Chemotactic Activity of Elastin-derived Peptides

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ABSTRACT Elastin-derived peptides, produced by digesting human aortic elastin and bovine ligament elastin with human neutrophil elastase, were tested for chemotactic activity. At 100 μg protein/ml, elastin digests were nearly as active for monocytes as saturating amounts of complement-derived chemotactic activity. Neutrophils and alveolar macrophages showed less response to elastin peptides than did monocytes. Fractionation of the digests by gel filtration chromatography disclosed that maximal chemotactic activity eluted in fractions corresponding to 14,000–20,000 mol wt containing most of the desmosine cross-links in the digests. Whole human serum and rabbit anti-elastin immunoglobulin inhibited the chemotactic activity. Purified desmosine also showed chemotactic activity for monocytes, maximal at 10 nM. These findings suggest that elastin-degradation products enriched in cross-linking regions recruit inflammatory cells in vivo and that elastin proteolysis, characteristic of emphysema, may be a signal for recruitment of mononuclear phagocytes into the lungs.

INTRODUCTION

Recently, collagen and collagen-derived peptides have been found to have chemotactic activity for monocytes and fibroblasts (1, 2). Fibronectin, another major extracellular component, also appears to be chemotactic for fibroblasts (3). These findings suggest that extracellular connective tissue macromolecules and their proteolytic fragments play a role in directing cell movement into tissues during injury, repair, and turnover. In this report, we demonstrate that peptides released from elastin by digestion with human neutrophil elastase (HLE)[1] have chemotactic activity, particularly for monocytes.

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[1]*Abbreviations used in this paper*: C5f, C5-derived chemotactic activity; E_{AB}, anti-elastin IgG.; ED_{50}, quantity of chemotactant for 50% maximal response; HLE, neutrophil elastase.

METHODS

Elastins. Human elastin was recovered by the hot-alkali method from normal aorta obtained at autopsy (4). Bovine ligament elastin, 100–400 mesh, was from Elastin Products Co., St. Louis, Mo. Purity of the elastins was confirmed by amino acid analysis using a Beckman 119C amino acid analyzer (Beckman Instruments Inc., Fullerton, Calif.) with a modified program for resolving elastin cross-linking amino acids (5).

Elastase. HLE was purified from human sputum by Dr. Edward J. Campbell, Washington University Medical Center, St. Louis, Mo. (6).

Elastin antiserum. Antiserum to bovine ligament elastin was raised in rabbits (7). The IgG fraction of the antiserum (E_{AB}) was prepared by ammonium sulfate precipitation and DEAE-cellulose chromatography (8).

C5-derived chemotactic factor activity. C5-derived chemotactic activity (C5f) was prepared by Dr. Donald L. Kreutzer, University of Connecticut Health Center, Farmington, Conn. from zymosan-activated human serum and standardized for 50% maximum chemotactic activity, (ED_{50}) against human monocytes (9). Twice the ED_{50}, in 25 μl, was used as a positive control in every experiment.

Desmosine. Desmosine was purified by Dr. Barry C. Starcher, Washington University Medical Center, St. Louis, Mo. (10).

Preparation of elastin peptides. Elastin was added to phosphate-buffered saline, 25 μg/μl (wt/vol), pH 8.0, containing HLE, 1 μg/μl (wt/vol). The mixture was incubated at 37°C for 24 h. Bovine elastin was completely solubilized, but a small residue of aortic elastin remained. The mixtures were centrifuged at 1,000 *g* for 10 min and the pellets discarded. Greater than 99% of the HLE was removed from the digests by affinity chromatography using Trasylol (Bayer AG, Wuppertal, West Germany)-Sepharose equilibrated with 0.15 M NaCl, 0.05 M Tris HCl, pH 8 (6).

Gel filtration chromatography of elastin digests. The protein-containing fractions in the fall-through peak from Trasylol-Sepharose were pooled, applied to a 2.5 × 27-cm column of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.), and eluted with 1 M ammonium bicarbonate, pH 8.3, at 100 ml/hr. Eluate fractions (4.8 ml) were collected, the absorbance measured at 230 nm, and the protein concentration determined by amino acid analysis.

Isolation of test cells. Mononuclear cell and neutrophil fractions of peripheral blood were obtained by Ficoll-Hypaque separation of peripheral venous blood collected from healthy volunteers (11). The cells were suspended in Eagle's minimum essential medium with L-glutamine (MEM/alpha modified, KC Biological Inc., Lenexa, Kans.), pH 7.2, containing 10% heat-inactivated fetal bovine serum at 2.5×10^6 mononuclear cells/ml and 1.5×10^6 neutrophils/ml. Alveolar

macrophages, obtained by bronchopulmonary lavage of healthy volunteers, were washed once with Neuman and Tytell medium, (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), then suspended at 1.2×10^6 cells/ml in chemotaxis assay medium (12).

Determination of chemotactic activity. Chemotaxis was determined in modified Boyden chambers (Ahlco Mfg. Co., Inc., Southington, Conn.) using a double membrane technique (13). The lower membrane was cellulose, 0.45 μm pores (Millipore Corp., Bedford, Mass.). The upper membrane was polycarbonate (Nucleopore Corp., Pleasanton, Calif.), pore size 2 μm for neutrophils, 5 μm for monocytes, and 8 μm for alveolar macrophages. Chambers were prepared in triplicate. After 60 or 120 min at 37°C (room air) for neutrophils and monocytes, respectively and 180 min at 37°C (5% CO_2 air) for macrophages, the membranes were removed and stained with hematoxylin and then coded, randomized, and read without reference to the code. Chemotaxis was quantified as the number of cells at the interface between the two membranes within an eyepiece grid under high power (×400). Five random grids were counted in each membrane pair. Results are expressed as the mean number of cells per grid for each set of triplicates corrected for random cell migration determined from chambers having only medium in the lower compartment.

Effect of E_{AB} and normal human serum on the chemotactic activity of elastin peptides. 50 μl of E_{AB} containing 524 μg protein/ml, was mixed with 250 μl of elastin digest or with double the ED_{50} of C5f, contained in 250 μl of buffer. Preliminary studies established that elastin digest cross-reacted with E_{AB}, as determined by inhibition of E_{AB} binding to radiolabeled bovine alpha-elastin (7). The mixtures were incubated at 37°C for 30 min, overnight at 4°C, then diluted with chemotaxis assay medium and assayed for chemotactic activity.

To determine the effect of whole human serum, 1 μl of serum was incubated at 37°C, 30 min, with 50–200 μl of chemotactically active elastin digest and then the mixtures were assayed for chemotactic activity.

Chemotactic activity of desmosine. Desmosine, dissolved in medium at varying concentrations, was assayed for chemotactic activity using monocytes.

RESULTS

Chemotactic activity of elastin digests for monocytes. Unfractionated elastin digests from bovine and human elastins, at comparable concentrations, displayed similar chemotactic activity for monocytes. At 100 μg/ml the activity nearly reached the C5f control (Fig. 1). The activity was clearly chemotactic, not simply chemokinetic, because putting elastin digest in the upper compartment with the cells only slightly stimulated movement, and cell migration above background was abolished by including identical concentrations of elastin digest in both compartments. Of note, elastin digests were not toxic to the cells as measured by trypan blue dye exclusion and lactic dehydrogenase release.

Gel filtration chromatography of bovine ligament and human aortic elastin digests showed virtually no peptides at the void volume, suggesting the elastins were degraded to peptides smaller than 25,000 mol wt (Fig. 2). Maximal chemotactic activity was found in fractions corresponding to 14,000–20,000 mol wt, fractions enriched in alanine and desmosine residues characteristic of the cross-linking domains of elastin (Fig. 2, Table I).

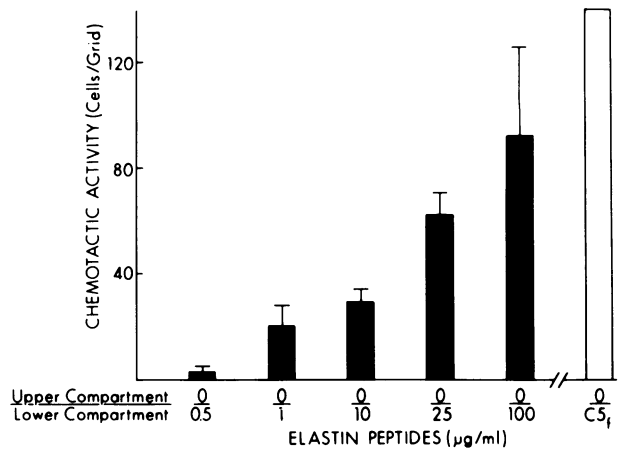

FIGURE 1 Chemotactic activity for monocytes of elastin peptides obtained by digesting bovine ligament elastin with HLE. The chemotactic activity of twice the ED_{50} of C5f is shown for comparison. The brackets show the range of triplicates.

Comparison of chemotactic activity of elastin-peptides for monocytes, macrophages, and neutrophils. Compared to monocytes, alveolar macrophages showed low levels of chemotaxis to both elastin peptides and C5f. Neutrophils were as active as monocytes to C5f, but showed minor chemotaxis to elastin peptides, suggesting that elastin peptides are chemotactic primarily for mononuclear phagocytic cells.

Effect of E_{AB} and serum on elastin-peptide chemo-

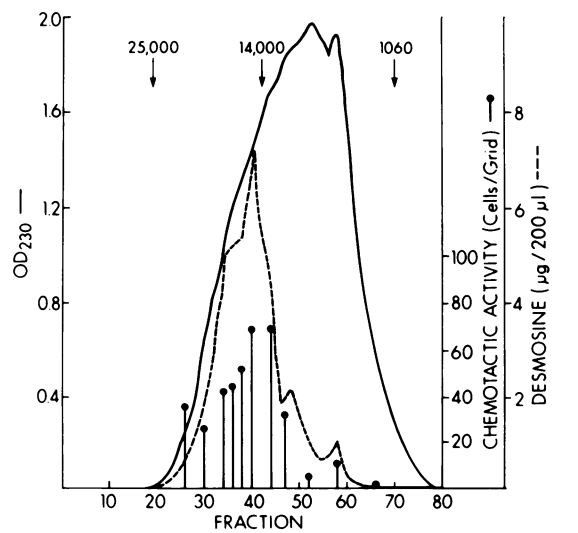

FIGURE 2 Relationships between the gel filtration profile, desmosine content and chemotactic activity for monocytes of bovine ligament elastin that has been digested with HLE. Column standards: chymotrypsinogen (25,000), lysozyme (14,000), and bradykinin (1,060).

TABLE I
Comparison of the Amino Acid Composition of Elastin-Peptide Fractions with and without Chemotactic Activity

	Fraction 40* Active	Fraction 66* Inactive
	Residues/1,000	
Lysine	6.7	0.2
Arginine	0.8	29.8
Hydroxyproline	10.2	2.0
Aspartic acid	14.1	3.5
Threonine	7.1	13.2
Serine	10.1	10.5
Glutamic acid	13.1	15.6
Proline	115.9	83.1
Glycine	304.4	291.5
Alanine	302.6	201.4
Valine	104.9	158.4
Isoleucine	16.6	39.3
Leucine	52.5	59.1
Tyrosine	2.9	0
Phenylalanine	27.3	86.5
Isodesmosine	4.27	trace
Desmosine	6.66	trace

Peptides were hydrolysed for 16 h at 105°C with constant boiling HCl. Values for serine and threonine are not corrected for losses due to hydrolysis.
* See Fig. 2 for identity of the fractions.

tactic activity. Preincubation of a chemotactically active elastin peptide fraction with E_{AB} abolished its chemotactic activity, but E_{AB} had no effect against C5f (Fig. 3). Whole human serum reduced C5f chemotactic activity > 80% and elastin-peptide chemotactic activity from 52 and 55 cells/grid to 10 and 5 cells/grid in two experiments, indicating that, as for C5f, serum has chemotactic inactivator activity for elastin peptides (9).

Chemotactic activity of desmosine. Desmosine had chemotactic activity that was maximal at 10 nM (Fig. 4). The cell migration to desmosine was almost completely abolished by putting equal concentrations

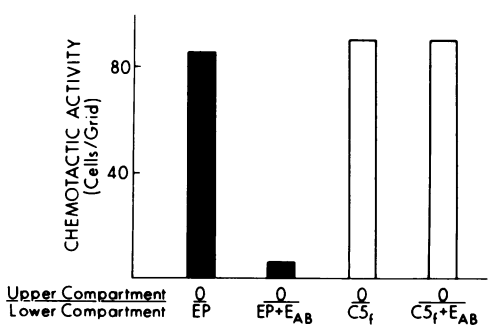

FIGURE 3 Effect of pre-incubating E_{AB} with chemotactically active elastin peptides (EP) and with twice the ED_{50} of C5f. Each bar is the average of triplicates.

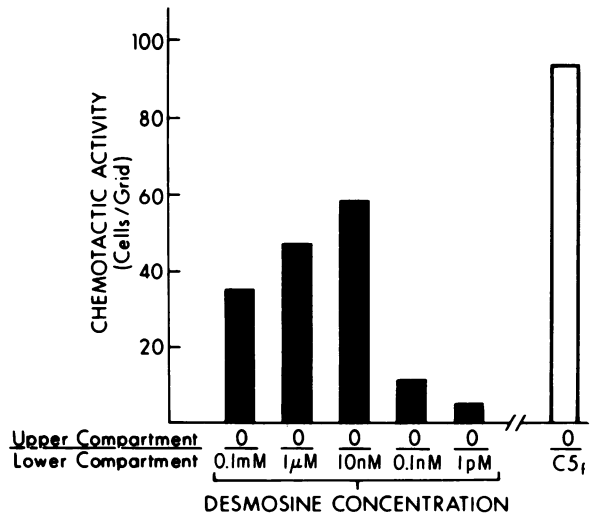

FIGURE 4 Relationship between desmosine concentration and chemotactic activity for monocytes. The chemotactic activity of twice the ED_{50} of C5f is shown for comparison. Each bar is the average of triplicates.

of desmosine in both compartments, although desmosine was not cytotoxic even at 0.1 mM.

DISCUSSION

This study demonstrates that HLE releases peptides from human and bovine elastins that are chemotactic for human inflammatory cells. The similarity of findings using human or bovine elastins supports the concept of homology of elastins among different species noted by radioimmunoassay (7).

HLE was used to degrade elastins to produce peptides similar to ones released in vivo by inflammatory cell elastases. Whether other elastases also release chemotactic peptides from elastin remains to be determined since different elastases release different peptides from elastin (14).

Among cells tested for chemotactic responses to elastin-derived peptides, monocytes responded best. Neutrophils responded less than monocytes although they moved as well as monocytes toward C5f. In this respect, elastin peptides resemble collagen and collagen peptides, reported to be chemotactic for monocytes but not for neutrophils (1). Alveolar macrophages responded sluggishly to both elastin peptides and C5f.

The chemotactic activity in mixtures of elastin peptides was found principally in peptides of 14,000–20,000 mol wt, having an increased ratio of alanine to glycine relative to the unfractionated digest, indicating enrichment in cross-links. Indeed, desmosine concentration was highest in fractions demonstrating maximal chemotactic activity. Other cross-linking amino acids in elastin might also serve as chemoattractants. Al-

lysine, dehydrolysinonorleucine, and dehydromerodesmosine occur in polyalanine regions of elastin identical to desmosine-containing sites. These cross-link amino acids were not detected in this study, however, because they are destroyed by acid hydrolysis (5). The role of cross-linking regions would be further elucidated by testing tropoelastin, a soluble form of elastin without cross-links but with the cross-linking site intact, and synthetic substrates for lysyl oxidase, the enzyme that initiates the formation of cross-links (15). The observation that IgG raised against elastin blocks the chemotactic activity of bovine elastin peptides, taken with the data relating chemotactic activity to cross-links, suggests that cross-link regions are involved in both the chemotactic activity and immunogenicity of elastin.

Whole serum contains inactivator activities against complement-derived and bacterial chemotactic factors (9). The present results show that serum chemotactic factor inactivator activity extends to elastin peptides.

Our findings suggest a new mechanism for inflammatory responses in elastin-containing tissues. Emphysema is notable in this respect because products of elastin degradation, a probable sequel of elastin proteolysis in emphysema, may provide one of the signals for recruitment of the large number of mononuclear phagocytes found in smokers' lungs (12). These cells, in turn, may influence the progress of emphysema by binding HLE and by attracting neutrophils through production of leukotactic factors (16, 17).

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REFERENCES

1. Postlethwaite, A. E., and A. H. Kang. 1976. Collagen and collagen peptide-induced chemotaxis of human blood monocytes. *J. Exp. Med.* **143:** 1299–1307.
2. Postlethwaite, A. E., J. M. Seyer, and A. H. Kang. 1978. Chemotactic attraction of human fibroblasts to type I, II and III collagens and collagen-derived peptides. *Proc. Natl. Acad. Sci. U. S. A.*. **75:** 871–875.
3. Postlethwaite, A. E., J. Keski-Oja, and A. H. Kang. 1979. Chemotactic attraction of human fibroblasts to fibronectin. *Clin. Res.* **27:** 646A. (Abstr.)
4. Lansing, A. I., T. B. Rosenthal, M. Alex, and E. W. Dempsey. 1952. The structure and chemical characterization of elastic fibers as revealed by elastase and by electron microscopy. *Anat. Rec.* **114:** 555–575.
5. Mecham, R. P., and J. A. Foster. 1979. Characterization of insoluble elastin from copper deficient pigs: its usefulness in elastin sequence studies. *Biochem. Biophys. Acta.* **577:** 147–158.
6. Martodam, R. R., R. J. Baugh, D. Y. Twumasi, and I. E. Liener. 1979. A rapid procedure for the large scale purification of elastase and cathepsin G from human sputum. *Prep. Biochem.* **9:** 15–31.
7. Mecham, R. P., and G. Lange. 1980. Measurement by radioimmunoassay of soluble elastins from different animal species. *Connect. Tissue Res.* In press.
8. Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1977. Methods in Immunology. W. A. Benjamin, Inc., Reading, Mass. 3rd edition. Chapters 24 and 26.
9. Kreutzer, D. L., W. D. Claypool, M. L. Jones, and P. A. Ward. 1979. Isolation by hydrophobic chromatography of the chemotactic factor inactivators from human serum. *Clin. Immunol. Immunopathol.* **12:** 162–176.
10. Starcher, B. C., and M. J. Galione. 1975. A large scale procedure for purification of desmosine and isodesmosine. *Prep. Biochem.* **5:** 455–460.
11. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97): 77–89.
12. Rodriguez, R. J., R. R. White, R. M. Senior, and E. A. Levine. 1977. Elastase release from human alveolar macrophages: comparison between smokers and nonsmokers. *Science (Wash. D. C.).* **198:** 313–314.
13. Campbell, P. B. 1977. An improved method for the in vitro evaluation of monocyte leukotaxis. *J. Lab. Clin. Med.* **90:** 381–388.
14. Senior, R. M., D. R. Bielefeld, and B. C. Starcher. 1976. Comparison of elastolytic effects of human leukocyte elastase and porcine pancreatic elastase. *Biochem. Biophys. Res. Commun.* **72:** 1327–1334.
15. Kagan, H. M., L. Tseng, P. C. Trackman, K. Okamoto, R. S. Rapaka, and D. W. Urry. 1980. Repeat polypeptide models of elastin as substrates for lysyl oxidase. *J. Biol. Chem.* **255:** 3656–3659.
16. Campbell, E. J., R. R. White, R. M. Senior, R. J. Rodriguez, and C. Kuhn. 1979. Receptor-mediated binding and internalization of leukocyte elastase by alveolar macrophages in vitro. *J. Clin. Invest.* **64:** 824–833.
17. Merrill, W. W., G. P. Naegel, R. A. Matthay, and H. Y. Reynolds. 1980. Alveolar macrophage-derived chemotactic factor. *J. Clin. Invest.* **65:** 268–276.