

MOLECULAR CLONING OF A MURINE FIBRONECTIN
RECEPTOR AND ITS EXPRESSION
DURING INFLAMMATION

Expression of VLA-5 Is Increased in Activated Peritoneal Macrophages
in a Manner Discordant from Major Histocompatibility
Complex Class II

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Macrophages secrete fibronectin (Fn)¹ (1-3), bind Fn (3-5), and have been reported to migrate in response to Fn fragments (6). In addition, the interaction of human macrophages with Fn provides a second signal that leads to enhanced complement and Fc receptor-mediated phagocytosis by these cells (7-14). The structure of the Fn receptor, or receptors, responsible for both adherence to Fn and Fn-mediated phagocytosis enhancement in macrophages is not completely understood. Previous studies have demonstrated membrane proteins on monocytes or culture-derived macrophages that interact with the 110-kD cell-binding domain of Fn (15). These proteins exhibit electrophoretic mobility typical of the recently described Fn receptor, termed VLA-5, purified from placenta, fibroblasts, the MG63 osteosarcoma cell line, erythroblasts, and platelets (reviewed in references 16-18). This receptor is a heterodimer consisting of an ~150-kD α chain and a 130-kD β chain under nonreducing conditions (19). Identification and sequence analysis of cDNA clones for both chains from a placental cDNA library has placed this receptor in the integrin family (18, 20), whose members function in cellular adhesion. The VLA antigens form a part of this family and are defined by having a common β chain that can noncovalently combine with at least six distinct α chains (21-24). We have recently demonstrated by Northern blot analysis the presence of mRNA that hybridizes with probes for both the VLA-5 α and the common VLA β chain in monocytes, as well as in the monocyte-like cell lines HL-60, THP, and U937 (25). In addition, partial cDNA clones that we have isolated from an HL-60 library have shown identical restriction

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¹ *Abbreviations used in this paper:* Fn, fibronectin, GITC/CsCl, Guanidine Isothiocyanate/Cesium chloride; PEC, peritoneal exudate cell.

endonuclease maps and derived amino acid sequence, thus far, when compared with VLA-5 (25), confirming that mononuclear phagocytes express this Fn receptor. However, several studies have identified other, less well-characterized, RGD-binding proteins that also may be involved in monocyte or macrophage binding to Fn (15, 26-29). Therefore, it appears likely that at least two, and possibly more, receptors for Fn are present on monocytes.

The specific role that VLA-5 plays in macrophage biology is not defined. Fn is increased in granulation tissue (30), in healing wounds (31), and in extravascular deposits in inflammatory foci (32). Mediation of adherence and/or chemotaxis (33) to these sites is one likely but unproven role for this receptor. To gain further insight into the *in vivo* biology of phagocyte Fn receptors, we have begun an analysis of the Fn receptors expressed in murine peritoneal macrophages. Here we present a partial sequence analysis of the α chain and complete sequence analysis of the β chain of the murine Fn receptor heterodimer that is the homologue of VLA-5. In addition, we have used three well-characterized models of macrophage activation in murine peritoneal exudate cells (PECs) to determine whether VLA-5 α and β chain mRNA and protein expression can be modulated in response to *in vivo* inflammatory stimuli.

Materials and Methods

Mice. Pathogen-free BALB/c retired breeders were obtained from Harlan-Sprague Dawley, Inc. (Indianapolis, IN) for use in resident, peptone, and thioglycollate-elicited experiments. BALB/c mice used for PEC elicitation using *Listeria monocytogenes* were obtained from Jackson Laboratories (Bar Harbor, ME), and 10-d *Listeria* infected, as well as uninfected, control mice were generously provided by Paul Allen (Washington University, St. Louis, MO).

Library Construction and Identification of Clones. To construct a cDNA library from peritoneal macrophages, cells elicited using thioglycollate were harvested from 60 BALB/c mice at day 3. Total RNA was isolated using the guanidine isothiocyanate, cesium chloride (GITC/CsCl) method (34). Poly(A)⁺ mRNA was isolated using oligo-dT-Sepharose chromatography. A cDNA library was constructed using the RNase H-Pol I method (35) and cloned into λ gt10 after size selection of >1.0 kb. Human cDNA Fn receptor α and β chain probes isolated from an HL-60 cDNA library (25) were labeled by the random primed method (36). The HL-60 cDNA clones were initially obtained by hybridization at high stringency with a partial cDNA provided by S. Argraves and E. Ruoslahti (La Jolla Cancer Research Foundation, La Jolla, CA) (37) for the VLA-5 α chain and at intermediate stringency with a chicken integrin probe provided by R. Hynes (Massachusetts Institute of Technology, Cambridge, MA) (38) for the VLA β chain. Approximately 150,000 amplified recombinant PEC phage clones were probed sequentially initially at high stringency in a buffer containing 5 \times SSC (1 \times = 0.15 M NaCl/0.015 M sodium citrate/0.05 M sodium phosphate, pH 6.5)/1 \times Denhardt's solution (1 \times = 0.02% Ficoll 400/0.02% polyvinylpyrrolidone /0.02% BSA)/50% (vol/vol) formamide/12.5% dextran sulfate/denatured herring sperm DNA (0.1 mg/ml) at 42°C. Blots were then washed at 56°C in a buffer containing 0.2 \times SSC/0.1% SDS, and autoradiographs were developed using Kodak XAR-5 film. The library was further analyzed with the same probes at a lower stringency, in the same conditions, except formamide (30%) and temperature (37°C) and was washed at 37°C in 2 \times SSC/0.1% SDS.

Subclone and Sequence Analysis. Phage clones hybridizing to probes were plaque purified and inserts subcloned into the Bluescript plasmid (Stratagene, San Diego, CA). Because some Eco RI ends were damaged during cloning, flanking Hind III and Bgl II sites in λ gt10 were used to isolate these particular inserts. Clones were sequenced using the Sanger method and modified T7 polymerase (Sequenase; U.S. Biochemical, Cleveland, OH). Specific sequencing oligonucleotides were constructed using the phosphoramidite method (39). Sequence analysis was performed using Microgenie software (Beckman Instruments, Inc., Palo Alto, CA).

Northern Blot Analysis. Total RNA was isolated from tissues and elicited cells using the GITC/CsCl method. Adherent peritoneal macrophages were first treated with GITC and then scraped completely. Northern blot electrophoresis was performed using 0.9% agarose/0.6% formaldehyde gels in a buffer containing 0.02 M 3-(*N*-morpholino) propanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA (disodium salt) in 0.03 μ g/ml ethidium bromide. To assure complete and equal transfer of RNA to the membrane, RNA was electrophoretically transferred to nylon membranes (Micron Systems, Inc., Honeyone Falls, NY). Completeness of transfer was assured by lack of residual RNA staining in the gel and the demonstration of transfer of ethidium bromide-stained RNA to the nylon. cDNA probes as noted below were labeled by the random primed method (36), and blots were hybridized and washed in the same high stringency buffers as above. Blots were dehybridized and reused to obtain quantitative data on relative expression. Quantitation of relative transcript expression was performed using laser densitometry (Ultrascan XL; LKB Instruments, Inc., Gaithersburg, MD), and densitometric values were normalized to 1.0 relative to resting total PECs or adherent macrophages for comparison studies.

cDNA Probes. Plasmids pBSMFNR α -1.4 and pBSMFNR β -3 are as described in results. The β -actin probe is the chicken β -actin cDNA (40) and was provided by J. Milbrandt (Washington University). The Ia probe utilized is the IA^d α chain cDNA of Davis et al. (41) and was provided by K. Murphy (Washington University). The CHO-B cDNA was isolated from Chinese hamster cells (42) and is a gene transcribed ubiquitously with no known modulators of transcription. This was provided by D. Dean (Washington University) and was isolated initially in the laboratory of R. Evans (Salk Institute, La Jolla, CA).

Generation and Isolation of Peritoneal Cells. To elicit peritoneal cells, either 1 ml of 10% peptone (Difco Laboratories Inc., Detroit, MI), 1 ml of 10% thioglycollate broth (Difco Laboratories Inc.), or 100,000 live *Listeria monocytogenes* were injected intraperitoneally at various time points before isolation of cells. Unless otherwise stated, peritoneal cells were isolated at 72–96 h using peptone or thioglycollate and at 10 d using *Listeria*. Resting and elicited peritoneal cells were isolated by irrigation of the peritoneum with sterile cold PBS with 1% FCS. Similar to previous reports, the total intraperitoneal cell population increased two- to eightfold upon addition of these inflammatory stimuli (43). Assessment of cell viability by trypan blue exclusion demonstrated >95% viability in all experiments and usually >99%. Adherent macrophages and nonadherent cells were separated by attachment of isolated PECs to polystyrene tissue culture dishes (Corning Glass Works, Corning, NY) for 2 h at 37°C in a buffer containing DME with 1% FCS in a 5% CO₂ incubator. Nonadherent cells were removed by vigorous rinsing with PBS three times.

Surface Iodination, Solubilization, and Immunoprecipitation. Resting and thioglycollate elicited macrophages were purified by adherence, removed by scraping, counted, and surface labeled with ¹²⁵Iodine (New England Nuclear, Boston, MA) using the lactoperoxidase method (44). 3 × 10⁷ cells of each were solubilized in PBS with 1% NP-40 and protease inhibitors as reported previously (44). After removal of insoluble material by centrifugation at 12,000 *g*, immunoprecipitation of equal cell number aliquots of solubilized membranes was performed. Ab33, a rabbit polyclonal antibody raised to the COOH-terminal 11 amino acids of the human VLA-5 α chain sequence, was used in these studies after affinity purification. Ab33 specifically and quantitatively immunoprecipitates VLA-5 from IMR-90 fibroblasts and localizes with Fn in immunofluorescent studies of these cells (44a). Control nonimmune rabbit Ig was used in equal quantities. Equal cell number aliquots of immunoprecipitated material was analyzed by reducing and nonreducing SDS-PAGE using 7.5% acrylamide followed by autoradiography. Reduction of samples was performed using 5% 2-ME at 80°C for 5 min.

Results

Mouse Fn Receptor α and β Chain cDNA Isolation. Using cDNA probes for both VLA-5 α and β chains that we had isolated from HL-60 cells, we examined a mouse thioglycollate-elicited PEC library at both high and intermediate stringency. Three α chain-related and three β chain-related cDNAs were identified by this method at high stringency, and no further clones were identified at a lower stringency.

Sequence analysis of the longest α chain-related clone, λ MFNR α -4, indicated that it was a partial cDNA (Fig. 1 a). The cDNA encoded an open reading frame that was highly homologous to the COOH-terminal 408 amino acids of the human placental VLA-5 (VLA-5 α) α chain sequence (20). The overall homology, when compared with the corresponding coding region of the human cDNA sequence (Fig.

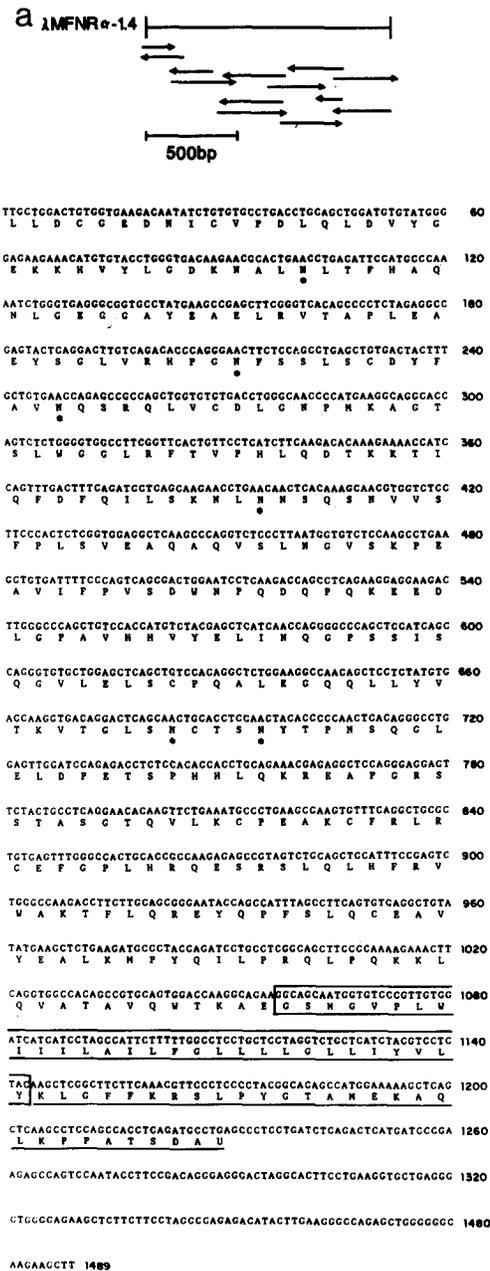


FIGURE 1. Continued on following page.

observation has been made when comparing β chain sequence across species (45, 46), this is the first demonstration of an α chain intracytoplasmic sequence identity across species. Taken together, this very high degree of homology is strong evidence that λ MFNR α -1.4 encodes the mouse homologue of VLA-5 α .

20 of the 42 sequence differences between the mouse and human VLA-5 α chain are clustered in 71 amino acids from the lysine at position 863 to the arginine at 932 (numbering by human sequence). These flank the presumed α chain cleavage site at the arginine-glutamic acid peptide bond at position 894–895. In spite of the sequence differences, comparison of hydrophobicity by the method of Kyte and Doolittle (47) about that site reveals no significant differences. Moreover, like human VLA-5 α , the mouse α chain undergoes a post-translational cleavage (see below).

Analysis of the longest β chain-related cDNA identified 3,491 nucleotides with a very high degree of homology to the human β (VLA β) chain sequence (20), which included the entire translated sequence with the exception of the initiating adenosine of the methionine codon (Fig. 2 *a*). Two potential polyadenylation sites are underlined, one near the 3' end and one \sim 600 nucleotides 5' of this. Comparison of mouse to human sequence in the translated region (Fig. 2 *b*) indicates a high degree of conservation in the coding region in both nucleotide (90%) and derived amino acid sequence (92%). 26 of the 67 amino acid differences are conservative, and there is one extra serine in the mouse sequence. All 56 cysteines are conserved, and the intracytoplasmic sequence is identical to the human VLA β as well as the chicken and *Xenopus laevis* integrin sequences (45). Also, all 12 potential *N*-linked glycosylation sites are conserved between the human and mouse VLA β chains. These data establish λ MFNR β -3 as a true VLA β chain clone.

Tissue Distribution. In man, VLA-5 is part of the VLA family and in some cells is absent or is a minor component of the total VLAs present. To assess relative expression of each chain in mouse tissue, total RNA was isolated from various organs of adult mice and analyzed by Northern blotting (Fig. 3). High level expression of the α chain was restricted to thioglycollate-elicited PECs and was undetectable by this method in other tissues. Using poly(A)⁺ RNA, VLA-5 α chain transcripts could be detected at low levels in heart, lung, spleen, kidney, and liver, but not brain (data not shown). VLA β chain message was expressed at relatively high levels in heart, kidney, brain, and lung. No VLA β chain message was detected by this method in mouse spleen although it could be seen in poly(A)⁺ RNA. Interestingly, two sizes of VLA β chain message were present (Figs. 3 and 4), a phenomenon not demonstrated in human cells. This most likely reflects alternate polyadenylation, as there is a second potential polyadenylation site \sim 600 bp 5' of the end of the cDNA. β -actin probing analysis of this blot (Fig. 3) shows variable levels as expected, but demonstrates no significant degradation of RNA that might account for the differences in transcript levels. In whole tissue, therefore, VLA β chain expression predominates and is likely to be associated with a mouse VLA α chain family similar to the human family.

Regulation of Expression in Elicited Macrophages. Because of the high level of VLA-5 expression in thioglycollate-elicited PECs, we examined the endogenous expression of VLA-5 in peritoneal macrophages and its induction by several inflammatory stimuli in vivo. Instillation of thioglycollate broth, peptone, or *Listeria monocytogenes* into

the mouse peritoneum is reported to lead to an increased number of peritoneal macrophages with an "activated" phenotype (reviewed in references 48 and 49). Activation refers to increased abilities to ingest and kill organisms, kill tumor cells, or increase Ia expression.

To perform our studies, equal amounts of total RNA of resident, peptone-elicited, and thioglycollate-elicited PECs were subjected to Northern blot analysis. Comparison of VLA-5 α and VLA β chain expression in total PECs demonstrated approximately a three- to fourfold increase in VLA-5 α chain expression in thioglycollate-elicited cells compared with resting peritoneal cells (data not shown). VLA β chain message did not change during thioglycollate-elicited inflammation. Because macrophages are only 50–80% of the total PEC population, we further purified them by adherence for 2 h, isolated total RNA, and repeated the Northern blot analysis using equal amounts of RNA in each lane. An example of these experiments is shown in Fig. 4, and results of relative levels of expression of the four probes used are summarized in Table I. Each experiment was repeated at least three times by independent isolation of cells and blot analysis. Relative expression was calculated using laser densitometry and normalizing values to 1.0 in resting cells for each probe.

The results demonstrate a 10-fold increase in VLA-5 α chain message in thioglycollate-elicited macrophages relative to steady state levels in resting peritoneal macrophages. This increase was specific for VLA-5 α , since β actin and Ia message levels were minimally altered or actually decreased. Interestingly, VLA β chain message levels were also unaltered in the thioglycollate-elicited cells, suggesting independent regulation of VLA-5 α and VLA β chain transcript levels. A reproducible approximately threefold increase in VLA-5 α chain message expression was seen in peptone-elicited cells. Time course experiments indicated that the VLA-5 α chain message increase was seen by 48 h in thioglycollate-elicited cells and remained constant through at least 96 h (data not shown). Because Ia expression decreased as VLA-5 α chain expression increased in the thioglycollate-elicited cells, we evaluated the effect of *Listeria* infection, a potent inducer of Ia expression, to determine whether VLA-5 α and Ia message levels were reciprocally regulated. As can be seen in Fig. 4 and is summarized in Table I, *Listeria* infection led to a sixfold increase in VLA-5 α chain message, while Ia expression was increased fourfold. This demonstrated that Ia and VLA-5 α are independently, rather than reciprocally, regulated. Since IFN- γ is the major cytokine responsible for enhancing Ia expression, it is unlikely that IFN- γ is a major factor in regulation of macrophage expression of VLA-5 α . As was seen in thioglycollate-induced inflammation, *Listeria* infection did not significantly alter VLA β chain message levels. There was also no significant alteration in the expression of CHO-B message in the thioglycollate-, peptone-, or *Listeria*-elicited macrophages compared with resting cells (data not shown).

Immunoprecipitation of Murine VLA-5. To determine whether VLA-5 surface protein expression on adherent macrophages was altered in parallel with the VLA-5 α chain message, we immunoprecipitated surface-labeled cells using Ab33. Because we had determined that the mouse intracytoplasmic sequence is identical to human sequence, we were able to use this specific anticytoplasmic domain peptide antibody to identify the receptor on PECs (Fig. 5). After surface labeling adherent macrophages with ^{125}I and immunoprecipitation of solubilized membranes with this an-

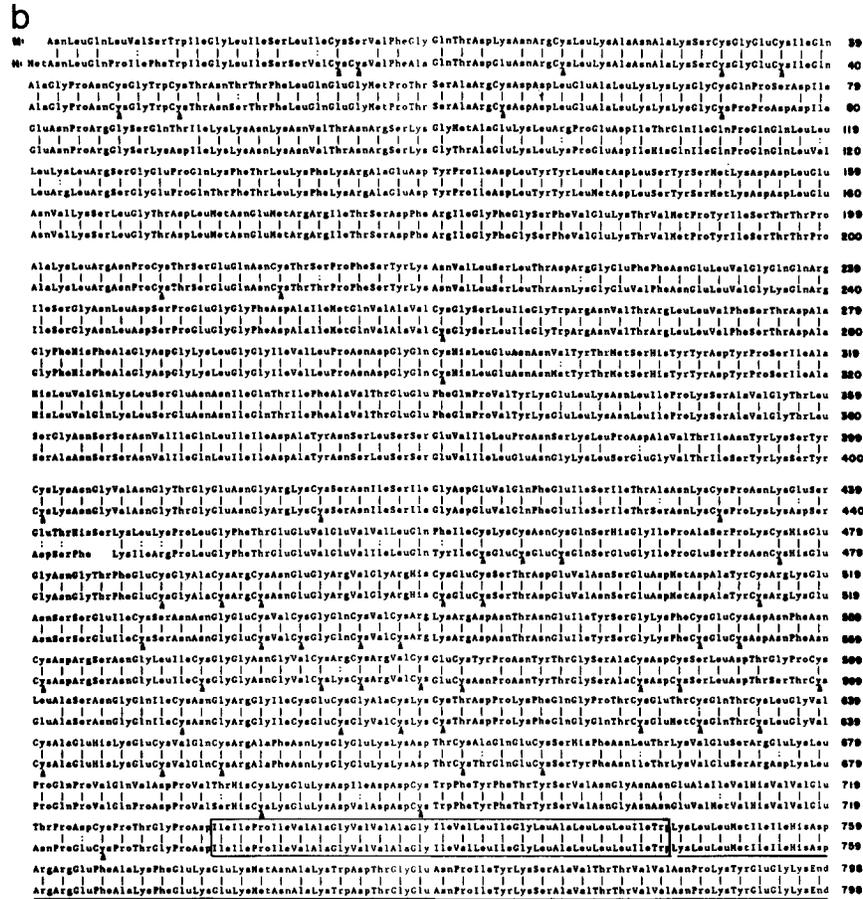


FIGURE 2. A restriction map and sequencing strategy of λ MFNR β -3 is shown above and the nucleotide and derived protein sequence below. The transmembrane sequence is boxed, and the intracytoplasmic sequence is underlined. Potential N-linked glycosylation sites are shown by asterisks. Potential poly-adenylation sites are underlined. (b) Sequence comparison between the human and mouse β chain sequences. The transmembrane region is boxed, and the identical intracytoplasmic sequences are shown. Conserved cysteines are shown by arrowheads. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00818.

tion has been primarily attributed to the release of a small COOH-terminal L chain fragment. This suggests that, despite the differences in amino acid sequence between mouse and human VLA-5 α chain around the proposed cleavage site, proteolytic processing of VLA-5 α occurs in murine cells. Decreased mobility of the VLA β chain is presumed due to reduction of disulfide bonds in the cysteine-rich region. The electrophoretic mobility in these cells appears similar to that of the Fn receptor detected in a mouse pre-B cell line (50).

In comparison of expression of VLA-5 on resident macrophages to thioglycollate-elicited macrophages (Fig. 5, lanes 2 and 6 vs. lanes 4 and 8), it is readily apparent

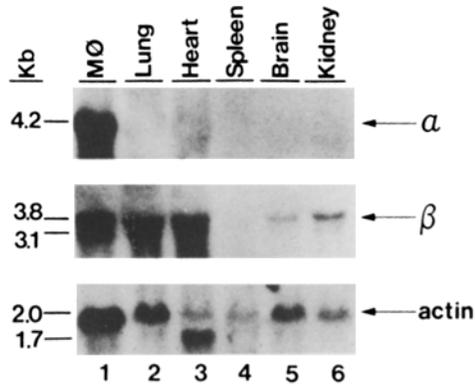


FIGURE 3. Tissue distribution Northern blot analysis using 15 μ g of total RNA per lane. Probes used sequentially on this single blot are the inserts of pBSMFNR α -1.4(α), pBSMFNR β -3(β), and β -actin (actin). The presence of two transcripts in lane 3 (heart) most likely represents crosshybridization of this cDNA probe to smaller cardiac α -actin transcripts, as has been previously demonstrated (58). Tissues of origin are: Lane 1; thioglycollate-elicited PECs; lane 2, lung; lane 3, heart; lane 4, spleen; lane 5, brain; and lane 6, kidney. Sizes of transcripts are shown at left.

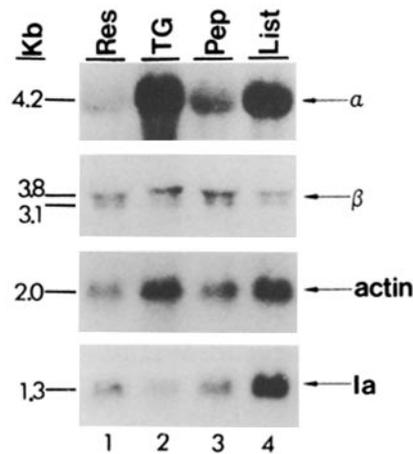


FIGURE 4. Northern blot analysis using 15 μ g of total RNA of adherent PECs isolated as resident (lane 1), thioglycollate elicited (lane 2), peptone elicited (lane 3), or *Listeria monocytogenes* elicited (lane 4). Probes used sequentially on this single blot are the same as Fig. 3 with the addition of an Ia probe (see Materials and Methods). Sizes of individual transcripts are shown at left.

TABLE I
Laser Densitometric Analysis by Northern Blot

Probe	Stimulus			
	Resident	Thioglycollate	Peptone	Listeria
pBSMFNR α -1.4	1.0	10.8 (6.9-18.3)	3.1 (2.0-4.9)	5.7 (4.9-6.6)
pBSMFNR β -3	1.0	1.1 (0.5-1.4)	1.7 (0.9-2.6)	1.1 (1.05, 1.09)
Ia	1.0	0.26 (0.1-0.5)	1.4 (0.9-2.1)	4.0 (3.2, 5.0)
β -actin	1.0	2.2 (1.5-2.6)	1.4 (1.3-1.5)	-

Data from laser densitometric analysis of relative expression of each gene by Northern blot analysis is summarized as the mean and range. $n \geq 3$ for all conditions except *Listeria* β chain and Ia expression. All resting macrophage values were normalized to 1.0 for these comparisons.

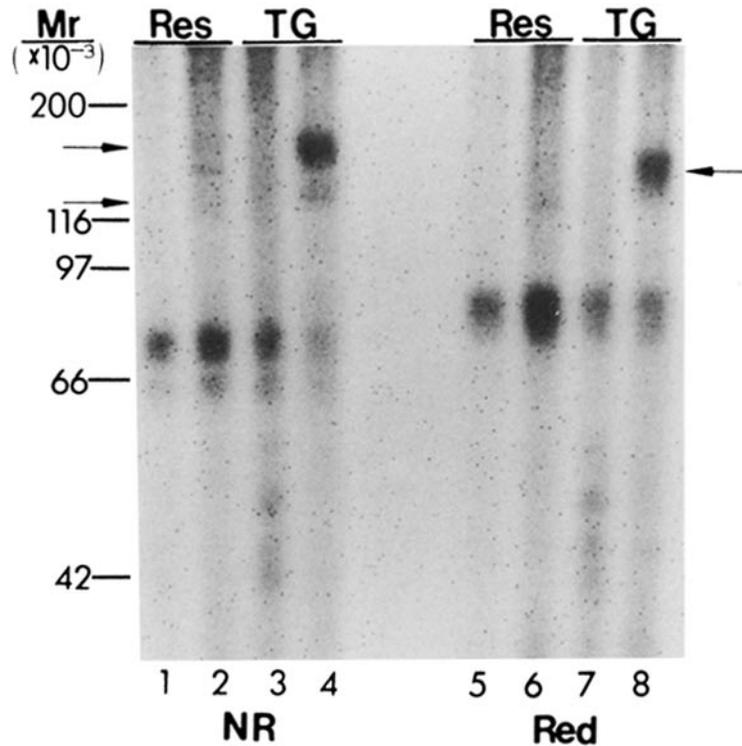


FIGURE 5. Immunoprecipitation of surface-labeled adherent resident (lanes 1, 2, 5, and 6) or thioglycollate elicited (lanes 3, 4, 7, and 8) macrophages. Ab33 was used in lanes 2, 4, 6, and 8 and the control antiserum in lanes 1, 3, 5, and 7. Precipitates were nonreduced in lanes 1-4 and reduced in lanes 5-8. Molecular weights are shown at the left. VLA-5 bands are demonstrated in lanes 4 and 8 by arrows.

that the differences in expression seen using Northern blot analysis are also reflected in surface expression of the receptor. In the resting state, little or no receptor is detectable by this method, whereas receptor is easily detected in immunoprecipitates from thioglycollate-elicited adherent cells.

Discussion

In this report, we have characterized a partial cDNA clone encoding the mouse VLA-5 α chain and a full coding clone of the mouse VLA β chain. Comparison of mouse to human VLA-5 α chain sequence demonstrates a striking complete conservation of the intracytoplasmic sequence. As this is also seen with β chain sequence, a strong evolutionary drive must be exerted to maintain this identity of both chains, suggesting that each may play an important role in VLA-5 function. The sequence conservation of α and β chains also likely indicates structural constraints on the evolution of the entire intracytoplasmic domain of both chains, presumably to interact with each other and/or with other highly conserved sequences of intracytoplasmic or membrane-associated proteins.

Although there is more sequence divergence about the putative VLA-5 α chain

cleavage site, α chain cleavage appears to occur in these cells. This is indicated by the appropriate increase in α chain mobility on reducing SDS-PAGE (Fig. 5, lane *B*). In some instances of protein cleavage (for example, signal peptide cleavage), hydrophobicity about the cleavage site plays a major role in the signal for cleavage. Analysis of hydrophobicity plots about the α chain cleavage site reveal no significant difference preceding the cleavage site between mouse and human VLA-5 α . The highly charged lysine-arginine dipeptide, which is immediately NH₂ terminal of the putative human cleavage site, is also conserved.

A comparison of VLA β chain sequence between mouse and human reveals a high degree of sequence homology as well as complete intracytoplasmic sequence identity with human, chicken, and *Xenopus* sequence. Interestingly, all of the potential *N*-linked glycosylation sites are conserved between mouse and human sequence. This may indicate some important role of glycosylation in the stable association of α and β chains with each other or with Fn. The overall high degree of homology of human and mouse β chains suggests the possibility that the human α chain could associate with the mouse β chain to form an intact VLA-5 protein, but this remains to be formally tested.

Comparison of our partial murine VLA-5 α chain sequence with other integrin α chain sequences reveals low but significant levels of homology (23–40%) (data not shown), as has been noted previously comparing these sequences to human VLA-5 α chain sequence (51–54). This is not surprising in light of the high degree of homology between the human and murine VLA-5 α chain sequence. Comparison of murine VLA β chain sequence with the other two integrin β chain sequences, human Mac-1 β chain and human gpIIIa (55–57), reveals ~45% homology to both (data not shown). This degree of homology has also been previously noted comparing these three human β chain sequences.

Of most interest is the increased expression of α chain mRNA induced by two nonspecific inducers of inflammation, thioglycollate and *Listeria*. A less pronounced but reproducible increase was also found using peptone, another inflammatory stimulus. This increase is seen without specific change in β chain message expression, in two other control genes (β -actin and CHO-B), and with an appropriate increase in Ia expression using *Listeria*. Immunoprecipitation analysis also demonstrates that surface expression of VLA-5 is increased in thioglycollate-elicited cells.

It is tempting to speculate that there is a correlation between the observed increase in mouse VLA-5 expression in thioglycollate-elicited peritoneal macrophages, the high phagocytic activity of these cells, and *in vitro* experiments demonstrating enhanced phagocytosis by human macrophages after interaction with Fn. Whether Fn-mediated phagocytosis enhancement is mediated via this receptor or whether other Fn receptors primarily mediate this effect is incompletely understood and is the subject of ongoing experiments in our laboratories. The role of increased murine VLA-5 expression in thioglycollate-elicited macrophages may be to mediate chemotaxis to Fn fragments, adherence to inflammatory sites, or combinations of these or other inflammatory functions. Further study should allow us to answer these questions using this *in vivo* model.

This alteration in VLA-5 α chain message without change in VLA β chain message leads us to conclude that the two genes are regulated differently and that, at

least for inflammatory stimuli, alteration of surface receptor expression correlates most closely with changes in VLA-5 α chain message. This may occur via two possible mechanisms. One possibility is that multiple VLAs are expressed on macrophages and that stimuli that enhance VLA-5 expression coordinately decrease expression of other, as yet unidentified, VLA α chains. This would leave the requirement for VLA β chain expression unchanged in resting and activated cells and thus might leave message levels unaffected. On the other hand, it is possible that there is a large intracellular pool of VLA β chains in resting cells that can combine with newly synthesized α chains, allowing surface expression of any particular VLA to be regulated exclusively by transcription of the relevant α chain gene. Further work is required to distinguish these possibilities, but biosynthetic experiments in fibroblasts demonstrating a large intracellular pool of VLA β chains (57) are consistent with the latter hypothesis.

Finally, our data suggest that VLA-5 expression is regulated independently of Ia. Since Ia expression is dependent on IFN- γ , it is unlikely that IFN- γ plays a major role in regulation of VLA-5. Whereas many other cytokines are potential regulators, our recent work suggests that VLA-5 α chain message expression can increase similarly during *in vitro* culture of resting peritoneal macrophages (data not shown). This points to the possible role of an autocrine or paracrine pathway related to macrophage secretion of cytokines such as IL-1 or TNF, or to the synthesis of Fn itself. The marked increase in VLA-5 α chain expression during inflammation points to a potentially important role for this receptor in the inflammatory process.

Summary

Human fibronectin receptor (VLA-5) α and β chain probes were used to identify their mouse homologues in a thioglycollate-elicited peritoneal exudate cell cDNA library. Sequence analysis of both α and β chain-related murine clones revealed ~90% homology to their human counterparts by both nucleotide and derived amino acid sequence comparisons. Detectable α chain transcripts were seen predominantly in total RNA of peritoneal macrophages. β chain expression, however, was detected at higher levels in lung, heart, brain, and kidney, suggesting the presence of a large murine VLA family similar to the human family. Analysis of levels of expression comparing resting peritoneal macrophages with macrophages elicited using inflammatory stimuli indicated that α chain message and surface VLA-5 expression were significantly increased using thioglycollate or *Listeria monocytogenes* as stimuli to elicit cells. Interestingly, β chain message was unaffected by these inflammatory stimuli, suggesting that VLA-5 expression is regulated by VLA-5 α chain message levels. These results indicate that macrophage VLA-5 expression can be modulated *in vivo* and may provide an important mechanism by which macrophages are recruited to or adhere to fibronectin in inflammatory foci.

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