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The primary structure of the α^4 subunit of VLA-4: homology to other integrins and a possible cell - cell adhesion function

Yoshikazu Takada, Mariano J.Elices, Carol Crouse and Martin E.Hemler

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA

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VLA-4 is a cell surface heterodimer in the integrin superfamily of adhesion receptors. Anti-VLA-4 antibodies inhibited cytolytic T cell activity, with inhibitory activity directed against the effector T cells rather than their targets. Thus, whereas other VLA receptors appear to mediate cell-matrix interactions, VLA-4 may have a cell-cell adhesion function. To facilitate comparative studies of VLA-4 and other integrins, cDNA clones for the human α^4 subunit of VLA-4 were selected and then sequenced. The 3805 bp sequence encoded for 999 amino acids, with an N-terminus identical to that previously obtained from direct sequencing of purified α^4 protein. The α^4 amino acid sequence was 17-24% similar to other integrin α chains with known sequences. Parts of the α^4 sequence most conserved in other α chains include (i) the positions of 19/24 cysteine residues, (ii) three potential divalent cation binding sites of the general structure DXDXDGXXD and (iii) the transmembrane region. However, α^4 stands apart from all other known integrin α subunit sequences because (i) α^4 has neither an inserted I-domain, nor a disulfide-linked C-terminal fragment, (ii) its sequence is the most unique and (iii) only α^4 has a potential protease cleavage site, near the middle of the coding region, which appears responsible for the characteristic 80 000 and 70 000 M_r fragments of α^4.

Key words: Integrin/cell adhesion/cytolytic T cell/cDNA sequence

Introduction

The cell surface heterodimer VLA-4 is highly expressed on thymocytes, peripheral blood lymphocytes, monocytes, T and B cell lines and myelomonocytic cell lines (Sanchez-Madrid *et al.*, 1986; Hemler *et al.*, 1987a,b) but has been found in only low amounts on most adherent cells and cell lines (Hemler *et al.*, 1987a). Thus VLA-4 is different from other VLA proteins (VLA-2, VLA-3, VLA-5, VLA-6) which are present in varying amounts on nearly all adherent cells and cell lines. Whereas the latter VLA proteins are receptors for the extracellular matrix components collagen, laminin and fibronectin (Hynes, 1987; Wayner and Carter, 1987; Gehlsen *et al.*, 1988; Sonnenberg *et al.*, 1988), a ligand for VLA-4 has not been discovered.

Recently, the mouse equivalent of VLA-4 was implicated as a lymphocyte homing receptor on Peyer's patch high endothelial venules (HEV) (Holzman *et al.*, 1988). This result suggests a possible cell-cell adhesion function for VLA-4.

The discovery of the integrin superfamily of cell adhesion receptors has greatly contributed to our understanding of cell adhesion mechanisms (Hynes, 1987). Within the integrin superfamily, there are at least 11 distinct receptors subdivided into three families known as (i) VLA proteins (Hemler *et al.*, 1987b, 1988), (ii) cytoadhesins (Ginsberg *et al.*, 1988), and (iii) LFA-1, Mac-1 and p150,95 proteins (Springer *et al.*, 1987). VLA-4 is known to be a member of the VLA/integrin family because the α^4 subunit associates with the common VLA β (β_1) subunit (Hemler *et al.*, 1987a,b) and the α^4 N-terminal amino acid sequence showed marked similarity to other integrin α subunit N-terminal sequences (Takada *et al.*, 1987). However, VLA-4 also differs from other integrin heterodimers because the VLA-4 α^4 subunit is only weakly associated with its β_1 subunit, and also the 150 000 M_r α^4 protein usually undergoes partial 'trypsin-like' cleavage to form 80 000 and 70 000 M_r fragments (Hemler *et al.*, 1987a). Among the human integrin α subunits, those from a fibronectin receptor (VLA-5) (Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987a), collagen receptor (VLA-2) (Takada and Hemler, 1988), vitronectin receptor (Suzuki *et al.*, 1987), platelet gpIIb/IIIa (Fitzgerald *et al.*, 1987a; Poncz *et al.*, 1987), Mac-1 (Arnaout *et al.*, 1988; Corbi *et al.*, 1988; Pytela, 1988) and p150,95 (Corbi *et al.*, 1987) have been sequenced and shown to have 20-60% conservation of amino acids. These six

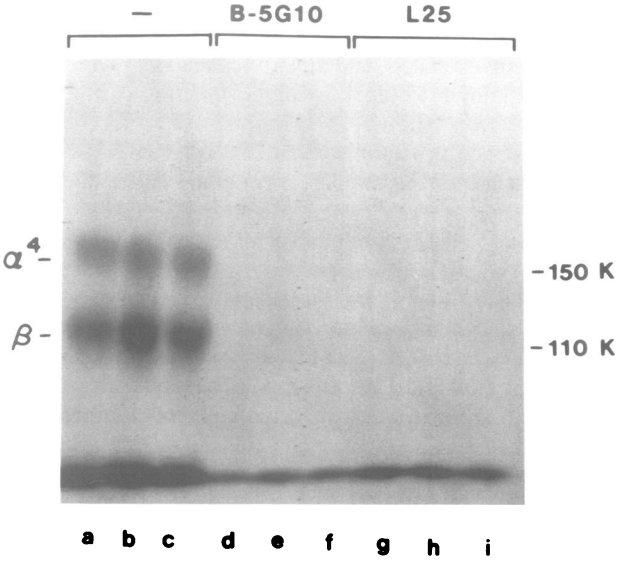

Fig. 1. Sequential immunoprecipitations using anti-VLA-4 antibodies. Extract from ^{125}I-labeled HPB-MLT cells was immunodepleted by successive incubations with control antibody, B-5G10 or L25 as indicated at the top of the figure. Then the remaining proteins were immunoprecipitated using B-5G10 (**lanes a, d and g**), B-5E2 (**lanes b, e and h**) or L25 (**lanes c, f and i**).

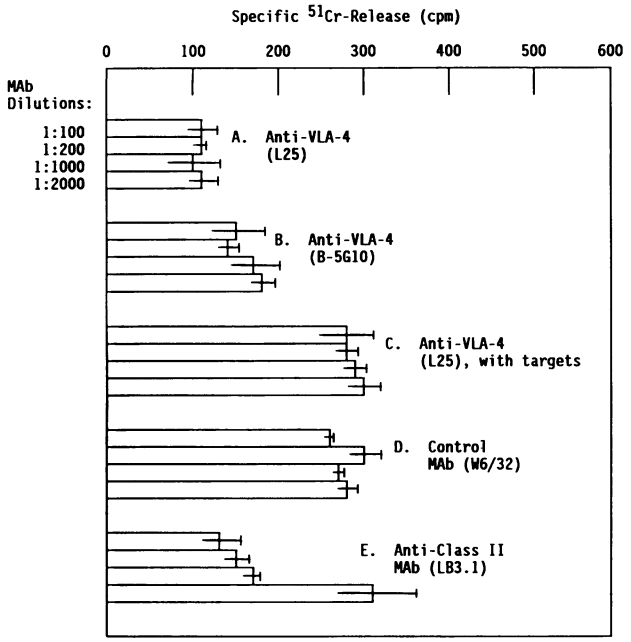

Fig. 2. Inhibition of cytolytic T cell function by anti-VLA-4 antibodies. The mAb L25 (**A**) and B-5G10 (**B**) were preincubated with cloned cytolytic T cells for 30 min as indicated and then specific ^{51}Cr release from target cells was determined in a cytolysis assay as described in Materials and methods. The anti-HLA class I mAb W6/32 was used as a negative control antibody (D) and gave results similar to no antibody (not shown). Also in some experiments L25 (**C**) or an anti-HLA class II mAb (**E**) was preincubated with target cells rather than effector T cells. Each experiment was done at four different dilutions of antibody ascites fluid, and at each dilution samples were analyzed in quadruplicate.

α subunits can be subdivided into two groups which have several distinguishing features (Takada and Hemler, 1988). Most prominently, there are (i) those containing an inserted I-domain and (ii) those which undergo protease cleavage, together with disulfide linkage of the cleaved C-terminal fragment.

In this paper, the role of VLA-4 in cytotoxic T lymphocyte function is investigated, and a mAb (called L25) which was previously known to block cytolytic function (Clayberger *et al.*, 1987), is shown to recognize VLA-4. Also, to facilitate detailed structural comparisons between VLA-4 and other integrins, the α^4 subunit was cloned and sequenced.

Results

Recognition of VLA-4 by the mAb L25

The mAb L25 was previously found to block cytolytic T cell function and also recognized a series of proteins of 150 000, 85 000 and 75 000 M_r (Clayberger *et al.*, 1987). Because the sizes of those proteins resembled the VLA-4 α^4 subunit and its cleaved fragments, the L25 mAb was directly tested for VLA-4 recognition. As shown (Figure 1), both L25 and the anti-VLA-4 mAbs B-5G10 and B-5E2 recognized proteins of similar sizes (lanes a–c). Furthermore, when the mAb B-5G10 was used to immunodeplete all VLA-4 (lanes d and e) from an HPB-MLT cell extract, material precipitated by L25 was also removed (lane f). Conversely, preclearing with the mAb L25 (lanes g–i) caused the disappearance of VLA-4 as detected by B-5G10 (lane g) or B-5E2 (lane i).

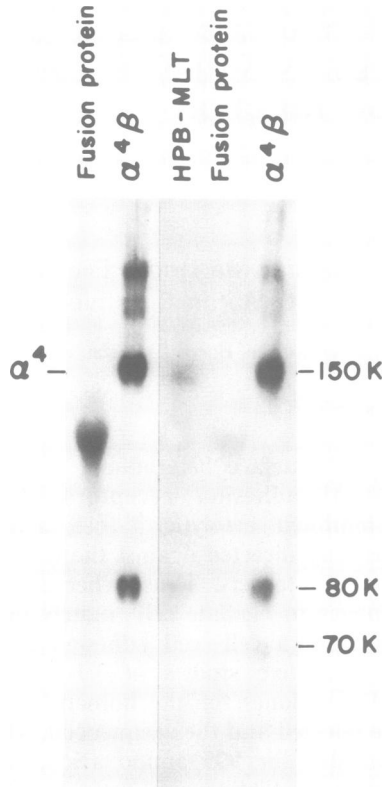

Fig. 3. Immunochemical crossreactivity between α^4 and a fusion protein made from α^4 cDNA. Rabbit anti-fusion protein antibodies were positively selected from crude anti α^4 antisera by using fusion protein–Sepharose as described in Materials and methods. The fusion-protein enriched sera was used to immunoblot the fusion protein itself (**lane a**) and purified VLA-4 protein (**lane b**). Also, un-enriched anti-α^4 sera was used to immunoblot whole cell lysate from HPB-MLT cells (**lane c**), fusion protein (**lane d**), and purified VLA-4 protein (**lane e**).

Inhibition of cytotoxic T cell function by anti-VLA-4 antibodies

To investigate further the potential role of VLA-4 in cytotoxic T cell function, the mAbs L25 and B-5G10 were tested for blocking effects. As shown (Figure 2), four different concentrations of L25 effectively blocked cytolytic T cell activity down to 30–40% of control levels. Another anti-VLA-4 mAb (B-5G10) was less potent, but also blocked cytolysis (down to 50–60% of control levels). When L25 was pre-incubated with target cells rather than with killer cells, it had no blocking effect. In another control experiment, an anti-HLA class II mAb (LB3.1) effectively blocked killing, as expected, since these cytolytic T cells were directed against class II targets. Similar blocking results were obtained using other CTL clones or uncloned CTL that recognize class I targets (data not shown). These results confirm the previous L25 anti-cytolytic blocking results (Clayberger *et al.*, 1987), and suggest that VLA-4 could have a cell–cell adhesion role during the association of cytolytic T cells and their targets.

Identification of cDNA encoding for the α^4 subunit

To allow detailed structural comparisons between VLA-4 and other integrin α subunits, the α^4 subunit was cloned and sequenced. Polyclonal anti-human VLA α^4 antisera was used to select multiple clones from a λgt11 library made

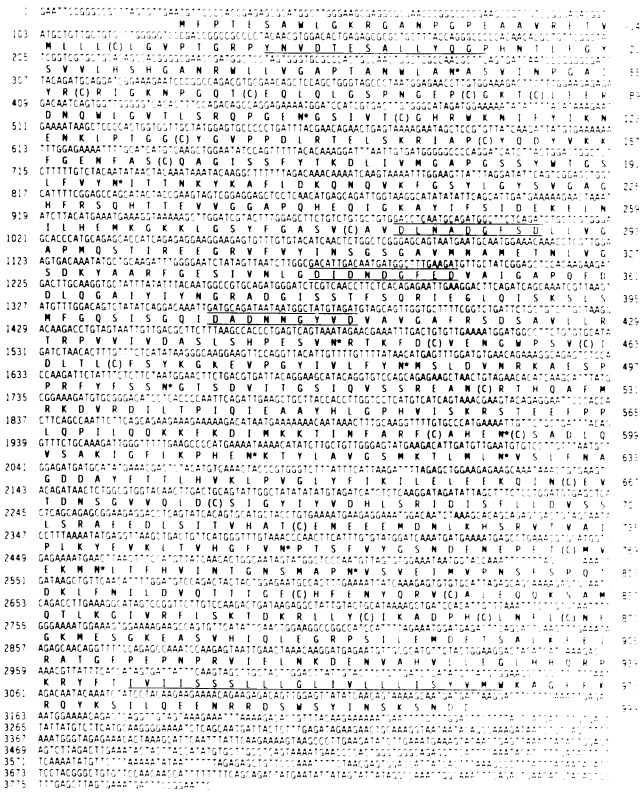

Fig. 4. Complete nucleotide sequence of an α^4 subunit cDNA and deduced amino acid sequence. The deduced N-terminal amino acid sequence (YNVDT...) which matches the N-terminal amino acid sequence from purified α^4 protein is underlined, and also the probable transmembrane domain is underlined. Three potential divalent cation binding domains are indicated by small boxes, possible N-glycosylation sites are marked with *, and cysteines are indicated by (C).

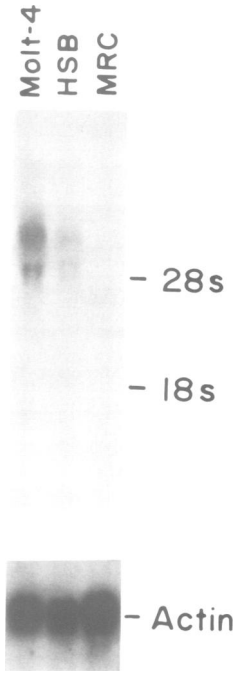

Fig. 5. RNA hybridization analysis. Total RNA (5 μg per lane) from the leukemic cell lines MOLT-4 and HSB and from the fibroblast cell line MRC was electrophoretically separated on 1% agarose−formaldehyde gels, transferred to nitrocellulose filters, then probed with α^4 cDNA clone 4.43. In a control experiment (below), the same RNA samples were probed with actin cDNA.

from HPB-MLT cells. Ten clones were identified as positive during the initial screen of 5×10^5 recombinant λgt11 plaques. Following plaque purification of these clones, they were digested with *Eco*RI, applied for agarose gel electrophoresis, transferred to nitrocellulose and examined for cross-hybridization of each insert. Two groups of four were found to cross-hybridize to each other. To provide preliminary evidence that some of the cDNA inserts contained authentic α^4 cDNA, fusion proteins synthesized by recombinant phage were examined. Fusion proteins made from two representative clones were coupled to Sepharose and then used for positive enrichment of anti-fusion protein antibodies from the anti-α^4 polyclonal antibody. After elution from the fusion protein−Sepharose, these antibodies were tested for immunoblotting of purified VLA-4 protein immobilized on nitrocellulose (Figure 3). Enriched anti-fusion protein 4.10 sera recognized itself (lane a) as well as the 150 000 M_r α^4 subunit and its 80 000 M_r fragment (lane b). Notably, the 70 000 M_r fragment of α^4 was not recognized, suggesting that clone 4.10 (~ 1.8 kb) coded for epitopes on the N-terminal half of the α^4 subunit. Un-enriched sera gave a weaker signal, but did bind to α^4 from a whole cell extract (lane c), 4.10 fusion protein (lane d) and purified α^4 (including both of its fragments, lane e). Of 10 clones originally selected, four contained α^4 cDNA inserts. Enriched antisera obtained using fusion protein−Sepharose from other clones did not bind to α^4 protein. Next the cDNA clone 4.10 was subcloned into pGEM-4 and partially sequenced. The amino acid sequence YNVDTESALLYQGPHN ... deduced from the nucleotide sequence was identical to the N-terminal sequence from purified α^4 peptide (Takada *et al.*, 1987). From this result, and the fusion protein results, we concluded that clone 4.10 is authentic cDNA for the VLA-4 α^4 subunit.

Nucleotide and amino acid sequence for α^4 cDNA

Because the cDNAs initially selected using anti-α^4 sera did not contain the entire α^4 sequence, additional overlapping clones with an overall length of 3.8 kb were obtained by screening a λgt10 library with radiolabeled 4.10 insert. A total of four different clones were analyzed, which overlapped as described in the Methods section. After the cDNA clones were subcloned into pGEM-4 plasmid, the complete nucleotide sequence was determined in both orientations from a series of overlapping deletion clones (Figure 4). Translation of the α^4 cDNA sequence yielded 1038 amino acids (encoded by 3114 bp) between the 5′ and 3′ untranslated regions of ~25 and 670 bp, respectively. Preceding the N-terminal sequence of the mature protein is a methionine followed by 39 amino acids which fulfill the requirements for a signal peptide (von Heijne, 1984). Thus the open reading frame (ORF) shown in Figure 4 encodes a mature protein of 999 amino acids predicted to be 111 000 M_r. The addition of 12 potential *N*-glycosylation sequences (Asn-Xaa-Ser/Thr, average 2500 M_r) to the core protein would result in an estimated size of 141 000 daltons. That value is close to the 140 000−150 000 M_r estimated from α^4 subunit migration of SDS−polyacrylamide gels.

Analysis of the N-terminal portion of the sequence revealed the presence of seven homologous repeating domains (domain I, residue 16−56; II, 92−133; III,

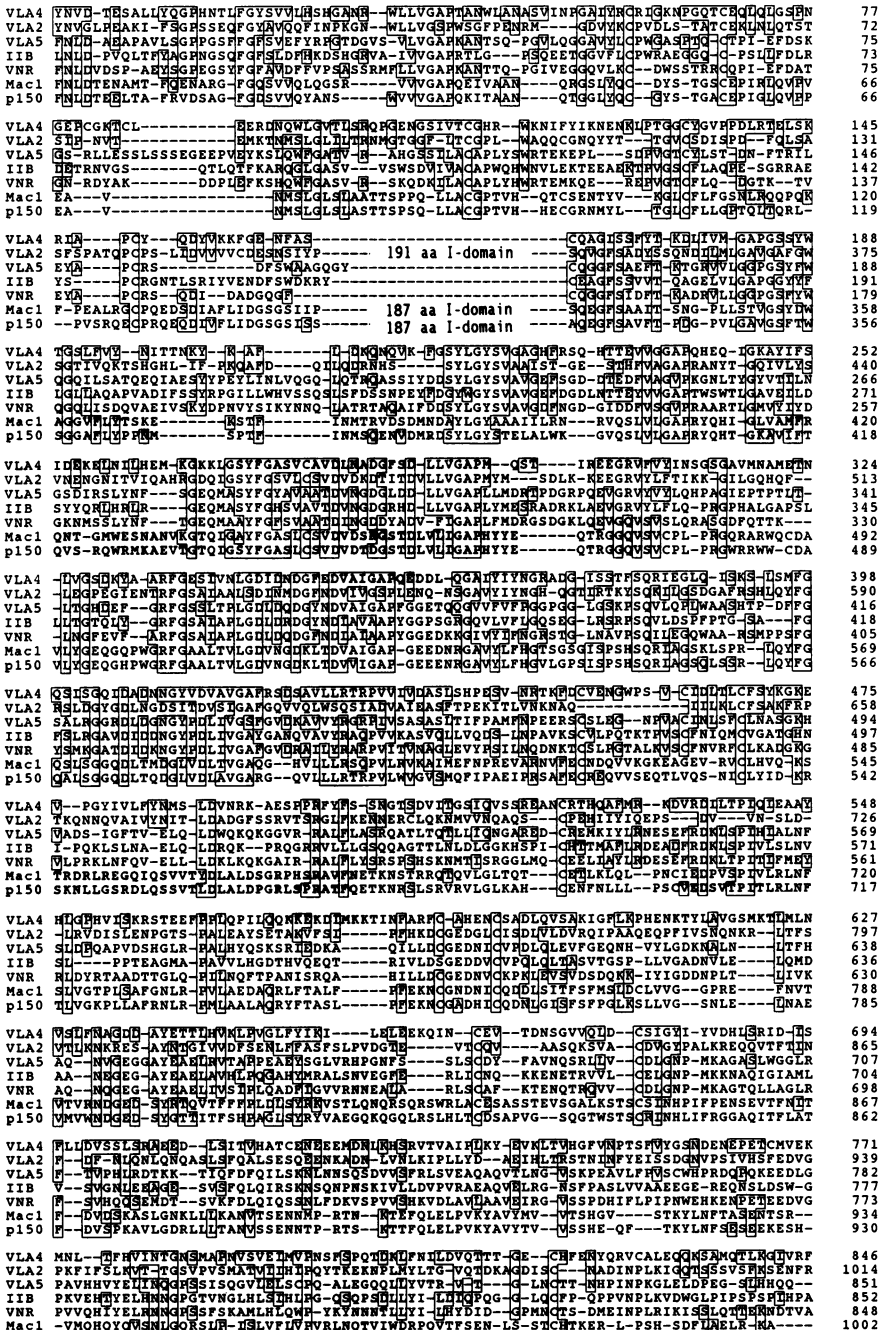

Fig. 6. Alignment of the α chain protein sequences of VLA-4 and other integrin α subunits. The α^4 sequence is compared to human integrin α subunit sequences from VLA-2 (Takada and Hemler, 1988), VLA-5 (Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987a), gp IIb (Poncz *et al.*, 1987), VNR (Suzuki *et al.*, 1987), Mac-1 (Arnaout *et al.*, 1988; Corbi *et al.*, 1988) and p150 (Corbi *et al.*, 1987). The 191 amino acid I-domain present in α^2 (residues 159−349), as well as corresponding 187 amino acid regions in p150 and Mac-1 have been omitted since there is no corresponding sequence in α^4. Residues in other sequences identical to those in α^4 are boxed.

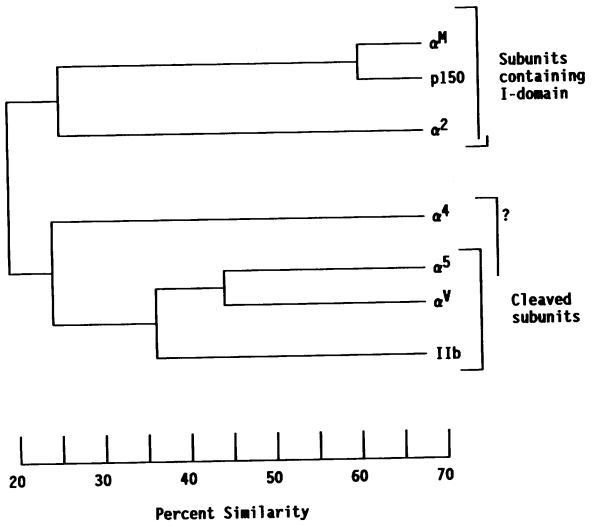

Fig. 7. Linkage map of relative similarities between integrin α subunits. Based on the alignment in Figure 6, percent similarities were calculated between pairs of α subunits (based on amino acid identity) and then linkage trees were determined by standard procedures using the average linkage values (Sneath and Sokal, 1973).

Table I. Comparison of subsets of α subunits which exclusively share residues at many positions

			Subunit comparison	No. of sites with shared residues	Common features
A	1	→	α^5,α^V,IIb	70	Cleaved subunits
	2	→	α^2,α^M,p150	42	I-domain subunits
	3		$\alpha^4\alpha^M$,p150	29	–
	4		α^M,IIb,p150	21	–
	5		α^4,α^5,α^V	20	–
	6		α^2,α^5,α^V	18	–
	7		α^5,α^M,p150	17	–
	8		α^V,α^M,p150	16	–
	9		α^4,α^V,IIb	10	–
	10		α^2,α^V,IIb	10	–
	11		α^2,α^4,IIb	8	–
	12		α^4,α^V,α^M	7	–
	13		α^5,IIb,p150	7	–
	14		α^2,α^5,IIb	6	–
	15	→	α^2,α^4,α^5	5	β_1-associated
B	1	→	α^4,α^5,α^V,IIb	29	–
	2		α^2,α^5,α^V,IIb	24	–
	3	→	α^4,α^2,α^M,p150	22	–
	4		α^4,α^2,α^5,IIb	10	–

[a]Sets of sequences (column 2), shown in order of prevalence (column 1), were derived from computer-generated lists of all amino acid sequence positions in which only three (Part A) or only four (Part B) identical amino acids are shared out of the total of seven at each position (from seven total sequences). The number of positions at which each set of sequences has identical amino acids is listed in column 3, and structural features shared within a set of sequences are listed in column 4. Sequence information from I-domains (residues 159–349 in α^2, 148–334 in α^M and 146–332 in p150) was omitted for these determinations.

165–190; IV, 216–249; V, 271–307; VI, 333–368; VII, 395–435) spaced 22–37 amino acids apart. These domains contain 30–41 amino acids and are 21–57% homologous to each other. Between repeating domains II and III of α^4 there is no large inserted 'I-domain' of the type found in VLA-2 or in the α subunits from the β_2 integrin family.

Table II. Comparison of structural features of α^4 and other integrin α subunits

Integrin α subunit	I-domain	Disulfide-linked fragment	Divalent cation sites	Residues at α^4 positions 119;278		Residues at α^4 positions 161;462	
α^4	No	No	3	F-119	C-278	C-161	C-462
α^2	Yes	No	3	C-110	C-467	S-350	--645
α^M	Yes	No	3	C-97	C-446	S-335	A-633
p150	Yes	No	3	C-97	C-444	A-333	--630
α^L	Yes	No	3	C-94	C-440	S-325	G-622
α^5	No	Yes	4	K-125	A-289	C-164	C-481
IIb	No	Yes	4	E-117	A-295	C-167	C-484
α^V	No	Yes	4	M-112	A-280	C-155	C-472
PS2α	No	Yes	4	T-157	A-337	C-198	C-536

Structural features of α subunits are from references listed in the legend for Figure 6, except for features of α^L (Larson *et al.*, 1989) and PS2α (Bogaert *et al.*, 1987). Each residue listed in the last two sets of columns exactly aligns with the others in the column based on the alignment shown in Fig. 6.

The repeated domains V, VI and VII each contain sequences of DX(D/N)X(D/N)GXXD which are similar to the EF-hand consensus metal binding domains of a number of calcium and magnesium binding proteins including calmodulin, troponin C, parvalbumin (Szebenyi *et al.*, 1981), thrombospondin (Lawler and Hynes, 1986), myosin light chain (Reinach *et al.*, 1986) and galactose binding protein (Vyas *et al.*, 1987). These potential divalent cation-binding sites are located in a region (between amino acids 280 and 414) devoid of cysteine residues and *N*-glycosylation sites. The presence of divalent cation-binding sites in the α^4 subunit is perhaps consistent with the recently noted divalent cation requirement for murine VLA-4 α–β chain association (Holzman *et al.*, 1988).

Northern blotting analysis

The distribution of mRNA for the α^4 subunit was studied by probing with cDNA clone 4.10. Two bands of ~5–6 kb were strongly present in Molt-4 RNA, more weakly in HSB RNA and absent from fibroblast RNA (Figure 5). These results are consistent with the known cell-surface expression of VLA-4 on these cells (Hemler *et al.*, 1987a). Hybridization of the same blot with a cDNA probe for the human actin gene gave comparable signals in all lanes. Since the RNA size (5–6 kb) was somewhat larger than the cDNA clone (3.8 kb), and since no poly(A) tail is present (Figure 4), it is assumed that the 3′ untranslated region (and perhaps also the 5′ end) is incomplete. While there is not yet an explanation for the appearance of two RNA bands, there is no evidence for diversity within the coding region.

Comparison of α^4 subunit sequence with other integrin α chains

The alignment of the α^4 subunit sequence with the α chain sequences of human VLA-2, VLA-5 (FNR), VNR, gpIIb/IIIa, Mac-1 and p150,95 shows that several structural characteristics are shared (Figure 6). For example, of the 24 α^4 cysteine residues, 19 are conserved in at least three of the other sequences, and 14 are conserved in all seven α subunit sequences. Also, there is 26–39% conservation in the 23 amino acid transmembrane region and 100%

conservation of the GFFKR sequence on the cytoplasmic side of the transmembrane domain. The most striking similarities are evident in the region of the α^4 homologous repeats in the N-terminal half of the molecule. As for α^4, each of the other integrin α subunits has also been noted to have seven homologous repeats with three or four potential divalent cation sites within repeats IV–VII (Argraves *et al.*, 1987; Corbi *et al.*, 1987, 1988; Fitzgerald *et al.*, 1987a; Suzuki *et al.*, 1987; Poncz *et al.*, 1987; Arnaout *et al.*, 1988; Pytela, 1988). In these repeat regions, the similarity between α^4 and the other α subunits ranged from 25–33% (in repeats I and II) to 39–52% (in repeats III–VII). The overall similarity between α^4 and the other integrin α subunits is 17–24%, or 20–24% if the I-domains for α^2, α^M and p150 are not included in the calculation. This is in contrast to the higher degree of similarity (~45%) between different human integrin β chains (Tamkun *et al.*, 1986; Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987b; Kishimoto *et al.*, 1987; Law *et al.*, 1987). The α^4 subunit did not contain a potential divalent cation binding site in repeat IV, and thus resembled α^2, α^M and p150 but differed from α subunits of VLA-5, VNR and IIb/IIIa.

Potential protease cleavage sites in the VLA-4 α^4 subunit

For three of the integrin α subunits (α^5, α^V, IIb), the C-terminal 15% of the amino acid sequence is cleaved, but then remains attached to the rest of the α chain by a disulfide linkage (Argraves *et al.*, 1987; Suzuki *et al.*, 1987; Loftus *et al.*, 1988). The α^4 sequence contains a Lys–Arg (at positions 852–853) which is in the same region as cleavage sites in other integrin α subunits. However, there is little additional homology and no evidence yet that this site is cleaved since the size of the α^4 subunit protein is appropriate for its sequence length. Also, the 150 000 M_r α^4 subunit does not diminish in size upon reduction (Hemler *et al.*, 1987b), suggesting that there are no disulfide-linked cleavage fragments. However, at another site in α^4 (residues 564–583) there is a 'KKEK' sequence which somewhat resembles the protease cleavage sites in other integrins (Argraves *et al.*, 1987; Suzuki *et al.*, 1987; Loftus *et al.*, 1988). Variable cleavage at this site would be consistent with the previously observed splitting of α^4 into 80 000 and 70 000 fragments (Hemler *et al.*, 1987a,b). Furthermore two residues after the 'KKEK', there is an α^4-specific 'MKKTI' insert, perhaps also involved in α^4-specific cleavage (see also Figure 6). However, unlike for the other cleaved integrin α subunits, cleavage of α^4 is variable and incomplete and there is no evidence for disulfide linkage of the 80 000 and 70 000 M_r cleaved α^4 fragments.

Relative similarities between integrin α subunits

The α subunits in the integrin superfamily have previously been subdivided into (i) those with I-domains and (ii) those with proteolytically cleaved, disulfide-linked C-terminal fragments (Takada and Hemler, 1988). Because the α^4 subunit does not have a disulfide-linked C-terminal fragment, and does not contain an I-domain, it was interesting to compare the overall similarity of this sequence with the other six previously established integrin α sequences (Figure 7). As shown, the α^4 sequence was a little more similar to the protease-cleaved subunits, than to the I-domain subunits, perhaps consistent with the anomalous protease cleavage mentioned above. Also Figure 7 shows that α^2, α^4 and α^5 did not group together despite being members of the same family and sharing the same β_1 subunit. Rather, α^2 was more similar to the two other subunits which contain an I-domain, and α^5 was more similar to the other subunits which undergo protease cleavage and have disulfide-linked C-terminal fragments.

To affirm further the linkages shown in Figure 7, patterns of amino acid conservation among sets of α subunits were examined. Thus a computer-generated list was compiled of all individual amino acid positions in which exactly three (out of seven) sequences shared residues. From that list it was determined that α^5, α^V and IIb most often shared amino acids (at 70 positions) and after that, α^2, α^M and p150 exclusively shared residues at 42 positions (Table I, Part A). The designation of these two groups by this method agrees with the results shown above in Figure 7 and previously discussed (Takada and Hemler, 1988). Residues were shared among other sets of three α subunits less frequently (at 29 positions or less). Notably, the VLA α subunits (α^2, α^4, α^5) shared residues at only five positions, and thus this group was only 15th-most prevalent in the list of possible groups of three α subunits. The 187–191 amino acids in the I-domains of α^2, α^M and p150 were excluded from the alignments used to obtain data in Table I. Because there are so few 'β_1-specific' amino acids it appears that the VLA α subunits do not depend on large numbers of conserved amino acids for β_1 association.

To address further the question of α^4 similarities to other integrin subunits, positions were enumerated in which exactly four (of the seven) sequences shared amino acids (Table I, Part B). The set of four sequences which most often exclusively shared residues was α^4, α^5 α^V and IIb (occurring 29 times). The next time α^4 appeared in the list (in third place) it was grouped with the 'I-domain' subunits α^2, α^M and p150 (occurring 22 times). This result emphasizes that the evidence moderately favors grouping of α^4 with the 'cleaved' subunits compared with grouping it with the 'I-domain' subunits, a result consistent with the grouping shown in Figure 7.

Disulfides in the α^4 sequence

The majority of cysteines in both integrin α and β chains are highly conserved, consistent with a major structural role for intrachain disulfide bonds in determining a conserved tertiary structure characteristic of integrins in general. However, in addition to the 14 cysteines conserved in nearly all integrin α subunits, it was previously noted that there was a pair of cysteine residues seen only in I-domain subunits (α^2, α^M and p150), and another pair seen only in cleaved α subunits (α^5, α^V and IIb) (Takada and Hemler, 1988). Notably, α^4 had cysteine residues at two positions (165 and 462 in the α^4 sequence) which matched the two positions where cysteines were previously found only in cleaved subunits. On the other hand, α^4 also had a cysteine in only one (position 178) of the two positions previously suggested to be I-domain specific (Table II). Thus with regard to 'subset-specific' cysteines, α^4 resembled both the cleaved subunits (α^5, α^V and IIb) and I-domain subunits (α^2, α^M and p150), but again α^4 appeared to be more related to the former than the latter.

Discussion

The ability of two separate anti-VLA-4 mAb (L25 and B-5G10) to block cytolytic T cell lysis of target B cells suggests that VLA-4 may function in cell−cell interactions. Thus VLA-4 appears to be distinct from other receptors (VLA-2, VLA-3, VLA-5 and VLA-6) in the VLA (integrin β_1) adhesion family which mediate cell−matrix adhesion functions. A cell−cell adhesion function for VLA-4 is consistent with VLA-4 being present on nearly all lymphocytes and monocytes, but absent from most adherent cells (Hemler *et al.*, 1987a). The mAb L25 had previously been shown to block class I and class II directed CTL function (Clayberger *et al.*, 1987), but the antigen was not identified as VLA-4. Now it is clear that the proteins of 150 000, 85 000 and 75 000 M_r previously immunoprecipitated by L25 (Clayberger *et al.*, 1987) are α^4 and its cleaved products. Most likely, a 130 000 M_r β_1 subunit was not previously seen in L25 immunoprecipitations (Clayberger *et al.*, 1987) because it had dissociated from α^4, as often occurs for VLA-4 subunits (Hemler *et al.*, 1987a).

Because VLA-4 expression is widespread on leukocytes, and because a wide variety of specific and non-specific receptors and ligands have been identified which assist T cell−target cell interaction (Martz, 1987), it appears most likely that VLA-4 would be an accessory molecule rather than a highly specific receptor in this process. Furthermore, VLA-4 not only appears to mediate T−B cell interaction, but another recent study has implicated mouse VLA-4 in lymphocyte−endothelial cell interaction. Specifically, anti-mouse VLA-4 mAb selectively blocked organ-specific homing to Peyer's patch high endothelial venules (Holzman *et al.*, 1988). At present it is difficult to understand how VLA-4 could have an organ-specific role in lymphocyte homing, considering the widespread distribution of VLA-4 on nearly all lymphocytes and its role also in T−B cell interaction.

Prior to recent studies of VLA-4, other integrin receptors known to have cell−cell interaction functions were found only in the integrin β_2 family. For example, LFA-1 on lymphocytes interacts with the ligand ICAM-1 on other lymphocytes, endothelial cells and other cells (Dustin *et al.*, 1988). Also LFA-1 appears to be involved as a non-organ-specific accessory molecule in lymphocyte homing (Hamann *et al.*, 1988; Pals *et al.*, 1988). Thus there is precedent for both T−B interactions and T−endothelial interactions mediated by the same integrin receptor. Also, the β_2 integrins Mac-1 and p150,95 mediate granulocyte and monocyte interaction with unknown ligand(s) on endothelial cells (Pohlman *et al.*, 1986; TeVelde *et al.*, 1987). In future studies, it will be interesting to identify the VLA-4 ligand(s) and determine if it/they have any resemblance to ICAM-1 or other β_2 integrin ligands.

An antibody (4B4) to the VLA β_1 subunit (also called (CD29) has previously been shown to subdivide lymphocytes into subpopulations correlating with different immunological functions (Morimoto *et al.*, 1985; Sanders *et al.*, 1988). However, since VLA-4 is evenly distributed on lymphocytes, with no evidence for subpopulations (Hemler *et al.*, 1987a), it is not clear that the immunological functions of VLA-4 would correlate with those previously defined subpopulation functions.

Although VLA-4 may functionally resemble the β_2 integrins, it is structurally quite distinct, and in fact, its primary α subunit structure is unlike any of the known integrin α subunits. The α^4 subunit has neither an I-domain, nor a typical cleaved, disulfide-linked, C-terminal fragment. Also α^4 stands apart in terms of overall sequence similarity with the other integrins, the location of certain characteristic cysteine residues, and the presence of an anomalous protease cleavage site near the middle of the sequence. In many of these aspects, α^4 is a little more like the cleaved subunits and less like the subunits with I-domains (see Tables I and II). Perhaps this is consistent with the unusual cleavage which occurs in α^4, resulting in partial conversion to 70 000 and 80 000 M_r fragments. As the list of VLA-4 functions begins to expand, the availability of an α^4 clone, and the accompanying sequence information should greatly aid future studies correlating VLA-4 structure and function.

Materials and methods

Monoclonal antibodies

The mAb B-5G10 and B-5E2 were produced as previously described (Hemler *et al.*, 1987a), and L25 (Clayberger *et al.*, 1987) was obtained from Dr B.McIntyre, M.D. Anderson Hospital, TX.

Analysis of cytolytic T cell function

Human cytolytic T cell clones and uncloned mixed lymphocyte cultures were derived as previously described (Brenner *et al.*, 1985). To assay cytolytic function, T cells were incubated with ^{51}Cr-labeled Epstein−Barr virus-transformed B cells for 4 h in U-bottom microtiter wells. The amount of ^{51}Cr was determined in quadruplicate for each sample and the specific killing was calculated using standard procedures (Brenner *et al.*, 1985). To determine inhibition, varying dilutions of mAbs were preincubated for 30 min with either the effector T cells or with the target cells.

Isolation of cDNA clones

Phage λgt10 and λgt11 cDNA libraries made from the T leukemic line HPB-MLT, were obtained from D.Dialynas (Dialynas *et al.*, 1986). For screening the λgt11 library, anti-α^4 antiserum was prepared by immunizing rabbits with the α^4 subunit of VLA-4 isolated from HPB-MLT cells (Hemler *et al.*, 1987a), and non-specific reactivity was removed by passage over SDS-denatured placenta extract coupled to Sepharose. Positive clones selected by antibody screening of 5×10^5 recombinant λgt11 (Young and Davis, 1983) were plaque-purified. The phage DNA was digested with *Eco*RI restriction enzyme and the inserts were tested for cross-hybridization by Southern blotting (Maniatis *et al.*, 1982). Two representative clones were selected to produce fusion proteins in lysogenic Y1089 *Escherichia coli* as described (Huynh *et al.*, 1985). The fusion proteins were purified by SDS−PAGE, electroeluted, coupled to Sepharose and then used to positively select anti-fusion protein antibodies from anti-α^4 rabbit serum. The initially selected clone (clone 4.10, bp 111−1827) did not contain the entire α^4 coding region, so additional clones were selected from the HPB-MLT λgt10 library by screening with ^{32}P-labeled 4.10 cDNA probe. These were clone 4.39 (bp 1−1827), clone 4.37 (bp 88−1660) and clone 4.43 (bp 1025−3805). Clone 4.43 was isolated by partial *Eco*RI digestion so that it would span the *Eco*RI site at bp 1827. In clone 4.10, to the 5′ side of the N-terminal amino acid codon (bp 142) there was a 30 bp sequence (not shown) which is not found in clones 4.39 and 4.37. At present it is not clear if there is variability in the leader sequence, or if the 30 bp at the 5′ end of clone 4.10 are a cloning artefact.

DNA sequencing

Phage DNAs were purified by the plate lysate method (Maniatis *et al.*, 1982), digested with *Eco*RI, and then the cDNA inserts were separated on agarose gels, electroeluted and subcloned into pGEM-4 plasmid (Promega Co.). The DNA sequences were determined by the dideoxynucleotide chain-termination method of Sanger *et al.*, 1977) using adenosine 5′-[α-thio]triphosphate labeled at the α-thio position with ^{35}S. To facilitate the sequencing, a series of overlapping deletion clones of both strands were made as described (Henikoff, 1984).

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