Peptide Sequences Selected by BA4, a Tropoelastin-Specific Monoclonal Antibody, Are Ligands for the 67-Kilodalton Bovine Elastin Receptor†

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ABSTRACT: A 67-kDa cell-surface elastin/laminin receptor is expressed by fetal bovine ligamentum nuchae fibroblasts and neutrophils. Two hexapeptides, VGVAPG and PGAIPG, contained within hydrophobic domains of tropoelastin are binding sites for this receptor. Studies of recombinant tropoelastin proteins and synthetic peptides demonstrated that a monoclonal antibody, BA4, recognized peptide sequences similar to those recognized by the 67-kDa receptor. Taking advantage of this similarity, an "epitope library" containing random hexapeptides was screened with BA4. Four BA4-selected peptides (VGAMPG, VGMAPG, VGSLPG, and VGLSPG) were synthesized; studies of fibroblast and neutrophil migration support the hypothesis that these peptides are ligands of the 67-kDa receptor present on ligamentum nuchae fibroblasts and neutrophils. Two additional, physically similar tropoelastin peptides, AGAIPG and PGAVGP, were also identified as peptide ligands, and hence potential binding sites within tropoelastin, of the elastin receptor. These data suggest that the 67-kDa elastin/laminin receptor may interact with a wide range of structurally similar peptides containing amino acid substitutions involving small nonpolar and uncharged amino acids.

Fetal bovine fibroblasts and chondrocytes, as well as circulating phagocytes, express a 67-kDa elastin receptor that is structurally identical to the 67-kDa high-affinity, metastasis-associated laminin receptor (Wrenn et al., 1988; Mecham et al., 1989a,b; Senior et al., 1989; Mecham, 1991). This protein is a peripheral membrane protein with antigenic and functional properties of a galactoside lectin (Hinek et al., 1988; Mecham et al., 1989a,b; Grosso & Scott, 1993). The interaction of elastin and tropoelastin with this receptor is responsible for directed cell migration to tropoelastin, proteolytic products of elastin, and synthetic tropoelastin peptides (Senior et al., 1984; Wrenn et al., 1986, 1988; Mecham et al., 1989a,b). In elastogenic cells, this receptor may play a role in directing or aiding the integration of tropoelastin into the extracellular matrix (Hinek et al., 1988).

Tropoelastin is a heterogeneous mixture of protein isoforms. While differing in primary structure, the isoforms maintain an alternating hydrophobic–hydrophilic domain structure (Boyd et al., 1991; Indik et al., 1991). Sequences within the hydrophobic domains are ligands for the elastin receptor (Boyd et al., 1991; Indik et al., 1991). The 67-kDa elastin receptor binds the hydrophobic hexapeptides VGVAPG[1] and PGAIPG contained in bovine and human tropoelastin (Senior et al., 1980, 1984; Grosso & Scott, 1993) and also binds a laminin peptide, LGTIPG (Mecham et al., 1989a,b). While these peptides are hydrophobic and physically similar, the difference in their chemical structure suggests that a range of conservative amino acid substitutions may be tolerated without interfering with the high-affinity binding interaction.

In recent studies, recombinant techniques have been used to produce deletion proteins of bovine tropoelastin that maintain the biological properties of the full-length protein (Grosso et al., 1991; Grosso & Scott, 1993). Here we report further characterization and studies utilizing these deletion proteins. Two such proteins, SE163 and SE173,[2] contained 20 kDa of tropoelastin protein sequence but lacked the VGVAPG hexapeptide that has been shown to define a receptor binding site (Grosso & Scott, 1993). SE163 and SE173, however contain a second peptide, PGAIPG, that binds to the 67-kDa elastin receptor (Grosso & Scott, 1993). Interestingly, these two recombinant elastin-derived proteins remained reactive with BA4, a monoclonal antibody that has been suggested to react with sequences similar to the binding site of the 67-kDa elastin/laminin receptor (Mecham & Lange, 1982; Senior et al., 1984; Wrenn et al., 1986; Grosso et al., 1991). Importantly, BA4 prevented fibroblasts attachment to SE163 and SE173. These findings, together with slot blot experiments with synthetic peptides, demonstrated that BA4 detected peptides that were ligands of the 67-kDa elastin receptor. To utilize the ability of BA4 to react with receptor recognition sequences, an "epitope library" containing random hexapeptides was screened with purified antibody (Parmley & Smith, 1988; Scott & Smith, 1990). Four candidate sequences, VGAMPG, VGMAPG, VGSLPG, and VGLSPG, were selected for further study. The chemotactic response of fibroblasts and neutrophils demonstrated that these peptides are ligands for the 67-kDa elastin receptor. Two additional tropoelastin peptides, AGAIPG and PGAVPG, selected because of structural similarity with identified ligands of the elastin receptor, were also shown to interact with the 67-kDa elastin receptor.

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[1] Hexapeptide designations use the one-letter amino acid code: V, valine; G, glycine; A, alanine; P, proline; I, isoleucine; L, leucine; T, threonine; M, methionine; S, serine.

[2] Abbreviations: FCL, late gestation bovine ligamentum nuchae fibroblasts (specified by estimated gestational age and passage number); SE163 and SE173, recombinant tropoelastin proteins produced by *E. coli* containing the plasmids pSE163 and pSE173; Protein A′, cyanogen bromide cleaved Protein A isolated from *E. coli* containing the plasmid pR1T2T; HPF, high power field; HPG, 5 × 5 grid superimposed on the high power field; PBS, phosphate-buffered saline.

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MATERIALS AND METHODS

Protein Isolation and Characterization and Screening of the Epitope Library. Recombinant tropoelastin proteins (SE163 and SE173) were isolated and purified from *Escherichia coli* strain N4830-1 containing the modified Protein A expression vectors pSE163 and pSE173 as described (Grosso & Scott, 1993). SDS–PAGE and Western blotting of recombinant proteins was as previously described (Grosso et al., 1991; Grosso & Scott, 1993).

An epitope library containing random hexapeptides inserted into the coat protein of filamentous bacteriophage was a generous gift of Dr. G. P. Smith (Parmley & Smith, 1988; Scott & Smith, 1990). Propagation, screening, and characterization of clonal isolates of bacteriophage were as described (Parmley & Smith, 1988; Scott & Smith, 1990). To corroborate the BA4 reactivity of selected bacteriophage, clonal isolates of bacteriophage were directly transferred to nitrocellulose and detected as described for Western blots (Grosso et al., 1991; Grosso & Scott, 1993).

Cell Isolation, Culture, and Migration Assay. Primary explants of late gestation fetal bovine ligamentum nuchae fibroblasts were established and maintained as described (Mecham et al., 1981; Senior et al., 1982, 1984). All experiments utilized fibroblasts from passages 1–3. Circulating neutrophils were isolated from peripheral blood as previously described (Senior et al., 1980, 1989).

Cell migration was assessed with modified Boyden chambers (Senior et al., 1980, 1982; Mecham, 1987). The wells (0.7 cm in diameter) were separated into upper and lower chambers by filter sandwiches composed of 8-μm Nucleopore (Costar, Cambridge, MA) and 0.25-μm Millipore (for fibroblasts) or 2-μm Nucleopore and 0.25-μm Millipore (for neutrophils). Fibroblasts (1.2×10^5 cells/mL) or neutrophils (1.5×10^6 cells/mL) contained in 0.34 mL of medium were placed in the top chamber. In some experiments, 20 mM lactose was added to the culture medium 18 h prior to assay and all subsequent buffers and culture medium contained lactose (Hinek et al., 1988; Grosso & Scott, 1993). For desensitization, isolated neutrophils were incubated with the desensitizing peptide for 30 min at room temperature (Senior et al., 1980, 1984, 1989). After washing to remove the test peptide, the migration of neutrophils to the second peptide was assessed. Migration was assessed after incubating in a humidified, 5% CO_2 atmosphere for either 6 h (fibroblast) or 1 h (neutrophil). At the end of the assay, the filter sandwich was removed and the entrapped and attached cells were fixed in ethanol and stained with hematoxylin. In each experiment, each peptide concentration was studied in three wells; five fields, chosen at random, were assessed per well. Quantification of the migrating cells (present between the filters) was with an Olympus binocular microscope using 400× fields (fibroblast) or a 5 × 5 grid superimposed on the 400× field (neutrophil). Net cell migration was the number of cells migrating to a stimulus minus the background migration to medium alone.

Fibroblast attachment was assayed as previously described (Grosso et al., 1991). All assays were performed at least twice.

Peptide Preparation, Purification, and Slot Blot. Peptides were synthesized with an Applied Biosystems peptide synthesizer using *t*-Boc chemistry; the sequence of each peptide was confirmed using an Applied Biosystems protein sequencer, Model 473. After cleavage the peptides were purified by HPLC on a semipreparative C_{18} HPLC column; elution was with a 0–60% acetonitrile gradient in H_2O containing 0.1% trifluoroacetic acid (Grosso & Scott, 1993). Peptide-containing fractions were pooled and lyophilized. The identity of the peptides was verified by determination of the amino acid composition after acid cleavage.

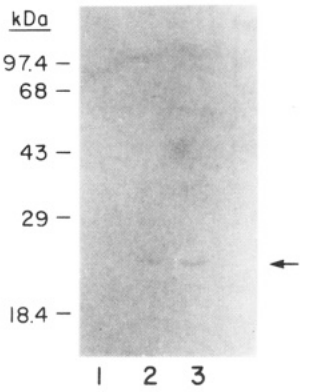

FIGURE 1: Western blot analysis SE163 and SE173. Isolated and cleaved tropoelastin proteins were electrophoretically separated (SDS–12% PAGE) and transferred to nitrocellulose. Detection was with BA4 and a goat anti-mouse alkaline phosphatase antibody. Lane 1 is Protein A′, lane 2 is SE163, and lane 3 is SE173.

For slot blot analysis, peptides were dissolved in phosphate-buffered saline (PBS) and transferred to 0.05-μm nitrocellulose (Schliecher and Scheull, Keene, NH) with a Bethesda Research Laboratories slot blot apparatus (Bethesda, MD). After transfer, the nitrocellulose filters were air-dried. Blocking of the filters and antibody detection was as described above for Western blotting (Grosso et al., 1991; Grosso & Scott, 1993).

RESULTS

The recombinant tropoelastin proteins, SE163 and SE173, were constructed by replacing the C-terminal portion of the coding sequence of bovine tropoelastin with a short duplex oligonucleotide containing stop codons (Grosso & Scott, 1993). The deleted region (the 3′ end of exon 23 and contiguous exons 24–36) includes the VGVAPG repeat of bovine tropoelastin (Grosso & Scott, 1993). Previously BA4, a monoclonal antibody generated to bovine elastin, had been shown to react with the VGVAPG hexapeptide (Wrenn et al., 1986). Since neither SE163 nor SE173 contains VGVAPG sequences, it was anticipated that SE163 and SE173 would not be detected by BA4 (Grosso & Scott, 1993). However, under conditions where Protein A′ was not detected, SE163 and SE173 showed reactivity with the BA4 antibody (Figure 1).

In addition to reacting with tropoelastin, BA4 greatly reduces the chemotactic response of fibroblasts to proteolytic products of tropoelastin and prevents fibroblast attachment to recombinant bovine tropoelastin (Mecham & Lange, 1982; Senior et al., 1984; Wrenn et al., 1986; Grosso et al., 1991). Previously we have shown that SE163 and SE173 support fibroblast attachment (Grosso & Scott, 1993). We, therefore, examined the effect of BA4 on fibroblast adhesion to SE163 and SE173. After the wells were coated with test proteins, but prior to introduction of fibroblasts, the wells were incubated with BA4. While incubation with BA4 did not affect cell attachment to fetal calf serum-coated wells, adhesion to SE163 and SE173 was dramatically reduced (Figure 2). This suggested that BA4 was interacting with SE163 and SE173 at or near the binding site for the elastin receptor and preventing fibroblast attachment.

Because SE163 and SE173 retained the ability to interact with the 67-kDa elastin receptor and were reactive with the

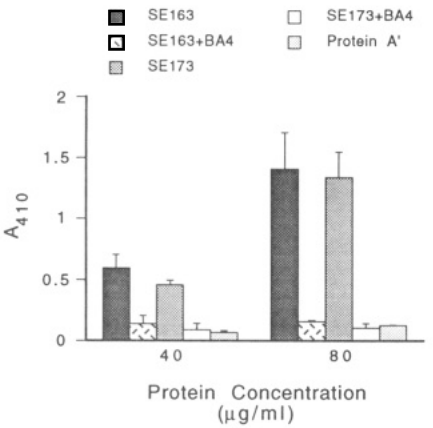

FIGURE 2: Ligamentum nuchae fibroblast adhesion to SE163 and SE173 is inhibited by BA4. The ELISA wells were coated with 100 μL of SE163, SE173, fetal calf serum (diluted 1:50 in PBS), or Protein A′. Prior to the introduction of fibroblasts (FCL-250, P2), the wells were incubated with 100 μL of either PBS or PBS containing 50 μg/mL BA4. After washing, fibroblast attachment was assayed as described. Attachment to fetal calf serum was 1.85 ± 0.2 when incubated with PBS and 1.79 ± 0.1 when incubated with PBS containing BA4. Data are the mean with standard error.

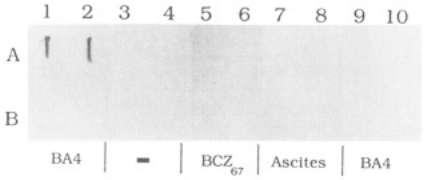

FIGURE 3: Slot blots of purified peptides. Purified peptides, dissolved in PBS, were transferred to 0.05 μM nitrocellulose using a slot blot apparatus. After being dried, the nitrocellulose membrane, containing 20 μg of peptide/slot, was sequentially incubated with the indicated antibody and goat anti-mouse alkaline phosphatase antibody. A1, A3, A5, and A7 are PGAIPG; A2, A4, A6, and A8 are VGVAPG; B1, B3, B5, and B7 are GAIPG; B2, B4, B6, and B8 are VGVPG; and A9, A10, B9, and B10 contain no peptides.

BA4 antibody, we investigated the possibility that BA4 was recognizing binding sites for the elastin receptor by determining whether BA4 would react with synthetic peptides containing VGVAPG-like sequences. After direct transfer of the peptides to nitrocellulose, the membranes were sequentially incubated with primary antibody and an alkaline phosphatase-conjugated detecting antibody. The chemotactic peptides (VGVAPG and PGAIPG), but not the inactive (VGVPG) or chemokinetic peptides (GAIPG), were detected by BA4 (Figure 3). If either BA4 or the peptides were absent or BA4 was replaced with either an irrelevant monoclonal antibody [BCZ_{67} (Mecham et al., 1988)] or antibodies isolated from control ascites, no signal was seen (Figure 3). BA4, therefore, appeared to have a specificity similar to that of the bovine elastin receptor.

Because the BA4 antibody seemed to recognize the same sequences as the elastin receptor, an epitope library containing random hexapeptides was screened with BA4 (Parmley & Smith, 1988; Scott & Smith, 1990). DNA sequencing of antibody-selected bacteriophage identified the reactive antigenic peptides.

Of the BA4-reactive peptides, 70% were of the xGxxPG form, and the remainder were of the xGxPGx form. Four of the identified peptides (VGAMPG, VGMAPG, VGSLPG, and VGLSPG) were synthesized and purified. These four peptides were selected because they contained noncharged amino acids. In addition, they were pairwise identical except for the reversal of the internal amino acids. To determine if these hexapeptides interacted with the 67-kDa elastin receptor, the migratory response of fetal bovine ligamentum nuchae

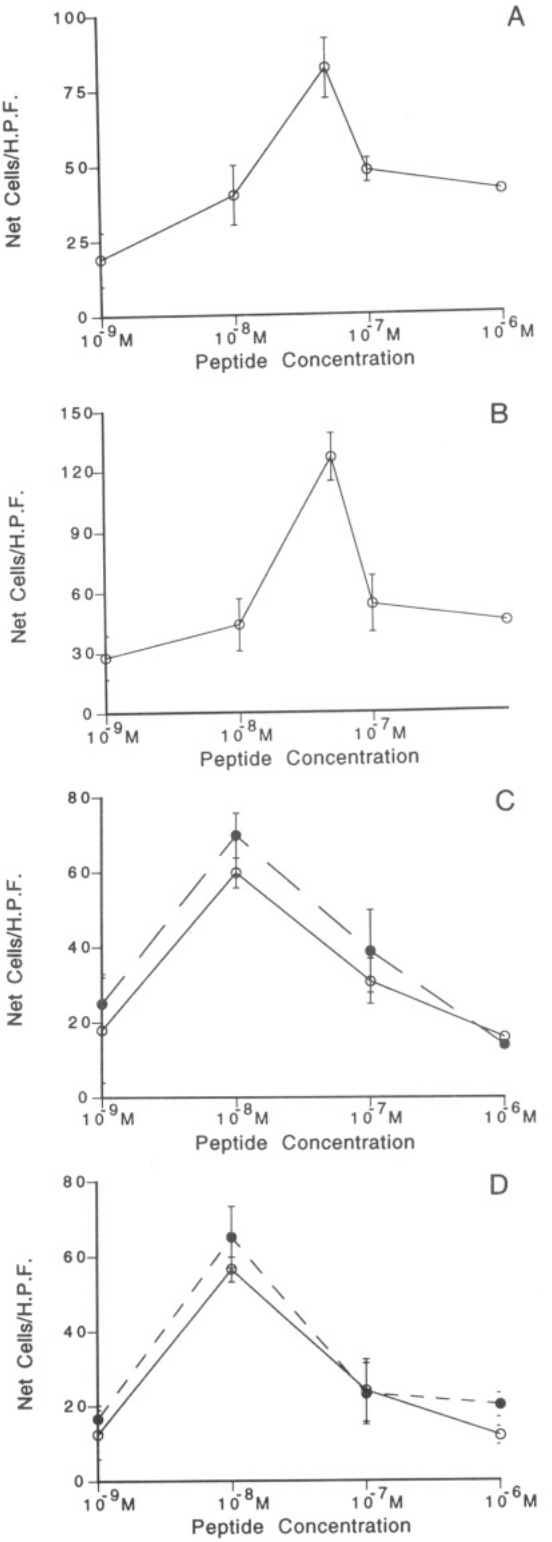

FIGURE 4: Fibroblast migration to purified peptides. (A) Migration to VGAMPG. FCL-240, P2; migration to VGVAPG (10^{-8} M) was 101 ± 5. (B) Migration to VGMAPG. FCL-240, P3; migration to VGVAPG (10^{-8} M) was 110 ± 14. (C) Migration to VGLSPG (●) and VGSLPG (○). FCL-270, P1; migration to VGVAPG (10^{-8} M) was 63 ± 23. (D) Migration to AGAIPG (●) and PGAVPG (○). FCL-300, P3; migration to VGVAPG (10^{-8} M) was 63 ± 3. Data are the mean with the standard error, n = 15.

fibroblasts and neutrophils was studied. While neither cell type migrated to VGVPG [a nonchemotactic, hydrophobic peptide (Grosso & Scott, 1993)], both cell types migrated to the BA4-selected peptides. The maximal response occurred at 5×10^{-8} M for VGMAPG and VGAMPG and at 1×10^{-8} for VGSLPG and VGLSPG (Figures 4 and 5). The maximal

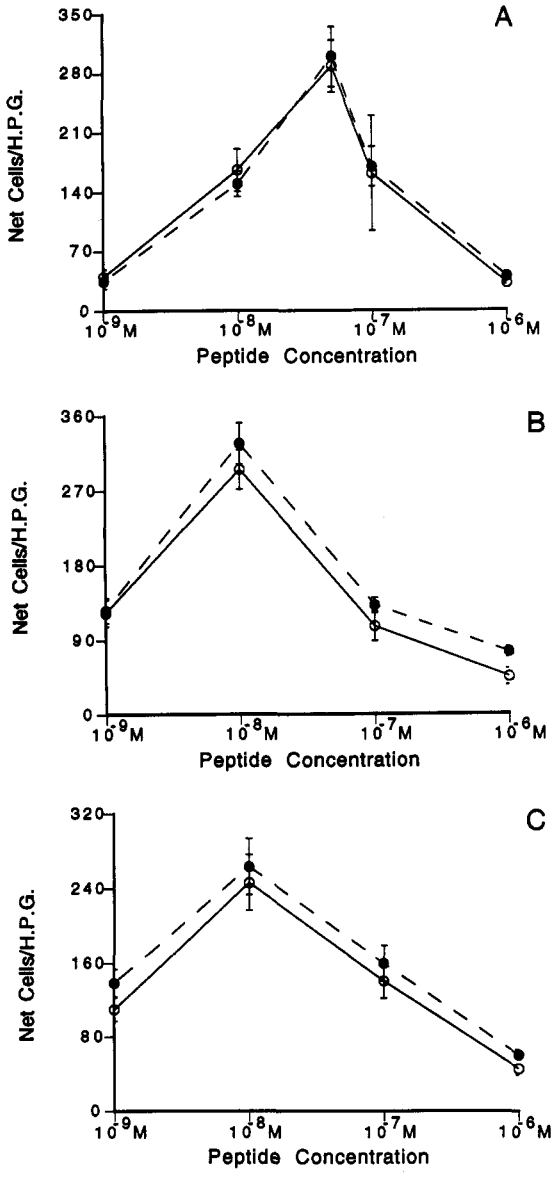

FIGURE 5: Neutrophil migration to purified peptides. (A) Migration to VGMAPG (●) and VGAMPG (○). Migration to VGVAPG (10^{-8} M) was 276 ± 6. (B) Migration to VGLSPG (●) and VGSLPG (○). Migration to VGVAPG (10^{-8} M) was 272 ± 15. (C) Migration to AGAIPG (●) and PGAVPG (○). Migration to VGVAPG (10^{-8} M) was 237 ± 11. Data are the mean with the standard error, n = 15.

Table I: Summary of Checkerboard Analysis of Fibroblast Migration to Synthetic Peptides

peptide	peptide concn (M)	medium/ peptide[a]	peptide/ medium	peptide/ peptide	cells
VGSLPG	1×10^{-8}	46 ± 2[b]	7 ± 1	7 ± 3	FCL-270, P3
VGLSPG	1×10^{-8}	47 ± 2	10 ± 1	10 ± 3	FCL-270, P3
VGAMPG	5×10^{-8}	82 ± 10	12 ± 8	11 ± 7	FCL-240, P2
VGMAPG	5×10^{-8}	127 ± 12	26 ± 4	27 ± 10	FCL-240, P3
PGAVPG	1×10^{-8}	48 ± 4	1 ± 1	2 ± 5	FCL-270, P2
AGAIPG	1×10^{-8}	44 ± 9	5 ± 1	5 ± 5	FCL-270, P2

[a] Top chamber/bottom chamber. [b] Net cells/HPF (mean with the standard error, n = 15).

number of cells migrating was identical to that of VGVAPG (used at its maximal effective concentration). Checkerboard analysis indicated that the peptides were chemotactic for both fibroblasts and neutrophils (summarized in Tables I and II).

To demonstrate that the peptides were inducing cell migration via the 67-kDa elastin receptor, the effect of lactose on fibroblast migration and the desensitization of neutrophil

Table II: Summary of Checkerboard Analysis of Neutrophil Migration to Synthetic Peptides

peptide	peptide concn (M)	medium/ peptide[a]	peptide/ medium	peptide/ peptide
VGSLPG	1×10^{-8}	296 ± 24[b]	39 ± 8	36 ± 7
VGLSPG	1×10^{-8}	327 ± 25	54 ± 27	46 ± 5
VGAMPG	5×10^{-8}	289 ± 31	51 ± 7	13 ± 13
VGMAPG	5×10^{-8}	374 ± 37	62 ± 11	26 ± 24
PGAVPG	1×10^{-8}	247 ± 32	32 ± 12	26 ± 7
AGAIPG	1×10^{-8}	264 ± 30	49 ± 13	37 ± 15

[a] Top chamber/bottom chamber. [b] Net cells/HPG (mean with the standard error, n = 15).

Table III: Inhibition of Peptide-Induced Fibroblast Migration by Lactose

peptide	peptide concn (M)	control[a]	lactose[b]	cells
VGAMPG	5×10^{-8}	192 ± 10[c]	14 ± 8	FCL-260, P1
VGMAPG	5×10^{-8}	166 ± 7	13 ± 8	FCL-270, P3
VGSLPG	1×10^{-8}	54 ± 6	3 ± 8	FCL-270, P2
VGLSPG	1×10^{-8}	52 ± 8	8 ± 1	FCL-270, P2
PGAVPG	1×10^{-8}	53 ± 6	9 ± 4	FCL-240, P3
AGAIPG	1×10^{-8}	48 ± 2	4 ± 3	FCL-240, P3

[a] Cell migration to peptides in medium lacking lactose. [b] Fibroblast migration to peptides in medium containing 20 mM lactose. [c] Net cells/HPF (mean with the standard error, n = 15).

Table IV: Desensitization of Neutrophils by Hexapeptides

	desensitizing peptide					
peptide	peptide	f-MLP	VGVAPG	PGAIPG	VGVPG	GAIPG
VGAMPG	4 ± 6[a]	95 ± 7	2 ± 8	2 ± 5	94 ± 9	86 ± 10
VGMAPG	1 ± 17	88 ± 10	1 ± 15	2 ± 14	91 ± 8	83 ± 13
VGSLPG	5 ± 9	94 ± 11	6 ± 13	4 ± 9	88 ± 15	92 ± 12
VGLSPG	3 ± 7	92 ± 14	7 ± 7	8 ± 4	94 ± 15	91 ± 17
PGAVPG	1 ± 11	93 ± 15	5 ± 12	7 ± 11	88 ± 13	82 ± 10
AGAIPG	9 ± 9	90 ± 13	5 ± 12	4 ± 11	93 ± 7	94 ± 12

[a] Percent of control neutrophil migration.

migration was studied. Exposure of fibroblasts to lactose inhibits the chemotactic response to peptide ligands of the elastin receptor. While no effect on fibroblast migration to PDGF was seen (data not shown), the chemotactic response of fibroblasts to VGAMPG, VGMAPG, VGSLPG, and VGLSPG was inhibited by lactose (Table III). Desensitization of neutrophils with VGAVPG and PGAIPG specifically desensitized neutrophils to migration induced by VGMAPG, VGAMPG, VGSLPG, and VGLSPG (Table IV). Additionally, fMLP, GAIPG, and VGVPG had no effect on neutrophil migration to these peptides (Table IV).

On the basis of the apparent ability of chemically distinct, but physically similar peptides to bind to the 67-kDa bovine elastin receptor, we compared the peptide sequences of human, rat, chicken, and porcine tropoelastin with the known peptide ligands (VGVAPG, PGAIPG, LGTIPG, VGAMPG, VGMAPG, VGSLPG, and VGLSPG). On the basis of their physical similarity to these peptides, two additional candidate peptide ligands, PGAVPG and AGAIPG, were identified. Both hexapeptides contain a single conservative (hydrophobic–hydrophobic) amino acid change with respect to the identified ligand PGAIPG. The ability of these peptides to induce lactose-sensitive chemotaxis in fetal bovine ligmentum nuchae fibroblasts and neutrophil chemotaxis that was desensitized by VGVAPG and PGAIPG confirmed that these peptides interacted with the elastin receptor (Figures 3 and 4; Tables I–IV).

DISCUSSION

Our goal is to delineate the structural features necessary for high-affinity binding to the 67-kDa elastin/laminin receptor. Previously peptide sequences in laminin (LGTIPG) and tropoelastin (VGVAPG and PGAIPG) have been shown to define epitopes for this receptor (Senior et al., 1984; Wrenn et al., 1986; Mecham et al., 1989a,b; Grosso & Scott, 1993). This receptor is expressed on ligamentum nuchae fibroblasts and circulating neutrophils and mediates the chemotactic response of fibroblasts and neutrophils to elastin (Senior et al., 1982, 1984, 1989; Wrenn et al., 1986). Fibroblast migration is inhibited by lactose and neutrophils are desensitized to migration by peptides interacting with the same receptor (Senior et al., 1982, 1984, 1989; Hinek et al., 1988). These criteria were used to evaluate whether specific peptides were interacting with the 67-kDa elastin receptor.

Using BA4 as the detecting antibody, Western analysis of recombinant proteins and slot blots of synthetic peptides demonstrated that the recognition site for the 67-kDa elastin receptor and the monoclonal antibody were similar. Because of the ease of production and purification of monoclonal antibodies, we wished to exploit this similarity. Recently, the construction and use of epitope libraries to characterize antibody recognition sites have been described (Parmley & Smith, 1988; Scott & Smith, 1990). After screening of an epitope library composed of random hexapeptides, four BA4-selected peptides, VGMAPG, VGAMPG, VGSLPG, and VGLSPG, were synthesized and purified. The migratory response of bovine ligamentum nuchae fibroblasts and neutrophils demonstrated that these peptides interacted with the 67-kDa elastin receptor.

Studies comparing the primary amino acid sequence of tropoelastin from several species have suggested that additional receptor binding sequences are present within tropoelastin (Boyd et al., 1991). On the basis of structural similarities with the identified binding sites, we selected two additional tropoelastin peptides, AGAIPG and PGAVPG, for analysis (Indik et al., 1991). The migratory response of neutrophils and fibroblasts indicate that both tropoelastin peptides interact with the 67-kDa elastin receptor.

The propensity of the 67-kDa elastin receptor to interact with several different sequences was unexpected. Initial work had identified a single highly repeated hexapeptide sequence, VGVAPG, as a ligand (Senior et al., 1984; Mecham et al., 1989a,b). Structurally similar peptides, including VGVPG, were shown not to be ligands. The use of a recombinant expression system, in conjunction with studies of synthetic peptides, identified a second peptide, PGAIPG, as a ligand for the receptor (Grosso & Scott, 1993). It should be noted that while amino acid substitutions can be made within the binding site, to date successful substitutions have involved only the small nonpolar or uncharged amino acids (alanine, leucine, isoleucine, valine, glycine, proline, methionine, threonine, and serine).

In many ways, the range of acceptable ligands for the elastin receptor resembles that of some of the chaperonins, heat shock, and major histocompatibility proteins (Flynn et al., 1989, 1991; Randall & Hardy, 1989; Randall et al., 1990; Gething & Sambrook, 1992; Latron et al., 1992). These proteins interact with many protein ligands in a similar fashion. The binding sites maintain general structural features and physical properties but do not contain a highly conserved amino acid sequence. Recently, the demonstration that major histocompatibility proteins interact extensively with the peptide backbone, in addition to amino acid side chains, has been proposed as an explanation of this binding behavior (Fremont et al., 1992; Matsumura et al., 1992). While less characterized, the ability of the chaperonins and heat shock proteins to bind to multiple sites within a single protein has been suggested to be responsible for the high-affinity binding of the protein ligand (Randall & Hardy, 1989; Randall et al., 1990). While neither of these features has been established for the elastin receptor, it is interesting that a major histocompatibility protein with a molecular weight of 67 kDa and multiple heat shock proteins in this size range have been identified.

Since the tropoelastin peptides VGVAPG, PGAIPG, PGAVPG, and AGAIPG interact with the 67-kDa receptor, the primary sequence of tropoelastin contains multiple, physically separated receptor binding sites. The physical locations, as well as the specific sequences, depend on the species. Regarding the tropoelastin–elastin receptor, we can only speculate about the significance of multiple binding sites. The elastin receptor has been suggested to act as a chaperonin and to direct tropoelastin to a site on an elastogenic cell surface for assembly into the adjacent matrix (Hinek et al., 1988; Prosser & Mecham, 1988; Mecham, 1991). At that site, the receptor-bound tropoelastin would be released, possibly using the lectin nature of the elastin receptor, and incorporated into the extracellular matrix. The multiple binding sites within tropoelastin might then serve to ensure that all isoforms (resulting from extensive alternative splicing) are capable of entering this delivery pathway. A second possibility concerns the physical properties of tropoelastin. At relatively high concentrations, tropoelastin coascervates at 37 °C (Wrenn et al., 1988). By binding to the elastin receptor within the secretory pathway of the cell, this aggregation problem may be alleviated. Again the presence of multiple, physically separated binding sites might ensure binding of all monomers as soon as they enter the secretory lumen.

Obviously, in studying the interaction of ligands with their receptors, affinities will need to be measured. In the case of the bovine elastin receptor, attempts to generate radioiodinated peptides for binding assays have produced biologically inactive peptides (unpublished data). Attempts are underway to prepare ligands for quantitative assessment of specific binding activities.

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CORRECTIONS

Recognition of $tRNA^{Cys}$ by *Escherichia coli* Cysteinyl-tRNA Synthetase, by George A. Komatsoulis and John Abelson*, Volume 32, Number 29, July 27, 1993, pages 7435–7444.

Page 7440. In Figure 3, panels B and C, the U at position 73 should be a C.