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Disruption of the Plasminogen Gene in Mice Abolishes Wound Healing after Myocardial Infarction

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The plasminogen system plays an important role in the proteolytic degradation of extracellular matrices during wound healing. In the present study we investigated the impact of the plasminogen system on cardiac wound healing and function after myocardial infarction. Myocardial infarction was induced in plasminogen-deficient mice (Plg−/−) and in wild-type controls (Plg+/+). Structural analysis 1, 2, and 5 weeks after infarction revealed that infarct healing was virtually abolished in Plg−/− mice, indicating that the plasminogen system is required for the repair process of the heart after infarction. In the absence of plasminogen, inflammatory cells did not migrate into the infarcted myocardium. Necrotic cardiomyocytes were not removed and the formation of granulation tissue and fibrous tissue did not occur. In these nonhealing infarcted hearts, LV dilatation was not altered. In addition, gelatinolytic activity of MMP-2 and MMP-9 was depressed in the Plg−/− infarcted hearts, suggesting that the plasmin effect on infarct healing may be mediated by MMPs. Surprisingly, cardiac function was only attenuated to a rather small extent in the Plg−/− infarcted mice when compared to the wild-types. This study provides direct prove that plasmin-mediated proteolysis plays a central role in cardiac wound healing after myocardial infarction in mice. ***(Am J Pathol 2000, 156:1865–1873)***

Myocardial infarction (MI) leads to necrosis of cardiomyocytes in the ischemic ventricle and is followed by a wound healing response. This response generally resembles that of parenchymatous tissue to ischemic injury, including migration of inflammatory cells into the affected myocardium, extracellular matrix degradation, fibroblast proliferation, and angiogenesis. On the other hand, cardiac wound healing has several unique characteristics such as the sustained presence of myofibroblasts in the infarcted myocardium[1] and the fact that cardiomyocytes are terminally differentiated cells that have lost the ability to divide. Myocardial healing is therefore independent of cardiomyocyte regeneration, but depends on the formation of granulation tissue. The mechanisms responsible for inflammatory and reparative phases of healing after myocardial infarction are not fully understood. However, recent evidence indicates an involvement of proteinases, including the plasminogen activators and metalloproteinase systems, in the process of extracellular matrix degradation and cell migration during cardiac wound healing.[2] Plasmin, generated from plasminogen, is the active enzyme of the plasminogen/plasmin system and degrades a variety of extracellular matrix (ECM) components.[3] A relevant feature of plasmin is the proteolytic amplification, which can be achieved by activating several matrix metalloproteinases (MMPs), which are also involved in the degradation of extracellular matrix components.[4] The generation of plasmin is controlled primarily by the balance between the plasminogen activators (t-PA and u-PA) and their physiological inhibitors, the plasminogen activator inhibitors (PAIs).[5] In a recent study, Heymans et al demonstrated that mice deficient in u-PA showed impaired infarct healing and were completely protected against cardiac rupture after induction of a myocardial infarction.[2] It is unknown whether this prominent effect of u-PA in infarct healing is mediated through the activation of plasminogen. In the present study we further investigated the role of the plasminogen system in infarct healing and function, in a model of chronic myocardial infarction in plasminogen-deficient mice.

Materials and Methods

Plasminogen-deficient (Plg−/−) mice were developed and characterized as described previously.[6] Plasmin activity was unmeasurable in Plg−/− mice (<5% of Plg+/+ mice), and neither plasminogen mRNA nor translation products could be identified by Northern or Western blot of liver extracts from Plg−/− mice.[6] The same strain of

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mice has been used in the present study, in which we did not measure plasminogen levels. Plg−/− mice survive embryonic development but develop spontaneous fibrin deposition due to impaired thrombolysis and suffer retarded growth and reduced fertility and survival.[6] In Plg−/− and in wild-type controls cardiac wound healing was studied, 1 week (n = 2 per group), 2 weeks (n = 8–10 per group), and 5 weeks (n = 5–7 per group) after induction of the infarct. Survival was not different between the two infarct groups, 70% and 77% in Plg−/− and Plg+/+ infarcted mice, respectively. Mortality was highest within the first hours after surgery. Only one wild-type animal died after this period, on day 5, due to cardiac rupture. Equal numbers of sham-operated Plg−/− and Plg+/+ animals served as controls. All experiments were performed according to the guidelines of the institutional animal care committee.

MI was induced surgically by permanent ligation of the main left coronary artery as recently described.[7] Tissue processing and architectural measurements were adapted from the same study.

Infusion of 5′-Bromo-2′-Deoxyuridine (BrdU)

To label DNA-synthesizing cells, all animals in the 2-week post-MI and sham group received BrdU (Serva, Heidelberg, Germany; infusion rate 13 mg/kg/day) from an osmotic minipump (Alzet 2001, Alza Corp., Palo Alto, CA), 7 days before sacrifice.

Immunohistochemistry

Immunohistochemistry was performed on the 1-, 2-, and 5-week groups, using conventional methods to identify macrophages (moma-2 monoclonal antibody),[8] endothelial cells (biotin-labeled lectin from *Bandeiraea simplicifolia*, Sigma Chemicals, St. Louis, MO),[9] smooth muscle cells and myofibroblasts (monoclonal anti-α-smooth muscle cell actin (α-sma), DAKO, Danmark), laminin (polyclonal anti-mouse laminin),[10] and DNA synthesis (monoclonal anti-BrdU, Harlan Sera-Lab, Loughborough, UK). For quantification of DNA synthesis, the total labeling fraction (LF; number of BrdU-positive/total numbers of counted nuclei × 100%) of BrdU-positive cells was calculated per 0.1 mm^2 in the center and border zones of the infarcts and in the non-infarcted septum. Apoptosis was studied in the 2-week postsurgery groups by means of the Terminal Transferase dUTP nick end labeling (TUNEL) assay as described by Kockx et al.[11] For quantification of apoptotic cells, total numbers of nuclei and percentage of TUNEL-positive nuclei were counted in the whole infarcted area. DNA synthesis and apoptosis were studied in the 2-week postsurgery groups.

Myocardial MMP Extraction and Zymography

A separate group of animals (6 Plg−/−, 4 Plg+/+) was sacrificed 2 weeks after surgery for myocardial MMP activity analysis. The hearts were perfused with saline and the LV free walls were rapidly frozen in liquid nitrogen and stored at −80°C. The frozen tissue was cut into small pieces, washed with cold saline, and incubated in 100 μl extraction buffer (10 mmol/L cacodylic acid, pH 5.0, 0.15 mmol/L NaCl, 1 μmol/L $ZnCl_2$, 20 mmol/L $CaCl_2$, 1.5 mmol/L NaN_3, and 0.01% Triton X-100) per 10 mg wet weight at 4°C with continuous agitation for 24 hours. This step was repeated with fresh buffer. The extraction buffer was collected and pH raised to 7.5 by addition of 1 mol/L Tris, pH 8.0. MMP activity was visualized using the zymography technique.[12] In short, 10 μg of myocardial protein extracts were loaded per lane onto electrophoretic gels (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) containing gelatin (0.66 mg/ml). After electrophoresis, gels were washed twice for 15 minutes with 2.5% Triton X-100 and incubated overnight at 37°C in substrate buffer (50 mmol/L Tris-HCl, pH 8.0, 5 mmol/L $CaCl_2$, 0.02% sodium nitrate). After incubation, gels were stained for 30 minutes in 0.05% Coomassie brilliant blue R250 in acetic acid:methanol:water (1:4:5) and destained in the same solvent. In each gel a reference sample was used to normalize scanned lytic activities between gels.

Hemodynamic Measurements

Cardiac output (CO) was evaluated in sham-operated and infarcted mice 2 weeks after surgery. In short, an electromagnetic flow-probe (1.2 mm; Skalar, Delft, The Netherlands) was placed on the ascending aorta, adjacent to its exit from the heart and connected to a Skalar MDL400 sine-wave flowmeter. An abdominal aorta catheter was connected to a pressure transducer (microswitch, model 156PC 156 WL, Honeywell, Amsterdam, The Netherlands). Signals were sampled at a rate of 2 kHz and fed into a personal computer for off-line analysis. After collecting baseline values of cardiac output (CO_{rest}) for 15 minutes, a rapid intravenous infusion (2.5 ml in 1 minute) of a warm (37°C) Ringer's solution was started. This procedure increased CO to a plateau value. In a pilot study in Swiss mice, we observed an increase in CO from 7.9 ± 0.4 to 21.1 ± 0.7 ml/minute in sham-operated mice and from 5.7 ± 0.4 to 15 ± 1.4 ml/minute in MI mice.

For pressure measurements, a separate group of animals (n = 5–6 per group) were anesthesized with urethane (2.1 mg/g body weight, subcutaneously, Sigma). A 1.4 French high-fidelity catheter tip micromanometer (SPR-671; Millar Instruments, Houston, TX) was inserted through the right carotid artery in the left ventricular cavity. After hemodynamic stabilization, left ventricular pressure was sampled with a computer at a rate of 2 kHz, and stored for analysis of left ventricular systolic pressure (LVSP), end-diastolic pressure (LVEDP), and maximal positive (dP/dt_{max}) rate of pressure development. Increasing amounts of dobutamine (0.5, 1.0, 1.5, 2, and 3 ng/g/minute; Dobutrix, Lilly, Brussels, Belgium) were administered for 2 minutes each by continuous infusion in the left external jugular vein, via a catheter connected to a Harvard microinjection pump (Harvard Apparatus Inc., Holliston, MA). Pressure measurements were performed after 90 seconds' dobutamine infusion.

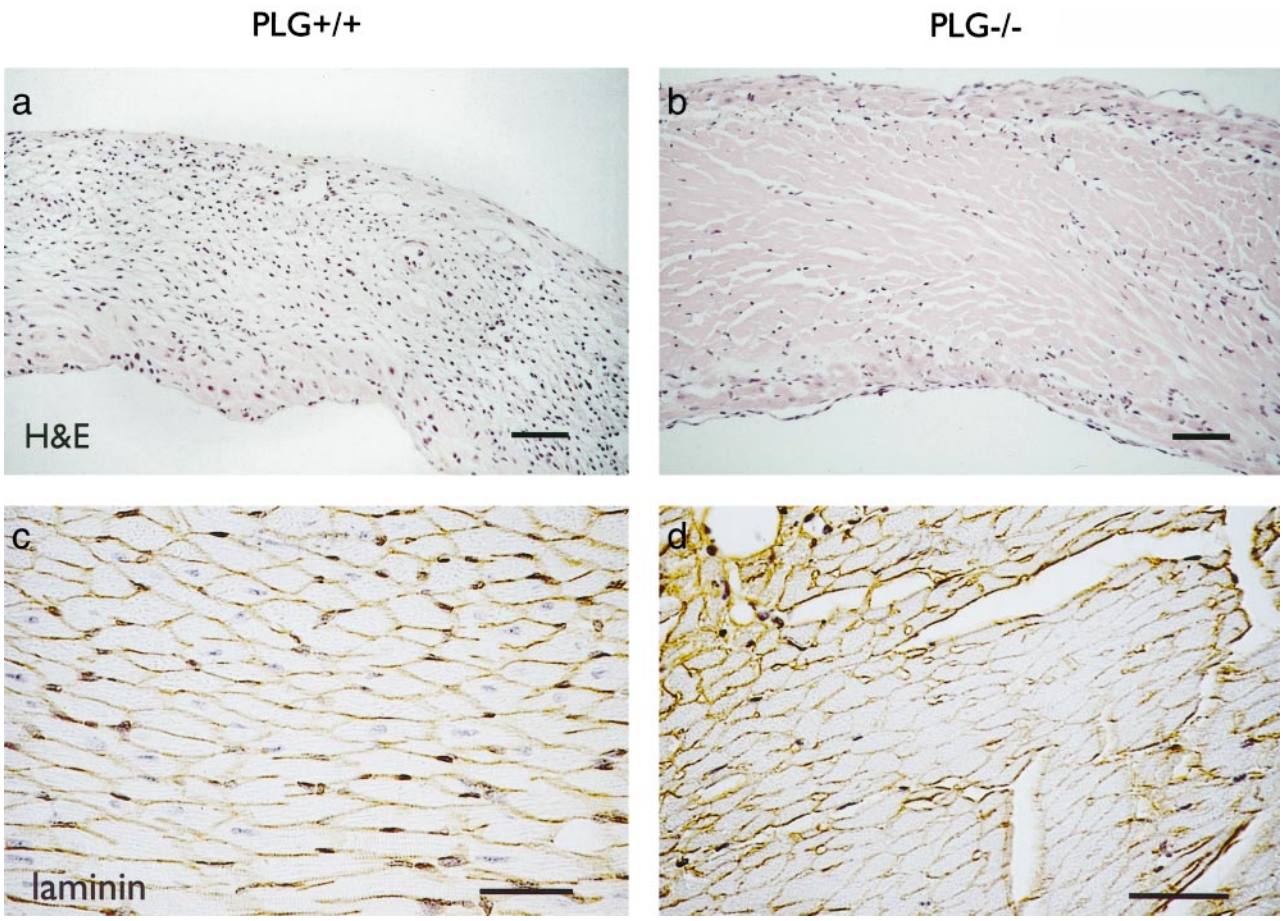

Figure 1. Structural composition of the infarcts. **a** and **b:** Hematoxylin-and-eosin-stained sections of Plg+/+ and Plg−/− infarcts 2 weeks after MI. In Plg+/+ infarcts, the necrotic cardiomyocytes are replaced by granulation tissue, whereas in Plg−/− infarcts wound healing is absent. **c** and **d:** laminin, a basement membrane component, encages normal cardiomyocytes in the non-infarcted myocardium (Plg+/+). In the center of 2-week-old Plg−/− infarcts, these basement membranes are still present. The intensity of the staining, however, decreases over time. Bars represent 50 μm.

Statistics

All above described parameters were measured without knowledge of the treatment group. Data are expressed as means ± SE. Means between groups were compared by the use of the Mann-Whittney *U* test. A *P* value <0.05 was considered statistically significant.

Results

Infarct Healing Is Absent in Plg−/− Mice

Histological examination of the Plg−/− infarcts demonstrated a complete absence of cardiac wound healing and the persistence of necrotic cardiomyocytes in the center of the Plg−/− infarcts, which remained present as ghost cells until at least 5 weeks after MI (Figure 1, a and b). In both Plg−/− and Plg+/+ infarcts, 1 to 2 cell layers of cardiomyocytes in endocardium and epicardium survived the MI. The basement membranes that surrounded the cardiomyocytes were still visible in the Plg−/− infarcts, as demonstrated by laminin immunohistochemistry 1, 2, and 5 weeks after MI (Figure 1, c and d). The intensity of the laminin staining around the necrotic cardiomyocytes in Plg−/− infarcts was slightly decreased in time when compared to normal cardiomyocytes. The absent wound healing in the Plg−/− mice was associated with a lack of influx of macrophages into the infarct. Also, myofibroblasts and endothelial cells were scarcely present in the center of 2- and 5-week-old Plg−/− infarcts.

In Plg+/+ infarcts, the necrotic cardiomyocytes had been replaced by granulation tissue, containing abundant macrophages (Figure 2, a and b), endothelial cells (lectin staining, data not shown), and α-smooth muscle actin-positive cells (eg, myofibroblast-like cells and smooth muscle cells; Figure 2, c and d).

Fibrous Tissue Formation Is Abolished in Plg−/− Infarcts

Sirius red staining demonstrated extensive collagen deposition in Plg+/+ infarcts, but not in Plg−/− infarcts, 2 weeks after surgery (Figure 3, a and b). Quantification revealed relative collagen positive areas of 48.0 ± 12.1% in the center of Plg+/+ infarcts and greatly reduced collagen deposition in Plg−/− infarcts (0.7 ± 0.3%, *P* =

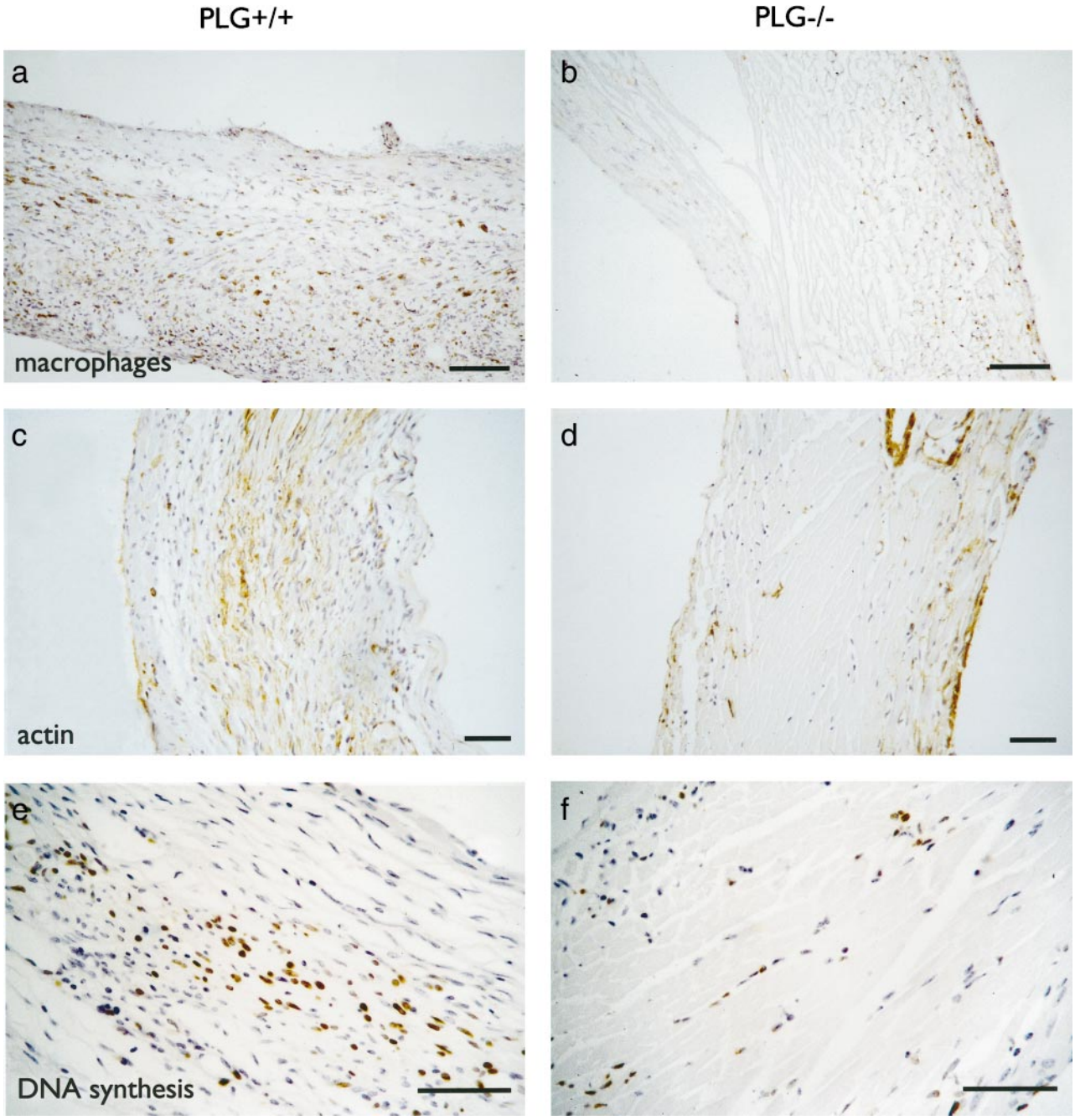

Figure 2. Cellular composition of the infarcts. **a** and **b:** Large numbers of macrophages infiltrate into 1-week-old Plg+/+ infarcts in contrast to the Plg−/− infarcts. **c** and **d:** α-Smooth muscle actin immunohistochemistry reveals myofibroblasts and smooth muscle cells in the center of the Plg+/+ infarcts. In the center of the Plg−/− infarcts, the only α-sma-positive cells were vascular smooth muscle cells. **e** and **f:** BrdU immunohistochemistry in the infarcted myocardium. The absolute number of BrdU-positive cells is higher in the Plg+/+, but the percentage of cells that are BrdU-positive is very similar in the Plg+/+ and Plg−/−. Bars represent 50 μm.

0.002, Figure 4), and comparable to that in the sham-operated animals. The border zones of Plg−/− infarcts revealed some collagen deposition, although much less than in the Plg+/+ infarcts. *In situ* hybridization using a type I collagen probe[13] demonstrated diffuse collagen expression throughout the entire Plg+/+ infarct. Type I collagen mRNA expression was restricted to the border zones and the endo- and epicardial layers in Plg−/− infarcts (Figure 3, c and d).

Cell Turnover Is Not Altered in Plg−/− Infarcts

Total cell numbers were lower in the center of 2-week Plg−/− infarcts (180 ± 130 cells per 0.1 mm^2) than of Plg+/+ infarcts (910 ± 160 cells, $P = 0.004$, Figure 5). Measurement of DNA synthesis by BrdU labeling (Figure 2, e and f) yielded similar percentages of BrdU-positive nuclei in the Plg−/− (23 ± 7%) and Plg+/+ (26 ± 5%, $P = 0.33$) infarcts, indicating that the proliferative capac-

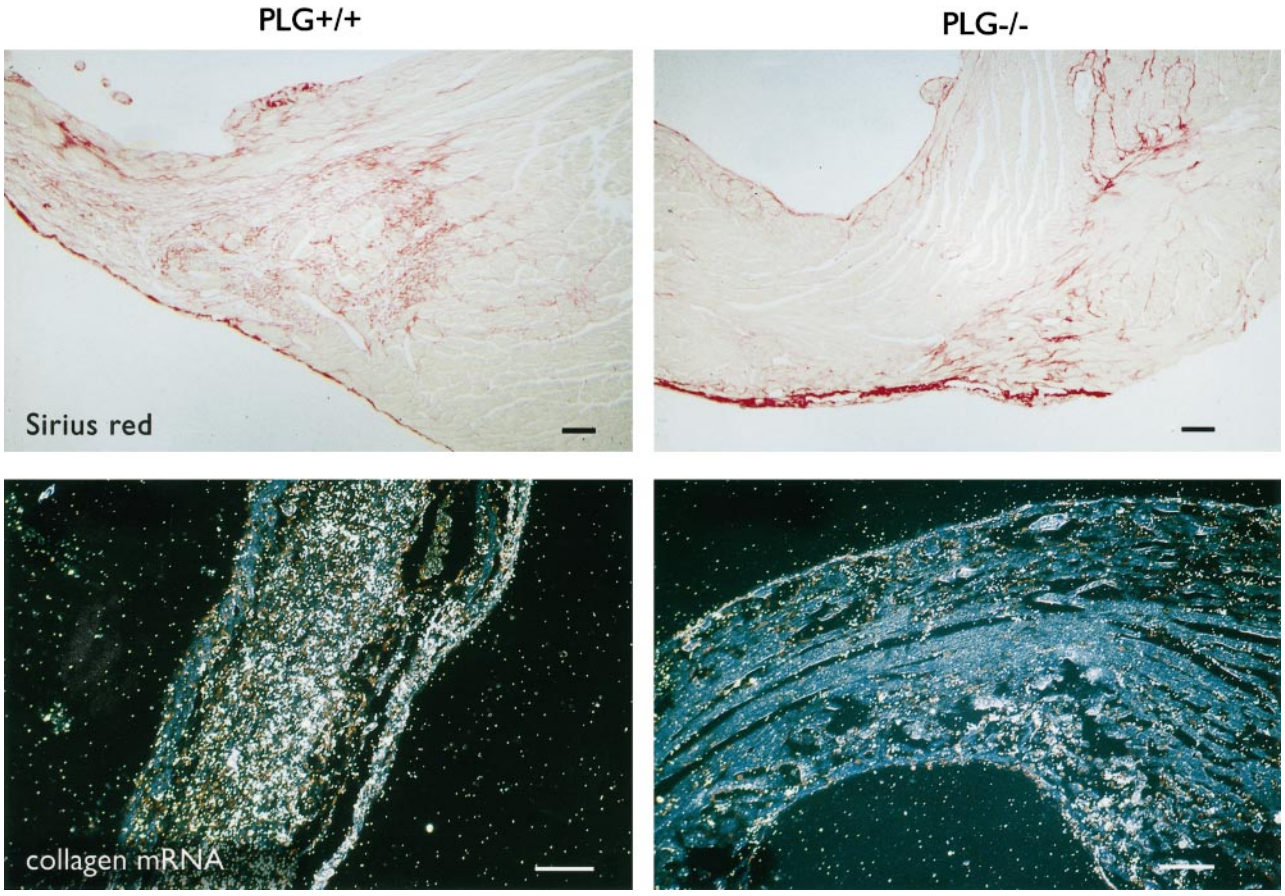

Figure 3. Collagen deposition and synthesis. **a** and **b:** Sirius red staining 2 weeks after MI shows extensive collagen deposition in the Plg+/+ infarcts and only a little in the Plg−/− infarcts. **c** and **d:** *In situ* hybridization shows that type I collagen mRNA expression is more pronounced in 2-week-old wild-type infarcts than in 2-week-old Plg−/− infarcts. Bars represent 50 μm.

ity of the Plg−/− cells is not reduced. Also, the percentage of apoptotic cells, measured by the TUNEL staining technique, was not significantly different in the Plg+/+ (0.4 ± 0.2%) and Plg−/− (0.4 ± 0.3%, $P = 0.64$) infarcts, 2 weeks after MI (Figure 5). Thus, the absent wound healing in Plg−/− mice is the consequence of abolished cell migration, not of cell turnover.

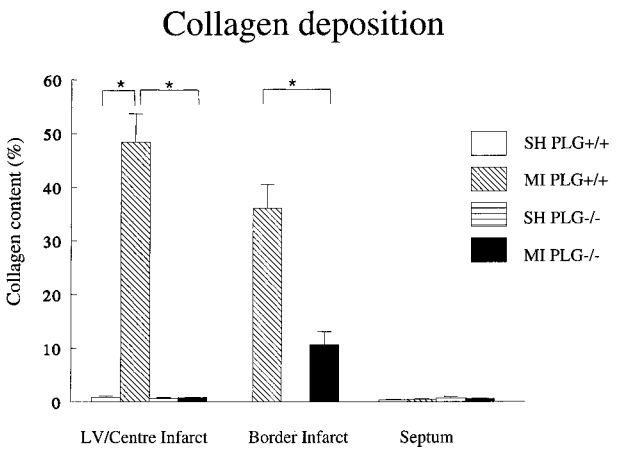

Figure 4. Quantification of the Sirius red staining demonstrates completely abolished collagen deposition in the center of the Plg−/− infarcts. MI, myocardial infarction; SH, sham operation.

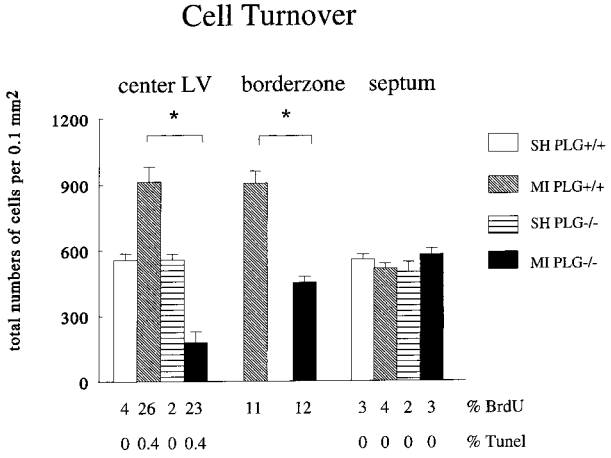

Figure 5. Cell turnover in Plg+/+ and Plg−/− hearts, 2 weeks after MI. Despite reduced absolute cell numbers (**bars**) in the Plg−/− infarcted hearts, the percentage of BrdU-positive cells is not significantly different from the Plg+/+ infarcts (23% and 26%, respectively; $P = 0.33$ by Mann-Whitney test). This was also seen for levels of apoptosis, as measured by the TUNEL staining technique. MI, myocardial infarction; SH, sham operation.

Table 1. Cardiac Dimensions in 2- and 5-week-old Plg−/− and Plg+/+ Infarcts and Shams

	2 weeks				5 weeks			
	Plg+/+		Plg−/−		Plg+/+		Plg−/−	
	SH	MI	SH	MI	SH	MI	SH	MI
inf %	0	47 ± 5	0	49 ± 10	0	41 ± 5	0	46 ± 9
Hw/Bw (mg/g)	15.8 ± 0.3	6.4 ± 0.2	6.1 ± 0.5	8.3 ± 0.4*†	5.7 ± 0.2	6.7 ± 0.3	5.6 ± 0.4	8.0 ± 0.2*†
LV th (mm)	1.2 ± 0.2	0.2 ± 0.03*	1.0 ± 0.07	0.5 ± 0.06*†	1.2 ± 0.37	0.2 ± 0.02*	0.9 ± 0.2	0.5 ± 0.04*†
LV lum (mm^2)	5.5 ± 1.0	10 ± 3.5*	5.1 ± 2.3	9.9 ± 4.4*	6.8 ± 1.9	12 ± 3.8*	7.0 ± 3.3	14 ± 2.1*
Sept th (mm)	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.2	1.2 ± 0.2*	1.0 ± 0.1	1.0 ± 0.2	1.2 ± 0.3	1.0 ± 0.2

MI, myocardial infarction; SH, sham operation; inf %, infarct percentage; Hw/Bw, heart weight corrected for body weight (in mg/g body wt); LV th, left ventricular thickness; LV lum, left ventricular lumen area; Sept th, septum thickness. Data presented as mean ± SE; *, different compared to matching sham ($P < 0.025$); †, different compared to Plg+/+ MI ($P < 0.025$, by Mann-Whitney test).

Architectural Changes of the Left Ventricle of Infarcted Hearts

The occurrence of architectural changes shortly after the onset of MI are well established: the infarcted left ventricle dilates, the infarcted wall becomes thinner, and the non-infarcted septum undergoes hypertrophy. These cardiac adaptations are normal compensatory mechanisms after infarction to maintain stroke volume at an adequate level.[14] In the present study, all these architectural changes were observed 2 and 5 weeks after MI, in both the Plg−/− and Plg+/+ infarcted hearts (Table 1). Infarct sizes, left ventricular lumen areas (measure for LV dilatation), and septum thicknesses were not significantly different between the two infarct groups. However, the infarcted wall of the Plg−/− hearts remained thicker than that of Plg+/+ hearts (0.45 ± 0.06 *vs.* 0.23 ± 0.03 mm, $P = 0.004$), as clearly shown in Figure 6. These thicker infarcted walls of the Plg−/− mice probably account for the increased heart weights, observed in the Plg−/− infarcts (Table 1).

Gelatinase Activity Is Decreased in Plg−/− Infarcts

Gelatin zymography on murine left ventricular tissue reveals four distinct proteins with gelatinase activity (Figure 7). The identity of these gelatinolytic bands in mouse tissue extracts has been described by others.[15] The top band (~97 kd) represents MMP-9. Three bands with molecular weights of ~72, ~66, and ~60 kd represent, respectively, the proenzyme form, intermediate form, and active forms of MMP-2. Quantification of the intensities of the bands demonstrated a down-regulation in the activity of MMP-9 (77%) and active MMP-2 (49%) in 2-week-old

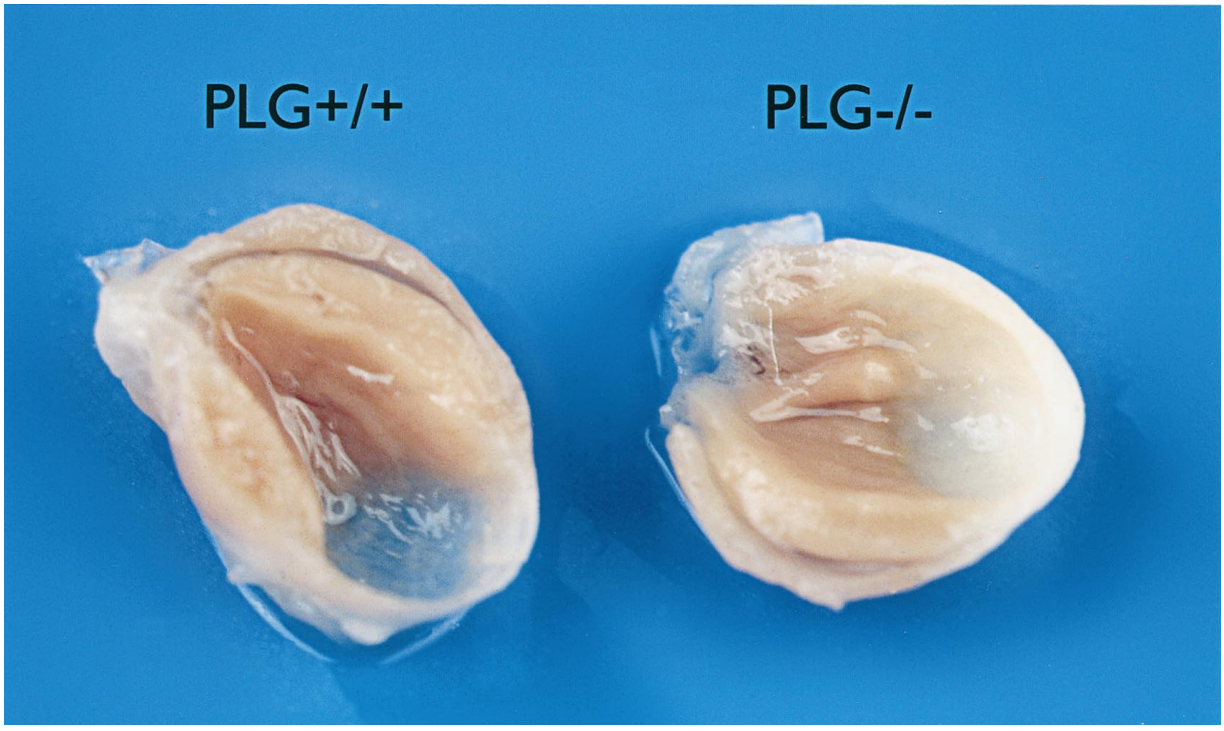

Figure 6. Infarcted hearts from Plg+/+ and Plg−/− mice, 5 weeks after surgery. Note the extensive infarct thinning in the wild-types, where the infarcted wall has almost become transparent.

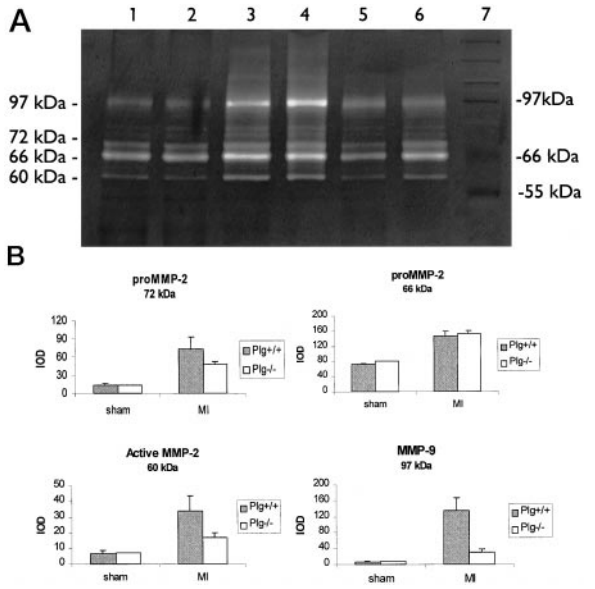

Figure 7. MMP-2 and MMP-9 activity in Plg−/− and Plg+/+ hearts. **A:** Gelatin zymography, demonstrating MMP-2 and MMP-9 activity in cardiac extracts, 2 weeks after surgery. **Lanes 1** and **2**, Plg−/− infarcts; **lanes 3** and **4**, Plg+/+ infarcts; **lane 5**, Plg+/+ sham; **lane 6**, Plg−/− sham; **lane 7**, marker. The 97-kd band corresponds to MMP-9. Three bands with molecular weights of ~72, ~66, and ~60 kd represent, respectively, two proenzyme forms and one activated form of MMP-2. **B:** Quantification of the scanned gelatinolytic bands.

Plg−/− infarcted hearts (Figure 7). We observed no differences in the intensity of the 66-kd band between the two strains and a 40% decrease in the activity of the 72-kd band in the Plg−/− mice. No differences in MMP activity were seen between sham-operated Plg−/− and Plg+/+ mice.

The Impact of Impaired Healing on Cardiac Function

Cardiac function was evaluated 2 weeks after surgery by measuring the rate of pressure development in the hearts (dP/dt_{max}) and cardiac output levels (CO). Under basal conditions, contractility (dP/dt_{max}) was similar in the infarcted Plg+/+ and Plg−/− hearts. However, after inotropic stimulation by dobutamine, dP/dt_{max} was blunted in the Plg−/− infarcted hearts as compared to the wild-types (Figure 8). Heart rate responses to dobutamine were similar in all groups (data not shown).

CO levels at rest were also comparable in the sham and infarct groups (Table 2). We therefore maximally challenged the hearts by a rapid intravenous fluid load. In rats, it has previously been shown that volume overload elevates cardiac output (CO) to maximal values during the last 10 to 15 seconds of a 1-minute fluid load and that infarction blunts the increase in CO proportional to the degree of heart failure.[14,16] After volume overload in Plg−/− and Plg+/+ mice, CO levels were comparably blunted in both infarct groups when compared to the shams (n = 3–5 per group, Table 2). Taken together, these measurements demonstrate impaired function in the Plg−/− infarcted hearts during mild stimulation and equalization of cardiac output in the two strains at extreme loads.

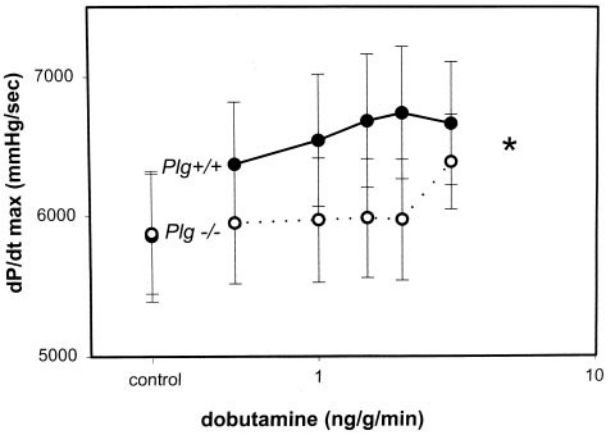

Figure 8. The cardiac contractile response to dobutamine infusion is blunted in the 2-week Plg−/− infarcts (n = 5–6 per group, P = 0.014 by two-way analysis of variance).

Discussion

Role of the Plasminogen System in Infarct Healing

Early events of tissue repair after MI include migration of inflammatory cells into the wound and degradation of the extracellular matrix and necrotic cardiomyocytes.[12] Inflammatory cells migrate into the wound by degrading the extracellular matrix surrounding the cardiomyocytes, using proteinases such as plasmin, MMPs, and cathepsins.[17] In the present study, the infiltration of macrophages, (myo)fibroblasts, and endothelial cells was found to be abolished in the absence of plasminogen for at least 5 weeks after MI. This indicates that plasmin is required for cellular infiltration into the infarct, either directly by extracellular matrix proteolysis, or indirectly through activation of MMPs. In fact, decreased activity of MMP-9 and active MMP-2 was found in the Plg−/− infarcts compared to the wild-types. The intensity of the latent MMP-2 band was suppressed to a smaller extent in Plg−/− infarcts. This indicates that *de novo* synthesis of MMP-2 is barely affected. The small effect of Plg deficiency on the amount of latent MMP-2 suggest that MMP-2 activity is decreased due to inhibited activation of pro-MMP-2, rather than to a reduced synthesis. This indicates also that the effect of plasmin on infarct healing is at least partly mediated by activation of MMP-9 and MMP-2. This is supported by the finding that mice deficient in MMP-9 showed a reduction in the infiltration of leukocytes into the infarct and were protected against cardiac rupture.[2] In the present study, we were unable to investigate the role of plasminogen in the development of left ventricular rupture, because cardiac rupture did not take place in Plg−/− and in only one Plg+/+ infarct.

The role of plasminogen in cardiac wound healing as shown here is consistent with its role in dermal and vascular wound healing.[18,19] However, the effects on car-

Table 2. Cardiac Output Elevation after 2.5 ml Volume Overload

	Plg+/+		Plg−/−	
	SH	MI	SH	MI
CO_{rest} (ml/min)	4.8 ± 1.0	4.8 ± 0.8	4.9 ± 0.7	3.2 ± 0.4
ΔCO (ml/min)	9.7 ± 2.9	6.8 ± 1.3	9.1 ± 1.2	6.8 ± 0.4

MI, myocardial infarction; SH, sham operation.
n = 3–5 per group.
Data are presented as mean ± SE. No statistical differences were observed between groups.

diac healing are more pronounced, in that vascular wound healing was delayed, but not completely abolished in plasminogen-deficient mice.[18] Dermal wound healing in Plg−/− mice was associated with impaired keratinocyte migration, but an intact inflammatory response.[19] In conclusion, impaired cardiac wound healing in both Plg−/− and u-PA−/− mice[2] indicate that plasmin proteolysis is needed for the normal repair process of the heart after infarction. The plasmin-mediated ECM degradation is initiated by enhanced activity of uPA. Although plasmin cannot directly degrade interstitial collagens, it can initiate collagenolysis by activation of latent MMPs.

LV Architecture and Performance in the Absence of Infarct Healing

Despite the absence of cardiac wound healing, architectural changes of the LV were comparable in Plg−/− infarcts and wild-type infarcts, except the less pronounced thinning of the infarcted wall in Plg−/− mice. This might be explained by the persistent presence of necrotic cardiomyocytes in the Plg−/− infarcts, which have larger volumes than the collagen fibers in the Plg+/+ infarcts. Larger heart weights in the Plg−/− infarcts cannot be explained by a hypertrophic response of the noninfarcted myocardium, since septum thicknesses are not different between the Plg−/− and Plg+/+ infarcts. The increased heart weights are more likely explained by the reduction in infarct thinning in the Plg−/− mice. Another remarkable observation was that left ventricular dilatation was not increased in the absence of healing, suggesting that the infarcted wall of the Plg−/− hearts, with its large number of necrotic cells, has a similar tensile strength as the infarcted wall of Plg+/+ hearts, where fibrous tissue is deposited to restore the structural integrity. The suggested maintenance of tensile strength in the infarcted Plg−/− wall may be due to preservation of the extracellular matrix and/or to the 1 to 2 cell layers of surviving cardiomyocytes in both endo- and epicardium.

The impact of impaired healing on left ventricular function was evaluated by measuring cardiac output and left ventricular pressure development. Both parameters provide information on global cardiac function. An impaired function was observed in the Plg−/− infarct group after submaximal stimulation (dobutamine). However, extreme challenge (volume overload) resulted in equalization of cardiac output between the two groups. The reduced response to dobutamine does not depend on differences in β-adrenoceptor signaling, since heart rates were similar in all groups. There is no *a priori* reason to assume a difference in contractility of cardiomyocytes in the noninfarcted myocardium between Plg−/− and Plg+/+ animals. The observed reduction of the response to dobutamine in the knockouts is compatible, however, with the observed structural changes in the infarcted area. In Plg−/− infarcts, reduced collagen deposition when compared to the wild-type infarcts, and the presence of necrotic cardiomyocytes increase the compliance of the infarct zone. This may result in mechanical disadvantages compared to the wild-types, where collagen deposition produces a virtually inextensible infarct.[20] However, at extreme volume loads, both infarcts are stretched to a maximum and stiffness of the two infarct types is no longer different. Under these conditions, pump function will exclusively be determined by the healthy part of the myocardium, which apparently is comparable between the two infarct types.

In conclusion, this study provides direct proof that plasmin-mediated proteolysis plays a central role in cardiac wound healing after myocardial infarction in mice.

Acknowledgments

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