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The very late antigen family of heterodimers is part of a superfamily of molecules involved in adhesion and embryogenesis

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ABSTRACT **The very late antigen (VLA) protein family contains at least five related heterodimers, including a fibronectin receptor structure, and probably other cell substrate adhesion receptors. These cell-surface VLA proteins were immunopurified from human placenta (VLA-1, VLA-3, and VLA-5), platelets (VLA-2), and Molt-4 cells (VLA-4) using a series of monoclonal antibody-Sepharose immunoaffinity columns. After further purification by gel electrophoresis, the N-terminal amino acid sequence for each of the five VLA α subunits was determined. In the first 14 positions, the five VLA α subunits showed an average of 42% homology to each other, rising to 59% including conservative amino acid substitutions. In addition, the α subunits from (*i*) the LFA-1, Mac-1 (CR-3), and p150,95 family of heterodimers, (*ii*) the vitronectin receptor-platelet GPIIb/IIIa family, and (*iii*) a position-specific (PS) antigen important in *Drosophila* embryogenesis each showed average homologies of 31–40% to individual VLA α sequences and 46–52% homology to VLA α subunits including conservative substitutions. Taken together, these results suggest that (*i*) the VLA proteins, (*ii*) the LFA-1, Mac-1, and p150,95 family, (*iii*) the GPIIb/IIIa, vitronectin receptor family, and (*iv*) the *Drosophila* PS antigens have evolved as four subgroups in a highly conserved supergene family of receptors involved in fundamentally important functions, such as cell adhesion, migration, and embryogenesis.**

The human very late antigen (VLA) heterodimers VLA-1 and VLA-2 were discovered on long-term activated T cells (1–3). Subsequently, additional related heterodimers, VLA-3, VLA-4, and VLA-5, were discovered (4), and VLA proteins were found to be expressed on nearly all cell types analyzed (2, 4, 5). Biochemical and immunochemical evidence established that the five VLA cell-surface heterodimers are composed of unique α subunits (140,000–200,000 M_r), each noncovalently associated with a common β subunit (110,000 M_r nonreduced) (4, 6). Recently, antiserum to the VLA β subunit was shown to block cell adhesion to fibronectin or laminin (7). Additional experiments (7) indicated that VLA-5 was identical to the human fibronectin receptor and VLA-3 was very similar to part of a chicken fibronectin/laminin receptor complex [also called CSAT antigen (8, 9), or integrin (10)].

The human fibronectin receptor and chicken fibronectin/laminin receptor(s) belong to a group of substrate adhesion receptors that recognize Arg-Gly-Asp sequences present in their ligand structures (9, 11–13). This group also includes the vitronectin receptor (110,000/95,000 M_r) (14) and the platelet GPIIb/IIIa complex (110,000/95,000 M_r) (15, 16). The latter two heterodimers have distinct α subunits but probably have identical β subunits (17).

Another family of cell-surface heterodimers is the LFA-1, Mac-1, and p150,95 family of leukocyte adhesion receptors. That group is also composed of unique α (180,000, 170,000, and 150,000 M_r) and common β (95,000 M_r) subunits (18). LFA-1 is expressed by most leukocytes and acts as a general adhesion protein in cellular interactions (19). During inflammation, increased surface expression of Mac-1 and p150,95 by monocytes and granulocytes leads to increased adhesiveness to endothelial cells and thus mediates localization of leukocytes to inflammatory sites (20, 21). Mac-1 (also called CR3) and p150,95 also appear to bind the iC3b fragment of complement (22, 23), and the Arg-Gly-Asp sequence present in iC3b may be of critical importance in its interaction with the Mac-1 (CR3) receptor (24). The importance of the LFA-1, Mac-1, and p150,95 family is emphasized by the immunodeficiency resulting from severely defective leukocyte adhesion in patients deficient in these proteins (25).

N-terminal sequences of α subunits of human and murine Mac-1, LFA-1, p150,95, vitronectin receptor, and GPIIb/IIIa have been shown to be markedly homologous to each other (17, 26–28). This evidence, together with their analogous $\alpha\beta$ subunit arrangements, and common recognition of Arg-Gly-Asp sequences, suggests that these two sets of heterodimers are subfamilies in a larger group of adhesive protein receptors. Another family of $\alpha\beta$ structures with distinct α subunits and common β subunits comprises the *Drosophila* position-specific (PS) antigens (29). Comparison of N-terminal sequences has revealed homology between the α subunit of PS1 and the α subunits of the vitronectin receptor, LFA-1, and Mac-1. The PS antigens are thought to be involved in specific cell recognition during embryogenesis (30) and have been suggested to be analogous to fibronectin receptors (31).

In the present study, N-terminal sequences of the α subunits of the five VLA proteins were determined for comparison with each other and also to those of Mac-1, LFA-1, p150,95, the vitronectin receptor, GPIIb/IIIa, and *Drosophila* PS1 antigen. The findings suggest that the VLA proteins, including the human fibronectin receptor structures, are an additional subgroup in a supergene family of adhesive protein receptors that are highly conserved in diverse animal species.

METHODS

Monoclonal Antibodies (mAbs). The mAbs TS2/7 (2), 12F1 (5), J143 (32), and B-5G10 (33) recognize the α^1, α^2, α^3, and α^4 subunits of VLA-1, VLA-2, VLA-3, and VLA-4 (4, 6, 33), respectively. The mAbs 12F1 and J143 were made available by K. Pischel and V. Woods (University of California, San Diego) and R. Kantor and L. Old (Sloan–Kettering Institute for Cancer Research, NY), respectively. The mAb A-1A5 specifically recognizes the β subunit common to all VLA proteins (4, 6). mAbs were purified from ascites fluid by 45% ammonium sulfate precipitation and then coupled to CNBr-activated Sepharose 4B (Pharmacia) at 3 mg of mAb per ml

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Abbreviations: mAb, monoclonal antibody, VLA, very late antigen; PS, position-specific; N-CAM, neural cell adhesion molecule.

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of packed beads according to the manufacturer's instructions.

Purification of VLA-1 and VLA-3. Fresh human placenta (from Brigham and Women's Hospital, Boston, MA) was extensively washed with ice-cold 0.9% NaCl, freed from amnion and chorion, and blotted dry. The tissue (100 g, wet weight) was diced and homogenized at 0°C in 5 vol of 10 mM Tris·HCl, pH 7.5/0.15 M NaCl/1 mM phenylmethylsulfonyl fluoride/1% Nonidet P-40 (buffer A) in a Waring blender (four 30-s bursts at 1-min intervals). The homogenate was centrifuged at 10,000 × *g* for 30 min, and the supernatant was loaded sequentially onto wheatgerm lectin-Sepharose (20 ml) and Ricin-Sepharose (40 ml) columns (Pharmacia). After rinsing with 10 column vol of buffer A, the bound material was eluted with buffer A containing 5% *N*-acetylglucosamine or 5% galactose, respectively. Combined lectin column eluate was passed through ovalbumin-Sepharose (10 ml) to clear nonspecifically adhering material, sequentially loaded onto TS2/7-Sepharose (5 ml) and J143-Sepharose (1 ml) columns, and then rinsed with 1 column vol of buffer A, 1 column vol of 50 mM NaCl/0.1% deoxycholate, and then 10 column vol of 10 mM Tris·HCl, pH 7.5/0.15 M NaCl/0.1% deoxycholate. VLA-1 and VLA-3 were eluted from the respective columns with 50 mM diethylamine/0.1% deoxycholate/5% glycerol, pH 11.5, and the pH was immediately neutralized with 0.1 vol of 1 M Tris·HCl (pH 6.8).

Purification of VLA-5, VLA-2, and VLA-4. After other VLA structures were depleted from human placenta glycoprotein preparations by TS2/7-, 12F1-, and J143-Sepharose chromatography, VLA-5 was purified from the remaining placenta glycoprotein fraction by β-specific A-1A5-Sepharose chromatography. VLA-2 was purified from 25 g of outdated platelets (from the Dana-Farber Institute blood bank and from the American Red Cross, Boston, MA) by 12F1-Sepharose or A-1A5-Sepharose chromatography, and VLA-4 was purified from 100 g of Molt-4 cells by B-5G10-Sepharose (5 ml) using the same procedures as described above.

Gel Separation and Sequence Analyses. Aliquots of column fractions containing up to 1–2 μg of protein were analyzed by $NaDodSO_4$/PAGE on 7% acrylamide gels; this was followed by silver staining (34) to assess yield and purity. Larger amounts of VLA protein (20–50 μg) were run on preparative 5% $NaDodSO_4$/PAGE gels, and aliquots of ^{125}I-labeled purified VLA proteins were run in adjacent lanes for use as markers to facilitate localization of separated subunits. Nonreducing preparative gels were run for VLA-3 and VLA-5 subunit separations (to prevent comigration of the α and β subunits), whereas reducing conditions were used for VLA-1, VLA-2, and VLA-4 subunit separation. After preparative $NaDodSO_4$/PAGE, gel slices containing α subunits from each VLA complex were excised and electroeluted as described (35), and about 30–100 pmol of each was sequenced with a gas-phase Applied Biosystems (Foster City, CA) 470A protein sequencer, carried out at the Harvard Microchemistry Facility (Cambridge, MA).

RESULTS

Purification of VLA Proteins. Human placenta extract was enriched for glycoproteins and then sequential immunoaffinity column purification yielded substantial amounts of the α and β subunits of VLA-1, VLA-3, and VLA-5, lesser amounts of VLA-2, and no VLA-4 (Fig. 1*A*). VLA-5 was purified using a β-chain-specific A-1A5-Sepharose column after the other VLA proteins were essentially depleted by TS2/7-, 12F1-, and J143-Sepharose chromatography. The identity of purified placenta VLA-5 was confirmed by testing its reactivity with mouse anti-VLA-5 serum (data not shown); that serum was prepared against VLA-5 from the cell line K-562, in the absence of any other VLA proteins (4, 7). Whereas only a small amount of VLA-2 was obtained from placenta, larger quantities of VLA-2 could be obtained from platelets, using A-1A5-Sepharose (Fig. 1*B*) or 12F1-Sepharose (not shown). Platelets are known to express mostly VLA-2, with a trace amount of VLA-3 (5). Following A-1A5-Sepharose immunoaffinity purification and prior to N-terminal sequencing, the VLA-2 α^2 subunit was readily separated from any contaminating α^3 subunit material by preparative $NaDodSO_4$/PAGE using reducing conditions. Though absent from placenta and platelets, VLA-4 could be purified from the T-lymphoblastoid cell line Molt-4 using B-5G10-Sepharose. In the result shown in Fig. 1*C*, the α^4 subunit was preferentially obtained, whereas the β subunit was presumably lost due to dissociation. This is consistent with other

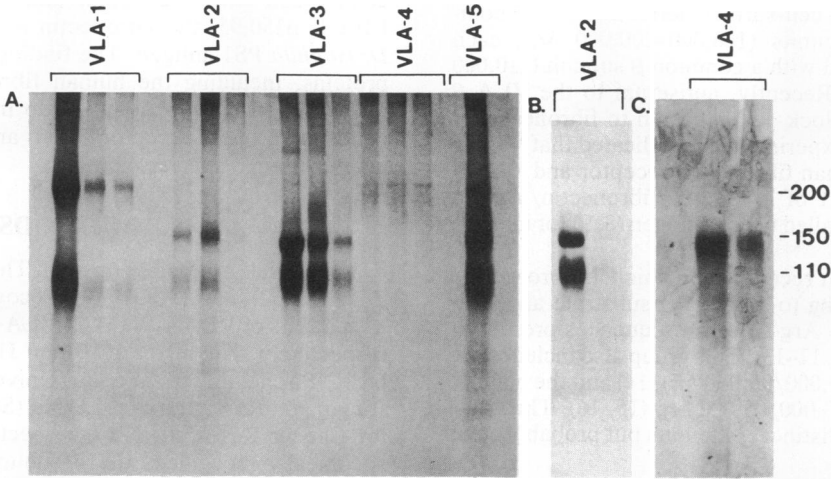

FIG. 1. Silver-staining analyses of purified VLA proteins from placenta, platelets, and Molt-4 cells. (*A*) Glycoproteins from placenta were passed sequentially over TS2/7-, 12F1-, J143-, B-5G10-, and A-1A5-Sepharose immunoaffinity columns and eluted. Each lane represents up to 1–2 μg of protein from a column elution fraction, separated by $NaDodSO_4$/PAGE and visualized by silver staining. (*B*) VLA proteins from platelets (which contain mostly VLA-2) were immunoaffinity purified using an A-1A5-Sepharose column. When a 12F1-Sepharose column (not shown) was used for VLA-2 purification from platelet extract, essentially identical results were obtained. (*C*) Glycoproteins from the T-lymphoblastoid cell line Molt-4 were immunoaffinity purified using a B-5G10-Sepharose column. As shown, the α^4 subunit was obtained with little or no β subunit, presumably due to β subunit dissociation (33). Molecular weights are shown as $M_r \times 10^{-3}$.

studies showing that the α^4 and β subunits of VLA-4 are easily dissociated (4, 33).

Comparisons of N-Terminal Amino Acid Sequences. The sequences of the first 14 N-terminal residues of each of the VLA α subunits were obtained (Fig. 2*A*). They showed striking homology, especially at positions 1–6 and 10–14. Homology between individual VLA α subunits (summarized in Table 1, group A) ranged from 21% (between α^1 and α^3) to 75% (between α^2 and α^5), with an average homology of 42% between any pair of α subunits. Allowing for conservative amino acid substitutions (defined as in the legend to Fig. 2), homology between different pairs of VLA α subunits is increased to an average of 59% (range, 43–75%). An 80,000 M_r peptide (not shown) that is often coexpressed with VLA-4 yielded an N-terminal sequence identical to that shown for the α^4 subunit (Fig. 2*A*). This result is consistent with other data suggesting that the 80,000 M_r peptide is derived from the VLA-4 α^4 subunit by proteolysis (33).

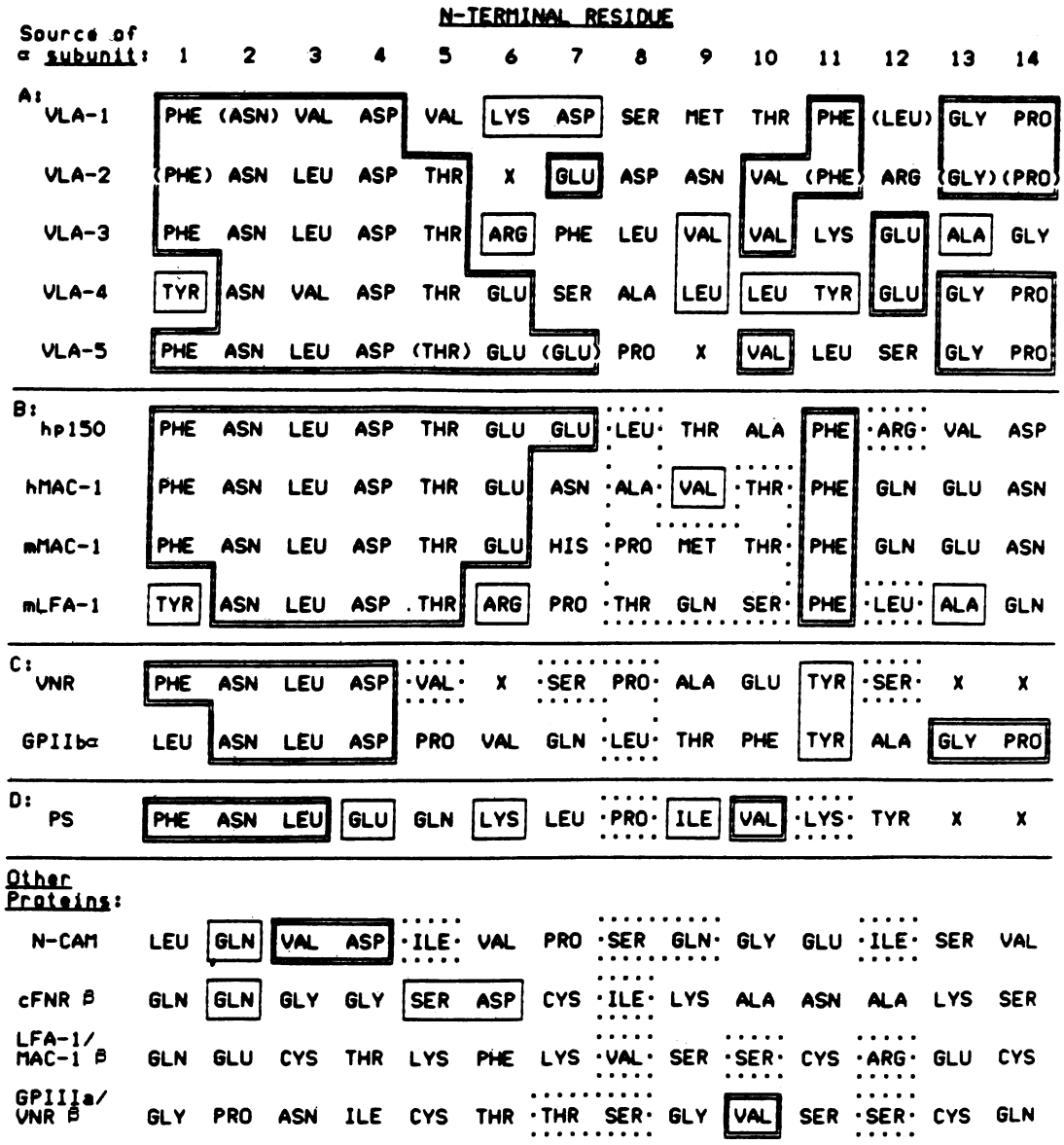

FIG. 2. Comparison of N-terminal sequences from VLA protein subunits and other related subunits. (*A*) N-terminal sequences for purified VLA α subunits were obtained (see text); homology within the VLA α subunit family is indicated by a double box around the appropriate residues. Homology allowing for conservative substitutions is indicated by a single box. The following amino acids were considered as conservative substitutions: threonine, serine; phenylalanine, tyrosine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; arginine, lysine; and alanine, glycine. (*B*) N-terminal sequences for α subunits from human p150,95 and Mac-1 (26) and mouse Mac-1 and LFA-1 (mMac-1, mLFA-1) (36) are shown. Residues from these α subunits are placed in a double box only if they are shared by at least two of the VLA α subunits. A single box is used to indicate homology if these residues are shared by at least two VLA α subunits, including conservative substitutions. A dotted box is used if these α subunits contain a residue (or a conservative substitution) that appears only once in the VLA α subunits. Homologies within these α-subunit families that are not also shared by VLA α subunits are not marked. (*C*) N-terminal sequences for human vitronectin receptor (VNR) α subunit (17, 28) and platelet GPIIb (17, 27) are shown, with homologies indicated as in *B*. (*D*) N-terminal sequence from the 116,000 M_r α subunit of the *Drosophila* PS1 antigen (37) is shown with homologies indicated as in *B*. (*Other proteins*) N-terminal amino acid sequences are shown for the neural cell adhesion molecule N-CAM (38) and for GPIIIa (27), which is probably the same as the VNR β subunit (17). Also, N-terminal amino acid sequences predicted from gene sequence information are shown for the band 3 subunit of the chicken fibronectin receptor (cFNR), also called integrin (10), and the β subunit from the LFA-1, Mac-1 family (39). In each part, an "X" indicates no determination, and parentheses indicate that the assignment is not definitive. For the VLA-5 α subunit, at both positions 5 and 7, a major alternative possibility is alanine.

Table 1. Percent homologies among VLA α subunits and comparisons to α and β subunits from other heterodimers

Group	Subunit	% homology				
		VLA-1	VLA-2	VLA-3	VLA-4	VLA-5
A	VLA α^1	—	46 (62)	21 (43)	36 (50)	38 (54)
	VLA α^2	46 (62)	—	46 (54)	38 (69)	75 (75)
	VLA α^3	21 (43)	46 (54)	—	28 (64)	46 (54)
	VLA α^4	36 (50)	38 (69)	28 (64)	—	46 (69)
	VLA α^5	38 (54)	75 (75)	46 (54)	46 (69)	—
B	hp150	29 (43)	62 (62)	46 (46)	29 (50)	54 (54)
	hMac-1 α	36 (43)	46 (46)	43 (43)	36 (64)	46 (46)
	mMac-1 α	43 (50)	46 (46)	36 (36)	29 (50)	54 (54)
	mLFA-1 α	29 (71)	38 (62)	43 (50)	29 (50)	31 (46)
C	VNR α	36 (55)	36 (45)	36 (36)	36 (55)	60 (60)
	GPIIb	29 (43)	38 (46)	29 (36)	36 (43)	38 (38)
D	PS1	25 (42)	36 (45)	42 (67)	8 (50)	45 (55)
Other proteins	N-CAM	21 (43)	8 (31)	7 (21)	14 (21)	8 (23)
	cFNR β	0 (7)	0 (15)	0 (21)	0 (21)	0 (23)
	LFA, Mac β	0 (7)	8 (8)	0 (7)	0 (0)	0 (0)
	GPIIIa/VNR β	7 (7)	8 (8)	7 (7)	0 (14)	15 (15)

The percent homology is calculated by comparing the 11–14 amino acid residues from each sequence shown in Fig. 2 (listed at the left) to the sequence for each individual VLA α subunit (listed at the top). Percent homologies including conservative amino acid substitutions are shown in parentheses.

The VLA protein α subunits also each showed striking homology to the α subunits of Mac-1, LFA-1, and p150,95 (Fig. 2*B*; Table 1, group B). The degree of homology averaged 40% (51% including conservative substitutions) and thus approached the level occurring within the VLA α subunit family. Notably, the homology illustrated in Fig. 2 *A* and *B* occurred at many of the same positions (compare positions 1–6 and 11). Although previous results have shown that mouse LFA-1 is 32–35% homologous with a segment of interferon α (36), the VLA α subunits were generally only 10–20% homologous to the same interferon α segments (not shown).

The α subunits of the vitronectin receptor and platelet GPIIb/IIIa protein (Fig. 2*C*; Table 1, group C) were also highly homologous to the VLA α subunits, showing an average homology of 37% (46% with conservative substitutions). Likewise, the N-terminal α-subunit sequence from a *Drosophila* PS antigen also was highly homologous to VLA α subunits (average, 31% homology, 52% with conservative substitutions), especially in the highly conserved positions 1–4. Another cell adhesion molecule, N-CAM, showed more limited homology to VLA proteins. However, in this structure as well, homology also occurred largely in positions (residues 2–4) that are most highly conserved among the other proteins listed.

Because the N-terminal amino acid of the human VLA β subunit was blocked, sequence could not readily be obtained. However, an avian analogue of the human VLA β subunit is the band 3 subunit of the chicken fibronectin receptor complex (also called integrin), and its N-terminal amino acid sequence, predicted from the nucleotide sequence (10), is shown (Fig. 2). Also, the N-terminal sequence predicted from the gene for the β subunit of the LFA-1, Mac-1 family is shown (39) as well as the actual N-terminal protein sequence for the β subunit shared by the vitronectin receptor and the GPIIb/IIIa complex (17, 27). Each of these sequences showed only random, low-level background homology to VLA α subunits. These data (Fig. 2; Table 1) support the concept that the α and β subunits are biochemically distinct.

DISCUSSION

The results in this paper (*i*) establish that the five distinct α subunits in the VLA family of heterodimers can be readily immunopurified on a preparative scale, using available mAbs, and (*ii*) confirm that there are at least five different VLA α subunits that are structurally distinct at the amino acid level. These findings are consistent with previous biochemical and immunochemical evidence for five distinct α subunits (4). Some of the VLA proteins (in particular, VLA-5 and VLA-3) have only recently been implicated in extracellular matrix adhesion functions (7). The striking homology seen here among the N-terminal amino acids for all of the VLA α subunits suggests that VLA-1, VLA-2, and VLA-4 may also eventually be shown to have similar substrate adhesion functions.

The level of homology seen within the VLA α subunits was roughly equivalent to the homology seen with α subunits from other families of heterodimers involved in cell or substrate adhesion processes. Like the VLA proteins, the VNR and GPIIb/IIIa proteins are involved in adhesion to extracellular matrix. Similarly, the LFA-1, Mac-1, and p150,95 proteins mediate adhesion functions, but this includes various leukocyte–cell adhesion functions rather than extracellular matrix adhesion. Nonetheless, the α subunits in the LFA-1, Mac-1 family had previously been shown to be homologous to the α subunits in the VNR and GPIIb/III subgroup (17, 26–28). The addition of the VLA proteins (which includes the fibronectin receptor) to this group now makes three subgroups in a superfamily of related structures. Other common features of these families of molecules in addition to (*i*) N-terminal sequence homology and (*ii*) adhesion functions are (*iii*) a pattern of unique α subunits that share a common β subunit within each family and (*iv*) at least one member of each group appears to recognize a site containing the Arg-Gly-Asp sequence in its respective ligand.

Consistent with their α subunit N-terminal sequence homology with VLA proteins, the *Drosophila* PS antigens (29, 30, 37) also have unique α subunits and a common β subunit that are arranged as $\alpha\beta$ heterodimeric structures, similar in size (110,000–150,000 M_r) to VLA proteins. Furthermore, their role in embryogenesis presumably involves cell migration, which would be analogous to known functions for fibronectin and laminin receptors (31). Specific evidence for Arg-Gly-Asp recognition has not yet been obtained for the PS antigens or for several of the other individual heterodimers listed in Fig. 2 (e.g., VLA-1, VLA-2, VLA-4, LFA-1,

p150,95). However, the general similarities within these groups of structures predict that each may eventually be shown to mediate variations of Arg-Gly-Asp recognition. In future studies, it will be important to determine the precise roles for the α and β subunits in the mechanism for Arg-Gly-Asp recognition.

The N-terminal sequence homologies in this paper show conservation in α-subunit structures among different families with diverse adhesion functions and also a high degree of conservation across widely divergent animal species. For example, as seen in Fig. 2, α subunits from human, mouse, and *Drosophila* are all conserved. It is not likely that this conservation is restricted to merely the N-terminus of the α subunits since (*i*) the β subunit common to human LFA-1, Mac-1, and p150,95 is 45% homologous to the avian CSAT (VLA homologue, also called integrin) β subunit (39) and (*ii*) the chicken CSAT complex is antigenically crossreactive with the human VLA antigens (7). The essential conservation of these $\alpha\beta$ heterodimers with diverse adhesion functions in such highly diverse organisms emphasizes their fundamental importance and suggests that they share a common evolution through gene duplication events.

Like the VLA proteins, the neural adhesion molecule N-CAM has multiple protein components in the 120,000–180,000 M_r size region (38). However, there has been no evidence for a N-CAM $\alpha\beta$ heterodimeric subunit structure, and, unlike the VLA proteins, each N-CAM protein component had an identical N-terminal sequence (38). Thus, N-CAM is a cell adhesion molecule that does not appear to be part of the "superfamily" of related adhesion structures. Despite these differences, the limited homology seen between VLA proteins and N-CAM in the highly conserved positions 2–4 suggests that it also could possibly have a common, but more distant, evolutionary origin.

To further investigate the homologies within the VLA α subunit group, and among the variety of α subunits from other homologous structures, the complete gene sequence of each must eventually be obtained. In this regard, the availability of the N-terminal sequence information in this paper will greatly facilitate future studies involving oligonucleotide probe screening as well as evaluating cloned genes for these structures. Also, synthetic peptides based on these N-terminal sequences could potentially be used to generate specific anti-α subunit antisera, which would be useful in a broad range of studies.

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