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A family of β_7 integrins on human mucosal lymphocytes

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ABSTRACT **The heterodimeric protein complex recognized by the human mucosal lymphocyte 1 (HML-1) monoclonal antibody is expressed on 95% of intraepithelial lymphocytes but on only 1–2% of peripheral blood lymphocytes [Cerf-Bensusson, N., Jarry, A., Brousse, N., Lisowska-Grospierre, B., Guy-Grand, D. & Griscelli, C. (1987) *Eur. J. Immunol.* 17, 1279–1285]. We purified the smaller HML-1 subunit (105 kDa under nonreducing conditions) from hairy-cell leukemia cells and determined the N-terminal amino acid sequence of this chain. The 17 residues determined were identical to the deduced amino acid sequence encoded by an integrin β_7 cDNA clone [Yuan, Q., Jiang, W.-M., Krissansen, G. W. & Watson, J. D. (1990) *Int. Immunol.* 2, 1097–1108]. Biochemical analysis of the larger HML-1 subunit (175 kDa under nonreducing conditions) suggested that it was a distinct member of the cleaved group of integrin α chains, which we designated α^E. The β_7 chain also was associated with the integrin α^4 subunit, suggesting that the HML-1 antigen ($\alpha^E\beta_7$) and $\alpha^4\beta_7$ constitute a β_7 integrin family on mucosal lymphocytes. Interestingly, regulation of the expression of the HML-1 antigen was reciprocal to that of lymphocyte function-associated molecule 1 in the presence of transforming growth factor β_1. We suggest that these β_7 integrins may play a specific role in mucosal localization or adhesion and that the expression of the HML-1 antigen might be regulated by transforming growth factor β_1 produced at or near epithelial tissues.**

Lymphocytes predominantly located in the intestinal, genitourinary, and upper respiratory tracts and mammary glands constitute the mucosal immune system. This compartment includes lymphocytes both in organized lymphoid tissues (such as Peyer's patches) and diffusely distributed in the epithelium. Intraepithelial lymphocytes (IELs) reside on the external face of the basement membrane adjacent to the basolateral surface of epithelial cells. Almost exclusively T lymphocytes, intestinal IELs are >80% $CD4^-$ $CD8^+$ (1) and have a restricted repertoire of T-cell receptor variable region gene segment usage relative to peripheral blood lymphocytes (2, 3). It is likely that these cells recognize antigens presented by cells in Peyer's patches or by epithelial cells (4) within the mucosal microenvironment. Thus, IELs appear to represent a distinct subpopulation of lymphocytes that may have special functions of importance in immunological defense and tolerance.

Mucosal memory lymphocytes recirculate through the bloodstream and then return to mucosal tissues through the process of homing (5). Like other lymphocytes, IELs may utilize cell surface molecules to localize to and to function in the epithelium. These may include adhesion molecules that would interact with ligands expressed on the surface of epithelial cells or in the adjacent basement membrane. The integrin superfamily of adhesion molecules are crucial in lymphocyte cell–cell and cell–extracellular matrix interactions (6) and are candidates for the tissue-specific adhesion molecules hypothesized to exist on IELs. These glycoproteins are composed of α and β chains that are noncovalently associated as heterodimers. The β chains are >90 kDa and characteristically migrate with a larger molecular mass after reduction, consistent with their cysteine-rich intrachain-disulfide-linked structure. The integrin α chains are divided into two groups based upon structural features. One group of α chains is characterized by an inserted region, or I domain. The other group of integrin α chains is cleaved during biosynthesis to produce a 120- to 135-kDa heavy chain and a 25- to 30-kDa light chain that remain associated by disulfide linkage. The integrins are classified into families based upon the use of particular β subunits, each of which can associate with several different α chains (6, 7).

Certain members of the β_1 and β_2 integrin families are expressed on B and T lymphocytes. Lymphocyte function-associated molecule 1 (LFA-1; $\alpha^L\beta_2$) binds to intracellular adhesion molecule 1 or 2 on other cells to promote cell–cell interactions, as well as extravasation of lymphocytes from the intravascular space in a broad range of tissues (6). The very late antigens (VLAs) [$\alpha^1\beta_1$ (VLA-1)–$\alpha^6\beta_1$ (VLA-6)] also are expressed on lymphocytes and predominantly function as extracellular matrix receptors (7). Although a β_3 integrin may be expressed on some lymphocytes and function as a vitronectin receptor (8), β_4–β_6 have not yet been identified on these cell types (6, 9). The cell surface expression of several of the β_1 and β_2 integrins is increased by lymphocyte activation (6, 10).

Little information is available about the adhesion/homing molecules on IELs. If particular integrins function in a site-specific manner in the epithelium, it seems likely those integrins might be expressed preferentially on lymphocytes at this location. Interestingly, a monoclonal antibody (mAb) called human mucosal lymphocyte 1 (HML-1) has been produced (11) that reacts with the surface of >95% of IELs and with 40% of lamina propria lymphocytes but with <2% of resting peripheral blood lymphocytes. This mAb immunoprecipitates a protein complex, the HML-1 antigen, which is composed of two predominant chains of 105 and 175 kDa under nonreducing conditions (11). In addition, HML-1 antigen expression is increased after stimulation of lymphocytes with mitogen or phorbol 12-myristate 13-acetate (12). These biochemical features and the increase in HML-1 antigen expression after T-cell stimulation suggested that it might be an integrin. Here, we determined the N-terminal amino acid sequence of the smaller (105-kDa under nonre-

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Abbreviations: TGF-β_1, transforming growth factor-β_1; HML-1, human mucosal lymphocyte 1; IEL, intraepithelial lymphocyte; LFA-1, lymphocyte function-associated molecule 1; NEPHGE, nonequilibrium pH gel electrophoresis; FACS, fluorescence-activated cell sorter; mAb, monoclonal antibody; PHA, phytohemagglutinin; 2D, two dimensional; IL, interleukin; MFI, mean fluorescence intensity.

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ducing conditions) HML-1 subunit. This sequence matched the deduced β_7 N-terminal sequence predicted from a cDNA clone (13, 14). This β_7-encoded protein was associated with two α chains, a cleaved α subunit (α^E, see note in Fig. 2) and α^4.

MATERIALS AND METHODS

Antibodies. mAbs used were HML-1 (mouse anti-human HML-1 antigen, IgG2A) (11), BerACT8 (mouse anti-human HML-1 antigen, IgG1) (15), B-5G10 (mouse anti-human α^4, IgG1) (16), A-1A5 (mouse anti-human β_1, IgG2b) (17), TS1/18 (mouse anti-human β_2, IgG1) (18), P3 (mouse control, IgG1) (19), and 187.1 (rat anti-mouse κ light chain) (20). A peptide was synthesized (Dana–Farber Cancer Institute Core Molecular Biology Facility) whose sequence (CRREYSRFEKEQQQLNWKQDS) is encoded by the β_7 gene (13) within the predicted intracytoplasmic domain with a cysteine added at the N terminus. This peptide was coupled to keyhole limpet hemocyanin using *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (Pierce) (37), and then rabbit antibodies were generated as described (38).

Derivation of Cell Lines. Human intestinal tissue was obtained from fatal accident organ donors or from adult patients undergoing resection for colorectal carcinoma from bowel sections at least 10 cm away from macroscopically detectable lesions. IELs were isolated as described (21, 22) except that the mucosal fragments were incubated with dithiothreitol for 15 min and the Percoll (Pharmacia) gradient utilized was a 40–75% step gradient with the cells suspended in the 40% solution. Once isolated, the IELs were stimulated with phytohemagglutinin-P (PHA; Difco) and irradiated feeder cells (80% peripheral blood mononuclear cells/20% JY B lymphoblastoid cells) in T-cell medium [TCM = Yssel's medium (23) containing 2 nM recombinant interleukin (IL) 2 (gift of Ajinomoto, Kawasaki, Japan), 5% (vol/vol) conditioned medium (24), 4% (vol/vol) fetal calf serum (HyClone), and 50 μM 2-mercaptoethanol]. Long-term cultures were maintained by intermittent stimulation with PHA and irradiated feeders.

Purification and Sequencing of the HML-1 β Subunit. Hairy-cell leukemia cells (15 g) expressing the HML-1 antigen were solubilized in 2% (vol/vol) Triton X-100 in lysis buffer [phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulfonyl fluoride, 8 mM iodoacetamide, 10 μM leupeptin, and aprotinin (1 unit/ml)]. After centrifugation to remove insoluble material, the lysate was precleared with nonspecific columns and passed over a 1.2-ml protein A-Sepharose column to which BerACT8 mAb was coupled by dimethyl suberimidate (Pierce). The BerACT8 column was washed with PBS/1% Triton X-100, 0.5% Triton X-100/5 mM EDTA, 0.5% Triton X-100/500 mM NaCl, and then 1% Triton X-100. The HML-1 protein was eluted with 50 mM diethylamine (pH 11) containing 0.1% deoxycholate and 5% (vol/vol) glycerol, and the fractions were neutralized. The protein-containing fractions were identified by SDS/PAGE followed by silver staining as described (25) and were then separated by SDS/PAGE under nonreducing conditions. The region of the gel containing the smaller HML-1 subunit (105 kDa) was excised and resolved in a second SDS/PAGE gel under reducing conditions. After electroblotting to poly(vinylidene difluoride) membrane (Problott; Applied Biosystems) in CAPS, pH 11/methanol buffer (26), the HML-1 subunit was identified by Coomassie blue staining and sequenced using an Applied Biosystems model 470A gas-phase sequencer equipped with a model 120A phenylhydantoin amino acid analyzer (Harvard Microsequencing Facility, Cambridge, MA).

Labeling and Immunoprecipitation. Cultured IELs were surface labeled with lactoperoxidase as described (24). For biosynthetic labeling, 2×10^7 IELs were cultured in TCM containing transforming growth factor β_1 (TGF-β_1) (2 ng/ml) for 2 days and labeled for 30 min with 1 mCi of [^{35}S]methionine and 1 mCi of [^{35}S]cysteine (1 Ci = 37 GBq) in methionine- and cysteine-free medium containing 2 nM recombinant IL-2, 10 mM Hepes (pH 7.3), 4% (vol/vol) dialyzed human serum, and 4% (vol/vol) dialyzed conditioned medium. The cells were then washed in ice-cold PBS, resuspended in TCM at 37°C without radiolabel, and incubated for 0 min, 15 min, 45 min, or 4 hr. The cells were then solubilized in 1% Triton X-100/lysis buffer and precleared, and lysate containing the equivalent of 1–3 $\times$ 10^6 cells was immunoprecipitated with 0.1 μl of HML-1, 0.1 μl of A-1A5, 0.2 μl of B-5G10, or 0.2 μl of P3 ascites fluid plus 75 μl of 187.1 culture supernatant (added to samples being precipitated with IgG1 mAbs), followed by protein A-Sepharose. The immunoprecipitates were washed five times with 1 ml of 0.1% Triton X-100/PBS and analyzed by SDS/PAGE under reducing or nonreducing conditions, as described (27).

Two-Dimensional (2D) Gels. IELs were cultured in TCM containing TGF-β_1 (2 ng/ml) for 6 days, iodinated, treated with neuraminidase (GIBCO; 166 international units/ml) at room temperature in PBS/0.1% bovine serum albumin/1 mM $CaCl_2$/glucose (1 mg/ml), washed, and solubilized in lysis buffer. After specific immunoprecipitation, the samples were resolved on 2D gels by nonequilibrium pH gel electrophoresis (NEPHGE) [20% (vol/vol) pH 3.5–10 and 80% (vol/vol) pH 4–6 ampholines (LKB)], followed by SDS/PAGE in 7% polyacrylamide gels as described (24).

Cytokines. In cytokine experiments, a cultured IEL line was incubated for 5 days with TGF-β_1 (gift of Celtrix, Palo Alto, CA; 3 ng/ml), interferon γ (Biogen; 250 units/ml), granulocyte/macrophage colony-stimulating factor (gift of the Genetics Institute, Cambridge, MA; 6.25 units/ml), IL-1α (gift of Hoffman–La Roche through the Biological Response Modifiers Program, National Cancer Institute; 10 ng/ml), IL-1β (gift of DuPont through the Biological Response Modifiers Program, National Cancer Institute; 10 ng/ml), IL-3 (gift of the Genetics Institute; 5 units/ml), or IL-4 (gift of Schering; 10 units/ml). In addition, the lines were cultured with each of these cytokines in the presence of TGF-β_1 (3 ng/ml).

Fluorescence-Activated Cell Sorter (FACS) Analysis. FACS analysis was performed as described (28). To determine the mean fluorescence intensity (MFI), the entire population was utilized, despite heterogeneity in samples stained with the HML-1 mAb.

RESULTS AND DISCUSSION

Purification and N-Terminal Sequence of the Smaller HML-1 Subunit. To determine the primary amino acid sequence of HML-1 polypeptides, the complex was purified by immunoaffinity chromatography and two stages of SDS/PAGE from hairy-cell leukemia cells, yielding ≈11 pmol of the smaller HML-1 subunit (105 kDa under nonreducing conditions). From the N terminus of this subunit a sequence of 17 amino acids was determined that matched the sequence deduced from a β_7 cDNA clone (13, 14) (Fig. 1). This

Determined Sequence:	ELDAK**I**PSTGDATE**WR**N
Predicted β_7 Sequence:	ES**L**LDAK**I**PSTGDATE**W**RN

FIG. 1. N-terminal sequence of the 105-kDa HML-1 subunit is encoded by the β_7 gene. The 105-kDa HML-1 chain was purified from hairy-cell leukemia cells [a tumor type often expressing the HML-1 antigen (29) by immunoaffinity chromatography]. The amino acids designated by the single-letter code in lightface type were identified with a high degree of confidence, whereas the amino acids represented in boldface type were possible determinations.

sequence revealed the subunit to be encoded by the human integrin β_7 gene, for which, to our knowledge, no protein product had been identified and defined the actual N terminus of the polypeptide.

Biochemical Characterization of HML-1 Versus β_1 and β_2 Integrins on IELs. To compare the HML-1 antigen to other integrins, a human IEL-derived T-cell line was surface-labeled by iodination, solubilized, and immunoprecipitated with control (P3), HML-1, anti-β_1 (A-1A5), or anti-β_2 (TS1/18) mAb. The HML-1 immunoprecipitation revealed two prominent species of 175 and 105 kDa in SDS/PAGE analysis under nonreducing conditions (Fig. 2*A*), as described (11). These were designated β_7, based upon the determined amino acid sequence, and α^E (epithelial), because of the restricted distribution of the HML-1 complex on lymphocytes within or adjacent to epithelial tissues (see below). To verify the identity of the HML-1 β subunit a rabbit anti-β_7 peptide antiserum was produced. Immunoprecipitations with this antiserum and the HML-1 mAb were strikingly similar (Fig. 2*B*), confirming that the HML-1 β subunit was encoded by the β_7 gene. Migration of β_7 was slower under reducing conditions than under nonreducing conditions (Fig. 2*A*, compare lanes B and C), consistent with the presence of intrachain disulfide bonds. Migration of the β_7 chain was clearly distinct from that of the β_2 subunit but was quite similar to that of the β_1 subunit (Fig. 2*A*, compare lane C to lanes D and E). The α^E chain migrated with a molecular mass of 175 kDa under nonreducing conditions (Fig. 2*A*, lane B). In contrast, under reducing conditions this subunit appeared to be 25 kDa smaller, associated with the appearance of a small radiolabeled species in the dye front (Fig. 2*A*, lane C, see arrow). This suggested that α^E was composed of two disulfide-linked polypeptides of 150 and <30 kDa, here called α^E heavy and α^E light chains, respectively, and this was confirmed by 2D nonreducing SDS/PAGE followed by reducing SDS/PAGE experiments. Two less-well-visualized species that were observed to migrate between the α^E and the β_7 subunits under nonreducing conditions (Fig. 2*A*, lane B) have not been characterized.

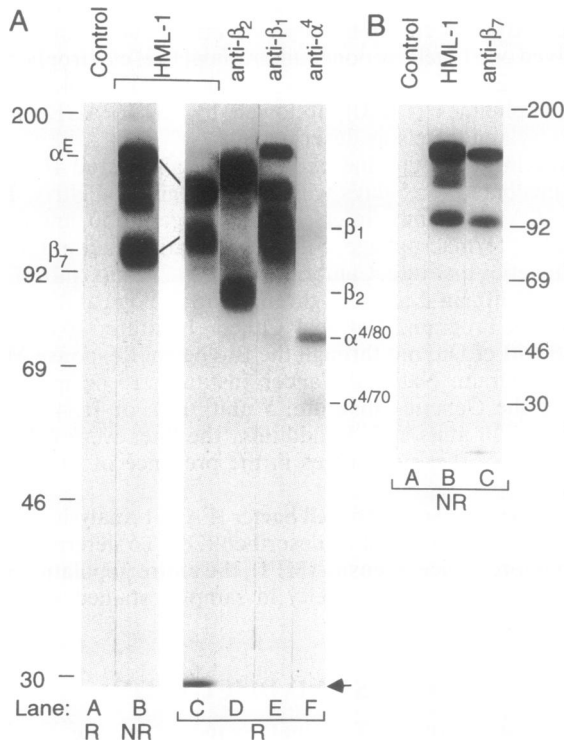

FIG. 2. Biochemical comparison of the HML-1 antigen to other integrins. (*A*) Surface-radiolabeled IELs were solubilized in 1% Triton X-100 and immunoprecipitated with control (P3, lane A), HML-1 (lanes B and C), anti-β_2 (TS1/18, lane D), anti-β_1 (A-1A5, lane E), or anti-α^4 (B-5G10, lane F) mAb and resolved in a 7% gel by SDS/PAGE under reducing (R) or nonreducing (NR) conditions. The positions of the β_7, α^E, β_2, β_1, and α^4 subunits are indicated [on this cell line, α^4 is expressed in the cleaved form (30), composed of an 80 ($\alpha^{4/80}$)- and a 70 ($\alpha^{4/70}$)-kDa subunit, which are noncovalently associated]. Lines between the HML-1 NR and R lanes indicate the change in molecular masses of the β_7 and the α^E subunits upon reduction. The arrow indicates the position of the α^E light chain in the dye front of lane C. (*B*) A surface-labeled IEL lysate was immunoprecipitated with preimmune rabbit serum (lane A), HML-1 mAb (lane B), or anti-β_7 peptide antiserum (lane C) and resolved in a 3–11% gradient gel by SDS/PAGE. Molecular masses are shown in kDa. Note that an α chain associated with β_4 was initially termed α^E before it was appreciated that this chain is identical to the α^6 subunit, which pairs with β_1 to form very late antigen 6. The α chain described and termed α^E in this report is distinct from α^6.

α^E Chain Was Cleaved During Biosynthesis. To evaluate whether the α^E heavy (150 kDa) and light (25 kDa) chains were independently derived or synthesized as a single precursor chain that was cleaved during biosynthesis, pulse–chase biosynthetic labeling was performed. In this study, the molecular mass under nonreducing condition of both the α^E and β_7 precursor chains (pα and pβ) increased with longer chase times, consistent with maturation of glycosylation as processing occurred (Fig. 3, lanes B–D). At the late 4-hr chase time point, the predominant species were the mature (mα and mβ) subunits. The predominant 175-kDa mα chain observed under nonreducing conditions (lane D) appeared to be composed of a 150-kDa subunit and a 25-kDa subunit (faintly visualized) under reducing conditions (lane H), like the mature polypeptides observed in surface labeling. Interestingly, under reducing conditions, the mature-sized α^E heavy chain (mα heavy) migrated more rapidly than any of the precursor α chains (pα), such as at the 15-min-chase time point (lanes F–H). Thus these data suggested that the α^E polypeptide was synthesized as a larger precursor protein, which was subsequently cleaved to form two chains that remained disulfide-linked in the mature subunit. The mature α^E chain (175 kDa under nonreducing conditions) was distinct as it was larger than any known human cleaved integrin α

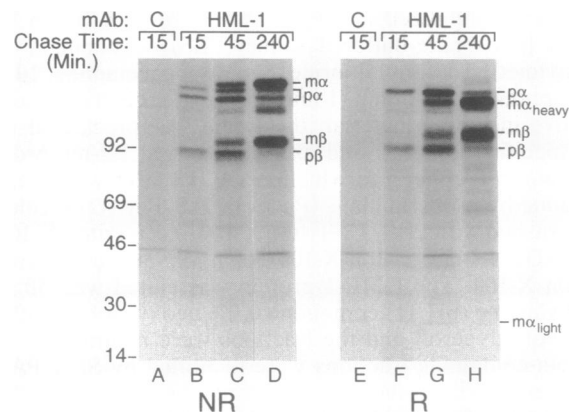

FIG. 3. HML-1 α chain appears to be cleaved during biosynthesis. IELs were labeled with [^{35}S]methionine and [^{35}S]cysteine for 30 min, washed, and then incubated without radiolabel for 0 min, 15 min, 45 min, or 4 hr. The cells were solubilized in 1% Triton X-100, immunoprecipitated with control (lanes C, P3) or with HML-1 mAb, and analyzed in a 3–11% gradient gel by SDS/PAGE under reducing (R) or nonreducing (NR) conditions. Bands visualized in the early time points were designated precursor (p), and those bands that migrated like the surface-radiolabeled HML-1 subunits were designated mature (m). Note that mβ migrated more slowly than the pβ bands, and the mα heavy band migrated faster than the pα species under reducing conditions, suggesting that it was cleaved during biosynthesis. Molecular masses are shown in kDa.

chain (7). An additional band was observed below the predominant mα and pα bands in lanes C and D. This band may correspond to the uncharacterized band migrating below α^E in Fig. 2*A*, lane B.

β_7 Also Associated with α^4. Anti-α^4 immunoprecipitation from a human IEL cell line yielded greater quantities of α^4 subunits than were observed to be coimmunoprecipitated with anti-β_1 (Fig. 2*A*, lanes F and E). This suggested that α^4 might be associated with a β chain other than the usual β_1 partner. However, one-dimensional gels were inadequate to determine if β_7 was associated with α^4, since the β_1 and β_7 subunits have similar migration patterns in one-dimensional SDS/PAGE (Fig. 2*A*, lanes C and E). Therefore, 2D gel conditions were determined that expanded the range of pH 4–6 to resolve β_1 and β_7 based upon charge after surface labeling. Anti-β_1 and HML-1 immunoprecipitations identified single β species, β_1 and β_7, respectively (Fig. 4 *A* and *E*). Note that the anti-α^4 immunoprecipitation revealed two β-chain species, which migrated with the same molecular mass but had distinct mobilities in NEPHGE (Fig. 4*C*). In the anti-α^4 mixed with anti-β_1 sample (Fig. 4*B*), the position of the $\alpha^{4/80}$ species permitted alignment of the gels in Fig. 4 *B* and *C*, allowing the positions of the β species to be compared. In the anti-α^4 immunoprecipitation the more-predominant β species aligned with the β_1 species (compare Fig. 4 *A* and *B* with *C*). Similarly, in the sample containing an anti-α^4 immunoprecipitation with an HML-1 immunoprecipitation (Fig. 4*D*), the positions of the β_1 species (to the right) and β_7 species (to the left) were resolved in the same gel. Importantly, the less-predominant β chain observed in the anti-α^4 immunoprecipitation migrated with the same charge as the β_7 species observed in the HML-1 mAb immunoprecipitation (compare Fig. 4 *C* with *D* and *E*). Thus, α^4 was observed to associate with two β species in 2D gels, one migrated with a pI value and a molecular mass identical to that of β_1 and the other migrated with a pI value and a molecular mass identical to that of β_7. The association of β_7 with both α^E and α^4 suggested that these β_7 complexes constitute a family of integrins. Because the HML-1 mAb failed to coimmunoprecipitate the $\alpha^4\beta_7$ integrin on these cells (Fig. 2*A*, lanes B and C), it appeared likely that this antibody recognized either an α^E-specific epitope or a combinatorial determinant within the $\alpha^E\beta_7$ (HML-1) complex. Therefore, the localization of the HML-1 antigen to epithelial tissues or tissues adjacent to the epithelium is likely to be a reflection of the tissue distribution of the α^E chain or of the specific $\alpha^E\beta_7$ heterodimer, rather than of the β_7 subunit, which may have a broader tissue distribution.

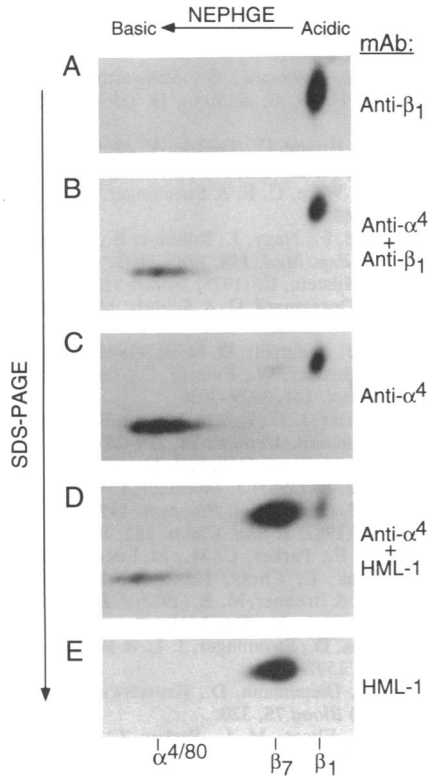

FIG. 4. β_7 associates with α^4. IELs were surface-radiolabeled, neuraminidase-treated, solubilized in Triton X-100, and immunoprecipitated with HML-1 (*E*), anti-β_1 (A-1A5) (*A*), or anti-α^4 (B-5G10) (*C*). Samples containing a single immunoprecipitation and samples containing a mixture of the anti-α^4 immunoprecipitation plus HML-1 immunoprecipitations (*D*) or containing the anti-α^4 immunoprecipitation plus anti-β_1 immunoprecipitations (*B*) were analyzed by 2D NEPHGE under reducing conditions followed by SDS/PAGE in a 7% polyacrylamide gel. Only the portion of the gels demonstrating the mobility of the β and the $\alpha^{4/80}$ subunits are shown. The position of these species in NEPHGE are indicated below *E*. In *B* no β_7 species was observable, possibly because it was below the level of detection of this autoradiogram with a slightly shorter exposure.

HML-1 Expression Is Induced by TGF-β_1. TGF-β_1 has been reported to affect the level of cell surface expression of integrins on many cell types (31). IELs are located adjacent to epithelial cells, a cell type that may produce TGF-β_1 (32, 33). Therefore, the effect of TGF-β_1 (3 ng/ml) on the surface expression of the HML-1 molecule was determined by FACS analysis of an IEL-derived T-cell line. When these IELs were cultured with TGF-β_1, a dramatic increase was observed in both the percentage of IELs expressing the HML-1 antigen (from 10 to 93%, Fig. 5*A*) and in the level of expression of the HML-1 antigen on these cells (MFI increased from 6 to 546; Fig. 5*B*). The percentage of IELs expressing the HML-1 antigen continued to increase for at least 27 days of continuous culture in TGF-β_1 (Fig. 5*A*). When TGF-β_1 was removed from these cultures, the percentage of HML-1-positive cells decreased over a period of 1–2 weeks back toward the baseline levels observed on IELs cultured in medium alone (Fig. 5*A*). The relatively slow fall in the levels

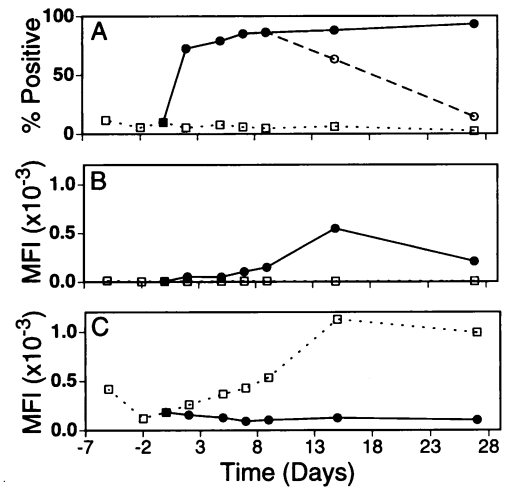

FIG. 5. TGF-β_1 stimulates an increase in the percentage of IELs expressing the HML-1 antigen but inhibits the activation-stimulated increase in LFA-1 expression. IELs were stimulated with PHA and cultured in medium alone for 5 days. At day 0 (solid square), TGF-β_1 was added to a portion of the cells, and the IELs were then cultured continuously in medium without (open squares) or with TGF-β_1 (solid circles) for the indicated period. After 10 days of culture with TGF-β_1, a portion of the TGF-β_1-treated cells was washed and cultured without TGF-β_1 (open circles) for the remainder of the period. The percentage of cells stained positively with the HML-1 mAb (*A*) and the mean fluorescence intensity (MFI) of the entire population after staining with the HML-1 (*B*) or anti-β_2 (TS1/18) (*C*) mAb was determined by FACS analysis and plotted versus time.

of the HML-1 antigen after the removal of TGF-β_1 may reflect the slow proliferation of the cells at this point 15 days after stimulation. Interestingly, culturing the IELs with TGF-β_1 appeared to prevent the increase in LFA-1 cell surface expression observed after PHA stimulation (Fig. 5*C*) but did not significantly affect the relatively low level of β_1-subunit expression (MFI < 75, data not shown). Thus TGF-β_1 had dramatic and discordant effects on integrin levels, stimulating an increase in HML-1 antigen expression but blocking an activation-related increase in LFA-1 levels. Other cytokines evaluated, including interferon γ, granulocyte/macrophage colony-stimulating factor, IL-1α, IL-1β, IL-3, and IL-4 had little or no effect on the HML-1 antigen levels and did not influence the increase in HML-1 antigen expression stimulated by TGF-β_1 (data not shown).

Related Mouse Integrins and Potential Functions for These β_7 Complexes. Two mouse integrins bear interesting structural relationships to the human β_7 integrins described here. The mouse lymphocyte Peyer's patch adhesion molecule 1 (LPAM-1) complex is composed of α^4 in association with a partially characterized non-β_1 integrin subunit called β_P (34). Thus LPAM-1 and $\alpha^4\beta_7$ share homologous α chains but determination of the primary sequence of β_P will be necessary to evaluate if these structures also use homologous β chains. Like LPAM-1 (34), $\alpha^4\beta_7$ might be a homing receptor that mediates binding to Peyer's patch high endothelial venules.

Another integrin that appears to be composed of the murine β_7 subunit (35) in association with a cleaved α chain (the M290 antigen) (36) may be related to human $\alpha^E\beta_7$ (the HML-1 antigen). The M290 antigen also has a relatively restricted tissue distribution on intestinal, pulmonary, and breast IELs and is increased by mitogen stimulation and by culture with TGF-β_1 (36). Although the finding here that the HML-1 β subunit is encoded by the human β_7 gene suggests that this chain is homologous to the M290 β subunit, determination of the primary sequences of the α^E and M290 α subunits will be necessary before concluding that these are homologous complexes. Like previously identified integrins, $\alpha^E\beta_7$ and the mouse M290 antigen also may perform adhesive function(s). Although these β_7-associated integrins can be induced on peripheral blood lymphocytes by activation (12) and are expressed on some tumors (29), their predominance *in vivo* on intraepithelial and lamina propria lymphocytes suggests that they might interact with particular ligands at the epithelium, such as epithelial cell surface or basement membrane molecules. It has been suggested that naive lymphocytes express a relatively nonspecific array of homing receptors. Once a lymphocyte has been activated in a given tissue, that tissue may cause a change in cell surface adhesion receptor expression of the lymphocyte that would mediate its homing to similar tissues (5). This hypothesis predicts that cellular interactions or exposure to cytokines within the specific microenvironment would result in an increase in the level of expression of adhesion receptors responsible for homing to or functioning in that tissue and might concomitantly cause a decrease in the level of broadly reactive adhesion receptors. With this hypothesis as a background, it is intriguing that TGF-β_1 not only stimulated increased expression of the mucosal lymphocyte integrins $\alpha^E\beta_7$ and M290 but also prevented the expected increase in LFA-1 expression on these IELs. It is tempting to speculate that the β_7 integrins play a specific role in mucosal localization or adhesion and that the expression of some β_7 integrins might be regulated by TGF-β_1 produced at this tissue site.

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