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Cloning, primary structure and properties of a novel human integrin β subunit

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The originally described integrin β subunits that define the three subfamilies of integrin heterodimers are β_1, β_2 and β_3. In this paper, we describe the isolation of a cDNA coding for a novel human integrin β subunit, designated as β_5. The β_5 cDNA was isolated from a human thymic epithelial cell library, using oligonucleotide probes that were designed from a region highly conserved among the known β_1, β_2 and β_3 sequences. The β_5 cDNA codes for 799 (or 796) amino acids, including a 23 amino acid leader sequence. There are 776 (or 773) amino acids in the mature protein, which includes a long extracellular domain of 696 amino acids, a transmembrane domain and an intracellular C-terminal domain of 57 amino acids. The β_5 sequence resembled the known β_3, β_1 and β_2 sequences by 55, 43 and 38%, respectively, including conservation of 56/56 cysteines. Rabbit antiserum was prepared against a 20 amino acid synthetic peptide predicted from the β_5 C-terminal sequence. This serum immunoprecipitated a β_5 protein that was 100 000 M_r (reduced) and 95 000 M_r (non-reduced). Only a single α subunit was detected in association with β_5, and that α subunit was immunochemically indistinguishable from the α^V subunit previously found as part of the vitronectin receptor complex. By immunoprecipitation, β_5 was most prevalent on carcinoma cell lines, was also present on hepatoma and fibroblast cell lines, and was absent from lymphoblastoid cells and platelets.

Key words: integrin/cell adhesion/vitronectin receptor/cDNA sequence

Introduction

The integrin family consists of at least 14 distinct cell surface heterodimers that are involved in cell−cell and cell−extracellular matrix adhesion functions (Ginsberg *et al.*, 1988; Kishimoto *et al.*, 1989b; Ruoslahti, 1988; Hemler, 1990). The integrins were originally divided into three subfamilies, each having a characteristic β chain that associates with multiple α subunits (Hynes, 1987). The VLA protein family (sharing a common β_1 subunit) has at least six members, including multiple receptors for collagen, laminin and fibronectin (Wayner *et al.*, 1988; Hemler, 1990); the LEUCAM family (sharing a common β_2 subunit) has three members involved in leukocyte cell−cell adhesion (Kishimoto *et al.*, 1989b); and the Cytoadhesin family (sharing the β_3 subunit) consists of the platelet IIb−IIIa complex and the vitronectin receptor, which adhere to a variety of extracellular matrix proteins (Ginsberg *et al.*, 1988).

Recently, the discovery of additional β subunits has added to the complexity within the integrin family. For example, the α^6 subunit associates not only with β_1, but also with a newly described β_4 subunit (Hemler *et al.*, 1989; Kajiji *et al.*, 1989). Compared to $\alpha^6\beta_1$, which has widespread distribution, $\alpha^6\beta_4$ is prevalent on normal neoplastic epithelial cell types (Hemler *et al.*, 1989) and aligns with basement membranes in many tissues (Kajiji *et al.*, 1987). Similarly, on a subset of mouse lymphocytes, the mouse α^4 subunit associated with β_P instead of β_1, and the $\alpha^4\beta_P$ complex functions as a receptor for high endothelial venules in Peyer's patches (Holzmann *et al.*, 1989; Holzmann and Weissman, 1989). While the $\alpha^4\beta_1$ (VLA-4) complex might also bind to Peyer's patches (Holzmann and Weissman, 1989), it has multiple other adhesive functions (Elices *et al.*, 1990). In another example, the α^V subunit of the vitronectin receptor was reported to associate with two additional β subunits besides β_3. A complex called $\alpha^V\beta_X$ was identified from lung carcinoma cells, using an anti-α^V monoclonal antibody (mAb) (Cheresh *et al.*, 1989). The β_X subunit was chemically distinguishable from the β_3 subunit which is usually associated with α^V on endothelial cells and other cell types (Cheresh *et al.*, 1989).On MG63 osteosarcoma cells and fibroblasts, a subunit called β_S was found to be associated with α^V, and the β_S subunit underwent a marked serine phosphorylation upon treatment of MG63 cells with a tumor promoter PMA (Freed *et al.*, 1989). Although β_S is antigenically and biochemically distinct from β_3, it is not yet certain that it is distinct from β_X. One problem has been that neither cDNA probes nor antibody reagents have been available for use in direct characterization of β_X (or β_S).

At this time the β_P, β_4, β_X (and β_S) subunits each are known to associate with only one known α subunit, and thus do not appear to define new integrin families. Instead, they are best regarded as alternative β subunits, replacing the prototype β_1 and β_3 heterodimers on certain cell types.

Among the β subunits, genes for human β_1 (Argraves *et al.*, 1987), β_2 (Kishimito *et al.*, 1987; Law *et al.*, 1987) and β_3 (Fitzgerald *et al.*, 1987; Rosa *et al.*, 1988) have been cloned and sequenced, and show 44−47% homology to each other, with complete conservation of all of their 56 cysteines. Also, the β_1 subunits from human (Argraves *et al.*, 1987), mouse (Tominaga, 1988; Holers *et al.*, 1989), chicken (Tamkun *et al.*, 1986) and frog (DeSimone and Hynes, 1988) show 82−90% homology, emphasizing the importance of this molecule throughout vertebrate evolution.

From their primary structures, it is evident that each of the integrin β subunits is a transmembrane protein, with a large extracellular domain and a short cytoplasmic tail. Within the extracellular domain, the region near amino acids 100−140 has a particularly high degree of conservation, and notably, RGD-peptide crosslinking studies have implicated that same region as a potential ligand binding site (D'Souza

et al., 1988; Smith and Cheresh, 1988).

The cytoplasmic domain of β_1 has been shown to bind to the cytoskeletal protein talin (Horwitz *et al.*, 1987) and a newly described protein called fibulin (Argraves *et al.*, 1989). These cytoskeletal interactions may be critical for the function of integrins as transmembrane receptors, linking extracellular matrix ligands with the cytoskeletal framework. The cytoplasmic domains of the known β subunits are highly dissimilar, suggesting that each interacts with the cytoskeleton in a specific manner.

In this study, oligonucleotide probes were designed from a region highly conserved among known β subunits, and have been used to isolate a cDNA clone encoding a new human β subunit which we designate as β_5. In addition, the β_5 protein, its associated subunit and its cell distribution have been characterized.

Results

Strategy for isolating cDNA clones for a new β subunit

Two oligonucleotides, a 32-fold degenerate 23mer and a 16-fold degenerate 21mer, were synthesized based on β_1, β_2 and β_3 subunit sequences from a highly conserved region (Figure 1). These oligonucleotides were then used as probes for screening a λgt11 cDNA library made from thymic epithelial cells. Phage clones that hybridized with both oligonucleotides, but not with a partial β_1 cDNA probe, were selected and further purified. Initial screening of 1.8×10^5 λgt11 recombinant phage plaques yielded six clones. Partial sequencing revealed that four of these clones had sequences identical to the integrin β_2 subunit, and one of them was identical to the N-terminal portion of the integrin β_1 sequence. A single clone of 3.0 kb size, designated clone 9.2, was found to be distinct from β_1, β_2 and β_3. As shown in Figure 1, this new clone matched oligonucleotide probe 1 in 23/23 positions and matched probe 2 in 18/21 positions.

Northern blotting

To obtain information regarding the message corresponding to clone 9.2, RNA from PEER (a T lymphoblastoid cell line), CCL 228 (a colon carcinoma cell line) and thymocytes was analyzed in a hybridization experiment (Figure 2). A single band of ~3.5 kb was observed in the lane containing RNA from CCL 228 cells, but was absent from thymocytes and PEER cells. Probing of the same blot with a control probe revealed that similar amounts of RNA were present in all three lanes (not shown). The results in Figure 2 confirmed that clone 9.2 was distinct from β_1 and β_2 for which messages are present in PEER cells and thymocytes, and also showed that clone 9.2 was distinct from β_4 which hybridizes with a 6–7 kb message from CCL 228 cells (C.Crouse and M.Hemler, unpublished).

Human β_5 cDNA sequence

Because the new β clone differed in sequence and/or message size from β_1, β_2, β_3 or β_4, it was designated β_5. The β_5 clone 9.2 appeared to be incomplete, so it was used as a probe for further screening of the λgt11 thymic epithelial library. Eight additional β_5 clones were obtained, including one which spanned the entire β_5 coding region (Figure 3). The complete β_5 sequence was determined in both directions from these clones, with the help of 13 oligonucleotide primers (15–17 bp each), synthesized at ~200 bp intervals. The complete sequence of the coding region, as well as 3′ and 5′ untranslated regions, is presented in Figure 4.

The GLNICT sequence in clone 5.1 is likely to be the N-terminus of the β_5 protein because it closely matched the GPNICT sequence at the N-terminus of β_3. Consistent with this assumption, direct N-terminal amino acid sequencing yielded a XLNICT sequence for β_X (D.Cheresh, personal

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BETA1    GACCTCTACTACCTTATGGACCTGTCTTATTCAATG
BETA2    GACCTGTACTATCTGATGGACCTCTCCTACTCCATG
BETA3    GACATCTACTACTTGATGGACCTGTCTTACTCCATG
BETA5    GACCTGTACTACCTGATGGACCTCTCCCTGTCCATG

OLIGO #1 GACCTCTACTACCTGATGGACCT   (23-mer, 32-fold degenerate)
           A G     TT T

OLIGO #2                ATGGACCTGTCTTACTCCATG  (21-mer, 16-fold degenerate)
                              C  C  T  A
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Fig. 1. Design of oligonucleotide probes used for selecting a new integrin β subunit. Two overlapping oligonucleotides were made from the cDNA region most highly conserved among β_1, β_2 and β_3 subunits, which is the region coding for the amino acid sequence DLYYLMDLSYSM.

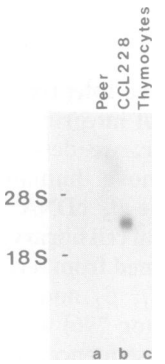

Fig. 2. Northern blot analysis of β_5 message. Total RNA from the leukemic cell line PEER, the colon carcinoma cell line CCL 228 and from normal human thymocytes (10 μg/lane) was separated by electrophoresis under denaturing conditions, and probed with a 1.8 kb *Sph*I fragment of cDNA derived from β_5 clone 9.2

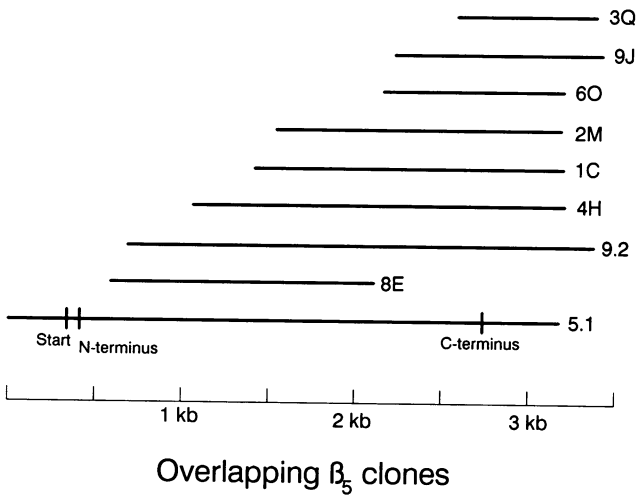

Fig. 3. Alignment of overlapping β_5 cDNA clones. The nine clones indicated have essentially the same sequences, except that the polyadenylation site observed in clone 60 is replaced by ~200 bp of unknown sequence in clones 9.2, 9J and 3Q. Other variations between clones are listed in Table II.

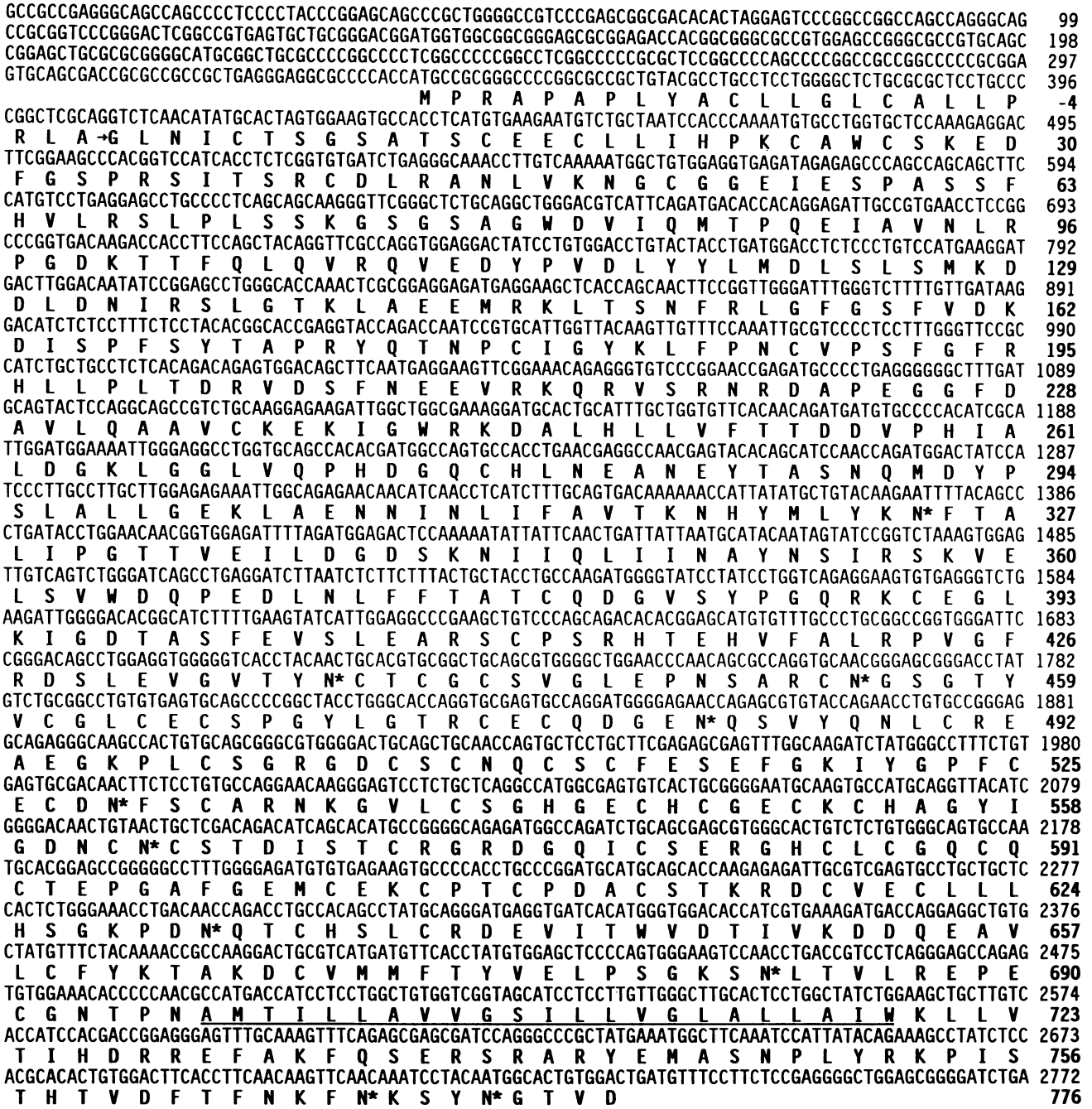

Fig. 4. Complete nucleotide sequence and the deduced amino acid sequence of human β_5 cDNA. The sequence is taken from clone 5.1. The putative N-terminus is indicated with an arrow. The transmembrane domain is underlined and the potential *N*-glycosylation sites are marked with *. The signal for a poly(A) tail is underlined. Although not found in clone 5.1, a poly(A) tail was found in clone 60, ~20 bp after the poly(A) signal.

communication), which may be related to or identical to β_5 (see below). The 5′ untranslated region in clone 5.1 (337 nucleotides) ends with a consensus CCACC sequence characteristic of a translational start site (Kozak, 1987). Following the predicted methionine initiation codon, there is an open reading frame of 2397 nucleotides corresponding to 799 amino acids. The 23 amino acids preceding the N-terminus are rich in hydrophobic residues as expected for a signal sequence (von Heijne, 1984), and are followed by 776/773 amino acids making up the mature protein. Another region of 23 hydrophobic amino acid residues (amino acids 697−719) corresponds to the transmembrane region. Between amino acids 438−592, β_5 contains four cysteine-rich motifs, each with eight cysteines in the pattern of

CxCyyyyCyyyyCyyyyCxCxxCxC, where x represents one amino acid and yyyy represents a stretch of 7–13 amino acids. CxCxxCxC forms the core of the motif, although the first core cysteine residue is absent in the first repeat. The β_5 sequence reveals eight potential *N*-glycosylation sites (Asn–X–Ser/Thr), not counting two such sites in the cytoplasmic domain of the molecule. At least six of these glycosylation sites (averaged 2500 M_r) may be utilized because the 86 000 M_r predicted size of β_5 (derived from the amino acid sequence of the mature protein) is ~15 000 M_r less than the 100 000 M_r size estimated by SDS–PAGE (see Figure 6 below).

Table I. Amino acid variations among β_5 clones

Clone designation	Amino acid positions			
	336–338	379–381	708	767–769 (FNK)
3Q	–	–	**G**	present
9J	–	–	I	absent
60	–	–	I	absent
2M	–	**K D E**	I	absent
1C	**G I R**	Q D G	I	absent
4H	I L D	Q D G	I	present
9.2	I L D	Q D G	I	absent
8E	I L D	Q D G	–	–
5.1	I L D	Q D G	I	present

Sequences differing from the prototype clone (5.1) are represented in bold letters. Dashes indicate sequences not determined because the clone does not span the corresponding region.

Clonal heterogeneity

Sequencing information from the eight available clones indicated that there were four sites where one or more of the clones differed from each other. These changes each involved multiples of three nucleotides thus resulting in changes in amino acid sequences (Table I). For example, at position 336–338, clone 1C expressed a Gly–Ile–Arg sequence, whereas four others had an Ile–Leu–Asp. Similarly, at position 379–381, five different clones expressed Gln–Asp–Gly, whereas clone 2M expressed Lys–Asp–Glu, and at position 708, all clones expressed an isoleucine, except clone 3Q, which had a glycine. In the cytoplasmic domain, immediately after the Phe–Asn–Lys sequence at position 764–766, a second Phe–Asn–Lys sequence was present in clones 3Q, 4H and 5.1 (amino acids 767–769), but not in five other clones. Thus far, clones 4H, 9.2, and 5.1 differ only in their Phe–Asn–Lys sequences, and otherwise appear to define prototype β_5 clones.

Comparison with other human β subunits

When compared with the other integrin β subunits that have been sequenced (Figure 5), β_5 was most related to β_3 (54.9% identity), and less similar to β_1 (43.1%) or β_2 (37.6%). All 56 cysteines present in the coding region of β_5 are conserved among each of the other β subunits, including the cysteines that make up the four repeated motifs in the cysteine-rich domain. In the cytoplasmic domain of β_5, which is 10 amino acids longer than the other β subunit cytoplasmic domains (Figure 5), there are two sites potentially meeting the requirements for tyrosine kinase

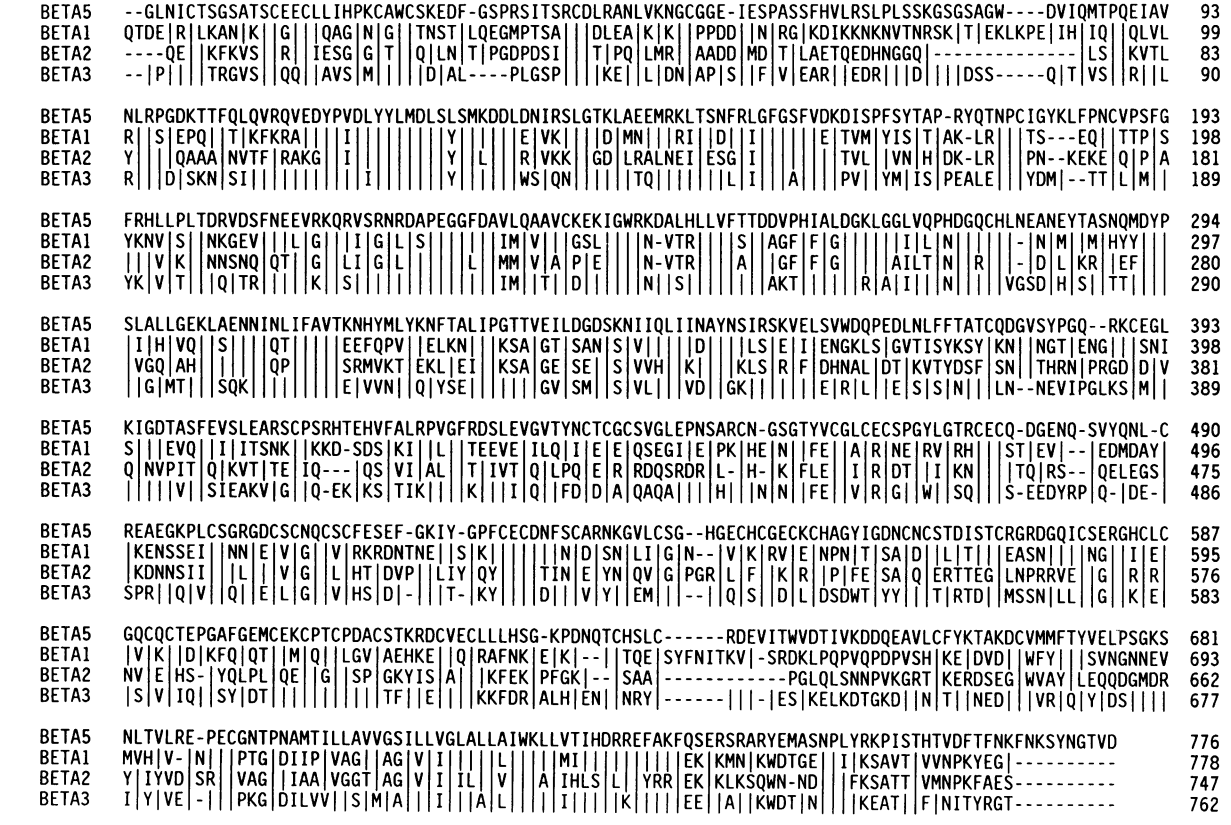

Fig. 5. Alignment of β_5 and other human integrin β subunits. The protein sequences of β_1 (Argraves *et al.*, 1987), β_2 (Kishimoto *et al.*, 1987; Law *et al.*, 1987) and β_3 (Fitzgerald *et al.*, 1987; Rosa *et al.*, 1988) are compared with that of β_5. The shared amino acids are indicated by vertical lines in the β_1, β_2 and β_3 sequences. Gaps (–) are introduced to maximize alignment.

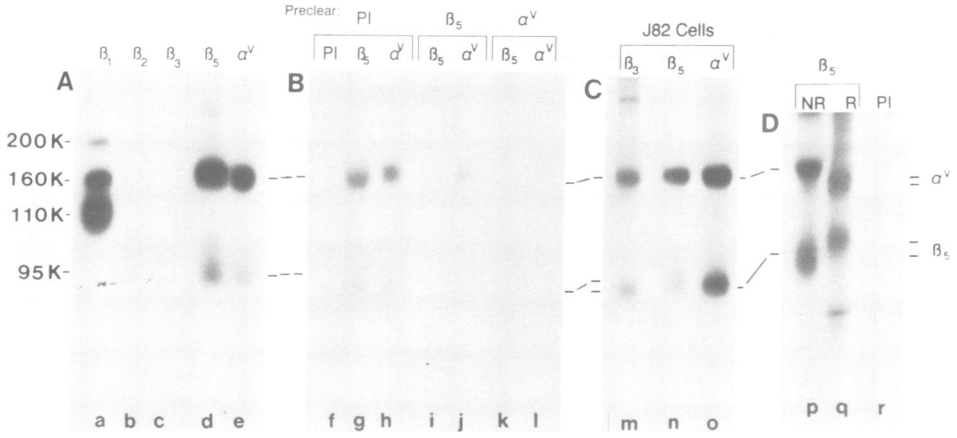

Fig. 6. Characterization of the β_5 protein and its associated α subunit. **(A)** ^{125}I-surface labeled CCL 228 cell extract was immunoprecipitated with monoclonal antibodies A-1A5 (lane a), TS1/18 (lane b), mAb 15 (lane c), rabbit anti-β_5 C-terminal peptide (lane d) and LM142 (lane e). **(B)** The CCL 228 extract was depleted of β_5 by pre-clearing with anti-β_5 C-peptide antiserum and then precipitated with the anti-β_5 antiserum (lane i) or with mAb LM142 (lane j). LM142 was also used for immunodepletion of α^V subunit from CCL 228 extract, followed by immunoprecipitation with anti-β_5 C-peptide antiserum (lane k) or with LM142 (lane l). Control pre-clearing was performed using pre-immune rabbit serum followed by precipitation with the same pre-immune serum (lane f), anti-β_5 C-peptide (lane g) or LM142 (lane h). **(C)** ^{125}I-surface-labeled J82 cell extract was immunoprecipitated with mAb 15 (lane m), anti-β_5 C-peptide antiserum (lane n) or LM142 (lane o). **(D)** The mobility of $\beta_5\alpha^V$ was determined by SDS−PAGE under non-reducing (lane p) and reducing (lane q) conditions. Control precipitation was done with rabbit pre-immune serum (lane r).

phosphorylation sites (Hunter and Cooper, 1985). The site near the tyrosine at position 751 is conserved in the β_1 and β_3 sequences, and resembles the EGF receptor tyrosine kinase site as noted previously (Tamkun *et al.*, 1986). Notably, the tyrosine at position 743 in β_5, which also satisfies the requirements for a tyrosine kinase site, is not conserved in other β subunits.

Identification of β_5 and its associated α subunit

An antiserum was raised against a synthetic peptide corresponding to the β_5 cytoplasmic domain (residues 757−776), and was then employed in immunoprecipitation experiments to analyze the β_5 protein and any associated α subunits. As shown (Figure 6A, lane d) the anti-β_5 antiserum yielded a pattern of two protein bands (165 000 and 95 000 M_r) from surface-labeled CCL 228 cells. This pattern was clearly distinct from the β_1 precipitation (lane a), and the blank β_2 and β_3 precipitations (lanes b and c). Because the integrin α^V subunit migrates at ~165 000 M_r, and is known to be present on carcinoma cells in association with a novel β subunit (Cheresh *et al.*, 1989), an anti-α^V immunoprecipitation was carried out for comparison. As shown, the anti-α^V monoclonal antibody (LM142) yielded a pattern of bands (lane e) with mobilities that closely resembled those seen in the anti-β_5 immunoprecipitation (lane d).

To better demonstrate the identity of the α subunit associating with β_5, immunodepletion experiments were carried out. When extract from surface-labeled CCL 228 cells was depleted of all α^V-reactive material (Figure 6B, lane 1), all β_5-reactive material was removed at the same time (lane k). Conversely, when all β_5-reactive material was depleted (Figure 6B, lane i) most, but not all of the 165 000 M_r protein recognized by LM142 was also removed. In undepleted extract (lanes g and h), substantial amounts of material were precipitated by the anti-β_5 and anti-α^V reagents. Together these results suggest that (i) all of the β_5 on CCL 228 cells is associated with the α^V subunit and (ii) a small proportion of α^V is not associated with the β_5 subunit (as defined using the anti-β_5 peptide antiserum), and thus perhaps could be associated with some other β subunit.

In another experiment, the mobilities of β_5 and β_3 were compared when immunoprecipitated from the same cell line (J82 bladder carcinoma cells). As shown (Figure 6C), the β_5 protein (lane n) had a slightly larger apparent size than β_3 (lane m). In the same experiment, the mobility of α^V was identical whether co-precipitated with β_3 or β_5 or precipitated directly (compare lanes m, n and o).

The substantial increase in the apparent size of β_5 upon reduction (Figure 6D, lane q) compared to non-reduced conditions (lane p) is characteristic of other integrin β subunits. Also upon reduction, the α^V subunit migrated faster due to the cleavage of a 25 000 M_r disulfide-linked C-terminal fragment, as previously described (Suzuki *et al.*, 1986).

Distribution of β_5

The distribution of β_5 on various cell types was studied by immunoprecipitation of surface ^{125}I-labeled cells. The β_5 subunit was most prevalent on various types of carcinoma cells. It was also present on cell lines of hepatoma and fibroblast origin, but was absent on lymphocytes and platelets (Table II). On some of the cell lines (CCL 228, A431, HepG2) β_5, but not β_3, was present in association with α^V, whereas on other cell lines (PHEC, JY), β_3 but not β_5 was present with α^V. On the J82 cell line, and perhaps also the MRC-5 fibroblast cells, both β_5 and β_3 were present in association with α^V. Platelets and the T cell line PEER lacked detectable levels of either α^V or β_5. The β_4 subunit, like β_5, was expressed on carcinoma cell lines and absent from platelets and lymphoid cells. Also, as expected, β_1 was present on all of the cell lines, and β_2 was only expressed on the lymphoid cell lines.

Discussion

A human integrin β subunit protein, named β_5, has been cloned, sequenced and found to be distinct from all other

Table II. Distribution of α^V, β_5 and other integrin β subunits

Name	Cell type	β_1	β_2	β_3	β_4	β_5	α^V
CCL228	colon carcinoma	++	−	−	++	+	+
A431	epidermoid carcinoma	+++	−	−	++	+/−	+
J82	bladder carcinoma	+++	−	+	+/−	+	++
HepG2	hepatoma	+++	−	−	+	+	+
MRC-5	fibroblast	+++	−	+/−	ND	+	+
PHEC	endothelial	++	−	+	+/−	−	+
JY	B cell	−	+	+	ND	−	+
PEER	T cell	++	++	−	−	−	−
Platelet		++	−	+++	−	−	−

Distribution of β and α^V subunits was determined by immunoprecipitation. Distribution of β_4 on PEER cells, PHEC and platelets was reported elsewhere (Hemler *et al.*, 1989). Expression of β_4 on JY and MRC-5 cells was not determined, but other B cell and fibroblast cell lines were negative (not shown).

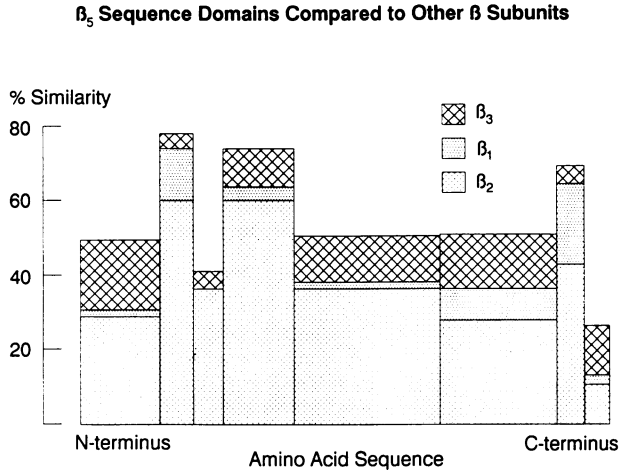

Fig. 7. Comparison of sequence domains in β_5 and other integrin β subunits. The amino acid sequence of β_5 was compared with other β subunit sequences based on the alignment shown in Figure 5. Domains of β_5 were divided into segments from amino acid 1−112, 113−162, 163−206, 207−315, 316−528, 529−694, 695−737 and 738−776.

known β subunits. An anti-β_5 rabbit serum was prepared against a synthetic C-terminal peptide of β_5, and this serum was used to directly demonstrate that (i) β_5 is a 95 000−100 000 M_r cell surface protein and (ii) that β_5 associates with the previously described integrin α^V subunit, but no other detectable α subunit.

The approach for selecting β_5 involved the use of two oligonucleotide probes, both prepared from a region of high sequence conservation among β_1, β_2 and β_3. The successful isolation of β_5 cDNA validates this approach, and suggests that it could be potentially useful for identification of additional integrin β subunits such as β_P (Holzmann and Weissman, 1989), β_4 (Kajiji *et al.*, 1989; Hemler *et al.*, 1989) or others not yet described.

Unlike β_1, β_2 and β_3, which each associate with multiple α subunits, β_5 only associated with a single α subunit, here identified as the integrin α^V subunit. Thus β_5 does not appear to define a new subfamily of integrins. Rather, β_5 is one of two or more distinct β subunits which can associate with the integrin α^V subunit. The α^V subunit was initially discovered in association with β_3, forming an $\alpha^V\beta_3$ complex that had vitronectin receptor activity (Pytela *et al.*, 1985). In subsequent studies, the $\alpha^V\beta_3$ complex was also found to bind fibrinogen, von Willebrand factor (Cheresh and Spiro, 1987), thrombospondin (Lawler *et al.*, 1988) and bone sialoprotein (Oldberg *et al.*, 1988). Later, α^V was found to associate with another β subunit, referred to as β_X, and the $\alpha^V\beta_X$ complex appeared to have both vitronectin and fibronectin receptor activity, but did not bind fibrinogen or von Willebrand factor (vWF; Cheresh *et al.*, 1989). The β_X subunit resembles the β_5 subunit described here in terms of size (both are slightly larger than β_3), and cell distribution (both are predominant on carcinoma cell lines), suggesting that the two proteins might be related or identical. In this regard, limited N-terminal amino acid sequencing of the β_X protein gave results (D.Cheresh, personal communication) that almost completely agree with the β_5 sequence reported here.

In addition to associating with β_3 and β_5, α^V might associate with yet another β subunit. On CCL 228 cells (which do not express β_3) we have detected α^V in association with a small amount of another β subunit that resembled β_5 in size, but was not recognized by antiserum to β_5 C-terminal peptide (see Figure 6B). However, in the absence of comparative sequence information, and/or antibody crossreactivity data, we cannot yet firmly conclude that there is a third β subunit that associates with α^V. Also in this regard, there is a recently described subunit called β_S, which associates with α^V on human fibroblasts, endothelial cells and on an osteosarcoma cell line (Freed *et al.*, 1989). Additional studies will be required to fully evaluate the relationship between β_S, β_5 and β_X.

The cell distribution of β_5 is like β_4 in that they are both present on carcinomas, but absent from lymphoid cells and platelets. Because the expression of β_4 in mouse (Falcioni *et al.*, 1986) and human cells (Kimmel and Carey, 1986) has been correlated with tumor cell aggressiveness, it will be interesting to see if future experiments show a similar correlation for β_5.

Although β_5 appears to be an alternative subunit, replacing β_3 in association with α^V, the expression of β_5 and β_3 is not mutually exclusive, since a bladder carcinoma cell line (J82) expressed both of the subunits in association with α^V.

Cloning and complete primary sequence determination revealed that β_5 most resembled β_3 (55% overall homology), and to a lesser extent, resembled β_1 (43% identity) and β_2 (38% identity). This trend is generally observed throughout the protein sequence (Figure 7). The highest levels of similarities (60−80%) are observed in two distinct regions in the N-terminal half of the molecule. Possibly one or both of these regions could be critical for $\alpha-\beta$ subunit association, since a deletion in this region of β_2 prevented α subunit association (Kishimoto *et al.*, 1989a). Also, RGD-crosslinking experiments have localized a putative ligand binding site (D'Souza *et al.*, 1988; Smith and Cheresh, 1988) in the vicinity of the most highly conserved region seen in Figure 7. Thus we would predict that these highly conserved domains seen in β_5 might be involved in subunit association and/or ligand binding.

Integrin β subunit cytoplasmic domains are thought to have a critical role in cytoskeletal interactions. For example, the β_1 cytoplasmic domain associates with cytoskeletal proteins

such as talin (Horwitz *et al.*, 1987) and fibulin (Argraves *et al.*, 1989), and is essential for integrin clustering into focal contacts (Solowska *et al.*, 1989). Consistent with this essential role, the cytoplasmic domain of the β_1 subunit sequence is one of the most highly conserved regions among human (Argraves *et al.*, 1987), chicken (Tamkun *et al.*, 1986), mouse (Holers *et al.*, 1989) and frog (DeSimone and Hynes, 1988) β_1 integrins.

In contrast, the C-terminal end of β_5 is the least conserved region between β_5 and the other β subunits, showing only 10–20% similarity (Figure 7). This dissimilarity includes 10 extra amino acids present in β_5 that are not present in the other β subunits. This striking variation among the cytoplasmic domains of β_5 and other integrin β subunits suggests that β_5 and the other β subunits may each interact with the cytoskeleton in a specialized manner.

A few variations were observed in the β_5 coding sequence when different β_5 clones were compared. Notably, in four sites there were changes in sequence involving multiples of three nucleotides, resulting in differences in amino acid sequence while maintaining the reading frame. Perhaps the most notable difference was observed in the cytoplasmic domain, where there was a repeated Phe–Asn–Lys sequence in three clones but only a single Phe–Asn–Lys was present in several other clones. Currently it is not known whether these amino acid variations are due to cloning artifacts or are genuine allelic forms, which might influence the function of the β_5 protein. In this regard, allelic variations have been found on at least one other β subunit (β_3), and are responsible for alloimmune reactions against platelets (Santoso *et al.*, 1987). Another possibility is that the β_5 subunit might undergo alternative splicing, such as has been found for β_3 (Van Kuppevelt *et al.*, 1989). However, it would be somewhat unusual to obtain such small alternatively spliced exons, accounting for only 1–3 amino acid differences.

Until recently, the organization of integrin subfamilies has been based on the simple concept of a few common β subunits, each associating with several different α subunits which provide ligand specificity. Now, however, it has been found that α^V can associate with two or more β subunits, resulting in changes in ligand specificity. For example $\alpha^V\beta_X$ bound to fibronectin but not fibrinogen or vWF, whereas $\alpha^V\beta_3$ bound vWF and fibrinogen, but not fibronectin (Cheresh *et al.*, 1989). Assuming that β_5 is identical to β_X (see above), then $\alpha^V\beta_5$ would bind to fibronectin and vitronectin. The absence of β_5 from lymphoid cells, but presence on most adherent cells tested, is consistent with a matrix-adherence function for this protein. The availability of β_5 cDNA will now allow expression in a well defined heterologous system to test the functional properties of this subunit.

Materials and methods

Isolation of cDNA encoding the β_5 gene

Recombinant phage plaques from a human thymic epithelial cDNA library in λgt11 (Clontech Laboratories, Inc.) were probed on duplicate filters with the two oligonucleotides described in Figure 1, using standard procedures. A third filter was probed with an insert corresponding to the 3′ half of the β_1 gene [base pairs 1133–3614 (Agraves *et al.*, 1987)]. Plaques positive for both oligonucleotide probes, but negative for the β_1 probe were selected, and then phage DNA was prepared, digested with *Eco*RI, and subcloned into pGem4 vector (Promega). Clone 9.2, containing a 3.0 kb β_5 insert, was used for secondary screening and eight additional phage plaques were selected, and those β_5 inserts were subcloned into the bluecript vector (Stratagene Co.).

DNA sequencing

Clones with β_5 cDNA inserts were sequenced on both strands by the dideoxy sequencing method. After sequencing the ends of the available β_5 clones (Figure 3), sequencing of the remaining gaps was facilitated by using synthetic primers of 15–17 bp prepared from known β_5 sequence.

Northern blot analysis

Total RNA (10 μg/lane) from CCL 228 cells, PEER cells and thymocytes was separated by electrophoresis in a 1% agarose gel containing formaldehyde, and then after blotting onto nitrocellulose, the RNA was probed with a ^{32}P-labeled *Sph*I fragment derived from clone 9.2.

Production of antibodies and immunoprecipitation

The peptide (CTHTVDFTFNKFNKSYNGTVD) was prepared by Multiple Peptide Systems (CA) and, except for the N-terminal cysteine, corresponds to the predicted 20 amino acids at the C-terminus of the β_5 cDNA sequence (Figure 4). Note that two repeating FNK sequences are present in the peptide, although several clones only had a single FNK sequence (Table I). The peptide was coupled to keyhole limpet hemocyanin (KLH) using *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (Pierce Chemical Co.) through the N-terminal cysteine residue as previously described (Kitagawa and Aikawa, 1976). The KLH-conjugated peptide was used to immunize rabbits, and after 3–4 injections at 2 week intervals, the resulting rabbit antiserum was of suitable titer for use in immunoprecipitation experiments.

Immunoprecipitations of integrin subunits from extracts of ^{125}I-surface-labeled cells were carried out as previously described (Hemler *et al.*, 1987). MAbs used for immunoprecipitation in this study include the anti-β1 mAb A-1A5 (Hemler *et al.*, 1984), anti-β2 mAb TS1/18 (Sanchez-Madrid *et al.*, 1983), anti-β3 mAb AP-5 (from Dr T.Kunicki), anti-β_3 mAb Ab-15 (from Dr M.Ginsberg), anti-β4 mAb 439-9B (Kennel *et al.*, 1989) and the anti-α^V mAb LM142 (Cheresh and Harper, 1987).

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