Mast Cells Play a Critical Role in the Pathogenesis of Viral Myocarditis

Hirokazu Higuchi, MD; Masatake Hara, MD, PhD; Kanjo Yamamoto, MD, PhD; Tadashi Miyamoto, MD, PhD; Makoto Kinoshita, MD, PhD; Tasuku Yamada, MD; Koji Uchiyama, MD; Akira Matsumori, MD, PhD

Background—Mast cells are powerful producers of multiple cytokines and chemical mediators playing a pivotal role in the pathogenesis of various cardiovascular diseases. We examined the role of mast cells in murine models of heart failure due to viral myocarditis, using 2 strains of mast cell–deficient mice.

Methods and Results—Two strains of mast cell–deficient mice, WBB6F1-KitW/W (W/WV) and WCB6F1-KitlSl/l-d (SI/SI4), were inoculated with 10 plaque-forming units of the encephalomyocarditis virus intraperitoneally. On day 14 after inoculation, survival of W/WV mice was significantly higher than that of their control littermates (77% versus 31%; P=0.03; n=13). On histological examination on day 7, myocardial necrosis and cellular infiltration were significantly less pronounced in W/WV and SI/SI4 mice than in their control littermates (area of infiltration, 7.6±3.5% versus 29.3±15.6%; P=0.002; area of necrosis, 7.6±3.5% versus 30.0±17.2%; P=0.003; n=10). Histological examination showed more severe changes in mast cell–reconstituted than in nonreconstituted W/WV and SI/SI4 mice. The gene expressions of mast cell proteases were upregulated in the acute phase of viral myocarditis and rose further in the subacute phase of heart failure. Their activation coincided with the development of myocardial necrosis and fibrosis and correlated with the upregulation of gene expression of matrix metalloproteinase-9. The histamine H1-receptor antagonist bepotastine improved encephalomyocarditis viral myocarditis.

Conclusions—These observations suggest that mast cells participate in the acute inflammatory reaction and the onset of ventricular remodeling associated with acute viral myocarditis and that the inhibition of their function may be therapeutic in this disease. (Circulation. 2008;118:363-372.)

Key Words: cardiomyopathy ■ heart failure ■ immunology ■ inflammation ■ myocarditis ■ viruses

Viral myocarditis is an important cause of congestive heart failure (CHF) and dilated cardiomyopathy,1 but its pathophysiology remains poorly understood. In recent years, mast cells have been implicated in the pathogenesis of cardiovascular and atherosclerotic disorders. In particular, we have observed that mast cells cause apoptosis of cardiac myocytes and proliferation of nonmyocytes in vitro.2 Furthermore, myocardial histamine and tryptase content and mast cell density are higher in CHF due to idiopathic dilated or ischemic cardiomyopathy than in control hearts.3 We showed that mast cells played a critical role in the progression of heart failure induced by pressure overload in mice.4

Clinical Perspective p 372

Mast cells are granulocytes known for their role in the pathogenesis of inflammatory diseases such as bronchial asthma, bacterial peritonitis,5,6 rheumatic diseases,7 and ulcerative colitis.8 They produce several cytokines, including interleukin (IL)-1, IL-3, IL-4, IL-5, IL-6, interferon-γ, and tumor necrosis factor-α, mediators that are central in the development of inflammatory reactions.9 Although they reside predominantly in tissues exposed to the outside environment, such as skin, intestinal tract, and trachea, mast cells are also normally present in the heart. They can be activated by several stimuli, including antibodies, cytokines, chemokines, and neuropeptides, eliciting a variety of responses, such as cell migration, the immediate release of inflammatory mediators, and selective cytokine production.10-12 Because they are widely distributed in the form of premature precursors, these multifunctional cells are poised to play a pivotal role in the immune system. To mature, mast cell precursors require stimulation by stem cell factor (SCF) via c-kit, a transmem-
brane receptor with intrinsic tyrosine kinase activity. The SCF–c-kit interaction stimulates them to migrate, proliferate, mature, and survive. SCF is expressed in a variety of tissue microenvironments, including the bone marrow, where mast cells normally begin their development. Therefore, the SCF–c-kit signal is key in the development of mast cell function.

The present study examined the role of mast cells in a mouse model of viral myocarditis using 2 strains of mast cell–deficient mice that have mutations in more upstream regulation, that is, SCF–c-kit mutations: (1) WBB6F1-Kit<sup>W/V</sup> (W/W<sup>V</sup>) mice, lacking the c-kit receptor, and (2) WCB6F1-Kit<sup>W/V</sup> (Sl/Sld) mice, lacking SCF. Furthermore, we reconstituted mast cells by 2 methods in these mutant mice and studied the myocarditis tissues by microscopy and by quantitative reverse transcriptase polymerase chain reaction (PCR) analysis.

**Methods**

### Animal Preparation

Genetically mast cell–deficient WBB6F1-Kit<sup>W/V</sup> (W/W<sup>V</sup>) mice, WCB6F1-Kit<sup>W/V</sup> (Sl/Sld) mice, and their congenic littermates were purchased from the Jackson Laboratory (Bar Harbor, Me.). Adult W/W<sup>V</sup> mice and Sl/Sld mice ordinarily contain 1.0% of the number of dermal mast cells present in the skin of congenic littermates and have no detectable mature mast cells in the heart or other anatomic sites.

### Experimental Myocarditis Model

Stocks of the myocardiotrophic variant of encephalomyocarditis virus (EMCV) were prepared as described previously and stored at −80°C. The 4-week-old male W/W<sup>V</sup> (n=13 for survival experiments, n=10 for histopathological experiments) and Sl/Sld mice (n=10) used in this study were treated in accordance with local institutional guidelines at all stages of the experiments. They were inoculated with 0.2 mL of EMCV intraperitoneally in phosphate-buffered saline diluted to a concentration of 50 plaque-forming units (pfu)/mL on day 0. The mice for survival experiments were observed daily for 14 days and were euthanized by cervical dislocation on day 7 for the histopathological experiments. The hearts were dissected, then 1 part was immediately frozen and stored at −80°C, and the other part was fixed in 10% formalin.

### Mast Cell Reconstitution in Sl/Sld Mice by Repair of the Deficient Ligand SCF

Twelve 6-week-old WCB6F1-Sl/Sld mice were treated daily with subcutaneous recombinant murine SCF (rmSCF) (30 μg/kg per day in 0.2 mL of sterile 0.9% NaCl containing 0.1% bovine serum albumin fraction V, fatty acid free) for 21 days by the slightly modified method of Zsebo et al<sup>17</sup> and Tsai et al<sup>18</sup>. As a control group, albumin fraction V, fatty acid free) for 21 days by the slightly modified method of Zsebo et al<sup>17</sup> and Tsai et al<sup>18</sup>. As a control group, 12 age-matched W/W<sup>V</sup> mice received the vehicle alone, consisting of 0.1% bovine serum albumin in 0.2 mL of Eagle’s medium intravenously. Mast cell–reconstituted W/W<sup>V</sup> mice were housed for 6 weeks; tissues from various organs were harvested from 2 treated mice in each group to confirm the reconstitution of mast cells. Inoculation with 10 pfu of EMCV was then performed in treated and control W/W<sup>V</sup> mice.

### Histopathological Examination

The hearts from surviving mice were harvested on day 7, fixed in 10% formalin, and embedded in paraffin. The left ventricles were sliced perpendicular to the long axis and stained with hematoxylin-eosin and Masson trichrome for light microscopic examinations. The extent of inflammatory cell infiltration and myocardial necrosis was evaluated by measuring the ratio (%) of inflammatory cell infiltration or myocardial necrosis area to total left ventricle area on a microscopic slide, with the use of the Scion Image program.

### Measurements of Viral Concentrations

Measurements of viral concentration in heart and brain harvested on day 7 were made by plaque assay methods as described previously. Each value represents the average of 2 experiments. Virus concentrations are expressed as log (pfu/g tissue).
Figure 2. Histological examination of EMCV myocarditis in W/Wv mice. A and B, Hematoxylin-eosin stain. C and D, Masson trichrome stain. Inflammatory cell infiltration and myocardial necrosis were less pronounced than in control littermates (B and D). E and F, Areas of inflammatory cell infiltration and myocardial necrosis in W/Wv mice (A and C) were less pronounced than in control littermates (4.3% vs 21.2% and 23.9% vs 12.1%, respectively; P=0.003). Values represent mean±SEM; n=10 in each group. WT indicates wild-type.

Quantitative Reverse Transcriptase Polymerase Chain Reaction Analysis

Total RNA was isolated from the left ventricles by the acid guanidinium thiocyanate-phenol-chloroform method, and the RNA concentration was measured spectrophotometrically. First-strand cDNA was synthesized with the use of the SUPERSCRIPT Premplification System for FirstStrand cDNA Synthesis (Gibco BRL). Real-time quantitative PCR (TaqMan PCR) with an ABI Prism 7700 Sequence Detection System and TaqMan PCR Core Reagent Kit (Perkin-Elmer Corp, Foster City, Calif) was performed according to the manufacturer’s protocol. We used 2 mL of the first-strand cDNA in the following assay. The following forward (F) and reverse (R) oligonucleotides and probes (P) were used for the quantification of mouse mast cell protease (mMCP)-4, mMCP-5, matrix metalloproteinase-9 (MMP-9), IL-6, and glyceraldehydes-3-phosphate dehydrogenase mRNA: mMCP-4 F, 5'-GAAGTGAAGCCCTGACCTGC-3'; mMCP-4 R, 5'-CATGCTTTGTTTGACCCCA AAG-3'; mMCP-5 R, 5'-TTGGCACCCTGTTAGAAA-3'; mMCP-5 F, 5'-AAGGCC-3'; GAPDH F, 5'-GGCATGGACTGTGGTCATGA-3'; GAPDH R, 5'-AAGTCATGGACTGTGGTCATGA-3'; GAPDH P, 5'-TGCATCTGACCACCAACTGCTTAG-3'.

The conditions for the TaqMan PCR were as follows: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Measurement of Active MMP-9 Activity in the Reconstituted W/Wv Mice Experiment

We measured active MMP-9 of the reconstituted W/Wv mice model using the Biotrak MMP-9 Activity Assay System from Amersham Biosciences (Piscataway, NJ). This assay is based on a 2-site enzyme-linked immunosorbent assay sandwich. Standards and samples are incubated in microtiter wells precoated with anti-MMP-9 antibody. Any MMP-9 present will be bound to the wells, whereas other components of the sample are removed by washing and aspiration. The total levels of free MMP-9 in a sample can be detected. To measure the total MMP-9 content, any bound MMP-9 in the sample is determined by interpolation from a standard curve.
Effect of Oral Histamine H1-Receptor Antagonist Bepotastine on EMCV Myocarditis in Mice

The histamine H1-receptor antagonist bepotastine besilate was obtained from Tanabe Seiyaku Co, Ltd, Osaka, Japan. The 4-week-old male DBA/2 mice were inoculated with EMCV as previously described. Bepotastine was dissolved in distilled water and given orally a dose of 10 mg/kg per day. Control mice were given distilled water. Survival (for 14 days, n=24 for each group) and histopathological changes on day 7 (n=12 for bepotastine group and n=8 for control) were examined as described previously.

Statistical Analysis

The survival rate of mice was analyzed by the Kaplan-Meier method, and survival differences between groups were tested by the log-rank test. Statistical comparisons of histological area and IL-6 were made by the unpaired 2-tailed Student t test. Comparisons of red blood cells and hemoglobin were made by the paired 2-tailed Student t test. Multiple comparisons among 3 groups were made by 1-way ANOVA and Newman-Keuls test for post hoc analysis. All values are presented as mean±SEM. Differences were considered statistically significant at probability values <0.05.

Figure 4. A, Mast cells in Sl/Sld mice reconstituted by rmSCF. Mast cells in dermis, tongue, and heart were detected by the metachromasia-positive granules (arrows). In control Sl/Sld mice (b), mast cells are absent. In Sl/Sld mice treated with rmSCF (c), mast cells are present, although their density is lower than in control littermates (a). B, Blood cell count in mast cell-reconstituted Sl/Sld mice. The red blood cell (RBC) count (a) and hemoglobin (Hb) (b) confirm a significant recovery from anemia with rmSCF. WT indicates wild-type.
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Murine Myocarditis in 2 Strains of Mast Cell–Deficient Mice

We inoculated W/W^v^ mice and Sl/Sld^d^ mice and their control littersates with 10 pfu of EMCV intraperitoneally. The survival rate of W/W^v^ mice on day 14 after inoculation (77%; P=0.03; n=13; Figure 1). On histological examination on day 7, myocardial necrosis and inflammatory cell infiltration were significantly less pronounced in both W/W^v^ (Figure 2A to 2F) and Sl/Sld^d^ mice (Figure 3A to 3F) than in their respective control littersmates.

Reconstitution of Mast Cells in Sl/Sld^d^ Mice by Treatment With SCF

We reconstituted the mast cells in Sl/Sld^d^ mice by daily subcutaneous injections of rmSCF (30 μg/kg per day) for 21 days. At 4 weeks after the initial injection, we euthanized the mice and examined the distribution of mast cells in all tissues. We showed mast cells in skin, tongue, and myocardium (Figure 4A, a through c). Although the density of mast cells was low compared with that found in control littersmates, we confirmed that they were distinctly reconstituted by the injections of rmSCF. Furthermore, examinations of the blood cell count showed a mean hemoglobin at 11.1±1.1 g/dL, in Sl/Sld^d^ mice treated with rmSCF versus 9.2±1.5 g/dL, in mice treated with the vehicle only (P=0.002; n=10 in each group; Figure 4B, b), consistent with a marked recovery from congenital macrocytic anemia from treatment with rmSCF.

We then inoculated both the treated Sl/Sld^d^ and the untreated Sl/Sld^d^ mice with 10 pfu of EMCV intraperitoneally. On day 7, myocardial necrosis and inflammatory cell infiltration were significantly more pronounced in reconstituted mast cells than in the untreated Sl/Sld^d^ mice (Figure 5A through 5F).

Transplantation of Bone Marrow–Derived Mast Cells to W/W^v^ Mice

We reconstituted the mast cells in W/W^v^ mice by transplanting bone marrow–derived mast cells (BMMC^c-kit^). Bone marrow cells harvested from both femurs of 6-week-old male white-type mice were left for 4 weeks in WEHI-3 medium to become mast cell progenitors. We verified by Alcian blue staining that >95% of cultured cells were mast cell progenitors (Figure 6A, a and b). No stem cell–like cells were detected in the preparation. Although mast cell progenitors contain acid polysaccharides that are dyed blue with Alcian blue, stem cell–like cells do not contain them. We could not confirm a significant change in the blood cell count before and after transplantation (hemoglobin before versus after transplantation, 12.8±0.7 versus 12.0±0.8 g/dL; P=NS; n=10 in each group). Mast cell progenitors were intravenously injected into 6-week-old W/W^v^ male mice, which were followed for 6 weeks. Microscopic confirmation of the whole body distribution of mast cells requires ≥26 weeks of observation.18 However, in our model of EMCV myocarditis, mice aged >12 weeks do not reliably develop myocarditis. Therefore, after a shorter period (M1), we inoculated with 10 pfu of EMCV intraperitoneally the mast cell–reconstituted and the untreated W/W^v^ mice. On histological examination on day 7, myocardial necrosis and cellular infiltration were significantly more pronounced in the mast cell–reconstituted than in the untreated W/W^v^ mice (Figure 6B, a through f).

Measurements of Viral Concentrations

On day 7, no significant difference was found in the mean myocardial viral concentration between Sl/Sld^d^ mice and their control littersmates (6.97±0.39 log [pfu]/g versus 7.17±0.43 log [pfu]/g; P=NS; n=10). Similar results were confirmed in the 2 reconstituted models (7.09±0.15 log [pfu]/g in Sl/Sld^d^ mice treated with rmSCF versus 6.99±0.24 log [pfu]/g in control mice; P=NS; n=10; 7.17±0.07 log [pfu]/g in mast cell–reconstituted W/W^v^ mice versus 7.18±0.04 log [pfu]/g in control W/W^v^ mice: P=NS; n=10). In addition, no significant difference was found in the mean brain viral concentration between W/W^v^ mice and control littersmates (3.59±0.54 log [pfu]/g versus 2.94±0.56 log [pfu]/g; P=NS; n=5). Mast cell did not have an effect on intramyocardial viral concentrations.
Gene Expressions of Mouse Mast Cell Protease-4 and -5 and MMP-9 in the Heart of the Selective Mast Cell Reconstitution Model With Viral Myocarditis

No significant difference at baseline in gene expressions of mMCP-4 and -5 could be observed between uninfected and infected W/WV mice (Figure 7A, a and b). In contrast, these gene expressions were significantly higher in the reconstituted than in the nonreconstituted W/WV mice (Figure 7A, a and b). This confirmed that the mast cell reconstitution by BMMC c-kit/H11001 transplantation was successful and that the reconstituted mast cells participated prominently in the pathological process of viral myocarditis. Similar results were confirmed in the assessment of MMP-9 gene expression (Figure 7A, c) and in the measurements of active MMP-9 (Figure 7A, d). Finally, in this mast cell–reconstituted model, the gene expressions of mMCP-4 and -5 and MMP-9 were highly correlated (mMCP-4/MMP-9, \( r^2 = 0.9027, P < 0.0001 \); mMCP-5/MMP-9, \( r^2 = 0.8665, P < 0.0001 \); Figure 7B, a and b).

Cytokine Gene Expression in Myocardial Tissue

The gene expression of the proinflammatory cytokine IL-6 was significantly lower in SI/SI² mice than in the control group (Figure 7C). In the model of mast cell reconstitution, it was significantly higher in mast cell–reconstituted W/WV than in control W/WV mice (Figure 7C).

Effect of Bepotastine on EMCV Myocarditis

Bepotastine improved survival and improved histopathological changes (Figure 8).

Discussion

In the study by El-Koraie et al., mast cells and their associated growth factor (SCF) and receptor (c-kit) were present within the interstitium of scarred human kidneys and played a role in the initiation and progression of renal interstitial fibrosis. Frangogiannis et al. reported an increase in the number of mast cells in areas of collagen deposition and proliferating cell nuclear antigen expression and concluded that mast cells played an important role in myocardial remodeling and fibrosis after myocardial ischemia. Miyamoto
Figure 7. A, Reverse transcriptase PCR analysis in mast cell–reconstituted W/WV mice. No significant difference was found in the gene expression of mMCP-4 and -5 between uninfected and infected W/WV mice (mMCP4/GAPDH [10⁻³], 0.5±0.2 vs 3.8±2.0; P=NS; mMCP-5/GAPDH [10⁻³], 0.6±0.2 vs 3.3±2.0; P=NS) (a and b). The gene expressions of mMCP-4 and -5 were significantly greater in infected reconstituted than in infected nonreconstituted W/WV mice (mMCP4/GAPDH [10⁻³], 13.0±10.0 vs 3.8±2.0; P=0.01; mMCP-5/GAPDH [10⁻³], 10.4±8.7 vs 3.3±2.0; P<0.05) (a and b). A similar result was observed in the gene expression of MMP-9 (noninfected W/WV mice vs infected nonreconstituted W/WV mice vs infected reconstituted W/WV mice; MMP-9/GAPDH [10⁻³], 0.065±0.26 vs 1.68±0.86 vs 6.64±5.01; P<0.05; n=10) (c) and its activity (activity of MMP-9 [ng/mL], 0.37±0.17 vs 1.51±0.33 vs 5.81±1.11; P<0.001; n=10) (d). B, Correlations between gene expression of mMCP-4 and -5 and MMP-9 in the reconstituted W/WV mice. The gene expression of mMCP-4 and -5 and that of MMP-9 were highly correlated (mMCP-4/MMP-9, r²=0.9027, P<0.0001; mMCP-5/MMP-9, r²=0.8665, P<0.0001) (a and b). C, Gene expression of the proinflammatory cytokine IL-6. It was significantly lower in mast cell–deficient mice (Sl/Sl d) than in control mice (IL-6/GAPDH [10⁻³], 0.6±0.5 vs 1.2±0.9; P=0.048; n=10) (a). On the other hand, it was significantly higher in mast cell–reconstituted W/WV mice than in control W/WV mice (IL-6/GAPDH [10⁻³], 1.6±1.5 vs 0.5±0.2; P<0.05; n=10) (b).
et al.24 observed that SCF mRNA was expressed in vitro and in vivo in human aortic endothelial and smooth muscle cells. This ability of endothelial and smooth muscle cells to interact with mast cells via SCF–c-kit receptor binding suggests that it plays a role in the metabolism of the arterial wall because SCF may be a most important factor influencing mast cell number, phenotype, and function in both health and disease. Kovanen et al.25 noted the local accumulation of mast cells in vivo in human aortic endothelial and smooth muscle cells. This ability of endothelial and smooth muscle cells to interact with mast cells via SCF–c-kit receptor binding suggests that SCF was a ligand for c-kit. Although macrocytic anemia were mitigated by active treatment beginning in the acute stage of murine viral myocarditis and rose further in the subacute phase of CHF.27 In end-stage dilated cardiomyopathy, the ventricular content of MMP-3 and -9 increases, whereas that of MMP-2 remains unchanged compared with controls.27 In cardiac remodeling, degradation and synthesis of the extracellular matrix occur simultaneously, resulting in ventricular chamber dilation. Mast cell chymase and tryptase, implicated in the degradation and synthesis of extracellular matrix, activate other MMPs including MMP-9.28 Thus, the products of activated mast cells provide alternative MMP activation pathways.29

We have reported that the gene expression of mast cell chymase and tryptase was upregulated in the acute phase of viral myocarditis and rose further in the subacute phase of CHF.26 This activation coincided with the development of myocardial necrosis and fibrosis and correlated with the upregulation of MMP-9 and type-I procollagen, suggesting that mast cell chymase and tryptase participate in the acute inflammatory reaction as well as the remodeling process associated with acute viral myocarditis.

The gene expression and activity of MMP-9 in mast cell–deficient mice was not significantly increased in the acute stage (day 7), although it was higher in mast cell–reconstituted W/WV mice. This indicates that cardiac remodeling began in the acute stage of murine viral myocarditis and that mast cells and mast cell proteases may participate in the pathology of viral myocarditis.

Evidence is growing that proinflammatory cytokines play an important role in modulating cardiovascular function and structure.30–32 Arteriovenous IL-6 spillover in the peripheral circulation increases with the severity of CHF, and an elevated level of plasma IL-6 was a predictor of mortality in

A multidisciplinary treatment to support the circulatory system in the acute stage of myocarditis is of critical importance, whereas, in the chronic stage, therapy should aim to prevent dilation of the cardiac chambers by limiting the development of cardiac fibrosis. MMPs, the endogenous system of extracellular matrix degradation and remodeling, are activated with the development of CHF.27 In end-stage dilated cardiomyopathy, the ventricular content of MMP-3 and -9 increases, whereas that of MMP-2 remains unchanged compared with controls.27 In cardiac remodeling, degradation and synthesis of the extracellular matrix occur simultaneously, resulting in ventricular chamber dilation. Mast cell chymase and tryptase, implicated in the degradation and synthesis of extracellular matrix, activate other MMPs including MMP-9.28 Thus, the products of activated mast cells provide alternative MMP activation pathways.29

We have reported that the gene expression of mast cell chymase and tryptase was upregulated in the acute phase of viral myocarditis and rose further in the subacute phase of CHF.26 This activation coincided with the development of myocardial necrosis and fibrosis and correlated with the upregulation of MMP-9 and type-I procollagen, suggesting that mast cell chymase and tryptase participate in the acute inflammatory reaction as well as the remodeling process associated with acute viral myocarditis.

The gene expression and activity of MMP-9 in mast cell–deficient mice was not significantly increased in the acute stage (day 7), although it was higher in mast cell–reconstituted W/WV mice. This indicates that cardiac remodeling began in the acute stage of murine viral myocarditis and that mast cells and mast cell proteases may participate in the pathology of viral myocarditis.

Evidence is growing that proinflammatory cytokines play an important role in modulating cardiovascular function and structure.30–32 Arteriovenous IL-6 spillover in the peripheral circulation increases with the severity of CHF, and an elevated level of plasma IL-6 was a predictor of mortality in

![Figure 8. Effect of histamine H1-receptor antagonist bepotastine on EMVC myocarditis. Bepotastine significantly improved survival (P=0.009) (A) and decreased cellular infiltration (8.3±3.1 vs 17.6±3.0; P=0.0003) (B) and myocardial necrosis (17.1±2.3 vs 11.0±1.4; P=0.029) (C).](http://circ.ahajournals.org/)

In the reconstitution model by subcutaneous administration of rmSCF to SI/SI14 mice, both mast cell deficiency and macrocytic anemia were mitigated by active treatment because SCF was a ligand for c-kit. Although macrocytic anemia was mitigated by the reconstitution process, the histopathological severity of the lesions was greater in the reconstituted than in the nonreconstituted SI/SI14 mice. This observation strongly suggests the participation of mast cells in viral myocarditis. In the second mast cell reconstitution method by BMMC–c-kit+ transplantation from wild-type mice, the histopathological changes were, once again, more severe in the reconstituted than in the control W/WV mice, further supporting the deleterious effects exerted by mast cells in viral myocarditis.

**Figure 8. Effect of histamine H1-receptor antagonist bepotastine on EMVC myocarditis. Bepotastine significantly improved survival (P=0.009) (A) and decreased cellular infiltration (8.3±3.1 vs 17.6±3.0; P=0.0003) (B) and myocardial necrosis (17.1±2.3 vs 11.0±1.4; P=0.029) (C).**
patients with CHF. In the present study, the gene expression of IL-6 by the myocardial tissue was significantly increased in the mast cell–reconstituted W/Wm mice.

On the basis of our present results and numerous reports in the literature, we formed a hypothesis that mast cells are triggered in viral myocarditis to promote myocardial remodeling, contributing to the pathogenesis leading to CHF. Although mast cells are bone marrow–derived hematopoietic cells, committed mast cell progenitors circulate in small numbers in blood and are thought to migrate to the heart tissue before undergoing the final stage of maturation, including the development of mature granules. Mast cell progenitors can change their characteristics depending on their location and the surrounding environment, for example, depending on the site of inflammation. One of the most important features of mast cells is where they reside long term, that is, in close association with blood vessels at the site that is most likely to be exposed to pathogens.

Mast cells can detect and respond to pathogens depending on a combination of direct mechanisms including toll-like receptor–mediated activations and indirect mechanisms including Fc receptor–mediated or complement receptor–mediated activation. In the heart tissue of our viral myocarditis model, activated mast cells release many proinflammatory cytokines such as IL-6 and tumor necrosis factor, mediators forming extracellular matrix such as MMPs, and fibrogenic mediators such as chymase and tryptase. Furthermore, these fibrogenic factors increase fibroblasts in the site of myocarditis and are suspected to produce SCF. These SCF can mature and differentiate more mast cell precursors in the heart. Thus, mast cells play a critical role in the pathogenesis of viral myocarditis.

No treatment, aside from circulatory support, currently exists for severe acute viral myocarditis. The functions of mast cells can be controlled by anti-asthmatic or antichemistry mediator drugs. In fact, a histamine H1-receptor antagonist improved EMCV myocarditis. Our study therefore offers hope that the control of mast cells, for example, the interaction between SCF and c-kit, or the control of mast cell proteases may be effective in the management of viral myocarditis and subsequent CHF.

Acknowledgments

We would like to thank M. Ozone, M. Hayashi, and M. Shimada for preparing the manuscript.

Sources of Funding

This work was supported in part by a research grant from the Japanese Ministry of Health, Labor, and Welfare and a grant-in-aid for general scientific research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Mast cells are multifunctional cells that contain various mediators such as cytokines, histamine, proteases, and leukotrienes. They are found in nearly all major organs of the body and are involved in many types of inflammation as well as allergic inflammation. Recently, we showed that the gene expressions of the mast cells chymase and tryptase were increased in the acute stages of heart failure and viral myocarditis, suggesting that viral infection may also activate mast cells. In the present study, survival of mice was better in mast cell–deficient mice infected with encephalomyocarditis virus and in association with less-pronounced myocardial necrosis, inflammation, and gene expressions of proinflammatory cytokines. Of note, all of these reactions were restored in mast cell–reconstituted mice. A histamine H1-receptor antagonist also alleviated viral myocarditis. These observations suggest that mast cells participate in the acute inflammatory reaction and the onset of ventricular remodeling associated with acute viral myocarditis and that the inhibition of their function may be therapeutic in this disease.
Mast Cells Play a Critical Role in the Pathogenesis of Viral Myocarditis
Hirokazu Higuchi, Masatake Hara, Kanjo Yamamoto, Tadashi Miyamoto, Makoto Kinoshita, Tasuku Yamada, Koji Uchiyama and Akira Matsumori

Circulation. 2008;118:363-372; originally published online July 7, 2008;
doi: 10.1161/CIRCULATIONAHA.107.741595
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/118/4/363

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/