A Role for Mast Cells in the Development of Adjuvant-Induced Vasculitis and Arthritis

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The objective of this study was to characterize the role of mast cells in the development of vasculitis and joint swelling in adjuvant-immunized rats. Leukocyte trafficking within mesenteric venules (rolling and adhesion) and mast cell activation (ruthenium red uptake) were examined in vivo. Elevated leukocyte trafficking was observed by 4 days after immunization, whereas joint swelling developed between days 10 and 12. Perivascular mast cells took up ruthenium red and appeared activated by electron microscopy at 4 but not 12 days after immunization. Treatment with the mast cell stabilizer cromolyn on days 1 to 4 after immunization blocked ruthenium red uptake at day 4 and reduced leukocyte rolling and adhesion by ~50%. This treatment also reduced rolling, adhesion, and joint swelling at day 12 by ~50%. Cromolyn treatment over days 9 to 12 reduced joint swelling but increased leukocyte emigration into the mesentery. Peritoneal mast cells isolated 4 days after immunization elicited significant neutrophil chemotaxis in vitro, whereas day 12 mast cells did not. Mast cell activation and vasculitis were absent in adjuvant-resistant Fisher/344 rats. These data suggest that mast cells play an early role in the initiation of vasculitis and may function by day 12 to limit infiltration of leukocytes from the vasculature. In the joint, however, mast cells appear to contribute to inflammation at early as well as later time points. (Am J Pathol 1998, 152:555–563)

Connective tissue mast cells, abundant in tissues such as the skin, peritoneum, and joints, have been implicated in the initiation of acute inflammatory responses and the recruitment of leukocytes to sites of injury.1-5 Mast cells contain numerous pro- and anti-inflammatory agents and are often closely apposed to the vasculature, placing them in an ideal position to influence leukocyte recruitment.1-5 Indeed, mast cells have been shown to play roles in the recruitment of leukocytes during immediate and late-phase hypersensitivity reactions,6,7 ischemia-reperfusion injury,8 and the clearance of microbial pathogens.9,10 Mast cell activation induced by IgE cross-linking or using mast cell degranulating agents such as CMP 48/80 can also elicit acute leukocyte recruitment in vivo.1,3 The inflammatory agents released from acutely activated mast cells are able to mediate the sequential leukocyte rolling and firm adhesion steps required for the recruitment of leukocytes from the vasculature.1,5 However, the role of mast cells in the development and maintenance of more persistent or chronic inflammatory responses is not clear.

Recently, we have characterized a model of vasculitis that develops in rats after immunization with Mycobacterium butyricum in adjuvant oil. The hallmarks of this vasculitis are profound increases in leukocyte rolling and adhesion within the mesenteric microvasculature.11-13 This increased leukocyte trafficking can be observed by 4 days after immunization and persists for at least 20 days. By day 12, when the vasculitis has reached maximal levels, the animals also develop adjuvant-induced arthritis. It is possible that a link may exist between the development of vasculitis and joint disease as systemic complications are often seen in both adjuvant arthritis and patients suffering from rheumatoid arthritis.14-16 However, the cells that contribute to the development and maintenance of vasculitis are unknown. The adjuvant-induced vasculitis model may be useful in studying the role of various inflammatory cells, including mast cells, in the development of chronic disease as the mesentery is optically clear and allows vascular leukocyte trafficking and mast cell activation to be directly examined in intact animals.

The purpose of this study was to examine the role of mast cells in the development of adjuvant-induced vasculitis. Several approaches were taken to address this objective. First, ruthenium red, a dye taken up specifically by activated mast cells, was used to elucidate the activation state of mast cells during the development of adjuvant-induced vasculitis in the mesentery. Second,
prevention of mast cell activation with mast cell stabilizers was used to determine the effects of this intervention on leukocyte trafficking within the mesentery and on joint swelling. Third, we directly assessed mast-cell-dependent leukocyte recruitment in vitro using cell co-culture systems. Finally, we characterized mast cell activation and leukocyte trafficking in Fisher/344 rats, a strain that is resistant to adjuvant-induced arthritis.

Materials and Methods

Adjuvant Immunization

Under light anesthetic (diethyl ether; BDH, Toronto, Ontario, Canada), male Sprague-Dawley rats (160 to 220 g) were injected subcutaneously at the base of the tail with a solution of heat-killed *M. butyricum* (Difco Laboratories, Detroit, MI) in Freund’s mineral oil adjuvant (Difco; 0.75 mg of *M. butyricum* in 0.1 ml of adjuvant). This protocol has been used as a model of arthritis in other laboratories and is described in detail elsewhere. Joint swelling became apparent 10 to 12 days after immunization. Previous experiments using intravital microscopy revealed a tremendous increase in leukocyte trafficking through mesenteric postcapillary venules 4 to 20 days after immunization.

Intravital Microscopy

Rats were maintained on a purified laboratory diet and fasted for 18 to 24 hours before surgery. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (55 mg/kg body weight). The right jugular vein was cannulated to maintain anesthetic, and the right carotid artery was cannulated to measure systemic arterial blood pressure (model P23XL pressure transducer, Viggo-Spectramed, Oxnard, CA, and model 7 physiological recorder, Grass Instruments Co., Quincy, MA). After laparotomy, rats were placed in a supine position on an adjustable Plexiglas microscope stage, and a segment of the mid-jejunum was exteriorized and prepared for intravital microscopy as previously described. The mesenteric preparation was observed through an intravital microscope (Optiphot-2, Nikon, Mississauga, Ontario, Canada) with a 25× objective lens (Wetzlar L25/0.35, E. Leitz, Munich, Germany) and a 10× eyepiece. A video camera (model 5100 HS, Panasonic, Osaka, Japan) mounted on the microscope projected the image onto a color monitor (model PVM 2030, Sony, Tokyo, Japan), and the images were recorded using a videocassette recorder (model AG-1790, Panasonic) for subsequent playback analysis. The final magnification of the image on the monitor was ×1800. Single unbranched mesenteric venules (25 to 50 μm in diameter) were selected for study. The same section of venule was observed throughout the experiment to control for variations between different regions. Venular diameter was measured on-line using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). Centerline red blood cell velocity was also measured on-line using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University).

The number of rolling and adherent leukocytes was determined off-line during video playback analysis. Leukocytes were considered adherent to the venular endothelium when they remained stationary for a period of time equal to or exceeding 30 seconds. Adhesion is expressed as cells per 100-μm venule length as variations in venular surface area were not significant between groups of experiments. Rolling leukocytes were defined as those white blood cells that moved at a velocity less than that of erythrocytes within a given vessel. The flux of rolling leukocytes was determined as the number of white blood cells that rolled past a fixed point in the venule during a 1-minute interval using frame-by-frame video analysis. Leukocyte emigration was measured as the number of extravascular leukocytes observed within a field of view (275 × 190 μm).

Experimental Protocols

Perivascular mast cell activation and leukocyte trafficking within mesenteric postcapillary venules were measured 4 and 12 days after *M. butyricum* immunization. On locating a mesenteric venule 25 to 50 μm in diameter, the image was recorded for 5 minutes. Preparations were then superfused continuously with a 0.001% solution of ruthenium red (Sigma Chemical Co., St. Louis, MO), a vital dye taken up specifically by activated mast cells. Additional 5-minute video recordings were made at 15-minute intervals over a 60-minute period and revealed little or no change in hemodynamic parameters or leukocyte kinetics throughout the experiment. To provide a positive control for mast cell staining at the end of each experiment, preparations were superfused for 30 minutes with the mast cell activator Compound 48/80 (CMP 48/80, 1 μg/ml; Sigma). To confirm adjuvant-induced changes in mast cell reactivity, some animals received an intravenous bolus of sodium cromoglycate (cromolyn, 5 mg/kg; Sigma) before laparotomy, a protocol that prevents mast cell activation induced by surgical manipulation.

The effects of mast cell stabilizers on the development of adjuvant-induced mast cell activation, leukocyte trafficking, and joint swelling were determined. Animals were immunized with *M. butyricum* and treated with oral administrations of the mast cell stabilizers sodium cromoglycate (cromolyn, 100 mg/kg/day; Sigma) or ketotifen (2 mg/kg twice a day; Sigma) over several time courses. A control group of immunized animals received an equal volume of vehicle (1 ml of sterile saline). In one group, animals received mast cell stabilizers every day for 4 days after immunization. Intravital microscopy experiments with ruthenium red staining were then performed at day 4. A second group of animals received mast cell stabilizers over the first 4 days after immunization, and intravital microscopy was performed at day 12. A final group of immunized animals received mast cell stabilization over the last 4 days (days 9 to 12) before experimentation at day 12.

Systemic blood samples were obtained at the beginning and end of each experiment for determination of total and
differential cell counts. A digital caliper (model CD-6BS, Mitutoyo Corp., Tokyo, Japan) was used to measure joint thickness at time points before and after immunization.

**Electron Microscopy**

Mesenteric tissue samples were fixed with 5% glutaraldehyde and prepared for transmission electron microscopy as previously described. Briefly, samples were post-fixed for 1 hour with 1% osmium tetroxide and subsequently dehydrated in a graded series of ethanol solutions. Tissues were then embedded in LX 112 (Polysciences, Warrington, PA), and ultrathin sections were obtained using an ultramicrotome (RMC 7000, RMC, Tucson, AZ) equipped with a diamond knife. Sections were stained with uranyl acetate and lead citrate before viewing with a JOEL 200CX electron microscope.

**In Vitro Chemotaxis Assays**

Neutrophils were purified from control and *M. butyricum*-immunized rats by dextran sedimentation, followed by hypotonic lysis and histopaque centrifugation as previously described. This procedure yielded neutrophils >85% pure and >97% viable. Mast cells were isolated from the same animals by peritoneal lavage followed by centrifugation of the peritoneal cell suspension through a two-step discontinuous Percoll gradient (30%/80%) as described previously. Mast cells obtained by this procedure were >90% pure and >97% viable.

Mast cells (1.1 × 10⁶ cells/ml) or buffer alone were added to the lower chamber of a Transwell co-culture kit (Corning Costar Corp., Cambridge, MA) and incubated with or without 1 μg/ml CMP 48/80 for 30 minutes at 37°C. The chambers were then assembled with 6.5-mm-diameter polycarbonate membrane inserts (3.0-μm pore size), and neutrophils (3.3 × 10⁵ cells/ml) were added to the upper surface of the insert. After 30 minutes at 37°C, neutrophils that migrated into the lower chamber were counted using a hemocytometer. Chemotaxis is represented as the percentage of the total neutrophils added to the upper chamber that migrated into the lower chamber.

**Statistical Analysis**

All values are reported as means ± SEM. An unpaired Student's t-test was used to compare between groups. Statistical significance was set at *P* < 0.05.

**Results**

**Mast Cells Are Activated in Adjuvant-Induced Vasculitis**

Figure 1 demonstrates that, under normal conditions, approximately 30 cells rolled through mesenteric venules per minute and only 5 cells adhered along a 100-μm length of venule during a 5-minute interval. Ruthenium red, a dye that permits quantitative analysis of mast cell activation on-line revealed very little perivascular mast cell activation. Only 3.0 ± 2.2% of the mast cells in control preparations took up dye (Figure 1C). As previously described in the adjuvant-induced vasculitis model, a profound increase in leukocyte rolling and leukocyte adhesion was noted 4 days after adjuvant administration (Figure 1, A and B). Associated with this increase in leukocyte trafficking, significant ruthenium red dye uptake was observed 4 days after immunization (Figure 1C), with 60.5 ± 11.4% of the mast cells staining with ruthenium red. Other cells and tissues in the preparation were not stained by the ruthenium red dye. Pre-treatment with 5 mg/kg intravenous cromolyn just before surgery did not block ruthenium red uptake at day 4 (data not shown), suggesting that mast cell activation was not an artifact of surgical manipulation.
surrounding a mast cell 4 days after *M. butyricum* immunization. Mast cell degranulation was apparent at day 4 even when the mesentery was fixed with glutaraldehyde before surgical exteriorization (Figure 2A). However, at 12 days after immunization, most mast cells were intact regardless of whether the mesentery was fixed before or after exteriorization (Figure 2B).

**Mast Cell Activation Is Related to Leukocyte Recruitment in Vasculitis**

As mast cell activation was observed 4 days after adjuvant immunization, we investigated the effects of mast cell stabilization on the development of adjuvant-induced vasculitis. In the first series of experiments, animals were given oral treatments with saline or the mast cell stabilizer cromolyn (100 mg/kg/day) for 4 days after immunization. Figure 3A demonstrates that this dose of cromolyn completely blocked adjuvant-induced mast cell activation at day 4. The daily cromolyn treatments also reduced leukocyte rolling flux and adhesion in the day 4 animals by 50 to 60% (Figure 3, B and C). Few extravascular leukocytes were observed in the mesentery (Figure 3D).

As this regimen of cromolyn treatment reduced leukocyte-endothelium interactions in animals observed 4 days after immunization, we examined whether mast cell stabilization during the early phase would be protective at later time points. In Figure 4, it can be seen that cromolyn treatment during the first 4 days after immunization significantly attenuated adjuvant-induced vasculitis (ie, leukocyte rolling flux and adhesion) at day 12. In this series of experiments, we also examined whether this regimen could impact on joint swelling; 4 days of cromolyn also attenuated adjuvant-induced joint swelling by ~50% (Figure 4D).

In the next series of experiments we examined whether administration of cromolyn after vasculitis was established (days 9 to 12) could also attenuate adjuvant-induced vasculitis and joint swelling (Figure 4). Interestingly, this treatment did not reduce leukocyte rolling flux or adhesion (Figure 4, A and B), suggesting that early events involving mast cells play a role in the development of the vasculitis. It also suggests that this concentration of cromolyn does not directly affect the ability of leukocytes to roll and adhere. In fact, administration of cromolyn from days 9 to 12 caused a threefold increase in the number of leukocytes in the interstitium of the mesentery on day 12 (Figure 4C), whereas administration of cromolyn for the first 4 days (days 1 to 4) did not elicit leukocyte emigration at day 12. Similar results were obtained when rats were treated with a different mast cell stabilizer, ketotifen (data not shown). However, joint swelling was reduced when animals received cromolyn over days 9 to 12 (Figure 4D), suggesting that interruption of mast cells at either the early or later time points could impact on the inflammatory processes in the joint.

Twelve days after immunization with *M. butyricum* (Figure 1) there was an even greater increase in the number of rolling cells (>300 cells/minute) and adherent cells (~30 cells/100 μm) within mesenteric venules. Despite this very large increase in leukocyte-endothelial cell interactions 12 days after immunization, there was little evidence of mast cell activation; ruthenium red dye uptake was not different from control preparations (Figure 1C). To ensure that the mast cells could be activated, the mast-cell-selective calcium ionophore CMP 48/80 was added at the end of each experiment. CMP 48/80 elicited ruthenium red uptake in all preparations (data not shown).

Histology of mesenteric preparations taken from separate groups of animals confirmed the in vivo ruthenium red data. Four days after immunization, 44.5 ± 8.5% (n = 5) of the mast cells were activated and releasing their granules, whereas only 13.8 ± 5.7% (n = 6) of the mesenteric mast cells exhibited some granule release 12 days after immunization (P < 0.05, day 4 versus day 12). The transmission electron micrograph in Figure 2A shows many extruded granules
Mast Cells Elicit Neutrophil Chemotaxis at Day 4 but Not Day 12

*In vitro* chemotaxis assays were used to evaluate the ability of mast cells from adjuvant-immunized rats to modulate leukocyte recruitment. Figure 5 demonstrates that approximately 15 to 25% of neutrophils spontaneously crossed polycarbonate filters toward mast cells harvested from untreated animals. This event was entirely independent of whether the neutrophils were harvested from normal or adjuvant-treated animals (4 or 12 days after immunization). Mast cells obtained at day 4 from *M. butyricum*-immunized animals elicited a significant increase in neutrophil chemotaxis through polycarbonate membranes compared with control mast cells (Figure 5A). However, this response occurred only with neutrophils isolated from adjuvant-treated animals (4 or 12 days); neutrophils isolated from control animals did not respond to mast cells from adjuvant-treated animals. Neutrophils from control animals did respond when mast cells were stimulated with CMP 48/80 (data not shown). Mast cells isolated from *M. butyricum*-immunized animals on day 12 did not elicit increases in neutrophil chemotaxis (Figure 5B), suggesting that these mast cells did not spontaneously release chemotactic substances. Alternatively, these mast cells may have released compounds that suppress leukocyte recruitment. Neutrophil chemotaxis could be elicited by stimulating the day 12 mast cells with CMP 48/80 (data not shown).
Rats Resistant to Adjuvant-Induced Arthritis Lack Vasculitis

It has been reported that the Fisher/344 strain of rats are resistant to the development of adjuvant arthritis. In contrast to Sprague-Dawley rats, Fisher/344 rats immunized with M. butyricum did not exhibit perivascular mast cell activation or vasculitis after immunization with M. butyricum (Figure 6); adjuvant immunization did not significantly increase leukocyte rolling flux or adhesion in the Fisher/344 rats (Figure 6, B and C). As previously reported, the Fisher/344 rats also failed to develop joint swelling after immunization (Figure 6D).

Adjuvant-sensitive Sprague-Dawley rats exhibited significantly elevated circulating leukocyte counts 4 and 12 days after immunization compared with control animals (Table 1). Cromolyn did not block the increase in the number of circulating leukocytes. Circulating leukocyte counts in the Fisher/344 rats did not increase after immunization with M. butyricum, suggesting that these animals are resistant in all aspects of the adjuvant treatment.

Discussion

In our model of adjuvant-induced vasculitis we report that connective tissue mast cells are activated during the development of this disease and contribute to the recruitment of leukocytes to the plasma-endothelial cell inter-

face. Mast cell activation in the mesentery appeared early and was transient (not evident at 12 days). However, functional inhibition of mast cell activation within the first 4 days significantly attenuated vasculitis and the progression of the disease. Surprisingly, inhibition of mast cell function at a later time point revealed a potential anti-inflammatory or repair role for mast cells as leukocyte emigration into the mesentery was increased. Although it remains unclear whether the vasculitis described herein is directly related to the joint injury observed in this animal model, or in patients with rheumatoid arthritis, the fact that early mast cell stabilization prevented both the vasculitis and joint swelling suggests some common elements between these two anatomically distinct sites. Similarly, the vasculitis and joint swelling appear to be linked

Figure 5. Mast-cell-induced neutrophil chemotaxis in vitro. Peritoneal mast cells isolated from control or M. butyricum-immunized rats were placed in culture wells. Neutrophils isolated from control or M. butyricum-immunized rats were placed in filter inserts (3-μm pores) over the mast cells. After 30 minutes of incubation, the proportion of neutrophils that migrated into the lower chamber was calculated. Day 4 mast cells (A) elicited migration of neutrophils from M. butyricum-immunized but not control animals. Day 12 mast cells (B) did not elicit migration of neutrophils from control or M. butyricum-immunized rats. Each data point represents the mean value from six to eight separate experiments. *P < 0.05 relative to the control mast cells; ^P < 0.05 relative to control neutrophils.

Figure 6. Fisher/344 rats do not develop adjuvant-induced inflammation. Perivascular mast cell activation (A), leukocyte rolling flux (B), leukocyte adhesion (C), and change in joint thickness (D) were measured in control and M. butyricum-immunized Sprague-Dawley and Fisher/344 rats (n = 5 per group). Leukocyte rolling flux and adhesion are represented as the average value observed during the first 60 minutes after exteriorization of the mesentery on day 4 or 12 after immunization. Mast cell activation was measured as the proportion of perivascular mast cells that had taken up 0.001% ruthenium red dye by 60 minutes after exteriorization. The change in joint thickness is the difference in joint diameter at day 12 compared with the initial diameter at day 0. *P < 0.05 relative to control group; ^P < 0.05 relative to Sprague-Dawley rats.
in that arthritis-resistant Fisher/344 rats also fail to develop vasculitis.

There are numerous ways in which mast cells may contribute to the vasculitis noted in this study. First, mast cells store a plethora of inflammatory mediators, including preformed vasoactive amines (histamine and serotonin) and cytokines (tumor necrosis factor (TNF)-α). Additionally, they can synthesize lipid mediators (leukotrienes, prostaglandins, and platelet-activating factor), cytokines (TNF-α, interferon (IFN)-γ, interleukin (IL)-4, IL-5, etc), and many other factors. Many of these mediators have been shown to play roles in leukocyte recruitment as they activate endothelial cells and/or leukocytes. Leukocyte recruitment consists of at least two distinct events, leukocyte rolling and leukocyte adhesion. In the vasculitis model used in this study, both neutrophils and mononuclear leukocytes traffic through mesenteric venules 4 and 12 days after immunization (and unpublished data), and these interactions appear to be dependent on contributions from both the selectin family of adhesion molecules (primarily L-selectin) and the α4/ß1 integrin. The most obvious scenario would be that perivascular mast cell activation in M. butyricum-immunized animals resulted in the release of various pro-inflammatory mediators. These mediators would impact directly on the endothelium to cause adhesion molecule expression and recruitment of leukocytes. Indeed, mast cell activation has been shown to stimulate the expression of selectins, ICAM-1 (ligand for CD11/CD18), and VCAM-1 (ligand for α4-integrin) on the surface of endothelium. The prevention of mast cell activation in this study significantly attenuated leukocyte rolling and adhesion, supporting a mast cell-endothelium axis of leukocyte recruitment.

It is also possible that mast cells directly affect leukocytes. We observed mast-cell-dependent neutrophil chemotaxis in vitro in the absence of endothelial cells. It was clear that day 4 mast cells released chemotactic products in the absence of exogenous stimuli. In vivo, the release of inflammatory mediators may contribute to the high level of adhesion observed after adjuvant immunization, but it did not elicit large-scale leukocyte diapedesis in the mesentery.

Mast cells may also contribute to injury in other models of arthritis. Experimental arthritis induced by intra-articular injection of IgE aggregates or arthritogenic factors are associated with mast cell activation. Mast cells have also been implicated in the flare reaction and cartilage damage in adjuvant-induced arthritis in mice. In streptococcal polysaccharide-induced arthritis, chronic mast cell degranulation with CMP 48/80 did not significantly attenuate the development of arthritis in rats. However, mast cells may still have synthesized and released inflammatory mediators that contributed to the arthritis.

In our model, mast cell stabilization with cromolyn during the first 4 days after immunization reduced vasculitis at day 12, suggesting that the mast cells play a critical role at an early time point in the initiation and development of adjuvant-induced vasculitis. Mast cells may be important in the early inflammatory cascade or initiation of the immune response that permits the full progression of chronic vasculitis. It is known that adjuvant arthritis is dependent on the early recruitment and activation of CD4+ αβ T lymphocytes as antibodies directed against these cells prevented the development of disease, even when the antibodies were given only during the first several days after immunization. If mast cell stabilization prevented the early recruitment of CD4+ αβ T lymphocytes critical for disease progression, then subsequent leukocyte recruitment at day 12 would also be impaired. Alternatively, mast cells may also be involved in antigen presentation and the initiation of the immune response. Stabilization of mast cells may prevent their activation and up-regulation of major histocompatibility complex class II and co-stimulatory molecules.

When mast cells were examined at days 4 and 12 after M. butyricum immunization, mast cell activation was evident only at day 4, and only day 4 mast cells recruited leukocytes in vitro, demonstrating a clear distinction in mast cell function at the two time points. An interesting but disconcerting observation was that mast cell stabilization over days 9 to 12 after immunization elicited an increase in leukocyte emigration into the connective tissue of the mesentery. One explanation could be that cromolyn directly promoted leukocyte recruitment. However, this seems unlikely as cromolyn does not affect leukocyte function in vitro and did not increase leukocyte infiltration when given over days 1 to 4. In addition, it is unlikely that leukocyte emigration was related to a nonspecific effect of cromolyn as leukocyte infiltration was also noted when animals were treated with ketotifen. Alternatively, mast cells in the mesentery may function by day 12 to actively suppress leukocyte emigration.


data table

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<th>Day after M. butyricum</th>
<th>Sprague-Dawley</th>
<th>Cromolyn</th>
<th>Fisher/344</th>
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<td>Control</td>
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<tr>
<td>Day 4</td>
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<td>Day 12</td>
<td>167.7 ± 11.7*</td>
<td>191.5 ± 21.6*</td>
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* P < 0.05 compared with control.
† P < 0.05 compared with Sprague-Dawley saline treated rats.
fate. Stabilization of the mast cells may prevent the release of potential anti-inflammatory compounds, thereby allowing increased leukocyte infiltration. Although the identity of the purported anti-inflammatory mediator produced in this model remains unknown, it would have to be a molecule that is not released via active degranulation as no ruthenium red uptake was noted at day 12, and mast cells appeared intact by electron microscopy. It is important to note that the release of some anti-inflammatory mediators does not depend on degranulation.

Although we cannot conclude that leukocyte trafficking in the mesentery is directly related to swelling in the joints, an accumulation of intravascular leukocytes has been observed in joint venules several days before the infiltration of leukocytes into the joint synovium. This suggests that vasculitis is common to both tissues. Another feature common to both mesenteric vasculitis and joint arthritis is an apparent role for mast cells; increases in mast cell number and activation have been observed within the synovial tissues in experimental arthritic and human rheumatoid arthritis. Our data demonstrate that unlike vasculitis, which could be attenuated only by mast cell stabilization during the early time point, joint swelling could be reduced by cromolyn treatments on days 1 to 4 or 9 to 12. This suggests different patterns of mast cell activation in these two tissues. In contrast to the early mast cell activation we observed in the mesentery, Gryfe et al. did not observe mast cell degranulation in the joint until days 10 to 11 after immunization. However, the observation that mast cell numbers in the joint were significantly elevated by day 5 after immunization may support an earlier time point of mast cell involvement, as mast cell activation is a potent stimulus for mast cell proliferation. Another noteworthy difference between the joint and mesentery is that the late mast cell activation in the joint is associated with a massive leukocyte infiltration (emigration) into the synovium 10 to 12 days after adjuvant administration. In the mesentery, we observed few emigrated leukocytes and little mast cell activation at day 12. It is possible that a later stage of mast cell degranulation in the joint is important in the initiation of joint-specific leukocyte recruitment and injury. This may be due to the presence of an inflammatory mediator that is absent in the mesentery and may be related to the fact that the joint bears weight and is exposed to a range of movements, whereas the mesentery is relatively stable. Reports have shown that joint immobilization will reduce leukocyte infiltration, supporting a role for joint motion in the inflammatory stage of adjuvant arthritis.

Another important difference between the joint and the mesentery in this study was the requirement for exteriorization and surgical preparation of the mesentery. We have previously demonstrated that surgical manipulation causes a subtle activation of mast cells that increases leukocyte rolling flux from approximately 10 to 30 cells/minute. However, this alone cannot explain the very significant increase in mast cell activation and the 200 to 300 rolling cells observed by 4 days after adjuvant administration, as mast cell stabilization immediately before surgery did not affect leukocyte trafficking, and mast cell degranulation was observed in tissue samples that were fixed just before exteriorization. Moreover, the increased reactivity of neutrophils and mast cells in the in vitro experiments also argues against surgical preparation modulating this response. Nevertheless, the possibility that exteriorization of the mesentery enhances vasculitis cannot be discounted.

Although it seems clear that the mast cell is not the only factor that contributes to adjuvant-induced vasculitis and arthritis (sequelae were reduced only 50% by mast cell stabilization protocols), the data clearly support a significant role for mast cells in the pathogenesis of this disease. Other contributing factors may include increased leukocyte responsiveness to stimuli as evidenced by the ability of peripheral blood neutrophils from M. butyricum-immunized rats, but not control rats, to migrate toward mast cells isolated from day 4 rats. Leukocyte priming is consistent with reports of increased effector functions of leukocytes isolated from peripheral blood of patients with rheumatoid arthritis. It is also possible that disseminated mycobacterial antigens may play a role in activating intravascular leukocytes. This may account for some of the leukocyte trafficking we observed even in the presence of mast cell stabilization. These factors clearly require further examination.

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