Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 263–268
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.845

JOURNAL OF PEPTIDE SCIENCE

Induction of macrophage migration through lactose-insensitive receptor by elastin-derived nonapeptides and their analog

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Received 16 October 2006; Revised 11 January 2007; Accepted 14 January 2007

Abstract: Elastin, one of the extracellular matrix components, is present in tissues requiring extensibility and resilience such as the aorta, lungs, ligaments and skin. Degradation of elastin is observed in diseases such as atherosclerosis, emphysema and metastasis. It has been suggested that degraded elastin-derived peptides interact with a variety of cell types and are involved in development of diseases. Two nonapeptides, Ala-Gly-Val-Pro-Gly-Leu-Gly-Val-Gly (AGVPGFGVG) and Ala-Gly-Val-Pro-Gly-Phe-Gly-Val-Gly (AGVPGFGVG), exist in hydrophobic regions of elastin. In this paper, we characterized these elastin-derived nonapeptides by macrophage migration assay. Both nonapeptides induced a maximal migration at 10^{-8} M and elicited the same degree of responsiveness. To investigate the role of the sixth residue of the nonapeptides, seven analog peptides in which Leu or Phe is substituted by Ile, Val, Ala, Gly, Pro, Lys or Glu were synthesized and their macrophage migration activity tested. Among the nonapeptide analogs, only Ala-Gly-Val-Pro-Gly-Ile-Gly-Val-Gly induced the migration of macrophages at the optimal concentration of 10^{-9} M and its responsiveness was the same as that of parent nonapeptide AGVPGFGVG. Results of the deactivation tests and the effect of lactose on macrophage migration showed that a lactose-insensitive receptor which mainly recognizes Ala-Gly-Val-Pro-Gly-Ile-Gly-Val-Gly is presumably present on the membrane of macrophages in addition to the elastin-binding protein (EBP) sensitive to lactose. These results suggest that Leu, Phe and Ile residues at the sixth position of elastin-derived nonapeptides are crucial for inducing macrophage migration and in particular, Ile residue is important for the recognition by receptor insensitive to lactose. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: elastin-derived nonapeptides; macrophages; migration; lactose-insensitive receptor

INTRODUCTION

Elastin is an insoluble protein found in tissues that require elasticity and extensibility like arteries, lungs and skin and imparts elasticity to these tissues. Elastin is synthesized as a soluble precursor protein, tropoelastin [1]. Tropoelastin molecules are crosslinked with the aid of lysyl oxidase to form elastin, the elastomeric core protein of elastic fibers. Elastin is known as a highly resistant protein to proteolysis because of its hydrophobicity and numerous crosslinks. In atherosclerotic lesions, however, the accumulation of macrophages is observed in the early stage and the degradation of elastin is noticed in the advanced stage. These events in atherosclerotic lesions suggest that the accumulation of macrophages resulted from the chemotactic responsiveness to chemoattractants and imply that elastase which is secreted by macrophages may degrade elastin. Recent studies have reported that the degraded fragments of elastin act as chemoattractants, interact with a variety of cell types to modulate cellular behavior and play roles in the development of atherosclerosis, emphysema and metastasis [2–5].

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Elastin has several repeating peptide sequences in its hydrophobic regions: a pentapeptide Val-Pro-Gly-Val-Gly, a hexapeptide Val-Gly-Val-Ala-Pro-Gly (VGVAPG) and two nonapeptides Ala^1-Gly^2-Val^3-Pro^4-Gly^5-Leu^6-Gly^7-Val^8-Gly^9 (AGVPGLGVG) and Ala^1-Gly^2-Val^3-Pro^4-Gly^5-Phe^6-Gly^7-Val^8-Gly^9 (AGVPGFGVG) [6–8]. These hydrophobic repeating peptides are parts of degraded elastin fragments, and among them, the hexapeptide has been identified as a chemoattractant for various cells including macrophages [9–13] and the nonapeptides have been characterized as chemoattractants for fibroblasts and endothelial cells [13,14]. In this study, we synthesized two nonapeptides and examined whether these nonapeptides can induce the migration of macrophages. In addition, we synthesized seven nonapeptide analogs in which Leu or Phe at position 6 is substituted by Ile, Val, Ala, Gly, Pro, Lys or Glu residue to investigate the importance of Leu and Phe residues for inducing the migration of macrophages. Since it is reported that lactose interacts with elastin-binding protein (EBP) on the surface of cells and inhibits the interaction between EBP and hexapeptide [15,16], the effect of lactose on macrophage migration in response to the nonapeptides was also examined. Moreover, deactivation tests were performed to study whether the nonapeptides are recognized by a single or multiple receptors on macrophages.

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MATERIALS AND METHODS

Peptide Synthesis

An elastin-derived hexapeptide, two elastin-derived nonapeptides and seven analogs were synthesized by solid-phase method using 9-fluorenylmethyloxycarbonyl (Fmoc) strategy. The growing peptide was subjected to mild base treatment using piperidine (20%) during Fmoc-group deprotection. For coupling reaction, 0.45 M 2-(benzotriazol-1-yl)-1,1,3,3-tetramthyluronium hexafluoro phosphate (HBTU)-1-hydroxybenzotriazole (HOBt) in DMF was used. The progress of each coupling reaction was monitored using the Keiser test. HBTU, HOBt and all amino acid derivatives were purchased from Watanabe Chemical Ind. Ltd. (Hiroshima, Japan). Other organic reagents were from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). After completion of the peptide chain elongation, the resin was cleaved with Reagent K [17]. The crude peptides were purified by gel filtration using Sephadex G-10, followed by HPLC. The molecular weights of peptides were confirmed by MALDI-TOF-MAS (Voyager-DE, Applied Biosystems, Foster City, CA) measurements.

Cell Preparation

Wister rats (9-week-old-males) were injected with 20 ml of 2% glycogen. After 4 days, macrophages were harvested from the peritoneal cavity by washing with 0.02 M phosphate-buffered saline (PBS) (pH 7.2) containing heparin (0.5 U/ml) and centrifuged at 1000 rpm for 10 min. The pellet was immediately suspended in sterile distilled H_2O followed by addition of PBS and centrifuged at 1000 rpm for 10 min. Then the pellet was resuspended in incubation medium, Gey's buffered saline solution ((GBSS): 1.5 mM $CaCl_2$, 5.0 mM KCl, 0.2 mM KH_2PO_4, 1.0 mM $MgCl_2$, 0.3 mM $MgSO_4$, 119.8 mM NaCl, 27.0 mM $NaHCO_3$, 0.8 mM Na_2HPO_4, 5.6 mM glucose, 20.0 mM HEPES, pH 7.2) supplemented with 2% bovine serum albumin (BSA) and adjusted to pH 7.02 (2% BSA-GBSS). The cells obtained were about 90% macrophages according to the criteria of morphology and nonspecific esterase activity [18] and its viability was found to exceed 95% by trypan blue dye exclusion. The cells at a density of 2×10^6 cells/ml were used in the experiment.

Migration Assay

Migration assay was conducted in a 48-well microchemotaxis chamber by following the method of Bar-Shavit [19]. A polyvinyl pyrrolidone (PVP)-free polycarbonate membrane with a pore size of 5 µm (Nucleopore, Pleasanton, CA) was placed between the upper and lower compartments. The lower compartment of the chamber was charged with 27 µl of two nonapeptides or seven nonapeptide analogs at concentrations ranging from 10^{-11} to 10^{-6} M or N-formyl-Met-Leu-Phe (fMLP); Peptide Institute Inc., Osaka, Japan) at 10^{-8} M in 2% BSA-GBSS medium or medium alone. fMLP was used as a positive control and 2% BSA-GBSS medium was used as a negative control. Macrophage suspension (2×10^6 cells/ml) in 2% BSA-GBSS was added to the upper compartment. After incubation at 37 °C in a humidified 5% CO_2 atmosphere for 3 h, cells were stained with Diff-Quick dye. The migrating cells were quantitated in three random high-power fields (h.p.f.; original magnification × 400) for each triplicate filter. Cell migration is expressed as the net number of cells migrated per h.p.f. obtained by subtracting the number of migrating cells in the control medium from the number of migrating cells in response to two nonapeptides, seven analogs or fMLP.

The deactivation tests for exploring whether the recognitions of the nonapeptides and their analog are by a single or multiple receptors on macrophages were performed by the method of Kamisato *et al.* [20]. After preincubating macrophages (2×10^6 cells/ml) in 2% BSA-GBSS with the hexapeptide at 10^{-8} M and the nonapeptides at 10^{-8} M or the nonapeptide analog at 10^{-9} M for 30 min at 37 °C, a suspension of these cells in 2% BSA-GBSS was added to the upper compartment of the chamber. To the lower compartment was added the same stimulus (hexapeptide, nonapeptides or nonapeptide analog). Migrated cells were counted in the same manner as described above after a 3-h incubation.

In order to investigate the effect of lactose on macrophage migration in response to the nonapeptides and the analog AGVPGIGVG, macrophages (2×10^6 cells/ml) were preincubated for 2 h with 10 mM lactose at 37 °C. A suspension of lactose-treated cells in 2% BSA-GBSS medium was added to the upper compartment of the chamber and the hexapeptide at 10^{-8} M and the nonapeptides at 10^{-8} M or the nonapeptide analog at 10^{-9} M in 2% BSA-GBSS were added to the lower compartment. Migrated cells were counted in the same manner as described above after incubation for 3 h.

RESULTS AND DISCUSSION

Macrophage Migration in Response to Elastin-derived Nonapeptides

Migration assays were performed over a broad range of concentrations (10^{-11}–10^{-6} M). As a result, both the nonapeptides AGVPGLGVG and AGVPGFGVG induced a maximal migration at the same concentration of 10^{-8} M and elicited the same degree of responsiveness (Figure 1). The net migrating cells in response to AGVPGLGVG and AGVPGFGVG were 63 and 62 cells per h.p.f., respectively, and their responsiveness was more active than the positive control, fMLP (40 and 53 cells, respectively). The optimal concentration of two nonapeptides at 10^{-8} M was the same as that of VGVAPG (10^{-8} M). It has been reported that the permutation peptides GLGVGAGVP and GFGVGAGVP of the nonapeptides have the same maximal activities at 8×10^{-8} M for aortic endothelial cells [13]. It has also been suggested that AGVPGFGVG and its permutation sequence GFGVGAGVP are chemotactic for ligamentum fibroblasts with the same optimal concentration of 10^{-8} M and the same degree of responsiveness [14]. Since the nonapeptides are repeated three or four times in a single sequence of an elastin molecule [6–8], it is reasonable that both the nonapeptides and their permutation sequences have the same chemotactic response. The biphasic aspect of the response curves of migration may be due to the possibility of saturation of the cell receptors that trigger the migration response

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DOI: 10.1002/psc

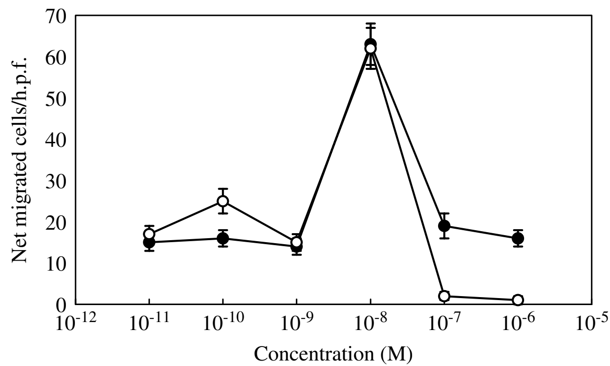

Figure 1 Macrophage migration in response to nonapeptides, AGVPGLGVG (closed circles) and AGVPGFGVG (open circles) at various concentrations. The net migrated cells per h.p.f. were obtained by subtracting the background migration (2% BSA-GBSS) from the number of migrated cells in response to nonapeptides or fMLP. fMLP at 10^{-8} M was used as a positive control and its net migration was 53 and 40 cells per h.p.f. for AGVPGLGVG and AGVPGFGVG, respectively. All values are expressed as means ± SD.

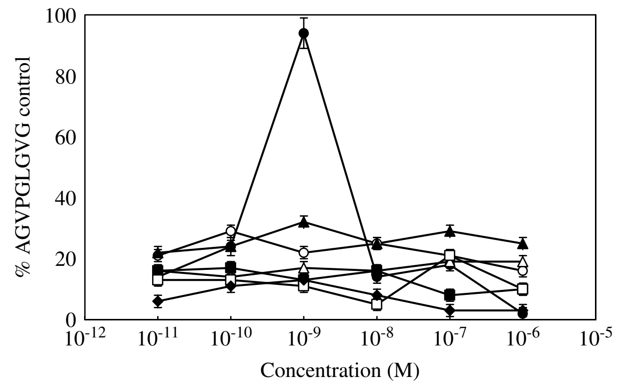

Figure 2 Macrophage migration in response to nonapeptide analogs, AGVPGXGVG where X is I (closed circles), V (open circles), A (closed triangles), G (open triangles), P (closed squares), K (open squares) and E (closed diamonds), at various concentrations. The responsiveness was expressed as a percentage of AGVPGLGVG, that is, the ratio of the net migration of cells in response to nonapeptide analogs to that in response to nonapeptide AGVPGLGVG. fMLP at 10^{-8} M was used as a positive control and its net migration was 59, 53, 67, 56, 55, 56 and 52 cells per h.p.f. for AGVPGXGVG where X is I, V, A, G, P, K and E, respectively. All values are expressed as mean ± SD.

[21] or of down regulation of receptors (internalization) [22]. These results suggest that the nonapeptide sequences AGVPGLGVG and AGVPGFGVG, as well as the hexapeptide sequence VGVAPG, may induce the migration of macrophages as parts of degraded elastin fragments in atherosclerotic lesions, causing the development of atherosclerosis.

Macrophage Migration in Response to Nonapeptide Analogs

The migration of macrophages in response to seven nonapeptide analogs in which Leu or Phe residue at position 6 in nonapeptides is substituted by Ile, Val, Ala, Gly, Pro, Lys or Glu residues was examined. As shown in Figure 2, the Ile residue in place of Leu^6 or Phe^6 induced the migration of macrophages, while the Val, Ala, Gly, Pro, Lys and Glu residues in place of Leu^6 or Phe^6 resulted in little or no responsiveness. The optimal concentration of Ile^6–nonapeptide AGVPGIGVG shifted to 10^{-9} M and its maximal response was the same as that of elastin-derived nonapeptide AGVPGLGVG. It has been suggested that two elastin-derived peptides (VGVAPG and PGAIPG) and a laminin-derived peptide (LGTIPG) containing the sequence XGXXPG (where X is a hydrophobic amino acid) showed chemotactic activity [8,23,24]. Other reports suggested that the XGXPG sequence of elastin peptide is needed for chemotactic and/or chemokinetic activity [25]. Although all nonapeptide analogs have XGXPG sequence at *N*-terminal portion, only AGVPGIGVG induced macrophage migration. From this fact, we next call attention to the hydrophobicity of amino acid residues at position 6 in nonapeptide.

The nonapeptides AGVPGLGVG and AGVPGFGVG are hydrophobic and have macrophage migration activity. The permutation sequences of the nonapeptides are also hydrophobic and chemotactic for fibroblasts and endothelial cells [13,14]. These facts suggest that the nonapeptide analogs also should have hydrophobic characteristics to exert their macrophage migration activities. The hydrophobicity of amino acid residues at position 6 in the nonapeptide is known as an order of Phe $>$ Ile $\geq$ Leu $\geq$ Val $>$ Ala $>$ Gly $>$ Pro $\gg$ Glu $>$ Lys [26]. The Phe residue is most hydrophobic and the hydrophobicity of Ile and Leu residues are nearly equal to that of Val residue but more than that of Ala residue. However, the side chains of Ile and Leu residues are sterically more bulky than the Val side chain. The hydrophobicity and steric bulkiness of side chains of amino acid residues at position 6 seem to be important for recognition by the receptors on macrophages. The CD spectrum of the nonapeptide analog AGVPGIGVG in trifluoroethanol was characteristic of a less ordered structure, as compared with other nonapeptide analogs (data not shown). The less ordered structure of AGVPGIGVG might be important for the responsiveness of migration, but the relationship between the structure and the activity is still unclear.

Deactivation of Macrophage Migration in Response to Nonapeptides and their Analog

The evidence that preexposure of cells to a chemotactic agonist results in a reduction in chemotactic responsiveness on reexposure to the same agonist is

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termed deactivation [27]. In order to examine whether the recognitions of the nonapeptides AGVPGLGVG and AGVPGFGVG and analog AGVPGIGVG by macrophages are the same or different from each other and whether their recognitions are the same as those of the hexapeptide VGVAPG or not, a deactivation test was carried out using VGVAPG, AGVPGLGVG, AGVPGFGVG and AGVPGIGVG as agonists. Results are shown in Figure 3. Macrophages exposed to VGVAPG lost appreciably their responsiveness of migration to the same agonist but not to AGVPGIGVG (Figure 3(a)). This responsiveness to both AGVPGLGVG and AGVPGFGVG diminished to ca. 50%. Preincubation of macrophages with AGVPGLGVG or AGVPGFGVG abolished their ability to migrate in response to the same agonist or VGVAPG to the extent of 20–50%, but this responsiveness to AGVPGIGVG was almost retained (Figure 3(b) and (c)). Preincubation of macrophages with AGVPGIGVG abolished appreciably their responsiveness of migration to the same agonist but not to VGVAPG (Figure 3(d)). This responsiveness to both AGVPGLGVG and AGVPGFGVG was retained to the extent of 60 and 70%, respectively. These results suggest the presence of multiple receptors for recognizing VGVAPG, AGVPGLGVG, AGVPGFGVG and AGVPGIGVG on the membrane of macrophages and imply that the receptor which recognizes AGVPGIGVG is not identical to that for VGVAPG.

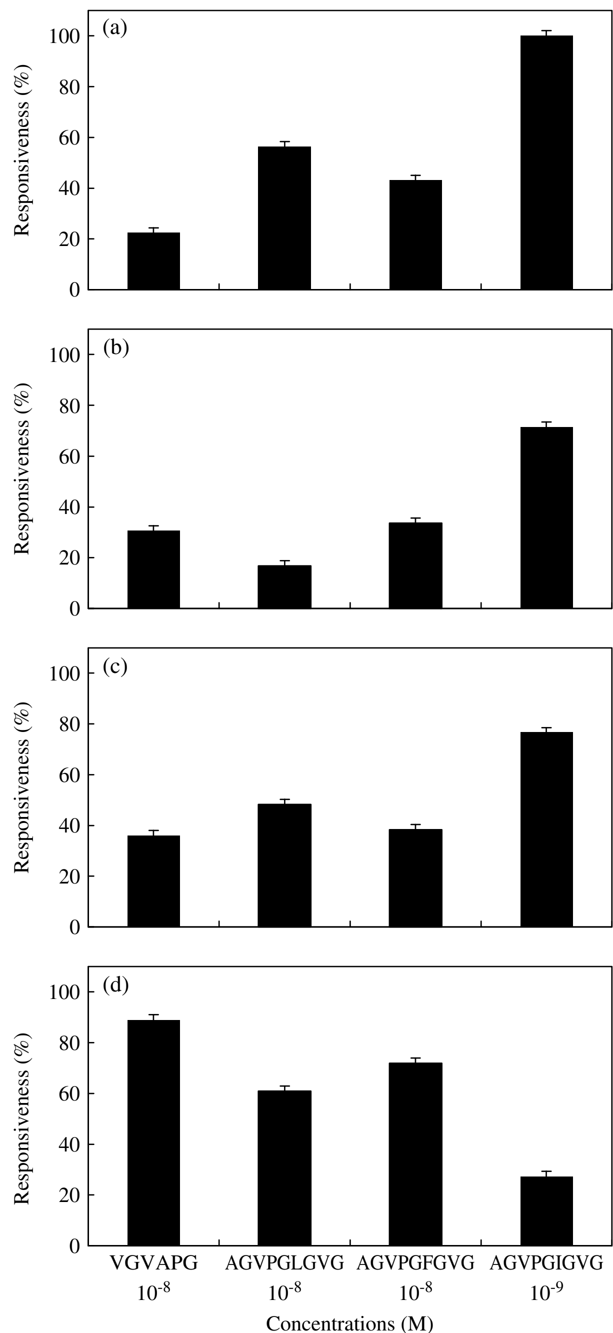

Figure 3 Deactivation of macrophage migration in response to hexapeptide VGVAPG at 10^{-8} M (a), nonapeptides AGVPGLGVG at 10^{-8} M (b) and AGVPGFGVG at 10^{-8} M (c) and nonapeptide analog AGVPGIGVG at 10^{-9} M (d). The concentration of the responsiveness (%) was expressed as the ratio of net migration of cells exposed to hexapeptide, nonapeptides or nonapeptide analog to that of unexposed cells. fMLP at 10^{-8} M was used as a positive control and its net migration was 81–115 per h.p.f. in experiments.

Effect of Lactose on Macrophage Migration in Response to Nonapeptides and their Analog

It has been suggested that EBP is identical to the 67-kDa enzymatically inactive, alternative spliced form of β-galactosidase [28]. The characteristic of EBP is that it contains two binding sites: one for elastin and the other for lectin [29]. The association between elastin and the 67-kDa EBP is allosterically abolished by the binding of β-galactosugars such as galactose and lactose to the lectin site of EBP [30,31]. Consistent with this, it was shown that lactose abolished the chemotactic response of numerous cell types to the hexapeptide VGVAPG [16,23]. Thus, we performed the migration assay in response to the nonapeptides AGVPGLGVG and AGVPGFGVG and their analog AGVPGIGVG in the presence of a 10-mM lactose, compared with VGVAPG. As shown in Figure 4, preexposure of macrophages to lactose abolished their responsiveness of migration to VGVAPG but not to AGVPGIGVG. However, this responsiveness to both AGVPGLGVG and AGVPGFGVG reduced to ca. 50%. Since it is known that the chemotactic receptor of VGVAPG is the 67-kDa EBP sensitive to lactose [23], these results suggest that AGVPGIGVG seems to interact with a receptor insensitive to lactose, which is distinct from the 67-kDa EBP. It is also indicated that AGVPGLGVG and AGVPGFGVG seem to interact with both the 67-kDa EBP that is sensitive to lactose and the receptor that is insensitive to lactose. As it is suggested that the chemotactic receptor sensitive to lactose could be activated by peptides with a more ordered conformation while the chemotactic receptor insensitive to lactose could be stimulated by the less ordered peptides

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DOI: 10.1002/psc

[32], the binding of AGVPGIGVG with less ordered conformation to the receptor insensitive to lactose may be reasonable.

CONCLUSION

Elastin-derived nonapeptides AGVPGLGVG and AGVPGFGVG induced macrophage migration, and among their analogs, only AGVPGIGVG showed migrating activity with the same degree with parent peptides. It was speculated from the results of deactivation and lactose treatment that besides EBP, at least one receptor, which recognizes two elastin-derived nonapeptides and their analog AGVPGIGVG, exists on the membrane of macrophages. As represented in the scheme of Figure 5, both AGVPGLGVG and AGVPGFGVG seem to be recognized not only by the 67-kDa EBP sensitive to lactose, which recognizes VGVAPG, but also by the receptor insensitive to lactose, competing with VGVAPG or AGVPGIGVG. This recognition may be done by an order of 67-kDa EBP > lactose-insensitive receptor. AGVPGIGVG appears to be recognized by the receptor insensitive to lactose alone and this recognition may be higher than that of AGVPGLGVG and AGVPGFGVG.

The accumulation of macrophages and the degradation of elastin are observed in the lesions of diseases such as atherosclerosis, aortic aneurysms and emphysema. The inflammatory response of macrophages to elastin degradation peptides is known to be important for the development of these diseases. However, the process of development of these diseases is still complicated and obscure. Besides EBP, which recognizes hexapeptide, and lactose-insensitive receptor on macrophages, which recognizes nonapeptides, both may play important roles in shedding light on these complicated and obscure processes. This expectation may promote the next step of identifying the receptor insensitive to lactose.

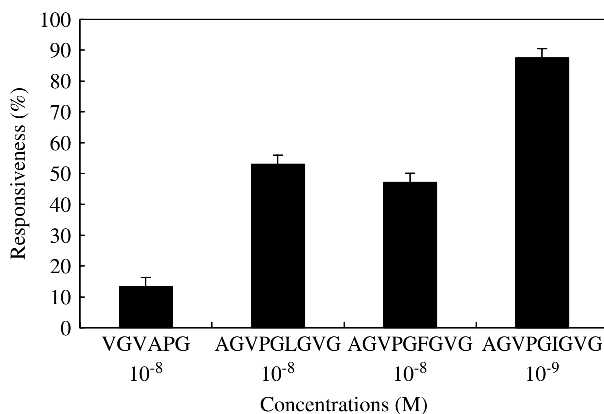

Figure 4 Effect of lactose on macrophage migration in response to VGVAPG, AGVPGLGVG, AGVPGFGVG and AGVPGIGVG. The concentration of lactose was 10 mM. The responsiveness (%) was expressed as the ratio of net migration of lactose-exposed cells to that of unexposed cells. fMLP at 10^{-8} M was used as a positive control and its net migration was 48, 62, 70 and 90 cells per h.p.f. for VGVAPG, AGVPGLGVG, AGVPGFGVG and AGVPGIGVG, respectively.

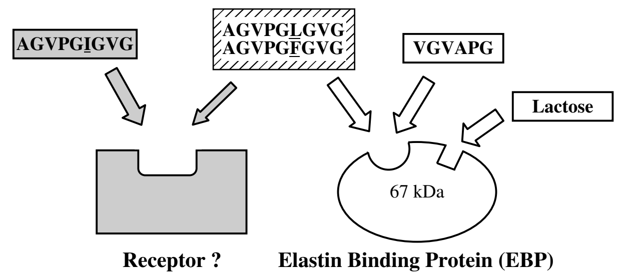

Figure 5 Diagrammatic representation of recognition by multiple receptors, at least two receptors. The model shows the recognition of the elastin-derived hexapeptide VGVAPG, the elastin-derived nonapeptides AGVPGLGVG and AGVPGFGVG and their analog AGVPGIGVG by the 67-kDa EBP sensitive to lactose and the other receptor insensitive to lactose.

REFERENCES

1. Smith DW, Weissman N, Carnes WH. Cardiovascular studies on copper deficient swine. XII. Partial purification of a soluble protein resembling elastin. *Biochem. Biophys. Res. Commun.* 1968; **31**: 309–315.
2. Robert L, Robert AM. Elastin, elastase and arteriosclerosis. Frontiers of matrix biology. In *Biology and Pathology of Elastic Tissues*, Robert L, Robert AM (eds). S. Karger Inc: Bâle, 1980; 130–173.
3. Hunninghake GW, Davidson JM, Rennard S, Szapiel S, Gadek JE, Crystal RG. Elastin fragments attract macrophage precursors to diseased sites in pulmonary emphysema. *Science* 1981; **212**: 925–927.
4. Houghton AM, Quintero PA, Perkins DL, Kobayashi DK, Kelley DG, Marconcini LA, Mecham RP, Senior RM, Shapiro SD. Elastin fragments drive disease progression in a murine model of emphysema. *J. Clin. Invest.* 2006; **116**: 753–759.
5. Yusa T, Blood CH, Zetter BR. Tumor cell interactions with elastin: implications for pulmonary metastasis. *Am. Rev. Respir. Dis.* 1989; **140**: 1458–1462.
6. Sandberg LB, Leslie JG, Leach CT, Alvarez VL, Torres AR, Smith DW. Elastin covalent structure as determined by solid phase amino acid sequencing. *Pathol. Biol. (Paris)* 1985; **33**: 266–274.
7. Raju K, Anwar RA. Primary structures of bovine elastin a, b, and c deduced from the sequences of cDNA clones. *J. Biol. Chem.* 1987; **262**: 5755–5762.
8. Indik Z, Yeh H, Ornstein-Goldstein N, Sheppard P, Anderson N, Rosenbloom JC, Peltonen L, Rosenbloom J. Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proc. Natl. Acad. Sci. U.S.A.* 1987; **84**: 5680–5684.
9. Senior RM, Griffin GL, Mecham RP, Wrenn DS, Prasad KU, Urry DW. Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. *J. Cell Biol.* 1984; **99**: 870–887.
10. Wrenn DS, Griffin GL, Senior RM, Mecham RP. Characterization of biologically active domains on elastin: identification of a monoclonal antibody to a cell recognition site. *Biochemistry* 1986; **25**: 5172–5176.

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DOI: 10.1002/psc

11. Briones MPP, Kozuru K, Maeda I, Kamisato S, Takami N, Okamoto K. Comparison of chemotactic activity of macrophages induced by permutations of elastin-derived hexapeptide VGVAPG and pentapeptide VGVPG. In *Peptide Science 2000*, Shioiri T (ed.). Protein Research Foundation: Osaka, 2001; 221–224.
12. Blood CH, Zetter BR. Membrane-bound protein kinase C modulates receptor affinity and chemotactic responsiveness of Lewis lung carcinoma sublines to an elastin-deroved peptides. *J. Biol. Chem.* 1984; **264**: 10614–10620.
13. Long MM, King VJ, Prasad KU, Freeman BA, Urry DW. Elastin repeat peptides as chemoattractants for bovine aortic endothelial cells. *J. Cell. Physiol.* 1989; **140**: 512–518.
14. Long MM, King VJ, Prasad KU, Urry DW. Chemotaxis of fibloblasts toward nonapeptide of elastin. *Biochim. Biophys. Acta* 1988; **968**: 300–311.
15. Mecham RP, Hinek A, Entwistle R, Wrenn DS, Griffin GL, Senior RM. Elastin binds to a multifunctional 67-kilodalton peripheral membrane protein. *Biochemistry* 1989; **28**: 3716–3722.
16. Hinek A. Nature and multiple functions of the 67-kDa elastin/laminin binding protein. *Cell Adhes. Commun.* 1994; **2**: 185–193.
17. King DS, Fields CG, Fields GB. A cleavage method which minimizes side reactions following Fmoc solid phase peptide synthesis. *Int. J. Pept. Protein Res.* 1990; **36**: 255–266.
18. Huang CK, Hill JM Jr, Bormann BJ, Mackin WM, Becker EL. Chemotactic factors induced vimentin phosphorylation in rabbit peritoneal neutrophil. *J. Biol. Chem.* 1984; **259**: 1386–1389.
19. Bar-Shavit R, Kahn A, Fenton JW, Wilner GD. Receptor mediated chemotactic response of a macrophage cell line (J774) to thrombin. *Lab. Invest.* 1983; **49**: 702–707.
20. Kamisato S, Uemura Y, Takami N, Okamoto K. Involvement of intracellular cyclic GMP and cyclic GMP-dependent protein kinase in α-elastin-induced macrophage chemotaxis. *J. Biochem.* 1997; **121**: 862–867.
21. Lauffenburger DA, Zigmond SH. Chemotactic factor concentration gradients in chemotaxis assay systems. *J. Immunol. Methods* 1981; **40**: 45–60.
22. Sullivan SJ, Zigmond SH. Asymmetric distribution of the chemotactic peptide receptor on polymorphonuclear leukocytes. *J. Cell Biol.* 1980; **85**: 703–711.
23. Mecham RP, Hinek A, Griffin GL, Senior RM, Liotta LA. The elastin receptor shows structural and functional similarities to the 67-kDa tumor cell laminin receptor. *J. Biol. Chem.* 1989; **264**: 16652–16657.
24. Grosso LE, Scott M. PGAIPG, a repeated hexapeptide of bovine tropoelastin, is a ligand for the 67-kDa bovine elastin receptor. *Matrix* 1993; **13**: 157–164.
25. Bisaccia F, Morelli MA, De Biasi M, Traniello S, Spisani S, Tamburro AM. Migration of monocytes in the presence of elastolytic fragments of elastin and in synthetic derivates. Structure-activity relationships. *Int. J. Pept. Protein Res.* 1994; **44**: 332–341.
26. Engelman DM, Steitz TA, Goldman A. Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. *Annu. Rev. Biophys. Biophys. Chem.* 1986; **15**: 321–353.
27. Donabedian H, Gallin GI. Deactivation of human neutrophil chemotaxis by chemoattractants: effect on receptor for the chemotactic factor f-Met-Leu-Phe. *J. Immunol.* 1981; **127**: 839–845.
28. Privitera S, Prody CA, Callahan JW, Hinek A. The 67-kDa enzymatically inactive alternatively spliced variant of beta-galactosidase is identical to the elastin/laminin-binding protein. *J. Biol. Chem.* 1998; **273**: 6319–6326.
29. Mecham RP, Whitehouse L, Hay M, Hinek A, Sheetz MP. Ligand affinity of the 67-kD elastin/laminin binding protein is modulated by the protein's lectin domain: visualization of elastin/laminin-receptor complexes with gold-tagged ligands. *J. Cell Biol.* 1991; **113**: 187–194.
30. Hinek A, Wrenn DS, Mecham RP, Barondes SH. The elastin receptor: a galactoside-binding protein. *Science* 1988; **239**: 1539–1541.
31. Hinek A, Boyle J, Rabinovitch M. Vascular smooth muscle cell detachment from elastin and migration through elastic laminae is promoted by chondroitin sulfate-induced "shedding" of the 67-kDa cell surface elastin binding protein. *Exp. Cell Res.* 1992; **203**: 344–353.
32. Castiglione Morelli MA, Bisaccia F, Spisani S, De Biasi M, Traniello S, Tamburro AM. Structure-activity relationships for some elastin-derived peptide chemoattractants. *J. Pept. Res.* 1997; **49**: 492–499.

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DOI: 10.1002/psc