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Rat cardiac mast cell maturation and differentiation following acute ventricular volume overload

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Abstract. *Objective:* Cardiac mast cell numbers increase significantly within 12 h following the creation of an aortocaval (AV) fistula in rats and play a central role in mediating adverse left ventricular remodeling. We studied whether this increase was related to maturation of resident immature mast cells.
Methods: We measured percentages of immature and mature cardiac mast cells at 1, 2 and 7 days following AV-fistula or sham surgery and in non-surgical control rats using the alcian-blue safranin reaction.
Results: Relative to sham-operated and control rats, there was a significant shift from immature to a greater percentage of mature cardiac mast cells at 1 day and 2 days post-fistula that returned to a normal distribution by 7 days.
Conclusions: We conclude that the acute increase in mast cell density following volume overload is due to a paracrine response in the heart that stimulates the maturation and differentiation of resident immature cardiac mast cells.

Key words: Mast cell phenotype – Arteriovenous fistula – Nedocromil – Alcian-blue safranin reaction

Introduction

In the infrarenal aortocaval (AV) fistula model of sustained chronic volume overload in rats, a rapid and significant increase in the number of cardiac mast cells occurs within 12 h that is prevented in animals treated with the mast cell membrane-stabilizing drug nedocromil [1–4]. Cardiac mast cell numbers are also known to increase in response to mitral regurgitation and following myocardial ischemia and reperfusion in the dog [5–6]. However, the source of this increased cardiac mast cell density has not been determined. Resident immature mast cells have been reported to be present in both the heart and peritoneal cavity [7–10]. We therefore hypothesized that this rapid increase in the number of toluidine blue stained mast cells was due to the maturation and differentiation of resident immature mast cells in the myocardium. Accordingly, the objective of this study was to determine the percentages of immature and mature mast cells resident in the myocardium following imposition of a sustained volume overload on the heart. To this end, the method of Yong et al. [7, 9] and Combs et al. [8] was used to determine the percentages of immature and mature mast cells at 1, 2 and 7 days following creation of an AV fistula. The results demonstrate a significant shift from immature to a greater percentage of mature cardiac mast cells occurring within 1 day, that had subsequently returned to a normal distribution by 7 days post-fistula. We attribute this induction of maturation in cardiac mast cells to a localized paracrine response.

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Materials and methods

These studies conformed to the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the protocol was approved by the University's Institutional Animal Care and Use Committee.

Healthy adult male Sprague-Dawley (Hsd:SD) rats (275 ± 57 g) which were deeply anesthetized with sodium pentobarbital (50 mg/kg) administered by intraperitoneal injection underwent AV fistula or sham-surgery. Postoperative analgesia was provided by subcutaneous buprenorphine HCl (0.025 mg/kg) administered at the time of surgery. Within 24 h following surgery, all rats were alert and exhibited normal activity. At the experimental endpoint, hearts were removed under deep anesthesia using a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) administered by intraperitoneal injection.

Experimental Design

Rats were randomized to the experimental groups which included AV fistula and sham-operated animals at 1, 2 and 7 days post-surgery (n = 5 per group). These were compared to a non-surgical control group (n = 5) and an AV fistula and sham-operated group that were treated with the mast cell membrane stabilizing drug, nedocromil (n = 5 per group), and sacrificed at 1 day post-surgery. Finally, an additional group of AV fistula and sham-operated rats were injected with a marker for cell proliferation, 5-bromo-2'-deoxyuridine (BrdU; n = 3 per group), and sacrificed at 1 day post-surgery. Prior to excision of the heart, the patency of each fistula was confirmed by palpation of the surgical site.

Surgical Protocol

An infrarenal AV fistula was created as previously described [1, 11–12]: A ventral abdominal laparotomy was performed exposing the aorta and caudal vena cava below the renal arteries. Following blunt dissection, an 18 gauge needle was inserted into the abdominal aorta and advanced obliquely through the medial wall into the vena cava. The needle was withdrawn and the aortic puncture site sealed with cyanoacrylate. A successful fistula was evident by observation of the pulsatile flow of oxygenated blood into the vena cava. The abdominal musculature and skin incisions were closed with absorbable suture and auto clips, respectively. Sham-operated rats underwent a similar surgical procedure, including blunt dissection, but without needle puncture of the vessels.

Nedocromil Pellet Implantation

Five days prior to sham or AV fistula surgery, 21-day time-release pellets delivering nedocromil sodium (30 mg/kg/day) were implanted subcutaneously via a 0.5 cm incision between the scapulae. The skin incision was closed with surgical staples.

Isolation of Rat Cardiac Mast Cells

Cardiac mast cells were isolated following mechanical and enzymatic dispersion of the whole rat heart as previously described [13]. The entire heart was utilized for mast cell isolation to ensure a sufficient yield of cells for the assessment of maturation and differentiation. Briefly, hearts were extirpated, placed in Hank's Balanced Salt Solution (HBSS) and minced into 1 mm^3 fragments. These fragments were incubated in enzymatic dispersion buffer consisting of 160 units/mL of collagenase type II, 100 units/mL of hyaluronidase, 1 unit/mL protease, and 304 units/mL of deoxyribonuclease I at 37 °C for 30 (incubation 1), 15 (incubation 2), and 15 (incubation 3) minutes. At the end of each incubation period, the supernatant from the partially dispersed tissue was filtered through a 70-*u*m pore nylon mesh cell strainer, diluted with HBSS, and washed three times. Cell pellets were then resuspended in HBSS and an aliquot of the cell isolate was stained with toluidine blue for 20 min to determine the number of mast cells. The average number (± SEM) of fully stained cardiac mast cells was $17.0 \pm 2.4 \times 10^4$ cells/mL.

Isolation of Rat Peritoneal Mast Cells

Immediately following excision of the heart, 20 mL of HBSS was injected into the peritoneal cavity. The abdomen was gently massaged for approximately 3 min and the peritoneal fluid aspirated via a small midline incision. The aspirate was diluted with HBSS, filtered through a 70-*u*m pore nylon mesh cell strainer, and centrifuged (4 °C). The resulting cell pellets were resuspended in HBSS and an aliquot of cells was stained with toluidine blue for 20 min to determine the number of mast cells. The average number (± SEM) of fully stained peritoneal mast cells was $15.0 \pm 3.8 \times 10^6$ cells/mL.

Determining Mast Cell Maturation Stages

Aliquots of isolated cardiac and peritoneal mast cells were transferred onto glass slides by cytocentrifugation for 10 min at 250 rpm. The cells were then fixed in Newcomers Fixative after air-drying for 30 min [14]. After fixation and hydration in progressively lower percentages of alcohol, the slides were incubated in alcian blue at pH 1.0 for 3 h. The slides were then rinsed briefly in 0.1 N HCl, stained with safranin at pH 1.0 for 30 min, dehydrated in graded alcohols and xylene, and cover slipped. A total of 200 to 500 mast cells from each heart were assessed in a blinded fashion (oil immersion 1000 X) to determine the stage of mast cell maturation and differentiation as defined in Results. The average mast cell diameter was also determined using an eyepiece micrometer.

Determining Mast Cell Proliferation

To evaluate whether the expansion of the mast cell population in the heart was due to proliferation, rats were injected subcutaneously with BrdU (40 mg/kg) 6 h before and 6, 12 and 18 h following AV-fistula or sham surgery. The BrdU was dissolved in DMSO and diluted (1:6.5) in sesame oil. Cardiac and peritoneal mast cells were isolated as previously described. Aliquots of cardiac and peritoneal mast cells were transferred onto glass slides using a cytocentrifuge or stained for flow cytometric analysis.

Immunocytochemistry

Slides were fixed in 4 % paraformaldehyde, incubated in 2 N HCl to denature mast cell DNA, and stained with a monoclonal anti-BrdU antibody tagged with a FITC-labeled secondary antibody. Slides were then counterstained with 0.00125 % toluidine blue.

Flow Cytometric Analysis

Cell isolates were first stained with a phycoerythrin-conjugated CD117 antibody to stem cell factor receptor, then fixed in paraformaldehyde and stained with a flurochrome-conjugated antibody to BrdU for flow cytometric analysis [15].

Statistical Analysis

All data analyses were performed using SigmaStat. The Z-Test was used to compare differences between percentages of immature and mature mast cell stages obtained from AV-fistula and sham-operated groups. One-way Analysis of Variance was used to compare cardiac or peritoneal mast cell diameter for stages I to IV (see Results). The level of statistical significance was taken to be $p \leq 0.05$.

Results

Heart weights did not differ between groups when referenced to body weight. A patent fistula was confirmed to be present in all of the rats with an AV-fistula.

Toulidine Blue Staining

Figure 1 depicts freshly isolated, non-fixed cardiac mast cells following 20 min of incubation with acidic toulidine blue dye. Panels a and b of Figure 1 are representative of cardiac mast cells that stain incompletely (i. e., immature stages), whereas, panels c and d demonstrate full metachromatic staining (i. e., mature stages). This variable pattern of staining is indicative of multiple stages of mast cell maturity in the isolate. Similar differential staining was seen in peritoneal mast cell isolates (not shown).

Alcian-Blue Safranin Reaction

The stages of maturation and differentiation in mast cells following the alcian-blue and safranin reaction are depicted in Figure 2. We previously confirmed that rat cardiac mast cells can be classified as "Connective Tissue-type" mast cells by

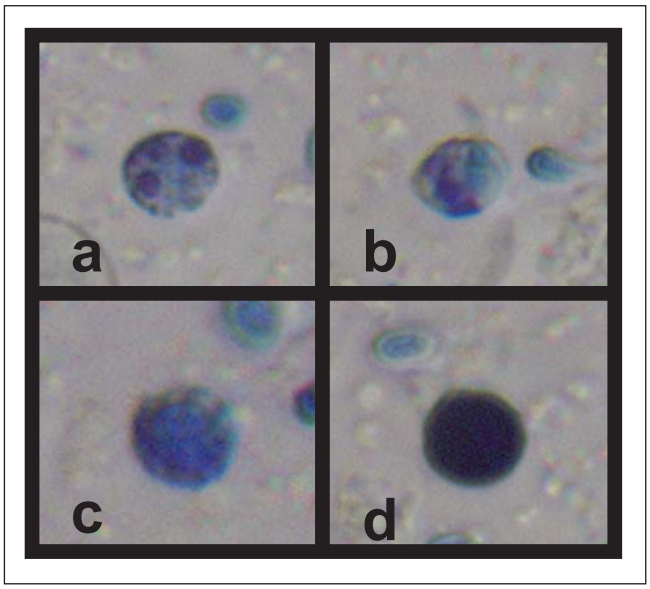

Fig. 1. Representative diffuse staining in freshly isolated, non-fixed cardiac mast cells following 20 min of incubation with acidic toluidine blue dye. Panels **a** and **b** demonstrate diffuse staining of immature "Connective Tissue-type" mast cells, while panels **c** and **d** demonstrate full metachromatic staining of mature "Connective Tissue-type" mast cells. Magnification = 1000X.

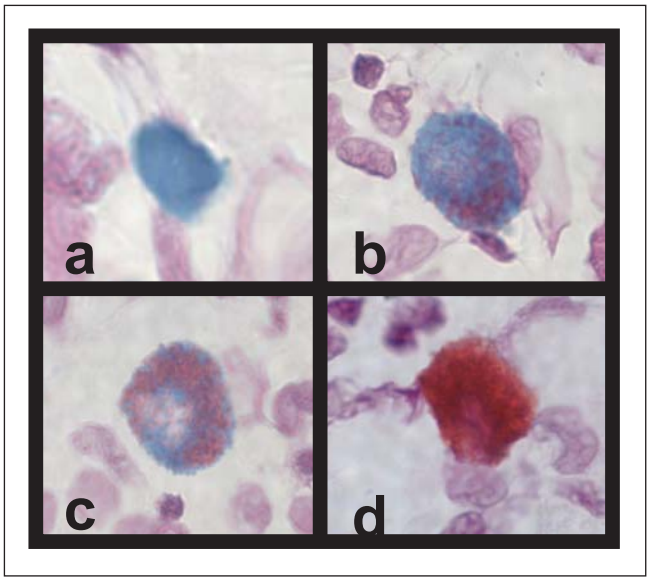

Fig. 2. Progressive stages of maturation and differentiation in fixed cardiac mast cell isolates following the alcian-blue and safranin reaction at ph 1.0. Panel **a** represents stage I mast cells containing a small number of cytoplasmic granules that absorb alcian-blue stain consistent with granule composition of predominately chondroitin sulfate mucopolysaccharides. Panel **b** represents stage II mast cells with up to 40 % of the granules staining positive for safranin. Panel c represents stage III mast cells with up to 60 % of the granules having affinity for safranin. Finally, the mature or Stage IV mast cells stain a brick-orange color consistent with heparin mucopolysaccharide synthesis as depicted in panel **d**. Magnification = 1000X.

the affinity of these cells for safranin staining [13]. Specific classification of the extent of maturation and differentiation of "Connective Tissue-type" granules was previously determined to consist of four morphological stages (I to IV) corresponding to a progression from predominately alcian-blue staining to safranin staining in mast cells from the heart and peritoneum of the rat [8–10]. These progressive changes in the percentage of granules staining with alcian-blue and safranin are related to alterations in mast cell proteoglycan synthesis during the maturation process. Stage I mast cells contain a small number of cytoplasmic granules composed primarily of the weakly sulfated polysaccharide, chondroitin sulfate, which absorbs alcian-blue stain (panel 2a). In Stage II mast cells, the number of granules progressively increase, with up to 40 percent staining positive for safranin (panel 2b). Due to an increase in heparin proteoglycan synthesis, Stage III mast cells contain up to 60 percent of safranin positive granules, producing a violet-orange color (panel 2c). Finally, the fully mature or Stage IV mast cells primarily synthesize heparin and stain a brick orange (panel 2d). Because the differences between stages I and II and between stages III and IV mast cells were subtle, the resulting numbers of stage I and II mast cells were combined into a single category designated as immature mast cells, while the resulting numbers of stage III and IV were combined into a single category designated as mature mast cells. The ability to consistently stage immature and mature mast cells was tested by re-reading slides at random. The percentages of cardiac and peritoneal mast cell stages obtained were reproducible within 10 %.

Cardiac Mast Cell Maturation/Differentiation and the Effects of Nedocromil

The percentages of immature and mature cardiac mast cells obtained from the non-surgical control and AV fistula and sham-operated groups at 1, 2 and 7 days post-surgery are presented in Table 1 and Figures 3–4. The proportion of

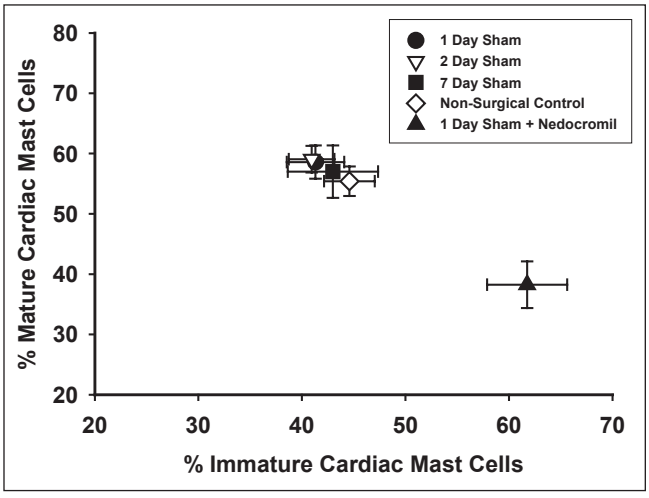

Fig. 3. Relationship between the proportions of mature versus immature cardiac mast cells obtained from the non-surgical control group, sham-operated rats at 1, 2 and 7 days, and the 1 day sham-operated group receiving nedocromil. Data points represent means ± SEM.

Table 1. Percent Immature and Mature Cardiac Mast Cells

Groups	Total (Mean ± SEM)	Percent Immature (Mean ± SEM)	Percent Mature (Mean ± SEM)
Non-Surgical Control	200 ± 44	45 ± 2	55 ± 2
1 Day Sham	408 ± 57	41 ± 3*	59 ± 3
1 Day Fistula	500 ± 01	34 ± 4	66 ± 4*
1 Day Sham + Nedocromil	463 ± 31	62 ± 4*#	38 ± 4#
1 Day Fistula + Nedocromil	478 ± 22	48 ± 5	52 ± 5*
2 Day Sham	467 ± 30	41 ± 2*	59 ± 2
2 Day Fistula	496 ± 02	29 ± 2	71 ± 2*
7 Day Sham	433 ± 34	43 ± 4	57 ± 4
7 Day Fistula	500 ± 01	46 ± 8	54 ± 8

* Indicates a significant difference in percent of immature or mature cardiac mast cells between sham and fistula-operated groups within each temporal period ($p \leq 0.001$).

# Indicates a significant difference in percent of immature or mature cardiac mast cells between non-surgical control and sham-operated groups ($p \leq 0.001$).

mature cardiac mast cells was significantly higher in the AV fistula rats following 1 and 2 days of volume overload ($p \leq 0.001$), while the relative proportions of immature and mature cardiac mast cells in sham-operated rats were not different from that of the non-surgical control group. This increase in the percentage of mature cardiac mast cells was transient, however, as following 7 days of volume overload, the percentages of immature and mature cardiac mast cells in the AV fistula group had returned to a normal distribution. These results are graphically demonstrated in Figures 3 and 4. The significant increase in the percentage of mature cardiac mast cells in the 1 and 2 day AV-fistula groups in Figure 4 is obvious, whereas, the 7 day AV-fistula group and the 1 day AV-fistula group treated with nedocromil remained clustered near the non-surgical controls. The percentages of immature and mature cardiac mast cells in the 1, 2 and 7 day sham-operated groups, depicted in Figure 3, also remained clustered near the non-surgical control group. Conversely, the sham-operated group treated with nedocromil developed a marked increase in the percentage of immature mast cells ($p \leq 0.001$). The combined mean diameters ± SEM of cardiac mast cells for each stage and experimental group are presented in Table 2. As can be seen, there was a consistent, significant increase in diameter associated with mast cell maturation.

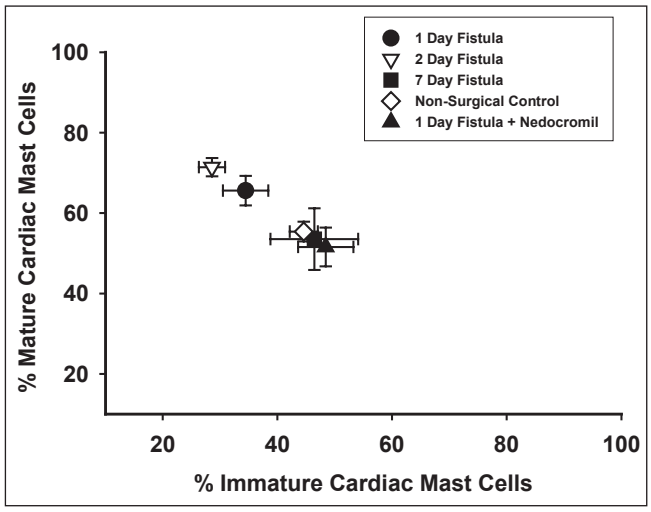

Fig. 4. Relationship between the proportions of mature versus immature cardiac mast cells obtained from the non-surgical control group, rats with an AV fistula at 1, 2 and 7 days, and the 1 day AV fistula group receiving nedocromil. Data points represent means ± SEM.

Peritoneal Mast Cell Maturation / Differentiation and The Effects of Nedocromil

The percentages of immature and mature peritoneal mast cells are presented in Table 3 and Figures 5–6. In contrast to the response in cardiac mast cells, the proportion of mature peritoneal mast cells was significantly higher in sham-operated rats at 1, 2 and 7 days post-surgery relative to the non-surgical control group ($p \leq 0.001$). The creation of an AV fistula produced a comparable increase in the percentage of mature peritoneal mast cells following 1 day of volume overload. However, by 2 days post-fistula, the percentage of mature peritoneal mast cells was significantly increased above that of the corresponding sham-operated group ($p \leq 0.001$). But similar to the cardiac mast cell response, the distribution of peritoneal mast cells had once again returned to normal in the AV fistula group by 7 days post-surgery. As can be seen from Figure 5, there was a significantly higher percentage of immature peritoneal mast cells in the 1 day sham-operated group treated with nedocromil relative to the non-surgical control group ($p \leq 0.001$), similar to the response in

Table 2. Mean Diameter for Cardiac and Peritoneal Mast Cells

Groups	Immature Stage Diameter		Mature Stage Diameter	
	Stage 1 *(um)*	Stage 2 *(um)*	Stage 3 *(um)*	Stage 4 *(um)*
Cardiac Mast Cells				
Sham Surgery (Mean ± SEM)	6.5 ± 0.1 (n = 176)	8.9 ± 0.1* (n = 2194)	9.2 ± 0.1* (n = 3827)	11.4 ± 0.8* (n = 30)
Fistula Surgery (Mean ± SEM)	6.9 ± 0.2 (n = 243)	8.4 ± 0.1* (n = 2331)	8.7 ± 0.1* (n = 4856)	12.7 ± 0.1* (n = 49)
Peritoneal Mast Cells				
Sham Surgery (Mean ± SEM)	8.1 ± 0.1 (n = 355)	10.2 ± 0.1* (n = 2338)	11.3 ± 0.1* (n = 4256)	15.6 ± 0.1* (n = 32)
Fistula Surgery (Mean ± SEM)	7.8 ± 0.1 (n = 339)	9.6 ± 0.1* (n = 1921)	10.7 ± 0.1* (n = 4633)	15.3 ± 0.3* (n = 86)
Peritoneal Mast Cells[a]				
Mean	8.3	11.0	12.7	16.0
Range	(6.7 - 10.3) (n = 45)	(9.2 - 12.2) (n = 200)	(11.0 - 14.0) (n = 200)	(13.6 - 17.0) (n = 200)

* Indicates a significant increase in mean cell diameter from that of the previous stage.

[a] Values Published by Yong et al., 1975.

n = Number of mast cells measured per stage.

cardiac mast cells. However, the AV-fistula group treated with nedocromil, despite having a slight immature shift, was clustered near the non-surgical control and 7 day AV-fistula groups (Fig. 6). These results demonstrate that mast cell stabilization with nedocromil does not attenuate peritoneal mast cell maturation and differentiation. Consistent with the findings in cardiac mast cells, the combined mean diameters ± SEM for peritoneal mast cells reported in Table 2 demonstrate a significant, progressive increase in cell diameter as a result of the maturation process. Peritoneal mast cell diameter as measured by Yong et al. [9] are also included in Table 2 for comparison purposes. Although in general the

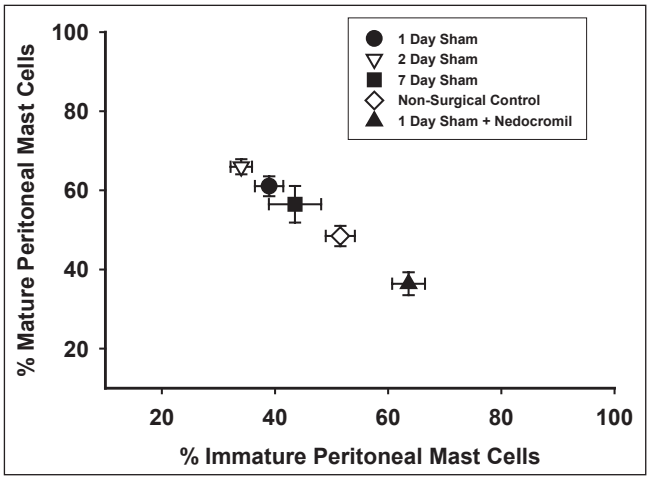

Fig. 5. Relationship between the proportions of mature versus immature peritoneal mast cells obtained from the non-surgical control group, sham-operated rats at 1, 2 and 7 days, and the 1 day sham-operated group receiving nedocromil. Data points represent means ± SEM.

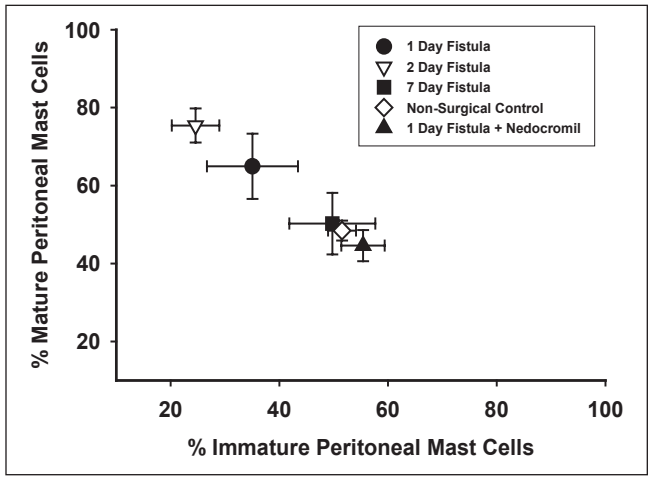

Fig. 6. Relationship between the proportions of mature versus immature peritoneal mast cells obtained from the non-surgical control group, rats with an AV fistula at 1, 2 and 7 days, and the 1 day AV fistula group receiving nedocromil. Data points represent means ± SEM.

Table 3. Percent Immature and Mature Peritoneal Mast Cells

Groups	Total (Mean ± SEM)	Percent Immature (Mean ± SEM)	Percent Mature (Mean ± SEM)
Non-Surgical Control	499 ± 01	52 ± 3	48 ± 3
1 Day Sham	500 ± 01	39 ± 3[#]	61 ± 3[#]
1 Day Fistula	449 ± 51	35 ± 8	65 ± 8
1 Day Sham + Nedocromil	500 ± 01	64 ± 3[*#]	36 ± 3[#]
1 Day Fistula + Nedocromil	478 ± 22	55 ± 4	45 ± 4[*]
2 Day Sham	496 ± 02	34 ± 2[*#]	66 ± 2[#]
2 Day Fistula	496 ± 02	25 ± 4	75 ± 4[*]
7 Day Sham	472 ± 29	44 ± 5[#]	56 ± 5[#]
7 Day Fistula	500 ± 01	50 ± 8	50 ± 8

[*] Indicates a significant difference in percent of immature or mature peritoneal mast cells between sham and fistula-operated groups at each temporal period ($p \leq 0.001$).

[#] Indicates a significant difference in percent of immature or mature peritoneal mast cells between non-surgical control and sham-operated groups ($p \leq 0.001$).

values obtained herein were slightly less than those reported by Yong et al. for Albino Wistar rats, the overall trends are the same.

Determination of Mast Cell Proliferation with Bromo-deoxyuridine Staining

Mast cells from the cardiac and peritoneal cell isolates did not incorporate BrdU in the stained slides. Further, less than one percent of mast cells stained for both the CD117 receptor (i.e., Stem Cell Factor receptor) and BrdU when evaluated by flow cytometery.

Discussion

Numerous studies have now shown increased cardiac mast cell density occurring secondary to various myocardial pathology [1–6]. Furthermore, this increase in the number of cardiac mast cells can occur very rapidly, as evidenced by the substantial increase in LV mast cell density during the initial hours following creation of an AV fistula [1]. These acute changes in cardiac mast cell morphology occur concurrently with remodeling of the extracellular matrix [3–5, 16]. Cardiac mast cell degranulation can also produce an acute depression in left ventricular function, development of myocardial edema, matrix metalloproteinase activation, reductions in collagen concentration, and alterations in fibroblast contraction [16–17]. While it is now clear that these activated mast cells are capable of mediating extracellular matrix degradation [1, 4], the origin of these additional mast cells in the myocardium remains in question. Thus, the objective of this study was to the determine the relative contribution of mast cell proliferation and/or maturation and differentiation to the expansion of cardiac mast cells occurring during the first 7 days of a sustained volume overload imposed on the heart. To this end, differential staining to identify alterations in the morphological stages of isolated cardiac mast cells and BrdU labeling to assess proliferation were performed in AV fistula and sham-operated rats.

While several studies have demonstrated proliferation in embryonic or neonatal mast cells via H^3-Thymidine [8, 18] or BrdU up-take [19], comparable studies in adult rat tissues have found that these mast cells do not incorporate H^3-Thymidine [8, 18]. Our finding of BrdU incorporation in <0.1 % of cardiac or peritoneal mast cells following 1 day of volume overload indicates that proliferation of mast cells did not contribute to the expansion of cardiac mast cell numbers post-fistula. These results are consistent with that reported by Frangogiannis et al. [6], who found that ischemia and reperfusion injury did not induce proliferating cell nuclear antigen expression in canine cardiac mast cells. A similar conclusion was reached by Ordeix et al. [20], who found that mature mast cells do not proliferate in the dermis of dogs symptomatic of atopic dermatitis.

In order to determine whether the increase in mast cell density could be attributed to maturation and differentiation of resident immature mast cells in the myocardium, differential staining of isolated mast cells was performed. The variable toluidine blue staining of the isolated cardiac mast cells (Fig. 1) demonstrates relative differences in the concentration of granule-associated mucopolysaccharide sulfation (i.e., chondroitin sulfate versus heparin). This is consistent with the report by Combs et al. [8] that chondroitin sulfate, a weakly sulfated mucopolysaccharide, is prevalent in immature "Connective Tissue-type" mast cells. Others have found that the degree of granule mucopolysaccharide sulfation determines the prominence of toulidine blue staining in mast cells [10, 21]. However, the differential staining affinity of immature mast cells for alcian-blue and mature mast cells for safranin more effectively delineates this difference in the mucopolysaccharide synthesis specific to a given stage of maturation. The prior studies by Yong et al. [7, 9] vali-

dated the alcian-blue/safranin methodology for characterization of the stages of mast cell maturation and differentiation. Consistent with our findings, Yong et al. [7] reported 37% of cardiac mast cells as immature (stages I and II) and 60% as mature mast cells (stages III and IV) in 30 day-old-rats. However, the results of the current study demonstrate a significant shift from immature to a greater percentage of mature cardiac mast cells during the first two days post-fistula, which then returns to a normal distribution within 7 days. Thus, we conclude that the increase in mature cardiac mast cell density occurring in the AV fistula model is derived primarily from the maturation of a resident population of immature mast cells. The subsequent normalization of immature and mature cardiac mast cell ratios at 7 days post-fistula may reflect a physiological process of negative feedback regulation in mast cells, such as induction of apoptosis in mature cardiac mast cells [22].

However, the induction of maturation appears to be differentially regulated at the tissue level. This is reflected in our study by the lack of an effect in the sham-operated rats on the percentages of immature and mature cardiac mast cells relative to that of the non-surgical control group. This is in contrast to the peritoneal mast cell population, where comparable increases in maturation occurred in both the AV fistula and sham-operated groups. These observations suggest that the AV fistula and abdominal surgical procedure each induce a localized paracrine effect on mast cell maturation and differentiation specific to that population of mast cells. However, because the extent of peritoneal mast cell maturation at 2 days post-fistula was significantly greater than that induced by the surgery alone, the possibility that the volume overload imposed on the heart produced a synergistic, systemic stimulus contributing to the induction of maturation must be considered.

Although our data indicate that cardiac mast cell maturation and differentiation post-fistula is mediated in a paracrine fashion, the release of this factor does not appear to be mast cell mediated. The evidence to this effect is derived from the results in the groups treated with the mast cell stabilizing compound, nedocromil. Mast cell stabilization with nedocromil in the sham-operated rats resulted in a significant increase in the proportion of immature mast cells relative to the untreated rats. This observation suggests that prevention of mast cell degranulation may interfere with the regulation of mast cell maturation and differentiation under basal conditions. However, despite starting from a different baseline, the extent of mast cell maturation induced by the sustained volume overload in the nedocromil treated AV fistua group was comparable to that seen in the untreated AV fistula group. Given that nedocromil prevents the release of mast cell proteases and cytokines [23], these results suggest the presence of redundant, differential signaling factors mediating mast cell maturation and differentiation in pathologic conditions, discrete from pathways which maintain the population of mature mast cells in normal organs and tissues [24].

One candidate molecule for induction of cardiac mast cell maturation in response to volume overload is fibroblast derived stem cell factor. This is supported by recent studies demonstrating the reversible expansion of cardiac and other organ mast cell populations following 14 to 21 day administration of exogenous recombinant stem cell factor in primates, rats and mice [25–26]. Frangogiannis et al. [6], reported a similar increase in cardiac mast cell numbers stimulated by the induction of stem cell factor after cardiac ischemia/reperfusion injury in the dog. These results, together with our findings, suggest that perturbations to the heart elicited by volume overload or ischemic insult, may regulate local stem cell factor production or release, thereby promoting maturation and differentiation of resident immature cardiac mast cells. The likelihood of a paracrine factor mediating the induction of cardiac mast cell maturation is further bolstered by the reports indicating increased cardiac mast cell numbers in rats following 2 days of inhalation exposure to diesel exhaust particles [27] and in free-roaming dogs chronically exposed to air pollutants [28].

Thus in summary, the increase in cardiac mast cell density during the acute response to myocardial volume overload is due to a paracrine response in the heart that stimulates the maturation and differentiation, but not proliferation, of a resident population of immature cardiac mast cells.

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