Postischemic inflammation: a role for mast cells in intestine but not in skeletal muscle

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Kanwar, Samina, Michael J. Hickey, and Paul Kubes. Postischemic inflammation: a role for mast cells in intestine but not in skeletal muscle. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G212–G218, 1998.—The objective of this study was to directly study a role for mast cells in ischemia-reperfusion (I/R)-induced mucosal and microvascular dysfunction. I/R was induced in the intestine and skeletal muscle (gastrocnemius and cremaster muscle) of wild-type mice and mast cell-deficient mice (W/Wv). Changes in mucosal permeability (blood-to-lumen clearance of 51Cr-EDTA), leukocyte infiltration (myeloperoxidase activity in the intestine and intravital microscopy in the cremaster muscle), and vascular permeability (tissue wet-to-dry weight ratio and FITC-albumin leakage) were measured as indexes of tissue dysfunction. In wild-type animals, intestinal I/R induced a significant increase in mucosal permeability, leukocyte infiltration, and vascular permeability. Mast cell-deficient animals were completely protected from I/R-induced mucosal dysfunction. However, skeletal muscle I/R induced a significant increase in leukocyte infiltration, FITC-albumin leakage, and edema formation to the same degree in both wild-type and mast cell-deficient animals. These data suggest that mast cells may be important mediators of I/R-induced mucosal and microvascular dysfunction in the intestine but not in microvascular dysfunction in skeletal muscle.

reperfusion injury; mucosal permeability; leukocyte recruitment

The reperfusion of tissues such as small intestine and skeletal muscle following a period of ischemia induces very similar inflammatory cascades that involve the production of oxidants, including superoxide and hydrogen peroxide (8, 14, 24). These oxidants, as well as various proinflammatory mediators, activate endothelium to rapidly express P-selectin, thereby tethering leukocytes to the lining of the vessel (18, 32, 34). The leukocytes then adhere and begin to emigrate out of the vasculature. During this physical process and perhaps due to the inappropriate release of oxidants and proteases from emigrating leukocytes, vascular dysfunction occurs (20, 26). The source of the various mediators has not been well established, but the directed movement of leukocytes out of the vasculature would suggest that at least some of the factors must be released from the extravascular space. Although there are numerous cell types in the parenchyma, there is a growing body of evidence that mast cells may contribute to reperfusion injury and leukocyte recruitment in the reperfused intestine (11, 12, 20). First, these cells degranulate at the time of reperfusion. This has been shown both histologically in the rat mesentery (20) and biochemically in the rat small bowel (11). The latter was based on increased plasma levels of rat mast cell protease II following intestinal ischemia-reperfusion (I/R). This protease is produced exclusively by rat mucosal mast cells and is an excellent indicator of mast cell degranulation. Other investigators (3) have demonstrated the release of histamine from postischemic intestine, also suggesting increased mast cell degranulation. Pharmacological inhibition of mast cell degranulation with the ostensibly specific mucosal mast cell stabilizer doxantrazole has been shown to reduce leukocyte recruitment and intestinal injury associated with I/R (11). A concern regarding this study is that it presumes that doxantrazole selectively stabilizes the microtubules of mast cells and not other cell types, an assumption that is difficult to accept or refute. To our knowledge, there is no I/R study to implicate a role for mast cells in nonintestinal tissue.

There are two types of mast cells, connective tissue and mucosal mast cells (2, 7). As their names imply, mucosal mast cells localize within mucosal tissues, including the lung and intestine, whereas connective tissue mast cells are located in the submucosa and muscularis layers of the gastrointestinal tract and in nonmucosal tissues such as skin and skeletal muscle. The two cell types are known to release very different mediators and may respond to very different stimuli (27). Another very obvious difference is that tissues directly in contact with the external environment, including the respiratory and gastrointestinal tract, harbor the greatest density of mast cells, whereas the internal organs such as skeletal muscle and heart have relatively few mast cells. Therefore, on the basis of the profound differences in mast cell characteristics and mast cell numbers in different tissues, it is conceivable that mast cells may have disparate degrees of importance during I/R in different tissues.

On the basis of the relatively indirect approaches used to implicate mast cells in I/R of the intestine, our first objective was to directly assess a role for mast cells in a model of I/R-induced leukocyte recruitment and mucosal dysfunction in the intestine using mast cell-deficient (W/Wv) mice. Our second objective was to examine whether mast cells also played a role in I/R-induced microvascular dysfunction in skeletal muscle, in which the mast cells are of a completely different phenotype (connective tissue mast cells) than those found in the intestine. We for the first time demonstrate a very profound reduction in I/R-induced mucosal dysfunction in mice lacking mast cells. More-
over, leukocyte recruitment into this tissue was dramatically reduced. By contrast, mice were far less responsive to similar or much longer episodes of I/R in skeletal muscle, and the leukocyte recruitment and microvascular injury that occurred appeared to be unrelated to the presence or absence of mast cells.

**METHODS**

All studies were performed in C57BL/6 mice (Charles Laboratories), WBB6F1/J +/+ mice (+/+; wild-type controls for the mast cell-deficient animals), and WBB6F1/J -/W- mice (W/-; mast cell-deficient animals) (Jackson Laboratory). All animals weighed between 25 and 40 g. The animals were anesthetized with an intraperitoneal injection of a cocktail of 10 mg/kg xylazine (MTC Pharmaceuticals, Cambridge, ON) and 200 mg/kg ketamine hydrochloride (Rogar/STB, Montreal, PQ). For all protocols, the left jugular vein was cannulated to administer additional anesthetic if necessary.

Intestinal I/R. After the animals were anesthetized and cannulated, a laparotomy was performed and both kidneys were immediately ligated to prevent excretion of 51Cr-EDTA (see below). The superior mesenteric artery was isolated, and a 5- to 10-cm loop of jejunum was exteriorized; blood vessels to the exteriorized segment of intestine remained intact. The loop of jejunum was fitted with inflow and outflow tubes to allow perfusion with warm Tyrode solution at a rate of 0.2 ml/min. Luminal perfusate samples were collected at 10-min intervals throughout the experiment. The intestinal segment and abdominal contents were kept moist with saline-soaked gauze and covered with a clear plastic sheet to minimize evaporation and tissue dehydration. The preparation was maintained at 36–38°C. Intestinal ischemia was induced by clamping the superior mesenteric artery for 30 min and then removing the clamp to allow for 60 min of reperfusion.

Mucosal permeability. Changes in mucosal permeability were assessed by measuring blood-to-lumen clearance of a radioactive marker, 51Cr-EDTA, as an index of intestinal I/R-induced mucosal dysfunction. 51Cr-EDTA was injected via the jugular vein and allowed to equilibrate across the vasculature for ~20–30 min. This was followed by a 30-min control period, 30-min ischemic period, and 30-min reperfusion period, during which luminal perfusate samples were collected at 10-min intervals. A single plasma sample (100 µl) was collected at the end of the experimental protocol via cardiac puncture. 51Cr-EDTA in plasma and luminal perfusate samples was measured in an LKB CompuGamma spectrometer. At the end of the experiment, the intestinal segment was removed, cut open along the mesenteric border, rinsed, and weighed. The plasma-to-lumen clearance of 51Cr-EDTA was calculated as \( \frac{cpm_t \times P_t \times 100}{cpm_p \times wt} \), where clearance of 51Cr-EDTA is given in milliliters per minute per 100 g of tissue, cpm\(_t\) is counts per minute per milliliter of the luminal perfusate, P\(_t\) is the perfusion rate (ml/min), cpm\(_p\) is counts per minute per milliliter of plasma, and wt is the weight of the intestinal segment in grams. No animal with a control mucosal clearance greater than 0.3 ml/min⋅100 g\(^{-1}\) or a clearance that increased with time over the control period was used. Less than 5% of animals were rejected on this basis. This technique has been used previously in our laboratory to quantify mucosal permeability in other species (11, 16).

Edema formation. Intestinal samples (~100 mg) were taken before and after I/R, and the wet weight was recorded. The tissues were then dried overnight using a heat lamp and weighed. The ratio of wet-to-dry weight was calculated and used as an index of vascular leakage or edema formation.

Leukocyte infiltration. We quantified leukocyte infiltration in the intestine by measuring myeloperoxidase (MPO) activity in jejunal samples (50–150 mg) before and after I/R. MPO is an enzyme found predominantly in granulocytes and is used as an index of neutrophil infiltration. Tissue MPO activity is measured using a standard assay as previously described (1, 11). Briefly, MPO is released from tissue biopsies by homogenization in detergent (hexadecytrimethylammonium bromide buffer). The MPO-containing supernatant is then reacted with a known amount of hydrogen peroxide and a hydrogen donor, o-phenylenediamine dihydrochloride. The reaction produces a colored compound, the appearance of which can be measured over time with a spectrophotometer to determine MPO content.

Skeletal muscle I/R. I/R of skeletal muscle was induced in two separate models of I/R. In the first series of animals, we directly visualized the microvasculature of the cremaster muscle in a similar model of I/R to that in the intestine (30-min and 60-min reperfusion). Briefly, an incision was made in the scrotal skin to expose the testes, which was then carefully removed from the associated fat pad. A lengthwise incision was made on the ventral surface of the cremaster muscle using a cautery. The testis and epididymis were separated from the underlying muscle and removed into the abdominal cavity. The muscle was then spread out over an optically clear viewing pedestal and secured along the edges with 5–0 suture. The exposed tissue was superfused with warm bicarbonate-buffered saline (pH 7.4) that was bubbled with a mixture of 5% CO\(_2\) and 95% nitrogen. The cremaster postcapillary venules were observed through an intravitral microscope (Nikon-Optiphot-2, Japan) with 25× objective lens (Leitz Wetzlar L25/0.35) and a 10× eyepiece. The images (1,400× magnification) were recorded at various time points during the experiment using a video camera (Panasonic-Digital 5100) and a video recorder (Panasonic NV-R9905).

Ischemia was induced for 30 min by clamping the main feeding artery perfusing the cremaster muscle. The clamp was then removed and the tissue was reperfused for 60 min. The degree of microvascular dysfunction was assessed as vascular albumin leakage from cremasteric venules. Briefly, 25 mg/kg FITC-labeled bovine albumin (Sigma Chemical, St. Louis, MO) was administered intravenously to animals 15 min before the start of the experimental procedure. Fluorescence intensity (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) was detected using a silicon-intensified fluorescence camera (model C-2400–08; Hammamatsu Photonics, Hamamatsu, Japn), and images were recorded for playback analysis using a videocassette recorder. The fluorescent intensity of FITC-albumin within a defined area (10 µm × 50 µm) of the venule under study and in the adjacent perivascular interstitium (20 µm from venule) was measured. This was accomplished using a video capture board (Vision-plus AT-OFG; Imaging Technology, Bedford, MA) and a computer-assisted digital imaging processor (Optimas; Bioscan, Edmonds, WA). The index of vascular albumin leakage (permeability index) was determined from the ratio (interstitial intensity – background)/(venular intensity – background), as previously reported (6, 23).

Leukocyte recruitment. As mast cells have previously been shown to induce P-selectin-dependent leukocyte rolling in postischemic mesenteric tissues, we examined leukocyte rolling in the mouse cremaster following reperfusion. Single, unbranched cremasteric venules (20–40 µm in diameter) were selected for each study. Venular diameter (D\(_v\)) was measured using a video caliper (Microcirculation Research Institute, Texas A & M University, College Station, TX).
Rolling leukocytes were defined as those leukocytes which rolled at a velocity slower than that of red blood cells. The flux of rolling cells was measured as the number of rolling cells passing by a given point in the venule per minute. A leukocyte was considered to be adherent if it remained stationary for at least 30 s, and total leukocyte adhesion was quantified as the number of adherent cells within a 100-µm length of venule. Leukocyte emigration was quantified as the total number of cells in the extravascular space within a 200 × 300 µm area.

This preparation also permitted recording of red blood cell velocity ($V_{BC}$) online using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A & M University). Venular blood flow was calculated from the product of cross-sectional area and mean $V_{BC}$ ($V_{mean} = V_{BC}/1.6$), assuming cylindrical geometry. Venular wall shear rate ($\gamma$) was calculated based on the Newtonian definition: $\gamma = 8 \times (V_{mean}/D_v)$, and venular shear stress was $\gamma \times$ blood viscosity, where blood viscosity was assumed to be 0.025 poise (9).

Because responses in the cremaster muscle did not differ between the mast cell-deficient and congenic control mice, in a second series of experiments, ischemia was induced by applying a tourniquet around the hindlimb for 120 min and then reperfusing the limb for 60 min. The contralateral limb was used as the control in these experiments. Tissue samples were taken for wet-to-dry weight as described above, as an index of vascular permeability.

Histology. Mast cells were counted in the cremaster muscle of both wild-type and mast cell-deficient animals following staining with a Giemsa-based technique (10). Briefly, whole cremaster preparations were mounted on glass slides and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. The tissue was washed three times in 0.1 M phosphate buffer containing 5% ethanol and subsequently stained with Giemsa stain (Sigma Chemical) at room temperature for 5–10 min. The stained tissues were then differentiated in 0.01% acetic acid. After the differentiation step, the tissue was washed in water, transferred to 75, 95, and 100% ethanol; and cleared in xylene. The slides were mounted using Permount and mast cells were counted in 10 random fields of view using a 40× objective.

Statistical analysis. Data are presented as means ± SE. A Student’s t-test with Bonferroni correction was used for multiple comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

I/R increases mucosal permeability in wild-type but not mast cell-deficient mice. Figure 1 illustrates blood-to-lumen clearance of $^{51}$Cr-EDTA in wild-type and mast cell-deficient animals under control conditions, 30 min of ischemia, and 60 min of reperfusion. The results from C57BL/6 and the appropriate control WBB6F1/J $+/+$ and $+/−$ mice were not significantly different; therefore, the data for the control mice were pooled. In wild-type animals under control conditions, $^{51}$Cr-EDTA clearance was $\sim 0.2$ ml·min$^{-1}$·100 g$^{-1}$. Ischemia for 30 min did not alter $^{51}$Cr-EDTA clearance. Within 10 min of reperfusion, however, there was a rapid and significant increase (−8-fold) in $^{51}$Cr-EDTA clearance, which remained elevated for the remainder of the experiment. In mast cell-deficient animals, $^{51}$Cr-EDTA clearance under control conditions was not different from wild-type animals, suggesting that baseline mucosal barrier function is relatively normal in mast cell-deficient animals. Furthermore, 30 min of ischemia did not alter $^{51}$Cr-EDTA clearance compared with control values. By 10 min of reperfusion, there was a slight (<2-fold) increase in $^{51}$Cr-EDTA clearance ($P < 0.05$). However, for the remaining 50 min of the experiment, no reperfusion-induced increase in $^{51}$Cr-EDTA clearance was observed in the mast cell-deficient mice. These data suggest that in the absence of mast cells mucosal barrier function is maintained following I/R.

Intestinal I/R-induced leukocyte infiltration and edema formation is inhibited in mast cell-deficient animals. Figure 2A represents the change in intestinal MPO activity (index of neutrophil influx) following I/R. In wild-type animals after I/R, there was a significant, ∼8-fold increase in tissue MPO activity. In mast cell-deficient animals, however, the MPO activity did not increase during reperfusion, suggesting that mast cells may play a critical role in I/R-induced leukocyte infiltration in the intestine. In addition, wet-to-dry weight ratios were measured as an index of vascular permeability. In wild-type animals, there was an approximately threefold increase in tissue wet-to-dry weight ratio following I/R. In mast cell-deficient animals, there was no increase in this parameter. However, due to the variability in both animal groups, the difference between wild-type and mast cell-deficient animals was not significant (Fig. 2B).

Skeletal muscle I/R induces an increase in various parameters independent of mast cells. Two hours of ischemia of the gastrocnemius muscle induced a significant increase in tissue wet-to-dry weight ratio at 1 h of reperfusion in both wild-type animals and mast cell-deficient animals. Figure 3 illustrates that the degree of increase in tissue wet-to-dry weight ratio was identical in both wild-type and mast cell-deficient animals, suggesting that mast cells were not involved in the edema formation associated with skeletal muscle I/R.
Although the increases look small, these are 30% increases in the total weight of the limb (includes bone). The hindlimb clearly looked edematous.

Previously, mast cells were thought to most profoundly affect leukocyte rolling (5), and so intravital microscopy was used to determine whether mast cells contribute to this parameter or to microvascular permeability directly in postcapillary venules, using the cremaster muscle. Figure 4 illustrates leukocyte rolling flux under control conditions and at 5, 30, and 60 min postreperfusion. In cremasteric postcapillary venules in wild-type mice under control conditions, there were ~30 cells rolling per minute. By 5 min of reperfusion, there was a significant increase in leukocyte rolling flux; however, this increase was relatively transient inasmuch as it returned to control levels by 30 and 60 min of reperfusion. I/R in the mast cell-deficient animals induced a very similar pattern of leukocyte rolling flux, and the values were not significantly different from wild-type animals. These observations suggested that mast cells were not likely to play a role in I/R-induced leukocyte rolling in skeletal muscle.

Interestingly, we have previously reported that mast cells play a critical role in leukocyte rolling under baseline conditions (19). In the current study, baseline leukocyte rolling flux in mast cell-deficient animals did not differ from wild-type controls, suggesting that at least in murine skeletal muscle, baseline leukocyte rolling occurs independently of mast cells. The responses in the mast cell-deficient animals were compared directly and only to their respective +/+ controls because leukocyte recruitment profiles were more pronounced in C57BL/6 mice than the +/+ wild-type control mice. Leukocyte adhesion and emigration and hemodynamic parameters did not differ between the +/+ control mice and W/Wv animals (data not shown).

Figure 5 illustrates FITC-albumin leakage from single venules of the postischemic cremaster microvasculature in wild-type animals and in mast cell-deficient mice. I/R induced a significant increase in microvascular permeability in both groups of animals. These data were consistent with the edema data obtained from whole hindlimb I/R (Fig. 3).

Histological analysis of the cremaster muscle of wild-type animals revealed ~40 mast cells/mm² in a whole mount of the cremaster muscle (~100 µm thick), many located adjacent to microvessels. Absolutely no mast cells were detected in the cremaster muscle of mast cell-deficient animals. These observations suggest that the absence of a role for mast cells in I/R-induced injury in the cremaster muscle of wild-type mice was not due to a lack of mast cells in this tissue.
DISCUSSION

In this study, we provide direct evidence supporting the concept that mast cells are critical for the pathogenesis of I/R injury in the small intestine. In particular, the critical disruption of the mucosal barrier that may permit bacterial translocation and distal organ injury is entirely absent in mast cell-deficient animals. On the basis of this observation, it is conceivable that distal organ injury and even multiorgan failure due to a highly permeable mucosal barrier may be attenuated or abolished in the absence of mast cells in the small bowel. In addition to the complete inhibition of mucosal barrier disruption associated with I/R, neutrophil influx and edema formation were not evident in mast cell-deficient mice. Because a very important component of the microvascular dysfunction observed following I/R in the intestine is thought to be the adhesion and emigration of neutrophils, it is tempting to postulate that the mast cell deficiency prevented the release of appropriate signals from the interstitium to recruit leukocytes into postischemic tissue and thereby prevented microvascular dysfunction. This thesis is supported by the fact that 1) mast cells release many potent neutrophil chemoattractants, 2) direct activation of mast cells with mast cell degranulating agents (compound 48/80) will recruit leukocytes, and 3) the mast cell-induced leukocyte recruitment contributes to the microvascular dysfunction (5, 7, 17).

Although mast cell activation is often associated with classic IgE-mediated inflammatory responses, these cells can no longer be regarded as cells that simply initiate acute allergic reactions. For example, mast cells have been implicated in many nonallergic models of inflammatory diseases associated with the gastrointestinal tract, including responses to various bacterial toxins (Clostridium difficile toxin A, cholera toxin), Helicobacter pylori, inflammatory bowel disease, and peptic ulcer disease (4, 13, 21, 22). Under these conditions, their activation may be associated with oxidant production, release of various neuropeptides (substance P), and various chemokines and would not require IgE-dependent activation. Our finding that mast cells contribute to the acute inflammatory response observed during I/R further supports a role for mast cells in IgE-independent inflammatory processes in the intestine.

There are commonalities in the molecular mechanisms that underlie reperfusion injury in the intestine versus other tissues. For example, neutrophils and oxidants have been invoked as mediators of tissue injury in cardiac and skeletal muscle, in lung, and in small intestine (8, 15, 28, 29, 33). In this study for the first time we demonstrate a clear difference between the postischemic mechanisms involved in tissue injury in small intestine versus skeletal muscle. Whereas mast cells appeared to play a critical role in each of the sequelae of I/R in small intestine, FITC-albumin leakage, edema formation, and neutrophil recruitment were not different in postischemic skeletal muscle between mast cell-deficient and normal mice. A number of possibilities exist to explain this difference, including 1) the difference in the type and number of mast cells between intestine and skeletal muscle, 2) the potential sensitivity of intestinal versus skeletal muscle mast cells, and 3) the severity of ischemia between the two tissues.

Generally, it is thought that there are two types of mast cells: 1) mucosal mast cells, found primarily at mucosal surfaces, e.g., in the intestine, respiratory tract, and urinary tract, and 2) connective tissue mast cells, found in the muscular layers of the intestine, skeletal muscle, and skin (2, 7). Therefore, on the basis of our data of intestine versus skeletal muscle, one might propose that mucosal but not connective tissue mast cells are responsive to I/R. This, however, does not appear to be the case, as we and others have previously observed that connective tissue mast cells in the mesenteric connective tissue are sensitive to I/R and contribute to leukocyte recruitment (12, 20). In this study, connective tissue mast cells in skeletal muscle appeared not to be sensitive to I/R. Perhaps defining mast cells in just two groups may be too simplistic when one considers the very different environmental conditions between lung, intestine, mesentery, and skeletal muscle. In fact, a recent report has identified many types of mast cells on the basis of their protease content and raises the possibility that each tissue has its own population of mast cells that may be sensitive to different stimuli and may potentially release a different variety of mediators (25).

Another important contributing factor may be the difference in the number of mast cells between tissues. Mast cell numbers are known to be highest in tissues such as skin and mucosal surfaces that are exposed to the inflammatory agents present in the external environment. Clearly this does not apply to skeletal muscle. However, our histological analysis shows that there are mast cells present in the cremaster muscle, many adjacent to postcapillary venules such that mast cell-derived mediators could readily affect the microvasculature to induce leukocyte recruitment. Despite mast cells being present, it is also possible that the difference...
in responsiveness between skeletal muscle and small intestine is due to large differences in tissue mast cell numbers. Previous studies using comparable histological techniques have shown that in murine skeletal muscle, the mast cell count is \( \sim 1/mm^2 \), whereas in uninfected murine jejunum it is \( \sim 10/mm^2 \) (30, 31). These data suggest that one possible explanation for the observation that mast cells appear to be important in postischemic injury in small intestine but not in skeletal muscle may be the large difference in the number of mast cells present in the tissue.

WW\(^\text{a}\) mice have a mutation at both copies of the c-kit locus and are therefore profoundly deficient in mast cells (<0.5% of normal). These mice have been used extensively to demonstrate that leukocyte recruitment and vascular dysfunction associated with IgE-dependent passive cutaneous anaphylactic reactions is indeed mast cell mediated. In this study, using the same mice, we demonstrate a direct role of mast cells in postischemic recruitment of leukocytes, increased vascular permeability, and induction of mucosal dysfunction in the small intestine, but not in skeletal muscle. The results of this study clearly demonstrate that despite the broad similarities in the events leading to the pathogenesis of reperfusion injury, the underlying mechanisms that occur in the intestine cannot be extrapolated indiscriminately to other tissues, including skeletal muscle.

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REFERENCES


28. Moore, T. M., P. Khimenko, W. K. Addkson, M. Miyasaka, and A. E. Taylor. Adhesion molecules contribute to ischemia and...


