Critical Role of Endogenous Thrombospondin-1 in Preventing Expansion of Healing Myocardial Infarcts

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Background—Matricellular proteins are extracellular matrix proteins that do not contribute directly to tissue integrity but are capable of modulating cell function. We hypothesized that the matricellular protein thrombospondin (TSP)-1, a potent inhibitor of angiogenesis and activator of transforming growth factor (TGF-β), is induced in healing myocardial infarcts and plays a role in suppressing the postinfarction inflammatory response, inhibiting local angiogenesis, and limiting expansion of granulation tissue into the noninfarcted area.

Methods and Results—We used a canine and a murine model of reperfused infarction. TSP-1 mRNA was induced in canine infarcts after 1 hour of ischemia and 3 to 7 days of reperfusion. TSP-1 protein showed a strikingly selective localization in the extracellular matrix, microvascular endothelium, and a subset of mononuclear cells of the infarct border zone after 5 to 28 days of reperfusion. Isolated canine venous endothelial cells showed low-level constitutive expression of TSP-1 mRNA, which was markedly induced by TGF-β1 and basic fibroblast growth factor. Murine infarcts also had marked TSP-1 deposition in the border zone. Infarcted TSP-1−/− mice exhibited sustained upregulation of the chemokines monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, and interferon-γ-inducible protein-10/CXCL10 and the cytokines interleukin-1β, interleukin-6, and TGF-β, suggesting an enhanced and prolonged postinfarction inflammatory response. In addition, TSP-1−/− mice had markedly increased macrophage and myofibroblast density in infarcts and in remodeling noninfarcted myocardial areas neighboring the myocardial scar, suggesting expansion of granulation tissue formation into the noninfarcted territory. TSP-1−/− animals had more extensive postinfarction remodeling than wild-type mice, although infarct size was similar in both groups.

Conclusions—The infarct border zone may be capable of modulating the healing process through its unique extracellular matrix content. The selective endogenous expression of TSP-1 in the infarct border zone may serve as a “barrier,” limiting expansion of granulation tissue and protecting the noninfarcted myocardium from fibrotic remodeling.

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Key Words: extracellular matrix ■ immunology ■ inflammation ■ myocardial infarction ■ pathology

Cardiac repair after myocardial infarction is a dynamic and complex biological process initiated by induction of acute inflammation.1,2 Debridement of the injured cells by leukocytes is followed by proliferation, migration, and activation of mesenchymal cells and deposition of extracellular matrix proteins. Infarct healing can be divided into 3 overlapping phases: the inflammatory phase, the proliferative phase, and the maturation phase. The inflammatory phase is characterized by plasma exudation and leukocyte infiltration into the infarct site. Marginated leukocytes contribute to removal of dead tissue through the release of proteolytic enzymes and produce fibrogenic and angiogenic mediators that are important for the formation of granulation tissue, leading to the proliferative phase of infarct healing. At this stage of the repair process, angiogenesis is a prominent event that is accompanied by accumulation and proliferation of phenotypically modulated fibroblasts capable of producing large amounts of extracellular matrix proteins.3,4 The maturation phase follows: The cellular content of the infarct decreases, vascular structures regress, and the collagen fibers become cross-linked, leading to formation of a scar composed of dense collagen bundles.

The inflammatory cascade is a prerequisite for healing of the infarcted myocardium. However, effective cardiac repair depends on mechanisms that suppress the inflammatory response after granulation tissue formation and that limit expansion of fibrosis to the noninfarcted myocardium. Multiple “points of control” may exist to ensure that the inflammatory response is contained (both topographically and temporally) to the area and time of injury.5 These suppressive
signals are critical for healing because they prevent a persistent, expanding inflammatory response. Although numerous studies have focused on the expression and role of inflammatory mediators in the infarcted heart, the cellular and molecular events responsible for downregulation and containment of the inflammatory cascade remain unknown.

In addition to their structural role, extracellular matrix proteins are capable of modulating cellular responses. In particular, the members of a group of extracellular matrix proteins called matricellular proteins do not contribute directly to tissue integrity but regulate cell function. Thrombospondin (TSP)-1 is a homotrimeric matricellular protein with potent angiostatic properties and a significant role in transforming growth factor (TGF-β) activation. We hypothesized that locally induced TSP-1 may act as a suppressive anti-inflammatory signal through TGF-β activation and inhibition of angiogenesis. Using both canine and murine models of reperfused infarction, we demonstrated that TSP-1 protein shows a striking pattern of localized deposition in the border zone of the healing infarct. Genetic disruption of TSP-1 resulted in prolonged postinfarction inflammation and extension of the fibrotic process into the neighboring noninfarcted myocardium, leading to extensive remodeling of the infarcted ventricle. We demonstrate that the infarct border zone, an area of direct interaction between surviving cardiomyocytes and newly formed granulation tissue, has a unique extracellular matrix composition and suggest that it serves as a barrier for expansion of the inflammatory/fibrotic response into the noninfarcted areas.

Methods

Canine Ischemia-Reperfusion Protocols

The Baylor College of Medicine Institutional Review Board approved all animal protocols. Healthy mongrel dogs (15 to 25 kg) of either sex were surgically instrumented as previously described. A hydraulically activated occluding device and a Doppler flow probe were secured around the circumflex coronary artery. After surgery, the animals were allowed to recover for 72 hours before occlusion. Ischemia-reperfusion protocols were performed as previously described. After 1 hour of coronary occlusion, the cuff was deflated and the myocardium was reperfused. Reperfusion intervals ranged from 72 hours to 28 days (72 hour, n = 3; 7 days, n = 5; 14 days, n = 4; 28 days, n = 4). After the reperfusion periods, hearts were stopped and sectioned from apex to base. Tissue samples were isolated from infarcted or normally perfused myocardium on the basis of visual inspection. Myocardial segments were fixed for histological analysis or snap frozen and stored at -80°C for RNA extraction. Duplicate adjacent samples were processed for blood flow determinations using radiolabeled microspheres as previously described. Samples described as ischemic were all from areas where ischemic blood flow was <25%.

Murine Ischemia-Reperfusion Protocols

Wild-type (WT) C57/BL/6 mice (purchased from Charles River) and TSP-1-/- mice were used for myocardial infarction experiments. TSP-1-/- animals were genotyped through the use of polymerase chain reaction (PCR) as previously described. Male and female WT and TSP-1--/ knockout (KO) C57BL/6 mice 8 to 12 weeks of age (body weight, 18.0 to 22.0 g) were anesthetized by an intraperitoneal injection of sodium pentobarbital (60 μg/g). A closed-chest mouse model of myocardial ischemia-reperfusion was used as previously described to avoid the confounding effects of surgical trauma and inflammation, which may influence the baseline levels of chemokines and cytokines. After 1 hour of coronary occlusion, the myocardium was reperfused for 3 hours to 7 days. At the end of the experiment, the chest was opened and the heart was immediately excised, fixed in Z-fix, and embedded in paraffin for histological studies or snap frozen and stored at -80°C for RNA or protein isolation. Sham animals were prepared identically without undergoing myocardial infarction protocols. Animals used for histology underwent 24-hour, 72-hour, and 7-day reperfusion protocols (8 animals per group). Mice used for RNA extraction underwent 3, 6, 24, and 72 hours of reperfusion (8 animals per group). Mice used for protein extraction underwent 24 hours of reperfusion (7 WT mice, 5 TSP-1--/-- animals).

Immunohistochemistry and Quantitative Histology

Samples from mouse myocardium were fixed in zinc-formalin (Z-fix, Anatech) and embedded in paraffin. Sections were stained immunohistochemically with the following antibodies: monoclonal anti-α smooth muscle actin (α-SMA) antibody (Sigma), mouse anti–TSP-1 antibody (Neomarkers), rat anti-mouse macrophage antibody clone F4/80 (Research Diagnostics Inc), and rat anti-mouse CD31 antibody (Pharmingen). Canine samples were fixed in B5 formalin, and sections were stained with the following antibodies: monoclonal anti-TSP-1 antibody Ab-4 (Neomarkers), mouse anti-human α-SMA antibody (Sigma), and mouse anti-human CD31 antibody (Dako). Staining of canine and murine sections was performed with a peroxidase-based technique with appropriate Vecstain ELITE kits (Vector). The Mouse on Mouse (MOM) kit (Vector) was used for α-SMA and TSP-1 immunohistochemistry in murine tissues. For CD31 staining, sections were pretreated with trypsin, and staining was performed with the Tyramide Signal Amplification kit (Perkin Elmer) as previously described. Dual immunohistochemical studies to identify vascular cells were performed using an alkaline phosphatase-based method for α-SMA (red) and a peroxidase-based technique for CD31 as previously described.

Quantitative assessment of macrophage density was performed by counting the number of F4/80-positive cells in the scar, neighboring noninfarcted myocardium, and remote control septum using Image Pro software as previously described. The neighboring noninfarcted myocardium was defined as the subepicardial and subendocardial area adjacent to the infarct. α-SMA percent staining was quantitatively assessed as an indicator of myofibroblast infiltration as previously described. Infarct microvascular density was assessed by counting the number of CD31-stained vascular profiles in infarcted murine hearts.

Perfusion Fixation and Assessment of Ventricular Volumes

For assessment of postinfarction remodeling, infarcted hearts after 7 days of reperfusion were used for perfusion fixation (n = 10 for WT and TSP-1--/KO animals) as previously described. The entire heart from base to apex was cross-sectioned at 250-μm intervals. Ten serial 5-μm sections were obtained at each interval. The left ventricular end-diastolic volume (LVEDV, expressed in mm3) was assessed with ImagePro software using methods developed in our laboratory. The size of the infarct was expressed as a percentage of the left ventricular volume.

RNA Extraction

Total RNA was isolated from whole mouse heart according to acid-guanidium-phenylthiocarbohydrazide extraction method. RNA from canine myocardial tissue segments and canine jugular vein endothelial cells was electrophoresed in 1% agarose gels containing formaldehyde and then transferred to a nylon membrane (Gene Screen Plus, New England Nuclear) by standard procedures.

Molecular Cloning, Northern Hybridization, and Ribonuclease Protection Assay

A specific canine cDNA clone for TSP-1 was prepared by reverse transcription (RT) using RNA extracted from TGF-β–stimulated endothelial cells. RT-PCR was performed with the following primers:

- TSP-1 sense primer: 5′-GCATCCAGGACACATTGAT-3′
- TSP-1 antisense primer: 5′-GTCCTCGGATGATCTTCTTGTA-3′

PCR amplification was performed using the following conditions: 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min for 35 cycles. The amplified products were then analyzed by agarose gel electrophoresis. The size of the amplified fragment was confirmed by sequencing.
ers: TSP-1 sense primer, 5′ TGAGGCAGATGAAAGAAGACC 3′; and TSP-1 antisense primer, 5′ TGGTAAAGCTGGAGCAGCCTT 3′. TSP-1 mRNA levels in the canine myocardium and in stimulated canine endothelial cells were assessed through Northern hybridization as previously described.14

TSP-1 mRNA levels and inflammatory gene expression in murine hearts were assessed using Ribonuclease Protection Assay as previously described.13 The mRNA expression levels of the chemokines monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP-1α), MIP-1β, MIP-2, and interferon-γ-inducible protein (IP)-10; the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, LIF, and IL-10; the growth factors TGF-β1, -β2, and -β3, SCF, GM-CSF, and M-CSF, and the adhesion molecules ICAM-1 and E-selectin were determined (RiboQuant, Pharmingen) according to the manufacturer’s protocol. Phosphorimaging of the gels was performed (Storm 860, Molecular Dynamics), and signals were quantified with Image QuaNT software (Molecular Dynamics) and normalized to the ribosomal protein L32 mRNA.

**Protein Isolation and Western Blot Analysis**

Protein isolation and Western blot analysis were carried out as described by Hao et al.20 WT (n=7) and TSP-1−/− (n=5) mice undergoing reperfused infarction protocols (1-hour ischemia–24-hour reperfusion) were used for protein extraction. Protein (30 μg) was separated on 10% SDS–polyacrylamide gels in a Tris/HCl buffer system, transferred onto nitrocellulose membranes, and blotted according to standard procedures with a polyclonal rabbit anti-Smad2 (1:1000) or a polyclonal rabbit anti-Phospho-Smad2 (Ser465/467, 1:200) antibody (both from Cell Signaling). The specific bands of target proteins were visualized by chemiluminescence, and band intensities were evaluated with ImageQuaNT. Membranes were then stripped and reblootted with monoclonal anti-GAPDH (1:10 000, Advanced ImmunoChemical) antibody. Target signals were normalized to GAPDH signal. The ratio of P-Smad2 to total Smad2 expression was used to indicate activation of the TGF-β signaling pathway.

**Canine Endothelial Cell Isolation and Stimulation**

Isolated canine jugular vein endothelial cells were obtained as previously described.13 Endothelial cells were incubated with recombinant human TNF-α, VEGF, basic fibroblast growth factor (bFGF), IP-10, and TGF-β (all from R&D) for 16 hours. At the end of the experiment, RNA extraction was performed and TSP-1 mRNA expression was assessed with Northern hybridization.

**Statistical Analysis**

Statistical analysis was performed with ANOVA, followed by a t test corrected for multiple comparisons (Student-Newman-Keuls). Data are expressed as mean±SEM. Statistical significance was set at \( P<0.05 \).

**Results**

**TSP-1 mRNA and Protein Expression in Canine Myocardial Infarcts**

The partial 428-bp cDNA clone for canine TSP-1, obtained with RT-PCR, demonstrated 90% identity with its human homologue. Northern hybridization studies indicated that segments from noninfarcted canine hearts had negligible TSP-1 mRNA expression. In contrast, canine myocardial infarction was associated with marked upregulation of TSP-1 mRNA synthesis. TSP-1 mRNA levels were significantly increased in ischemic segments after 5 days of reperfusion (Figure 1A and 1B).

**TSP-1 Localization in the Border Zone of Canine Infarcts**

TSP-1 immunoreactivity was first noted in healing canine infarcts after 72 hours of reperfusion (Figure 2A). After 7 days of reperfusion, injured cardiomyocytes were replaced with granulation tissue, and an organized temporary matrix was created. Although this matrix network was prominent in both the center and the border of the healing infarct, TSP-1 protein showed a striking pattern of localized deposition in the infarct border zone after 7 to 28 days of reperfusion, clearly demarcating the infarcted area from the noninfarcted myocardium (Figure 2B and 2C). TSP-1 deposition was noted in the extracellular matrix of the border zone (Figure 2E through 2G). In addition, the microvascular endothelium (Figure 2I through 2L) and some cells with macrophage morphology also stained for TSP-1 (Figure 2F and 2H). Significant deposition of TSP-1 was noted in the adventitia of mature pericyte-coated vessels (Figure 2D and 2H), formed in the mature scar after 28 days of reperfusion.

**Infarct Healing in TSP-1−/− Mice: TSP-1–KO Infarcts Exhibit Enhanced and Prolonged Cytokine and Chemokine Expression**

We studied the functional role of endogenous TSP-1 expression in infarct healing using a murine model of reperfused infarction, developed and extensively characterized by our laboratory.17 In the murine model, TSP-1 mRNA was mark-
edly induced after 6 to 24 hours of reperfusion (Figure 4A). TSP-1 protein was noted after 24 hours to 7 days and was localized in the infarct border zone after 3 to 7 days of reperfusion (Figure 4). TSP-1 immunoreactivity was noted in the extracellular matrix of the infarct border zone; in addition, cells with mononuclear morphology and endothelial cells also demonstrated TSP-1 expression (Figure 4B and 4C). Control and sham-operated TSP-1–KO hearts demonstrated no evidence of active inflammation, exhibiting no inflammatory leukocyte infiltration and minimal mRNA expression of inflammatory mediators. Microvascular, arteriolar, venular, and macrophage densities were similar in control TSP-1/–/– and WT hearts. Furthermore, sham-operated TSP-1/–/– and WT animals showed comparable cytokine (IL-1, IL-6, TNF-α, and IL-10) and chemokine (MCP-1, MIP-1α, MIP-1β, MIP-2, and IP-10) mRNA expression. mRNA levels of the adhesion molecule E-selectin in TSP-1/–/– animals were low (<3% of the housekeeping gene L32) but higher than in WT animals (ratio to L32: TSP-1/–/–, 0.026±0.007 versus 0.006±0.002; P<0.01). Both TSP-1/–/– and TSP-1/–/– sham-operated hearts showed negligible expression of ICAM-1.

TSP-1/–/– mice undergoing reperfused infarction protocols showed mortality rates comparable to those of WT animals (mortality rate, 8.2% in TSP-1/–/– mice versus 5.6% in WT animals). However, infarcted TSP-1–KO animals demonstrated enhanced and prolonged expression of inflammatory genes. TSP-1/–/– hearts exhibited increased mRNA expression of the chemokines MCP-1 (Figure 4D), MIP-1α, and IP-10 (not shown) and the cytokines IL-6, IL-1β, and TGF-β1 (Figure 5) after 24 and 72 hours of reperfusion. In contrast, TNF-α, IL-10, TGF-β2, and TGF-β3 levels were comparable in TSP-1–KO and WT infarcts (data not shown).

TSP-1–KO Infarcts Show Expansion of Granulation Tissue Formation Into the Noninfarcted Myocardial Area

Abundant macrophages and myofibroblasts infiltrate healing murine infarcts and are contained mostly within the infarcted zone. In WT mice, macrophage and myofibroblast densities peaked after 72 hours and decreased significantly after 7 days of reperfusion. In contrast, TSP-1/–/– mice showed increased and prolonged macrophage and myofibroblast infiltration into the infarct (Figures 6 and 7). Importantly, after 7 days of reperfusion, macrophage and myofibroblast density was markedly increased in noninfarcted myocardial areas neighboring the myocardial scar (Figures 6D and 7D), suggesting...
expansion of granulation tissue formation into the noninfarcted territory. In contrast, the remote posterior septum had comparable macrophage and myofibroblast infiltration in KO and WT mice.

The vascular network in infarcted hearts had similar features in KO and WT animals. Capillary and arteriolar density were comparable in WT and TSP-1/H11546/H11546 mice after 7 days of reperfusion (not shown).

**Activation of the Smad2/3 Signaling Pathway in WT and TSP-1−/− Myocardial Infarcts**

To evaluate the role of TSP-1 in activation of TGF-β signaling pathways, we examined Smad2/3 phosphorylation by measuring the ratio of p-Smad2 to total Smad-2 protein expression in WT and TSP-1−/− infarcts. After 24 hours of reperfusion, there was a trend for a higher ratio in WT infarcts compared with TSP-1−/− mice (P<0.10) (Figure 8A).

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**Figure 3.** TSP-1 mRNA expression by stimulated canine jugular vein endothelial cells. A, Unstimulated canine endothelial cells show little TSP-1 expression. TGF-β stimulation markedly induces TSP-1 expression (A), whereas TNF-α somewhat decreases TSP-1 expression levels. B, Angiogenic factors VEGF (V) and bFGF (bF) induce TSP-1 mRNA expression in canine venous endothelial cells. Interestingly, IP-10 (i), an angiostatic CXC chemokine known to inhibit bFGF-induced angiogenesis, significantly increases bFGF-mediated endothelial TSP-1 mRNA upregulation. Incubation conditions: 1, 2, control; 3, VEGF 10 ng/mL; 4, VEGF 50 ng/mL; 5, VEGF 100 ng/mL; 6, VEGF 100 ng/mL + IP-10 (500 ng/mL); 7, VEGF 100 ng/mL + IP-10 (1 μg/mL); 8, bFGF 10 ng/mL; 9, bFGF 50 ng/mL; 10, bFGF 100 ng/mL; 11, bFGF 100 ng/mL + IP-10 (500 ng/mL); and 12, bFGF 100 ng/mL + IP-10 (1 μg/mL).

**Figure 4.** A, TSP-1 mRNA expression in murine infarcts peaks after 3 to 6 hours of reperfusion (**P<0.01 vs sham). B, TSP-1 immunoreactivity is noted in mouse infarct after 72 hours of reperfusion. Cells with morphological characteristics of mononuclear cells express TSP-1 (arrows). C, After 7 days of reperfusion, TSP-1 deposition is found in matrix of infarct border zone. D, TSP-1−/− animals exhibit enhanced and prolonged MCP-1 mRNA expression after myocardial infarction (**P<0.001 vs WT). Scale bar, 40 μm.

**Figure 5.** TSP-1−/− animals have increased and prolonged cytokine expression after myocardial infarction. A, Quantitative analysis of IL-1β expression in healing infarcts (**P<0.01 vs WT). B, Quantitative analysis of IL-6 mRNA expression in reperfused mouse infarcts (**P<0.01 vs WT). C, Quantitative analysis of TGF-β1 mRNA levels in healing mouse infarcts (**P<0.01 vs WT).
TSP-1 Deficiency Results in Adverse Remodeling of the Murine Myocardium

Myocardial infarction results in remodeling of the ventricle, the extent of which depends on infarct size. After 7 days of reperfusion, TSP-1−/− mice had higher LVEDV (P<0.01) than WT animals (Figure 8B through 8D), although the 2 groups had comparable infarct size (WT, 10.14±0.93%; TSP-1 KO, 13.7±2.27%; P=0.17).

Discussion

Unlike the various structural proteins of the extracellular matrix, matricellular proteins do not contribute directly to tissue integrity but influence cell function by modulating cell-matrix interactions.11,21,22 TSP-1 is a 450-kDa extracellular calcium-binding glycoprotein with a complex functional profile. TSP-1 binds the αvβ3, αvβ1, and αvβ5 integrins, the integrin-associated protein, and CD36 on the cell surface.11 Through these interactions, TSP-1 modulates cellular phenotype; however, its specific effects in regulating in vivo processes remain poorly understood. Evidence suggests that TSP-1 suppresses proliferation and migration of endothelial cells in vitro23 and inhibits neovascularization in vivo.24 In addition, TSP-1 plays a critical role in TGF-β activation.12 Although TSP-1 upregulation in skin wounds has been reported,25 its role in the wound healing process remains controversial. Treatment of skin wounds with TSP-1 antisense oligonucleotides resulted in delayed repair and decreased rate of reepithelialization,25 however TSP-1 overexpression in the skin suppressed granulation tissue formation and wound healing in mice.26 Furthermore, TSP-1−/− mice had an impaired inflammatory response in excisional skin wounds.27 We have used a canine and a murine model to clarify the role of TSP-1 in healing myocardial infarcts. We found a striking pattern of localized deposition of TSP-1 in the infarct border zone. In addition, using TSP-1−/− mice, we demonstrated a crucial role for TSP-1 in preventing expan-

Figure 6. In TSP-1−/− infarcts, macrophages infiltrate neighboring noninfarcted myocardium. F4/80 immunohistochemistry of representative sections from WT (A, C) and KO (B, D) mice after 3 (A, B) and 7 (C, D) days of reperfusion. In addition, macrophages in KO animals infiltrate neighboring remodeling noninfarcted myocardium (D, arrows), whereas WT mice exhibit relatively well-demarcated inflammatory infiltrate (C). E, Quantitative analysis demonstrates that TSP-1−/− infarcts exhibit increased macrophage density (cells/mm²) after 3 and 7 days of reperfusion. F, TSP-1−/− animals exhibit significantly higher macrophage infiltration in scar (**P<0.01 vs WT) and neighboring noninfarcted myocardium (nei; *P<0.05 vs WT). In contrast, remote noninfarcted septum (rem) contains comparable number of macrophages in both groups (P=NS). Scale bar, 40 µm.

Figure 7. In absence of TSP-1, infarct myofibroblasts infiltrate neighboring noninfarcted area. A, α-SMA immunohistochemistry identifies myofibroblasts (spindle-shaped extravascular cells; arrows) and smooth muscle cells (located in vascular wall). WT animals show clearly demarcated infarct, whereas TSP-1−/− mice (B) have large number of myofibroblasts extending into neighboring noninfarcted area (arrows). C, Quantitative analysis of α-SMA percent staining demonstrates that TSP-1−/− mice have increased α-SMA expression in infarct after 7 days of reperfusion. TSP-1−/− mice show significantly higher α-SMA expression in scar (**P<0.01 vs WT scar) and neighboring noninfarcted myocardium (nei; *P<0.05 vs WT) compared with WT mice. In contrast, α-SMA percent staining is similar in remote noninfarcted septum (rem; P=NS). Scale bar, 40 µm.
sion of granulation tissue formation into the noninfarcted areas and decreasing postinfarction ventricular remodeling. We suggest that selective expression of inhibitory mediators such as TSP-1 in the infarct border zone may serve as a “barrier,” limiting extension of the inflammatory response into the noninfarcted areas.

Canine infarcts demonstrated selective deposition of TSP-1 in the extracellular matrix of the border zone after 7 to 14 days of reperfusion (Figure 2). Many cell types are capable of producing TSP-1; we have identified a subset of endothelial and mononuclear cells as the main source of TSP-1 in the healing infarct (Figure 2). Growth factors such as TGF-β, VEGF, and bFGF markedly induce endothelial TSP-1 mRNA synthesis (Figure 3); in contrast, proinflammatory cytokines such as TNF-α and IL-1β suppress TSP-1 expression28 (Figure 3). Release and activation of TGF-β in the infarct border zone have previously been demonstrated20 and may explain the selective localization of TSP-1.

TSP-1−/− animals showed no evidence of spontaneous cardiac inflammation, suggesting that TSP-1 does not have a crucial role in cardiac homeostasis. However, after myocardial infarction, TSP-1−/− KO mice had enhanced and prolonged chemokine and cytokine induction (Figures 4 and 5). Increased expression of inflammatory mediators in TSP-1−/− mice was associated with extensive infiltration of the cardiac interstitial space of the neighboring noninfarcted area with macrophages (Figure 6) and myofibroblasts (Figure 7), resulting in expansion of granulation tissue formation. As a result, TSP-1−/− KO mice demonstrated increased adverse left ventricular remodeling, although the size of their infarcts was similar to that of WT animals (Figure 8). Thus, in the absence of TSP-1 from the infarct border zone, the inflammatory process was enhanced and expanded into the noninfarcted area, leading to increased fibrosis of the neighboring remodeling myocardium. In contrast, the remote septum did not exhibit significant macrophage and myofibroblast accumulation in both KO and WT animals (Figures 6 and 7).

The effects of TSP-1 in limiting expansion of the inflammatory/fibrotic process in healing myocardial infarcts may be due to its TGF-β activating or its angiostatic effects. TSP-1 has been shown to be one of the major activators of TGF-β1 in vivo.12,29 TGF-β activation is mediated by a WXXW sequence that is present in each of the type I repeats of TSP-1, but it also requires an interaction with a KRFK sequence located between the first and second type I repeats.30 The physiological significance of TSP-1–mediated TGF-β activation has been supported by the phenotype exhibited by TSP-1−/− KO mice. In WT animals, both TSP-1 and active TGF-β1 are highly expressed in the bronchial epithelium. TSP-1−/− mice show inflammatory changes in the lung and pancreatic parenchyma,13 which are corrected by administration of the TGF-β–activating peptide KRFK.12 However, TSP-1 is not the sole activator of TGF-β. In the absence of injury, the brain, heart, kidney, spleen, stomach, intestines, aorta, and liver of TSP-1−/− mice show no major abnormalities.15 Furthermore, we found that control canine and murine hearts do not express significant amounts of TSP-1, and mRNA expression of chemokines and cytokines was similar in KO and WT animals, indicating that TSP-1 does not play a role in maintaining a normal phenotype and function in the heart. In infarcted hearts, however, an intense inflammatory reaction is triggered, leading to marked TGF-β1 mRNA upregulation.17 We found that infarcted TSP-1−/− hearts demonstrate a trend toward decreased phosphorylation of Smad2, a critical intracellular effector of TGF-β signaling, compared with WT infarcts (Figure 8A). This is consistent with an important role of TSP-1–mediated TGF-β activation in the healing infarct. In the absence of TSP-1, impaired TGF-β activation may result in accentuation and expansion of the postinfarction inflammatory response into the neighboring remodeling myocardium, leading to extensive fibrosis and adverse left ventricular remodeling (Figures 6 through 8).

Not all effects of TSP-1 deficiency can be explained through the TGF-β–activating actions of the molecule. Although TGF-β is an important factor in myofibroblast differentiation, TSP-1−/− mice exhibit increased numbers of α-SMA–positive myofibroblasts in the infarct (Figure 7). TSP-1 may regulate infarct healing through alternative mechanisms involving inhibition of matrix metalloproteinase activity31 and sequestration of matrix-associated growth factors. A recent study demonstrated that TSP-1 promotes the mobilization of matrix-bound FGF-2, generating an inactive TSP-1/FGF-2 complex, thus decreasing bioavailability of the growth factor.32
angiogenesis and may prevent expansion of the inflammatory response by locally suppressing neovessel formation in the infarct border zone. Although we did not find a significant difference in the number of microvascular profiles between WT and KO animals, inhibition of angiogenesis may be an important mechanism responsible for suppressing granulation tissue expansion in healing infarcts.

The postinfarction inflammatory response is important for removal of irreversibly injured cells and for healing of the wound. However, optimal repair requires timely suppression of inflammation and containment of granulation tissue formation into the infarcted area. Our investigation was not designed to establish the protective role of TSP-1 in the infarcted myocardium, but it introduces a novel concept in our understanding of infarct healing and left ventricular remodeling. We suggest that the infarct border zone may be capable of modulating the healing process through its unique extracellular matrix content. The selective endogenous expression of TSP-1 in the infarct border zone may serve as a barrier, limiting expansion of granulation tissue and protecting the noninfarcted myocardium from fibrotic remodeling. TSP-1 may locally suppress inflammatory cytokine and chemokine synthesis through its TGF-β-activating effects, preventing extension of the inflammatory process into the neighboring remodeling myocardium.

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References

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