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Immunologic and structural relatedness of the integrin $\beta_7$complex and the human intraepithelial lymphocyte antigen HML-1

Geoffrey W. Krissansen[1], Cristin G. Print[1], Ross L. Prestidge[1], Daniel Hollander[2], Qian Yuan[1], Wei-meng Jiang[1], David R. Jenkins[1], Euphemia Leung[1], Paul Mead[1], Rita Yong[1], Rohan V. Ameratunga[1], Nadine Cerf-Bensussan[3] and James D. Watson[1]

[1]*Department of Molecular Medicine, School of Medicine, University of Auckland, New Zealand,* [2]*Department of Medicine, University of California, Irvine, USA* and [3]*Group of Paediatric Immunology and Rheumatology, INSERM U132, Hopital des Enfants Malades, Paris, France*

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We recently cloned the newest human integrin β subunit, termed β_7, from a cDNA library constructed from SEA-activated T lymphocytes. In this communication, we report on the structure of the human integrin β_7 protein complex determined using a rabbit anti-β_7 peptide antibody raised to an N-terminal 22 amino acid residue sequence deduced from the human β_7 subunit cDNA. The β_7 subunit (M_r 116 000) expressed on PHA lymphoblasts associates with a single major α subunit (α_{II}) that is distinct from the prominent T cell marker, integrin α_4. The α_{II} subunit (M_r 180 000 nonreduced) displays a distinctive shift in size on reduction to an apparent M_r of 150 000. We show that these structural properties of the integrin β_7 complex are shared with the cell surface antigen HML-1 found highly expressed on T cells which populate the intestinal epithelium and are proposed to be involved in mucosal immunity. Sequential immunoprecipitation and Western blotting demonstrate identity or close homology between the $\alpha_{II}\beta_7$ and HML-1 proteins.

Integrin; β_7 subunit; HML-1; Protein structure

1. INTRODUCTION

T lymphocyte activation in response to immunologic challenge is accompanied by a rapid transition between adherent and nonadherent states (reviewed in [1]). This results from an array of activation-dependent adhesion mechanisms involving an ensemble of adhesion receptors displayed on resting T lymphocytes. There follows an increase in surface expression of adhesion molecules including CD2 (T11), CD44 (Hermes) and the integrins, lymphocyte function-associated antigen-1 (LFA-1), very late antigen (VLA)-4, -5, -6, during the subsequent differentiation process [1]. This change in adhesive character may account for the preferred localisation of memory cells to mucosal surfaces and distinct lymphoid microenvironments, and may enhance the sensitivity of memory cells to antigens [1].

We have described the cloning of a novel activation-dependent integrin β subunit with a presumed adhesive function, termed integrin β_7, from both human [2] and mouse lymphocytes [3]. The deduced N-terminal sequence of the mouse β_7 subunit was found to be identical to the N-terminus of the β subunit of the M290 antigen, a surface molecule found highly expressed on mouse intestinal intraepithelial lymphocytes (IEL), and on a majority of T cells in the gut lamina propria [3]. The M290 antigen which is composed of multiple subunits (100 000–170 000 kDa [4] seems superficially similar to other IEL surface differentiation markers identified in rat (100 000–200 000 kDa) and humans (105 000–150 000 kDa) by the RGL-1 [5] and HML-1 [6] mAbs. The curious constitutive expression of these markers on IEL in the small intestine and their de novo inducibility on peripheral blood T cells following cell activation has drawn several suggestions for their functions. The M290 antigen is thought to perform an adhesive function which regulates effector cell activity during IEL defence of the mouse gut mucosa [4]. It was speculated that this antigen might be involved with lymphocyte homing to epithelial surfaces, but it was considered more likely that it was induced locally by inflammatory cytokines when lymphocytes actually reached the gut [4]. It was postulated that HML-1 positive T lymphocytes in the intestinal lamina propria represent a specialised memory T cell subset since they express CD45RO, but only partially express the memory T cell marker CD29 [7]. HML-1 was considered unlikely to represent a homing receptor since all lymphocyte subsets in gut-

Abbreviations: BSA, bovine serum albumin; IEL, intraepithelial lymphocytes; LPAM-1, lymphocyte Peyer's patch adhesion molecule-1; PBL, peripheral blood lymphocyte; PHA, phytohaemagglutinin; VLA, very late antigen; VnR, vitronectin receptor.

Correspondence address: G.W. Krissansen, Department of Molecular Medicine, School of Medicine, University of Auckland, Auckland, New Zealand. Fax: (64) (9) 735 215.

associated lymphoid tissues use common migratory pathways, yet only 40% of lamina propria lymphocytes expressed HML-1, and there was no exclusive restriction in expression between T cell types [7]. Finally, HML-1 and RGL-1 may define differentiation antigens expressed on a population of non-B lymphocytes belonging to a common-mucosae associated lymphoid system [6].

The similar structures, cellular distributions, regulatory properties and putative functions for the RGL-1, HML-1, and β_7-related M290 antigens indicate that these 3 complexes are probably species homologues, but this has not been formally proven since the various antibodies reacting with these molecules do not react across species. Here we report for the first time the characterisation of the human integrin β_7 protein complex expressed on phytohaemagglutinin (PHA) lymphoblasts and compare its structure and immunochemistry with that of the human IEL antigen, HML-1.

2. MATERIALS AND METHODS

2.1. *Synthetic peptide*

The β_7 synthetic peptide (amino acid sequence DAKIPSTGDATEWRNPHLSMLGCG) corresponding to N-terminal amino acid residues 5–26 deduced from the human β_7 cDNA [2] was kindly synthesized by the Immunex Corp., Seattle, USA. The peptide sequence was chosen on the basis of its predicted immunogenicity [8] and because it forms part of an aminoterminal extension that is unique to the β_7 subunit [2]. An anchoring cysteine residue and a glycine spacer were incorporated at the C-terminus. The peptide was coupled to porcine thyroglobulin using *m*-maleimidoenzoyl *N*-hydroxysuccinimide ester (MBS) at a ratio of approximately 100 molecules of peptide to 1 molecule of carrier.

2.2. *Antibodies*

Antiserum was raised in a New Zealand White rabbit by priming with a mixture of 150 μg of peptide coupled to thyroglobulin and 500 μg of free peptide emulsified in Freund's complete adjuvant, and by subsequent injections using incomplete Freund's. Antibody was purified by affinity chromatography on Protein A-Sepharose and coupled directly to Sepharose CL-4B beads (4 mg antibody per ml beads) by the method of Kumel et al. [9]. The control anti-human VLA-4 mAb (B-5G10) [10] was a kind gift from Dr M. Hemler (Dana-Farber Cancer Institute, Boston, MA). Mouse anti-human vitronectin receptor (VnR) mAb (13C2) [11] was generously provided by Dr M. Horton (Haemopoiesis Research Group, Imperial Cancer Research Fund, London, UK). Some of the HML-1 mAb used for immunoprecipitation was purchased from Immunotech (distributed by Haem Pty Ltd, Victoria, Australia). Control antibodies were similarly coupled to Sepharose CL-4B.

2.3. *Preparation of PHA lymphoblasts*

PBL were isolated from fresh heparinized venous blood of healthy donors by centrifugation on Ficoll-Hypaque density gradients (Pharmacia). Cells were washed in RPMI 1640 to reduce platelet contamination, and cultured for 3 days in RPMI 1640 medium containing penicillin (50 U/ml), streptomycin (50 μg/ml), 7.5% (v/v) foetal calf serum (Gibco BRL, New Zealand) and 5 μg/ml PHA at 37°C in a 6% CO_2 incubator.

2.4. *Surface iodination and immunoprecipitation*

PHA lymphoblasts were labelled at the cell surface by lactoperoxidase-catalyzed iodination essentially as described previously [12] and solubilised for 45 min in 1% (v/v) Nonidet P-40 in 10 mM Tris-HCl buffer, pH 7.4, 150 mM sodium chloride containing 20 mM iodoacetamide, 2 mM phenylmethylsulphonyl fluoride, and 1 mg/ml bovine serum albumin. Immunoprecipitation was carried out using antibody-Sepharose matrices and analyzed by SDS-PAGE on 7.5% slab gels as described [13]. Labelled proteins were detected by autoradiography at −70°C using Kodak XAR-5 film with intensifying screens (Cronex Lightning Plus). Apparent molecular weights were calculated by reference to the mobilities of the following proteins: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (94 kDa) and bovine serum albumin (67 kDa).

2.5. *Immunoblotting*

Immunoprecipitates were electrophoresed by SDS-PAGE on 7.5% slab gels and proteins transferred electrophoretically for 18 h at 30 V in a Tris-glycine-methanol buffer [14] to nitrocellulose (Schleicher and Schuell). Filters were blocked overnight at 4°C with 2% (w/v) bovine serum albumin (BSA), 1% dried skim-milk powder, 0.05% Tween 20 and 0.02% sodium azide in phosphate buffered saline (PBS). Immunoreactive proteins were revealed by incubating the filter overnight at 4°C with anti-β_7-peptide rabbit antibody (32 μg/ml) in PBS containing 2% (w/v) BSA and 0.02% sodium azide. After washing 3-times in PBS, 0.05% Tween 20, the filter was incubated for 1 h with a 1:100 dilution of biotinylated goat anti-rabbit IgG (Vector Laboratories Inc.) and washed as above. The filter was finally incubated for 1 h with avidin-biotinylated horse-radish peroxidase conjugate (Vector laboratories) and after washing was visualised with chloronaphthol (0.3 μg/ml) and hydrogen peroxide (0.03%) in 50 mM Tris-HCl, pH 7.6.

3. RESULTS AND DISCUSSION

The anti-β_7-peptide rabbit antibody immunoprecipitated from surface-labelled PHA lymphoblasts a protein complex with characteristics that typify the $\alpha\beta$ heterodimeric integrin receptors (Fig. 1). Noncovalently associated high-molecular-weight components of 180 and 116 kDa, here designated the α_H and β_7 subunits respectively, were resolved under nonreducing conditions. In addition, two minor 170 and 150 kDa bands were consistently seen. When reduced, the α_H subunit shifted to 150 kDa, whereas there was negligible change in mobility of the β_7 subunit. The shift of the α_H subunit on reduction is typical of many of the integrin α subunits which are composed of disulphide-linked heavy and light chains [15]. Despite the dramatic shift, no labelled α light chain was resolved on a higher percentage acrylamide gel, suggesting that the light chain may be primarily intracellular and not labelled (data not shown). In some experiments the α_H subunit exhibited a minor breakdown product at 95 kDa, and the β_7 subunit a product at 80 kDa (data not shown).

As mentioned above, the mouse integrin β_7 subunit shares N-terminal amino acid sequence identity with the β subunit of the M290 IEL antigen, which in turn bears a gross structural similarity to a human IEL antigen, termed HML-1. The HML-1 antigen was immunoprecipitated to directly compare its structural relationship with the $\alpha_H\beta_7$ protein complex. The pattern of bands obtained under both nonreducing and reducing conditions was identical to that observed with the anti-β_7-peptide antibody (Fig. 1). The distinctive pattern of the nonreduced α subunits displaying the major 180 kDa band and minor bands at 170 and 150 kDa which may

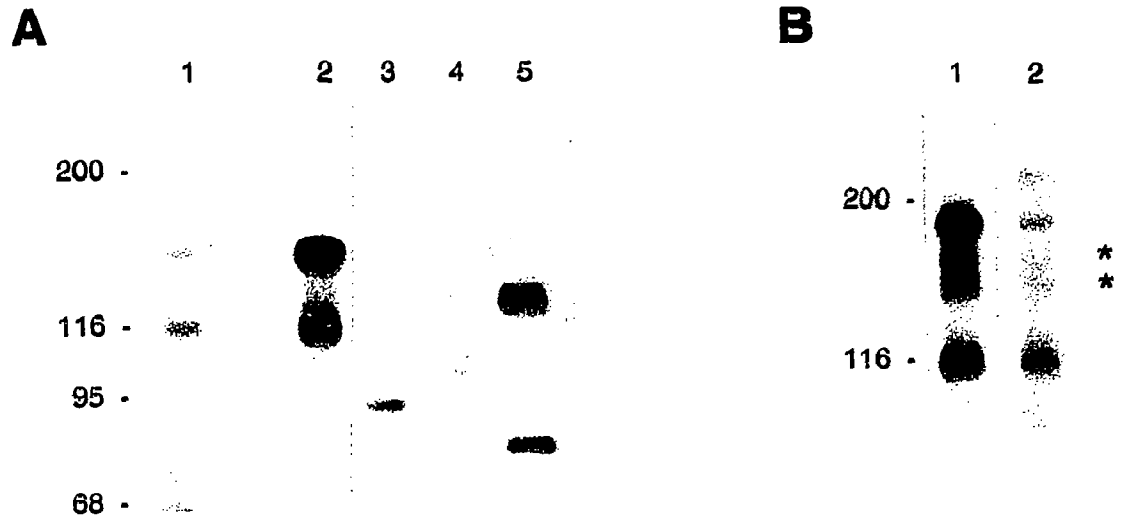

Fig. 1. Comparison of the β_7 and HML-1 protein complexes by SDS-PAGE analysis. Cell lysates of ^{125}I-labelled PHA lymphoblasts were immunoprecipitated with (A) anti-β_7-peptide antibody (lane 1); HML-1 mAb (lane 2); normal rabbit immunoglobulin negative control (lane 3); anti-VnRα (lane 4); anti-VLAα_4 (lane 5); and (B) HML-1 mAb (lane 1); anti-β_7-peptide antibody (lane 2). Immunoprecipitates were analysed on 7.5% SDS-polyacrylamide gels under reducing (A) and non-reducing (B) conditions. The relevant portion of the nonreducing gel (B) has been shown to highlight the distinctive pattern of bands shared between the β_7 and HML-1 protein complexes. The minor α-related bands are indicated by asterisks. The band >200 kDa (Fig. 1B, lane 2) was sometimes observed in immunoprecipitates with normal rabbit immunoglobulin (data not shown). Molecular weights are shown in the left hand margins in kDa.

represent partially reduced forms is evidence that the HML-1 and β_7-associated α chains are very similar or identical. Completely different profiles of radiolabelled proteins were precipitated with control antibodies. Anti-VnRα gave a β_3 chain at 95 kDa and an α_v chain at 130 kDa. The anti-VLA α_4 profile was also different giving the reported 145 (α_4) (faint), 130 (β_1) and 80 kDa (α subunit breakdown product) bands [10]. In the mouse VLAα_4 associates with the alternative β_1 and β_p subunits [16,17]. The $\alpha_4\beta_p$ lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1) complex is present on the surface of certain T lymphocytes and has a role in lymphocyte homing to Peyer's patches located in the terminal ileum of the small intestine [17]. LPAM-1 has so far been detected on certain high endothelial venule (HEV)-binding T cell lymohoma lines, mesenteric lymph node lymphocytes and most normal lymphocytes [16,17]. Since the 'integrin β_7/M290' antigen is constitutively expressed in the small intestine it is not unreasonable to conclude that the β_7 subunit is β_p. PHA lymphoblasts expressed moderate levels of the VLA α_4 and β_7 subunits, yet no α_4 subunit bands were detectable in anti-β_7-peptide precipitates, and vice versa the β_7 subunit band was not detected in VLA α_4 precipitates. We conclude

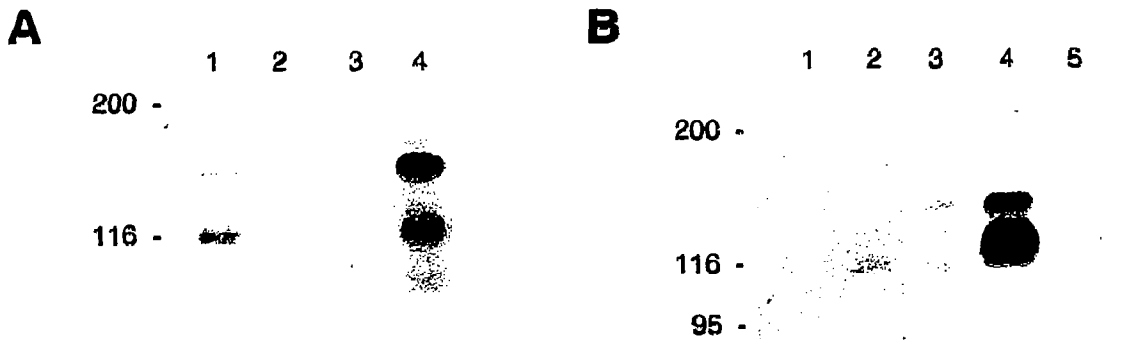

Fig. 2. Immunologic relationship of the β_7 and HML-1 protein complexes. (A) Sequential immunoprecipitation study. PHA lymphoblasts were radioiodinated, lysed, and immunoprecipitated 2 successive times with the HML-1 mAb (lanes 1 and 2) and then tested with the anti-β_7-peptide antibody (lane 3). The original lysate was also immunoprecipitated with the anti-β_7-peptide antibody (lane 4). Immunoprecipitates were analysed on 7.5% SDS-polyacrylamide gels under reducing conditions. The relevant portion of the autoradiogram is illustrated. Variations in the proportions of the respective α and β subunits recovered as visualised in this experiment were often observed. Molecular weights are shown in the left margin in kDa. (B) Immunoblotting analysis of the HML-1 β subunit. Immunoprecipitated HML-1 (lanes 2 and 3) or VLA $\alpha_4\beta_1$ (lanes 4 and 5) from radioiodiniated PHA lymphoblasts were subjected to reducing SDS-PAGE and transferred to nitrocellulose. The transferred proteins were probed with the anti-β_7-peptide antibody (lanes 2 and 5) or subjected to autoradiography (lanes 3 and 4). The molecular weights of marker proteins developed with India ink are shown in the left margin in kDa. Lane 1 is a negative control of the HML-1 mAb preparation probed with the anti-β_7-peptide antibody.

that the β_7 and β_p subunits are either distinct protein entities, or that the association of VLAα_4 with β_7 in PHA lymphoblasts is very weak and easily disrupted. A third possibility is that β_7 (β_p) can associate with either αHML-1 or α_4, but that association with αHML-1 is preferred because the resulting complex may be inherently more stable.

To further verify that the β_7 subunit and HML-1 antibodies were precipitating the same antigen, sequential immunoprecipitation experiments were carried out. Preclearing the lysate with the HML-1 antibody removed the labelled proteins reactive with the β_7-peptide antibody (Fig. 2).

The experiments described above strongly indicate that the HML-1 and β_7-associated α chains are structurally related or identical. To explore an immunological relationship between α_7 and the HML-1 β subunit, we used immunoblotting. The anti-β_7-peptide antibody reacted with the 116-kDa β subunit band contained in an HML-1 immunoprecipitate resolved by SDS-PAGE (Fig. 3). A sharp band at 100 kDa was also visualized and may represent an intracellular β subunit precursor form since this band was not ^{125}I-labelled. Notably, none of the VLA$_4$ bands were visualised with the anti-β_7 antibody. Thus, the β subunits comprising HML-1 and $\alpha_H\beta_7$ are immunologically related.

In summary, an antibody raised against a peptide sequence deduced from the human β_7 cDNA clone has facilitated the identification of the encoded protein product. Taken together, the immunological and structural relatedness of the $\alpha_H\beta_7$ and HML-1 components indicates that the respective protein complexes are probably identical. The observed relationships of both the mouse M290 and human HML-1 antigens to the integrin $\alpha_H\beta_7$ complex is in accord with the suggestion that the M290 and HML-1 IEL antigens are species homologues. The 'HML-1/$\alpha_H\beta_7$' receptor is the only integrin to be rapidly synthesized de novo on activation of resting lymphocytes reaching half maximal levels within 3 days. Thus, this receptor may perform a functional role on antigen-specific peripheral blood lymphocytes which arrive at a site of inflammation within hours following activation. Now that the M290 and HML-1 antigens have been shown to be closely similar or identical to the $\alpha_H\beta_7$ integrin, it will be possible to devise specific experiments based on our knowledge of integrin function to explore the role of these activation antigens in the gut and their involvement in inflammatin.

NOTE ADDED IN PROOF

After this communication was submitted for publication, Kilshaw and Murant [18] reported that in the mouse β_7 was present on most lymph node lymphocytes in association with α_4, rather than αM290. Stimulation of mouse lymph node T cells with a combination of anti-CD3 and TGF-β induced the expression of αM290 which preferentially associated with the β_7 subunit. Chelation of divalent cations had little effect on the stability of αM290 β_7, whereas the $\alpha_4\beta_7$ complex was completely dissociated, suggesting that the former complex may be thermodynamically more stable. The results of our own study which suggests preferential association of αHML-1 and β_7 in human lymphoblasts are in accord with the above findings. Kilshaw and Murant [18] refer to unpublished work by K. Micklem et al. which independently confirms our contention that the HML-1β subunit and β_7 are identical. This work provides evidence that the N-termini of β_7 and HML-1β are identical.

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