpha 5\beta 1$, but lack of a function blocking anti-chick $\alpha 5$ antibody made it unfeasible to test this possibility. In conclusion, $\alpha\beta 1$, together with unidentified $\beta 1$ and $\alpha v$ integrins, promotes neurite outgrowth by dorsal root ganglion neurons on FN120.

**DISCUSSION**

The major objective of this study was to identify ligands for integrin $\alpha\beta 1$. We conclude that $\alpha\beta 1$ is a functionally important FN receptor that recognizes the RGD site within the central cell binding domain of FN. This conclusion is based on the following observations: 1) FN adhesion of K562 transfectants expressing integrin $\alpha\beta 1$ and $\alpha\beta 1$ (Figure 1) was completely inhibited by an anti-$\beta 1$ MAb (Figures 3 and 4). Adhesion was also strongly inhibited by a combination of anti-$\alpha 5$ and anti-$\alpha 8$ antibodies but not by either antibody alone (Figures 3 and 4). 2) Adhesion of $\alpha 8$ transfectants to a FN fragment spanning the central cell binding domain was dependent on integrin $\alpha\beta 1$ and $\alpha\beta 1$ and completely inhibited by RGD peptide (Figures 8 and 9). 3) $\alpha 8$ transfectants spread substantially better on FN then parental K562 cells. Spreading was inhibited by anti-$\beta 1$ or anti-$\alpha 8$ but not by anti-$\alpha 5$ antibodies (Figures 5 and 6). This suggests that $\alpha\beta 1$ but not $\alpha 5\beta 1$ promotes substantially cell spreading of K562 cells. 4) Neurite outgrowth of primary sensory neurons on FN was partially dependent on integrin $\alpha 8$ (Table 2).

$\alpha\beta 1$-dependent adhesion to FN had a similar requirement for specific divalent cations as has been described for other $\beta 1$ class integrins heterologously expressed in K562 cells (Arroyo et al., 1992; Chan and Hemler, 1993; Delwel et al., 1993). Minimal adhesion was observed in the presence of Mg$^{2+}$ or Ca$^{2+}$. Adhesion was strongly enhanced by Mn$^{2+}$ or by the $\beta 1$-specific–activating antibody TS2/16 (Figures 2-4). Interestingly, cell adhesion in the presence of Mn$^{2+}$ was inhibited by a combination of anti-$\alpha 5$ and anti-$\alpha 8$ antibodies but not by either antibody alone (Figure 3). In contrast, adhesion in the presence of TS2/16 was partially sensitive to anti-$\alpha 5$ MAb (Figure 4). This observation is consistent with the interpretation that MAb TS2/16 preferentially activated integrin $\alpha 5\beta 1$ whereas Mn$^{2+}$ activated $\alpha 5\beta 1$ and $\alpha\beta 1$ equally well. Previous studies have shown that $\beta 1$-specific Mabs can affect individual integrin heterodimers differently. For example, a MAb to chicken $\beta 1$ inhibits cell adhesion to

<table>
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<th>cont</th>
<th>$\alpha 8$</th>
<th>$\alpha v$</th>
<th>$\beta 1$</th>
<th>$\alpha 8 + \alpha v$</th>
<th>$\alpha v + \beta 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN</td>
<td>100</td>
<td>60 ± 1</td>
<td>65 ± 15</td>
<td>36 ± 9</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>FN120</td>
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<td>51 ± 9</td>
<td>25 ± 6</td>
<td>33 ± 9</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>FN40</td>
<td>100</td>
<td>99 ± 4</td>
<td>113 ± 7</td>
<td>55 ± 11</td>
<td>103 ± 7</td>
<td>54 ± 8</td>
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Sensory dorsal root ganglion neurons were isolated from E8 chicken embryos and cultured on intact FN, FN120, or FN40. To assay effects of various antibodies, the fraction of neurons with neurites extending at least two cell diameters was quantified. Antibodies and antibody concentration were as follows: anti-$\alpha 8$ (58X, 20 µg/ml); anti $\alpha v$ (CHA V, 100 µg/ml); and anti-$\beta 1$ (W1B10, 50 µg/ml).
VN but promotes adhesion to laminin and collagen types I and IV (Neugebauer and Reichardt, 1991).

Adhesion to FN in the presence of Mn\textsuperscript{2+} was inhibited strongly but not completely by a combination of \(\alpha_5\) and \(\alpha_8\) antibodies. Several criteria support the fact that the residual adhesion in the presence of these antibodies was not caused by expression of an additional integrin. First, adhesion to FN was completely inhibited by an anti-\(\beta_1\) MAb (Figures 3 and 4); \(\alpha_5\beta_1\) and \(\alpha_8\beta_1\) were the only \(\beta_1\)-class integrins expressed (Figure 1). Expression of other potential FN binding integrins such as \(\alpha_3\beta_1\), \(\alpha_4\beta_1\), or \(\alpha_5\beta_1\) was not detectable by us or by other groups (Figure 1 and our unpublished observations; Arroyo et al., 1992; Chan and Hemler, 1993; Delwel et al., 1993; Pasqualini et al., 1993). Second, we observed low level expression of integrin \(\alpha\nu\) (our unpublished observations), which presumably forms a heterodimer with \(\beta_5\) as previously described for K562 cells (Pasqualini et al., 1993). Antibodies to integrin \(\alpha\nu\) (our unpublished observations) or \(\alpha\nu\beta_5\) (Figures 3 and 4) did not affect adhesion to FN, even in the presence of anti-\(\alpha_5\) antibodies (Figure 3), suggesting that \(\alpha\nu\)-containing integrins were not involved. We can currently only speculate on the nature of the residual adhesion. Clearly, the anti-\(\alpha_5\) antibody can completely inhibit \(\alpha_5\) function (Figures 3 and 4). This suggests that the anti-\(\alpha_8\) antibody may only partially block interaction of \(\alpha_8\) with the substrate. Integrins are known to exist in multiple different conformational states that are recognized with different affinity by integrin antibodies (reviewed in Loftus et al., 1994). It is possible that in the presence of Mn\textsuperscript{2+}, \(\alpha_8\beta_1\) may exist in multiple different conformers, some of which may be recognized with low affinity by the \(\alpha_8\)-specific antibody. Interestingly, in the presence of the \(\beta_1\)-activating antibody TS2/16, adhesion was completely inhibited (Figure 4), suggesting that TS2/16 may induce an \(\alpha_8\beta_1\) conformation preferentially recognized by the antibody. Further studies are necessary to resolve these issues.

In some assays we observed \(\alpha_8\beta_1\)-dependent adhesion of \(\alpha_8\) transfectants to VN (Table 1). Adhesion was clearly sensitive to \(\alpha_8\) and \(\beta_1\) antibodies and it was inhibited by RGD peptides, suggesting that \(\alpha_8\beta_1\) binds to the RGD site within VN (our unpublished observations); however, adhesion to VN was only observed with some but not other preparations of VN. The cause for this variability is as yet not clear. The VN preparations were not contaminated with FN as analyzed by Western blotting, and no other major contaminants were detected by staining gels after electrophoretic separation of VN preparations (our unpublished observations). As one possibility, the RGD site within VN may have a very defined conformation that is sensitive to denaturation during purification. \(\alpha_8\beta_1\) may not recognize the denatured RGD site. Further experiments are necessary to address this point.

\(\alpha_8\beta_1\) is an additional member of the extensive family of integrins that bind to FN. One may ask why there are so many different receptors for a single ECM ligand. Functional redundancy between receptors cannot be excluded, but recent observations suggest that different integrins may have different functions. For example, Zhang et al. (1993) showed that the integrin \(\alpha_5\beta_1\) can promote attachment, matrix assembly, and migration of CHO cells on FN whereas the integrin \(\alpha\nu\beta_1\) functions only as an adhesion receptor. In another study, the integrin \(\alpha_5\beta_1\) and an \(\alpha\nu\)-containing heterodimer were shown to contribute to cell adhesion and spreading of endothelial cells on FN, but a rise in intracellular Ca\textsuperscript{2+} was only observed for \(\alpha\nu\)-mediated interactions (Schwartz and Denninghoff, 1994). Our data suggest that binding of \(\alpha_5\beta_1\) or \(\alpha_8\beta_1\) to FN may lead to receptor-specific cellular responses: integrin
a8β1 but not integrin α5β1 induced substantial spreading of K562 cells (Figures 5 and 6). It seems likely that integrin α5 and α8 cytoplasmic domains may specifically interact with or modulate the activity of different cytoplasmic components. Indeed, different integrin cytoplasmic domains have been shown to execute distinguishable functions. Chan et al. (1992) expressed in RD cells integrin chimeras containing the extracellular and transmembrane domain of α2 linked to different α cytoplasmic domains. The cytoplasmic domain of integrin α4 conferred enhanced motility to the cells while cytoplasmic domains of α2 and α5 allowed enhanced collagen gel contraction. Pasqualini and Hemler (1994) showed by a similar approach that the β1 cytoplasmic domain promotes adhesion, proliferation, and focal adhesion localization but not migration of CHO cells on FN; in contrast, the β5 cytoplasmic domain promotes adhesion and migration but not focal adhesion localization or proliferation. It will be interesting to construct chimeras between integrin α5 and α8 to analyze possible functional differences caused by the cytoplasmic domains of these receptors.

During embryonic development integrin α8 is highly expressed in the developing nervous system on a number of projection axons. Expression is also observed in several epithelial cells adjacent to basal laminae where FN may be its primary ligand (Bossy et al., 1991). FN is found in the pathway of migrating neural crest cells and supports migration of these cells (Newgreen and Thiery, 1980; Krotoski et al., 1986; Dufour et al., 1988). FN also supports neurite outgrowth of numerous peripheral neurons when analyzed in vitro (Rogers et al., 1983; Tomaselli et al., 1986; Humphries et al., 1988). It is up-regulated at sites of nerve injury, suggesting a possible function in regeneration (Lefcort et al., 1992). Our data suggest that embryonic sensory neurons use multiple integrins, including the integrin α8β1 to extend neurites on FN (Table 2); however, FN is a rather poor substrate for in vitro neurite outgrowth of many neurons derived from the central nervous system (Rogers et al., 1983). The striking axonal expression pattern of αβ in the central nervous system (Bossy et al., 1991) has tempted us to analyze possible interactions of α8β1 with members of the Ig-superfamily known to be expressed on fasciculating axons (reviewed in Sonderegger and Rathjen, 1992). We did not observe an interaction of α8β1 with any of the Ig-superfamily members tested (Table 1). Recent experiments in our laboratory show that α8β1 can promote neurite outgrowth of dorsal root ganglion neurons and motor neurons on tenascin-C (Varnum-Finney et al., 1995). Tenascin is highly expressed in the central nervous system (Grumet et al., 1985; Chuong et al., 1987), suggesting that α8β1 may mediate some of the interactions of central neurons with this ECM glycoprotein. Studies are in progress to identify additional ligands.

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