Adhesion of a Chicken Myeloblast Cell Line to Fibrinogen and Vitronectin through a $\beta_1$-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 $\beta_1$ 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 $\beta_1$ family functions as a receptor for fibrinogen. While the $\alpha_\beta_1$ 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 $\beta_1$ subunit associates with different, unidentified $\alpha$ 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 $\alpha_2\beta_1$ and $\alpha_\beta_1$ associated with an unidentified 100-kD $\beta$ 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.

The 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 $\alpha$ and $\beta$ subunit noncovalently associated with one-to-one stoichiometry, and the particular combination of $\alpha$ and $\beta$ subunits determines the ligand-binding specificity of the dimer. Our knowledge of $\alpha$ and $\beta$ subunit diversity and the complexity of dimer function has expanded in recent years to include at least 12 $\alpha$ subunits, eight $\beta$ 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 $\beta_6$ and $\alpha_6$ (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 integrins whose activity is regulated by cell activation (Beug et al., 1979; Brown and Goodwin, 1988; Shaw and Mercurio, 1988; 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) (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 $\beta_1$-class integrins, while nonplatelet receptors for VN and FN are composed of $\alpha$, in association with $\beta_1$, $\beta_3$, or $\beta_5$ (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 $\beta_1$ and $\alpha$ subunits. Three of these mAbs are function blocking: CSAT to the $\beta_1$ subunit, Chav-1 to $\alpha_\alpha$, and CSAT to the $\beta_1$ subunit, Chav-1 to $\alpha_\alpha$, and

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Abbreviations used in this paper: LPS, lipopolysaccharide; FN, fibronectin; Col I, collagen type I; VN, vitronectin; FBG, fibrinogen.
LMD609 to the $\alpha_3\beta_1$ complex (Buck et al., 1986; Cheresh, 1987; Neugebauer et al., 1991). In addition, the TASC mAb binds to a unique epitope on the $\beta_3$ subunit and inhibits $\beta_3$-mediated adhesion to VN while simultaneously promoting adhesion to other $\beta_3$-class integrin ligands (Neugebauer and Reichardt, 1991). The results in the present study provide evidence that a previously unidentified $\beta_3$-class integrin heterodimer(s) promotes cell adhesion to FBG and VN.

**Materials and Methods**

**Cells**

The HDI1 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 identified, cells were stimulated with 5 ng/ml lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) for 24 h before use.

**Antibodies**

Hybridoma cells secreting the CSAT mAb (integrin $\beta$-specific) were the generous gift of Dr. A. F. Horwitz (University of Illinois, Urbana, IL). LMD609 (anti-$\beta_3$-specific) ascites fluid was kindly provided by Dr. D. A. Cheresh (Research Institute of the Scripps Clinic, La Jolla, CA). The TASC (anti-$\beta_3$) and Chav-l (anti-$\alpha_3$) mAbs were isolated in this laboratory as described (Neugebauer and Reichardt, 1991; Neugebauer et al., 1991). CSAT, LMD609, Chav-l, 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 diazylated against calcium- and magnesium-free PBS (CMF-PBS; 200 mg/liters KCl, 200 mg/liters K$_2$SO$_4$, 8 g/liters NaCl, and 2.16 g/liter Na$_2$HPO$_4$·7H$_2$O, 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 VN 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 $\mu$g/ml and 50 $\mu$g/ml, respectively, in CMF-PBS. FBG was diluted to 20 $\mu$g/ml in Ca$^{++}$/Mg$^{++}$-containing PBS (above solution plus 1 mM CaCl$_2$ and 1 mM MgCl$_2$). Col I was diluted to 20 $\mu$g/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. HDI1 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$_{540}$ (Boehringer Mannheim, Indianapolis, IN). Each determination was made in triplicate, and values represent the mean ± SEM. By the Student's t test, LPS treatment results in significant differences from control for Col I ($P < 0.002$), VN ($P < 0.005$), and FBG ($P < 0.005$).

![Figure 1](https://example.com)  
**Figure 1.** Attachment of HDI1 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 ± SEM. By the Student's t test, LPS treatment results in significant differences from control for Col I ($P < 0.002$), VN ($P < 0.005$), 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 \( \alpha_i \) subunit, Chav-1 to the integrin \( \alpha_i \) subunit, and LM609 to the \( \alpha_i \beta_i \) 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 \( \beta_i \) subunit all precipitated the \( \beta_i \) subunit of 120,000 \( M_i \), in association with at least three apparent \( \alpha_i \) subunits of 160,000, 145,000, and 140,000 \( M_i \), from metabolically labeled cells (Fig. 2, lanes 1–3). A 100,000-\( M_i \) band of variable intensity was observed upon precipitation of \( \beta_i \)-class integrins. Since two similar bands were detected in \( \beta_i \)-specific immunoblots of HD11 cell extracts (see Fig. 3 b), the 100,000-\( M_i \) protein probably represents a degraded or precursor form of the \( \beta_i \) subunit (Akiyama et al., 1990). Immunoblots of HD11 cell extracts with antibodies specific for the cytoplasmic domains of the integrin \( \alpha_i \) subunits 1–8 indicate that these cells express the \( \alpha_1, \alpha_3, \alpha_4, \alpha_5 \), and \( \alpha_6 \) subunits (data not shown). Thus, the HD11 cells express many, but not all, of the integrin \( \alpha_i \) subunits that associate with \( \beta_i \), consistent with the observed ability of these cells to attach to many distinct ECM substrates.

Multiple \( \alpha_i \)-containing integrins are also expressed by HD11 cells. LM609 (Fig. 2, lane 4) immunoprecipitated two bands, \( \alpha_i \) at 150,000 \( M_i \), and \( \beta_i \) at 95,000 \( M_i \). Chav-1 (lane 5) and a polyclonal antibody against the cytoplasmic domain of the \( \alpha_i \) subunit (data not shown) precipitated a similar pattern of proteins, except that the lower band was more prominent and broader (95,000–100,000 \( M_i \)). In addition to \( \beta_i \), \( \alpha_i \) has been shown to dimerize with \( \beta_3, \beta_3, \beta_4, \) and \( \beta_8 \) 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 \( \beta_i \) subunit(s) additional to \( \beta_3 \), an immunodepletion experiment was performed (Fig. 3 a). Repeated precipitation of a metabolically labeled extract with LM609 depletes \( \alpha_i \beta_i \) from the extract (lanes 1–3), and subsequent precipitation with Chav-1 reveals a distinct dimer composed of \( \alpha_i \) in association with a 100,000-\( M_i \) \( \beta_i \) subunit. The latter protein has not been identified as the chick homologue of one of other \( \beta_i \) subunits associated with \( \alpha_i \) on mammalian cells (\( \beta_3, \beta_3, \) or \( \beta_8 \)) that have similar molecular weights (Cheresh et al., 1989; Moyle et al., 1991). Therefore, the 100,000-\( M_i \) \( \beta_i \) subunit could potentially be \( \beta_3, \beta_6, \beta_8, \) or a novel \( \beta_i \) subunit.

To determine whether the \( \beta_1 \) subunit associates with \( \alpha_i \) on HD11 cells, Chav-1 (anti-\( \alpha_i \)) precipitable material was subjected to immunoblotting with a polyclonal antiserum to the \( \beta_1 \) subunit (Fig. 3 b). No \( \beta_i \)-reactive bands were present in the Chav-1 (\( \alpha_i \)) or LM609 (\( \alpha_i \beta_i \)) precipitates. The lane containing parallel quantities of \( \beta_i \)-specific (TASC mAb) immunoprecipitate did contain a readily detected \( \beta_1 \)-reactive band. Conversely, polyclonal antibodies against the \( \alpha_i \) subunit recognized an appropriate band in the Chav-1 (\( \alpha_i \)) precipitate, but not in lanes containing immunoprecipitates derived using either of two \( \beta_i \)-specific antibodies (CSAT or TASC). Thus, the \( \alpha_i \beta_1 \) heterodimer cannot be detected on HD11 cells. Furthermore, the 100-\( kD \) \( \beta_i \) subunit that does associate with \( \alpha_i \) on these cells is distinct from \( \beta_1 \) 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

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**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 \( \beta_1 \) subunit (Tomasselli et al., 1988), (lane 2) CSAT (\( \beta_1 \)), (lane 3) TASC (\( \beta_1 \)), (lane 4) LM609 (\( \alpha_i \beta_i \)), and (lane 5) Chav-1 (\( \alpha_i \)). Antigen-antibody complexes were resolved by SDS-PAGE under nonreducing conditions on 6% gels.
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 β1-specific mAb CSAT on HDII cell attachment to FN120 (Fig. 4 A) and Col I (Fig. 4 B) indicate that HDII cell interactions with both ligands can be attributed to the action of integrin β1-class heterodimers. Consistent with this, the integrin β1-specific mAb TASC which enhances β1-dependent cell binding to many ligands (Neugebauer and Reichardt, 1991) increased HDII cell attachment to FN120 twofold and to Col I 2.7-fold. The β1-specific mAb CSAT blocks all of the attachment measured in the presence of TASC, demonstrating the specificity of TASC’s effects. Neither αv (Chav-I) nor αvβ3 (LM609) antibodies inhibited attachment to FN120 or to Col I, indicating that the αv-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 HDII cell attachment to VN reflects the activity of at least two different integrin receptors, αvβ3 and a β1-containing heterodimer, both of which are sensitive to the RGD peptide. Although TASC antibody promotes β1-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 β3 or with a 100-kD β subunit expressed by avian neurons, demonstrating the specificity of TASC’s effects for β1-class heterodimers (Neugebauer et al., 1991). The β1-specific monoclonal antibodies CSAT and TASC reduced HDII 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 β1 to produce VN binding. The αv-specific mAb Chav-1 reduced attachment by ~80% of control, suggesting that an αv-containing heterodimer(s) plays a prominent role in VN binding. αvβ3 is likely to account for some of this activity, since the αvβ3-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%.
Comparison of results using Chav-1 (anti-α1) and LM609 (anti-α5β3) suggests that an additional α1-containing heterodimer (e.g., α1β3) must be expressed by these cells. This is consistent with biochemical results in Fig. 3a, indicating that an additional β subunit, β100K, associates with α1 on HD11 cells. An integrin α1β1100K 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 β1- and α1-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 β1-specific mAb CSAT as well as the α1β1-specific mAb LM609 completely blocked attachment to FBG. Chav-1 also dramatically reduced attachment to FBG by 80%. Conversely, the β1-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 β1-class integrin ligand (e.g., FN) that was adsorbed to the BSA-blocked plastic surface or to FBG itself. In addition, neither LM609 (α5β3) nor Chav-1 (α1) significantly reduced the TASC-enhanced attachment, suggesting that fully activated β1-class FBG receptors are sufficient to mediate cell attachment in the absence of α1-dependent activity. In general, however, both α5β3 and the β1-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 β1 subunit. The β1-associated α subunit has not been identified but is very unlikely to be α1, since the α1β3 heterodimer was not detectable on these cells. To our knowledge, this is the first evidence that a β1-class integrin receptor mediates FBG binding. (b) HD11 adhesion to VN is also partially dependent on the function of a β1-class integrin heterodimer that is also unlikely to be α1β1. (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 β1 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 β1-dependent, as the CSAT mAb completely removed the cells from either substrate. Previously described integrin receptors that bind FN include α5β1, α5β3, α5β1, α1β1, α1β3, and α1β3 (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 α1β1, α1β3, and α2β1 heterodimers (see Akiyama et al., 1990; Elices et al., 1991). The lack of inhibition by anti-α1 or anti α1β1 on FN120 and Col I makes it unlikely that α1, in association with β1, β3, or a β subunit variant accounts for binding

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

| Ligand  | Integrin receptor(s) |
|---------|----------------------|
| FN-120  | All β1-class          |
| Col I   | All β1-class          |
| VN      | α2β1, α2β3, α3β1     |
| FBG     | α1β1 and α1β3        |

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 antibodies against α5 (160 K) and α3 (140 K) recognize HD11 dimers, the appropriate molecular weight in the chicken, α5β1, and α3β1 heterodimers 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 β1-class integrins also play a major role in HD11 cell binding to VN and FBG. The function-blocking mAb CSAT completely inhibited attachment to FBG and partially inhibited attachment to VN. The β1 mAb TASC enhanced attachment to FBG and inhibited attachment to VN, suggesting that FBG, like other β1-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, α5β1, α5β2, α5β3, and α5β1 have been shown to bind FBG and VN (see Pytel et al., 1986; Cheresh and Sprio, 1987; Languino et al., 1989; Smith et al., 1990). A recent report describes the function of αβ1 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 mAb Chav-1 did not inhibit HD11 cell attachment to FBG when β1-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., αβ1). Similarly, embryonic chick retinal neurons do not express α5β1, also attach to VN through a β1-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 β1 subunit in association with an unknown α subunit(s). It remains to be determined whether the αβ1 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 αβ specificity (see Elices and Hemler, 1989).

The integrin α5 subunit was shown to be expressed on HD11 cells in noncovalent association with β1 and another β subunit of ~100,000 M, possibly β3 (Table I). Indeed, α5β1 has been detected on primary human monocytic cells which also express α5β1 (Kriessansen et al., 1990). The α5 mAb Chav-1 inhibited HD11 cell attachment to VN to a greater extent than did the α5β1 mAb LM609, indicating that both α5β1 and α5β10 K are VN receptors on these cells. In support of this interpretation, Chav-1 is known to inhibit the function of an α5β10 K in neuronal attachment to VN (Neugebauer et al., 1991). In addition to binding VN, the α5β1 heterodimer functioned as a FBG receptor on HD11 cells, consistent with its binding specificity on other cells (see Cheresh and Sprio, 1987). Interestingly, α5β1 functions on primary human macrophage to mediate the specific killing of dying neutrophils (Savill et al., 1990), raising the possibility that α5β1 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 Spriio, 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 β1-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 β1-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.

The authors thank Drs. D. Cheresh and Z. Ruggeri for their generous gifts of reagents and several helpful conversations. We are grateful to Dr. K. Klassing for sending us the HD11 cells, which originated in the laboratory of Dr. T. Graf. We thank Marion Meyerson for her expert assistance with the manuscript.

This work was supported by the National Institutes of Health (grant NS 16033) and the Howard Hughes Medical Institute. L. F. Reichardt is an investigator of the Howard Hughes Medical Institute.

Received for publication 22 February 1991 and in revised form 19 August 1991.

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