Negative Regulation of Monocyte Adhesion to Arterial Elastic Laminae by Signal Regulatory Protein α and Src Homology 2 Domain-containing Protein-Tyrosine Phosphatase-1*

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Elastic laminae are extracellular matrix constituents that not only contribute to the stability and elasticity of arteries but also play a role in regulating arterial morphogenesis and pathogenesis. We demonstrate here that an important function of arterial elastic laminae is to prevent monocyte adhesion, which is mediated by the inhibitory receptor signal regulatory protein (SIRP) α and Src homology 2 domain-containing protein-tyrosine phosphatase (SHP)-1. In a matrix-based arterial reconstruction model in vivo, elastic laminae were resistant to leukocyte adhesion and transmigration compared with the collagen-dominant arterial adventitia. The density of leukocytes within the elastic lamina-dominant media was about 58–70-fold lower than that within the adventitia from 1 to 30 days. An in vitro assay confirmed the inhibitory effect of elastic laminae on monocyte adhesion. The exposure of monocytes to elastic laminae induced activation of SIRP α, which in turn activated SHP-1. Elastic lamina degradation peptides extracted from arterial specimens could also activate SIRP α and SHP-1. The knockdown of SIRP α and SHP-1 by specific small interfering RNA diminished the inhibitory effect of elastic laminae, resulting in a significant increase in monocyte adhesion. These observations suggest that SIRP α and SHP-1 potentially mediate the inhibitory effect of elastic laminae on monocyte adhesion.

Arterial elastic laminae have long been considered a structure that determines the strength and elasticity of blood vessels (1–6). Recent studies, however, have demonstrated that arterial elastic laminae also participate in the regulation of arterial morphogenesis and pathogenesis (7–12). An important contribution of elastic laminae is to confine smooth muscle cells (SMCs) to the arterial media by inhibiting SMC proliferation (8, 9) and migration (10), thus preventing intimal hyperplasia under physiological conditions. Arterial elastic laminae also exhibit thrombosis-resistant properties. When implanted in an artery, elastic lamina scaffolds are associated with significantly lower leukocyte adhesion and thrombosis compared with collagen matrix scaffolds (10).

These observations suggest an inhibitory role for elastic laminae relative to collagen matrix. Although such a role is well documented, the mechanisms remain poorly understood.

Leukocytes are known to express the inhibitory receptor SIRP α (also known as Src homology 2 domain-containing tyrosine phosphatase substrate)-1, a transmembrane glycoprotein receptor that exerts an inhibitory effect on cell mitogenic (13–18) and inflammatory (19, 20) activities. Upon ligand binding, SIRP α transmits inhibitory signals through tyrosine phosphorylation of its intracellular immunoreceptor tyrosine-based inhibitory motif (15–18, 21, 22). The phosphorylation of the immunoreceptor tyrosine-based inhibitory motif initiates the recruitment of SHP homology 2 domain-containing protein-tyrosine phosphatase (SHP)-1 to SIRP α, which is known as a substrate of SHP-1 (21, 22). The recruitment of SHP-1 also localizes and activates SHP-1 (23), which in turn dephosphorylates protein kinases, possibly including receptor tyrosine kinases (23–25), the Src family protein-tyrosine kinases (26), phosphatidylinositol 3-kinase (26), and the Janus family tyrosine kinases (27, 28). These activities potentially suppress inflammatory and mitogenic responses (29–32). Because the inhibitory effect of elastic laminae coincides with the activity of the inhibitory receptor in leukocytes, it is conceivable that, upon contacting leukocytes, elastic laminae may interact with SIRP α and activate SHP-1, leading to the inhibition of leukocyte adhesion. In this study, we test the possibility that SIRP α and SHP-1 mediate the inhibitory effect of elastic laminae.

MATERIALS AND METHODS

Matrix-based Aortic Reconstruction—We have established a matrix-based aortic reconstruction model to test the inhibitory effect of elastic laminae on leukocyte transmigration relative to collagen matrix. Aortic substitutes were constructed with three types of aortic matrix scaffold as follows: NaOH-treated matrix scaffolds with an elastic lamina blood-contacting surface, untreated matrix scaffolds with a basal lamina blood-contacting surface, and NaOH-treated matrix scaffolds with an adventitial blood-contacting surface.

The first type of aortic substitute was created by treating fresh rat aortic specimens with 0.1 M NaOH at 20 °C for 2 h, followed by washing in distilled water for 12 h with vigorous agitation. Such a treatment removes the cellular components, basal lamina, and medial collagen matrix, leaving an aortic matrix scaffold with medial elastic laminae and collagen-dominant adventitia, as detected by immunohistochemistry (10).

The aortic substitutes with untreated matrix scaffolds and a basal lamina blood-contacting surface were constructed by freezing (–76 °C) and thawing (20 °C) fresh aortic specimens for three cycles, followed by
washing in distilled water with vigorous agitation for 12 h. Such a treat-ment removes endothelial cells and destroys other cell types, leaving a matrix scaffold with intact matrix and a basal lamina blood-contacting surface. The removal of endothelial cells was verified by immunohistochemistry with an anti-factor VIII antibody (10). The exposure of the basal lamina was verified by using an anti-collagen type IV antibody (10).

To create aortic substitutes with an adventitial blood-contacting sur-face, fresh rat aortic specimens were treated with 0.1 M NaOH at 20 °C for 2 h, followed by washing in distilled water for 12 h with vigorous agitation, which removes cells and proteoglycans in the adventitia. The aortic specimens were then turned outside in, resulting in aortic substitutes with an adventitial blood-contacting surface. The presence of the collagen blood-contacting surface was verified by immunohistochemistry with an anti-collagen type III antibody (10).

To create an aortic reconstruction model, a rat (Sprague-Dawley, male, 300–350 g) was anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital. Two aortic substitutes, either a pair with elastic lamina (NaOH-treated) and basal lamina (untreated) blood-con-tacting surfaces or a pair with elastic lamina (NaOH-treated) and adventi-tial surfaces, were anastomosed together in a series and grafted into the host rat abdominal aorta by using a method established previously (33). Such an arrangement ensured that each matrix substitute within the pair was in contact with the host aorta at one end. It should be pointed out that it is possible to graft all three types of matrix substitutes in a series in each animal. With such an arrangement, however, one of the three substitutes will not be in contact with the host aorta. Because cell migration from the host aorta to the substitutes contribute to inti-mal hyperplasia (34), a model with a series of three aortic substitutes will result in conditions of inconsistent controls. Observations were carried out at 1, 5, 10, 20, and 30 days with five rats at each observation time. Experimental procedures were approved by the Animal Care and Use Committee of Northwestern University.

**Measurement of Leukocyte Transmigration in Vivo**—To measure leukocyte transmigration in matrix substitutes, a rat was anesthetized as described above at each observation time. Specimens were collected from all types of arterial substitutes, fixed in 4% formaldehyde in phos-phate-buffered saline, cut into transverse cryosections of 10 μm in thickness, incubated with phycerothrin-conjugated anti-CD11b/c antibody (Caltag) and Hoechst 33258 (for cell nucleus labeling), and observed by fluorescence microscopy. The density of CD11b/c-positive cells in the elastic lamina-dominant media and collagen-dominant adventitia was measured and compared between different specimens.

**Measurement of Monocyte Adhesion to Elastic Lamina, Basal Lamina, and Adventitia in Vitro**—We used an in vitro monocyte adhesion assay to demonstrate the mechanisms of the inhibitory effect of elastic laminae. To collect monocytes for the in vitro assay, rats were anesthe-tized as described above. A blood sample of ~10 ml was collected from the vena cava of each rat and mixed with 20% acid/mg/liter sodium citrate, 110 mmol/liter glucose, and 80 mmol/liter citric acid. Monocytes were collected by using a monocyte enrichment kit (Stemcell Technologies Inc., Vancouver, British Columbia, Canada) according to the manufacturer’s instructions. Enriched monocytes were suspended in 15% fetal bovine serum/Dulbecco’s modified Eagle’s medium at a cell count ~6 × 10⁶ cells/ml, supplemented with 100 μg/ml streptomycin and 100 units/ml penicillin.

Aortic matrix specimens were prepared with four different surfaces for interacting with monocytes as follows: NaOH-treated elastic lamina, untreated elastic lamina, basal lamina, and adventitia. Specimens with NaOH-treated elastic lamina, basal lamina, and adventitial surfaces were prepared as described under “Matrix-based Aortic Reconstruction.” Matrix specimens with an untreated elastic lamina surface were prepared as follows. Fresh aortic specimens were collected and sepa-rated between the media and adventitia with fine tweezers under a sur-gical microscope. The media were further separated between interior elastic laminae to expose the surface of elastic laminae. Separated medial specimens were frozen (~76 °C) and thawed (20 °C) for three cycles. The surface of exposed elastic laminae was scraped with a fine metal wire along the elastic fiber direction to remove cell debris and other matrix components, including medial collagen and proteoglycans. The medial specimens were washed in distilled water for 12 h with vigorous agitation. Medial specimens with an elastic lamina surface were selected by immunohistochemistry. All prepared elastic lamina specimens were incubated consecutively with four antibodies, including anti-collagen type III (Chemicon), anti-collagen type IV (Chemicon), anti-vascular proteoglycans (US Biological), and anti-SMC α-actin (Chemicon) antibodies. Hoechst 33258 was used for labeling cell nuclei. Specimens without collagen type III, collagen type IV, proteoglycans, SMC α-actin filaments, and cell nuclei at the elastic lamina surface were selected by fluorescence microscopy and used as untreated elastic laminae. Fig. 1 shows the presence of elastic lamina and the absence of collagen matrix and SMCs at the surface of prepared specimens.

Enriched monocytes were incubated in culture media at 37 °C in the presence of the four types of matrix specimen for 3, 6, 12, and 24 h with gentle agitation. At each observation time, samples from the four types of matrix specimen were collected, fixed in 4% formaldehyde in phosphate-buffered saline, incubated with an anti-CD 14 antibody and Hoechst 33258, and observed with a fluorescence microscope for meas-uring the density of monocytes adhered to the matrix specimens. Spec-imens from five rats were used for statistical analyses at each time point.

**Detection of the Role of Elastic Laminae in Regulating the Activity of SIRP α and SHP-1**—To test whether the exposure of monocytes to elastic laminae induces activation of SIRP α and SHP-1, enriched mono-cytes were cultured in dishes coated with four types of matrix specimens as follows: NaOH-treated elastic lamina, untreated elastic lamina, basal lamina, and adventitia, which were prepared as described above. At culture times of 3, 6, and 12 h, monocytes were collected from each of the four types of matrix coatings and prepared for detecting the expres-sion and phosphorylation of SIRP α and SHP-1. Enriched monocytes were lysed in lysis buffer containing 1% Triton X-100, 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM sodium orthovanadate, 1 mM EDTA, and a protease inhibitor mixture (1 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml pepstatin, and 1 mM phenylmethylsulfonyl fluoride). The lysates were measured for total protein concentration, precloned with protein A-conjugated agarose beads (10% packed beads, Upstate), immunoprecipitated with an anti-SIRP α antibody (4 μg/ml, sc-17803; Santa Cruz Biotechnology) at 4 °C for 4 h, and incubated with protein A-agarose beads (10% packed beads) at 4 °C for 4 h. The agarose beads were col-lected and treated with protein sample buffer at ~100 °C for 5 min. Immunoprecipitates were resolved by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with the anti-SIRP α antibody (0.5 μg/ml). The relative level of protein was examined by secondary peroxidase-IgG labeling and chemiluminescent detection of peroxidase activity (35). The nitrocellulose membrane was stripped and immuno-blotted consecutively with an anti-SHP-1 antibody (sc-287; Santa Cruz Biotechnology) and an anti-phosphotyrosine antibody (clone 4G10, 05-321; Upstate).
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Detection of the Role of SIRP α and SHP-1 in Regulating Monocyte Adhesion to Elastic Laminae—To test the role of SIRP α and SHP-1 in regulating monocyte adhesion to elastic laminae, small interfering RNA (siRNA) specific to SIRP α and SHP-1 mRNA was used to degrade these mRNAs and thus knock down the expression of SIRP α and SHP-1, respectively. Briefly, SIRP α- and SHP-1-specific siRNAs were prepared according to the provider’s instruction (Santa Cruz Biotechnology). Enriched monocytes were transfected with siRNA for SIRP α (0.05 μM) or siRNA for SHP-1 (0.05 μM) by the mediation of siRNA transfection reagent (0.5%, Santa Cruz Biotechnology) at 37 °C for 48 h. Monocytes transfected with a nontargeting scrambled siRNA control, which does not degrade known mRNAs, were used as a control (36, 37). Note that the transfection medium contains 8.8% fetal bovine serum without antibiotics. Following siRNA transfection, fetal bovine serum was added to the culture to make the final serum concentration of 15%. Matrix specimens with NaOH-treated and untreated elastic lamina, basal lamina, and adventitia, prepared as described above, were applied to the culture with siRNA-transfected monocytes. Cells were subsequently cultured with the matrix specimens for 3, 6, 12, and 24 h. At each time, five matrix specimens were collected and prepared for measuring the density of monocytes adhered to the matrix surface by using methods described above.

The effectiveness of siRNA treatment was tested by immunoprecipitation and immunoblotting for each type of siRNA. At 48 h of culture with an siRNA, monocytes were collected and prepared for detecting the expression of SIRP α and SHP-1 by immunoprecipitation and immunoblotting with anti-SIRP α and SHP-1 antibodies, respectively, as described above (two assays done for each siRNA). Furthermore, monocytes were transfected with a fluorescein-conjugated nontargeting siRNA and observed by fluorescence microscopy for the verification of positive siRNA transfection. In addition, cell viability was tested by estimating the total cell number in each culture dish before and after the siRNA treatment (four samples tested for each siRNA and the control siRNA). Note that after 48 h of culture, a fraction of cells adhered to the culture base. The total cell number after the siRNA treatment was the sum of the adherent and suspended cells.

Detection of Elastic Lamina Degradation Peptide Binding to SIRP α—To test the possibility that elastic laminae interact with SIRP α in monocytes, a reasonable approach is to detect whether components from elastic laminae bind to SIRP α. To achieve such a goal, we prepared elastic lamina degradation peptides from rat aortic specimens by using a method established previously (38). Briefly, aortic specimens were collected from rats. The media of the aorta were manually separated from the adventitia under a surgical microscope with a pair of fine surgical forceps. The media were collected, minced, and treated with 1 N KOH/ethanol (80:20, v/v) at 37 °C for 1 h. The resulting mixture was centrifuged, and the supernatant was discarded. Samples were randomly selected from the remaining insoluble fraction and used for verifying the removal of medial cells, collagen fibers, and proteoglycans by immunohistochemistry as described above. Note that the KOH treatment removed all medial components except the elastic laminae. The insoluble elastic lamina fraction was treated with the KOH/ethanol mixture at 37 °C for 1 h. The supernatant was collected and neutralized with perchloric acid to pH 7.4, and the resulting precipitate was discarded. Elastic lamina degradation peptides were collected from the remaining supernatant.

Monocytes were cultured in the presence of 10 μg/ml elastic lamina degradation peptides, collected at 0.5, 1, and 3 h, and lysed in lysis buffer as described above. Lysates were processed for immunoprecipitation with an anti-elastin antibody (Elastin Products), resolved by SDS-PAGE, and probed with an anti-SIRP α antibody (sc-17803; Santa Cruz Biotechnology) and an anti-phosphotyrosine antibody (clone 4G10; Upstate) as described above.

Detection of SIRP α and SHP-1 Phosphorylation in the Presence of Elastic Lamina Degradation Peptides—To detect whether the binding of elastic lamina degradation peptides induces SIRP α phosphorylation and whether SIRP α phosphorylation induces SHP-1 recruitment, monocytes were cultured in the presence and absence of elastic lamina degradation peptides (10 μg/ml), collected at 0.5, 1, 3, and 6 h, and lysed in lysis buffer. Lysates were processed for immunoprecipitation with an anti-SIRP α antibody (sc-17803; Santa Cruz Biotechnology) and for consecutive immunoblotting by using anti-SIRP α, anti-SHP-1 (sc-287;
Santa Cruz Biotechnology), and anti-phosphotyrosine (clone 4G10; Upstate) antibodies with antibody stripping after each immunoblotting reaction.

**Cytometry Confirmation of Elastic Lamina Degradation Peptide Binding to SIRP α**—To confirm the binding of elastic lamina degradation peptides to SIRP α, the influence of an anti-SIRP α antibody (sc-17803; Santa Cruz Biotechnology), developed with the extracellular domain of SIRP α (1–300 amino acids) as an antigen, on the relative binding of elastic lamina degradation peptides was detected by flow cytometry. Monocytes were treated with 0, 5, and 10 μg/ml anti-SIRP α antibody separately at 37 °C for 1 h and subsequently incubated with 10 μg/ml fluorescein-conjugated elastic lamina degradation peptides at 37 °C for 1 h. Monocytes incubated with an unrelated fluorescein-conjugated secondary antibody (10 μg/ml) without elastic lamina degradation peptides were used as controls. In addition, an anti-CD11b antibody (10 μg/ml; MLDP5; R & D Systems) was used as a control in the presence of 10 μg/ml elastic lamina degradation peptides. Monocytes were detected for fluorescent intensity by flow cytometry (Beckman Coulter Epics XL-MCL).

**Statistical Analyses**—Means and standard deviations were calculated for each measured parameter at each observation time. The Student’s t test was used for difference comparisons between two groups. A difference is considered statistically significant if *p* < 0.05.

### RESULTS

**Role of Elastic Laminae in Preventing Leukocyte Transmigration in Vivo**—We used an in vivo matrix-based arterial reconstruction model to observe the role of elastic laminae in preventing leukocyte transmigration through the arterial media. As shown in Fig. 2, many cells found in the matrix of aortic substitutes were CD11 b/c-positive leukocytes (predominantly monocytes/macrophages and granulocytes), especially during the early period. Although a large number of leukocytes migrated into the collagen-dominant adventitia, few leukocytes were found within the elastic lamina-dominant media of the matrix-based aortic substitutes. The density of leukocytes in the media was 58–70-fold lower than that in the adventitia from 1 to 30 days after surgery, whereas no significant difference was detected in the media between NaOH-treated (with an elastic lamina blood-contacting surface) and untreated matrix (with a basal lamina blood-contacting surface) substitutes (Fig. 2). At the end of the elastic lamina-dominant media, leukocytes were not able to migrate into the gaps between the elastic laminae, even though the gaps were apparently larger than the diameter of leukocytes (Fig. 2A, Day 10°). However, at locations with aneurysm-like changes (induced possibly by excessive mechanical stretch because of surgical damage to the adventitia), leukocytes migrated into the medial wall, where elastic laminae were largely destroyed (Fig. 2A, Day 10° ). These observations demonstrate that intact and NaOH-treated elastic laminae exert an inhibitory effect on leukocyte transmigration relative to the adventitial collagen matrix.

**Inhibitory Effect of Elastic Laminae on Monocyte Adhesion in Vitro**—We carried out an in vitro monocyte adhesion assay to observe the inhibitory effect of elastic laminae on monocyte adhesion relative to that of the arterial basal lamina and adventitia. As shown in Fig. 3, the exposure of monocytes to NaOH-treated and untreated elastic laminae induced monocyte adhesion ranging from 9 to 20 and 13 to 16 cells/mm², respectively, from 3 to 24 h. In contrast, exposure to the basal lamina and adventitia resulted in a more than 11- and 98-fold increase in monocyte adhesion, respectively, compared with elastic lamina. These observations verify the inhibitory role of elastic laminae on monocyte adhesion relative to collagen-containing matrix.

**Activation of Monocyte SIRP α and SHP-1 in Response to Exposure to Elastic Laminae in Vitro**—Tyrosine phosphorylation of SIRP α is required for its inhibitory effect and for the recruitment and activation of SHP-1. To test whether the interaction of monocytes with matrix components induces SIRP α phosphorylation and SHP-1 recruitment, we examined co-immunoprecipitation of SIRP α with SHP-1 and the relative phosphorylation of these molecules in monocytes reacted with NaOH-treated and untreated matrix scaffolds. Differences were significant (*p* < 0.0001) between elastic laminae and adventitia at all observation times except time 0, at which time no CD11b/c-positive cells were found. No significant difference was detected between NaOH-treated and untreated elastic laminae at any observation time (*p* > 0.05). Means ± S.D. are presented (*n* = 5 for each group).

### FIGURE 2

**Leukocyte transmigration in the elastic lamina-dominant media and collagen-dominant adventitia of matrix-based aortic substitutes in vivo.** A, transverse fluorescent micrographs showing the distribution of CD11 b/c-positive leukocytes in the media and adventitia of matrix-based aortic substitutes. Note that leukocytes did not migrate into the gaps between the elastic laminae at the end of the aortic matrix substitutes (Day 10°). However, leukocytes migrated into the media of aortic matrix substitutes when the elastic laminae were largely destroyed at locations with aneurysmatic changes (Day 10°* ). Red, antibody-labeled CD11 b/c. Green, elastic lamina. Blue, Hoechst 33258-labeled cell nuclei. Solid arrow, blood-contacting surface of aortic matrix substitutes. Open arrow, aneurysmatic change. Scale, 100 μm. B, density of CD11 b/c-positive cells within the elastic lamina-dominant media and collagen-dominant adventitia of NaOH-treated and untreated matrix scaffolds. Differences were significant (*p* < 0.0001) between elastic laminae and adventitia at all observation times except time 0, at which time no CD11b/c-positive cells were found. Data are mean ± S.D. (n = 5 for each group).
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stimulates the activation of SIRP α, which induces the recruitment and phosphorylation of SHP-1.

Role of SIRP α and SHP-1 in the Negative Regulation of Monocyte Adhesion to Elastic Laminae in Vitro—We used an siRNA approach to knock down the expression of SIRP α and SHP-1 and thus to demonstrate the role of these molecules in mediating the inhibitory effect of elastic laminae on monocyte adhesion. As shown in Fig. 5A, transfection with SIRP α-specific siRNA apparently reduced the expression of SIRP α in monocytes. Such a treatment diminished the inhibitory effect of elastic laminae on monocyte adhesion, resulting in a significant increase in the density of monocytes on the surface of NaOH-treated and untreated elastic laminae (Fig. 5B). However, such a treatment did not significantly influence monocyte adhesion to the basal lamina and adventitia (Fig. 5B). Similar results were observed for the transfection with SIRP-1-specific siRNA. These observations suggest that SIRP α and SHP-1 serve as potential mediators for the inhibitory effect of elastic laminae on monocyte adhesion.

We have also tested the influence of siRNA transfection on the cell viability by estimating the total cell numbers before and after siRNA transfection. Among the four groups tested were a control group with culture medium only, a group with siRNA transfection reagent in culture medium, and two groups with siRNA for SIRP α and SHP-1 in culture medium supplemented with transfection reagent. The total cell numbers (in 2 ml of culture medium used for each assay) for the four groups were 1.15 × 10^6 ± 1.83 × 10^5 (culture medium only), 1.21 × 10^6 ± 2.13 × 10^5 (transfection reagent), 1.20 × 10^6 ± 1.80 × 10^5 (siRNA for SIRP α), and 1.19 × 10^6 ± 1.64 × 10^5 (siRNA for SHP-1) before siRNA transfection (n = 4 for each group). After 48 h of siRNA transfection, the total cell numbers (sum of suspended and adherent monocytes) for the same groups were 1.17 × 10^6 ± 1.14 × 10^5 (culture medium only), 1.18 × 10^6 ± 2.2 ± 10^5 (transfection reagent), 1.18 × 10^6 ± 1.52 × 10^5 (siRNA for SIRP α), and 1.14 × 10^6 ± 1.01 × 10^5 (siRNA for SHP-1). Although the total cell numbers were reduced for the groups with the transfection reagent and siRNA after siRNA transfection, no statistical significance was detected.

Binding of Elastic Lamina Degradation Peptides to SIRP α in Vitro—To test whether components from elastic laminae bind to SIRP α, we prepared elastic lamina degradation peptides and examined the co-immunoprecipitation of elastic lamina degradation peptides with SIRP α. As shown in Fig. 6A, in monocytes reacted with elastic lamina degradation peptides (10 μg/ml), SIRP α could be co-immunoprecipitated with elastic lamina degradation peptides by using an anti-elastin antibody, which was shown to react with elastin peptides in an immunoblotting analysis. In a flow cytometry test, treatment with an anti-SIRP α antibody (5 and 10 μg/ml) developed with the extracellular domain of SIRP α as an antigen, competitively reduced the binding of fluorescein-conjugated elastic lamina degradation peptides (10 μg/ml) to monocytes (Fig. 6B). The relative fluorescent intensity at the peak distribution of the tested monocytes, a relative index for the level of ligand binding, was reduced by 50 ± 15% and 63 ± 12% in the presence of 5 and 10 μg/ml anti-SIRP α antibody, respectively (n = 3). In contrast, an anti-CD11b antibody did not apparently influence the binding of the elastic lamina degradation peptides. These observations suggest that elastic lamina degradation peptides can bind to SIRP α in monocytes.

Activation of Monocyte SIRP α and SHP-1 in the Presence of Elastic Lamina Degradation Peptides—To test whether elastic lamina degradation peptides influence the activity of SIRP α and SHP-1, we examined...
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The inhibitory effect of arterial elastic laminae—Biological activities are often regulated by coordinated stimulatory and inhibitory signaling mechanisms so that the activities can be controlled precisely as needed. Extracellular matrix components participate in the regulation of cellular processes such as cell adhesion, proliferation, and migration. Collagen-containing matrix has long been known to stimulate these cellular processes. However, the inhibitory aspect of extracellular matrix has been poorly understood. Several recent studies have suggested a role for the arterial elastic laminae in the negative regulation of vascular SMC proliferation and migration (9, 10, 39, 40). Such a role is supported by several lines of experimental evidence. Genetically induced deficiency of elastin in a mouse model is associated with enhanced SMC proliferation, intimal hyperplasia, and arterial stenosis during the fetal stage, resulting in animal death shortly after birth (9). Incomplete development of arterial elastic laminae in human genetic disorders, such as Williams syndrome and supravalvular aortic stenosis, is associated with similar pathological changes in large arteries (39, 40). In a model of arterial implantation, decellularized elastic laminae prevent SMC migration into the elastic lamina-dominant media (10). These observations have demonstrated that elastic laminae exert an inhibitory effect on the mitogenic activities of vascular SMCs. Such an inhibitory effect may counterbalance the stimulatory effect of collagen matrix.

The present study demonstrates that arterial elastic laminae are resistant to leukocyte adhesion and transmigration compared with collagen-dominant adventitia. It is interesting to note that the density of leukocytes within the elastic laminae was 58–70-fold lower than that within the collagen-dominant adventitia in the in vivo aortic reconstruction model. Furthermore, in vitro tests showed that the density of monocytes adhering to elastic laminae was about 11- and 98-fold lower than that adhering to the basal lamina and adventitia, respectively. These observations suggest an inhibitory effect of elastic laminae on leukocyte adhesion relative to collagen-containing matrix. It is often
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observed that the arterial media are associated with a relatively low-level of inflammatory reaction compared with the intima and adventitia. The inhibitory effect of elastic laminae may contribute to such a phenomenon.

Although elastic laminae are resistant to inflammatory activities, we have observed monocyte infiltration in the arterial media with atherosclerosis. A possible mechanism is that elastin degradation occurs in arterial atherosclerosis because of the up-regulation of elastolytic matrix metalloproteinases such as MMP-2 and -9 (41), and the resulting elastin degradation peptides may serve as chemotactic factors, which induce monocyte infiltration into the arterial media (42). Although elastin degradation peptides may contribute to monocyte infiltration in atherosclerotic arteries, it may also be possible that the destruction of elastic laminae diminishes the protective effect of elastic laminae, thus allowing monocyte transmigration. This possibility is supported by the present observation that monocytes are found in the media of aortic substitutes with apparently destroyed elastic laminae but not in the media with NaOH-treated and untreated elastic laminae (Fig. 2A, Day 10°). It is important to note that leukocytes are capable of producing and releasing matrix metalloproteinases that degrade matrix components, a process facilitating leukocyte migration. The inhibition of leukocyte adhesion to elastic laminae potentially prevents the degradation of arterial medial matrix, thus protecting the media from further leukocyte infiltration.

The discovery of the inhibitory effect of arterial elastic laminae suggests that elastic laminae may be potentially used for constructing the blood-contacting surface of arterial substitutes. During the past decades, investigators have been searching for ideal arterial substitutes that possess the structural and mechanical features of a natural artery as well as inflammation-resistant properties. A number of polymeric and biological materials, including nonbiodegradable and biodegradable polymers (43–48), collagen matrix (49–51), and fibrin matrix (52), have been characterized and tested in experimental or clinical studies. Although each of these biomaterials exhibits characteristics suitable for the construction of arterial substitutes, the potency of biomaterial-based arterial substitutes remains problematic because of inflammation and thrombogenesis (53). Endothelial cell seeding of biomaterials has been proposed and used for reducing the thrombogenicity of biomaterials (54). However, difficulties in cell retention during and after arterial reconstruction hinder the application of such an approach (55). Although autogenous vein grafts offer satisfactory results (33, 34, 56), not all patients possess veins available for arterial reconstruction. Thus a pressing issue in arterial reconstruction is to develop arterial substitutes that possess the structural and mechanical properties of natural arteries. Given the natural features, inhibitory properties, inert immunogenicity, and availability, arterial elastic laminae may be used as a blood-contacting material for arterial reconstruction.

Potential Mechanisms for the Inhibitory Effect of Elastic Laminae—Leukocyte activation is a critical process that contributes to thrombogenesis and atherogenesis. Two opposing mechanisms, protein-tyrosine kinase-induced phosphorylation and protein-tyrosine phosphatase-induced dephosphorylation, are potentially involved in the regulation of leukocyte activities (29–32). Whereas the activation of certain protein-tyrosine kinases may initiate and promote leukocyte activities, the activation of corresponding protein-tyrosine phosphatases may exert an opposite effect. Protein-tyrosine phosphatases thus play a critical role in the prevention of excessive inflammatory activities.

Leukocytes express the inhibitory receptor SIRP α (13–15), which can recruit and activate protein-tyrosine phosphatase SHP-1 (21–23). Activated SHP-1 in turn dephosphorylates or deactivates substrate protein-tyrosine kinases, thus inhibiting inflammatory activities (23–32). In SHP-1-deficient mice, profound activation of macrophages and neutrophils occurs in association with excessive inflammatory reactions in the lung, liver, joint, and dermis (57). These observations confirm the role of SHP-1 in suppressing leukocyte activities. Thus, the SIRP α–SHP-1 signaling pathway provides a potential mechanism that may mediate the inhibitory effect of arterial elastic laminae on monocyte adhesion.

The present study demonstrates that the exposure of monocytes to arterial elastic laminae induces activation of SIRP α, which further recruits and activates SHP-1. These activities were consistent with the inhibitory effect of elastic laminae on monocyte adhesion relative to the basal lamina and adventitial collagen matrix. When the expression of SIRP α or SHP-1 was suppressed by transfection with specific siRNA, the inhibitory effect of elastic laminae was reduced, resulting in a significant increase in monocyte adhesion to elastic laminae. In contrast, SIRP α- or SHP-1-specific siRNA did not significantly influence monocyte adhesion to the basal lamina and adventitial specimens. These observations suggest that SIRP α and SHP-1 mediate the inhibitory effect of elastic laminae but do not significantly influence the interaction of monocytes with collagen-dominant matrix.

Further investigations have shown that the degradation peptides of elastic laminae are capable of binding to SIRP α, suggesting interactions of SIRP α with elastic lamina components. Similar to the reaction of monocytes with elastic laminae, treatment with elastic lamina degradation peptides activates SIRP α and subsequently SHP-1. These observations further support the role of SIRP α and SHP-1 in mediating the inhibitory effect of elastic laminae on monocyte adhesion.

Our flow cytometry tests have demonstrated that the blocking antibody for SIRP α only partially blocks the binding of elastic lamina degradation peptides to SIRP α. This observation suggests that components from elastic laminae may interact with not only SIRP α but also other types of receptor. A possible candidate is the laminin/elastin receptor, to which elastin degradation peptides can bind (1, 3, 58). Another possibility is that the blocking antibody may not be able to completely block the interaction of elastic lamina degradation peptides with SIRP α.

As reported previously, elastin degradation peptides from human atherosclerotic arteries interact with and activate the laminin/elastin receptor of monocytes, stimulating monocyte chemotactic activities (42). Furthermore, the binding of elastin degradation peptides to the laminin/elastin receptor has been shown to activate mitogenic signaling mechanisms and to promote the proliferation of cultured SMCs (58). However, other studies have demonstrated that elastin degradation peptides exert an inhibitory effect on SMC proliferation and migration (59). These observations suggest that stimulatory and inhibitory receptors may co-exist for elastin degradation peptides and may coordinate in the regulation of cell activities. Elastin degradation peptides may selectively interact with these receptors, depending on the relative strength of the receptors. Various factors, such as the type and state of cells and experimental conditions, may influence the relative receptor strength. It is important to note that although experimental observations on elastin degradation peptides are controversial, the inhibitory effect of elastic laminae has been consistently observed in human and experimental investigations (9, 10, 12, 39, 40). These observations suggest that the inhibitory components may be dominant at the surface of the elastic laminae.

In summary, the present observations suggest that, compared with collagen-dominant matrix, arterial elastic laminae are resistant to monocyte adhesion. Such an effect is potentially mediated by the inhibitory receptor SIRP α and SHP-1. The interaction of elastic laminae with...
monocytes may activate SIRP α- and SHP-1-related signaling pathways that potentially suppress pro-adhesion mechanisms. The inhibitory effect of elastic laminae may potentially counterbalance the stimulatory effect of the collagen matrix, contributing to coordinated regulation of inflammatory activities in the wall of arteries. The inhibitory feature renders elastic lamina a potential blood-contacting material for arterial reconstruction.

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