Influence of Mast Cells on Structural and Functional Manifestations of Radiation-Induced Heart Disease

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Abstract

Radiation-induced heart disease (RIHD), characterized by accelerated atherosclerosis and adverse tissue remodeling, is a serious sequela after radiotherapy of thoracic and chest wall tumors. Adverse cardiac remodeling in RIHD and other cardiac disorders is frequently accompanied by mast cell hyperplasia, suggesting that mast cells may affect the development of cardiac fibrosis. This study used a mast cell–deficient rat model to define the role of mast cells in RIHD. Mast cell–deficient rats (Ws/Ws) and mast cell–competent littermate controls (+/+ or +/−) were exposed to 18 Gy localized single-dose irradiation of the heart. Six months after irradiation, cardiac function was examined by echocardiography and Langendorff-perfused isolated heart preparation, whereas structural changes were assessed using quantitative histology and immunohistochemical analysis. Mast cell–deficient rats exhibited more severe postradiation changes than mast cell–competent littermates. Hence, mast cell–deficient rats exhibited a greater upward/leftward shift in the left ventricular (LV) diastolic pressure-volume relationship ($P = 0.001$), a greater reduction in in vivo LV diastolic area (from 0.50 ± 0.02 cm in age-matched controls to 0.24 ± 0.03 cm after irradiation; $P = 0.006$), and a greater increase in LV posterior wall thickness (from 0.13 ± 0.00 cm in age-matched controls to 0.15 ± 0.00 cm after irradiation; $P = 0.04$). Structural analysis revealed more pronounced postradiation accumulation of interstitial collagen III but less myocardial degeneration in hearts from mast cell–deficient rats. These data show that the absence of mast cells accelerates the development of functional changes in the irradiated heart, particularly diastolic dysfunction, and suggest that, in contrast to what has been the prevailing assumption, the role of mast cells in RIHD is predominantly protective. (Cancer Res 2005; 65(8): 3100-7)

Introduction

Radiation-induced heart disease (RIHD) is an underdiagnosed but potentially lethal side effect of radiation therapy (1). The manifestations of RIHD usually become clinically apparent several years after irradiation and may include chronic pericarditis, accelerated atherosclerosis, conduction abnormalities, valvular changes, and, notably, pericardial and myocardial fibrosis. The symptoms and signs of RIHD are, for the most part, indistinguishable from those encountered in patients with heart disease of other etiologies. However, clinical trials and epidemiologic studies show the adverse impact of RIHD on the outcome of long-term cancer survivors. For example, radiation therapy for breast cancer may reduce the risk of recurrent cancer, but this benefit may be offset by a >2-fold increase in cardiac mortality compared with comparable breast cancer patients who have not received radiation therapy (2, 3). Survivors of childhood cancers are at particular risk of treatment-induced cardiac disorders, with 5% to 10% of patients suffering from severe chemotheraphy-induced or radiation-induced cardiotoxicity (4, 5). The fact that cardiac abnormalities develop in 50% to 75% of survivors of Hodgkin lymphoma (6) and cardiovascular disease is the leading cause of death in these patients (7) also illustrates the clinical significance of RIHD. Technical advances in radiation treatment planning and delivery may lead to a reduction of the incidence of RIHD in patients with tumors in the breast and mediastinum. On the other hand, the same technical advances may also lead to increased long-term survival, and thus increased incidence of RIHD, in the large cohort of patients with lung cancer.

Several lines of evidence support a role for mast cells in cardiac remodeling. First, mast cell hyperplasia is a common feature in human conditions associated with coronary atherosclerosis and myocardial fibrosis (8, 9) and in many animal models of heart disease (10), including RIHD (11, 12). Second, pharmacologic modulation of mast cell function in animals is consistent with a role for mast cells in cardiac tissue remodeling (13, 14). Third, mast cells contain a plethora of profibrogenic and antifibrogenic mediators that regulate tissue remodeling in various physiologic and disease states, including radiation-induced tissue remodeling.

Although mast cells regulate fibrosis development in some other organs, including irradiated intestine (15, 16), direct evidence supporting a role for mast cells in RIHD is lacking. The rat model used here is, for all practical purposes, completely devoid in cardiac organs, including heart, from other significant phenotypic alterations and thus provides the opportunity to directly examine the role of mast cells in the development of structural and functional changes in the heart in response to ionizing radiation. Surprisingly, postradiation cardiac dysfunction and structural evidence of remodeling was strikingly exacerbated in mast cell–deficient rats compared with mast cell–competent controls. These results refute the prevailing notion that mast cells have an adverse impact on the cardiac radiation response. The data also point to a need to reassess the role of mast cells in other cardiac disorders characterized by adverse remodeling and a need for studies addressing specific mast cell mediators in terms of their influence on the cardiac radiation response and their potential for therapeutic modulation aimed at reducing the incidence of RIHD in patients.

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Table 1. Heart and body weights of mast cell–deficient and mast cell–competent rats at 6 months after irradiation or control treatment (n = 4-5/group)

<table>
<thead>
<tr>
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<th>Nonirradiated</th>
<th>Irradiated</th>
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<tr>
<td></td>
<td>Deficient</td>
<td>Competent</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>434 ± 36</td>
<td>440 ± 32</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>1,323 ± 63</td>
<td>1,292 ± 64</td>
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<tr>
<td>Heart/body ratio (mg/g)</td>
<td>3.08 ± 0.16</td>
<td>2.96 ± 0.12</td>
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NOTE: There was no statistically significant difference in body weight, heart weight, and heart/body weight ratio between nonirradiated mast cell–deficient and nonirradiated mast cell–competent rats. Similarly, although there was a trend toward lower values after irradiation, the effect of irradiation on these gross variables was not significant.

Materials and Methods

Animal model and radiation. All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Male mast cell–deficient (Ws/Ws) rats and mast cell–competent littermates (+/+) were obtained from Japan SLC (Hamamatsu, Japan) and maintained in our Division of Laboratory Medicine on a 12:12-hour light/dark cycle with free access to food and water. At the age of 3 months, animals were anesthetized with isoflurane. Local heart irradiation was done according to a method described previously (17, 18). Rats were irradiated with a Seifert Isovolt 320 X-ray machine (Seifert X-Ray Corp., Fairview Village, PA) operated at 250 kV and 15 mA with 3 mm aluminum and 1.85 mm copper added filtration, at a dose rate of 1.17 Gy/min. A single dose of 18 Gy was given locally on the heart using parallel opposed fields (anterior/posterior 1:1) with a 19 mm diameter, while the rest of the animal was shielded with lead plates. A single dose of 18 Gy to the heart corresponds to the effective dose of a fractionated irradiation schedule commonly used in patients (30 × 2 Gy).

In vivo echocardiography. Echocardiographic images of the rat hearts were obtained using the HP Sonos 5500 system (Philips Medical Systems, Eindhoven, the Netherlands) with a S12 probe (5-12 MHz), while the animals were lightly anesthetized with a volatile agent. Fractional area change (FAC) was calculated as: [left ventricular (LV) diastolic area − LV systolic area] / LV diastolic area.

Ex vivo Langendorff-perfused hearts. Hearts were isolated from rats in each of the four groups and perfused via the aorta with an oxygenated Krebs-Henseleit solution (37°C) of the following composition: 118.0 mmol/L NaCl, 27.1 mmol/L NaHCO₃, 3.7 mmol/L KCl, 1.8 mmol/L CaCl₂, 1.2 mmol/L MgCl₂, 1.0 mmol/L KH₂PO₄, and 11.1 mmol/L glucose. The flow rate was set at 9.0 mL/g heart/min, a value similar to that observed when flow is examined at a constant pressure of 80 mm Hg; coronary pressure was monitored continuously by a Statham pressure transducer. Both atria were removed, and the ventricles were paced electrically at 250 bpm by platinum contact electrodes positioned on the interventricular septum. A fluid-filled balloon catheter (connected to a pressure transducer) was placed in the LV to measure intraventricular pressure, and the heart was enclosed in a humidified, temperature-controlled chamber. Cardiac function was monitored by measuring diastolic pressure, peak pressure, +dP/dt max, −dP/dt max at various preload balloon volumes (20-500 μL, a range that elicited maximum contractility in all preparations). In addition to a polygraph recording, all data were digitized and analyzed with the use of acquisition and analysis software (CODAS, DataQ Instruments, Akron, OH).

Histology. For histologic examination, hearts were fixed in methanol Carnoy’s solution and engrossed to provide three transverse sections called level 1 (near the base of the heart), level 2 (at the equator of the heart), and level 3 (near the apex of the heart). Five μm sections were stained with standard Masson trichrome. Sections from each of the three levels were scored for myocardial degeneration (myocardial necrosis accompanied by inflammation) by two independent observers (J.W. and M.R.) using a graded scale between 0 and 3, with 0 representing no degeneration and 3 representing area of degeneration >0.15 mm². Scores of the two observers were averaged.

For determination of mast cell numbers, 5 μm sections were stained with 0.5% toluidine blue in 0.5 N HCl for 20 minutes followed by a 10-minute incubation in 0.7 N HCl.

Immunohistochemistry and computed image analysis. Quantitative immunohistochemistry was used to determine collagen I and III. This method has been extensively used and validated in our laboratory (19, 20). Sections were incubated with goat anti–collagen I and goat anti–collagen III (Southern Biotechnology Associates, Birmingham, AL) in a 1:100 dilution at 20°C for 2 hours. Binding of biotin-labeled rabbit anti–goat IgG (Vector Laboratories, Burlingame, CA) was visualized with a standard avidin-biotin-peroxidase complex staining technique (Vector Laboratories). Computerized image analysis with the ImagePro Plus software (Media Cybernetics, Silver Spring, MD) was used to determine areas of collagen I and III. Intersitial collagen I and III areas in each of the three levels of the LV were determined in 12 rectangular fields at a ×40 magnification, and collagen was expressed as area per 100 μm² LV. Perivascular collagen I and III areas were determined for arteries with a luminal diameter 10 to 200 μm (10-21 arteries per heart), with collagen being expressed as area per μm² area of the artery lumen.

Statistical analysis. Data were evaluated by one-way or two-way ANOVA as appropriate, with the Student’s-Newman-Keuls’ post hoc test, using Sigmastat (SPSS, Inc., Chicago, IL) and NCSS 2000 (NCSS, Kaysville, UT). Degeneration scores were evaluated with a nonparametric two-sample test, stratified on level in the heart (1, 2, or 3). The criterion for significance was P < 0.05. Data are reported as means ± SE.

Results

Animal model. No mast cells were observed in nonirradiated and irradiated Ws/Ws rat hearts after toluidine blue staining. In nonirradiated +/+ hearts, the average mast cell density was 0.75 ± 0.25 per mm². After irradiation, the mast cell density increased to 2.30 ± 0.44 per mm² (F = 9.26, ν = 1; P = 0.016).

Figure 1. Effects of balloon volume on LV diastolic pressure in Langendorff-perfused hearts isolated from mast cell–competent and mast cell–deficient rats. The curve is slightly shifted leftward/upward for nonirradiated mast cell–deficient hearts (●, n = 4) compared with nonirradiated mast cell–competent hearts (○, n = 4). Radiation induced a markedly greater leftward/upward shift in mast cell–deficient hearts (■, n = 5) compared with mast cell–competent hearts (○, n = 4).
As shown in Table 1, body weight, heart weight, and heart/body weight ratio did not differ between the two nonirradiated groups. After irradiation, values for body weight, heart weight, and heart/body weight ratio tended to be smaller in both rat types; however, this was not significant.

**Effects of irradiation on left ventricular diastolic function.** As shown in Fig. 1, radiation injury shifted the ex vivo diastolic pressure-volume relationship leftward/upward in both mast cell–competent and mast cell–deficient groups; however, the magnitude of this radiation-induced shift was much greater in mast cell–deficient compared with mast cell–competent rats. For example, diastolic pressures at a balloon volume of 300 μL were 17.2 ± 3.0, 42.4 ± 5.7, 34.8 ± 4.7, and 97.0 ± 16.7 mm Hg (F = 16.10, ν = 3; P = 0.001) in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively. In addition, as reported previously (21), it is interesting to note that the curve was shifted leftward/upward in mast cell–deficient control (i.e., nonirradiated) compared with mast cell–competent control hearts.

Radiation-associated changes in −dP/dt max were observed in mast cell–competent but not mast cell–deficient animals. As indicated in Fig. 2, maximum observed values for −dP/dt max were not significantly different among the four groups (1,654 ± 83, 1,786 ± 89, 1,872 ± 107, and 1,859 ± 110 mm Hg/s in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively). However, at balloon volumes between 100 and 180 μL, values for −dP/dt max were less in mast cell–competent nonirradiated hearts compared with the other three groups (e.g., at a balloon volume of 120 μL, values for −dP/dt max were 1,157 ± 103, 1,583 ± 74, 1,586 ± 147, and 1,567 ± 88 mm Hg/s in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively; F = 4.09, ν = 3; P = 0.032). Essentially, the slope of the curve was less in the mast cell–competent nonirradiated compared with the other groups.

**Effects of irradiation on left ventricular systolic function.** Maximum systolic function was not different among the four experimental groups. As shown in Fig. 3, maximum observed values for developed pressure (peak systolic-diastolic) were 104.5 ± 4.2, 117.2 ± 4.1, 109.2 ± 5.6, and 105.2 ± 6.4 mm Hg in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively. Values in the mast cell–deficient control group tended to be greater than those in the mast cell–competent control group; however, this was not statistically significant. Similar to −dP/dt max, developed pressure at intermediate balloon volumes (100-140 μL) was smaller in the mast cell–competent nonirradiated compared with the other groups (e.g., at 120 μL, developed pressures were 73.4 ± 5.0, 100.1 ± 3.9, 92.3 ± 5.5, and 91.7 ± 6.5 mm Hg in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively; F = 3.61, ν = 3; P = 0.046).

Values for +dP/dt max (Fig. 4) showed similar differences in terms of systolic function. There were no significant differences in the maximum observed values for +dP/dt max (3,009 ± 165, 3,477 ± 210, 3,459 ± 184, and 3,235 ± 290 mm Hg/s in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively). However, values observed at intermediate balloon volumes (100–140 μL) were smaller in the mast cell–competent nonirradiated compared with the other groups (e.g., at 120 μL, +dP/dt max was 2,090 ± 201, 3,072 ± 80, 2,728 ± 192, and 3,004 ± 223 mm Hg/s in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively; F = 3.86, ν = 3; P = 0.038).

**Effects of irradiation on coronary pressure.** Coronary perfusion pressure in the Langendorff-perfused preparation was increased by radiation treatment in both mast cell–competent and mast cell–deficient hearts (56.5 ± 4.8, 62.4 ± 4.7, 80.2 ± 3.5, and 83.5 ± 8.2 mm Hg in mast cell–competent control, mast cell–deficient control, mast cell–competent irradiated, and mast cell–deficient irradiated hearts, respectively; F = 6.42, ν = 1; P = 0.042).

**Echocardiographic variables.** Table 2 shows echocardiographic variables in mast cell–deficient and mast cell–competent rats at 6 months after irradiation or control treatment. Irradiation induced a decrease in in vivo LV diastolic area (F = 84.41, ν = 1;
Effects of balloon volume on left intraventricular +dP/dtmax in Langendorff-perfused hearts isolated from mast cell–competent and mast cell–deficient hearts. At balloon volumes between 100 and 140 μL, +dP/dtmax was significantly less in nonirradiated mast cell–competent hearts (○, n = 4), irradiated mast cell–competent hearts (●, n = 4), and irradiated mast cell–deficient hearts (□, n = 5).

Figure 4.

P < 0.001) and LV systolic area (F = 84.09, v = 1; P < 0.001) and an increase in FAC (F = 29.17, v = 1; P < 0.001). Changes in LV dimensions were more extreme in mast cell–deficient compared with mast cell–competent rats (LV diastolic area: F = 8.55, v = 1; P = 0.006; LV systolic area: F = 6.23, v = 1; P = 0.017). Moreover, irradiation induced an increase in LV posterior wall diastolic thickness (F = 56.04, v = 1; P < 0.001) that was more severe in mast cell–deficient compared with mast cell–competent rats (F = 4.77, v = 1; P = 0.036).

Histology and collagen deposition. Histopathologic changes in irradiated hearts included myocardial degeneration (myocardial necrosis, accompanied by inflammation and fibrosis, as shown in Fig. 5A). No myocardial degeneration was observed in rat hearts after sham irradiation (Fig. 5B). Table 3 shows the average scores for degeneration in the LV as scored by two observers. Mast cell–deficient rats exhibited less radiation-induced myocardial degeneration than mast cell–competent rats. Hence, a nonparametric two-sample test, stratified on level in the heart (1, 2, or 3), showed significantly lower degeneration scores for irradiated mast cell–deficient compared with mast cell–competent hearts (P = 0.005).

Figure 6 shows the area of interstitial collagen I and III in sections at three different levels in irradiated hearts. Radiation induced a significant increase in areas of collagen I (F = 74.43, v = 1; P < 0.001) and collagen III (F = 369.84, v = 1; P < 0.001) in both types of hearts. Larger collagen III areas were determined across the three levels of irradiated mast cell–deficient hearts compared with mast cell–competent hearts after irradiation (F = 7.47, v = 1; P = 0.012). ANOVA did not show a significant difference in collagen I among all three levels. Univariate analysis, however, showed a borderline significant difference in collagen I between mast cell–deficient and mast cell–competent hearts at level I (F = 6.50, v = 1; P = 0.043). The lower collagen I deposition in mast cell–deficient hearts at level 1 likely reflects less replacement fibrosis, because less myocardial degeneration is found at this level.

Table 4 shows perivascular collagen I and III areas per lumen area of coronary arteries in irradiated and nonirradiated, mast cell–deficient and mast cell–competent rat hearts. Perivascular collagen III was lower in control mast cell–deficient hearts compared with control mast cell–competent hearts. This difference, however, was not significant. Irradiation induced a significant increase in perivascular collagen I (F = 30.99, v = 1; P < 0.001) and collagen III (F = 15.73, v = 1; P = 0.0011); however, there was no significant difference between the two types of rat.

Table 2. Echocardiographic variables in mast cell–deficient and mast cell–competent rats at 6 months after irradiation or control treatment (n = 10/group)

<table>
<thead>
<tr>
<th></th>
<th>Nonirradiated</th>
<th>Competent</th>
<th>Irradiated</th>
<th>Deficient</th>
<th>Competent</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diastolic area (cm²)</td>
<td>0.50 ± 0.024</td>
<td>0.53 ± 0.014</td>
<td>0.24 ± 0.032* (P = 0.006)</td>
<td>0.35 ± 0.022*</td>
<td></td>
</tr>
<tr>
<td>LV systolic area (cm²)</td>
<td>0.19 ± 0.011</td>
<td>0.21 ± 0.017</td>
<td>0.06 ± 0.012* (P = 0.017)</td>
<td>0.10 ± 0.013*</td>
<td></td>
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<tr>
<td>FAC</td>
<td>0.60 ± 0.018</td>
<td>0.60 ± 0.027</td>
<td>0.78 ± 0.019* (not significant)</td>
<td>0.70 ± 0.032*</td>
<td></td>
</tr>
<tr>
<td>LV posterior wall diastolic thickness (cm)</td>
<td>0.13 ± 0.003</td>
<td>0.12 ± 0.004</td>
<td>0.15 ± 0.003* (P = 0.036)</td>
<td>0.14 ± 0.003*</td>
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</table>

*P < 0.001, statistical significance for the differences between irradiated mast cell–deficient and mast cell–competent hearts. All differences between irradiated and nonirradiated rats were highly statistically significant.

Discussion

Mast cells, in addition to their well-known roles in allergy and hypersensitivity, have been implicated in various physiologic and pathologic conditions associated with extracellular matrix deposition, such as wound healing and radiation-induced fibrosis (16, 22). In the heart, mast cell hyperplasia coincides temporally and spatially with tissue remodeling in various disease states (e.g., myocardial infarction, heart transplant rejection, hyperhomocysteinemia, and RIHD; refs. 10, 11, 23, 24). Despite ample correlative evidence suggesting that mast cells are involved in the radiation response in various organs, to our knowledge, only one study has investigated radiation injury in mast cell–deficient rats (16). That study showed significant differences in intestinal mucosal injury and fibrosis between mast cell–deficient and mast cell–competent rats, thus supporting a role for mast cells in the regulation of the intestinal radiation response.

The rat model is well suited for studies of radiation-induced cardiac remodeling. Although the rat is less prone to atherosclerosis than humans, cardiac tissue remodeling in rats after local heart irradiation is similar to that observed in human myocardium several years after thoracic radiotherapy. The Ws/Ws rat is a...
powerful model to study the role of mast cells in tissue remodeling. Ws/Ws rats are homozygous for a 12-base deletion in the tyrosine kinase domain of the c-kit receptor gene (i.e., the so-called white-spotting locus). The c-kit receptor induces mast cell proliferation and differentiation when activated by stem cell factor. Homozygous Ws/Ws rats lack functional melanocytes, mast cells, and interstitial cells of Cajal (ICC) in the intestine (25, 26). In contrast to mast cell–deficient mice, adult mast cell–deficient rats are not anemic (27); thus, mast cell reconstitution is generally not considered necessary for validation.

Although the consequences of the c-kit mutation in the rat model are less than in the equivalent mouse model, there are nevertheless deficiencies other than mast cells that may potentially influence the cardiac radiation response. These include differences in c-kit-expressing vascular endothelial cells, a subset of natural killer cells, and possible differences in cardiac progenitor cells that may be involved in regeneration and repair of the myocardium (28, 29). Moreover, the defect in ICC may affect heart rate secondarily through vagal reflex mechanisms (30), although the absence of ICC in Ws/Ws rats is highly unlikely to be involved in development of radiation-induced structural and functional alterations that are the focus of this study.

Depletion of cardiac mast cells in Ws/Ws rats was confirmed by toluidine blue staining. In hearts from the +/+ rats, similar increases in mast cell density were seen after irradiation as reported previously in various other rat strains (11, 12). The present study clearly showed more pronounced functional changes and interstitial collagen III deposition in hearts from Ws/Ws rats than from +/+ controls, consistent with the notion that mast cells protect against radiation-induced cardiac structural and functional changes or, conversely, that the absence of mast cells accelerates adverse cardiac remodeling in RIHD. On the other hand, the observation that coronary perfusion pressure and perivascular collagen deposition increased to a similar extent in mast cell–deficient and mast cell–competent rats suggest that mast cells are not involved in the development of postradiation changes in cardiac vessels, at least not within the first 6 months after irradiation.

The definitive experiment to determine whether mast cells play a role in a particular response would be to reconstitute the mast cell population in mast cell–deficient animals. This has been accomplished in mast cell–deficient mice by injecting precursor cells from mast cell–competent bone marrow (31). In contrast, the original deletion in the c-kit receptor gene in the rat model was a spontaneous mutation, and Ws/Ws and +/+ rats are the F2 generation of inbred rat strains. Hence, long-term reconstitution with +/+ bone marrow is not feasible in the Ws/Ws mast cell–deficient rat model (32, 33), and reconstitution with injection of +/+ mast cells (34) is of short duration and not useful in studies focused on long-term disease processes, such as RIHD. Moreover, the role of mast cells in fibrotic processes in the mouse is different than in the rat and less comparable to the human situation, so data from mast cell–deficient mice would be unlikely to be directly comparable to and possibly less relevant than those from the present study. Studies to identify the role of specific mast cell mediators in our rat model of RIHD using pharmacologic inhibitors is another way to approach this problem and are currently under way in our laboratory.

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The present study clearly showed more pronounced functional changes and interstitial collagen III deposition in hearts from Ws/Ws rats than from +/+ controls, consistent with the notion that mast cells protect against radiation-induced cardiac structural and functional changes or, conversely, that the absence of mast cells accelerates adverse cardiac remodeling in RIHD. On the other hand, the observation that coronary perfusion pressure and perivascular collagen deposition increased to a similar extent in mast cell–deficient and mast cell–competent rats suggest that mast cells are not involved in the development of postradiation changes in cardiac vessels, at least not within the first 6 months after irradiation.

The results from the present study are in accordance with a report of renal fibrosis in mast cell–deficient rats, in which Ws/Ws rats exhibited more severe fibrosis after puromycin aminonucleoside injection than mast cell–competent littermates (15). In contrast, the majority of studies that have used mast cell degranulation inhibitors to investigate the role of mast cells in fibrosis show a reduction in the formation of fibrosis (35, 36).
Possible reasons for these apparently conflicting results include the possibility that mast cell degranulation inhibitors do not block constitutive synthesis of extragranular mast cell mediators and that degranulation inhibitors not only block the release of granule contents but also may preserve physiologic (putative beneficial) mast cell functions. These possibilities may be addressed as novel inhibitors of specific mast cell mediators become available.

It is interesting that myocardial degeneration was somewhat more pronounced in mast cell–competent hearts despite lesser changes in diastolic area, diastolic function, and collagen content. However, this observation is in accordance with in vitro studies showing that although mast cells promote apoptosis of cardiac myocytes they induce proliferation of other cell types present in the myocardium (37).

Clinical studies consistently show a decrease in LV diameter after mediastinal radiotherapy (6, 38) that is attributed to cardiomyopathy and/or myocardial fibrosis. The leftward/upward shift in the LV diastolic pressure-volume relationship of irradiated hearts in the present study might in part be explained by a similar decrease in LV diastolic diameter and increase in LV wall thickness. However, irradiated mast cell–competent hearts and nonirradiated mast cell–deficient hearts had similar diastolic pressure-volume curves but different LV diastolic diameters in vivo. In addition, nonirradiated mast cell–deficient hearts showed a shift in the diastolic pressure-volume curve compared with nonirradiated mast cell–competent hearts (21), but echocardiography variables did not differ between these hearts. Therefore, additional factors are likely involved in the mechanisms underlying the diastolic dysfunction observed in this study. The severe increase in the pressure-volume relationship in mast cell–deficient hearts after irradiation might result from changes in extracellular matrix composition. Indeed, in our study, irradiated mast cell–deficient hearts exhibited more collagen III accumulation than irradiated mast cell–competent hearts. The slightly lower collagen I near the base of irradiated Ws/Ws hearts than in irradiated +/+ hearts likely reflected a lower incidence of reparative fibrosis, because this zone also showed less myocardial degeneration.

The increase in echocardiographically determined FAC after irradiation seems inconsistent with the developed pressure-volume curves acquired ex vivo, because the latter suggests no major increase in systolic function after irradiation. However, because the in vivo LV diastolic area showed large changes after irradiation, FAC might not represent true systolic function in these hearts. In addition, neurohumoral and hemodynamic factors might affect in vivo but not ex vivo heart function.

| Table 4. Perivasculare collagen area (μm²) per lumen area (μm²) of coronary arteries in nonirradiated and irradiated mast cell–competent and mast cell–deficient rat hearts (n = 4-5/group) |
|-----------------------------------------------|----------------|----------------|---|
|                                               | Nonirradiated | Irradiated     |   |
|                                               |               |                |   |
|                                               | Deficient     | Competent      |   |
| Collagen I                                    | 2.33 ± 0.39   | 2.73 ± 0.29    |   |
| Collagen III                                  | 2.77 ± 0.25   | 3.41 ± 0.12    |   |
|                                               | 4.24 ± 0.37   | 4.48 ± 0.24    | <0.001* |
|                                               | 3.95 ± 0.35   | 4.35 ± 0.29    | 0.001*  |

*Statistical significance for the differences between irradiated and nonirradiated rats. Differences between mast cell–deficient and mast cell–competent rats were not statistically significant.
Mast cells express a wide variety of mediators that may contribute to the regulation of tissue remodeling. Some of these mediators are preformed and stored in granules ready for immediate release, whereas others are synthesized on demand (22). Possible explanations for how mast cells may affect connective tissue deposition and/or degradation include altered expression of procollagens and matrix metalloproteinases by cardiac fibroblasts (39, 40); formation of heparin and transforming growth factor-β; formation of chymases, which generate the profibrogenic hormone angiotensin II from its precursor angiotensin I (α chymases), activate transforming growth factor-β and endothelin (41), but may also lead to decreased levels of angiotensin II (β chymases; ref. 42); and release of tryptase, the major serine protease in mast cells that activates protease-activated receptor 2 on other cell types to induce proliferation and collagen production (43–45). Recent studies in our laboratory suggest that protease-activated receptor 2 may be important in the radiation response of normal tissues (20, 46). Further studies are needed to determine how the various mediators, alone and in concert, influence the cardiac radiation response.

Besides affecting the amount of collagen in the heart tissue, mast cells may also influence collagen cross-linking and thereby affect cardiac function and compliance (47, 48). Moreover, changes in the expression of myofilaments, especially titin, might play a role in cardiac function and myocardial compliance (49–51). The differing slopes of the $\frac{dP}{dV}$max–$\frac{dP}{dV}$max and developed pressure curves that were observed in Langendorff-perfused heart preparation and that cannot be fully explained at present may reflect differences in extracellular matrix and myofilaments. This is in accordance with a study of Yarom et al. (12), who reported a beneficial effect of captopril on radiation-induced fibrosis but no effect on heart function, suggesting that intramyofiber derangements may be involved in long-term functional changes in the heart after radiation. Future studies should examine collagen cross-linking as well as the deposition of extracellular matrix components other than collagen in these models.

In conclusion, the absence of mast cells accelerates the development of functional and structural changes in the irradiated heart. Thus, these data, in contrast to the prevailing assumption based on correlative studies, show a protective role for mast cells in experimental RIHD. More research is needed to delineate the roles of individual mast cell mediators in postradiation cardiac tissue remodeling and to develop strategies to specifically target mast cell mediators for the purpose of reducing the risk of RIHD in patients.

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