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Cloning and expression of mouse integrin $\beta_p(\beta_7)$: A functional role in Peyer's patch-specific lymphocyte homing

(adhesion molecule/cell adhesion/DNA sequence/high endothelial venule)

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ABSTRACT **Lymphocytes express integrin receptors, termed lymphocyte Peyer's patch high endothelial venule (HEV) adhesion molecules (LPAMs), that mediate their organ-specific adhesion to specialized HEVs found in mucosal lymphoid organs (Peyer's patches). LPAM-1 consists of a murine integrin α_4 noncovalently associated with integrin β_p. Here, we describe the cloning and expression of a mouse cDNA encoding β_p, which is an 806-amino acid transmembrane glycoprotein. The genomic Southern blot analysis indicates that β_p is the murine homologue of human β_7. The function of $\alpha_4\beta_7$ as a Peyer's patch-specific adhesion molecule was tested directly by expression of the murine β_7 cDNA in an $\alpha_4^+\beta_7^-$ B-cell line or coexpression of the α_4 and β_7 cDNAs in an $\alpha_4^-\beta_7^-$ T-cell line. The transfected cells exhibited a new Peyer's patch-specific adhesive phenotype that could be specifically blocked by monoclonal antibodies against α_4 and β_7. Moreover, an anti-β_7 monoclonal antibody specifically blocked binding of normal lymphocytes to Peyer's patch HEV but did not inhibit their binding to peripheral lymph node HEVs, indicating that β_7 is a unique component of the Peyer's patch-specific homing receptor.**

The recirculation of lymphocytes throughout the body, from blood through lymphoid organs and back into the bloodstream (1), plays a critical role in the normal function of the immune system by maximizing interactions of lymphocytes with antigen and by increasing collaborative interactions between many disparate cell types. The organ-specific interactions of lymphocytes with specialized high endothelial venules (HEVs) in lymphoid organs (2) are believed to be controlled by homing receptors on lymphocytes and vascular addressins on HEVs (3, 4). Thus far, at least three functionally distinct lymphocyte–HEV recognition systems governing the homing of lymphocytes to peripheral lymph nodes (PLNs), mucosal lymphoid organs [Peyer's patches (PPs) and appendix], and inflamed synovium have been identified (5, 6).

Murine lymphocyte PP HEV adhesion molecule 1 (LPAM-1) involved in the organ-specific adhesion of lymphocytes to PP HEVs has been identified as an integrin receptor (7, 8). The integrin receptors are engaged in cell–cell adhesion and interactions with extracellular matrix components (9). The functional role of LPAM-1 as a lymphocyte homing receptor for PP is confirmed by *in vivo* migration studies in the rat (10). LPAM-1 consists of a murine integrin α_4 homologous to human VLA-4α and an integrin β chain, designated as β_p, that is invariantly involved in lymphocyte adhesion to PP HEVs (8). In the rat, however, a high endothelial binding factor specific for Peyer's patch high endothelium (HEBFpp) defines a PP adhesion molecule that is an ≈80-kDa single-chain protein (11) and distinct from LPAM-1. Whether this molecule operates concurrently with LPAM-1 to mediate adhesion of lymphocytes to PP HEVs remains to be established. Here, we describe the isolation and characterization of a mouse full-length cDNA clone encoding this β_p subunit.‡ Expression of this cDNA in lymphoid cells induces a new PP-specific adhesive phenotype, indicating that β_p is a distinct component of the PP-specific homing receptor.

MATERIALS AND METHODS

Isolation and Characterization of the cDNA Clone for β_p. A TK1 cDNA library was constructed in λ_{ZAP} as described (12). Approximately 2×10^6 recombinants were screened with the preadsorbed antisera against LPAM-1 (8). Positive phage were selected and transferred to pBluescript SK− (Stratagene) with the helper phage R408.

Northern Blot and Genomic Southern Blot Analyses. Northern blot analysis was performed by the standard protocol (13). For genomic Southern blot analysis, 10 μg of each mouse or human high molecular weight DNA was digested with endonucleases, electrophoresed, transferred to a Zeta-Probe membrane (Bio-Rad), and hybridized with either the mouse β_p or the human β_7 probe.

Stable Expression of β_7 cDNA in 38C13 Cells and Stable Coexpression of α_4 and β_7 cDNAs in BW5147 Cells. pLβ_pSN was constructed by cloning the murine β_7 cDNA into the retroviral vector pLXSN (14) and recombinant retroviruses were prepared from the producer line GP+E-86 (15). 38C13 cells were infected with LXSN or pLβ_pSN retroviruses and selected in medium containing G418 (1 mg/ml). BW5147 cells were electroporated with pRSVtkhyg^R/α_4, which contains the α_{4m} cDNA (12) in the expression vector pRSVtkhyg^R, and hygromycin-resistant clones expressing cell-surface α_4 were isolated. The β_7 cDNA was subcloned into the expression vector pEF-BOS (16). A BW5147/α_4^+ clone was cotransfected with pEF-BOS/β_7 and pSV$_2$neo DNA and G418-resistant clones were isolated.

Immunoprecipitation and Western Blot Analysis. For Western blot analysis, cells were lysed in C-IPB buffer as described (7), immunoprecipitated with monoclonal antibody (mAb) R1-2, subjected to SDS/PAGE under reducing conditions, transferred to nitrocellulose, and probed with either preimmune or anti-β_7 rabbit antisera. For immunoprecipitation, cells were cell-surface labeled with ^{125}I using the glucose oxidase–lactoperoxidase method, lysed, precleared, and precipitated with either preimmune or anti-β_7 antisera as described (7).

***In Vitro* HEV Binding Assay.** This assay was performed as described (7). Relative adherence ratios (RARs) were calcu-

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Abbreviations: HEV, high endothelial venule; PP, Peyer's patches; LPAM, lymphocyte Peyer's patch HEV adhesion molecules; mAb, monoclonal antibody; MLN, mesenteric lymph node; PLN, peripheral lymph node; PMA, phorbol 12-myristate, 13-acetate; RAR, relative adherence ratio; VCAM-1, vascular cell adhesion molecule 1.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M95632).

lated as described (7) and are presented as the mean with standard deviation.

RESULTS

Cloning and Characterization of the cDNA Clone for β_p. To isolate cDNA clones for β_p, a λ_{ZAP} cDNA library prepared from poly(A)$^+$ RNA isolated from a PP-binding T-cell line TK1 was screened using anti-LPAM-1 antiserum (8) and followed by a reverse Northern blot analysis (data not shown). To examine whether these β_p clones contain cDNA of the full-length RNA transcript, equal amounts of poly(A)$^+$ RNA from TK1 ($\alpha_4^+\beta_p^+$), RAW112 ($\alpha_4^+\beta_p^-$), and two control T-cell lines, TK5 and BW5147 ($\alpha_4^-\beta_p^-$), were analyzed on a Northern blot using the β_p cDNA T30 clone as a probe. As shown in Fig. 1*A*, a single band of ≈2.7 kilobases was evident in the lane containing RNA from TK1 (lane 2) but was negative in RNAs from RAW112, TK5, and BW5147 (lanes 3–5). Since the length of the T30 clone was ≈2.7 kilobases, this result indicated that it contained a cDNA at or near the full-length RNA transcript of β_p. To confirm that 38C13, a non-PP-homing B-cell line, did not express β_p or β_1 (see below), equal amounts of poly(A)$^+$ RNA from 38C13, TK1, and RAW112 were analyzed on Northern blots using either the β_p or mouse β_1 cDNA as a probe. The results show that 38C13 expressed neither β_p (Fig. 1*B*) nor β_1 (Fig. 1*C*).

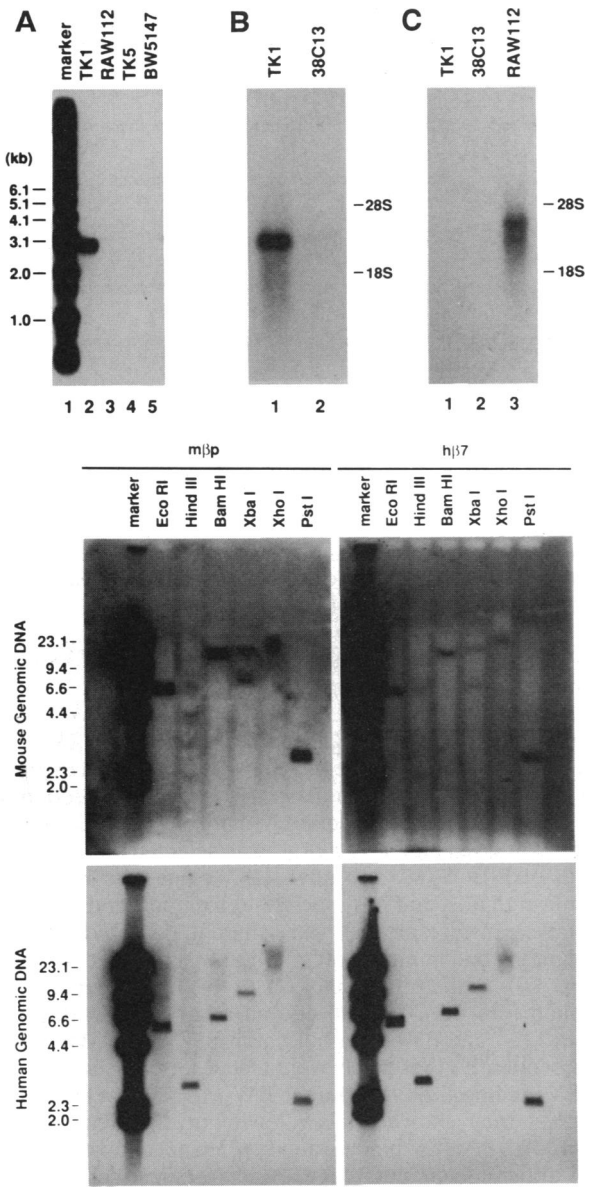

FIG. 1. Northern and genomic Southern blot analyses. (*A* and *B*) Poly(A)$^+$ RNAs from cells as indicated were probed with β_p cDNA, under high-stringency conditions. (*C*) Similar analysis with a mouse β_1 cDNA probe. (*D*) Each genomic DNA was digested with the indicated restriction enzymes, and probed with either a mouse β_p cDNA (mβp) or a human β_7 cDNA (hβ7) as indicated. Molecular sizes in kilobases are indicated to the left in *A* and *D*.

The nucleotide sequence of 2628 base pairs (bp) contains a 32-bp 5′ untranslated region followed by a single open reading frame of 2418 bp encoding a polypeptide of 806 amino acids, and followed by a 178-bp 3′ untranslated region that contains the polyadenylylation signal at position 2600 (Fig. 2). After the initiation codon ATG, there is a stretch of 19 hydrophobic amino acids with the characteristics of a signal peptide (17). Thus, the predicted mature β_p protein contains an extracellular region of 705 amino acids, a transmembrane domain of 21 amino acids, and a cytoplasmic domain of 61 amino acids. On the extracellular region, there are eight potential N-linked glycosylation sites having the consensus sequence N-X-S/T. In the cytoplasmic domain, there are three tyrosine residues at positions 752, 757, and 777.

The deduced amino acid sequences of mouse β_p and human β_7 (18, 19) show 86% identity, suggesting that β_p is the murine homologue of β_7. However, there is an unusual difference between these two sequences. Although most of the cytoplasmic domain sequences are highly conserved, the C-terminal sequences are quite distinct (data not shown). To test whether the mouse genome contains sequences that are β_7-like in addition to β_p, a genomic Southern blot analysis was performed. Mouse genomic DNA showed that the pattern of restriction fragments hybridized to either the mouse β_p probe (mβ_p) or the human β_7 probe (hβ_7) was virtually identical (Fig. 1*D*). Similarly, human genomic DNA showed the same hybridization pattern of restriction fragments with either the mβ_p or the hβ_7 probe (Fig. 1*D*), indicating that β_p is indeed the murine homologue of β_7 (hereafter called murine β_7).

Stable Expression of β_7 cDNA in 38C13 Cells Confers the β_7-Specific Antigenic Epitopes in These Cells and Mediates the Organ-Specific Adhesion to PP HEVs. To obtain the B-cell lymphoma 38C13 that stably expresses $\beta_p(\beta_7)$ (hereafter called 38-β_p), 38C13 cells were infected with Lβ_pSN recombinant retroviruses. As a control, 38C13 cells were infected with retroviruses carrying a pLXSN vector that does not contain the β_7 cDNA (hereafter called 38-LXSN). All vectors conferred G418 resistance to infected cells. Most of the infected cells expressed high levels of cell surface β_7 (Fig. 3*f*), whereas the parental cells 38C13 (Fig. 3*d*) and the control LXSN retrovirus-infected cells (Fig. 3*e*) were clearly negative for β_7 surface expression. Infection with these retroviruses did not change surface expression of other adhesive receptors, including L-selectin (Fig. 3*g–i*), lymphocyte function-associated antigen (Fig. 3*j–l*), and CD44 (Pgp-1) (Fig. 3*m–o*). To examine whether β_7 associated with α_4 to form heterodimers in 38-β_p, lysates from these cells were first immunoprecipitated with mAb R1-2, in the presence of Ca^{2+} and analyzed with β_7-specific antisera on a Western blot. The immunoblot in Fig. 4*A* shows that a β_7 polypeptide of ≈130 kDa is present in 38-β_p but not in 38C13 or 38-LXSN cells.

The binding capacity of 38-β_p for HEVs in PP or PLNs was examined in an *in vitro* HEV binding assay (2). The specific binding of 38-β_p to mouse PP HEVs is illustrated in Fig. 5*A*. As shown in Fig. 5*C*, although 38-β_p had no significant alteration in PLN HEV binding ability, they showed a highly significant increase (at least 20-fold) in PP HEV binding ability as compared with 38C13 or 38-LXSN. To assess the binding specificity of 38-β_p, mAbs R1-2, M301 (20), MEL-14, and R7D4 were tested for their blocking ability on cell adhesion to HEVs in either PP or PLNs. The results showed that preincubation of 38-β_p with anti-α_4 R1-2 reduced their binding to PP

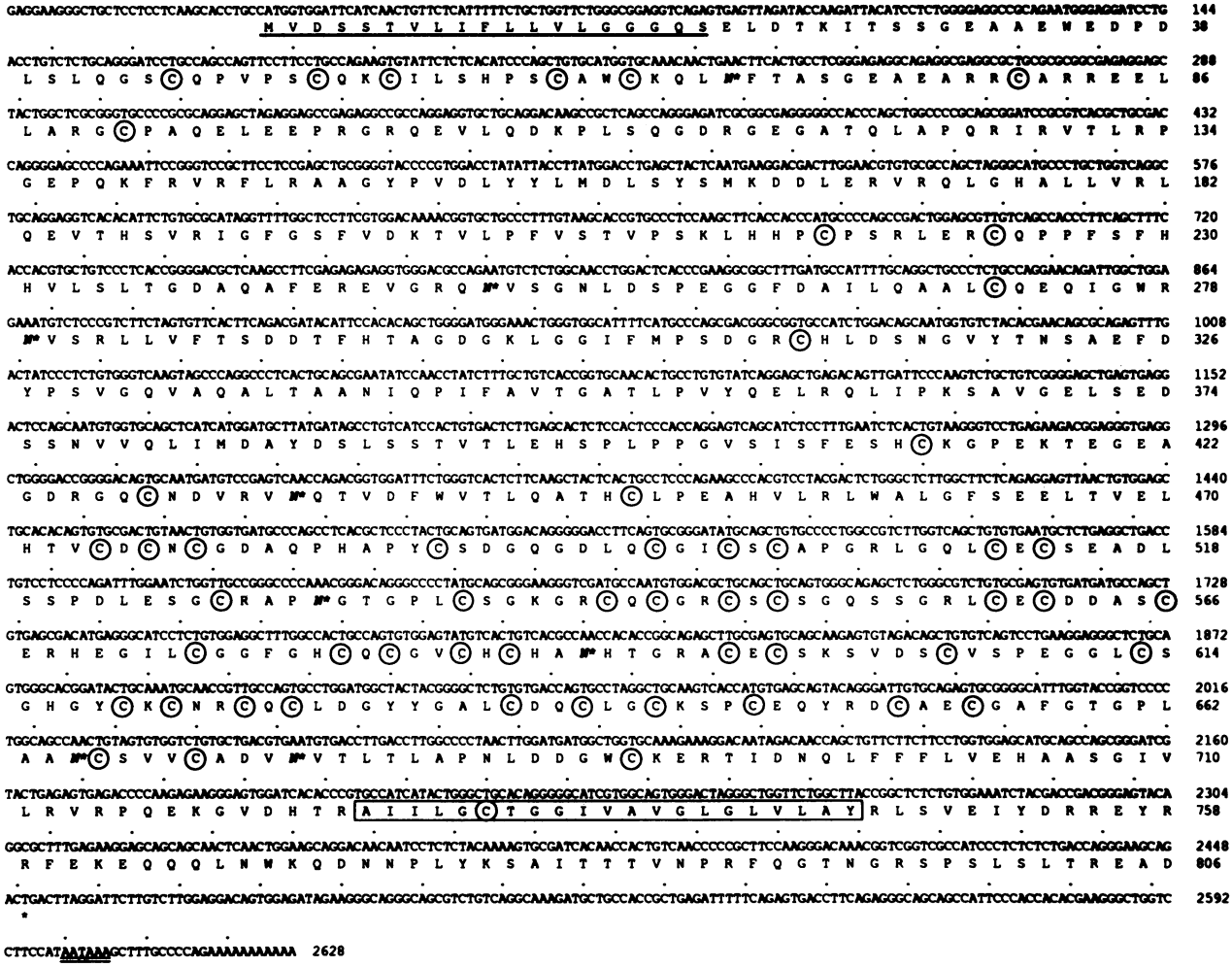

FIG. 2. Sequence of the mouse β_p cDNA. The derived amino acid sequence is indicated below the first nucleotide of each codon and the termination codon is marked with an asterisk. The predicted signal peptide is underlined, the cysteine residues are circled, the potential N-glycosylation sites are highlighted in boldface type as N*, and the transmembrane domain is boxed.

HEVs by 80–90% but did not affect their binding to PLN HEVs. Similarly, preincubation of these cells with anti-β_7 M301 reduced their binding to PP HEVs by ≈50% but did not alter their ability to bind PLN HEVs. In contrast, preincubation of these cells with MEL-14 did not change their ability to bind PP HEVs but reduced their binding to PLN HEVs by 90–95%. Adhesion of these cells to PP or PLN HEVs was not inhibited by the control mAb R7D4.

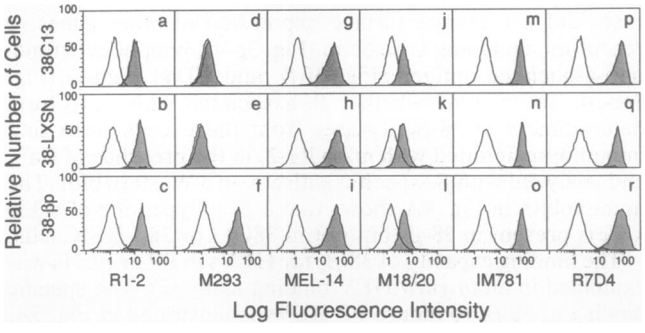

FIG. 3. Flow cytometry analyses. Cells were stained with either an isotype-matched control mAb 30H12 (unshaded histograms) or one of the several mAbs (shaded histograms) directed against the cell surface adhesion receptors as indicated. Rat mAbs used included M293 (anti-*mouse* β_7) (20), MEL-14 (anti-L-selectin), IM781 (anti-Pgp-1), M1/70 (anti-Mac-1 α), M18/20 (anti-lymphocyte function-associated antigen 1 β_2), and R7D4 recognizing the idiotype surface immunoglobulin of 38C13.

Stable Coexpression of α_4 and β_7 cDNAs in BW5147 Cells Mediates Low-Level but Specific Adhesion to PP HEVs. Stable expression of $\alpha_4\beta_7$ was achieved by the sequential expression of α_4 and β_7 cDNAs in BW5147. After transfection with pRSVtkhyg^R/α_4 and selection with hygromycin B, one clone that expressed high levels of α_4 was selected and transfected with pEF-BOS/β_7, and an $\alpha_4^+\beta_7^+$ clone was isolated. To confirm that α_4 and β_7 associate to form heterodimers in the double transfectant, they were characterized by cell-surface iodination and immunoprecipitation with β_7-specific antisera (Fig. 4*B*). In the presence of Ca^{2+}, this antisera immunoprecipitates an ≈133-kDa β_7 and an associated 160-kDa α_4 in both TK-1 and BW-$\alpha_4\beta_p$, but not in BW5147 or BW-α_4 cells. Subsequently, these cells were tested for differences in their HEV binding ability. Although BW-$\alpha_4\beta_p$ did not bind to PLN HEVs, they showed a low level but significant increase (4-fold) in PP HEV binding ability (data not shown). Note that it is difficult to quantitate RAR values of these cells since they may form aggregates during HEV binding assay.

Anti-β_7 mAb Specifically Blocks Binding of Normal Lymphocytes to PP HEVs. To confirm that β_7 uniquely contributes to the homing function of PP, normal lymphocytes isolated from mouse mesenteric lymph nodes (MLNs) were tested for binding to HEVs of PP and PLNs. Most normal MLN lymphocytes express β_7, α_4, and L-selectin (data not shown) and bind equally

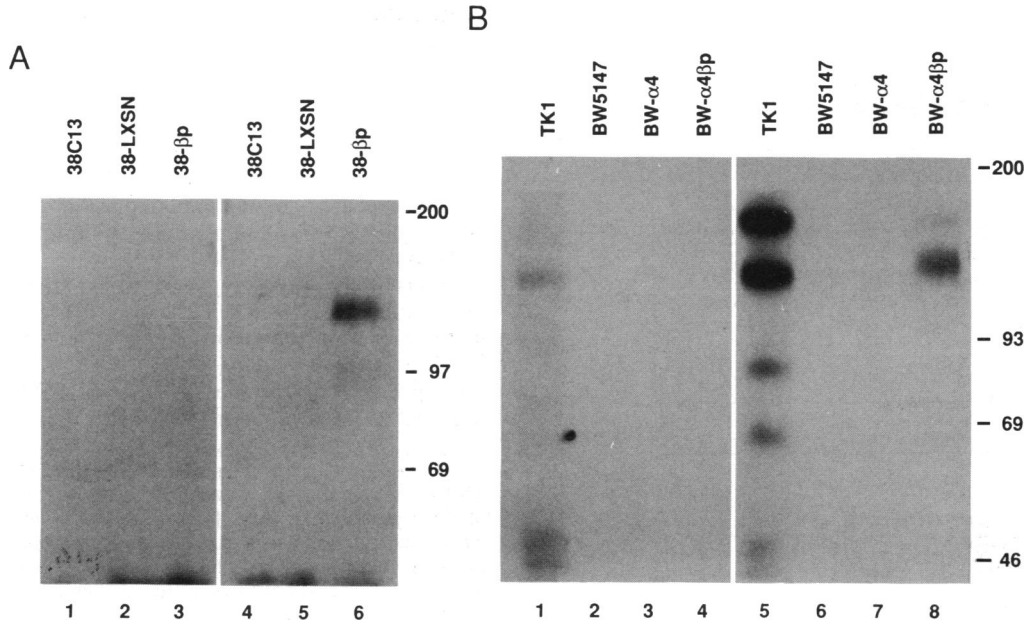

FIG. 4. Western blot analysis and immunoprecipitation of β_7. (*A*) β_7 immunoprecipitated from infected and control 38C13 cells. Lysates from the cells indicated were immunoprecipitated with R1-2, resolved by SDS/PAGE under reducing conditions, and probed with preimmune (lanes 1–3) or rabbit anti-β_7 antisera (lanes 4–6) followed by the ECL Western detection system (Amersham). (*B*) Immunoprecipitation of cell-surface-iodinated β_7 from cells as indicated with either preimmune (lanes 1–4) or rabbit anti-β_7 antisera (lanes 5–8), under reducing conditions.

well to PP HEVs and PLN HEVs (Fig. 6). Preincubation of normal MLN lymphocytes with anti-β_7 M301 reduced their binding to PP HEVs by 70% but did not alter their ability to bind to PLN HEVs, whereas MEL-14 specifically blocked their binding to PLN HEVs. Similarly, preincubation of these cells with mAb R1-2 reduced their binding to PP HEVs by 80%, in accordance with the previous data (7). Thus, anti-β_7 mAb specifically blocked binding of normal lymphocytes to PP HEVs but did not inhibit their binding to PLN HEVs.

All Known PP HEV-Binding Lymphomas Express β_7. We have shown (8) that TK50 was the only PP HEV-binding lymphoma that was thought to express $\alpha_4\beta_1$ but not $\alpha_4\beta_7$, as analyzed by coimmunoprecipitation with mAb R1-2 (8). $\alpha_4\beta_1$ was, therefore, proposed to be a possible receptor involved in binding to PP HEVs. However, $\alpha_4\beta_1$ is predominantly expressed on lymphomas that do not bind to PP HEVs, suggesting that $\alpha_4\beta_1$ alone is not sufficient to mediate this adhesive event. Flow cytometry analysis with anti-β_7 mAb showed that TK50 also expressed β_7 (data not shown), indicating that all known PP HEV-binding lymphomas express β_7. It is not known, however, why β_7 was not detected in TK50 by immunoprecipitation with mAb R1-2 (8).

DISCUSSION

Our results establish the complete sequence and a major function of murine integrin β_7. The expression data demon-

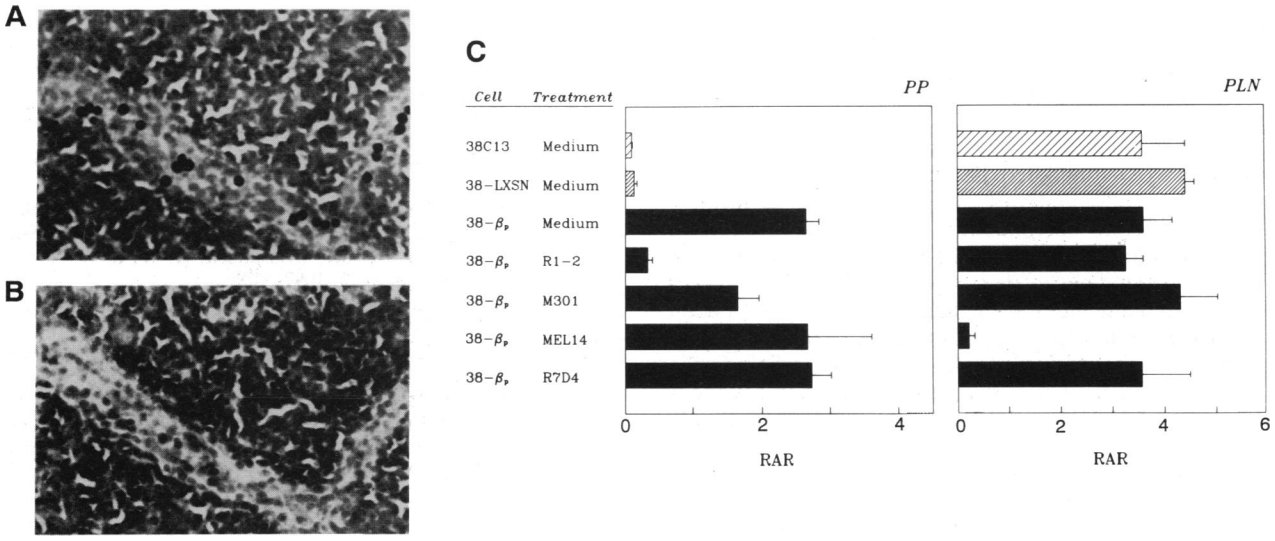

FIG. 5. Adhesion of 38-βp cells to PP HEVs. Cells were incubated on frozen sections of a mouse PP (7) and cells bound to HEVs were fixed to the section. (*A*) 38-βp cells are the round dark circles lying above the plane of tissue. (×630.) The HEV is delineated by a distinct poorly stained basement membrane with toluidine blue. (*B*) No 38C13 cells are bound to PP HEVs. (*C*) Cells were incubated with the indicated mAbs, washed, and mixed with an internal standard population of fluorescein isothiocyanate-labeled MLN lymphocytes. Cell suspensions were incubated on frozen sections of PP or PLNs.

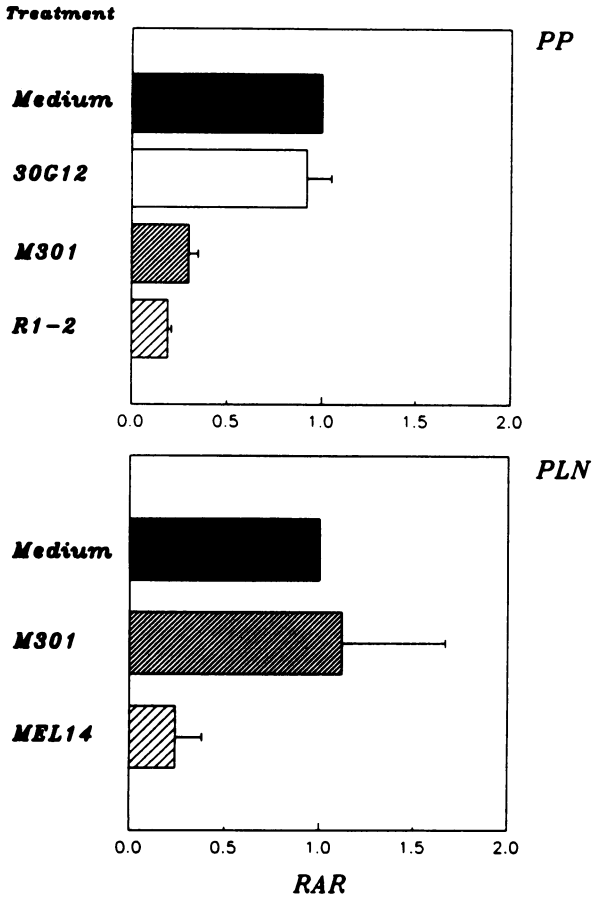

FIG. 6. Adhesion of normal lymphocytes to PP HEVs. Normal lymphocytes isolated from mouse MLNs were incubated with medium alone or the indicated mAbs, washed, mixed with fluorescein isothiocyanate-labeled MLN lymphocytes, and incubated on frozen sections of PP or PLNs. mAb 30G12 was a control antibody.

strate that β_7 is capable, along with α_4, of mediating organ-specific adhesion to PP HEVs. This β_7-mediated organ-specific binding can be blocked specifically by anti-β_7 mAb. The TK50 lymphoma also expresses β_7, indicating that all PP HEV-binding lymphoma cells tested express β_7. Moreover, anti-β_7 mAb specifically blocks binding of normal lymphocytes to PP HEVs but does not inhibit their binding to PLN HEVs. Thus, β_7 is a unique component of a PP-specific homing receptor.

It has been shown recently that phorbol 12-myristate 13-acetate (PMA)-stimulated TK1 cells can bind to the CS-1 region of fibronectin and vascular cell adhesion molecule 1 (VCAM-1), whereas unstimulated TK1 cells do not bind to either fibronectin or VCAM-1 (21). We proposed that the binding of unstimulated $\alpha_4\beta_7$ receptors to PP HEVs is very different from binding of PMA-stimulated $\alpha_4\beta_7$ to fibronectin and VCAM-1. First of all, $\alpha_4\beta_7$-positive TK1 cells can bind to PP HEVs constitutively without stimulation, whereas the same cells cannot bind to fibronectin and VCAM-1 without stimulation. Furthermore, PMA stimulation significantly decreases binding of normal lymphocytes to PP HEVs (22), whereas PMA stimulation markedly increases the binding of TK1 cells to fibronectin and VCAM-1, suggesting that PMA stimulation may alter the conformation of $\alpha_4\beta_7$ and/or the expression of other adhesion molecules. For instance, stimulation of T cells with PMA dramatically increased the expression of CD2 and CD44 (22, 23). More importantly, evidence against the role of $\alpha_4\beta_7$ in these interactions has been addressed with human cells, where it has been shown very recently that $\alpha_4\beta_7$ does not play a major role in binding to fibronectin or VCAM-1 when PMA-stimulated cells were used (24). In contrast, $\alpha_4\beta_1$ (VLA-4) can bind to fibronectin and VCAM-1 constitutively without stimulation, and its binding affinity to fibronectin and VCAM-1 can be rapidly augmented upon stimulation with PMA (24, 25). Thus, the binding specificity of unstimulated $\alpha_4\beta_7$ receptor to PP HEVs is distinct from the binding of PMA-stimulated $\alpha_4\beta_7$-positive cells to fibronectin and VCAM-1.

In this manuscript we have referred to the LPAM-1 β chain as β_p and then as β_7 when evidence was provided for β_7–β_p identity. Hereafter, we propose that only the β_7 nomenclature be used. The appellation LPAM-1 for $\alpha_4\beta_7$ remains appropriate, but the designation of LPAM-2 should be dropped in favor of its original name, VLA-4.

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1. Gowans, J. & Knight, E. (1964) *Phil. Trans. R. Soc. London Ser.* B **159**, 257–282.
2. Stamper, H. B., Jr. & Woodruff, J. J. (1976) *J. Exp. Med.* **144**, 828–833.
3. Streeter, P. R., Berg, E. L., Rouse, B. T. N., Bargatze, R. F. & Butcher, E. C. (1988) *Nature (London)* **331**, 41–46.
4. Streeter, P. R., Rouse, B. T. N. & Butcher, E. C. (1988) *J. Cell Biol.* **107**, 1853–1862.
5. Gallatin, M., St. John, T. P., Siegelman, M., Reichert, R., Butcher, E. C. & Weissman, I. L. (1986) *Cell* **44**, 673–680.
6. Jalkanen, S., Reichert, R. A., Gallatin, W. M., Bargatze, R. F., Weissman, I. L. & Butcher, E. C. (1986) *Immunol. Rev.* **91**, 39–60.
7. Holzmann, B., McIntyre, B. W. & Weissman, I. L. (1989) *Cell* **56**, 37–46.
8. Holzmann, B. & Weissman, I. L. (1989) *EMBO J.* **8**, 1735–1741.
9. Hynes, R. O. (1992) *Cell* **69**, 11–25.
10. Issekutz, T. B. (1991) *J. Immunol.* **147**, 4178–4184.
11. Chin, Y. H., Rasmussen, R. A., Woodruff, J. J. & Easton, T. G. (1986) *J. Immunol.* **136**, 2556–2561.
12. Neuhaus, H., Hu, M. C.-T., Hemler, M. E., Takada, Y., Holzmann, B. & Weissman, I. L. (1991) *J. Cell Biol.* **115**, 1149–1158.
13. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
14. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**, 980–990.
15. Markowitz, D., Goff, S. & Bank, A. (1988) *J. Virol.* **62**, 1120–1124.
16. Mizushima, S. & Nagata, S. (1990) *Nucleic Acids Res.* **18**, 5322.
17. von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4690.
18. Yuan, Q., Jiang, W.-M., Krissansen, G. W. & Watson, J. D. (1990) *Int. Immunol.* **2**, 1097–1108.
19. Erle, D. J., Rüegg, C., Sheppard, D. & Pytela, R. (1991) *J. Biol. Chem.* **266**, 11009–11016.
20. Kilshaw, P. J. & Murant, S. J. (1991) *Eur. J. Immunol.* **21**, 2591–2597.
21. Rüegg, C., Postigo, A. A., Sikorski, E. E., Butcher, E. C., Pytela, R. & Erle, D. J. (1992) *J. Cell Biol.* **117**, 179–189.
22. Bührer, C., Berlin, C., Jablonski-Westrich, D., Holzmann, B., Thiele, H.-G. & Hamann, A. (1992) *Scand. J. Immunol.* **35**, 107–120.
23. Geppert, T. D. & Lipsky, P. E. (1991) *J. Immunol.* **146**, 3298–3305.
24. Chan, B. M. C., Elices, M. J., Murphy, E. & Hemler, M. E. (1992) *J. Biol. Chem.* **267**, 8366–8370.
25. Shimizu, Y., Van Seventer, G. A., Horgan, K. J. & Shaw, S. (1990) *Nature (London)* **345**, 250–253.