Stem Cell Factor Induction Is Associated With Mast Cell Accumulation After Canine Myocardial Ischemia and Reperfusion

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Background—Myocardial infarction is associated with an intense inflammatory reaction leading to healing and scar formation. Because mast cells are a significant source of fibrogenic factors, we investigated mast cell accumulation and regulation of stem cell factor (SCF), a potent growth and tactic factor for mast cells, in the healing myocardium.

Methods and Results—Using a canine model of myocardial ischemia and reperfusion, we demonstrated a striking increase of mast cell numbers during the healing phase of a myocardial infarction. Mast cell numbers started increasing after 72 hours of reperfusion, showing maximum accumulation in areas of collagen deposition (12.0±2.6-fold increase; P<0.01) and proliferating cell nuclear antigen (PCNA) expression. The majority of proliferating cells were identified as α-smooth muscle actin–positive myofibroblasts or factor VIII–positive endothelial cells. Mast cells did not appear to proliferate. Using a nuclease protection assay, we demonstrated induction of SCF mRNA within 72 hours of reperfusion. Immunohistochemical studies demonstrated that a subset of macrophages was the source of SCF immunoreactivity in the infarcted myocardium. SCF protein was not found in endothelial cells and myofibroblasts. Intravascular tryptase–positive, FITC-avidin–positive, CD11b-negative mast cell precursors were noted in the area of healing and in the cardiac lymph after 48 to 72 hours of reperfusion.

Conclusions—Mast cells increase in number in areas of collagen deposition and PCNA expression after myocardial ischemia. The data provide evidence of mast cell precursor infiltration into the areas of cellular injury. SCF is induced in a subset of macrophages infiltrating the healing myocardium. We suggest an important role for SCF in promoting chemotaxis and growth of mast cell precursors in the healing heart. (Circulation. 1998;98:687-698.)

Key Words: cells ■ collagen ■ myocardial infarction ■ reperfusion ■ growth substances

Mast cells are recognized effector cells in allergic inflammatory responses. Recent findings indicate that mast cells can influence biological processes through the production of cytokines and growth factors.1 The suggestion that mast cells may participate in the fibrotic process is supported by studies demonstrating that mast cells are capable of producing a wide variety of mediators, such as histamine, tryptase, TNF-α,1 bFGF,2 and TGF-β,3 which can modulate fibroblast phenotypic characteristics and extracellular matrix synthesis. The association of inflammation with myocardial infarction has been recognized for more than a century4 and is properly considered part of the healing process. Our laboratory has concentrated on characterizing the biological basis for reperfusion injury in a canine model of myocardial infarction.5,6 In the companion article,7 we present evidence suggesting that the cardiac mast cell is the primary source of preformed TNF-α in the canine heart and, through degranulation and TNF-α release, initiates a cytokine cascade involving IL-6 induction in infiltrating mononuclear cells and subsequent intercellular adhesion molecule-1 induction in cardiac myocytes. The present study was designed to investigate cardiac mast cells during the healing phase of an experimental canine myocardial infarction. We found that during the healing phase, mast cells were markedly increased in number in the ischemic segments of canine myocardium, accumulating in areas of collagen deposition and cell proliferation. We present the first in vivo demonstration of mast cell precursor influx into an evolving tissue injury. In addition, we present the first evidence for induction of SCF,4 a potent tactic and growth factor for mast cells, in a subset of infiltrating macrophages after myocardial ischemia. We postulate that SCF may attract circulating mast cell precursors in the healing myocardium and promote their maturation and growth.
Methods

Ischemia/Reperfusion Protocols

Healthy mongrel dogs (15 to 25 kg) of either sex were surgically instrumented as previously described with a hydraulic occluder secured around the circumflex coronary artery and a cannula placed in the cardiac lymph duct. After surgery, the animals were allowed to recover for 72 hours before occlusion. Ischemia-reperfusion protocols were performed in awake animals as described. Coronary artery occlusion was achieved by inflating the coronary cuff occluder until mean flow in the coronary vessel was zero as determined by the Doppler flow probe. At the end of 1 hour, the cuff was deflated and the myocardium was reperfused. Reperfusion intervals ranged from 1 hour to 7 days. During the experiment, the cardiac lymph was collected. The samples were centrifuged, and the supernatant was collected and immediately frozen at $-80^\circ$C. The pelleted cells were fixed in Carnoy’s fixative and used for histological studies. After reperfusion, hearts were stopped by the rapid intravenous infusion of 30 mEq of KCl and removed from the chest for sectioning. Tissue samples were isolated from infarcted or normally perfused myocardium on the basis of visual inspection. Myocardial segments were fixed in 10% buffered formalin, Carnoy’s fixative, Mota’s fixative, or B*5 fixative for histological analysis or were immediately frozen, homogenized, and processed for RNA extraction. Duplicate adjacent samples were also processed for blood flow determinations with radiolabeled microspheres as previously described. The presence of a myocardial infarct was based on light-microscopic examination of hematoxylin-eosin–stained tissue sections by findings of contraction bands, “wavy fibers,” interstitial edema, and neutrophil infiltration, all in segments displaying markedly reduced blood flow during the ischemic period. For experiments lasting ≥24 hours after the start of the ischemic insult, the presence of histological elements characteristic of myocyte necrosis and fibrosis was added to the required criteria. Samples described as ischemic were all from areas in which ischemic blood flow was <25%. Samples of control tissues had normal blood flow during coronary occlusion. Data from 25 experiments, all showing evidence of ischemia based on blood flow determinations and light-microscopic examination, were analyzed ($n=5$ for 1 hour of ischemia and 3 hours of reperfusion, $n=4$ for 1 and 24 hours, $n=5$ for 1 and 72 hours, $n=6$ for 1 and 120 hours, and $n=5$ for 1 and 144 hours).

Immunohistochemistry and Histology

For histological studies, sections were fixed in 10% phosphate buffered formalin, Carnoy’s fixative, or B*5 fixative and embedded in paraffin. Sequential 3- to 5-μm sections were cut by microtomy.

Figure 1. Mast cell number increase after myocardial ischemia and reperfusion. A, Control canine myocardium stained with FITC-avidin to identify mast cells, located predominantly along arterioles (a) and venules (v) ($\times100$). B, Ischemic section of canine myocardium after 1 hour of ischemia and 7 days of reperfusion stained with FITC-avidin. Section shows area of scar formation (bottom) where myocytes have been replaced by fibrotic tissue and area where myocyte architecture is relatively preserved. A striking accumulation of mast cells is noted in healing area ($\times160$).
Immunostaining was performed with the ELITE rabbit or mouse kit (Vector Laboratories). The following primary antibodies were used for immunohistochemistry: mouse monoclonal antibody to CD11b MY904 (a clone from the American Type Culture Collection), mouse monoclonal antibody to human α-smooth muscle actin (Sigma), monoclonal mouse anti-PCNA antibody (clone PC10) (Dako), rabbit anti-human polyclonal antibody to factor VIII (Dako), monoclonal anti–smooth muscle myosin antibody (kindly donated by Dr C.L. Seidel, Baylor College of Medicine, Houston, Tex), monoclonal anti–canine SCF antibody (generously donated by Dr H.J. Deeg, University of Washington, Seattle), and monoclonal anti–macrophage antibody AM-3K (a generous gift from Dr K. Takahashi, Kumamoto University, Japan). Fluorescent immunohistochemistry was done with appropriate rhodamine- or Cy3-labeled secondary antibodies (Sigma). Appropriate positive and negative controls were used for each antibody. Dual immunohistochemistry was performed by combining peroxidase-based immunostaining for PCNA with fluorescent immunohistochemistry for α-smooth muscle actin or factor VIII with rhodamine-labeled secondary antibodies. A similar technique was used for SCF/α-smooth muscle actin and SCF/factor VIII immunostaining. Fluorescent labeling of mast cells with FITC-avidin was performed as previously described. The histochemical staining technique for tryptase was performed as previously described by Caughey et al. Quantitative analysis of mast cell numbers in canine heart was performed by counting FITC-labeled mast cells in sections from control and ischemic areas. Mast cells from 10 random fields were counted, and at least 2 different sections from 2 different tissue samples from each experiment were used for quantitative analysis. Samples labeled as ischemic had evidence of coronary ischemia based on histological analysis and blood flow quantification by use of radiolabeled microspheres. For studies on mast cell accumulation in areas of collagen deposition, sections from ischemic samples were stained for

![Graph](image)

**Figure 2.** Time course of mast cell numbers after myocardial ischemia/reperfusion. Note statistically significant increase in number of mast cells in ischemic areas after 72 hours (1.69-fold increase, \( P < 0.05 \)), 5 days (3.3-fold, \( P < 0.01 \)), and 7 days (3.39-fold, \( P < 0.01 \)) of reperfusion vs respective numbers in control areas from same experiment by ANOVA followed by \( t \) test with Bonferroni correction. For shorter reperfusion intervals, mast cell numbers in control and ischemic sections were comparable. Numbers of animals used for statistical analysis: \( n = 5 \) for 3 hours, \( n = 4 \) for 24 hours, \( n = 5 \) for 72 hours, \( n = 6 \) for 5 days, and \( n = 5 \) for 7 days of reperfusion. \(* P < 0.05, ** P < 0.01.**

![Images](image)

**Figure 3.** Mast cells accumulate in areas of collagen deposition and cell proliferation. a, c, and e, Serial 5-μm sections from totally fibrotic area of ischemic section of canine myocardium after 1 hour of coronary occlusion and 7 days of reperfusion; b, d, and f, serial sections from border zone containing fibrotic area (right) and area with relatively intact myocardium (left). Sections were stained for tryptase to identify mast cells (a, b), with picrosirius red to demonstrate collagen (c, d), and immunostained for PCNA (e, f) to label nuclei of proliferating cells. Note that tryptase-positive mast cells are located in areas with marked collagen deposition and PCNA expression, whereas the relatively intact area in b, d, and f does not show evidence of mast cell accumulation (×400). These observations are representative of 11 consecutive experiments of myocardial infarction with reperfusion intervals ranging from 120 to 144 hours.
tryptase. At least 15 random fields (×400) were photographed, and the number of tryptase-positive mast cells was counted in each field. Sections were then incubated overnight in 10% Tween 20 to erase the tryptase stain. Subsequently, picrosirius red staining was performed to visualize collagen fibers. The same fields were photographed again and on the basis of the presence of fibrotic tissue were classified as fibrotic (where myocytes were fully replaced by scar), partially fibrotic (where myocytes were partially replaced by fibrotic tissue), and nonfibrotic (where no evidence of scar formation was present). The mast cell numbers in the fibrotic and partially fibrotic areas were normalized on the basis of the corresponding numbers for nonfibrotic areas (nonfibrotic = 1). Mast cell numbers were statistically analyzed by Student’s t test (two-tailed) for 5 different experiments of myocardial ischemia, with reperfusion intervals ranging from 96 to 144 hours. Cell proliferation studies were performed by use of immunohistochemistry with the antibody to PCNA. Sections from canine bowel were used as positive controls. To correlate mast cell numbers with the number of proliferating cells, sections were stained with FITC-avidin and photographed. The number of FITC-avidin-positive mast cells was counted in each field. Subsequently, sections were immunostained for PCNA, and the numbers of PCNA-positive cells in the same fields were counted. Correlation studies between the number of mast cells and the number of PCNA-positive nuclei were performed for 5 experiments, with reperfusion intervals ranging from 96 to 144 hours.

Molecular Cloning

A cDNA clone for canine SCF was prepared by RT with RNA extracted from the ischemic and reperfused myocardium. RT-PCR was performed with the antisense primer 5’-TTGCAACATACTTATCCATC-3’ and the sense primer 5’-ATGAAGAAGACTCAGAAGAGCACAAACTTTGATTA-3’. The nucleotide sequence of the primers
was based on the published sequence for canine SCF and corresponded to base pairs 1 through 25 and 770 through 795 of the sequence. RT protocols were performed with 5 μg of total RNA. After first-strand synthesis, primed with the anti-sense primer, aliquots of the RT reaction were amplified with 5 U Taq DNA polymerase (Promega Corp) for 30 cycles of 93°C, 1 minute; 55°C, 2 minutes; and 72°C, 3 minutes. The resulting 795-bp fragment was purified, cloned in the PCR vector (Invitrogen), and sequenced.

RNA Isolation
RNA isolation from myocardial segments was performed by the acid guanidinium–phenol-chloroform procedure. RNA (30 μg) was electrophoresed in 1% agarose gels containing formaldehyde, then transferred to a nylon membrane (Gene Screen Plus, New England Nuclear) by standard procedures.

Nuclease Protection Assay
SCF mRNA expression in samples from the ischemic and reperfused myocardium was studied with an RNAse protection assay. The vector containing the 795-bp canine SCF insert was linearized by restriction digestion of the unique EcoRV site in the polylinker at the 5′ end of the insert. Antisense 32P-labeled transcripts were synthesized at 37°C in a reaction containing 1 μg of linearized template DNA; 1 mmol/L each of ATP, GTP, and UTP; 70 μCi [α-32P]CTP (400 Ci/mmol); 10 U Sp6 RNA polymerase; and standard transcription buffer. The probe was purified by 2 rounds of precipitation with ammonium acetate and ethanol. RNAse protection was performed with the RNAse protection kit (Boehringer Mannheim) or the Riboquant kit (Pharmingen) according to the manufacturer’s instructions. Briefly, 30 μg of total RNA was hybridized with 300 000 cpm 32P-labeled riboprobe overnight at 50°C followed by RNAse digestion. As a control reaction, the riboprobe was hybridized with yeast tRNA followed by RNAse digestion. The protected fragments were resolved on a 6% denaturing polyacrylamide gel and quantified with a GS-363 molecular imager system (Biorad). Size determinations of protected fragments were made by comparison to the full-length riboprobe and a DNA sequencing ladder. The predicted sizes of the full-length riboprobe and the protected fragment are 893 and 795 nucleotides, respectively. The riboprobe contains some linker sequences that are not protected. Loading of RNA was monitored with ethidium bromide staining as well as by probing of the nylon membranes with canine GAPDH.

Statistical Analysis
ANOVA followed by Student’s t test, corrected for multiple comparisons (Bonferroni), was used to test the significance of differences between mean values. Mean values are given with SEM. Correlation studies were performed for 5 consecutive experiments of myocardial infarction, with reperfusion intervals ranging from 120 to 144 hours. For this and all earlier time periods, immunohistochemical experiments were performed in at least 5 different experiments from each time point. The results described were consistently reproduced in all experiments.

Results
Mast Cells Increase in Number After an Experimental Canine Myocardial Infarction
Staining with FITC-labeled avidin (Figure 1) demonstrated a 3.39-fold increase of mast cell numbers in the ischemic sections after experiments of 1 hour of ischemia and 7 days of reperfusion, compared with control sections from the same experiments (P<0.01, n=5) (Figure 2). The increase in mast cell numbers was first noted after 72 hours of reperfusion (1.65-fold; P<0.05, n=5). We demonstrated the presence of the mast cell–specific proteinase tryptase in the cytoplasm of these cells (Figure 3a and 3b).
Mast Cells Accumulate in Areas of Collagen Deposition and PCNA Expression

Serial sections from tissue samples obtained from ischemia/reperfusion experiments were stained for tryptase to identify mast cells (Figure 3a and 3b), for collagen with picrosirius red (Figure 3c and 3d), and immunohistochemically for PCNA (Figure 3e and 3f) to identify proliferating cells. Mast cells appeared to accumulate in areas of cell proliferation and collagen deposition (Figure 3). Dual staining by the histochemical staining technique for tryptase followed by staining for collagen demonstrated that mast cells accumulated in areas with evidence of fibrosis (Figure 4a through 4d). Figure 4e demonstrates that mast cell numbers in fibrotic areas in which myocytes were fully replaced by scar were markedly higher than the numbers from areas of the same section that showed intact myocardium (12.0±2.6-fold increase; P<0.01, n=5). Furthermore, partially fibrotic areas had significantly higher mast cell numbers than nonfibrotic areas (6.1±2.2-fold increase; P<0.05, n=5). Further experiments combining FITC-avidin staining with immunostaining for PCNA (Figure 5A and 5B) showed an excellent correlation between the numbers of tryptase-positive cells and PCNA-positive nuclei in the same field (r=0.75, P<0.001) (Figure 5C).

Most PCNA-Positive Cells Are Identified as Myofibroblasts and Endothelial Cells

The number of PCNA-positive cells in the healing scar peaked after 3 to 5 days of reperfusion. The majority of these cells demonstrated cytoplasmic staining with an antibody to α-smooth muscle actin (Figure 6) but were smooth muscle myosin-negative (Figure 7). Other proliferating cells were identified as factor VIII-positive endothelial cells (Figure 8).

Absence of PCNA-Positive Mast Cells in the Healing Myocardium

Dual staining combining immunostaining for PCNA and FITC-avidin labeling (Figures 3 and 5) did not demonstrate significant numbers of PCNA-positive mast cells (<1%).

Cloning of Canine SCF

A specific canine clone for SCF was obtained by use of RT-PCR techniques encoding nucleotides 1 to 795 of the previously published sequence for canine SCF (data not shown).

SCF mRNA Is Induced in the Ischemic and Reperfused Myocardium

Expression of SCF mRNA was studied in the ischemic and reperfused myocardial segments with a nuclease protection
assay (Figure 9). Low levels of constitutive SCF mRNA expression were noted in several experiments. Significant upregulation of SCF mRNA occurred in the ischemic and reperfused samples after reperfusion intervals of 72 to 120 hours. No significant induction of SCF mRNA was noted with shorter reperfusion intervals (3 to 48 hours of reperfusion).

SCF Protein Localization in the Ischemic and Reperfused Myocardium

Immunohistochemical studies using a monoclonal antibody to canine SCF were performed to localize SCF protein in the healing myocardium. Sections of canine liver were used as a positive control and showed staining of the bile ducts in accordance with previous reports (Figure 10a). In the ischemic and reperfused heart, a significant increase of SCF immunoreactive cells was identified within 96 hours of reperfusion (Figure 10b). Serial sections were immunostained for SCF and the macrophage-specific antibody AM-3K, demonstrating that the source of SCF is a subset of macrophages (Figure 11). Dual immunohistochemistry did not show SCF immunoreactivity in factor VIII–positive endothelial cells or α-smooth muscle actin–positive myofibroblasts (data not shown).

Evidence for the Presence of Mast Cell Precursors in the Healing Heart

Staining for tryptase identified several intravascular tryptase-positive cells in the healing heart after 72 to 144 hours of reperfusion (Figure 12a and 12b). Studies using the cells isolated from the postischemic cardiac lymph demonstrated the presence of occasional metachromatric tryptase-positive cells that appeared only after 48 to 72 hours of reperfusion (12c and 12d). No tryptase-positive cells were present in the preischemic cardiac lymph. Furthermore, dual staining demonstrated the presence of a number of intravascular FITC-avidin–positive cells that did not stain for the basophil marker CD11b (Figure 13A through 13C). These findings suggested the attraction of mast cell precursors to the healing myocardium. The presence of these cells in the cardiac lymph demonstrates that these mast cell precursors can migrate through the venular endothelium and enter the tissue.

Discussion

Myocardial ischemia and reperfusion are followed by an acute inflammatory response associated with leukocyte infiltration. Tissue repair follows, with removal of cellular and
tissue debris by phagocytes, repair and remodeling of extracellular matrix by connective tissue cells, and new vessel formation. In this report, we provide evidence that mast cell accumulation occurs in healing areas of an experimental myocardial infarction. Our findings indicate that macrophage-derived SCF is induced in the postischemic heart and may be responsible for chemotaxis of mast cell precursors and mast cell growth in the myocardium. The presence of mast cells in the heart has been described in several species: in recent reports, human heart mast cells have been isolated and characterized.17 Although the role of cardiac mast cells remains unclear, the discovery that mast cells are an important source of cytokines and growth factors suggests new ways in which mast cell activation may be involved in myocardial inflammation and repair.

Mast Cells Accumulate in Areas of Cell Proliferation and Collagen Deposition

Our data demonstrate significant accumulation of FITC-avidin–positive, tryptase-positive mast cells in the ischemic and reperfused segments of the canine myocardium. Mast cells start accumulating after 72 hours of reperfusion (Figure 2) and are concentrated predominantly in areas of collagen synthesis (Figures 3 and 4) and cell proliferation (Figures 3 and 5). Several studies have suggested that mast cells and their mediators may play an active role in wound healing and fibrosis.18,19 Mast cell degranulation products have been demonstrated to induce fibroblast18,19 as well as endothelial cell proliferation20,21 and angiogenesis.22 When activated mast cells were cocultured with fibroblasts, they were found to increase collagen synthesis and stimulate fibroblast proliferation, indicating a direct involvement of mast cells in the fibrotic process.18 Studies of specific mast cell mediators have provided information on their effects in fibroblast activation and proliferation. Histamine has been shown to stimulate fibroblast growth and collagen synthesis in vitro,23,24 an effect mediated by H2 receptors. Tryptase, the most abundant of the proteases found in mast cell granules, is a potent mitogen for fibroblasts.25 Nanomolar concentrations of dog tryptase strongly stimulate thymidine incorporation in Chinese hamster lung and rat-1 fibroblasts and markedly potentiate DNA synthesis stimulated by epidermal growth factor and bFGF.25 Recent studies demonstrated that tryptase directly stimulates fibroblast chemotaxis and induces procollagen mRNA synthesis.26 Furthermore, mast cells have recently been recognized to be important sources of bFGF2 and TGF-β factors that can regulate fibroblast growth and modulate extracellular matrix metabolism. Mast cell products may also have a role in metalloproteinase activation and subsequent matrix degradation, an important component of wound healing. Cleutjens and colleagues27 demonstrated increased MMP-1, MMP-2, and MMP-9 activity in the rat myocardium after myocardial infarction. They suggested that posttranslational activation of latent collagenase (MMP-1) played a greater role in the wound-healing response than transcription of collagenase mRNA. Recently, canine mast cells have been found to secrete a 92-kDa gelatinase28 (MMP-9), which was extracellularly activated by chymase. This provided the first demonstration of a cell that activates an MMP it secretes by
cosecreting an activating enzyme. These findings suggest that mast cells may have an important role in myocardial remodeling and fibrosis after myocardial ischemia.

Cellular Identity of Proliferating Cells
Dual immunohistochemical studies identified a significant number of proliferating cells as α-smooth muscle actin–positive myofibroblasts (Figure 6). These phenotypically altered fibroblasts develop several ultrastructural and biochemical features of smooth muscle cells, including the expression of α-smooth muscle actin. Recent studies suggest the importance of α-smooth muscle actin–expressing myofibroblasts in wound healing because of their ability to alter the contractile properties of the affected tissues, to manifest heightened collagen gene expression, and to elaborate cytokines. TGF-β is a significant factor in mediating the development of myofibroblastic features. Willems and colleagues have previously identified nonvascular spindle-shaped α-smooth muscle actin–positive cells in healing myocardial scars. These cells were shown to be the main source of type I and type III procollagen mRNA in the infarcted myocardium. Our studies clearly established the myofibroblast-like phenotype of these cells, which do not express smooth muscle myosin (Figures 6 and 7). Other proliferating cells were identified as factor VIII–positive endothelial cells (Figure 8), suggesting active angiogenesis in the healing myocardium. Mast cell–derived TGF-β and bFGF may have a role in mediating the fibroblast phenotypic modulation and the angiogenic process.

Mast Cells Do Not Appear to Proliferate: Mast Cell Precursors Infiltrate the Ischemic Heart
Our findings failed to demonstrate significant mast cell proliferation in the ischemic myocardium. Although the contribution of cell proliferation cannot be ruled out, we believe that chemotaxis of circulating mast cell precursors in the healing myocardium may be the predominant mechanism responsible for mast cell accumulation in the ischemic myocardium. Mast cells originate from CD34 stem cells in the bone marrow and circulate as immature precursor cells in the peripheral blood. However, information on the phenotypic characteristics of these precursor cells is scarce. Rodewald and colleagues recently identified a cell population in murine fetal blood that fulfills the criteria of progenitor mastocytes. It is defined by the phenotype Thy-1 (lo) c-kit (hi), contains cytoplasmic granules, and expresses RNAs encoding mast cell–associated proteases but lacks expression of the high-affinity IgE receptor. Our studies identified intravascular cells expressing the mast cell–specific protease tryptase in sections from the ischemic and reperfused myocardium (Figure 12a and 12b). Furthermore, tryptase-
positive metachromatic cells could be identified in the postischemic cardiac lymph as early as 48 hours after reperfusion (Figure 12c and 12d). The expression of tryptase, a specific mast cell marker not found in other cell types, by these intravascular cells suggests that they represent mast cell precursors infiltrating the healing myocardium. Further experiments demonstrated the presence of intravascular FITC-avidin-positive cells that do not express the basophil marker CD11b (Figure 13). These findings support our hypothesis, identifying cells with phenotypic characteristics of mast cell progenitors in the healing heart.

SCF Is Induced in Areas of Healing
The factors responsible for mast cell recruitment in areas of fibrosis have yet to be defined. In vitro studies have implicated SCF as a potent mast cell chemotactant that stimulates directional motility of both mucosal- and connective tissue-type mast cells. SCF along with the anaphylatoxins C3a and C5a are the only chemotactic factors shown to induce migration of human mast cells. Recently, several angiogenic factors (platelet-derived growth factor-AB, vascular endothelial growth factor, bFGF) have also been demonstrated to promote murine mast cell chemotaxis in vitro. Galli and colleagues showed that subcutaneous administration of recombinant human SCF to baboons produced a striking expansion of the mast cell population, which was reversed when administration of the cytokine was discontinued. These experiments provided the first evidence that a specific cytokine can regulate mast cell development in vivo. However, the significance of this finding in the fibrotic process remains unclear. We present evidence demonstrating significant SCF mRNA upregulation in ischemic segments of the canine myocardium. SCF mRNA induction was first noted after 1 hour of ischemia and 72 hours of reperfusion (Figure 9); at the same time point, an increase in mast cell numbers is noted in the ischemic myocardium. Anaphylatoxins, such as C5a, cannot represent an important migratory stimulus for mast cells, because they are generated in the injured heart early after myocardial necrosis and are not active after 3 to 6 hours. In addition to being a mast cell chemotactant, SCF critically regulates the maturation and survival of mast cells by suppressing mast cell apoptosis, enhancing mast cell maturation, and inducing mast cell adhesion to fibronectin. SCF is also an important factor affecting mast cell activation and histamine release. Furthermore, SCF is capable of upregulating TNF-α mRNA in canine mastocytoma cells. All these actions may be important in promoting mast cell growth and activity after myocardial ischemia. Recent observations suggest that the ability of SCF to support certain stages of mast cell differentiation is influenced by interactions with specific cofactors, such as IL-3, IL-4, and IL-10. Experiments from our laboratory have demonstrated induction of IL-10 mRNA in the infarcted myocardial...
potential to secrete SCF and may promote the recruitment of mast cells in areas of fibrosis. However, immunohistochemical studies using a monoclonal antibody to canine SCF produced a surprise: SCF immunoreactivity was localized predominantly in a small subset of macrophages infiltrating the healing myocardium identified by immunohistochemistry with the macrophage-specific antibody AM-3K (Figures 10 and 11). SCF protein was not found in α-smooth muscle actin–positive myofibroblasts or factor VIII–positive endothelial cells.

**Significance of Hypothesized Cellular Interactions in Cardiac Healing**

Previous studies from our laboratory suggest that the potent mononuclear cell chemoattractant MCP-1 is induced in the ischemic and reperfused myocardium. MCP-1 appears to be an important factor in promoting mononuclear cell influx after 3 hours of reperfusion. It is interesting to speculate that under the influence of a variety of factors, mononuclear cells mature to macrophages, which may have a significant role in scar formation through the production of growth factors. It appears that a small subset of macrophages is responsible for production of SCF, promoting mast cell accumulation in the healing myocardium. Mast cell secretagogues may be important in mediating the phenotypic modulation and proliferation of fibroblasts and endothelial cells in the healing myocardium.

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