Adhesion of a Chicken Myeloblast Cell Line to Fibrinogen and Vitronectin through a β_1-class Integrin

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Abstract. The adhesive interactions of circulating blood cells are tightly regulated, receptor-mediated events. To establish a model for studies on regulation of cell adhesion, we have examined the adhesive properties of the HD11 chick myeloblast cell line. Function-perturbing antibodies were used to show that integrins containing the β_1 subunit mediate HD11 cell attachment to several distinct extracellular matrix proteins, specifically fibronectin, collagen, vitronectin, and fibrinogen. This is the first evidence that an integrin heterodimer in the β_1 family functions as a receptor for fibrinogen. While the $\alpha_v\beta_1$ heterodimer has been shown to function as a vitronectin receptor on some cells, this heterodimer could not be detected on HD11 cells. Instead, results suggest that the β_1 subunit asso-

ciates with different, unidentified α subunit(s) to form receptors for vitronectin and fibrinogen. Results using function-blocking antibodies also demonstrate that on these cells, additional receptors for vitronectin are formed by $\alpha_v\beta_3$ and α_v associated with an unidentified 100-kD β subunit. The adhesive interactions of HD11 cells with these extracellular matrix ligands were shown to be regulated by lipopolysaccharide treatment, making the HD11 cell line attractive for studies of mechanisms regulating cell adhesion. In contrast to primary macrophage which rapidly exhibit enhanced adhesion to laminin and collagen upon activation, activated HD11 cells exhibited reduced adhesion to most extracellular matrix constituents.

T HE integrins are a family of cell surface, heterodimeric receptors that mediate a variety of responses to extracellular ligands including cell adhesion, motility, and the regulation of gene expression (see Akiyama et al., 1990; Hemler, 1990). Each heterodimer is composed of an α and β subunit noncovalently associated with one-to-one stoichiometry, and the particular combination of α and β subunits determines the ligand-binding specificity of the dimer. Our knowledge of α and β subunit diversity and the complexity of dimer function has expanded in recent years to include at least 12 α subunits, eight β subunits, and a large number of secreted and cell surface ligands. Despite these advances, the establishment of receptor–ligand relationships at the biochemical and cellular levels is ongoing. Moreover, new integrin subunits, such as β_6 and α_8 (Sheppard et al., 1990; Bossy et al., 1991), have been identified by structural homology, yet their ligand-binding specificities are unknown.

The usefulness of stable cell lines in the study of integrin structure and function has long been appreciated. Although the chicken embryo has been a convenient system for studies in primary culture, no such avian cell line has been available. The chicken myeloblast cell line HD11 is an attractive candidate, since human macrophage express a broad range of inte-

grins whose activity is regulated by cell activation (Beug et al., 1979; Brown and Goodwin, 1988; Shaw and Mercurio, 1989; Krissansen et al., 1990; Savill et al., 1990; Shaw et al., 1990). HD11 cells share surface antigens with myeloblasts rather than mature macrophage and express hematopoietic growth factors upon stimulation with lipopolysaccharide (LPS)[1] (Beug et al., 1979; Leutz et al., 1984). As a first step toward characterizing the HD11 cell line as a useful model system, we show here that HD11 cells attach to collagen type I (Col I), vitronectin (VN), fibrinogen (FBG), and the cell-binding domain of fibronectin (FN), and that binding to the first three proteins is down regulated by LPS treatment.

Previously characterized cell surface receptors for collagen I, VN, FBG, and FN include a number of integrin heterodimers. Responses to collagen and FN are commonly mediated by β_1-class integrins, while nonplatelet receptors for VN and FN are composed of α_v in association with β_1, β_2, or β_5 (see Akiyama et al., 1990; Bodary and McLean, 1990; Smith et al., 1990). To identify receptors involved in HD11 cell adhesion to these ligands, therefore, we have used a panel of function-perturbing mAbs, focusing on the shared β_1 and α_v subunits. Three of these mAbs are function blocking: CSAT to the β_1 subunit, Chav-1 to α_v, and

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1. *Abbreviations used in this paper:* LPS, lipopolysaccharide; FN, fibronectin; Col I, collagen type I; VN, vitronectin; FBG, fibrinogen.

LM609 to the $\alpha_v\beta_3$ complex (Buck et al., 1985; Cheresh, 1987; Neugebauer et al., 1991). In addition, the TASC mAb binds to a unique epitope on the β_1 subunit and inhibits β_1-mediated adhesion to VN while simultaneously promoting adhesion to other β_1-class integrin ligands (Neugebauer and Reichardt, 1991). The results in the present study provide evidence that a previously unidentified β_1-class integrin heterodimer(s) promotes cell adhesion to FBG and VN.

Materials and Methods

Cells

The HD11 chicken myeloblast cell line, originally named LSCC-MC/MA1 (Beug et al., 1979), was provided by Dr. K. Klassing of the University of California (Davis, CA), and cells were maintained under a 5% CO_2 atmosphere in RPMI plus 10% FBS, penicillin, and streptomycin. When indicated, cells were stimulated with 5 μg/ml lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) for 24 h before use.

Antibodies

Hybridoma cells secreting the CSAT mAb (integrin β_1-specific) were the generous gift of Dr. A. F. Horwitz (University of Illinois, Urbana, IL). LM609 ($\alpha_v\beta_3$-specific) ascites fluid was kindly provided by Dr. D. A. Cheresh (Research Institute of the Scripps Clinic, La Jolla, CA). The TASC (anti-β_1) and Chav-1 (anti-α_v) mAbs were isolated in this laboratory as described (Neugebauer and Reichardt, 1991; Neugebauer et al., 1991). CSAT, LM609, Chav-1, and TASC IgGs were purified from ascites fluid by 50% ammonium sulfate precipitation, followed by chromatography on protein A-Sepharose Cl-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as per Ey et al. (1978). IgGs were dialyzed against calcium- and magnesium-free PBS (CMF-PBS; 200 mg/liters KCl, 200 mg/liters K_2SO_4, 8 g/liters NaCl, and 2.16 g/liter $Na_2HPO_4\cdot7H_2O$, pH 7.4) and stored at $-70°C$.

Adhesive Proteins

VN was purified from FBS by chromatography on Heparin Sepharose Cl-6B (Pharmacia Fine Chemicals) as described (Yatogo et al., 1988). FBG was the generous gift of Dr. Z. Ruggeri of the Research Institute of the Scripps Clinic (La Jolla, CA). Col I was purchased from Collaborative Research, Inc. (Bedford, MA). The cell-binding, 120-kD chymotryptic fragment of FN was obtained from Calbiochem-Behring Corp. (San Diego, CA).

Cell Adhesion Assays

Cell attachment was measured as described in Neugebauer et al. (1991). VN and FN120 were diluted to 2 μg/ml and 50 μg/ml, respectively, in CMF-PBS. FBG was diluted to 20 μg/ml in Ca^{2+}/Mg^{2+}-containing PBS (above solution plus 1 mM $CaCl_2$ and 1 mM $MgCl_2$). Col I was diluted to 20 μg/ml in 0.1 M glacial acetic acid. Note that each protein-coated well was incubated with 1% BSA in PBS for 3 h at 37°C to eliminate nonspecific binding. Antibodies and peptides were added to the wells in serum-free medium at twice the final concentration and allowed to equilibrate in the incubator while the cells were being prepared. HD11 cells were harvested with 0.05% trypsin/0.2% EDTA, spun down, resuspended in RPMI, and seeded at 100,000 cells per well. After incubation at 37°C of varying duration, nonadherent cells were washed from the substrate. Adherent cells were fixed overnight at 4°C in 2.5% glutaraldehyde in PBS, stained with 0.5% crystal violet, solubilized with 1% SDS, and quantitated by A_{540} (Bodary et al., 1989) in a microtiter plate reader (Flow Laboratories Inc., McLean, VA). In each experiment, all determinations were done in triplicate.

Immunoprecipitation

HD11 myeloblasts were metabolically labeled with 100 μCi/ml ^{35}S-methionine/cysteine (New England Nuclear, Boston, MA) for 16–20 h. Cells were mechanically removed from their plates with a rubber policeman, centrifuged, and resuspended in an equal volume of ice-cold Ca^{2+}/Mg^{2+}-containing PBS plus 1% Triton X-100 and 2 mM PMSF. After 15 min of incubation on ice, the extracts were spun at 10,000 rpm in a refrigerated microfuge. The supernatants were divided into 200-μl aliquots, and 20 μg

of each antibody was added. Samples were rocked 1 h to overnight at 4°C and then precleared twice with 50 μl of Sepharose Cl-4b (Pharmacia Fine Chemicals). Antibody-antigen complexes were precipitated with 75 μl packed protein A-Sepharose Cl-4B (Pharmacia Fine Chemicals) and washed extensively with 0.05 M Tris, pH 8.0/150 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/0.5% Triton X-100. For immunodepletion studies, the extract supernatant was repeatedly precipitated with 20 μg of the depleting antibody. An additional protein A-Sepharose adsorption step was performed before adding the second antibody to ensure that all of the depleting antibody had been removed from the extract. The resulting supernatant was divided in half and precipitated with 20 μg of the second or test antibodies. Pellets were extracted in sample buffer and separated by SDS-PAGE on 6% gels (Laemmli, 1970). Gels were fixed, stained, treated with EN[3]HANCE (New England Nuclear), dried, and exposed to film (X Omat AR; Eastman Kodak Co., Rochester, NY). Molecular weight standards were from Bio-Rad Laboratories (Richmond, CA).

Immunoblots of immunoprecipitated material were performed by precipitating 4 mg unlabeled HD11 cell extract with the indicated antibodies as above, except that protein G-Sepharose was used. Antibody-antigen complexes were run under reducing conditions on 6% gels so that the reduced antibody bands would not run in the same molecular weight range as the antigens. Nitrocellulose blots of these gels were incubated with polyclonal antisera raised to either the chicken β_1 subunit (Damsky et al., 1985) or the extracellular domain of the chicken integrin α_v subunit (Bossy and Reichardt, 1990) and then probed with alkaline-phosphatase-conjugated second antibodies (Promega Biotec, Madison, WI). Reactive bands were visualized by developing with NBT and BCIP (Sigma Chemical Co.).

Results

As a first step towards characterizing the responses of the chicken myeloblast cell line HD11 to purified extracellular matrix molecules, HD11 cell attachment to the cell binding fragment of FN120, Col I, VN, FBG, and poly-D-lysine was measured. Compared to poly-D-lysine as a positive control, 100% of the cells attached to FN120 and VN, ~50% attached to Col I, and ~25% attached to FBG in a 40-min assay (Fig. 1). Since treatment of these cells with LPS results in their assumption of a macrophage-like phenotype (Beug et al., 1979), it seemed possible that their responses would change with LPS treatment. LPS-stimulated HD11 cells continued to attach well to FN120, but attachment to Col I, VN, and FBG was significantly decreased (Fig. 1). In the studies that follow, only unstimulated HD11 cells were used, since these were generally more adhesive.
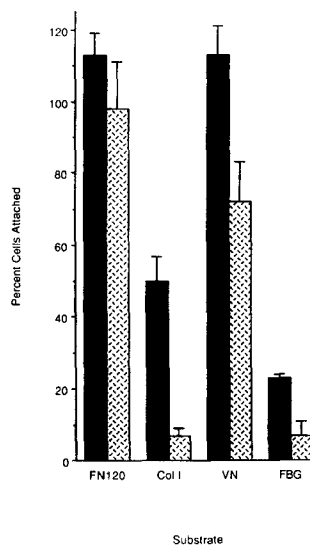

Figure 1. Attachment of HD11 cells to FN120, Col I, VN, and FBG with (*stippled bars*) and without (*solid bars*) prior stimulation by LPS. Attachment to each substrate after 40 min of incubation is normalized to that measured on poly-D-lysine, taken to represent 100%. Each determination was made in triplicate, and values represent the mean $\pm$ SEM. By the t test, LPS treatment results in significant differences from control for Col I ($P < 0.002$), VN ($P < 0.05$), and FBG ($P < 0.005$).

To identify receptors that mediate attachment to these purified glycoproteins, we used a panel of mAbs which specifically alter the function of members of the integrin family of extracellular matrix receptors. These include CSAT and TASC to the integrin β_1 subunit, Chav-1 to the integrin α_v subunit, and LM609 to the $\alpha_v\beta_3$ complex (Buck et al., 1986; Cheresh, 1987; Neugebauer and Reichardt, 1991; Neugebauer et al., 1991). Immunoprecipitation analysis showed that HD11 cells express all three antigens (Fig. 2). CSAT, TASC, and a polyclonal antibody against the cytoplasmic domain of the β_1 subunit all precipitated the β_1 subunit of 120,000 M_r in association with at least three apparent α subunits of 160,000, 145,000, and 140,000 M_r from metabolically labeled cells (Fig. 2, lanes 1-3). A 100,000-M_r band of variable intensity was observed upon precipitation of β_1-class integrins. Since two similar bands were detected in β_1-specific immunoblots of HD11 cell extracts (see Fig. 3 b), the 100,000-M_r protein probably represents a degraded or precursor form of the β_1 subunit (Akiyama et al., 1990). Immunoblots of HD11 cell extracts with antibodies specific for the cytoplasmic domains of the integrin α subunits 1-8 indicate that these cells express the α_1, α_3, α_4, α_5, and α_6 subunits (data not shown). Thus, the HD11 cells express many, but not all, of the integrin α subunits that associate with β_1, consistent with the observed ability of these cells to attach to many distinct ECM substrates.

Multiple α_v-containing integrins are also expressed by HD11 cells. LM609 (Fig. 2, lane 4) immunoprecipitated two bands, α_v at 150,000 M_r and β_3 at 95,000 M_r. Chav-1 (lane 5) and a polyclonal antibody against the cytoplasmic domain of the α_v subunit (data not shown) precipitated a similar pattern of proteins, except that the lower band was more prominent and broader (95,000-100,000 M_r). In addition to β_3, α_v has been shown to dimerize with β_1, β_5, β_6, and β_8 on specific cell types, such as chick embryo fibroblasts (Cheresh et al., 1989; Freed et al., 1989; Bodary and McLean, 1990; Vogel et al., 1990; Bossy and Reichardt, 1990; D'Souza et al., 1991; Moyle et al., 1991). To determine whether the broad lower band in the Chav-1 immunoprecipitate includes a β subunit(s) additional to β_3, an immunodepletion experiment was performed (Fig. 3 a). Repeated precipitation of a metabolically labeled extract with LM609 depletes $\alpha_v\beta_3$ from the extract (lanes 1-3), and subsequent precipitation with Chav-1 reveals a distinct dimer composed of α_v in association with a 100,000-M_r β subunit. The latter protein has not been identified as the chick homologue of one of other β subunits associated with α_v on mammalian cells (β_5, β_6, or β_8) that have similar molecular weights (Cheresh et al., 1989; Moyle et al., 1991). Therefore, the 100,000-M_r β subunit could potentially be β_5, β_6, β_8, or a novel β subunit.

To determine whether the β_1 subunit associates with α_v on HD11 cells, Chav-1 (anti-α_v) precipitable material was subjected to immunoblotting with a polyclonal antiserum to the β_1 subunit (Fig. 3 b). No β_1-reactive bands were present in the Chav-1 (α_v) or LM609 ($\alpha_v\beta_3$) precipitates. The lane containing parallel quantities of β_1-specific (TASC mAb) immunoprecipitate did contain a readily detected β_1-reactive band. Conversely, polyclonal antibodies against the α_v subunit recognized an appropriate band in the Chav-1 (α_v) precipitate, but not in lanes containing immunoprecipitates
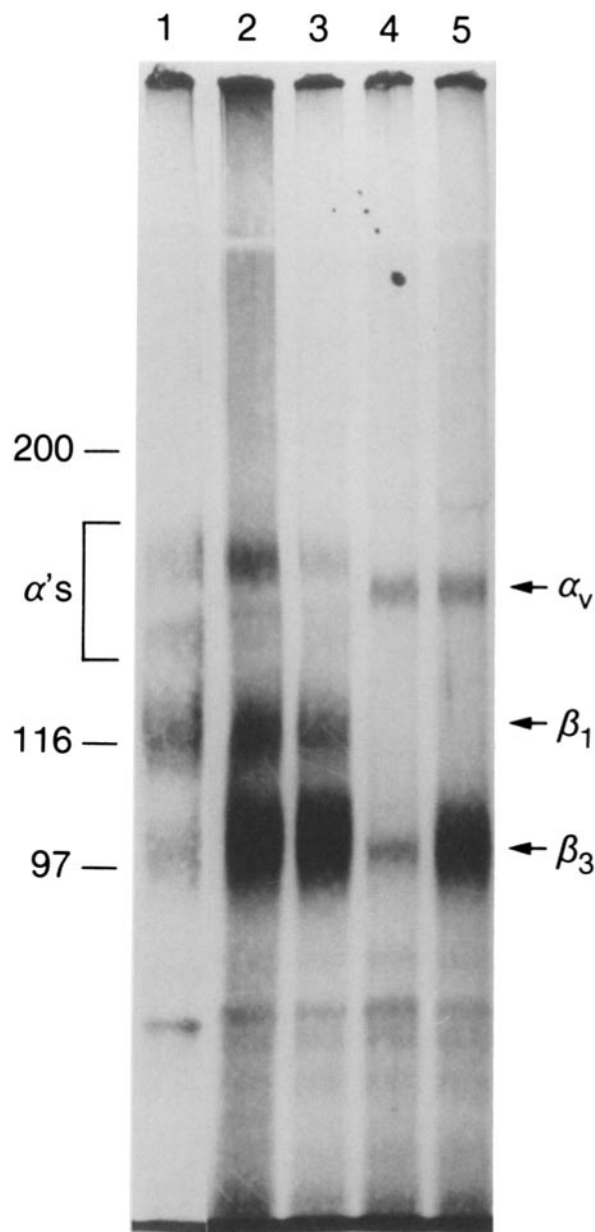

Figure 2. Immunoprecipitation of HD11 integrins visualized by autoradiography. ^{35}S-methionine/cysteine–labeled HD11 cells were extracted and immunoprecipitated with (lane 1) a polyclonal antiserum against a cytoplasmic peptide predicted from the sequence of the human β_1 subunit (Tomaselli et al., 1988), (lane 2) CSAT (β_1), (lane 3) TASC (β_1), (lane 4) LM609 ($\alpha_v\beta_3$), and (lane 5) Chav-1 (α_v). Antigen–antibody complexes were resolved by SDS-PAGE under nonreducing conditions on 6% gels.

derived using either of two β_1-specific antibodies (CSAT or TASC). Thus, the $\alpha_v\beta_1$ heterodimer cannot be detected on HD11 cells. Furthermore, the 100-kD β subunit that does associate with α_v on these cells is distinct from β_1 in both size and antigenicity.

Attachment of FN120 and Col I was studied, using the above antibodies and the arg-gly-asp–containing hexapeptide, GRGDSP, to perturb integrin function in short-term assays. Consistent with the behavior of previously characterized integrin receptors for these ligands, the RGD peptide
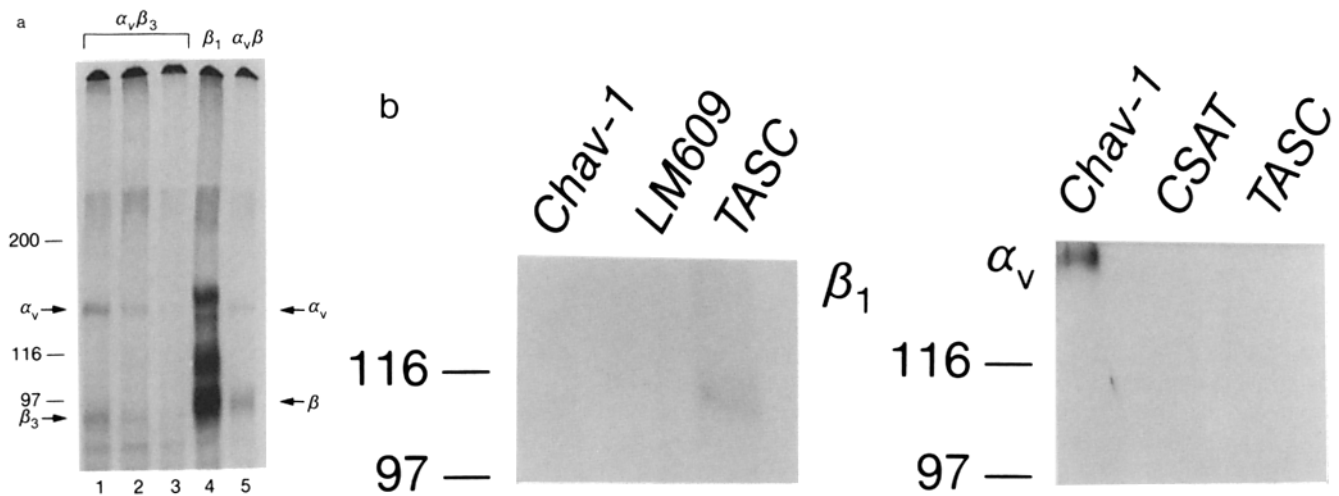
Figure 3. Analysis of α_v-containing integrin dimers on HD11 cells. (*a*) Depletion of $\alpha_v\beta_3$ from a ^{35}S metabolically labeled HD11 cell extract using the $\alpha_v\beta_3$-specific mAb LM609 (lanes *1–3*) was followed by precipitation with the β_1-specific mAb CSAT (lane *4*) or the α_v-specific mAb Chav-1 to α_v (lane *5*). Note that the 100,000-M_r β subunit is not depleted by the $\alpha_v\beta_3$-specific mAb and has a size clearly distinct from that of β_3. (*b*) β_1 and α_v immunoblots of immunoprecipitated, reduced material from HD11 cell extracts. The precipitating antibody is indicated at the top of each lane. Polyclonal antibodies against the β_1 subunit detect two bands at 105 and 120 kD in the β_1 (*TASC*) immunoprecipitate, but not in the α_v (*Chav-1*) or $\alpha_v\beta_3$ (*LM609*) precipitates. Polyclonal antibodies against the heavy chain of the chicken α_v subunit detect their 130-kD antigen in the α_v (*Chav-1*) immunoprecipitate but not in either of two β_1-specific (*TASC* or *CSAT*) precipitates.

effectively inhibited attachment to FN120 but not to Col I (Pierschbacher and Ruoslahti, 1984; Elices et al., 1991). The strong inhibitory effects of the integrin β_1-specific mAb CSAT on HD11 cell attachment to FN120 (Fig. 4 *A*) and Col I (Fig. 4 *B*) indicate that HD11 cell interactions with both ligands can be attributed to the action of integrin β_1-class heterodimers. Consistent with this, the integrin β_1-specific mAb TASC which enhances β_1-dependent cell binding to many ligands (Neugebauer and Reichardt, 1991) increased HD11 cell attachment to FN120 twofold and to Col I 2.7-fold. The β_1-specific mAb CSAT blocks all of the attachment measured in the presence of TASC, demonstrating the specificity of TASC's effects. Neither α_v (Chav-1) nor $\alpha_v\beta_3$ (LM609) antibodies inhibited attachment to FN120 or to Col I, indicating that the α_v-containing heterodimers expressed by these cells do not contribute significantly to attachment of these cells to either ligand.

Data presented in Fig. 5 show that HD11 cell attachment to VN reflects the activity of at least two different integrin receptors, $\alpha_v\beta_3$ and a β_1-containing heterodimer, both of which are sensitive to the RGD peptide. Although TASC antibody promotes β_1-class integrin–mediated cell attachment to other substrates, it was isolated for its ability to inhibit neuronal attachment to VN (Neugebauer and Reichardt, 1991). The TASC mAb did not crossreact with β_3 or with a 100-kD β subunit expressed by avian neurons, demonstrating the specificity of TASC's effects for β_1-class heterodimers (Neugebauer et al., 1991). The β_1-specific monoclonal antibodies CSAT and TASC reduced HD11 cell attachment to VN by 42 and 59%, respectively, and the combination of CSAT plus TASC reduced attachment by 85% of control. The simplest explanation for the additive behavior of these two antibodies (used at concentrations well above saturation) is that both antibodies partially inhibit the function of the same heterodimer. We do not know the identity of the α subunit(s) that associates with β_1 to produce VN binding. The

α_v-specific mAb Chav-1 reduced attachment by ~80% of control, suggesting that an α_v-containing heterodimer(s) plays a prominent role in VN binding. $\alpha_v\beta_3$ is likely to account for some of this activity, since the $\alpha_v\beta_3$-specific antibody LM609 had significant, though weaker, effects on cell attachment (Fig. 5). While LM609 alone inhibited attachment to VN by only 25%, the combination of CSAT and LM609 was more effective, reducing attachment by 75%.
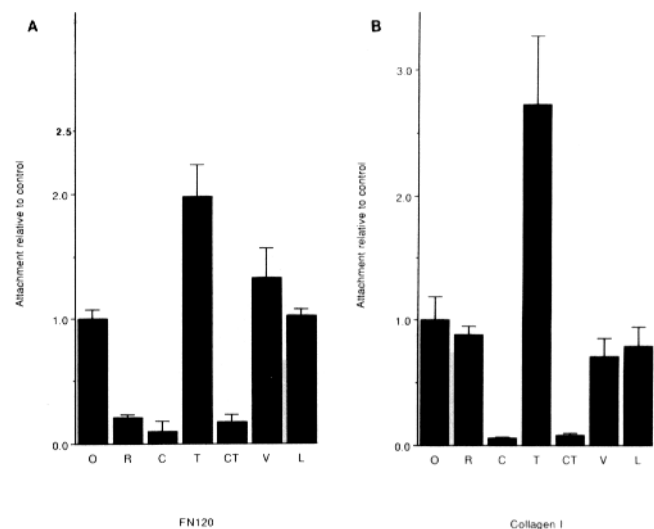

Figure 4. Effects of function-blocking monoclonal antibodies and the GRGDSP peptide on HD11 cell attachment in a 5-min assay to FN120 (*A*) or Col I (*B*). (*O*) No antibody added; (*R*) 100 μg/ml GRGDSP; (*C*) 50 μg/ml CSAT IgG; (*T*) 200 μg/ml TASC IgG; (*CT*) 50 μg/ml CSAT IgG plus 200 μg/ml TASC IgG; (*V*) 100 μg/ml Chav-1 IgG; (*L*) 100 μg/ml LM609 IgG. Each determination was made in triplicate, and values represent the mean ± SEM normalized to the attachment measured in the absence of antibody.
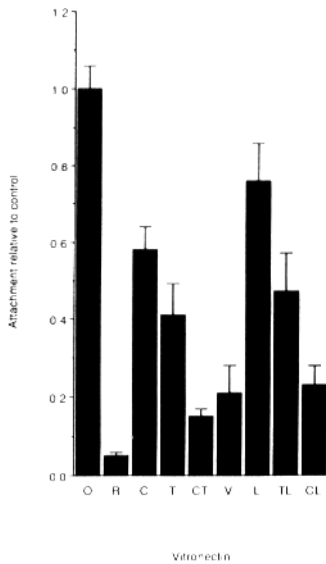
Figure 5. Effects of function-blocking mAbs and the GRG-DSP peptide on HD11 cell attachment to VN in 5 min. Abbreviations as in Fig. 4, except TL, 200 µg/ml TASC IgG plus 100 µg/ml LM609 IgG; and CL, 50 µg/ml CSAT IgG plus 100 µg/ml LM609 IgG. All values are normalized to the attachment measured in the absence of antibody (O). Each determination was made in triplicate, and values represent the mean ± SEM normalized to the attachment measured in the absence of antibody.
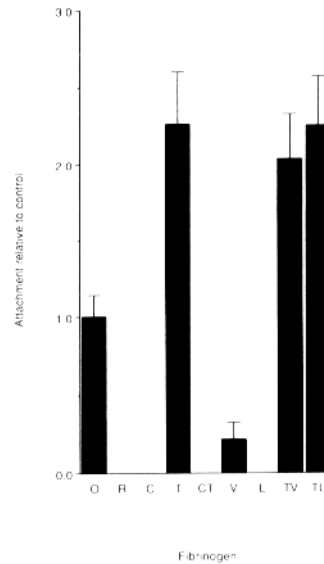

Figure 6. Effects of function-blocking mAbs and the GRG-DSP peptide on HD11 cell attachment to FBG in a 40-min assay. Abbreviations as in Fig. 3, except TV, 200 µg/ml TASC IgG plus 100 µg/ml Chav-1 IgG. All values are normalized to the attachment measured in the absence of antibody (O). Each determination was made in triplicate, and values represent the mean ± SEM normalized to the attachment measurement in the absence of antibody.

Comparison of results using Chav-1 (anti-α_v) and LM609 (anti-$\alpha_v\beta_3$) suggests that an additional α_v-containing heterodimer (e.g., $\alpha_v\beta_5$) must be expressed by these cells. This is consistent with biochemical results in Fig. 3 a, indicating that an additional β subunit, $\beta_{100\,K}$, associates with α_v on HD11 cells. An integrin $\alpha_v\beta_{100\,K}$ dimer also appears to be a major VN receptor on embryonic chick retinal neurons (Neugebauer et al., 1991).

HD11 cell attachment to FBG was RGD dependent and appeared to require the activity of both β_1- and α_v-containing integrin heterodimers (Fig. 6). In this case, the assay was allowed to proceed for 40 min, since attachment to FBG developed more slowly than on the other substrates. The β_1-specific mAb CSAT as well as the $\alpha_v\beta_3$-specific mAb LM609 completely blocked attachment to FBG. Chav-1 also dramatically reduced attachment to FBG by 80%. Conversely, the β_1-specific mAb, TASC, promoted attachment 2.3-fold. The TASC mAb also promoted adhesion of HD11 cells to FBG in a 5-min assay in which attachment in the absence of TASC was not detectable (data not shown), arguing that the HD11 cells did not secrete a β_1-class integrin ligand (e.g., FN) that was adsorbed to the BSA-blocked plastic surface or to FBG itself. In addition, neither LM609 ($\alpha_v\beta_3$) nor Chav-1 (α_v) significantly reduced the TASC-enhanced attachment, suggesting that fully activated β_1-class FBG receptors are sufficient to mediate cell attachment in the absence of α_v-dependent activity. In general, however, both $\alpha_v\beta_3$ and the β_1-class FBG receptor appear to play critical roles of HD11 cell attachment to FBG under the conditions of our assay, since either LM609 or CSAT can abolish adhesion to FBG when added alone. As described above, cell attachment to FBG requires a comparatively long time. Thus, each receptor may mediate comparatively weak interactions with FBG, while the combination of the two yields detectable attachment in our assay.

Discussion

The present characterization of the integrins expressed and active on the HD11 chicken myeloblast cell line has led to three interesting observations. (a) HD11 cell adhesion to

FBG is dependent on the integrin β_1 subunit. The β_1-associated α subunit has not been identified but is very unlikely to be α_v, since the $\alpha_v\beta_1$ heterodimer was not detectable on these cells. To our knowledge, this is the first evidence that a β_1-class integrin receptor mediates FBG binding. (b) HD11 adhesion to VN is also partially dependent on the function of a β_1-class integrin heterodimer that is also unlikely to be $\alpha_v\beta_1$. (c) LPS treatment down-regulates adhesive responses to Col I, FBG, and VN. The identification of integrins active on HD11 cells (summarized in Table I) and the observation that responses to specific ligands are differentially regulated by LPS suggest that the HD11 cell line will provide a useful system for future studies on the regulation of the expression and activity of extracellular matrix receptors.

Function-perturbing mAbs to the integrin β_1 subunit inhibited partially or completely HD11 cell attachment to all of the adhesive substrates tested (Table I). HD11 cell binding to Col I and the 120-kD chymotryptic cell binding fragment of FN was entirely β_1-dependent, as the CSAT mAb completely removed the cells from either substrate. Previously described integrin receptors that bind FN include $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_6$ (see Akiyama et al., 1990; Charo et al., 1990; Vogel et al., 1990; D'Souza et al., 1991). Binding to collagens has been attributed to $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$ heterodimers (see Akiyama et al., 1990; Elices et al., 1991). The lack of inhibition by anti-α_v or anti $\alpha_v\beta_3$ on FN120 and Col I makes it unlikely that α_v in association with β_1, β_3, β_6, or a β_1 subunit variant accounts for binding

Table I. Summary of HD11 Cell Receptor Interactions with Various Ligands

Ligand	Integrin receptor(s)
FN-120	All β_1-class
Col I	All β_1-class
VN	$\alpha_v\beta_3$, $\alpha_v\beta_{100\,k}$, $\alpha_7\beta_1$
FBG	$\alpha_v\beta_3$ and $\alpha_7\beta_1$

Summary of the integrin receptors involved in HD11 cell attachment to FN120, Col I, VN, and FBG. See text for results and rationale.

to either ligand as has been recently reported on other cell types (Charo et al., 1990; Dedhar and Gray, 1990; Vogel et al., 1990; D'Souza et al., 1991). Since subunit-specific antisera against α_5 (160 K) and α_3 (140 K) recognize HD11 proteins of the appropriate molecular weight in the chicken, $\alpha_5\beta_1$ and $\alpha_3\beta_1$ heterodimes may contribute to HD11 cell attachment to FN120 and Col I (Venstrom, K., and L. F. Reichardt, unpublished results; Hynes et al., 1989). The observation that LPS stimulation of these cells decreases their responsiveness particularly to Col I (Fig. 1) provides a compelling reason to identify and further study these heterodimers.

Results presented here suggest that β_1-class integrins also play a major role in HD11 cell binding to VN and FBG. The function-blocking β_1 mAb CSAT completely inhibited attachment to FBG and partially inhibited attachment to VN. The β_1 mAb TASC enhanced attachment to FBG and inhibited attachment to VN, suggesting that FBG, like other β_1-class integrin ligands, may bind a domain on an integrin heterodimer that is distinct from the site recognized by VN (Neugebauer and Reichardt, 1991). In platelets, placenta, and endothelial cells, $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ have been shown to bind FBG and VN (see Pytela et al., 1986; Cheresh and Spiro, 1987; Languino et al., 1989; Smith et al., 1990). A recent report describes the function of $\alpha_v\beta_1$ in the attachment of the human embryonic kidney cell line 293 to VN (Bodary and McLean, 1990), but HD11 cells do not appear to express this heterodimer (Fig. 3). Moreover, the α_v mAb Chav-1 did not inhibit HD11 cell attachment to FBG when β_1-class integrin function was enhanced by the mAb TASC (Fig. 6). This argues that the two mAbs are not acting on the same heterodimer (i.e., $\alpha_v\beta_1$). Similarly, embryonic chick retinal neurons do not express $\alpha_v\beta_1$, also attach to VN through a β_1-class integrin, but do not attach to FBG (Neugebauer and Reichardt, 1991; Neugebauer et al., 1991). Thus, HD11 cell interactions with VN and FBG are likely to be mediated by an integrin heterodimer composed of the β_1 subunit in association with an unknown α subunit(s). It remains to be determined whether the $\alpha\beta_1$ VN receptor is biochemically equivalent in HD11 and retinal cells and whether HD11 cell attachment to FBG depends on the expression of either a distinct α subunit or a cell-specific factor that modulates $\alpha\beta$ specificity (see Elices and Hemler, 1989).

The integrin α_v subunit was shown to be expressed on HD11 cells in noncovalent association with β_3 and another β subunit of $\sim$100,000 M_r, possibly β_5 (Table I). Indeed, $\alpha_v\beta_5$ has been detected on primary human monocytes which also express $\alpha_v\beta_3$ (Krissansen et al., 1990). The α_v mAb Chav-1 inhibited HD11 cell attachment to VN to a greater extent than did the $\alpha_v\beta_3$ mAb LM609, indicating that both $\alpha_v\beta_3$ and $\alpha_v\beta_{100\ K}$ are VN receptors on these cells. In support of this interpretation, Chav-1 is known to inhibit the function of an $\alpha_v\beta_{100\ K}$ in neuronal attachment to VN (Neugebauer et al., 1991). In addition to binding VN, the $\alpha_v\beta_3$ heterodimer functioned as a FBG receptor on HD11 cells, consistent with its binding specificity on other cells (see Cheresh and Spiro, 1987). Interestingly, $\alpha_v\beta_3$ functions on primary human macrophage to mediate the specific killing of dying neutrophils (Savill et al., 1990), raising the possibility that $\alpha_v\beta_3$ has a cellular ligand as well. The HD11 cell line may provide a useful probe for the identification of such a ligand.

The regulation of integrin-mediated cell adhesion is possible at several levels: (a) changes in transcription of specific integrin subunit genes (see Heino and Massague, 1989; de Curtis et al., 1991); (b) heterodimer assembly and release from intracellular stores (see Cheresh and Spiro, 1987; Phillips et al., 1988); and (c) rapid modulation at the cell surface (see Phillips et al., 1988; Springer, 1990; Neugebauer and Reichardt, 1991). Recent reports have described the activation-dependent increase in β_1-class integrin laminin and collagen receptor function on primary murine macrophages and human lymphocytes (Shaw and Mercurio, 1989; Shaw et al., 1990; Shimizu et al., 1990). In these systems, integrin activation is associated with increased levels of receptor phosphorylation and cytoskeletal association (Burn et al., 1988; Shaw et al., 1990). We have shown that HD11 cells adhere poorly to Col I, VN, and FBG after treatment with LPS, suggesting that certainly β_1-class receptors and possibly others are targets for down-regulation by HD11 cell activation. Since attachment to FN120 was unaffected by LPS, the changes must be heterodimer specific. The observed reductions in integrin receptor function upon LPS treatment, especially on collagen, suggest that the HD11 cell line will be an excellent model system for studies of regulatory mechanisms that act on distinct sets of integrin heterodimers.

References

Akiyama, S. K., K. Nagata, and K. M. Yamada. 1990. Cell surface receptors for extracellular matrix components. *Biochim. Biophys. Acta.* 1031:91–110.

Beug, H., A. von Kirschbach, G. Doderlein, J.-F. Conscience, and T. Graf. 1979. Chicken hematopoetic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. *Cell.* 18:375–390.

Bodary, S. C., and J. W. McLean. 1990. The integrin β_1 subunit associates with the vitronectin receptor α_v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. *J. Biol. Chem.* 265:5938–5941.

Bodary, S. C., M. A. Napier, and J. W. McLean. 1989. Expression of recombinant platelet glycoprotein IIbIIIa results in a functional fibrinogen-binding complex. *J. Biol. Chem.* 264:18859–18862.

Bossy, B., and L. F. Reichardt. 1990. Chick integrin α_v subunit molecular analysis reveals high conservation of structural domains and association with multiple β subunits in embryo fibroblasts. *Biochemistry.* 29:10191–10198.

Bossy, B., E. Bossy-Wetzel, and L. F. Reichardt. 1991. Characterization of the integrin α_8 subunit: a new integrin β_1-associated subunit, which is prominently expressed on axons and on cells in contact with basal laminae in chick embryos. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2375–2385.

Brown, E. J., and J. L. Goodwin. 1988. Fibronectin receptors of phagocytes. *J. Exp. Med.* 167:777–793.

Buck, C. A., E. Shea, K. Duggan, and A. F. Horwitz. 1986. Integrin (the CSAT antigen): functionality requires oligomeric integrity. *J. Cell Biol.* 103:2421–2428.

Burn, P., A. Kupfer, and S. J. Singer. 1988. Dynamic membrane-cytoskeletal interactions: specific association of integrin and talin arises in vivo after phorbol ester treatment of peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. USA.* 85:497–501.

Charo, I. F., L. Nannizzi, J. W. Smith, and D. A. Cheresh. 1990. The vitronectin receptor $\alpha_v\beta_3$ binds fibronectin and acts in concert with $\alpha_5\beta_1$ in promoting cellular attachment and spreading on fibronectin. *J. Cell Biol.* 111:2795–2800.

Cheresh, D. A. 1987. Human endothelial cells synthesize and express an arg-gly-asp-directed adhesion receptor involved in attachment to fibrinogen and

von Willebrand factor. *Proc. Natl. Acad. Sci. USA.* 84:6471-6475.

Cheresh, D. A., and R. C. Spiro. 1987. Biosynthetic and functional properties of an arg-gly-asp-directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen, and von Willebrand factor. *J. Biol. Chem.* 262:17703-17711.

Cheresh, D. A., J. W. Smith, H. M. Cooper, and V. Quaranta. 1989. A novel vitronectin receptor integrin $(\alpha_v\beta_x)$ is responsible for distinct adhesive properties of carcinoma cells. *Cell.* 57:59-69.

Damsky, C. H., K. A. Knudsen, D. Bradley, C. A. Buck, and A. F. Horwitz. 1985. Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. *J. Cell Biol.* 100:1528-1539.

de Curtis, I., V. Quaranta, R. N. Tamura, and L. F. Reichardt. 1991. Laminin receptors in the retina: sequence analysis of the chick integrin α_6 subunit; evidence for transcriptional and posttranslational regulation. *J. Cell Biol.* 113:405-416.

Dedhar, S., and V. Gray. 1990. Isolation of a novel integrin receptor mediating ARG-GLY-ASP-directed cell adhesion to fibronectin and type I collagen from human neuroblastoma cells. Association of a novel β_1-related subunit with α_v. *J. Cell Biol.* 110:2185-2193.

D'Souza, S., M. H. Ginsberg, and E. F. Plow. 1991. Arginyl-glycyl-aspartic acid (RGD): a cell adhesion motif. *Trends Biochem. Sci.* 16:246-250.

Elices, M. J., and M. E. Hemler. 1989. The human integrin VLA-2 is a collagen receptor on some cells, and a collagen/laminin receptor on others. *Proc. Natl. Acad. Sci. USA.* 86:9906-9910.

Elices, M. J., L. A. Urry, and M. E. Hemler. 1991. Receptor functions for the integrin VLA-3: fibronectin, collagen, and laminin binding are differentially influenced by arg-gly-asp peptide and by divalent cations. *J. Cell Biol.* 112:169-181.

Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-sepharose. *Immunochemistry.* 15:429-436.

Freed, E., J. Gailit, P. van der Geer, E. Ruoslahti, and T. Hunter. 1989. A novel integrin β subunit is associated with the vitronectin receptor a subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:2955-2965.

Heino, J., and J. Massague. 1989. Transforming growth factor- beta switches the pattern of integrins expressed in MG-63 human osteosarcoma cells and causes a selective loss of adhesion to laminin. *J. Biol. Chem.* 264:21806-21811.

Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* 8:365-400.

Hynes, R. O., E. E. Marcantonio, M. A. Stepp, L. A. Urry, and G. H. Yee. 1989. Integrin heterodimer and receptor complexity in avian and mammalian cells. *J. Cell Biol.* 109:409-420.

Krissansen, G. W., M. J. Elliott, C. M. Lucas, F. C. Stomski, M. C. Berndt, D. A. Cheresh, A. F. Lopez, and G. F. Burns. 1990. Identification of a novel integrin β subunit expressed on cultured monocytes (macrophages). *J. Biol. Chem.* 265:823-830.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680-684.

Languino, L. R., S. Colella, A. Zanetti, A. Andrieux, J. J. Ryckewaert, M. H. Charon, P. C. Marchisio, E. F. Plow, M. H. Ginsberg, G. Marguerie, and

E. Dejana. 1989. Fibrinogen-endothelial cell interaction in vitro: a pathway mediated by an arg-gly-asp recognition specificity. *Blood.* 73:734-742.

Leutz, A., H. Berg, and T. Graf. 1984. Purification and characterization of cMGF, a novel chicken myelomonocytic growth factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3191-3197.

Moyle, M., M. A. Napier, and J. W. McLean. 1991. Cloning and expression of a divergent integrin subunit β_8. *J. Biol. Chem.* 266:19650-19658.

Neugebauer, K. M., and L. F. Reichardt. 1991. Cell surface regulation of β_1-integrin activity on developing retinal neurons. *Nature (Lond.).* 350:68-71.

Neugebauer, K. M., C. J. Emmett, K. A. Venstrom, and L. F. Reichardt. 1991. Vitronectin and thrombospondin promote retinal neurite outgrowth: developmental regulation and role of integrins. *Neuron.* 6:345-358.

Phillips, D. R., I. F. Charo, L. V. Parise, and L. A. Fitzgerald. 1988. The platelet membrane glycoprotein IIb-IIIa complex. *Blood.* 71:831-843.

Pierschbacher, M. D., and E. Ruoslahti. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature (Lond.).* 309:30-33.

Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Ruoslahti. 1986. Platelet membrane glycoprotein IIb/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. *Science (Wash. DC).* 231:1559-1562.

Savill, J., I. Dransfield, N. Hogg, and C. Haslett. 1990. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature (Lond.).* 343:170-173.

Shaw, L. M., and A. M. Mercurio. 1989. Interferon γ and lipopolysaccharide promote macrophage adherence to basement membrane glycoproteins. *J. Exp. Med.* 169:303-308.

Shaw, L. M., J. M. Messier, and A. M. Mercurio. 1990. The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the $\alpha_6\beta_1$ integrin. *J. Cell Biol.* 110:2167-2174.

Sheppard, D., C. Rozzo, L. Starr, V. Quaranta, D. J. Erle, and R. Pytela. 1990. Complete amino acid sequence of a novel integrin β subunit (β_6) identified in epithelial cells using the polymerase chain reaction. *J. Biol. Chem.* 265:11502-11507.

Shimizu, Y., G. A. Van Seventer, K. J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (β_1) integrin receptors on T cells. *Nature (Lond.).* 345:250-253.

Smith, J. W., D. J. Vestal, S. V. Irwin, T. A. Burke, and D. A. Cheresh. 1990. Purification and functional characterization of integrin $\alpha_v\beta_5$. *J. Biol. Chem.* 265:11008-11013.

Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425-434.

Tomaselli, K. J., C. H. Damsky, and l. F. Reichardt. 1988. Purification and characterization of mammalian integrins expressed by a rat neuronal cell line (PC12): evidence that they function as α/β heterodimeric receptors for laminin and type IV collagen. *J. Cell Biol.* 107:1241-1252.

Vogel, B. E., G. Tarone, F. G. Giancotti, J. Gailit, and E. Ruoslahti. 1990. A novel fibronectin receptor with an unexpected subunit composition ($\alpha_v\beta_1$). *J. Biol. Chem.* 265:5934-5937.

Yatogo, T., M. Izumi, H. Kashiwagi, and M. Hayashi. 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. *Cell Struct. Funct.* 13:281-292.