Direct and Regulated Interaction of Integrin $\alpha_E\beta_7$ with E-Cadherin

Jonathan M.G. Higgins,* Didier A. Mandlebrot,† Sunil K. Shaw,* Gary J. Russell,* Elizabeth A. Murphy,* Yih-Tai Chen,‡ W. James Nelson,¶ Christina M. Parker,* and Michael B. Brenner*

*The Lymphocyte Biology Section, Division of Rheumatology, Immunology and Allergy and †Renal Division, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115; ‡Combined Program in Pediatric Gastroenterology and Nutrition, and Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts 02115; and ¶Department of Molecular and Cellular Physiology, Beckman Center, Stanford University School of Medicine, Stanford, California 94305

Abstract. The cadherins are a family of homophilic adhesion molecules that play a vital role in the formation of cellular junctions and in tissue morphogenesis. Members of the integrin family are also involved in cell to cell adhesion, but bind heterophilically to immunoglobulin superfamily molecules such as intracellular adhesion molecule (ICAM)–1, vascular cell adhesion molecule (VCAM)–1, or mucosal addressin cell adhesion molecule (MadCAM)–1. Recently, an interaction between epithelial (E-) cadherin and the mucosal lymphocyte integrin, $\alpha_E\beta_7$, has been proposed. Here, we demonstrate that a human E-cadherin–Fc fusion protein binds directly to soluble recombinant $\alpha_E\beta_2$, and to $\alpha_E\beta_7$ solubilized from intraepithelial T lymphocytes. Furthermore, intraepithelial lymphocytes or transfected JY’ cells expressing the $\alpha_E\beta_7$ integrin adhere strongly to purified E-cadherin–Fc coated on plastic, and the adhesion can be inhibited by antibodies to $\alpha_E\beta_7$ or E-cadherin.

The binding of $\alpha_E\beta_7$ integrin to cadherins is selective since cell adhesion to P-cadherin–Fc through $\alpha_E\beta_7$ requires >100-fold more fusion protein than to E-cadherin–Fc. Although the structure of the $\alpha_E$-chain is unique among integrins, the avidity of $\alpha_E\beta_7$ for E-cadherin can be regulated by divalent cations or phorbol myristate acetate. Cross-linking of the T cell receptor complex on intraepithelial lymphocytes increases the avidity of $\alpha_E\beta_7$ for E-cadherin, and may provide a mechanism for the adherence and activation of lymphocytes within the epithelium in the presence of specific foreign antigen. Thus, despite its dissimilarity to known integrin ligands, the specific molecular interaction demonstrated here indicates that E-cadherin is a direct counter receptor for the $\alpha_E\beta_7$ integrin.

The cadherins constitute a family of cell surface adhesion molecules that are involved in calcium-dependent homophilic cell to cell adhesion (Takeichi, 1990). The best studied human cadherins, E-, P-, N-, and VE-cadherin, have a restricted tissue distribution: E- and P-cadherin are expressed in epithelial tissues (Nose and Takeichi, 1986; Shimoyama et al., 1989a), N-cadherin is found mainly on neural cells (Hatta et al., 1987), and VE-cadherin is found on vascular endothelium (Lampugnani et al., 1992). Homophilic binding between cadherins on adjacent cells is vital for the maintenance of strong cell to cell adhesion in these tissues. For example, E-cadherin is required for the formation of adherens junctions between mature epithelial cells (Boller et al., 1985; Gumbiner et al., 1988) and is involved in Langerhans cell adhesion to keratinocytes (Tang et al., 1993), and VE-cadherin is needed for the maintenance of lateral association between endothelial cells (Lampugnani et al., 1992). During development cadherins are critically involved in the cell sorting required for tissue morphogenesis (Takeichi, 1995), and loss of E-cadherin function contributes to the metastasis of a variety of carcinomas (Ben-Ze’ev, 1997). The extracellular regions of mature mammalian cadherins are comprised five “CAD” modules of $\sim$110 amino acids. Crystallographic and biochemical studies indicate that cadherins probably form dimers on the cell surface (Shapiro et al., 1995; Nagar et al., 1996), and that interaction with dimeric cadherins on opposing cell surfaces can lead to the formation of “zipper-like” cell junctions (Shapiro et al., 1995; Tomschey et al., 1996).

The integrins are a second family of transmembrane adhesion molecules that are involved in both cell to cell and cell to matrix interactions. At least 15 $\alpha$ chains associate with 8 $\beta$ chains to form a large number of heterodimeric integrins that can be classified into several major subfamilies based on their shared use of a particular $\beta$ chain.
(Hynes, 1992). Members of three such subfamilies, the \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \) integrins, are commonly found on leukocytes. The expression of \( \beta_1 \) integrins is widespread (for example, \( \alpha_5\beta_1 \), CD49e/CD29, is found on T cells, granulocytes, platelets, fibroblasts, endothelium, and epithelium), whereas the \( \beta_2 \) and \( \beta_3 \) integrins have a restricted pattern of expression. For example, \( \alpha_\beta_2 \) (CD11a/CD18) is expressed on most lymphocytes and many myeloid cells but not on other cell types, and \( \alpha_\beta_2 \) (CD103/uncoupled) is found on >95% of intestinal intraepithelial lymphocytes (iIEL)\(^1\) and on other mucosal T cells, macrophages, and mast cells, but on only 2% of peripheral blood lymphocytes (Cepek et al., 1994; Karecla et al., 1995). The major ligands of the integrins fall into two categories: cell surface molecules that are members of the immunoglobulin superfamily (such as vascular cell adhesion molecule [VCAM]–1, intracellular adhesion molecule [ICAM]–1, 2, 3, and mucosal adhesion cell adhesion molecule [MadCAM]–1) and a variety of large extracellular proteins (such as fibronectin, vitronectin, fibrinogen, and complement component iC3b; Hynes, 1992). A common feature of both groups is the presence of exposed acidic amino acids crucial for integrin binding (Bergelson and Hemler, 1995). Many integrins exist in states of low or high avidity for their ligands, and these states can be regulated from within the cell (Hynes, 1992). For example, the avidity of \( \alpha_5\beta_1 \) on resting T cells can be increased by stimulation through the T cell receptor (TCR) with anti-CD3 mAbs (Dustin and Springer, 1989; van Kooyk et al., 1989).

Recently, we reported that E-cadherin on human epithelial cells may be a ligand for the mucosal lymphocyte integrin, \( \alpha_\beta_7 \), and a similar interaction has been suggested in the mouse (Cepek et al., 1994; Karecla et al., 1995). mAbs to E-cadherin or to \( \alpha_\beta_7 \)–block IEL adherence to epithelial cells, and transfection of cells with \( \alpha_\beta_7 \)–confers upon them the ability to adhere to cells transfected with E-cadherin (Cepek et al., 1994). E-cadherin has been defined extensively as a homophilic adhesion molecule, and its sequence is not related to known cell surface or extracellular integrin ligands. Thus, the concept that \( \alpha_\beta_7 \) and E-cadherin are counter receptors is at variance with current knowledge of both integrin and cadherin interactions. Moreover, as we and others have pointed out, the indirect methods used in the studies to date are insufficient to conclusively demonstrate a direct physical interaction between these two adhesion receptors (Cepek et al., 1994; Erle, 1995; Karecla et al., 1995; Takeichi, 1995). For example, it is clear that antibodies to one integrin (e.g., \( \alpha_\beta_7 \)) can cause specific transdominant inhibition of the function of another integrin (e.g., \( \alpha_\beta_2 \); Blystone et al., 1995; Díaz-González et al., 1996) or could result in steric hindrance of an adjacent receptor. Furthermore, expression of an exogenous gene in a transfected cell can have profound effects on the surface expression of a variety of other proteins (Marks et al., 1996), and the transfection of constructs containing integrin \( \beta_1 \) tails into fibroblasts alters the function of endogenous integrins (LaFlamme et al., 1994). Such effects can lead to erroneous conclusions about the interactions that are directly involved in adhesion.

Given that the interaction of \( \alpha_\beta_7 \) on intraepithelial lymphocytes with its receptor on epithelial cells is likely to be crucial for the normal development, function, and/or retention of lymphocytes in the epithelium (Cepek et al., 1993; Erle, 1995), we sought to determine if \( \alpha_\beta_7 \) and E-cadherin are directly interacting counter receptors. In addition, we investigated whether this interaction is specific among cadherins and whether it can be regulated through alterations in \( \alpha_\beta_7 \) avidity.

### Materials and Methods

#### Materials

DNA manipulating enzymes were purchased from New England Biolabs Inc. (Beverly, MA). Oligonucleotides were obtained from Oligotech (Bost on, MA). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). ICAM–1-Fc (the entire extracellular region of human ICAM–1 fused to the hinge and FC portion of human IgG1) was a generous gift of Dr. Lloyd Klickstein (Brigham and Women’s Hospital, Boston, MA). Purified human IgG1 was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA).

#### mAbs

The mAb used (all mouse IgG against human antigens) were as follows: E4.6 (anti-E-cadherin, IgG1; Cepek et al., 1994), HECD-1 (anti-E-cadherin, IgG1; Shimoyama et al., 1989b), NCC-CAD299 (anti-P-cadherin, IgG1; Shimoyama et al., 1989b), HML-1 (anti-\( \alpha_\beta_5 \), IgG2a; Cerf-Bensussan et al., 1987), BerACT-8 (anti-\( \alpha_\beta_5 \), IgG1; Kruschwitz et al., 1991), oE7-1 (anti-\( \alpha_\beta_5 \), IgG2a; Russell et al., 1994), oE7-2 (anti-\( \alpha_\beta_5 \), IgG1; Russell et al., 1994), oE7-3 (anti-\( \alpha_\beta_5 \), IgG1; Russell et al., 1994), D6.21 (anti-\( \alpha_\beta_5 \), IgG1; Cepek et al., 1993), TSI/22 (anti-\( \alpha_\beta_5 \), IgG1; Sanchez-Madrid et al., 1982), TSI/18 (anti-\( \beta_1 \), IgG1; Sanchez-Madrid et al., 1983), ACT-1 (anti-\( \alpha_\beta_7 \), IgG1; Lazarovits et al., 1984), BS5G10 (anti-\( \alpha_\beta_7 \), IgG1; Hemler et al., 1987), 4B4 (anti-\( \beta_1 \), IgG1; Morimoto et al., 1985), RRI/1 (anti-ICAM-1, IgG1; Rothlein et al., 1986), SPVT3b (anti-CD3, IgG2a; Spits et al., 1983), OKT4 (anti-CD4, IgG2b; obtained from American Type Culture Collection [ATCC], Rockville, MD), OKT8 (anti-CD8a, IgG2a; ATCC), W6/32 (anti-MHCI, IgG2a; Barnstable et al., 1978), TCR-81 (anti-TCR-\( \gamma \), IgG1; Band et al., 1987), P3 (control IgG1; Kohler and Milstein, 1975), RPC5.4 (control IgG2a; Mohit and Fan, 1971).

### Cells

Human iIEL were isolated as previously described (Roberts et al., 1993; Russell et al., 1996). The iIEL were stimulated with PHA-P (Difco Laboratories Inc., Detroit, MI) and irradiated feeder cells (80% PBMC and 20% JY lymphoblastoid cells) in 2 mM IL–2, 4% (vol/vol) heat-inactivated fetal calf serum, and 10% (vol/vol) heat-inactivated FBS (Hyclone Laboratories Inc., Logan, UT). 50 μM 2-mercaptoethanol, and Yssel’s medium at 10% CO\(_2\) (Russell et al., 1996). The iIEL lines 496 and 194 were maintained by periodic restimulation as described and were used in adherence assays after 2–8 wk. At the time of assay, IEL496 was 100% CD3\(^{+}\), 90% CD8\(^{+}\), 10% CD4\(^{+}\), 95% \( \alpha_\beta_2 \)\(^{+}\) (MFI ∼400), 97% \( \alpha_\beta_5 \)\(^{+}\) (MFI ∼700), and IEL194 was 100% CD3\(^{+}\), 60% CD8\(^{+}\), 40% CD4\(^{+}\), 95% \( \alpha_\beta_5 \)\(^{+}\) (MFI ∼500), 80% \( \alpha_\beta_7 \)\(^{+}\) (MFI ∼400) by FACScan\(^\text{®} \) analysis. Both lines maintained expression of \( \alpha_\beta_7 \) without addition of exogenous TGF-\( \beta_1 \).

A subline of the human B lymphoblastoid cell line, JY, that expresses the \( \alpha_\beta_7 \) integrin (JY\( \alpha_\beta_7 \)), was kindly provided by Dr. Martin Hemler (Dana-Farber Cancer Institute, Boston, MA; Chan et al., 1992) and was maintained in 10% (vol/vol) heat-inactivated FBS (Hyclone Laboratories Inc., RPMI-1640 [GIBCO BRL, Gaithersburg, MD] at 37°C, and 5% CO\(_2\). Human embryonic kidney HEK293 cells (obtained from ATCC) were maintained in 10% (vol/vol) heat-inactivated FBS (Hyclone Laboratories Inc.) and DME Medium (GIBCO BRL) at 37°C in 10% CO\(_2\), COS-7 cells (obtained from ATCC) were grown in 10% (vol/vol) NuSerum (Collaborative Research, Inc., Waltham, MA), 10 mM Hepes, 2 mM L-glutamine, and DMEM (GIBCO BRL) at 10% CO\(_2\). Human breast epithelial 16E6.A5 cells were maintained as described (Cepek et al., 1993).

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1. Abbreviations used in this paper: ICAM, intracellular adhesion molecule; iIEL, intestinal intraepithelial lymphocytes; MadCAM, mucosal adhesion cell adhesion molecule; MFI, mean fluorescence intensity; PBL, peripheral blood lymphocytes; TCR, T cell receptor; VCAM, vascular cell adhesion molecule.
Construction of E- and P-Cadherin–Fc Expression Vectors

E-cadherin–Fc. A double-stranded DNA adapter containing a 5′ Ngo MI cohesive end, the final five codons of the human E-cadherin extracellular region, and a 3′ XhoI cohesive end was produced by annealing the complimentary oligonucleotides JON1 (5′-GGGGCTCTGAAGAGGAGC-3′) and JON2 (5′-TGGACGCCTCAAGACGC-3′). This adapter was then ligated to the 3′-end of an EcoRV–NgoMI fragment encoding the rest of the extracellular region of human E-cadherin derived from the plasmid pEER-1 (kindly provided by Dr. David Rimm, Yale University, New Haven, CT; Rimm and Moore, 1994). After removal of excess adapters by centrifugation through a Centricon-100 filter (Amicon Corp., Danvers, MA), the resulting EcoRV–XhoI fragment was introduced into frame, upstream of the coding for the hinge and Fc region of human IgG1 from a derivative of pCDM8 (pCDM8/Fc: Chen and Nelson, 1996) also cleaved with EcoRV and XhoI. The sequence of the junctional region is shown in Fig. 1 a. To confirm the integrity of the construct, the nucleotide sequence of the junctional region of the E-cadherin–Fc construct was determined by double-stranded sequencing using the Sequenase kit (United States Biochemical Corp., Cleveland, OH) according to the manufacturer’s protocol. Finally, the E-cadherin–Fc cDNA was excised from pCDM8 using EcoRV and NotI and ligated into the expression vector pCEP4 (Invitrogen Corp., Carlsbad, CA) cleaved with PvuII and NotI.

P-Cadherin–Fc. An adapter fragment encoding the final 93 codons of the human P-cadherin extracellular region was generated by PCR from a human P-cadherin cDNA in pBR322 (kindly provided by Dr. S. Hirohashi, National Cancer Center Research Institute, Tokyo, Japan; Shimoyama et al., 1989b) using the primers JON2 (5′-GGGGTTCAACCTACCTTATCAT-3′) and JON5 (5′-TTTTTTCGAGTCGGAGGTCTGTCGAC-3′) and cloned plaque-forming unit (PFU) polymerase (Stratagene, La Jolla, CA) according to the manufacturer’s recommendations, with 25 cycles of 94°C 1 min/55°C 1 min/72°C 1 min. After digestion with BsaBI and XhoI, this adapter was ligated to the 3′-end of a HindIII–BsaBI fragment encoding the rest of the extracellular region of human P-cadherin. After digestion of the ligation products with HindIII and XhoI to generate a 180-bp fragment from the end of the E-cadherin–Fc construct was determined by double-stranded sequencing using the Sequenase kit (United States Biochemical Corp., Cleveland, OH) according to the manufacturer’s protocol. Finally, the E-cadherin–Fc cDNA was excised from pCDM8 using EcoRV and NotI and ligated into the expression vector pCEP4 (Invitrogen Corp., Carlsbad, CA) cleaved with PvuII and NotI.

Production of Cadherin–Fc Proteins

HEK293 cells (10^6 cells per 75-cm² flask) were stably transfected with 25 µg plasmid DNA using the Mammalian transfection kit (Stratagene Inc.). After growth for 24 h in nonselective medium the cells were transferred to 96-well tissue culture plates and incubated in selective medium containing 300 µg/ml hygromycin B. After 15 d, supernatants from wells containing resistant colonies were assayed for fusion proteins by ELISA.

To produce the cadherin–Fc proteins, transfected cells were grown in triple-layer 500-mm² flask (Nunc, Roskilde, Denmark) in 10% (vol/vol) Ultralow Ig FBS ( Gibco BRL), 300 µg/ml hygromycin B, and DMEM. After 5–10 d of culture, the medium was harvested and filtered through a 0.2-µm membrane. The E- and P-cadherin–Fc fusion proteins were then purified on separate, previously unused GammaBind G-Sepharose columns (Pharmacia Biotech Sverige, Uppsala, Sweden). The columns were washed with TBS and 1 mM CaCl₂, pH 7.4, and then eluted with 0.2 M glycine and 1 mM CaCl₂. Fractions containing purified fusion protein were dialyzed into TBS and 1 mM CaCl₂, pH 7.4 and then stored at −20°C. The purity of fusion protein was assessed by SDS-PAGE and Coomassie blue staining, and the concentration was determined by Bradford assay using BSA as a standard (Bio-Rad Labs., Hercules, CA).

Production of Soluble 35S-labeled Recombinant α7β1 Integrin

Soluble recombinant α7β1 was produced by COS-7 cells after transient transfection using DEAE-dextran (Coligan et al., 1994, Unit 10-14) with the plasmids pAPRM8/α7Es and pAPRM8/β7Es. Control transfections were carried out with the antisense constructs pAPRM8/α7Es and pAPRM8/β7Es. After incubation for 48 h in complete medium, the cells were washed once with PBS and then 1 mM 3′-Express (Dupont–NEB, Boston, MA) in 6 ml 10% (vol/vol), dialyzed PBS, 5% DMEM, 85% methionine- and cysteine-free DMEM (GIBCO BRL), 10 mM Heps, and 2 mM l-glutamine was added. After incubation at 37°C for 24 h, the medium, containing labeled secreted proteins, was filtered through a 0.2-µm membrane.

Generation of JY-α7β1 and JY-7 Vector Cell Lines

To produce cells expressing cell surface α7β1, JY′ cells that express endogenous β1 integrin chain were transfected by electroporation with 4 µg pSRα-neo/αE or with the pSRα-neo vector alone as a control. Then transfected cells were selected by culture in 0.5 mg/ml G418. To generate the JY–α7, JY–β1, and JY–α7β1-expressing transfectants were isolated by positive selection with the anti-α7β1 mAb BerACT-8 on magnetic goat anti–mouse immunoglobulin dynabeads according to the manufacturer’s recommendations (Dynal A.S., Oslo, Norway) and by flow cytometric sorting. FACS® was carried out as previously described (Parker et al., 1990). The clone J6.7 was finally isolated by limiting dilution. The α7β1-expressing JY′-α7β1 cells was indistinguishable from that on IEL when immunoprecipitated from 35S-labeled surface-labeled cells with the anti-α7β1 mAb, HML-1 (not shown).

Adhesion Assays

Unless otherwise stated, the wells of Linbro 96-well microtiter plates (ICN Flow Laboratories, Horsham, MA) were coated with human IgG1 Fc-containing proteins in 50 µg/well TBS and 1 mM CaCl₂, pH 7.4, for 18 h at 4°C. The wells were subsequently washed twice with 20 mM Heps, 137 mM NaCl, and 3 mM KCl, pH 7.4 (HBS), with 1 mM CaCl₂, and was then blocked with 1% BSA (Calbiochem-Novabiochem Corp.), HBS, and 1 mM CaCl₂ for 2 h at room temperature. In assays in which the effects of divalent cations were assessed, coating and blocking was carried out in HBS and 10 mM EDTA. In assays in which adhesion to E- and E-cadherin–Fc was compared, the wells were coated with 1 µg/well goat anti-human IgG
polyclonal antibody (Zymed, San Francisco, CA) in 100 μl TBS, pH 7.4, and blocked as described above before addition of Fc fusion proteins.

IEL or transfected JY' cells were labeled with B3EFG-AM (Molecular Probes, Eugene, OR) as previously described (Cepek et al., 1993). During labeling of JY' cells, 10% (vol/vol) heat-inactivated normal human serum was included to block Fc receptors. Adhesion assays were carried out in 0.1% BSA and HBS with combinations of MnCl$_2$, MgCl$_2$, CaCl$_2$, or 1 mM EGTA, as indicated (see text). In antibody blocking experiments, cells or wells were preincubated with mAbs for 10 min at 4°C as described in the text. For cell activation experiments, cells were preincubated with antibodies or 50 ng/ml PMA at 4°C for 15 min. Adhesion assays were carried out as described previously (Cepek et al., 1993) with the following modifications. Labeled cells were brought into contact with the microtiter plate wells by centrifugation at 60 g for 2 min (IEL) or 1 min (JY'). After incubation at 37°C for 10 min, nonadherent cells were removed by washing with 1 mM MnCl$_2$, 1 mM MgCl$_2$, 1 mM CaCl$_2$, and HBS at 37°C unless the effect of divalent cations was being assessed, in which case HBS alone was used. Since in these assays the fluorescence of input cells was quenched to some degree by the presence of adhesion buffer, but the percent bound was determined after removing the buffer, some apparent readings of >100% are obtained.

Homophilic adhesion assays were carried out as described above with the following modifications. 16E6.A5 cells were released from culture dishes using 0.02% (wt/vol) trypsin, 2 mM CaCl$_2$, and HBS to minimize proteolysis of cadherins. After adding 2 vol of 0.04% (wt/vol) soy bean trypsin inhibitor, HBS, and washing twice with HBS, the cells were resuspended in 0.1% BSA, HBS, and 1 mM CaCl$_2$ and allowed to settle onto the microtiter plate wells for 10 min at 4°C. After incubation at 37°C for 30 min, and washing twice with HBS and 1 mM CaCl$_2$, the percentage of bound cells was determined using a fluorogenic assay of endogenous cellular phosphatase activity (Tolosa and Shaw, 1996).

Surface Labeling of iIEL with $^{125}$I

Cultured iIEL (4 × 10$^5$) were isolated by centrifugation on Ficoll-Paque (Pharmacia Biotech Sverige) and subjected to cell surface labeling with 2 mCi Na$^{125}$I (Dupont-NE) using the lactoperoxidase method (Coligan et al., 1994, Unit 8-11). The labeled cells were then lysed in 0.5% Triton X-100, TBS, and 4 mM iodosocamidine. 1 mM phenylmethylsulfonyl fluoride for 4 h at 4°C. Insoluble material was removed by centrifugation at 12,000 g for 20 min.

Immunoadsorption

Batches of $^{125}$I-labeled IEL lysates or $^{35}$S-labeled transfected COS-7 medium were supplemented with divalent cations (see text) and precleared twice with 0.4% (vol/vol) normal rabbit serum, 1.5% (vol/vol) protein A-Sepharose (Pharmacia), and 1.5% (wt/vol) Pansorbin (Calbiochem-Novabiochem Corp.) for a total of 24 h at 4°C. Subsequently, aliquots were incubated with mouse mAbs or human IgG1 Fc-containing proteins for 3 h at 4°C. Then, 10 μl protein A-Sepharose resin was added to each tube, and the incubation at 4°C was continued for a further 3 h. For immunoprecipitations using mouse IgG1 mAbs, protein A–Sepharose precoated with rabbit anti-mouse immunoglobulin polyclonal antibody (Cappel, West Chester, PA) was used. Then the immobilized complexes were washed six times with TBS containing the same concentration of divalent cations used during the adsorption steps. For immunoadsorption from IEL lysates, 0.1% Triton X-100 was also present during washing. Proteins bound to the resin were eluted by boiling in 5% (wt/vol) SDS, 10% (vol/vol) glycerol, 50% (wt/vol) urea, and 60 mM Tris, pH 7, before analysis by SDS-PAGE.

SDS-PAGE

SDS-PAGE on 7.5% (wt/vol) polyacrylamide gels (Protegel; National Diagnostics, Atlanta, GA) was carried out as described (Coligan et al., 1994, Unit 8-4). Samples were reduced by the inclusion of 25 mM dithiothreitol. Radiolabeled proteins were visualized by autoradiography using Biomax MR and MS film (Kodak, Rochester, NY) and quantitated using phosphorimaging and the ImageQuant package (Molecular Dynamics Inc., Sunnyvale, CA).

Statistical Analysis

$P$ values testing the hypothesis that two populations had equal means were calculated using a two-tailed Welch $t$ test (which assumes the popu-
ence of disulfide bonds in the Fc region (Fig. 1a), possibly similar to cadherin dimers on the cell surface (Shapiro et al., 1995; Nagar et al., 1996).

After purification on protein G-Sepharose, SDS-PAGE in reducing conditions revealed the presence of proteins of the expected size for both E- and P-cadherin–Fc monomers (~120 kD, Fig. 2). Minor species of 135 or 130 kD were also present in preparations of the E- and P-cadherin fusion proteins, respectively. Since cadherins are synthesized as proproteins and the sizes of these bands match those predicted for the immature forms, it is likely that a small proportion of partially processed procadherin is present in each case. In nonreducing conditions both fusion proteins migrate at ~240 kD (Fig. 2) as expected for dimeric fusion proteins linked through disulfide bonds in the hinge of the Fc region. A small proportion of monomeric cadherin fusion protein is also present in each case. In an ELISA, E-cadherin–Fc but not P-cadherin–Fc is recognized by the antihuman E-cadherin mAb E4.6, and P-cadherin–Fc but not E-cadherin–Fc is recognized by the antihuman E-cadherin mAb E4.6, while adhesion to ICAM-1–Fc was not affected (Fig. 3b and data not shown). In contrast, the anti-β2 integrin mAb, TS1/18, the anti-αLβ2 mAbs, TS1/22 and D6.21, and the anti–ICAM-1 mAb RR1/1 all prevented IEL adhesion to ICAM–1–Fc, but not to E-cadherin–Fc. The anti-β1 integrin mAb, 4B4, blocked adhesion of IEL to human fibronectin (not shown) but not to E-cadherin–Fc or ICAM–1–Fc. A blocking mAb to αEβ7 (ACT-1) also did not inhibit IEL adhesion to E-cadherin–Fc or ICAM–1–Fc (Fig. 3b). Adhesion of IEL to P-cadherin–Fc was also completely blocked by the anti–αEβ7 mAb, αE7-2, but was unaffected by the anti–αLβ2 mAb, D6.21 (data not shown). Thus, adhesion of IEL to E- or P-cadherin–Fc involves αEβ7, and adhesion to ICAM–1–Fc involves αLβ2.

**E-Cadherin–Fc Supports Adhesion of IEL**

Cell surface E-cadherin has been proposed to be a ligand for the IEL integrin αEβ7 (Cepek et al., 1994; Karecla et al., 1995). To test the capacity of the cadherin–Fc fusions to support adhesion of IEL, we immobilized the proteins via antihuman IgG antibody on polystyrene microtiter plate wells. Binding of IEL to wells coated with subnanogram quantities of E-cadherin–Fc could be detected in the presence of 1 mM MnCl2, 1 mM MgCl2, and 1 mM CaCl2 (Fig. 3a). At 1 ng/well, ~40% of the IEL adhered, and maximal adhesion occurred at 10 ng/well of E-cadherin–Fc. Thus, a dose-dependent adhesion of IEL to the E-cadherin fusion protein was clear. Indeed, for IEL expressing similar levels of αEβ7 and αLβ2, adhesion to human E-cadherin–Fc was similar or greater than that seen to human ICAM–1–Fc (data not shown and see Fig. 3b). In contrast, adhesion to P-cadherin–Fc required >100-fold more fusion protein and did not reach 100% at any coating concentration. 40% adhesion was seen to P-cadherin–Fc at 500 ng/well. No adhesion could be detected to wells coated with 500 ng/well human IgG1 (Fig. 3a).

Adhesion of PHA-activated peripheral blood lymphocytes (PBL) to E-cadherin–Fc could not be detected (data not shown). This is consistent with the fact that only 2–5% of PBL express αEβ7 (Cerf-Bensussan et al., 1987). The E- and P-cadherin–Fc proteins support similar levels of adhesion of 16E6.A5 epithelial cells (Cepek et al., 1993) that express similar levels of E- and P-cadherin, suggesting that both fusion proteins are equally able to support cadherin-mediated homophilic adhesion (Fig. 3c).

**Antibodies to αEβ7 and E-Cadherin Block IEL Adhesion to E-Cadherin–Fc**

To determine if lymphocyte αEβ7 was responsible for adhesion of IEL to E-cadherin–Fc, we attempted to block the interaction with mAbs to human lymphocyte surface integrins. The binding of IEL to E-cadherin–Fc coated directly on plastic was completely blocked by anti-αEβ7 mAbs HML-1, BerACT8, αE7-1, and αE7-2 and by the anti–E-cadherin mAb E4.6, while adhesion to ICAM–1–Fc was not affected (Fig. 3b and data not shown). In contrast, the anti-β2 integrin mAb, TS1/18, the anti-αLβ2 mAbs, TS1/22 and D6.21, and the anti–ICAM-1 mAb RR1/1 all prevented IEL adhesion to ICAM–1–Fc, but not to E-cadherin–Fc. The anti-β1 integrin mAb, 4B4, blocked adhesion of IEL to human fibronectin (not shown) but not to E-cadherin–Fc or ICAM–1–Fc. A blocking mAb to αEβ7 (ACT-1) also did not inhibit IEL adhesion to E-cadherin–Fc or ICAM–1–Fc (Fig. 3b). Adhesion of IEL to P-cadherin–Fc was also completely blocked by the anti–αEβ7 mAb, αE7-2, but was unaffected by the anti–αLβ2 mAb, D6.21 (data not shown). Thus, adhesion of IEL to E- or P-cadherin–Fc involves αEβ7, and adhesion to ICAM–1–Fc involves αLβ2.

**JY Cells Transfected with αE Adhere to E-Cadherin–Fc**

To confirm that expression of exogenous αEβ7 would confer upon a cell the ability to adhere to E-cadherin–Fc, adhesion assays were performed with JY’ cells transfected with a full-length human αE-encoding cDNA construct. JY’ cells, or JY’ cells transfected with vector only (JY’-vector), expressed the αEβ7 integrin, very little β2 integrin, but no αLβ2, FACS®. JY’ transfected with αE (JY’-αE) had levels of αE and β2 integrins similar to untransfected or JY’-vector cells, but now also expressed αEβ7 (99% αEβ7+, MFI ~80). Both JY’-vector and JY’-αE transfectants also expressed similar levels of αLβ2 (Fig. 4a).

In the presence of manganese, JY’ cells transfected with vector alone did not adhere to E-cadherin–Fc coated directly on microtiter plate wells at any concentration tested. However, under the washing conditions used, 40% of JY’-αE cells adhered to 600 ng/well E-cadherin–Fc (Fig. 4b). In contrast, both cell lines adhered to ICAM–1–Fc (not shown) while neither adhered to the control FC protein human IgG1 (Fig. 4b). The higher percent binding observed for IEL to E-cadherin–Fc when compared with transfected JY’ cells probably reflects the higher surface ex-
expression of αEβ7 on IEL (on IEL, mean fluorescence intensity [MFI] ~600; on JY-αE, MFI ~80).

The adhesion of JY-αE cells to E-cadherin–Fc was blocked from 40% cells bound to <5% (the level seen for JY-vector cells) by mAbs to αEβ7 (αE7-2 and BerACT8) and E-cadherin–Fc (E4.6), but not by blocking mAbs to α1β3, α4β7, β1, or β2 integrins or to ICAM-1 (Fig. 4c). In contrast, the adhesion of JY-αE cells to ICAM-1–Fc was blocked by antibodies to αiβ2 (TS1/22 and D6.21), β2 integrins (TS1/18), and ICAM-1 (RR1/1), but not by antibodies to αEβ7, αEβ1, or β1 integrins (data not shown). These mAb-blocking experiments further confirm that the cell adhesion measured is mediated by αEβ7 binding to E-cadherin–Fc, or by αiβ2 binding to ICAM-1–Fc.

**E-Cadherin–Fc Binds Directly to 125I-labeled αEβ7 from a Lysate of IEL**

To demonstrate that E-cadherin molecules interact directly with αEβ7 molecules, the proteins that bound to E-cad-
herin–Fc from a ¹²⁵I-labeled IEL lysate in the presence of 1 mM MnCl₂, 1 mM MgCl₂, and 1 mM CaCl₂ were analyzed. Since the fusion protein contains a human IgG1 Fc region that binds to protein A, a standard immunoadsorption procedure was used. Proteins of the expected size for αEβ₇ (175, 135, and 110 kD) and αLβ₂ (160 and 100 kD) were visible on SDS-PAGE in nonreducing conditions after immunoprecipitation with anti–αEβ₇ and anti–αLβ₂ mAbs, respectively (Fig. 5). Remarkably, E-cadherin–Fc bound to radiolabeled species identical in size and relative intensity to those seen with the anti–αEβ₇ mAb (Fig. 5, compare lanes 1 and 3), but distinct from those seen with the anti–αLβ₂ mAb (Fig. 5, compare lanes 3 and 8). In contrast, no radiolabeled species were detected binding to the P-cadherin–Fc or IgG1 proteins. After immunoadsorption with the ICAM-1–Fc, proteins of the expected size for αLβ₂ could be visualized only on overexposed phosphor-images (not shown). While the same number of cell equivalents were used in each of the human IgG1 containing protein-binding experiments, ~40-fold fewer cell equivalents were required to immunoadsorb an equal amount of radiolabeled αEβ₇ with the anti–αEβ₇ mAb compared with E-cadherin–Fc (see legend to Fig. 5). This is consistent with the expected lower affinity of a cell adhesion receptor interaction compared with that of an antibody–antigen interaction.

Figure 4. Analysis of JY' cells transfected with αE cDNA. (a) Flow cytometric analysis of the cell surface expression of integrins on JY' cells transfected with pSRα-neo vector alone (JY'-vector) or with pSRα-neo/αE (JY'-αE). The staining with control mAb P3 is shown unshaded. Staining with αE7-2 (anti-αEβ₇), 4B4 (anti-β₁), BSG10 (anti-α₄), ACT-1 (anti-α₄β₇), and TS1/22 (anti-αLβ₂) is shown shaded. All mAbs were mouse IgG1. (b) Adhesion of transfected JY' cells to E-cadherin–Fc. Microtiter plate wells were coated directly with serial dilutions of E-cadherin–Fc or human IgG1, and blocked with BSA. The adhesion of JY'-vector and JY'–αE cells was determined in the presence of 1 mM MnCl₂, 1 mM MgCl₂, 1 mM CaCl₂, 0.1% BSA, HBS, pH 7.4, as described in Materials and Methods. The results are expressed as the mean percent bound ± 1 SD (n = 3). (c) Inhibition of adhesion of JY'–αE cells to E-cadherin–Fc by mAbs. Micortiter plate wells were coated directly with 0.63 μg/well of E-cadherin–Fc or human IgG1, and blocked with BSA. The adhesion of transfected JY' cells in the presence of various mAbs was determined as described for IEL in Fig. 3 b. The purified mAbs αE7-2, and BerACT8 (anti-αEβ₇), ACT-1 (anti-α₄β₇), 4B4 (anti-β₁), D6.21 (anti-α₄β₇), E4.6 (anti–E-cadherin), and RR1/1 (anti–ICAM-1) were used at 10 μg/ml. Ascites fluid containing TS1/18 (anti-β₂) was used at a dilution of 1:100. All mAbs were mouse IgG1. The results are expressed as the mean percent bound ±1 SD (n = 4). Adhesion of JY'-vector and JY'–αE cells to human IgG1 in this experiment was <3%.
To further confirm that the proteins bound by anti-α7β7 and the E-cadherin fusion protein were the same, batches of lysate were precleared with mAbs to α7β7 (αE7-1, αβ72 (TS1/22), or the control mAbs RPC5.4 and P3, before exposure to E-cadherin–Fc (Fig. 5). Prior immunodepletion with the anti-α7β7 mAb prevented the subsequent binding of material from the IEL lysate to E-cadherin–Fc. Preclearing with the other mAbs had no such effect. Thus, the predominant 125I-labeled protein from the IEL cell surface that interacts with E-cadherin–Fc in these conditions is α7β7.

**E-Cadherin–Fc Binds to Soluble Recombinant α7β7**

To produce a soluble form of α7β7, stop codons were introduced immediately upstream of the transmembrane coding regions in the cDNAs encoding the human α7 and β7 proteins (see Fig. 1 b, and Materials and Methods). Supernatant containing 35S-labeled soluble α7β7 was produced by transient transfection of COS-7 cells followed by metabolic labeling. Proteins in the supernatant were then subjected to immunoprecipitation with a panel of anti-α7β7 antibodies that recognize at least three distinct α7 epitopes (Russell et al., 1994). In the medium from cells transfected with plasmids encoding truncated α7 and β7 in the sense orientation, all the anti-α7β7 mAbs tested (αE7-1, αE7-2, αE7-3, HML-1, and BerACT8), but not a control mAb (TCR-81), precipitated two major bands of 140 and 105 kD on reducing SDS-PAGE. On nonreducing SDS-PAGE, two bands of 170 and 100 kD were observed (Fig. 6 and data not shown). These sizes correspond to those expected for the truncated α7 and β7 chains, respectively. No such proteins were precipitated from the medium of COS-7 cells transfected with constructs containing truncated α7 and β7 cDNAs in the antisense orientation. These results confirm the secretion of a soluble form of α7β7 that retains all of the epitopes of cell surface α7β7 that were tested.

We then sought to demonstrate binding of recombinant soluble α7β7 to the human E-cadherin–Fc fusion. Both the anti-α7β7 mAb αE7-1 and the E-cadherin–Fc protein were able to bind proteins of the expected size for soluble α7β7 (Fig. 6). No detectable soluble α7β7 bound to the control mAb RPC5.4, or the fusion proteins P-cadherin–Fc and ICAM-1–Fc. Furthermore, these proteins were not bound by E-cadherin–Fc in medium from COS-7 cells transfected with either α7 and β7 constructs in the sense or in the antisense orientation were metabolically labeled with 35S amino acids as described in the text. The medium was then made 1 mM with respect to MnCl2 and subjected to immunoadsorption with antibodies or Fc-fusion proteins. Samples were resolved on 7.5% SDS-PAGE in nonreducing conditions, and visualized by autoradiography. The precipitations with αE7-1 and RPC5.4 mAbs (both mouse IgG2a) represent the material obtained using 0.1 μl ascites from 7 × 10^6 cell equivalents, for TS1/22 and P3 mAbs (both mouse IgG1, with rabbit anti-mouse IgG) using 0.5 μl ascites from 7 × 10^6 cell equivalents, and for E-cadherin–Fc, P-cadherin–Fc, ICAM-1–Fc and human IgG1 using 5 μg fusion protein from 3 × 10^9 cell equivalents. For the preclearing experiments, batches of 1.5 × 10^9 cell equivalents were preabsorbed with the stated antibodies and protein A–Sepharose, before immunoadsorption with 2.5 μg E-cadherin–Fc.
cells transfected with the antisense \( \alpha_E \) and \( \beta_7 \) constructs (Fig. 6). Thus, E-cadherin–Fc interacts with soluble \( \alpha_E \beta_7 \) in the absence of other cellular proteins that are present in the IEL lysate used previously.

**The Avidity of Cell Surface \( \alpha_E \beta_7 \) Is Regulated**

Although the avidity of many integrins is regulated from within the cell, similar regulation of \( \alpha_E \beta_7 \) has not previously been reported. Furthermore, the structure of the \( \alpha_E \) chain is unique among integrins due to the presence of an extra domain in the extracellular region, membrane distal of the A domain, that is cleaved in the mature protein (the “X” domain, see Fig. 1 b; Shaw et al., 1994). This raises the possibility that regulation of the avidity of \( \alpha_E \beta_7 \) due to conformational changes could be different from other integrins. Therefore, studies were performed to investigate the regulation of \( \alpha_E \beta_7 \) avidity on \( \text{JY}^7 \)-\( \alpha_E \) cells and IEL, and to compare it to the well studied \( \alpha_B \beta_2 \) integrin.

\( \text{JY} \) cells transfected with \( \alpha_E \) adhered poorly to E-cadherin–Fc in the presence of 1 mM MgCl\(_2\) and 1 mM CaCl\(_2\) in the absence of manganese. The addition of 1 mM manganese caused a 12-fold or greater rise in the adhesion of \( \text{JY}^7 \)-\( \alpha_E \) cells to E-cadherin–Fc. PMA stimulation of \( \text{JY}^7 \)-\( \alpha_E \) also caused a sevenfold increase in binding to E-cadherin–Fc (Fig. 7 a). Adhesion of \( \text{JY}^7 \)-\( \alpha_E \) cells to ICAM-1–Fc was also enhanced by manganese or PMA, suggesting that both \( \alpha_E \beta_7 \) and \( \alpha_B \beta_2 \) can be activated by similar means. Relative to the effect of manganese, PMA was better able to stimulate adhesion of \( \text{JY}^7 \)-\( \alpha_E \) to ICAM-1–Fc than to E-cadherin–Fc. The reason for this difference is unclear, but valid comparisons are difficult since \( \alpha_E \beta_7 \) is expressed at a higher level than \( \alpha_B \beta_2 \) on \( \text{JY}^7 \)-\( \alpha_E \) cells (Fig. 3 a).

In contrast to \( \text{JY} \) transfectants, >90% of IEL adhered to E-cadherin–Fc in 1 mM MgCl\(_2\) and 1 mM CaCl\(_2\) and the addition of manganese or PMA had little enhancing effect on adhesion (not shown). Thus IEL, which are maintained in culture in the presence of IL-2 with periodic PHA-stimulation, have constitutively active \( \alpha_B \beta_2 \), suggesting that both \( \alpha_B \beta_2 \) and \( \alpha_B \beta_2 \) can be activated by similar means.

To study regulation of \( \alpha_E \beta_7 \) on IEL, we carried out assays in 0.05 mM MgCl\(_2\) and 1 mM CaCl\(_2\). In these conditions of limiting MgCl\(_2\), just under 40% of IEL adhere to E-cadherin–Fc (Fig. 7 b). The addition of manganese or PMA causes an almost twofold increase in the percentage of IEL adhering to E-cadherin–Fc. Moreover, cross-linking of the TCR complex and a mouse anti–CD3 mAb, followed by anti–mouse immunoglobulin polyclonal antibody, also causes a significant increase in IEL adhesion to E-cadherin–Fc (\( P < 0.0001 \), by Welch’s alternate t test, see Fig. 7 b). Similar treatment of IEL with antibodies to CD8\(\alpha\) or MHC class I had no such effect. Cross-linking of the TCR on IEL also increases the avidity of \( \alpha_E \beta_7 \) for ICAM-1, and of \( \beta_1 \)-integrins for fibronectin (data not shown), as has been reported for other T cells (Dustin and Springer, 1989; Shimizu et al., 1990; van Kooij et al., 1989).

It is known that calcium is required for the rigidification of the structure of E-cadherin (Nagar et al., 1996; Pokutta et al., 1994) and for E-cadherin–mediated homotypic cell to cell adhesion (Hyafil et al., 1981; Yoshida and Takeichi, 1982). However, in the presence of manganese, even when calcium has been depleted with EGTA, both \( \text{JY}^7 \)-\( \alpha_E \) cells (Fig. 7 a) and IEL (not shown) adhere to E-cadherin–Fc.

Thus, the heterophilic interaction of E-cadherin appears to be independent of calcium, at least in this system. It is unlikely that this is due to restriction of conformational changes within plate-bound E-cadherin–Fc, since similar results were found whether the fusion protein was immobilized directly on plastic or through its Fc region on anti-immunoglobulin antibodies (not shown). In contrast, the
adhesion of E-cadherin–expressing 16E6.A5 epithelial cells to E-cadherin–Fc is dependent on calcium (not shown). Also, since mouse E-cadherin does not have appreciable affinity for magnesium (Hyafil et al., 1981), and magnesium cannot support homophilic adhesion to E-cadherin–Fc (not shown), it is unlikely that magnesium substitutes for calcium in the cadherin structure. The results also suggest that calcium is not required for the function of α₃β₇ integrin. This is also the case for α₁β₂ binding to ICAM-1 (Fig. 7a; Shimizu and Mobley, 1993; Stewart et al., 1996).

In summary, α₃β₇, like α₁β₂, can exist in both high and low avidity states on the cell surface. In common with α₁β₂–mediated adhesion to ICAM-1, treatment of cells expressing α₃β₇ in a low avidity state with manganese, with PMA, with anti–CD3 antibodies, or by removing calcium and elevating magnesium, leads to increased adhesion to E-cadherin–Fc.

The Direct Binding of E-Cadherin–Fc to Solubilized α₃β₇ Is Modulated by Divalent Cations

We wished to determine if the effects of divalent cations on cell adhesion to E-cadherin–Fc were due to a direct influence on the binding of α₃β₇ molecules to E-cadherin–Fc molecules. The binding of E-cadherin–Fc to α₃β₇ in an IEL lyse was analyzed by immunoadsorption as described in Fig. 5, but in the presence of various concentrations of divalent cations (Fig. 8). No binding of E-cadherin–Fc to α₃β₇ was detected in the presence of 5 mM EDTA, confirming the cation dependency of the interaction (Fig. 8b). Binding of E-cadherin–Fc to α₃β₇ was clear in the presence of 1 mM MgCl₂ and 1 mM CaCl₂, but was increased eightfold by the addition of 1 mM MnCl₂ (Fig. 8b). Approximately equal quantities of α₃β₇ were immunoadsorbed by the anti–α₃β₇ mAb αE7-1 in all conditions, confirming that the differences seen in binding to the E-cadherin–Fc fusion protein were not due to dissociation or degradation of the α₃β₇ chains (Fig. 8a). Furthermore, since cadherins are known to be protease sensitive in the absence of calcium, we confirmed that an equal quantity of E-cadherin–Fc protein was present in each condition by Coomassie Blue staining of the immunoadsorbed material (Fig. 8b, ii).

Thus, the cation dependency of α₃β₇–mediated cell adhesion to E-cadherin–Fc is paralleled by the cation dependency of α₃β₇ binding directly to E-cadherin–Fc. Also, the association of α₃ with β₇ chains is not dependent on calcium or magnesium, as found for mouse α₃β₇ (Kilshaw and Murant, 1991), but in contrast to mouse α₃β₇, an integrin that requires calcium for heterodimer formation in similar experiments (Holzmann et al., 1989).

Discussion

Here, we demonstrate that an E-cadherin fusion protein binds directly both to α₃β₇ from solubilized intraepithelial lymphocytes, and to a soluble recombinant form of α₃β₇. Furthermore, purified E-cadherin fusion protein can serve as a potent substrate for cell adhesion through the α₃β₇ integrin. Thus, although E-cadherin is classically thought to be a homophilic adhesion molecule and is unlike known integrin ligands, it is a direct counter receptor for the α₃β₇ integrin.

We found that α₃β₇ exhibits selectivity in binding to cadherins. The adhesion of IEL cells to P-cadherin–Fc requires >100-fold more fusion protein than adhesion to E-cadherin–Fc. In both cases the adhesion is dependent on α₃β₇, since it is abolished by antibodies to α₃β₇. Thus, although human epithelial cells express both E- and P-cadherin, it is likely that E-cadherin is the primary epithelial receptor for α₃β₇ in vivo. The idea that α₃β₇ is selective in binding cadherins is consistent with the proposed role of α₃β₇ in tissue-specific leukocyte adhesion, and with the finding that IEL do not adhere to all cadherin-expressing cells. For example, IEL do not adhere to endothelial cells that possess VE-cadherin (Cepek et al., 1994).
Regulation of $\alpha_E\beta_7$ avidity for its counter receptor has not previously been reported. An increase in IEL adhesion to epithelial cells in the presence of manganese has been observed (Cepek et al., 1993; Karecla et al., 1995). However, it is not clear that this change is due to regulation of $\alpha_E\beta_7$ binding to E-cadherin since other receptors, including $\alpha_E\beta_7$ and ICAM-1, are involved in this complex cell to cell interaction (Cepek et al., 1993; Roberts et al., 1993). Here, the adhesion of cells expressing both $\alpha_E\beta_7$ and $\alpha_E\beta_7$ to purified E-cadherin–Fc or ICAM-1–Fc allowed us to compare the regulation of $\alpha_E\beta_7$ avidity with that of $\alpha_E\beta_7$ in a system in which only a single integrin counter receptor was available in each case. The $\alpha_E\beta_7$ integrin on freshly isolated PBL has low avidity for immobilized ICAM-1 in the presence of 1 mM MgCl$_2$ and 1 mM CaCl$_2$. However, in the presence of manganese ions PBL adhere strongly to purified ICAM-1 (Dransfield et al., 1992). Changes in the concentrations and presence of divalent cations are thought to have a direct effect on the conformation of the integrin at the cell surface, leading to increased ligand binding through an increase in the affinity of the integrin and/or changes in clustering of the integrin in the membrane. In addition, signaling from inside the cell can lead to increased integrin avidity. For example, PMA stimulation of resting PBL leads to increased adhesion to ICAM-1 through $\alpha_E\beta_7$. This type of regulation may involve changes in integrin clustering on the cell surface due to alterations in integrin association with the cytoskeleton (Lub et al., 1995, 1997; Stewart et al., 1996). In contrast to resting PBL, $\alpha_E\beta_7$ on a proportion of IL-2/PHA-activated PBL has high avidity for ICAM-1 (Lub et al., 1997). Furthermore, the avidity state of integrins transfected into different cell types can vary. For example, $\alpha_E\beta_7$ expressed on K562 cells is in a constitutively inactive state, while $\alpha_E\beta_7$ expressed on L cells is constitutively active (Lub et al., 1995).

We find that in 1 mM MgCl$_2$ and 1 mM CaCl$_2$, $\alpha_E$-transfected JY' cells exhibit poor adhesion to both E-cadherin–Fc and ICAM-1–Fc. Thus, on these cells, both $\alpha_E\beta_7$ and $\alpha_E\beta_7$ exist in a low avidity state. However, these cells can be induced to adhere to both integrin ligands by the addition of 1 mM MnCl$_2$. Since parallel changes in the direct binding of solubilized $\alpha_E\beta_7$ to E-cadherin–Fc were also observed, it is likely that this difference in cell adhesiveness is due to a direct effect of manganese on the conformation of the $\alpha_E\beta_7$ integrin. Thus, changes in extracellular cations can regulate the affinity of $\alpha_E\beta_7$ for its ligand. In contrast, cultured IEL, which are maintained in IL-2 with periodic stimulation with PHA and feeder cells, adhere avidly to both E-cadherin–Fc and ICAM-1–Fc in 1 mM MgCl$_2$ and 1 mM CaCl$_2$, even in the absence of manganese. Both $\alpha_E\beta_7$ and $\alpha_E\beta_7$ on IEL appear to be maintained in a constitutively active state, like $\alpha_E\beta_7$ on a proportion of IL-2/PHA-stimulated peripheral blood T cells.

We also show that signaling from inside the cell is able to increase the avidity of $\alpha_E\beta_7$. The addition of the phorbol ester, PMA, which acts intracellularly to upregulate protein kinase C, leads to increased adhesion of $\alpha_E$-transfected JY' cells to E-cadherin–Fc or ICAM-1–Fc. In the presence of a suboptimal concentration of magnesium, PMA also enhanced IEL adhesion to E-cadherin–Fc. The physiological triggers for such changes in lymphocyte integrin avidity in vivo remain unclear. In vitro, chemokines such as MCP-1, MIP-1$\beta$, and RANTES can activate lymphocyte integrins (Campbell et al., 1996; Carr et al., 1996; Lloyd et al., 1996), and cross-linking of components of the T cell receptor can boost the adhesion of resting PBL to ICAM-1 through $\alpha_E\beta_7$ (Dustin and Springer, 1989; van Kooyk et al., 1989) or to fibronectin and laminin through $\beta_7$-integrins (Shimizu et al., 1990). Here, we report that antibody cross-linking of cell surface CD3 is similarly able to increase IEL adhesion to E-cadherin–Fc, while cross-linking of MHC class I or CD8 receptors had no such effect. Thus, recognition by an IEL of an antigen presented by an epithelial cell or a professional antigen-presenting cell expressing E-cadherin (such as a Langerhans cell; Tang et al., 1993) could trigger increased adhesion to that cell through an upregulation of $\alpha_E\beta_7$ avidity. This interaction may also provide costimulation to the T cell, since anti-$\alpha_E\beta_7$ antibodies, in common with anti-$\alpha_E\beta_7$ antibodies, are known to increase T cell proliferation in the presence of suboptimal anti-CD3 (Russell et al., 1994; Sarnacki et al., 1992; Wacholtz et al., 1989), and ICAM-1 on an antigen-presenting cell can costimulate through $\alpha_E\beta_7$ (Dubey et al., 1995). Such events may be important in arresting lymphocytes within the epithelium when a specific antigen is recognized. In addition, since IEL contact more than one cell when resident within the epithelium, localized upregulation of $\alpha_E\beta_7$ avidity within an IEL could aid in polarizing lymphocyte interactions toward the relevant antigen-presenting cell.

X-ray crystallography and NMR studies have recently revealed that cadherin modules adopt a tertiary structure rather like immunoglobulin domains (Overduin et al., 1995; Shapiro et al., 1995; Nagar et al., 1996). Thus E-cadherin has a structure resembling that of the known cellular integrin ligands and can now be placed within the family of immunoglobulin-like, integrin-binding proteins. E-cadherin may share another feature of well-defined integrin ligands; the presence of a solvent-exposed acidic residue vital for integrin binding (Bergelson and Hemler, 1995). Mutation of an aspartate or glutamate residue in the CD-loop region in domain 1 of the immunoglobulin superfamily integrin ligands VCAM-1 (Osborn et al., 1994; Renz et al., 1994; Vonderheide et al., 1994; Jones et al., 1995; Wang et al., 1995), ICAM-1, (Staunton et al., 1990; Holness et al., 1995), and MadCAM-1 (Briskin et al., 1996; Viney et al., 1996) abolishes integrin binding. The tenth immunoglobulin-like FN III repeat of fibronectin also has an acidic residue within the RGD sequence on the FG-loop that is involved in integrin binding (Main et al., 1992). In mouse E-cadherin, the BC loop of domain 1 contains a glutamate residue required for adhesion of $\alpha_E\beta_7$-expressing lymphocytes to E-cadherin–transfected L cells (Karecla et al., 1996). Since this residue is conserved in human E-cadherin and we demonstrate that E-cadherin is a direct counter receptor for $\alpha_E\beta_7$, it is likely that this amino acid contributes directly to the $\alpha_E\beta_7$-binding site on E-cadherin, in a manner similar to that proposed for other integrin ligands. It is interesting to note that while three different families of immunoglobulin-like structures with exposed acidic amino acids serve as integrin ligands, the face of the module involved appears to be different in each case. It is also intriguing that both E-cadherin and ICAM-1 are likely to be
dimeric on the cell surface (Miller et al., 1995; Reilly et al., 1995; Nagar et al., 1996), and that both are ligands of integrins that contain an A domain in the α chain (αEβ7 and αLβ2, respectively). Since it has been proposed that integrin β-chains also contain a ligand-binding A domain (Lee et al., 1995; Puzon-McLaughlin and Takada, 1996), it is possible that αEβ7 and αLβ2 actually possess two A domains each. As suggested for αLβ2 binding to ICAM-1 (Miller et al., 1995), it is tempting to speculate that the binding of dimeric E-cadherin by αEβ7 could involve both these domains.

Although E-cadherin is a calcium-binding molecule, we find that E-cadherin–Fc binding to αEβ7 is independent of calcium. It seems that calcium is not directly involved in maintenance of the αEβ7 binding site on E-cadherin. Studies with recombinant soluble forms of mouse E-cadherin and Xenopus C-cadherin suggest that homophilic cadherin interactions do require the presence of calcium (Brieher et al., 1996; Tomsky et al., 1996). Interestingly, in their NMR studies of the first module of mouse E-cadherin, Overduin et al. (1995) find that the addition of calcium leads to a large shift in the orientation of histidine-79 within the HAV motif implicated in homophilic E-cadherin interaction (Blaschuk et al., 1990; Shapiro et al., 1995). In contrast, no such shift is found in any of the residues in or around the BC-loop implicated in αEβ7 interaction (Overduin et al., 1995). Thus it is possible that changes in extracellular calcium levels (for review see Maurer et al., 1996) would differentially regulate E-cadherin binding to αEβ7 versus other E-cadherin molecules. However, since calcium is required to rigidify and extend E-cadherin (Pokutta et al., 1994; Nagar et al., 1996) and to protect it from proteolysis (Hyafil et al., 1981; Yoshida and Takeichi, 1982), the lack of calcium dependence for αEβ7-mediated adhesion to the E-cadherin–Fc fusion protein may not hold true on the cell surface.

Although the role of αEβ7 binding to E-cadherin in vivo has yet to be established, the direct, specific, and regulated binding of αEβ7-expressing cells and of αEβ7 itself to the E-cadherin fusion protein very strongly suggests a physiological function for this interaction. Although there is an increase in the number of β3-integrin positive iIEL in chimeric mice with a defect in intestinal E-cadherin expression (Hermiston and Gordon, 1995), this is perhaps not surprising since the observed disruption of the epithelial layer and accompanying infectious and inflammatory response is likely to result in the attraction of many leukocytes into the intestine. In contrast, recently developed αE knockout mice have reduced numbers of intraepithelial IEL (Parker, C.M., unpublished results).

The role in vivo of the low but detectable αEβ7-mediated adhesion of IEL to human P-cadherin is more difficult to assess. The relative abundance of E- and P-cadherin on epithelial cells depends upon the state of cell differentiation (Hirai et al., 1989a,b), but this may not be the sole determinant of αEβ7-mediated interactions. On a single cell type, cadherins can display differential association with the cytoskeleton and thus exist in distinct pools on the cell surface (Salomon et al., 1992), and E- and P-cadherin are found in separate complexes on A431 cells (Johnson et al., 1993). Cell adhesion through homophilic cadherin–cadherin interactions requires their association with the cytoskeleton through intracellular catenins (Nagafuchi and Takeichi, 1988). However, while E-cadherin lacking its cytoplasmic tail is unable to associate with the catenins and cannot support strong homophilic adhesion, it is able to support adhesion of lymphocytes expressing αEMβ7 (Karecla et al., 1996). So, since different pools of cadherins may differ in their availability for interaction with different counter receptors, it is difficult to predict whether or not a high local concentration of a distinct population of P-cadherin on the cell surface could contribute to αEβ7-mediated adhesion.

Like human, mouse, canine, and Xenopus E-cadherin, human P-cadherin possesses an acidic residue at the tip of the BC loop in the first cadherin module (Shimoyama et al., 1989b). Thus human E- and P-cadherin may share a similar mode of binding to αEMβ7. In contrast, mouse P-cadherin does not possess an acidic residue at this position (Nose et al., 1987), and it has been reported that murine lymphocytes expressing αEMβ7 do not adhere to L cells transfected with mouse P-cadherin (Karecla et al., 1996). Although this cell to cell adhesion assay is likely to be less sensitive than the cell to fusion protein assay used here, these findings further complicate the question of the potential importance of αEβ7 binding to P-cadherin. It is possible that in some respects the function of P-cadherin may differ in the two species, and this may be reflected in the different expression pattern of P-cadherin in human and mouse (Shimoyama et al., 1989a,b).

Recently, a second direct heterophilic noncadherin ligand of a cadherin has been identified. The Listeria surface protein internalin binds to E-cadherin and invasion of cells expressing E-cadherin by Listeria in vitro is inhibited by anti–E-cadherin antibodies (Mengaud et al., 1996). Together with studies suggesting that different cadherins can bind to each other (for example N- and R-cadherin) (Takeichi, 1995), it is now clear that the biology of cadherin function is not limited to homophilic interactions, and is more complex than previously imagined.

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