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Elafin-overexpressing mice have improved cardiac function after myocardial infarction

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Ohta, Kunio, Takanori Nakajima, Alexander Y. L. Cheah, Syed H. E. Zaidi, Nilo Kaviani, Fayez Dawood, Xiao-Mang You, Peter Liu, Mansoor Husain, and Marlene Rabinovitch. Elafin-overexpressing mice have improved cardiac function after myocardial infarction. *Am J Physiol Heart Circ Physiol* 287: H286–H292, 2004. First published December 23, 2003; 10.1152/ajpheart.00479.2002.—Elevated serine elastase activity after myocardial infarction can contribute to remodeling associated with left ventricular dilatation and dysfunction. We therefore assessed the effects of overexpressing the selective serine elastase inhibitor elafin in transgenic mice in which a myocardial infarction was caused by ligation of the left anterior descending coronary artery (LAD). Elevated serine elastase activity was observed in nontransgenic littermates as early as 6 h after LAD ligation and persisted at 4 and 7 days but not in sham-operated or elafin-overexpressing transgenic mice. Myeloperoxidase activity (index of inflammatory cells) and matrix metalloproteinase 2 were also increased but only at 4 and 7 days and only in nontransgenic mice ($P < 0.05$ for both comparisons), and this increase correlated with inflammatory cell infiltration. Echocardiographic study at 4 days revealed indexes of diastolic dysfunction in nontransgenic versus elafin-overexpressing mice ($P < 0.05$). Morphometric and biochemical analyses at 28 days indicated impairment in cardiac performance, with greater scar thinning and infarct expansion in nontransgenic versus elafin transgenic littermates ($P < 0.05$ for all comparisons). Thus serine elastase inhibition appears to suppress inflammation, cardiac dilatation, and dysfunction after myocardial infarct.

elastase; metalloproteinase; myocardial infarction; remodeling; transgenic mice

THE SEVERITY OF ventricular dilatation and impaired cardiac performance after myocardial infarction (MI) is related to the magnitude of the initial ischemic damage and the subsequent repair process (20). The repair process is associated with proteolytic activity and alterations in extracellular matrix (ECM) components, ultimately reflected in replacement of myocardial tissue by collagen (1). Studies in human (14) and animal models (2, 3, 6, 7, 18, 22, 25) suggest that early local activation of proteolytic enzymes such as serine elastases and matrix metalloproteinases (MMPs) may play a pivotal role in the subsequent inflammatory response leading to changes in left ventricular (LV) structure and function. Elevated plasma levels of MMP-2 and MMP-9 correlate with cardiac dilatation after acute MI in patients (14) and experimental animals (3), and treatment of pigs (18) and mice (22) with MMP inhibitors attenuates early LV dilatation after MI. Serine elastase inhibitors also reduce MI size in rats (25).

A variety of transgenic mice have been used to identify the relative roles of serine proteinases and MMPs in mediating changes in cardiac structure and function after MI. For example, attenuated cardiac dilatation was observed in MMP-9 null mice post-MI (2), but there was also reduced collagen accumulation associated with impaired healing. Deletion of MMP-2 in mice resulted in better survival after MI, associated with reduced cardiac dilatation and incidence of cardiac rupture (6). In plasminogen activator null mice, increased cardiac rupture and decreased vascularization indicated that serine proteinases such as plasmin play an important role in the healing process related to vasculogenesis (7).

Elastases, which, in addition to elastin, can degrade a wide variety of ECM components including collagen, can also activate MMPs (19) and inactivate tissue inhibitors of MMPs (TIMPs) (12). We created a transgenic mouse that overexpresses the serine elastase inhibitor elafin under the regulation of the preproendothelin promoter (29). These mice are protected against the cardiac dysfunction and fibrosis after acute viral myocarditis (29) and against intimal thickening after wire injury of the carotid artery (28). The purpose of this study was, therefore, to use this mouse to determine how enhanced endogenous expression of a selective inhibitor of serine elastases could influence cardiac structure and function after MI induced by left anterior descending coronary artery (LAD) ligation.

We now report that an experimentally produced MI in mice results in an early increase in serine elastase and MMP-9 activity that precedes MMP-2 expression and the inflammatory response. Suppression of all these features except MMP-9 occurs in the elafin transgenic mice and is correlated with a reduction in both early cardiac dilatation and diastolic dysfunction and protects against the later impairment in cardiac function (dP/d*t*). Collagen accumulation is similar in elafin transgenic and nontransgenic mice post-MI, but the elafin transgenic group exhibits reduced scar thinning and infarct expansion, features associated with impaired cardiac contractility and propensity to cardiac rupture.

METHODS

Study design. Creation and genotyping of the B6/SJL-CD1 transgenic mouse in which the mouse preproendothelin promoter was used to target overexpression of FLAG-tagged human elafin to the cardio-

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vascular system are provided elsewhere (29). These mice have a normal vasculature, and we have previously documented serine elastase inhibitory activity in the heart, after induction of myocarditis (29), and in arteries, after wire injury (28). Equal numbers of male and female mice matched from three different litters underwent ligation of the LAD or sham operation at 9–12 wk of age according to a protocol approved by the University of Toronto Committee on Animal Care. The animals were anesthetized with 10 mg/ml ketamine (MTC Pharmaceuticals; Cambridge, Ontario, Canada) and 10% xylazine (10 mg/kg; Bayer; Toronto, Ontario, Canada), intubated, and ventilated with room air using a pressure control ventilator (Kent Scientific; Litchfield, CT). The thorax and pericardium were opened, and the heart was exposed. With the use of a 7-0 silk suture (Deknatel; Fall River, MA), the LAD was ligated, the chest was closed, and the animal was allowed to recover. For consistency, ligation of the LAD was performed by one investigator (F. Dawood). The mice were killed at various time points ranging from 6 h to 28 days after LAD ligation. Hearts were removed and rinsed quickly in ice-cold PBS (pH 7.4). Tissues used for enzymatic assays were immersed in liquid nitrogen and stored at −70°C, whereas tissue for histology was fixed overnight in 10% formalin. Where possible, we tried to use the same animals for different assessments so that the results could be better correlated. The animals were divided between those used for histology and those for enzyme assays at 6 h after LAD ligation. At 4 days, the animals used for echocardiography were also used for enzyme assays and for histology. At 14 days, the hearts were all used for histology, and at 28 days the hearts from animals used for hemodynamic studies (including those that were incomplete for technical reasons) were either used for biochemical assessment of collagen or were evaluated by histological morphometry.

Elastase, myeloperoxidase, and MMP activity extracted from entire heart. Determination of elastase activity was carried out at 6 h, 4 days, and 7 days after LAD ligation according to a modification of a method previously described (16). Frozen myocardial tissue was extracted, dialyzed, lyophilized, and incubated with 2 μl of 20 mM fluorescent elastin peptide substrate, AFC091 (Enzyme Systems Products; Livermore, CA) with the volume adjusted to 1 ml with Tris acetate buffer. The change in absorbance at 505 nm after excitation at 400 nm was monitored for 10 min using a fluorescence spectrophotometer (model F9000, Hitachi America; Tarrytown, NY) and compared with a standard curve generated using 1–4 ng of human leukocyte elastase (HLE) (Elastin Products; Owensville, MO) with a specific activity of 800 U/mg. Each sample was normalized for protein content using a kit (Bio-Rad Laboratories), and all values are expressed as HLE units per milligram of protein.

Myeloperoxidase (MPO) activity assessed by spectrophotometry as previously described (16) and was used as an index of inflammatory response in myocardial tissue harvested 6 h, 4 days, and 7 days after LAD ligation. Human MPO (Sigma-Aldrich Canada) was used to generate a standard curve, and 1 unit of MPO activity represented the degradation of 1 μM of hydrogen peroxide per minute at 25°C. MMPs were extracted from myocardial tissue at 6 h, 4 days, and 7 days after LAD ligation and assessed by gelatin zymography using a standard procedure (3). The gels were dried and scanned using a charge-coupled device camera densitometer (Bio-Rad imaging densitometer), and the band intensities were analyzed and normalized to a standard (MMP-2) on the same gel. To assess the number of inflammatory cells in histological sections of the heart at 6 h and 4 days, an anti-MPO antibody (1:50 dilution) was used.

Echocardiography. Transthoracic echocardiographic studies were carried out 4 days after LAD ligation to assess LV dimensions and diastolic function (23). M-mode and Doppler exhocardiographic studies were performed under anesthesia using 10 mg/ml ketamine and 10% xylazine (10 mg/kg) with an S12 (model 21380A) ultraband linear array transducer on a SONOS 5500 echocardiographic imaging system (Agilent Technologies). A customized rubber standoff filled with Aquasonic 100 ultrasound transmission gel (Parker Laboratories; Orange, NJ) was attached to the transducer so that the distance between the transducer and mouse chest wall was 2–3 cm. The S12 emits an M-mode echo signal at 12 MHz and a Doppler signal at 5 MHz. For Doppler measurements, the pulse repetition frequency was dynamic and calculated automatically by the imaging system based on the returning frequency phase shift. For mitral inflow velocities, we used a Doppler sampling volume of 60 micrometer with a pulse repetition frequency of 89 Hz. M-mode measurements of LV septal and posterior wall thickness and LV end-diastolic and end-systolic diameter (LVEDd and LVESd) were made in all animals at the midventricular level, defined as being that point at which the papillary muscles are visualized. LVEDd was defined as the maximal diastolic diameter, whereas LVESd was measured at the point at which the posterior wall achieved maximum anterior excursion. An average of three measurements was taken for each parameter in each animal. Percent fractional shortening was calculated as $\%FS = (LVEDd - LVESd)/LVEDd \times 100$. All measurements were recorded at the same midventricular level in each mouse, so nonuniform changes in ventricular geometry (i.e., differences between scar and noninfarcted wall thickness) were not analyzed by echocardiography.

Histological and biochemical assessment of collagen. To assess the extent of myocardial fibrosis at 14 and 28 days after LAD ligation, fixed heart tissues were embedded in paraffin and 10-μm sections were stained by the trichrome method and examined using a microscope (Nikon Eclipse E1000, Nikonl Westbury, NY). With the use of a ×2 objective lens, the complete LV myocardial cross-section area was digitized and the area related to collagen, which appeared blue-green, was planimeterized using NIH Image version 1.5 (National Institutes of Health), a software package for morphometric analysis, which allows setting of a color threshold (16). The hydroxyproline content of the myocardium was also used as an index of collagen content at 28 days after LAD ligation as assessed by a spectrophotometric method previously described (16).

Histological assessment of infarct size, scar thickness, and infarct expansion. From the histological sections at *days 14* and *28*, we were able to measure the circumferential length as an index of infarct size (4) as well as infarct expansion (9) and scar thickness (5). Increased infarct expansion and decreased scar thickness have been correlated with poor myocardial performance and propensity to cardiac rupture (18). For circumferential length, the sections were stained with picrosirius red as previously described, and infarct length to total myocardial length was calculated as a percentage (4). In addition, we calculated the index of infarct expansion as previously described (i.e., LV cavity/total heart area × uninfarcted septum thickness/infarcted LV free wall thickness) (9). We measured scar thickness as the infarct thickness divided by normal wall thickness, i.e., the average of three equidistant points over the infarcted area of the heart divided by the average of three equidistant points on the uninfarcted area of the heart (5).

Assessments of systemic pressure and dP/dt. At 28 days after MI, anesthetized mice underwent assessment of dP/dt and systolic blood pressure using a microcatheter in the carotid artery and LV (model 300, Micromed; Louisville, KY) as described in our previous studies (29). The studies that were successfully completed were recorded, and the hearts (including those in which satisfactory hemodynamic evaluation could not be performed owing to technical difficulties) were then harvested and subjected to assessment of collagen or histological analyses.

Statistical analysis. The number of tissue samples used in each assessment is recorded in the figures. The values presented are expressed as means ± SE. Fisher's protected least-significant difference test was used to establish which groups were different at each time point. Statistical significance was defined as a value of $P < 0.05$.

RESULTS

Elastase, MPO, and MMP activity. We observed a 20% mortality rate within the first 24 h after surgery with no difference when comparing elafin transgenic and nontransgenic littermates. Late cardiac rupture was not observed in either group. We observed an increase in serine elastase activity in the myocardial extracts of nontransgenic mice as early as 6 h after LAD ligation and initiation of the MI. This elastase activity was completely inhibited by elafin. It was not, however, detected in the myocardium of sham-operated or elafin transgenic mice. These features persisted at 4 and 7 days after LAD ligation (Fig. 1*A*). We next determined whether the elevation in serine elastase activity correlated with an inflammatory response in the myocardium as judged by MPO activity (Fig. 1*B*). We could not show an elevation in MPO activity at 6 h after LAD ligation in the nontransgenic mice, suggesting that elastase activity could not be explained by an influx of inflammatory cells. Nor were inflammatory cells detected by MPO staining of the tissues. At 4 and 7 days after LAD ligation, an elevation in MPO activity was apparent in the nontransgenic mice but not in the littermates that overexpressed the elastase inhibitor elafin ($P < 0.02$ and $P < 0.04$, respectively), in which values approximated those in sham-operated animals. This correlated with the number of MPO-positive cells observed in the tissue limited to the infarct area, 48 ± 9.4 in the nontransgenic versus 18.3 ± 5 in the elafin transgenic mice.

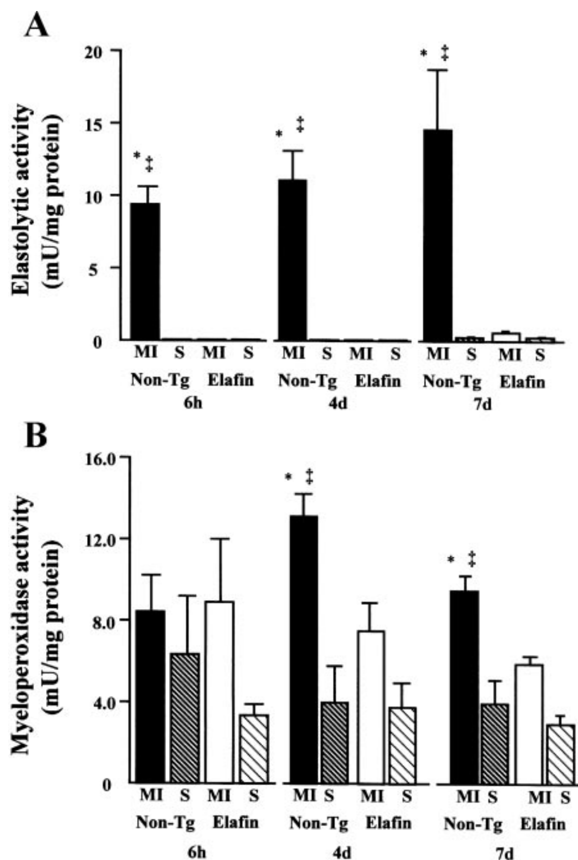

Fig. 1. *A*: elastolytic activity in the entire heart after left anterior descending coronary artery (LAD) ligation. Bars depict means ± SE; $n = 3$ mice at 6 h and 4 days (4d) and $n = 5$ mice at 7 days (7d). All values are expressed as human leukocyte elastase (HLE) units per milligram of protein. *$P < 0.05$, myocardial infarct (MI) nontransgenic (non-TG) mice compared with sham-operated (S) mice. *B*: myeloperoxidase (MPO) activity in the entire heart after LAD ligation. Bars depict means ± SE; $n = 3$ mice for 6 h and 4 days and $n = 5$ mice for 7 days. *$P < 0.05$, MI non-TG mice compared with sham-operated mice; ‡$P < 0.05$, MI non-TG mice compared with MI elafin TG mice.

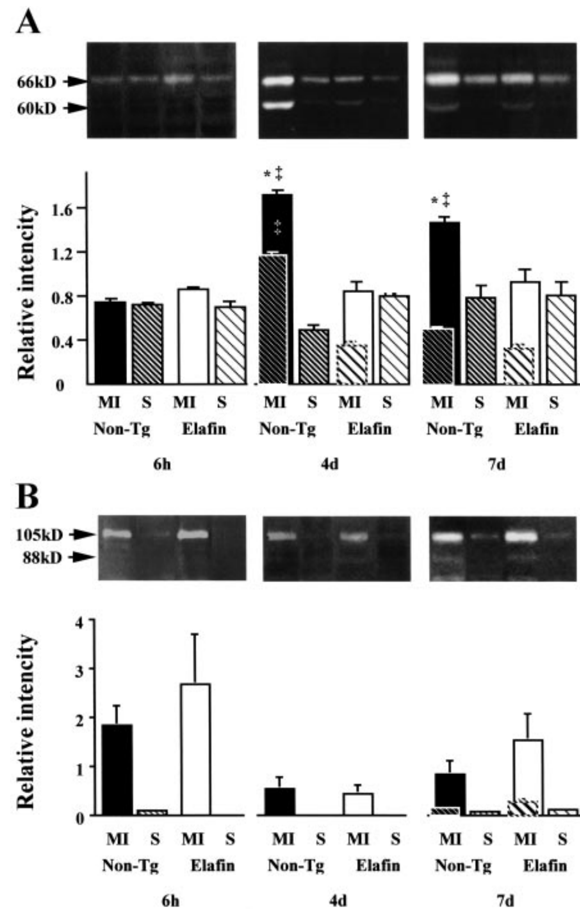

Fig. 2. Matrix metalloproteinase (MMP) activity in the entire heart after LAD ligation. MMP activity in myocardial extracts was examined using gelatin as a proteolytic substrate. To quantify the activities of the detected enzymes, zymograms were digitally scanned and the band intensities were analyzed and expressed as the ratio of a standard (proMMP-2) on the same gel. *A*, *top*: the lytic band at 66 kDa represents the pro form of MMP-2, and the band at 60 kDa represents the active form of MMP-2. *B*, *top*: the bands at 105 and 88 kDa represent the pro and active forms of MMP-9, respectively. *A* and *B*, *bottom*: bars depict means ± SE; $n = 3$ mice. *$P = <0.05$, MI non-TG mice compared with sham-operated mice; ‡$P < 0.05$, MI non-TG mice compared with elafin TG mice.

To determine the temporal relationship between the elevation in serine elastase activity and an increase in MMPs, we carried out gelatin zymography on the myocardial tissues (Fig. 2). At 6 h after the MI, only the pro form of MMP-2 was observed on the zymogram and there were no differences when comparing the bands in transgenic, nontransgenic, or sham-operated control mice. However, at 4 days in nontransgenic mice, LAD ligation and creation of the MI markedly enhanced the 66-kDa pro form of MMP-2 ($P < 0.001$) and upregulated the 60-kDa active form of MMP-2, which was not detectable in sham operated mice. Both forms of MMP-2 were, however, suppressed in elafin transgenic mice compared with nontransgenic mice ($P < 0.003$). An increase in the pro but not the active form of MMP-2 was present in the nontransgenic but not in the elafin transgenic mice at *day 7* after MI ($P < 0.003$). In

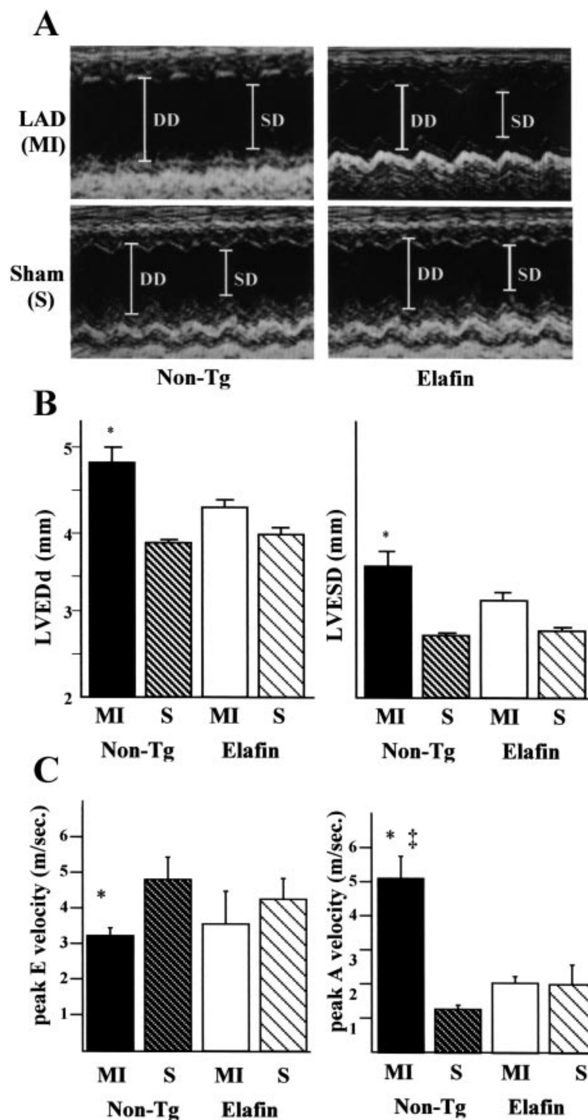

Fig. 3. Transthoracic echocardiography. *A*: representative views by M-mode of a sham-operated heart with a small systolic cavity, but in the non-TG mouse 4 days after MI, the left ventricular (LV) cavity was enlarged in both systolic (SD) and diastolic dimensions (DD). In the elafin TG mice, however, SD and DD were similar to those in sham-operated mice. *B*: quantitative results depicted graphically as the LV end-diastolic dimension (LVEDd) and LV end-systolic dimension (LVESd). Bars depict means ± SE; $n = 6$ mice. *$P < 0.05$, MI non-TG mice compared with sham-operated mice. *C*: diastolic function. *A* and *E* peaks 4 days after LAD ligation. Bars depict means ± SE; $n = 5$ or 6 mice. *$P < 0.05$, MI non-TG mice compared with sham-operated mice; ‡$P < 0.05$, MI non-TG mice vs. MI elafin TG mice.

the nontransgenic and transgenic mice, similar increases compared with the sham-operated controls in the pro form of MMP-9 were observed at 6 h and 4 days, and at 7 days there was also an increase in the active form of MMP-9.

Echocardiographic assessment of ventricular function. To investigate the relationship between elevation in elastase and MMP activity after MI to ventricular function at *day 4*, M-mode (Fig. 3, *A* and *B*) as well as Doppler (Fig. 3*C*) transthoracic echocardiographic assessments were carried out. Compared with sham-operated mice, the creation of an MI in the nontransgenic group resulted in an enlarged LV with elevated LVEDd and LVESd ($P < 0.05$ for both compared with sham-operated animals). In the elafin transgenic mice, however, normal systolic and diastolic dimensions were preserved. Pulsed Doppler flow measurements were performed to assess LV diastolic function. In nontransgenic mice, mitral valve peak A velocity was increased in the MI versus sham-operated group ($P < 0.001$) and peak E velocity was decreased ($P < 0.005$), whereas in elafin transgenic mice after MI, values were similar to those in sham-operated mice.

Assessment of myocardial fibrosis. To determine whether the presence of early cardiac dilatation and diastolic dysfunction at 4 days would be associated with the later development of myocardial fibrosis, morphometric analyses on myocardial tissue sections were carried out (Fig. 4*A*). We observed a ~30% decrease in the area of fibrosis localized to the LV wall and related to the infarct expressed as a percentage of total LV myocardial area in elafin transgenic compared with nontransgenic littermates ($P < 0.03$) at *day 14* after MI with a similar trend at *day 28* ($P = 0.085$). Collagen content assessed by hydroxyproline was, however, similarly elevated in nontransgenic and elafin transgenic littermates relative to sham-operated controls at *day 28* (Fig. 4*B*).

Infarct size, infarct expansion, scar thickness, and hemodynamic study. Infarct size assessed by the percentage of circumferential length relative to total myocardial length was similar in variability in the nontransgenic and elafin transgenic mice at

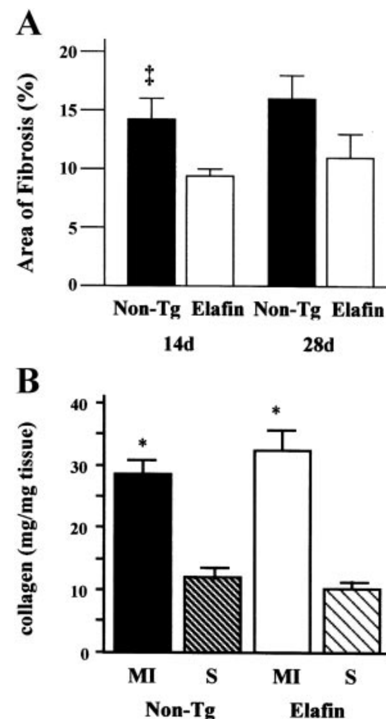

Fig. 4. Morphometric and biochemical assessment of ventricular fibrosis. Cross-sections of LV myocardium stained by the trichrome method were digitized using Image-Pro Plus software, and the total area of fibrosis localized to the LV wall was measured and expressed as a percentage of the total myocardial area (*A*). Bars depict means ± SE; $n = 7$ non-TG mice and 8 elafin TG mice at 14 d and $n = 4$ non-TG mice and 7 TG mice at 28 days. ‡$P < 0.03$, MI non-TG mice compared with MI elafin TG mice. *B*: myocardial collagen content assessed by hydroxyproline assay on *day 28* after LAD ligation. Bars depict means ± SE; $n = 8$ MI non-TG mice, 5 sham-operated non-TG mice, 9 MI elafin TG mice, and 7 sham-operated elafin TG mice. *$P < 0.05$ vs. sham-operated mice.

28 days. The expansion index was, however, elevated in the nontransgenic versus transgenic mice ($P < 0.05$) and the scar was thinner ($P < 0.02$; Fig. 5*A*). Figure 5*B* shows representative photomicrographs illustrating these features.

To determine whether the differences in structural remodeling at *day 28* after LAD ligation in both transgenic and nontransgenic mice is reflected in perturbation of function, we carried out catheter measurements in anesthetized mice. In the

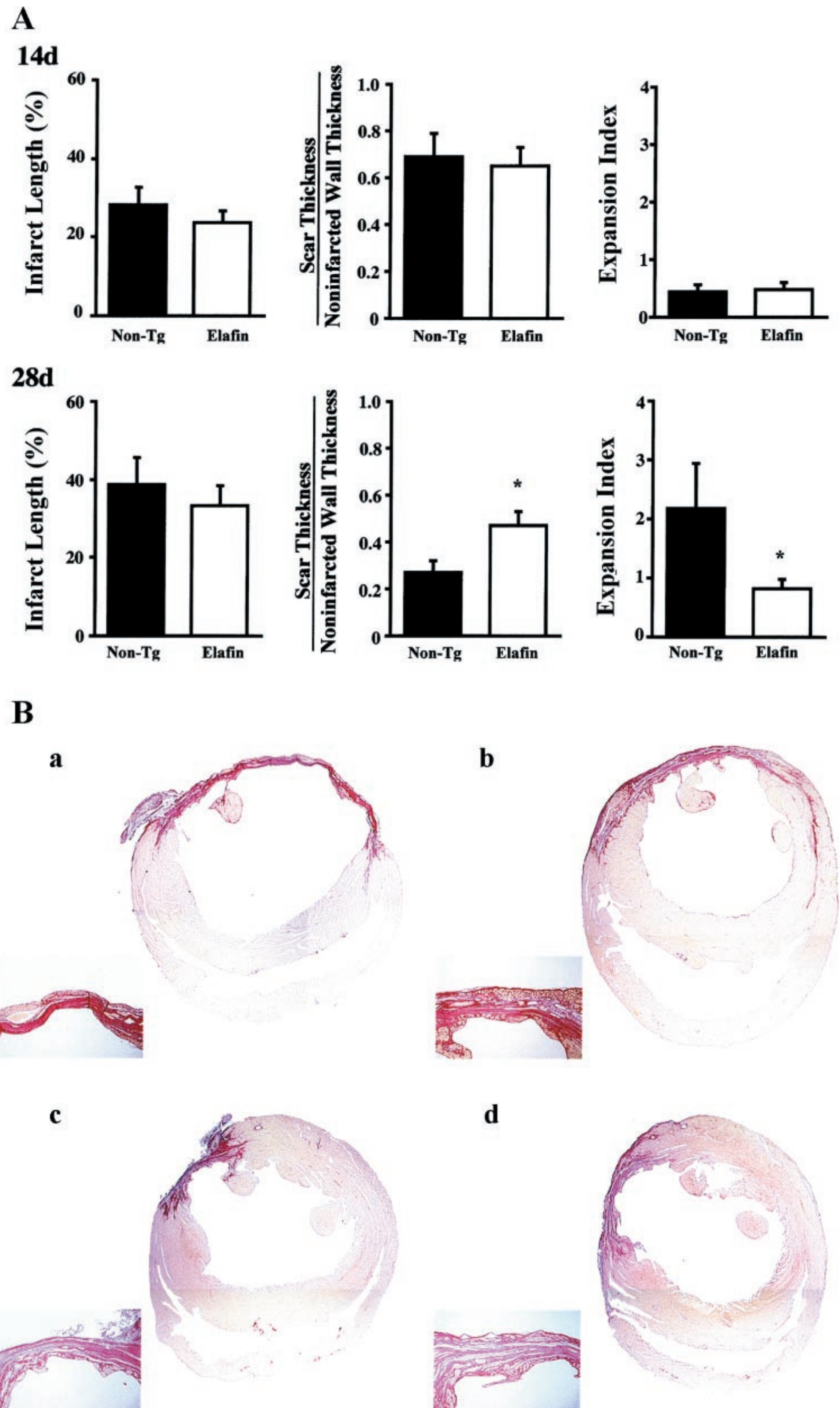

Fig. 5. Circumferential length of scar/muscle and scar thickness/noninfarcted wall thickness and expansion index. *A*: morphometric analyses were carried out as described in the text. Images were digitized using Image Pro Plus software. Bars depict means ± SE of the 14-day experiment, where $n = 7$ non-TG mice and 8 elafin TG mice, and of the 28-day experiment, where $n = 4$ non-TG mice and 7 elafin TG mice. *$P \leq 0.05$, elafin TG mice compared with non-TG mice. *B*: representative sections of hearts with further magnification (*insets*) of the infarcted area at 28 days after LAD ligation. In a non-TG (*a*) and elafin TG mouse heart (*b*), similar large infarcts are shown, but the expansion index is greater and scar thickness is less than in the non-TG mouse. Similar findings are apparent in a relatively small infarct in the non-TG (*c*) and elafin TG (*d*) mouse heart. Original magnification is ×20 and ×100 for the *insets*.

nontransgenic group after MI, LV systolic pressure was decreased ~25% compared with sham-operated mice (69 ± 3 vs. 94 ± 8 mmHg, $P < 0.05$) and LV function assessed by +dP/d*t* was also reduced by ~35% (2,828 ± 176 vs. 4,280 ± 497 mmHg/s, $P < 0.05$; Fig. 6). In the elafin transgenic mice, however, no significant reduction in these features was observed (77 ± 4 vs. 88 ± 4 mmHg for LV systolic pressure and 3,402 ± 406 vs. 4,258 ± 424 mmHg/s for +dP/d*t*). LV end-diastolic pressure and maximal negative rate of pressure development (−dP/d*t*) were similar in the transgenic, nontransgenic, and sham-operated mice (Fig. 6).

DISCUSSION

In this study, we experimentally produced a MI in the mouse and demonstrated an early induction of serine elastase activity in extracts from myocardial tissue. This elastase activity correlated with the subsequent inflammatory response, heightened MMP-2 activity, early cardiac dilatation and diastolic dysfunction, late scar thinning, infarct expansion, and impaired function as judged by +dP/d*t*. Moreover, these features were attenuated or not observed in mice that overexpressed the serine elastase inhibitor elafin. MMP-9 activity was, however, similarly elevated in transgenic and nontransgenic mice.

Elevated myocardial elastolytic and MMP-9 activity at 6 h preceded inflammatory cell infiltration as assessed by MPO. This suggests that the serine elastase and MMP-9 are produced by noninflammatory cells as has been previously reported for MMP-2 as well (3). Inhibition of serine elastase activity by elafin was associated with a reduction in the inflammatory influx into the myocardium as assessed by MPO activity at 4 days. Elastases could contribute to inflammatory cell migration through the release of growth factors (24) such as PDGF (21), which induce chemokines, as well as via highly chemotactic degradation products, elastin and fibronectin peptides (8). The elevation in MMP-9 suggests that it is not dependent on elastase activity. Elevated active MMP-2 is consistent with elastase-mediated activation of the pro to latent form of the enzyme or the destabilization of TIMP-MMP complexes (12). In addition, elastases can produce elastin or fibronectin peptides or release ECM-incorporated fibroblast growth factor-2, all of which increase MMP transcription (17, 26). This function is consistent with elafin-mediated suppression of both the pro as well as the active form of MMP-2. Other studies in pigs (3) have, however, shown that interstitial MMP-2 activity is elevated within hours after MI, so it is also possible that we have missed a transient elevation in MMP-2 or that there is a species specific difference in levels of activity as judged by zymography. Because MMP-9 null mice are protected against an adverse outcome after MI (2), our data would suggest that an elevation in both MMP-2 and -9, as well as serine elastase activity, are all necessary for this pathological process.

Cardiac ECM components that are degraded by elastases and MMPs include proteolglycans and mucopolysaccharides such as hyaluronan, molecules that become highly hydrophilic when structurally uncoiled (11). Moreover, relatively minor elevation in interstitial water content can influence cardiac function (15). Degradation of collagen might also play a role in cardiac myocyte dysfunction because vascular myocytes show profound alterations in phenotype when exposed to denatured or depolymerized collagen (13).

There is always the concern about inducing infarcts of similar size in mice. While there was individual variability in infarct size, there was no significant difference when comparing the elafin transgenic and nontransgenic groups. There were no late deaths or cardiac rupture over the time frame of the study in either the elafin transgenic or nontransgenic groups, indicating that we had not produced a severe infarct. The area of fibrosis 14 days after MI as judged by trichrome staining appeared mildly reduced in elafin transgenic versus nontransgenic mice, but at 28 days after LAD ligation, structural and biochemical assessments of collagen were similar in elafin transgenic and nontransgenic mice. However, at 28 days, the scar was thinner and associated with infarct expansion in the nontransgenic compared with elafin transgenic mice. We can only speculate about features that may have influenced scar thinning and infarct expansion, such as changes in the relative abundance of different types of collagen or in other components of the ECM associated with collagen (10, 27). These changes could be responsible for alterations in cardiac myocyte phenotype and cell-cell interactions that influence cardiac function as judged by lower blood pressure and +dP/d*t*. This would indicate that changes can occur in the myocardium in association with a relatively modest infarct that result in cardiac dilatation and dysfunction. The protective effect of a selective serine elastase inhibitor such as elafin in preserving the functional integrity of the myocardium would seem to merit further investigation as a potential therapy.

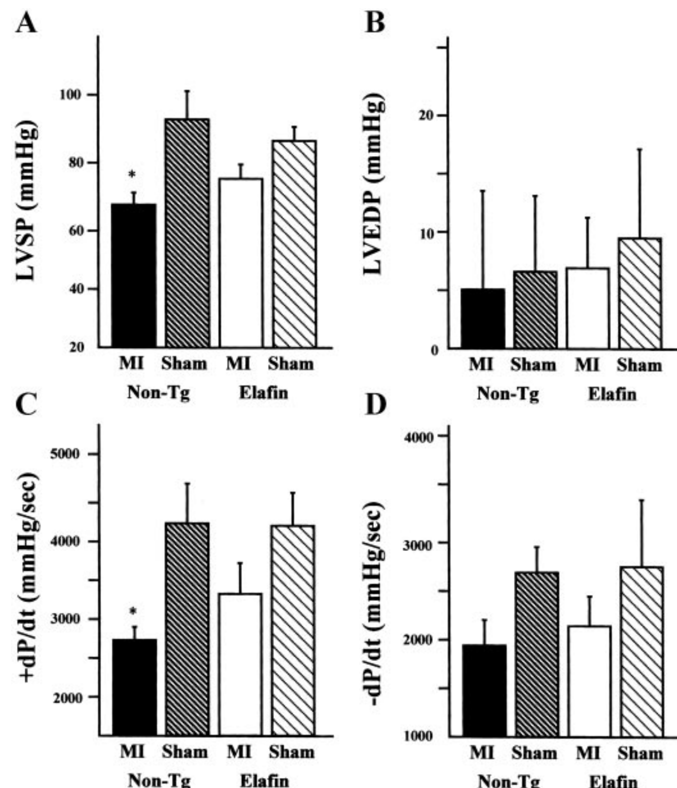

Fig. 6. LV systolic pressure (LVSP; *A*), LV end-diastolic pressure (LVEDP; *B*), +dP/d*t* (*C*), and −dP/d*t* (*D*) at 28 days after LAD ligation and MI. Bars depict means ± SE; $n = 6$ MI non-TG mice, 5 non-TG sham-operated mice, 10 MI elafin TG mice, and 6 sham-operated elafin TG mice. *$P < 0.03$, non-TG mice after MI compared with sham-operated mice.

GRANTS

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REFERENCES

1. **Cleutjens JP, Verluyten MJ, Smiths JF, and Daemen MJ.** Collagen remodeling after myocardial infarction in the rat heart. *Am J Pathol* 147: 325–338, 1995.
2. **Ducharme A, Frantz S, Aikawa M, Rabkin E, Linsey M, Rohde LE, Kelly RA, Werb Z, Libby P, and Lee RT.** Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest* 106: 55–62, 2000.
3. **Etoh T, Joffs C, Deschamps AM, Davis J, Dowdy K, Hendrick J, Baicu S, Mukherjee R, Manhaini M, and Spinale FG.** Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. *Am J Physiol Heart Circ Physiol* 281: H987–H994, 2001.
4. **Fraccarollo D, Hu K, Galuppo P, Gaudron P, and Ertl G.** Chronic endothelin receptor blockade attenuates progressive ventricular dilation and improves myocardial function in rats with myocardial infarction. *Circulation* 96: 3963–3973, 1997.
5. **Hammerman H, Koner RA, Schoen FJ, Brown J, EJ, Hale S, and Braunwald E.** Indomethacin-induced scar thinning after experimental myocardial infarction. *Circulation* 67: 1290–1295, 1983.
6. **Hayashidani S, Tsutsui H, Ikeuchi M, Shiomi T, Matsusaka H, Kubota T, Imanaka-Yoshida K, Itoh T, and Takeshita A.** Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction. *Am J Physiol Heart Circ Physiol* 285: H1229–H1235, 2003.
7. **Heymans S, Luttun A, Nuyens D, Theilmeier G, Creemer E, Moons L, Dyspersin GD, Cleutjens JP, Shipley M, Angellilo A, Levi M, Bube O, Baker A, Keshet E, Lupu F, Herbert JM, Smits JF, Shapiro SD, Baes M, Borgers M, Collen D, Daemen MJ, and Carmeliet P.** Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. *Nat Med* 5: 1135–1142, 1999.
8. **Hinek A, Molossi S, and Rabinovitch M.** Functional interplay between interleukin-1 receptor and elastin binding protein regulates fibronectin production in coronary artery smooth muscle cells. *Exp Cell Res* 225: 122–131, 1996.
9. **Hochman JS and Choo H.** Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage. *Circulation* 75: 299–306, 1987.
10. **Iimoto DS, Covell JW, and Harper E.** Increase in cross-linking of type 1 and type III collagens associated with volume-overload hypertrophy. *Circ Res* 63: 399–408, 1988.
11. **Iozzo RV.** Matrix proteoglycans: from molecular design to cellular function. *Annu Rev Biochem* 67: 609–652, 1998.
12. **Itoh Y and Nagase H.** Preferential inactivation of tissue inhibitor of metalloproteinases-1 that is bound to the precursor of matrix metalloproteinase 9 (progelatinase B) by human neutrophil elastase. *J Biol Chem* 270: 16518–16521, 1995.
13. **Jones P, Crack J, and Rabinovitch M.** Regulation of tenascin-C, a vascular smooth muscle cell survival factor that interacts with the αvb3 integrin to promote epidermal growth factor receptor phosphorylation and growth. *J Cell Biol* 139: 279–293, 1997.
14. **Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, Ueno T, Sugi K, and Imaizumi T.** Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 32: 368–372, 1998.
15. **Laine GA and Allen SJ.** Left ventricular myocardial edema. Lymph flow, interstitial fibrosis, and cardiac function. *Circ Res* 68: 1713–1721, 1991.
16. **Lee JK, Zaidi SH, Liu P, Dawood F, Cheah AY, Wen WH, Saiki Y, and Rabinovitch M.** A serine elastase inhibitor reduces inflammation and fibrosis and preserves cardiac function after experimentally-induced murine myocarditis. *Nat Med* 4: 1383–1391, 1998.
17. **Miyake H, Yoshimura K, Hara I, Eto H, Arakawa S, and Kmidono S.** Basic fibroblast growth factor regulates matrix metalloproteinases production and in vitro invasiveness in human bladder cancer cell lines. *J Urol* 157: 2351–2355, 1997.
18. **Mukherjee R, Brinsa TA, Dowdy KB, Scott AA, Baskin JM, Deschamps AM, Lowry AS, Escobar GP, Lucas DG, Yarbrough WM, Zile MR, and Spinale FG.** Myocardial infarct expansion and matrix metalloproteinase inhibition. *Circulation* 107: 618–625, 2003.
19. **Okada Y and Nakanishi I.** Activation of matrix metalloproteinase 3 (stromelysin) and matrix metalloproteinase 2 ("gelatinase") by human neutrophil elastase and cathepsin G. *FEBS Lett* 249: 353–356, 1989.
20. **Pfeffer MA and Braunwald E.** Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 81: 1161–1172, 1990.
21. **Raines EW.** The extracellular matrix can regulate vascular cell migration, proliferation, and survival: relationships to vascular disease. *J Exp Pathol* 81: 173–182, 2000.
22. **Rohde FG, Ducharme A, Arroyo LH, Aikwa M, Sukhova GH, Lpez-Anaya A, McClure KE, Mitchell PG, Libby P, and Lee RT.** Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation* 99: 3063–3070, 1999.
23. **Tanaka N, Dalton N, Mao L, Rockman HA, Peterson KL, Gottshall KR, Hunter JJ, Chien KR, and Ross J, J.** Transthoracic echocardiography in models of cardiac disease in the mouse. *Circulation* 94: 1109–1117, 1996.
24. **Thompson K and Rabinovitch M.** Exogenous leukocyte and endogenous elastases can mediate mitogenic activity in pulmonary artery smooth muscle cells by release of extracellular-matrix bound basic fibroblast growth factor. *J Cell Physiol* 166: 495–505, 1996.
25. **Tiefenbacher CP, Ebert M, Niroomand F, Batkai S, Tillmanns H, Simmerman R, and Kubler W.** Inhibition of elastase improves myocardial function after repetitive ischaemia and myocardial infarction in the rat heart. *Pflügers Arch* 433: 563–570, 1997.
26. **Werb Z, Tremble PM, Behrendtsen O, Crowley E, and Damsky CH.** Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J Cell Biol* 109: 877–889, 1989.
27. **Willems IE, Arends JW, and Daemen MJ.** Tenascin and fibronectin expression in healing human myocardial scars. *J Pathol* 179: 321–325, 1996.
28. **Zaidi SH, You XM, Ciura S, O'Blenes S, Husain M, and Rabinovitch M.** Suppressed smooth muscle proliferation and inflammatory cell invasion after arterial injury in elafin-overexpressing mice. *J Clin Invest* 105: 1687–1695, 2000.
29. **Zaidi SHE, Hui CC, Cheah AYL, You XM, Husain M, and Rabinovitch M.** Targeted overexpression of elafin protects mice against cardiac dysfunction and mortality following viral myocarditis. *J Clin Invest* 103: 1211–1219, 1999.