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Mast Cell Degranulation Alters Lymphatic Contractile Activity Through Action of Histamine

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ABSTRACT

Objective: Mast cells reside in most tissues and in close association with blood vessels and nerves, areas where lymphatic vessels are also present. Mast cells and lymphatic vessels are two important players in the development of the inflammatory process. This study was designed to examine the effects of mast cell degranulation on the contractile activity of mesenteric lymphatic vessels.

Methods: Lymphatic vessel contractile activity was assessed in vitro by video microscopy of the mesentery of cow's milk-sensitized guinea pigs upon application of β-lactoglobulin and compared to the response measured in sham animals.

Results: Application of 5–10 μM β-lactoglobulin increased lymphatic vessel constriction frequency and decreased constriction amplitude ($n = 12$). This effect was not seen in sham-treated animals ($n = 16$) and was not due to an increased number of mast cells in the mesentery of the milk-sensitized animals, as revealed by histological examination. Two known mast cell-derived mediators, histamine and thromboxane A_2, via stable mimetic U46619 also altered lymphatic pumping in a similar manner, but only pretreatment with the histamine H_1 receptor antagonist pyrilamine (1 μM) could reduce the β-lactoglobulin-induced response. The thromboxane A_2 receptor antagonist, SQ 29548, and the 5-lipoxygenase inhibitor, caffeic acid, were without significant effect.

Conclusion: In the in vitro mesenteric preparation, mast cell degranulation altered lymphatic contractile activity via the release of a mediator suggested to be histamine and the subsequent activation of H_1 receptors. This action could potentially interfere with the expected ability of lymphatic vessels to reduce edema during inflammation.
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KEY WORDS: β-lactoglobulin, inflammation, lymphatic vessel, video-microscopy

The lymphatic system is essential for preservation of fluid homeostasis within tissues. It achieves this function through a network of fine lymphatic vessels, which extends throughout the body and actively pumps excessive interstitial fluid away from tissues. Alteration of this lymphatic pumping function may lead to edema, which is also a consequence of increased vascular permeability observed during inflammation. Importantly, many inflammatory mediators, which may gain entry to or are present within the environment of lymphatic vessels, have been shown to regulate lymphatic contractile activity (reviewed in 16). Modulators of lymphatic pumping may increase or decrease the frequency and/or amplitude of constrictions, resulting in altered drainage of lymph. Mast cells are present in the mesentery, an area where lymphatic vessels are densely distributed. Mast cells play an important role in the vascular response during inflammation (4,8,19). When activated, mast cells release mediators, such as histamine and serotonin, stored in preformed granules, and simultaneously synthesize new ones, including prostaglandins and leukotrienes.

Although the importance of both mast cells and lymphatic vessels is appreciated during inflammation, whether mast cell activation may alter lymphatic

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pumping has not been investigated. In the present study, we used a milk-sensitized guinea pig model to assess in vitro the effect of mast cell degranulation within the mesentery of the small intestine on lymphatic vessel contractile activity.

METHODS

Animal Sensitization

Guinea pigs were sensitized to the cow's milk protein β-lactoglobulin as described by others (1,2). Male guinea pigs (5–7 days of age) were fed with cow's milk ad libitum instead of water and dry guinea pig chow supplemented with vitamin C for 21 days. After the 21-day period, the animal drinking supply was switched to water for 3 days. Age-matched control animals were given water for the entire period.

Tissue Preparation

Animals were sacrificed by decapitation during deep anesthesia induced by inhalation of halothane. This procedure has been approved by the University of Calgary Animal Care and Ethics Committee and conforms to the guidelines established by the Canadian Council on Animal Care. The small intestine with its attached mesentery was rapidly dissected and placed in a physiological saline solution (PSS) of the following composition (mM): $CaCl_2$, 2.5; KCl, 5; $MgCl_2$, 2; NaCl, 120; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose, 11. The pH was maintained at 7.4 by constant bubbling with 95% O_2/5% CO_2.

Vessel Constriction Measurements

Lymphatic tissue was prepared as previously described (9,17). Briefly, a small piece of mesentery (1–2 cm^2) containing collecting lymphatic vessels (diameter < 350 μm) together with associated artery and vein was dissected from the jejunal or ileal regions. The mesentery was used to pin out the tissue on the Sylgard-coated base of a 2-mL organ bath. The bath was mounted on the stage of an inverted microscope (CK40, Olympus) and continuously superfused at a flow rate of 3 mL min^{-1} with PSS heated to 36°C. To induce a consistent rate of vessel constrictions, the vessel lumen was perfused through a fine glass micropipette inserted into a cut opening of the vessel, across the first valve. This procedure provided a reasonably tight seal around the perfusion cannula, as no leakage at the perfusion site could be visualized using dye in the perfusate (von der Weid, unpublished observation). Thus, under these conditions, most of the perfusion fluid flows through the vessel. The cannula was connected to an infusion pump via Teflon tubing allowing the vessel lumen to be perfused in the direction of the valves at a flow rate of 2.5 μL min^{-1}. This flow rate was very reliable in inducing a regular rhythmical contractile activity in lymphatic vessels in the range of diameters used in the study (8). At this perfusion rate the vessel constricted at about 80% of its maximum rate for the duration of the experiment (typically 3–4 h). As the Ca^{2+} concentration in normal PSS tended to block the cannula, a low-calcium solution, in which 0.3 mM $CaCl_2$ was substituted for 2.5 mM was used for vessel perfusion. This solution did not alter vessel contractile activity or endothelial responsiveness (9,17).

Lymphatic vessel chambers or lymphangions were observed by video microscopy, with diameter changes and constriction frequency continuously measured with a video dimension analyzer (Model V94, Living Systems Instrumentation, Burlington, VT). This device, designed to sense the optically denser wall of the vessel, at a chosen scan line seen on the monitor, followed any change in vessel diameter with a rapid (<20-ms) time resolution. Data were then recorded on a computer via an analog-to-digital converter (PowerLab/4SP, ADInstruments, Mountain View, CA). Preparations were allowed a 30-min equilibration period prior to the first agonist application.

β-Lactoglobulin or other agonist treatments were performed only on vessels with a consistent pumping frequency of at least 4–5 constrictions/min during the minimal 30-min equilibrium period. A 5-min control period of contractile activity was recorded prior to the addition of β-lactoglobulin or other agonists. β-Lactoglobulin was added only once to each mesenteric lymphatic preparation, as a response could not be elicited with additional applications (1). When used, inhibitors were present for at least 15-min in the superfusion before the preparation was challenged with β-Lactoglobulin or other agonists. For each drug application, experimental traces were analyzed for the 5 min preceding the treatment (control), the 5-min treatment period, and 5 min of washout. Constriction frequencies, diastolic and systolic diameters, and amplitudes of constrictions were obtained from the diameter tracings. Indices for stroke volume and flow of lymph through a section of a mesenteric lymphatic vessel were calculated according to Benoit et al. (3). Time course histograms were expressed as a percentage of the mean of the 5-min control

value (min 1–5). Vessel pumping parameters were also assessed by comparing the mean rate reached during the 3 min showing the greatest response to β-lactoglobulin application (min 7–9) to the mean of the 5-min control period.

Mast Cell Detection and Staining

Whole-mount preparations of mesentery were fixed and stained with a solution of 50% ethanol, 5% acetic acid, 10% formaldehyde, and 2% toluidine blue followed by mounting in glycerol on poly-L-ornithine-coated slides (1,12). For each preparation (5 sham-treated, 5 sensitized), mast cell counts were performed at a magnification of 400× in 10 fields of view of 250 μm^2 each, where a lymphatic vessel was visible, and in 10 fields of view taken from the mesentery in the middle of the mesenteric arcades where no lymphatic vessel was in view (i.e., at least 300 μm away from the closest lymphatic vessel).

Chemicals and Drugs

Caffeic acid, β-lactoglobulin, pyrilamine, SQ 29548, ketotifen, and cimetidine were obtained from Sigma-Aldrich (Oakville, ONT, Canada). Histamine was purchased from ICN (Costa Mesa, CA) and U46619 from Cayman Chemical (Ann Arbor, MI). Drugs were dissolved in deionized, distilled water to give 10 mM stock solutions. U46619 was diluted in ethanol from the solution provided by the manufacturer to a 1 mM stock solution. Stock solutions were then diluted in PSS to achieve the appropriate concentration. The final concentration of ethanol was always $\leq$0.1% (v/v), a concentration that had no effect on lymphatic contractile functions.

Statistical Analysis

Data are expressed as means ± one standard error of the mean (SE mean). Statistical significance was assessed using a two-tailed paired or unpaired Student's t test, or one-way analysis of variance (ANOVA) followed by a Tukey post hoc test (as specified in the text), with $p < .05$ being considered significant.

RESULTS

Detection of Mast Cells in the Guinea Pig Mesentery

Mast cells could easily be identified in whole-mount mesenteric preparation after exposure to toluidine blue (Figure 1). They were evenly distributed throughout the connective tissue, with some of them positioned along the lymphatic vessels. To determine if cow's milk sensitization affects the number of mast cells recruited within the mesentery and in the vicinity of the lymphatic vessels, in particular, mast cell counts were separated into fields of view containing or devoid of lymphatic vessels (see Methods). Counts of mast cell number did not reveal any significant difference between sham-treated and sensitized animals in area close to lymphatic vessels, with mast cell numbers of 27 ± 2 and 29 ± 1 per 250 μm^2, respectively. A difference was noted, however, in mesentery devoid of lymphatics between sham-treated and sensitized animals with 27 ± 1 and 33 ± 1 mast cells per 250 μm^2, respectively (Figure 1).

Effect of β-Lactoglobulin Challenge on Lymphatic Pumping Parameters

During intraluminal perfusion, mesenteric lymphatic vessels rhythmically constricted at frequencies of 8 ± 3 min^{-1}. Application of 5–10 μM β-lactoglobulin in the superfusate for 5 min increased the frequency and decreased the amplitude of the vessel constrictions ($n = 12$). Substantial variation in the extent of the response to β-lactoglobulin was noted among the different vessels, some responding moderately (Figure 2A), while others displayed strong responses with very rapid constrictions of small amplitude, comparable to vessel "fibrillation" (Figure 2B). Application of β-lactoglobulin to vessels from sham animals, which were never exposed to milk, did not cause changes in vessel contractile activity ($n = 16$) (Figure 2C). The changes in amplitude and frequency reached a maximum during the third and fourth minute of β-lactoglobulin application, and then decreased to return to control values in the subsequent 5–10 min (Figure 3A, B). The decrease in amplitude led to a decrease in the calculated stroke volume index (Figure 3C). As a result of the opposing changes in stroke volume and constriction frequency, the index of lymph flow was only marginally increased during β-lactoglobulin administration (Figure 3D).

Effect of Mast Cell Mediators on Lymphatic Pumping

Histamine and the stable thromboxane A_2 (TXA_2) mimetic U46619 increased lymphatic constriction frequency and decreased constriction amplitude in sensitized guinea pigs ($n = 4$), (Figure 4A, C). The histamine-induced increase in pumping was inhibited by the H_1 receptor antagonist pyrilamine

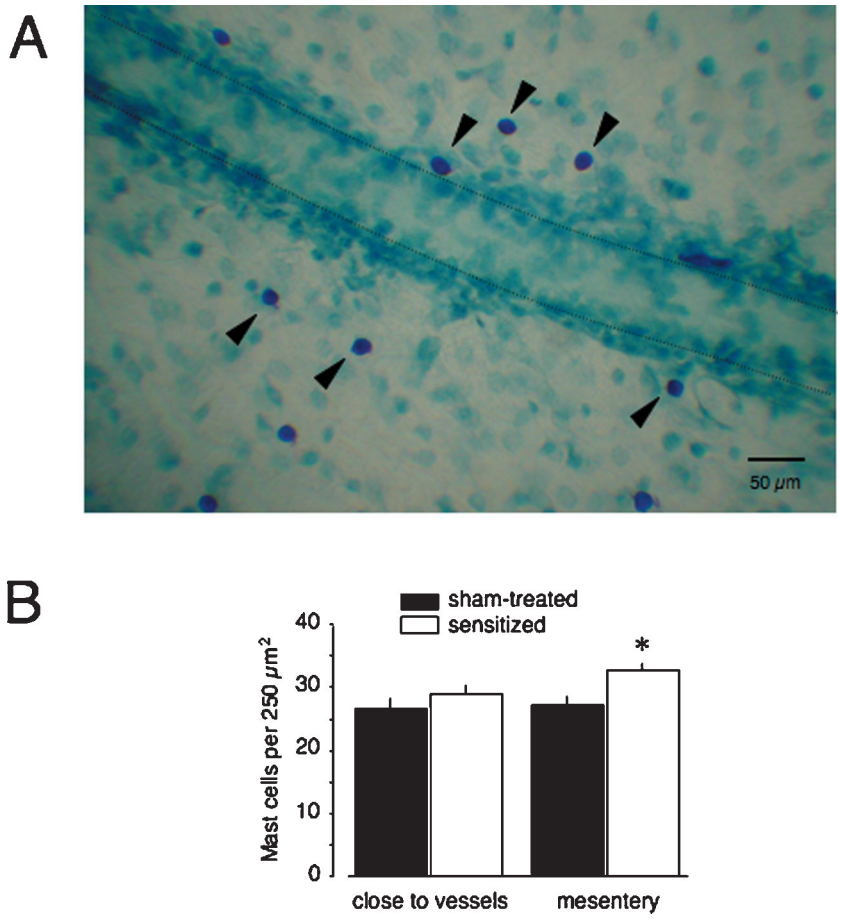

Figure 1. Detection of mast cells in the guinea pig mesentery. (A) Light micrograph showing the close relationship between mast cells stained with toluidine blue (arrowheads) and a lymphatic vessel, delineated by the dotted lines. (B) Numbers of mast cells detected in 250-μm^2 areas of the mesentery with (close to vessel) or devoid of lymphatic vessels (mesentery) in sham and sensitized guinea pigs. *$p < .05$ vs. sham (one-way ANOVA, with Tukey post hoc test).

(1 μM), while the response to U46619 was blocked by the thromboxane A_2 receptor antagonist SQ 29548 (1 μM) (Figure 4B, D).

In some vessels (10 out of 24), histamine administration induced only a weak increase, no change in contractile response, or even a decrease in activity in several cases. In addition, as illustrated in Figure 4B, administration of pyrilamine unmasked a histamine-induced decrease in constriction frequency. A similar observation was already reported in a previous study (9) and shown to be consequent to a concomitant action of histamine on H_2 receptors, causing a decrease in contractile activity that could be blocked by the H_2 receptor antagonist cimetidine. Indeed, in 8 vessels where histamine was shown to decrease constriction frequency in the present study, the response could be significantly blocked by cimetidine (30 μM, not shown). Thus, in most vessels, the overall resulting response to histamine was a combination of these two effects.

Effect of Inhibitors of Mast Cell Mediators on β-Lactoglobulin-Induced Changes in Lymphatic Pumping Parameters

To investigate which mediator was responsible for the β-lactoglobulin-induced response, β-lactoglobulin was administered to preparations superfused with the mast cell stabilizing drug and histamine antagonist ketotifen (30 μM) and with pyrilamine (1 μM). The response to β-lactoglobulin was strongly decreased in the presence of each of these blockers, with amplitude and frequency of constrictions not significantly different from control and different from the responses caused by β-lactoglobulin alone (Figure 5A,B). The calculated stroke volume index reflected

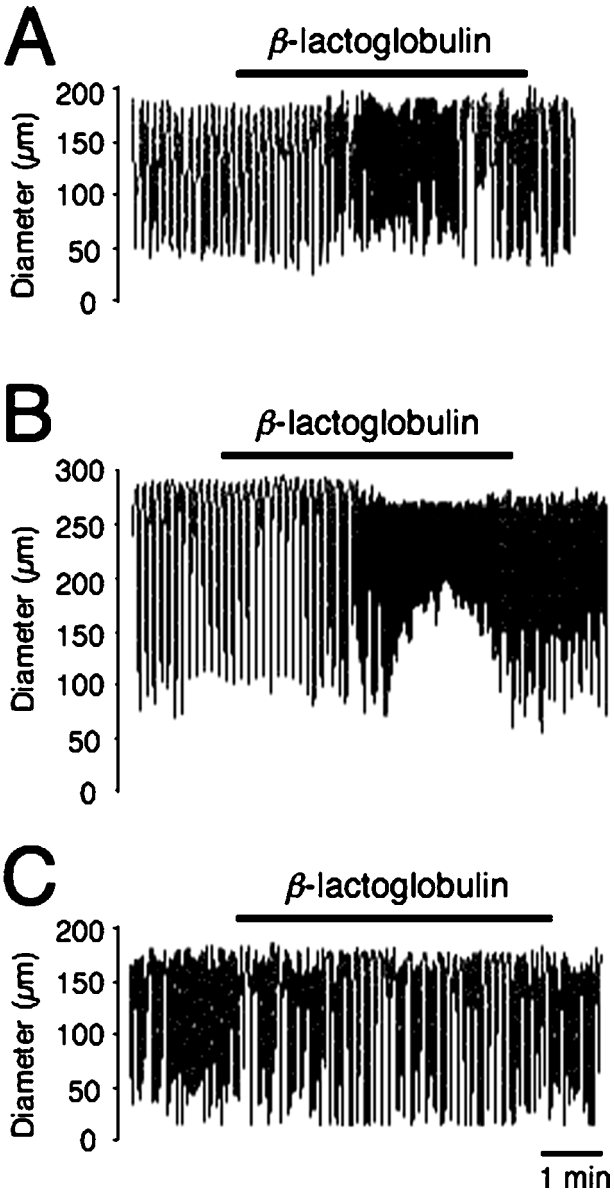

Figure 2. Effect of β-lactoglobulin on the contractile activity of perfused lymphatic vessels. Representative traces of vessel diameter changes, during administration of β-lactoglobulin (5 μM, horizontal bars), where downward deflections represent constrictions. β-Lactoglobulin caused a moderate (A) or strong (B) increase in the frequency and decrease in the amplitude of constrictions in the sensitized vessels, but had no effect in the vessel of the sham-treated animal (C).

changes in amplitude with the same inhibition trend (Figure 5C) and none of the blockers could affect calculated lymph flow, which as already presented was not significantly altered by the β-lactoglobulin administration (Figure 5D).

The role of TXA_2 was assessed next using SQ 29548. At a concentration of 1 μM, sufficient to inhibit U46619-induced response (see Figure 4C), SQ 29548 did not significantly inhibit the β-lactoglobulin-induced changes in pumping parameters (Figure 5). Similarly, addition of the 5-lipoxygenase inhibitor caffeic acid was ineffective in blocking β-lactoglobulin-induced response (Figure 5).

DISCUSSION

The cow's milk-sensitized guinea pig has been extensively used as a model of food allergy to study the functional interactions between mast cells and various tissues, such as the intestinal mucosa (2), submucosal arterioles (1), and neurons (15), as well as parasympathetic cardiac neurons (13). The lymphatic system extends to most parts of the body as a dense network of fine vessels. This widespread distribution suggests that, like blood vessels and nerves, lymphatic vessels are in close contact with mast cells. Such apposition suggests possible interactions between mast cells and lymphatic vessels, which may be of importance during the inflammatory process. In the present study we documented the presence of mast cells close to lymphatic vessels in the guinea pig mesentery and demonstrated that mast cell degranulation in response to an antigenic challenge with β-lactoglobulin in cow's milk-sensitized guinea pigs altered lymphatic contractile activity. This alteration of pumping was characterized by a marked increase in constriction frequency, accompanied by a decrease in constriction amplitude, which was particularly obvious at high constriction frequency. Importantly, lymph flow, calculated from these two antagonistic effects, appears to be unchanged. Furthermore, our pharmacological assessment suggests that the response to β-lactoglobulin is mediated in large part by histamine acting on H_1 receptors located in the lymphatic vessel wall.

As reported in earlier studies using the same sensitized animal model, only the initial application of β-lactoglobulin was able to evoke a response, probably reflecting a maximal release of mast cell mediators to the antigenic challenge (1). Response to β-lactoglobulin was totally absent in animals that were not sensitized to milk and was markedly inhibited by ketotifen, a mast cell stabilizing drug (10). These findings suggest an essential involvement of mast cells in the β-lactoglobulin-induced alteration of lymphatic contractile activity. This response is not due to an increased recruitment of mast cells close

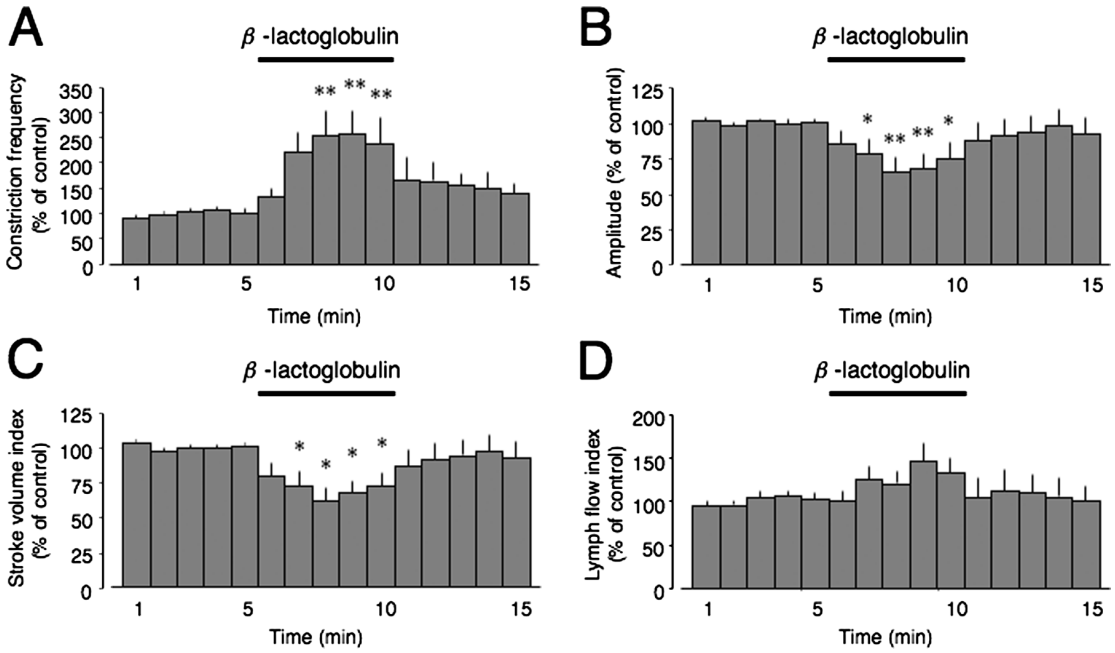

Figure 3. Effect of β-lactoglobulin on pumping parameters of mesenteric lymphatic vessels from cow's milk-sensitized guinea pigs. Time-course histograms summarizing the effect of β-lactoglobulin on constriction frequency (A), constriction amplitude (B), calculated indices of stroke volume (C) and lymph flow (D). Bars represent means ± SEM of 12 experiments. $^{*,**}p < .05$ and $p < .01$, respectively, vs. the mean of 5 min of control (paired Student's t test).

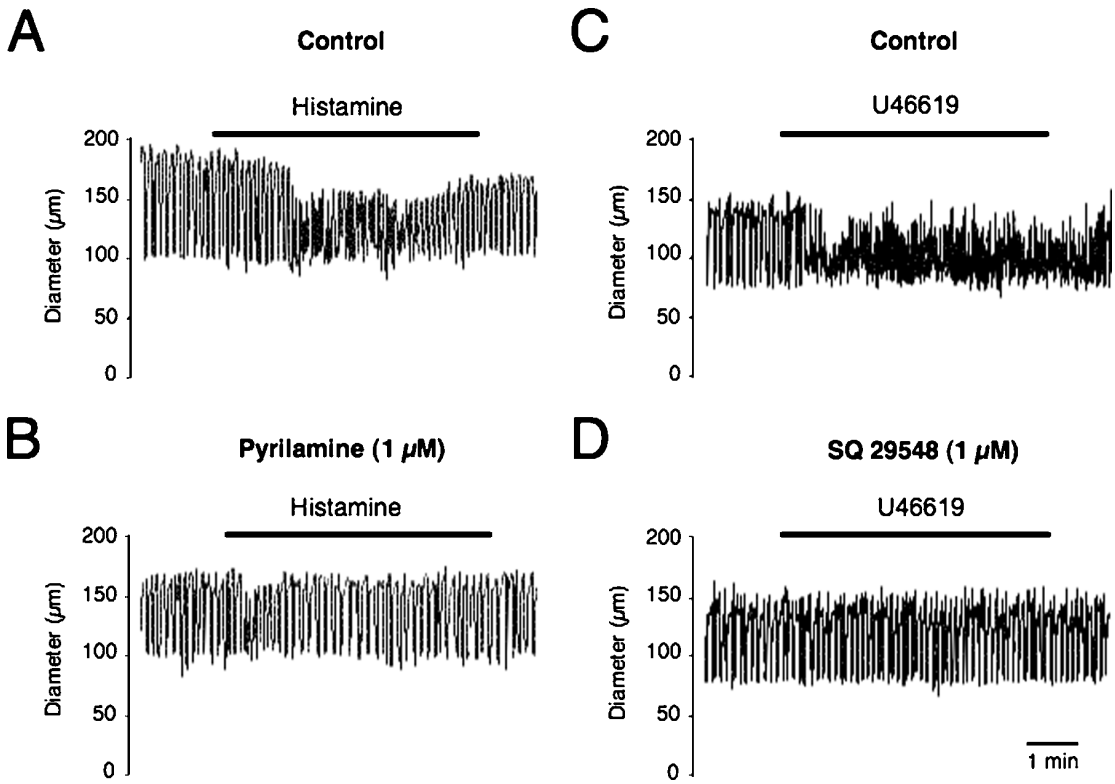

Figure 4. Effect of histamine and U46619 on the contractile activity of sensitized guinea pig lymphatic vessels. Representative traces of vessel diameter changes, during administration (horizontal bars) of histamine (1 μM), (A) and U46619 (0.1 μM), (C), and abolition of these responses in the presence of pyrilamine (B) and SQ29548 (D).

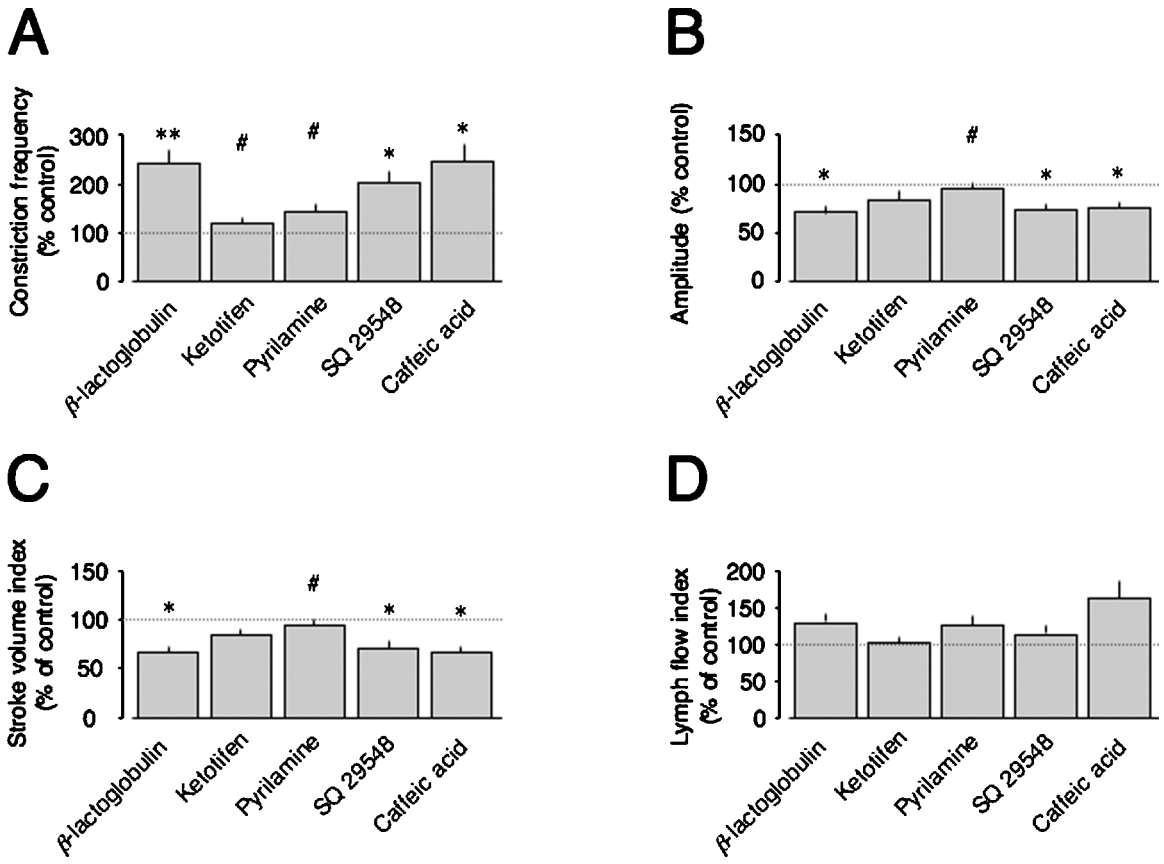

Figure 5. Effect of mast cell stabilizer and mast cell mediator blockers on the β-lactoglobulin-induced changes in pumping parameters in sensitized guinea pig lymphatic vessels. Summary histograms of changes in constriction frequency (A), amplitude (B), calculated indices of stroke volume (C), and lymph flow (D) in response to β-lactoglobulin in control conditions ($n = 12$) and in the presence of ketotifen ($n = 4$), pyrilamine ($n = 8$), SQ29548 ($n = 8$), caffeic acid ($n = 5$). Bars represent the mean ($\pm$ SEM) of the data obtained during the 3 minutes of maximum effect to β-lactoglobulin application. $^{*}p < .05$ vs. mean of 5 min of control (paired Student's t test) and $^{\#}p < .05$ vs. β-lactoglobulin responses in control conditions (one-way ANOVA, with Tukey post hoc test).

to the lymphatic vessels of sensitized animals, as the mast cell counts we obtained from sham-treated and sensitized animals were not different. Our interpretation is similar to that made by Atwood et al. (1) in the early characterization of this model.

Among the well-characterized preformed and membrane-derived mast cell mediators that are suggested to be released following β-lactoglobulin exposure, many have already been shown to directly affect lymphatic contractile function. We have demonstrated in earlier studies using the same guinea pig mesenteric preparation that 5-HT, PGI_2, and PGE_2 were potent inhibitors of lymphatic pumping (6,7). On the other hand, U46619, a TXA_2 mimetic, and histamine were shown to increase lymphatic constriction frequency (9,18), making histamine and thromboxanes likely candidates for the mediation of the β-lactoglobulin-induced response. The action of these mediators has also been reported in other lymphatic vessels from various species (reviewed in 16). Leukotrienes (B_4, C_4, and D_4), another family of mast cell mediators, have been reported to be amongst the most potent activators of pumping in bovine mesenteric lymphatics (11). However, their effects on guinea pig lymphatic vessels have not been reported. U46619 was shown to increase pumping in lymphatic vessels of cow's milk-sensitized animals, a response inhibited by SQ 29548. However, SQ 29548 did not significantly decrease β-lactoglobulin-evoked response, suggesting a minimal, if any, role for thromboxanes in this response. Likewise, leukotrienes were not thought to be involved, as the 5-lipoxygenase inhibitor caffeic acid failed to block or significantly

decrease the response. Although partial involvement of these arachidonic acid derivatives could not totally be ruled out, the present data suggest a predominant role for histamine in the β-lactoglobulin-evoked alteration of lymphatic pumping, as this response was strongly inhibited by the H_1 receptor antagonist, pyrilamine. The finding that ketotifen potently inhibited the response may also support this interpretation, as ketotifen has also been described as a H_1 receptor antagonist (reviewed in 10). Involvement of histamine in mast cell-mediated actions in the cow's milk-sensitized guinea-pig is consistent with reports by Powers et al. (14), which described a histamine-mediated, mast cell-dependent stimulation of intracardiac neurons, and by Atwood et al. (1), which observed submucosal arteriolar dilations mediated by the same mechanism. In further support of the role of histamine in mast cell-mediated actions, a study by Bytautiene et al. (5) reported that challenge with the mast cell degranulating agent 48/80 stimulated cervical tissue contraction via activation of H_1 receptors in pregnant and nonpregnant female guinea pigs.

Determination of the location of the H_1 receptors, as well as the signaling pathway(s) activated by these receptors in lymphatic vessels, were issues beyond the scope of the present study. However, we have previously reported (9) that the H_1 receptors were located on the smooth muscle and proposed that their activation led to an increase in lymphatic pumping via the phospholipase C/inositol–trisphosphate pathway, Ca^{2+} release from intracellular Ca^{2+} stores and increase in cytosolic Ca^{2+}, an action similar to the response of H_1 receptors to histamine in most smooth muscle examined (13). In the same study, we also showed that in addition to H_1 receptors, about 50% of the guinea pig mesenteric lymphatic vessels possessed H_2 receptors, as they responded to dimaprit application with a decrease in the perfusion-induced constriction rate. This finding was confirmed in the present study, where we observed that some vessels experienced a decrease in pumping in response to histamine that could be blocked by cimetidine, while others exhibited a weak histamine-induced activation in constriction rate that was converted to a decrease in pumping in the presence of pyrilamine. Intriguingly, a decrease in pumping was rarely observed in response to β-lactoglobulin, even in the presence of H_1 antagonists.

These observations made in both sensitized and control animals suggest no obvious changes in histamine receptor expression following cow-milk sensitization. The wide range of lymphatic contractile responses to histamine could explain the important variation in the responses to β-lactoglobulin observed in the sensitized vessels. Such disparity could also be a consequence of a variable number of mast cells in the vicinity of a particular vessel or the extent of degranulation these mast cells had achieved upon β-lactoglobulin challenge. However, this suggestion could not be verified in our study.

In conclusion, we have demonstrated a mast cell-dependent, histamine-mediated alteration of lymphatic contractile activity in response to antigenic challenge in the cow's milk-sensitized guinea pig. This finding is of importance with respect to inflammation, where both mast cell activation and lymphatic drainage play important roles. In light of our data, mast cell degranulation seems to interfere with the drainage capability of the lymphatic vessels, as despite the marked increase in constriction frequency elicited, the concomittant decrease in amplitude results in a calculated lymph flow not significantly increased. Consequently, the lymphatic system may not be able to drain away the augmented interstitial fluid experienced during inflammation. Moreover, under very high stimulation, when strong and sustained vessel constrictions ("fibrillation") occur, fluid drainage is expected to be even lower, resulting in a failure to reduce inflammation-associated edema.

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