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# Involvement of Intracellular Cyclic GMP and Cyclic GMP-Dependent Protein Kinase in α-Elastin-Induced Macrophage Chemotaxis

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**α-Elastin with an average molecular mass of 70 kDa, an oxalic acid fragmentation product of highly purified insoluble elastin, induced the migration of macrophages, with maximum activity at $10^{-1}$ μg/ml. Relative to the positive control of $10^{-8}$ M *N*-formylmethionyl-leucyl-phenylalanine (fMLP), the responsiveness of macrophages to α-elastin was nearly the same. Checkerboard analysis demonstrated that the cell movement is chemotaxis and not chemokinesis. A homologous deactivation test showed the possibility of the existence of α-elastin-recognizing sites on macrophages. In connection with macrophage chemotaxis in response to α-elastin, the intracellular signaling pathway was examined. The guanosine 3′,5′-cyclic monophosphate (cGMP) level was enhanced in macrophages stimulated by α-elastin, whereas the adenosine 3′,5′-cyclic monophosphate (cAMP) level was not. Chemotaxis assaying of macrophages treated with 8-Br cGMP- and dibutyryl cAMP-loaded macrophages indicated that cGMP promotes cell movement and cAMP suppresses cell locomotion. The possible involvement of protein kinases in the α-elastin signaling pathway was explored by use of inhibitors specific for cGMP-dependent protein kinase (PKG), cAMP-dependent protein kinase (PKA), protein kinase C (PKC), and tyrosine kinase. The macrophage chemotactic response to α-elastin was inhibited by the PKG inhibitor, but not by the PKA, PKC, or tyrosine kinase inhibitor. These results suggested that the increase in the cGMP level and the activation of PKG in macrophages are involved in α-elastin-induced macrophage chemotaxis.**

**Key words: chemotaxis, cyclic GMP, cyclic GMP-dependent protein kinase, α-elastin, macrophage.**

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Elastin, the core protein of the elastic fibers in such tissues as arterial walls, lungs, and ligaments, provides the resilience or restorative force of these tissues. The rupture and fragmentation of elastin have been morphologically observed at sites of atherosclerotic and emphysematous lesions, and this elastin degradation is thought to play an important role in the pathological processes of such diseases (*1, 2*). Elastin and its degradation products interact with a variety of cell types to modulate cellular functions physiologically, and in several pathological processes, such as atherosclerosis, emphysema, and metastasis (*1-11*). For example, elastin has been shown to mediate the adhesion of monocytes, fibroblasts, and tumor cells (*3-5*), and elastin degradation products have been shown to be chemoattractants for monocytes, fibroblasts, and smooth muscle cells (*6-10*). Although elastin degradation products have been recognized as potentially important extracellular matrix-derived chemoattractants, so far little is known concerning the mechanism underlying the chemotactic responsiveness.

The present investigation was undertaken to assess the chemotactic potential of α-elastin with an average molecular mass of 70 kDa, a degradation product of elastin, as to macrophages, and to clarify the signal transduction pathway associated with the chemotactic response to α-elastin. The results demonstrate that α-elastin is chemotactic for macrophages, implying the possibility of the existence of α-elastin-recognizing sites on macrophages, and suggest that an increase in the level of guanosine 3′,5′-cyclic monophosphate (cGMP) and the activation of cGMP-dependent protein kinase (PKG) are involved in the α-elastin signaling pathway.

## MATERIALS AND METHODS

*Materials*—The adenosine 3′,5′-cyclic monophosphate (cAMP) and cGMP radioimmunoassay kits were purchased from Yamasa, Chiba. Dibutyryl cAMP sodium, 8-Br cGMP, lipopolysaccharide (LPS), and genistein were from Wako Pure Chemical, Osaka. Gamma interferon (IFN-γ) was from JCR Pharmaceuticals, Kobe. KT5720, KT5823, and C-3030 were from LC Laboratories, Woburn, MA, USA. Diff-Quick dye was from International Reagents, Hyogo. *N*-Formylmethionyl-leucyl-phenylalanine (fMLP) was supplied by Prof. Kondo (Saga University).

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Abbreviations: BSA, bovine serum albumin; cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; fMLP, *N*-formylmethionyl-leucyl-phenylalanine; GBSS, Gey's buffered saline solution; IFN-γ, gamma interferon; LPS, lipopolysaccharides; NO, nitric oxide; $NO_2^-$, nitrite; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase.

*Preparation of $\alpha$-Elastin*—Insoluble elastin was prepared from bovine ligamentum nuchae with a hot alkaline solution (*12*). Its purity was checked by amino acid analysis. The highly purified insoluble elastin was partially hydrolyzed by treatment with oxalic acid and fractionated by the method of Partridge *et al.* (*13*) to yield $\alpha$-elastin with an average molecular mass of 70 kDa.

*Cell Preparation*—Elicited macrophages were harvested from the peritoneal cavities of Wistar rats (>8 weeks of age) 4 days after the injection of 1% glycogen (*14*). The cells were washed once with 0.02 M phosphate-buffered saline (pH 7.2). The cells obtained were about 90% macrophages according to the criteria of morphology and non-specific esterase activity (*15*). The viability of the cells was over 95%, as assessed by trypan blue dye exclusion.

*Chemotaxis Assaying*—Chemotaxis assays were performed in 48-well microchemotaxis chambers according to the method of Bar-Shavit *et al.* (*16*). The lower compartment of a chamber was charged with 27 $\mu$l of $\alpha$-elastin at concentrations ranging from $10^{-4}$ to $10^2$ $\mu$g/ml or fMLP at $10^{-8}$ M. A polycarbonate filter (pore size: 5 $\mu$m) was used to separate the upper and lower compartments. An aliquot (50 $\mu$l) of a macrophage suspension ($2\times10^6$ cells/ml) in 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) containing 2% bovine serum albumin (BSA) was added to the upper compartment. The chamber was incubated at 37°C for 3-h in a 95% humidified incubator containing 95% air and 5% $CO_2$. The filter was removed from the chamber, fixed in methanol, and then stained with Diff-Quick dye. The net number of cells that migrated completely through the 5-$\mu$m pores was determined in three random high-power fields (×400) for each of triplicate filters. fMLP was a positive control and GBSS containing 2% BSA (2% BSA-GBSS) was a negative control.

*Checkerboard Analysis*—Checkerboard analysis was performed in 48-well microchemotaxis chambers according to the method of Long *et al.* (*17*). A macrophage suspension ($2\times10^6$ cells/ml) in 2% BSA-GBSS was added to the upper compartment of a chamber. $\alpha$-Elastin in the concentration range of $10^{-3}$ to $10^{-1}$ $\mu$g/ml was added to the lower compartment, to the upper compartment containing the cell suspension, or to both compartments to set up positive, negative and zero gradients, respectively. After 3-h incubation at 37°C in a humidified incubator containing 95% air and 5% $CO_2$, the migrating cells were counted in the same manner as described under "*Chemotaxis Assaying.*"

*Deactivation Test*—A deactivation test was performed in 48-well microchemotaxis chambers by the method of Sozzani *et al.* (*18*). After the preincubation of macrophages ($2\times10^6$ cells/ml) in 2% BSA-GBSS with $\alpha$-elastin at $10^{-1}$ $\mu$g/ml or fMLP at $10^{-8}$ M for 30 min at 37°C, the cells were washed with 2% BSA-GBSS, resuspended in the same buffer, and then added to the upper compartment of the chamber. To the lower compartment was added the same stimulatory compound ($\alpha$-elastin or fMLP). After 3-h incubation at 37°C in a humidified incubator containing 95% air and 5% $CO_2$, the migrating cells were counted in the same manner as described under "*Chemotaxis Assaying.*"

*Measurement of the cAMP and cGMP Levels*—For time-course experiments, macrophages ($2\times10^6$ cells/ml) in 2% BSA-GBSS were incubated with $\alpha$-elastin at $10^{-1}$ $\mu$g/ml at 37°C for the indicated times (0, 5, 15, 30, and 60 min). For concentration-dependent experiments, macrophages ($2\times10^6$ cells/ml) in 2% BSA-GBSS were incubated with $\alpha$-elastin at concentrations ranging from $10^{-3}$ to 10 $\mu$g/ml at 37°C for 15 min. At the end of the incubation, ice-cold trichloroacetic acid was added to the cell suspension to a concentration of 6%. After the mixture had been homogenized with an ultrasonic homogenizer at 4°C, the precipitate was removed by centrifugation at $1,000\times g$ for 10 min at 4°C. Then, the trichloroacetic acid was removed by three successive extractions with diethyl ether saturated with water, and the resultant solution was lyophilized. cAMP and cGMP were succinylated and quantitated by means of the radioimmunoassay of Honma *et al.* (*19*) with Yamasa cAMP and cGMP assay kits, respectively.

*Chemotaxis Assaying of Macrophages Treated with Dibutyryl cAMP and 8-Br cGMP*—Macrophages ($2\times10^6$ cells/ml) in 2% BSA-GBSS were preincubated with 0.5 mM dibutyryl cAMP or $10^{-6}$ M 8-Br cGMP for 30 min at 37°C. Then, the cells were washed in 2% BSA-GBSS and resuspended in the same buffer, and then added to the upper compartment of a 48-well microchemotaxis chamber. To the lower compartment was added $\alpha$-elastin at $10^{-1}$ $\mu$g/ml or fMLP at $10^{-8}$ M. After 3-h incubation at 37°C in a humidified incubator containing 95% air and 5% $CO_2$, the migrating cells were counted in the same manner as described under "*Chemotaxis Assaying.*"

*Measurement of Nitrite ($NO_2^-$) Production*—A macrophage suspension was adjusted to $5\times10^5$ cells/ml in RPMI 1640 containing 10% fetal bovine serum. Aliquots (0.9 ml) of the macrophage suspension were exposed to 0.1 ml of $10^{-1}$ $\mu$g/ml $\alpha$-elastin, $10^{-8}$ M fMLP, or a mixture of 100 ng/ml LPS and 100 U/ml IFN-$\gamma$ at 37°C for 3, 24, 48, and 72-h in a humidified incubator containing 95% air and 5% $CO_2$, and then centrifuged at $700\times g$ for 7 min at 4°C. The centrifuged media were assayed for $NO_2^-$ by means of the Griess reaction (*20*). Briefly, aliquots (500 $\mu$l) of the centrifuged media were incubated with 500 $\mu$l of Griess reagent (250 $\mu$l of 1% sulfanilamide/$H_2O$, 250 $\mu$l of 0.1% naphthylethylenediamine dihydrochloride/2.5% $H_3PO_4$) at room temperature for 10 min. Then, the absorbance at 550 nm was measured. $NO_2^-$ was quantified by comparison with $Na(NO_2)$ as a standard.

*Effects of Protein Kinase Inhibitors*—Solutions of KT5720 (*22*), KT5823 (*21*), and C-3030 (*22*) in DMSO, and genistein (*23*) in ethanol were diluted with 2% BSA-GBSS to give the desired concentrations. A macrophage suspension ($2\times10^6$ cells/ml) in 2% BSA-GBSS was preincubated in a solution containing the inhibitor at the desired concentration at 37°C for 10 min, and then assayed, using 48-well microchemotaxis chambers, as to migration to $\alpha$-elastin at $10^{-1}$ $\mu$g/ml or fMLP at $10^{-8}$ M, in the same manner as described under "*Chemotaxis Assaying.*" The concentrations of DMSO (<1.5%) and ethanol (<4%) used in the assays were not toxic for the cells (cell viability >93%, trypan blue dye exclusion).

## RESULTS

The macrophage migration in response to $\alpha$-elastin in a concentration gradient ranging from $10^{-4}$ to $10^2$ $\mu$g/ml is shown in Fig. 1. $\alpha$-Elastin stimulated the migration of



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macrophages, with the optimal response occurring at $10^{-1}$ $\mu$g/ml. Relative to the positive control of $10^{-8}$ M fMLP, the responsiveness to $\alpha$-elastin was nearly the same. Checkerboard analysis was performed to determine whether the macrophage migration in response to $\alpha$-elastin is chemotaxis (directed cell movement that depends on a concentration gradient) or chemokinesis (random cell locomotion that is not gradient-dependent). As shown in Table I, macrophage migration occurred only when a positive gradient of $\alpha$-elastin existed, *i.e.* it did not when a negative gradient or no gradient of $\alpha$-elastin existed.

It has been demonstrated that the exposure of cells to a chemotactic agonist results in a reduction in chemotactic responsiveness, termed deactivation, on reexposure to the same agonist (*24*). For this reason, a homologous deactivation test was carried out to determine the effect of preincubation with the optimal $\alpha$-elastin ($10^{-1}$ $\mu$g/ml) and fMLP ($10^{-8}$ M) concentrations on macrophage chemotaxis. As shown in Table II, preincubation of macrophages with $\alpha$-elastin or fMLP abolished their ability to migrate in response to the same stimulatory compound.

The time-courses of the cAMP and cGMP levels in macrophages incubated with $10^{-1}$ $\mu$g/ml $\alpha$-elastin are

TABLE II. **Deactivation of macrophage chemotactic response to $\alpha$-elastin and fMLP.**

| Stimulus | Macrophage migration after preincubation with | | |
|---|---|---|---|
| | Buffer[a] | $\alpha$-Elastin ($10^{-1}$ $\mu$g/ml) | fMLP ($10^{-8}$ M) |
| Buffer | 7±3[b] | 9±5 | 10±4 |
| $\alpha$-Elastin ($10^{-1}$ $\mu$g/ml) | 82±5 | 22±1 | 86±4 |
| fMLP ($10^{-8}$ M) | 90±7 | 94±8 | 14±5 |

Macrophages ($2\times10^6$ cells/ml) were preincubated with or without $\alpha$-elastin ($10^{-1}$ $\mu$g/ml), or fMLP ($10^{-8}$ M) at 37°C for 30 min, and then assayed as to their migration to the same stimulatory compound ($\alpha$-elastin or fMLP) in the same manner as described in the text. [a]2% BSA-GBSS was used as the buffer. [b]Migrating cells per h.p.f. are expressed as means±SD.

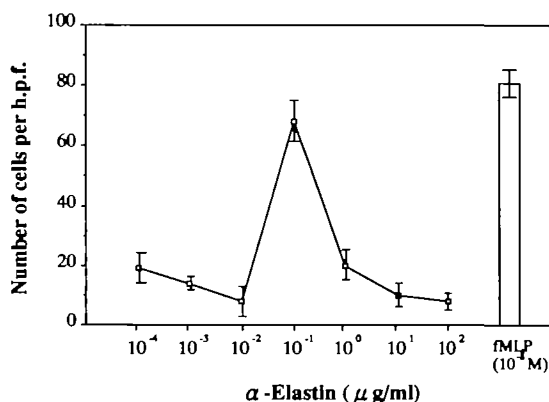


Fig. 1. **Macrophage migration in response to $\alpha$-elastin.** Macrophages ($2\times10^6$ cells/ml) were incubated with $\alpha$-elastin at concentrations ranging from $10^{-4}$ to $10^2$ $\mu$g/ml for 3 h at 37°C. The background migration (buffer alone) was 18 cells per high-power field (h.p.f.). The net fMLP migration at $10^{-8}$ M was 78 cells per h.p.f. All values are expressed as means±SD.

TABLE I. **Checkerboard analysis of macrophage migration to $\alpha$-elastin.**[a]

| $\alpha$-Elastin ($\mu$g/ml), lower compartment | $\alpha$-Elastin ($\mu$g/ml), upper compartment | | | |
|---|---|---|---|---|
| | 0 | $10^{-3}$ | $10^{-2}$ | $10^{-1}$ |
| 0 | (21)0[b] ±0 | 1 ±1 | 1 ±1 | 6 ±4 |
| $10^{-3}$ | 10 ±3 | 7 ±3 | 13 ±2 | 10 ±3 |
| $10^{-2}$ | 30 ±4 | 11 ±2 | 5 ±1 | 10 ±4 |
| $10^{-1}$ | 62 ±1 | 22 ±2 | 31 ±2 | 4 ±5 |

[a]The diagonal column in the chart indicates the migration of cells when no gradient of the attractant existed. The areas above and below the diagonal column indicate migration in the presence of a negative gradient and a positive gradient of $\alpha$-elastin, respectively. [b]The value of (21) 0 for zero concentration means that the background (buffer alone) was 21 cells per h.p.f. Accordingly, 21 was substracted from all other values to give the net numbers of cells. All values are expressed as means±SD.

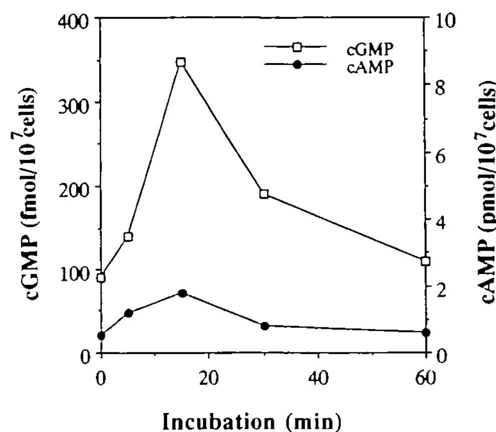


Fig. 2. **Time-courses of the cAMP and cGMP levels in macrophages incubated with $\alpha$-elastin.** Macrophages ($2\times10^6$ cells/ml) were incubated with $\alpha$-elastin ($10^{-1}$ $\mu$g/ml) at 37°C for the indicated times (0, 5, 15, 30, and 60 min).

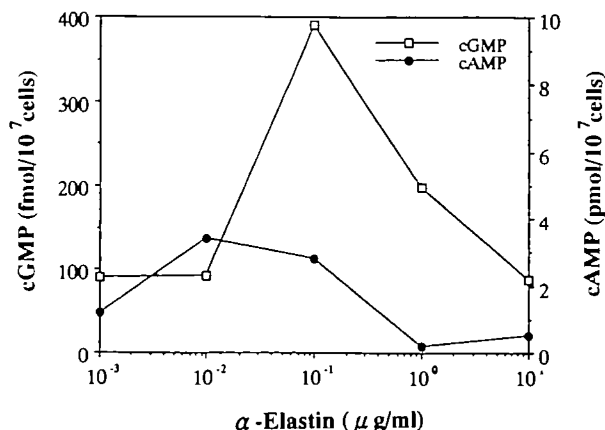


Fig. 3. **Changes in the levels of cAMP and cGMP in macrophages incubated with $\alpha$-elastin.** Macrophages ($2\times10^6$ cells/ml) were incubated with $\alpha$-elastin at concentrations ranging from $10^{-3}$ to 10 $\mu$g/ml for 15 min at 37°C. The background levels of cAMP and cGMP (buffer alone) were 1.3 pmol/$10^7$ cells and 32 fmol/$10^7$ cells, respectively.

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shown in Fig. 2. The cGMP level was significantly increased, showing a peak at 15 min, and then decreased to the basal level, while the cAMP level showed no significant increase at any time during the incubation. Figure 3 shows the changes in the levels of cAMP and cGMP in macrophages incubated with α-elastin at concentrations ranging from $10^{-3}$ to 10 μg/ml for 15 min. The cGMP level was enhanced in macrophages, the maximum level being observed at $10^{-1}$ μg/ml α-elastin. This optimal concentration was the same as the optimal chemotactic concentration ($10^{-1}$ μg/ml), and the response curve was also similar to the chemotactic curve in Fig. 1. However, the cAMP level was not increased at any concentration of α-elastin. The effects of dibutyryl cAMP and 8-Br cGMP on macrophage chemotaxis in response to α-elastin were examined (Table III). Dibutyryl cAMP and 8-Br cGMP are analogs to the parent cyclophosphates, cAMP and cGMP, respectively. The preincubation of macrophages with $5\times10^{-4}$ M dibutyr-

TABLE III. **Effects of dibutyryl cAMP and 8-Br cGMP on the macrophage chemotactic response to α-elastin and fMLP.**

| Preincubation | Macrophage migration in response to | |
|---|---|---|
| | α-Elastin ($10^{-1}$ μg/ml) | fMLP ($10^{-8}$ M) |
| Buffer[a] | 51±1[b] | 62±1 |
| Dibutyryl cAMP ($5\times10^{-4}$ M) | 4±4 | 68±6 |
| 8-Br cGMP ($1\times10^{-6}$ M) | 95±3 | 61±2 |

Macrophages ($2\times10^6$ cells/ml) were preincubated with and without dibutyryl cAMP ($5\times10^{-4}$ M), or 8-Br cGMP ($1\times10^{-6}$ M) at 37°C for 30 min, and then assayed as to their migration to α-elastin ($10^{-1}$ μg/ml) or fMLP ($10^{-8}$ M) in the same manner as described in the text. [a]2% BSA-GBSS was used as the buffer. [b]Migrating cells per h.p.f. are expressed as means±SD.

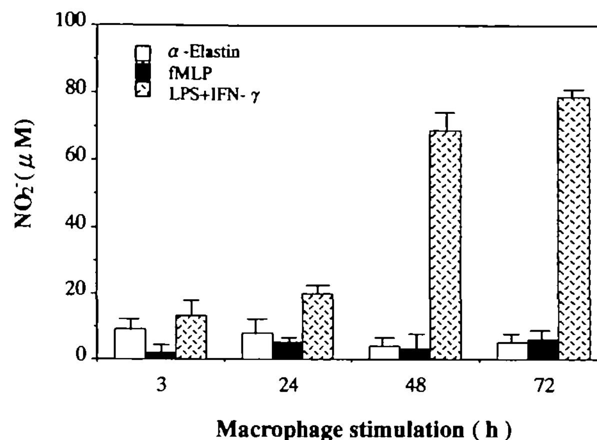


Fig. 4. **$NO_2^-$ production by macrophages after treatment with α-elastin, fMLP, or LPS and IFN-γ.** Macrophages ($5\times10^5$ cells/ml) were incubated with α-elastin ($10^{-1}$ μg/ml), fMLP ($10^{-8}$ M), or LPS (100 ng/ml) and IFN-γ (100 U/ml) at 37°C. Determinations were carried out after 3, 24, 48, and 72 h incubation.

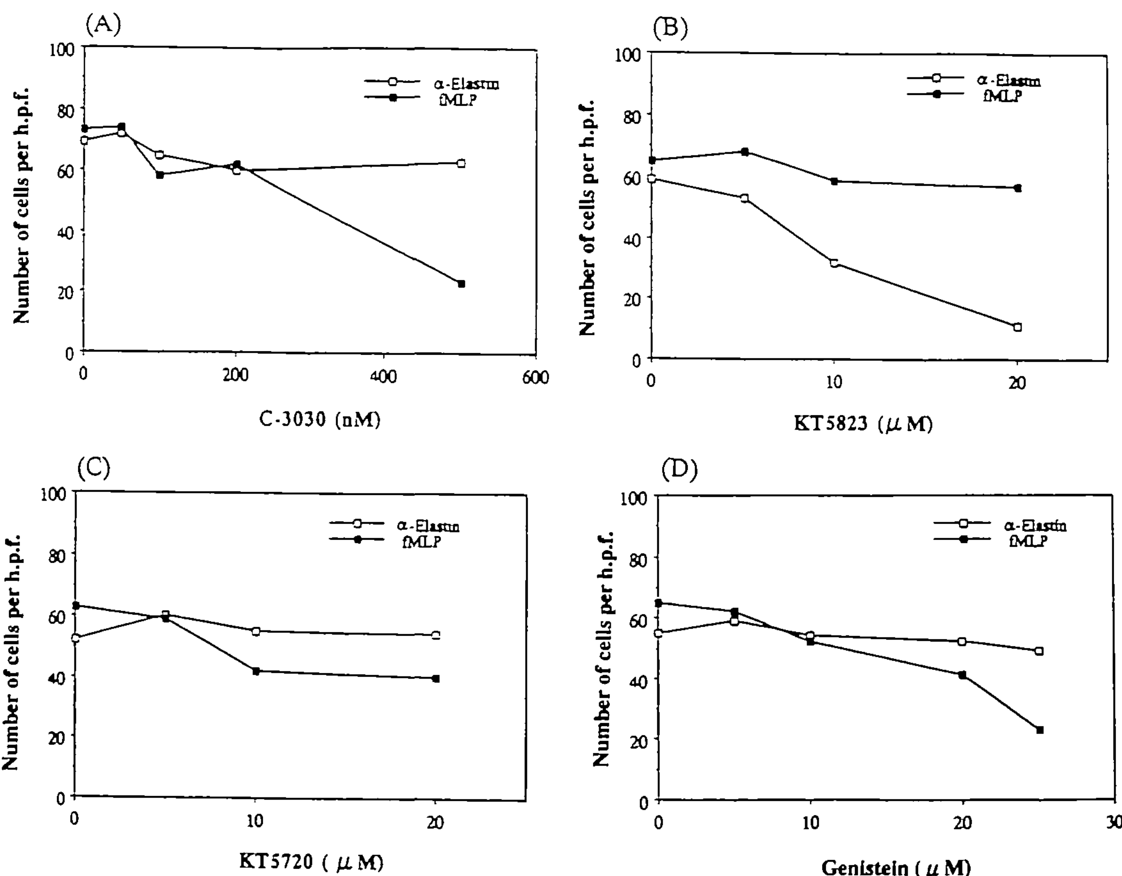


Fig. 5. **Effects of C-3030, KT5823, KT5720, and genistein on macrophage chemotaxis in response to α-elastin or fMLP.** Macrophages ($2\times10^6$ cells/ml) were preincubated with the indicated concentrations of (A) C-3030, (B) KT5823, (C) KT5720, and (D) genistein at 37°C for 10 min, and then assayed as to their migration to α-elastin ($10^{-1}$ μg/ml) or fMLP ($10^{-8}$ M) in the presence of each inhibitor in the same manner as described in the text.



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yl cAMP depressed the chemotactic responsiveness of the macrophages to $\alpha$-elastin, while preincubation with $10^{-6}$ M 8-Br cGMP enhanced the macrophage chemotactic responsiveness to $\alpha$-elastin. Neither dibutyryl cAMP nor 8-Br cGMP was effective on macrophage migration in response to fMLP.

The enhancement of the intracellular cGMP level is dependent upon the activation of either cytosolic guanylate cyclase or membrane-bound guanylate cyclase. Nitric oxide (NO) generated by activated cells is known to elevate the cGMP level by activating cytosolic guanylate cyclase (*25*). In order to determine whether the cGMP increase in macrophages stimulated by $\alpha$-elastin is caused by the activation of membrane-bound guanylate cyclase or cytosolic guanylate cyclase, the generation of NO by macrophages treated with $\alpha$-elastin was measured. Because NO is a short-lived intermediate, the production of $NO_2^-$, a stable oxidative degradation product of NO, was measured after macrophage treatment with $\alpha$-elastin, fMLP, or LPS and IFN-$\gamma$, as shown in Fig. 4. IFN-$\gamma$ and LPS, which are known as a primer and a trigger of $NO_2^-$ (*26*), dramatically enhanced the $NO_2^-$ production by macrophages. However, $NO_2^-$ production by macrophages treated with $10^{-1}$ $\mu$g/ml $\alpha$-elastin was scarcely observed at any time during the incubation. Treatment of macrophages with $10^{-8}$ M fMLP also did not induce $NO_2^-$ production.

The possible involvement of protein kinases in the $\alpha$-elastin signaling pathway associated with macrophage chemotaxis was investigated by use of various kinds of inhibitors (Fig. 5). As shown in Fig. 5B, KT5823, an inhibitor specific for PKG, inhibited the macrophage migration to $\alpha$-elastin in a dose-dependent manner, but not that to fMLP. A total 50% inhibition was observed at 13 $\mu$M. On the other hand, C-3030, an inhibitor specific for protein kinase C (PKC), effectively inhibited the macrophage migration to fMLP, but not that to $\alpha$-elastin (Fig. 5A). Genistein, an inhibitor of tyrosine kinase, also reduced the macrophage migration to fMLP, but not that to $\alpha$-elastin (Fig. 5D). Furthermore, KT5720, an inhibitor specific for cAMP-dependent protein kinase (PKA), also reduced the macrophage migration to fMLP, but its inhibitory effect was weaker than those of C-3030 and genistein (Fig. 5C). However, the macrophage migration to $\alpha$-elastin was not affected by KT5720 at concentrations up to 20 $\mu$M.

## DISCUSSION

$\alpha$-Elastin showed chemotactic activity toward macrophages at $10^{-1}$ $\mu$g/ml. Its chemotactic response curve is biphasic because of the possibility of the saturation of cell-surface receptors that trigger the chemotactic response (*27*) or of the possibility of the down regulation of the receptors (*28*). Although the existence of elastin receptors on macrophages has not yet been reported, it is suggested by the results of a homologous deactivation study that $\alpha$-elastin specific receptors may exist on macrophages.

In conjunction with the chemotactic response to $\alpha$-elastin, the signal transduction pathway used by $\alpha$-elastin was investigated. It is known that cell responses induced by fMLP, the positive control in the present experiments, are involved in the phosphoinositol cascade, leading to the enhancement of the intracellular $Ca^{2+}$ level and the production of $O_2^-$ (*29*). However, $\alpha$-elastin induced neither elevation of the intracellular $Ca^{2+}$ level nor $O_2^-$ production (data not shown). This suggests that the phosphoinositol cascade is not involved in the chemotactic response of macrophages to $\alpha$-elastin. Estensen *et al.* (*30*) have demonstrated that cell locomotion is controlled by cAMP and cGMP. Furthermore, Rivkin *et al.* (*31*) have reported that agents which increase the intracellular cAMP level inhibit cell locomotion, and Sandler *et al.* (*32*) have shown that agents which elevate the cGMP level enhance cell locomotion. Since cAMP and cGMP are thought to exert antagonistic regulatory effects on cell locomotion, we measured the intracellular cAMP and GMP levels after treatment with $\alpha$-elastin. As a result, it was found that $\alpha$-elastin increased the cGMP level, the maximal concentration being the same as that of $\alpha$-elastin-induced chemotaxis. Moreover, the curve of the change in the level of cGMP was biphasic, similar to the chemotactic-response curve. This biphasic nature suggests the possibility that down regulation of the $\alpha$-elastin and receptor complex was taking place. Therefore, the reason why the curve decreased with higher concentrations than $10^{-1}$ $\mu$g/ml is that the coupling of the receptor to its signaling pathway, leading to the activation of guanylate cyclase or an increase in the cGMP level, is strongly decreased by the down regulation. Since dibutylyl cAMP and 8-Br cGMP, less-polar derivatives of the parent cyclophosphates, are believed to penetrate cells more easily and to be active in cells, we investigated the effects of dibutyryl cAMP and 8-Br cGMP on macrophage chemotaxis induced by $\alpha$-elastin. Preincubation of macrophages with 8-Br cGMP enhanced macrophage chemotaxis. This suggests that cGMP is an important determinant of cell locomotion. On the other hand, the cAMP level remained to unaffected over the range of $\alpha$-elastin concentrations examined. Moreover, preincubation of macrophages with dibutyryl cAMP depressed the chemotactic responsiveness induced by $\alpha$-elastin. These reciprocal results for cAMP and cGMP are consistent with the previous proposal that cGMP might provide a "go" signal and cAMP a "stop" one (*30*). There are two types of guanylate cyclase which produce cGMP; one is $Ca^{2+}$-dependent cytosolic guanylate cyclase and the other is $Ca^{2+}$-independent membrane-bound guanylate cyclase. The cytosolic guanylate cyclase is known to be activated by NO (*25, 33*). The fact that $\alpha$-elastin induced neither the elevation of NO (Fig. 4) nor an increase in the intracellular $Ca^{2+}$ level (data not shown) in macrophages implies that $\alpha$-elastin might increase the cGMP level through the $Ca^{2+}$-independent membrane-bound guanylate cyclase. In an effort to explore further the signaling pathway used by $\alpha$-elastin, we used a series of protein kinase inhibitors. It was found that inhibitors of PKA, PKC, and tyrosine kinase had no effect on $\alpha$-elastin-induced chemotaxis. In contrast, a PKG inhibitor caused marked inhibition of $\alpha$-elastin-induced chemotaxis. The results obtained suggest that PKG is involved in the $\alpha$-elastin-induced signaling pathway.

In the present study, we investigated peritoneal macrophages as a potential reflection of inflammatory responsive macrophages at the sites of atheroscleotic and emphysematous lesions, in which elastin is degraded (*1, 2*), and have demonstrated that $\alpha$-elastin, one of the extracellular matrix components, induces macrophage chemotaxis, and that its chemotactic responsiveness is due to the elevation

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of the cGMP level and the activation of PKG. The interaction of $\alpha$-elastin with macrophages could be of importance in the inflammatory response associated with injury to elastin-rich organs such as arterial walls and lungs.

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