Role of Mast Cells in Ion Transport Abnormalities Associated with Intestinal Anaphylaxis

Correction of the Diminished Secretory Response in Genetically Mast Cell-deficient W/W^v Mice by Bone Marrow Transplantation

Mary H. Perdue,* Stephen Masson,* Barry K. Wershil,‡§ and Stephen J. Galli‡

**Intestinal Disease Research Unit and Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5; ‡Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215; and §the Combined Program in Pediatric Gastroenterology and Nutrition, Harvard Medical School, Boston, Massachusetts 02215*

Abstract

To investigate the role of mast cells in transport abnormalities during intestinal anaphylaxis, we examined responses to antigen in isolated intestinal preparations from ovalbumin-sensitized genetically mast cell-deficient WBB6F$_1$-W/W^v (W/W^v) mice and congenic normal WBBGF$_1$-+/+ (+/+) mice. Changes in ion transport (primarily secretion of chloride ions) were indicated by increases in short-circuit current (Isc). In tissues from +/+ mice, antigen caused increases in Isc which were significantly inhibited by antagonists to histamine (diphenhydramine) and serotonin (ketanserin), by a cyclooxygenase inhibitor (piroxicam) and by a neurotoxin (tetrodotoxin). In preparations from W/W^v mice, antigen-stimulated responses were ~ 30% of that in +/+ mice and were inhibited only by piroxicam. Responses to electrical transmural stimulation of nerves were ~ 50% in W/W^v versus +/+ mice, and were inhibited by antagonists of mast cell mediators in +/+ but not W/W^v mice. Reconstitution of mast cells in W/W^v mice by intravenous injection of +/+ bone marrow cells restored the normal responses to both antigen and nerve stimulation. Our results indicate that mast cell-dependent mechanisms are primarily responsible for the ion secretion associated with intestinal anaphylaxis, but that other cells are also involved. In addition, our data provide evidence for the functional importance of bidirectional communication between nerves and mast cells in the regulation of ion transport in the gastrointestinal tract. (*J. Clin. Invest.* 1991. 87:687–693.) Key words: intestinal epithelium • hypersensitivity • mast cells

Introduction

Anaphylactic reactions affecting the gastrointestinal tract are associated with significant changes in intestinal transport (1–3). Several lines of evidence indicate that mast cells are a critical source of mediators responsible for the functional abnormalities (reviewed in reference 2). IgE is clearly essential to the pathogenesis of certain anaphylactic reactions (4), and mast cells express surface receptors that bind IgE with high affinity (4, 5). Upon exposure to specific bivalent or multivalent antigen, IgE-primed mast cells release a variety of mediators that theoretically are capable of altering intestinal transport (6, 7). For example, anaphylaxis elicited in vivo results in release of mediators from mast cells, some of which can be detected systemically (8), and is associated with abnormal transport of fluid and electrolytes (1, 9, 10).

The potential importance of mast cell mediator release in the production of intestinal transport abnormalities is supported by pharmacological studies with drugs that influence either mast cell activation or the end organ effects of mast cell-associated mediators (9, 11–14). Recent morphological, pharmacological, and electrophysiological evidence indicates that interactions between enteric nerves and mast cells may importantly participate in some of these responses (11, 13–16). Finally, in vitro studies have demonstrated that chloride ion secretion is the force responsible for the changes in fluid and electrolyte transport observed during intestinal anaphylaxis (17–19) and that activated mast cells or mast cell lysates can induce secretion from intestinal epithelial cells maintained in tissue culture (20, 21).

However, even taken in aggregate, such evidence does not constitute proof that mast cells are responsible for the transport changes associated with intestinal anaphylactic responses. A variety of cell types that can elaborate mediators similar or identical to those of mast cells, such as basophils, monocytes/macrophages, platelets, and eosinophils (22, 23), can also bind IgE or aggregates of IgE (23). In addition, genetically mast cell-deficient mice can express systemic anaphylactic responses resulting in death after appropriate sensitization and challenge with specific antigen (24–26). At least some of these anaphylactic responses have been shown to be IgE dependent (26). Finally, it has been shown that the intestinal tissues of genetically mast cell-deficient and congenic normal mice develop indistinguishable spasmogenic responses upon challenge with a wide variety of agonists, including the anaphylatoxin C5a that can induce mast cell mediator release in parallel with the spasmogenic responses (27). Taken together, findings such as these raise the possibility that at least some of the physiologic changes in the intestine of mice experiencing anaphylactic reactions may occur independently of the mast cell activation associated with these responses.

To examine more directly the actual contribution of mast cells to the secretory changes associated with intestinal anaphylaxis, we tested intestinal tissues derived from genetically mast cell-deficient WBB6F$_1$-W/W^v (W/W^v)[1] mice and the congenic

Address correspondence and reprint requests to Dr. Mary Perdue, Intestinal Disease Research Unit, HSC-3N5C, McMaster University, 1200 Main Street, Hamilton, Ontario, Canada L8N 3Z5.

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1. *Abbreviations used in this paper:* BM, bone marrow; G, conductance; Isc, short-circuit current; NSE, neuron-specific enolase; OA, ovalbumin; W/W^v or +/+, WBB6F$_1$-W/W^v mice or WBB6F^1-+/+ mice.

normal WBB6F$_1$-+/+ (+/+) mice (28–30). *W/W^v* mice exhibit a profound mast cell deficiency as a result of mutations involving the putative tyrosine kinase receptor *c-kit* (31–33). The skin of adult *W/W^v* mice contains < 0.3% the number of mast cells as the congenic +/+ mice, and no mast cells at all (of either the "connective tissue" or "mucosal" type) are observed in the peritoneal cavity, the gastrointestinal tract, or multiple other anatomical sites of *W/W^v* mice (28, 29). However, the mast cell deficiency of the *W/W^v* mice can be repaired by intravenous injection of normal bone marrow cells derived from the congenic +/+ animals (28, 29). Notably, *W/W^v* mice have normal levels of circulating platelets (29), basophils (24), and other leukocytes (29), and are not defective in their ability to generate an IgE response to appropriate sensitization (25).

A recent preliminary report indicated that intestinal preparations from *Trichinella*-infected *W/W^v* mice may be deficient in their ability to express ion transport changes in response to parasite antigen (34). However, no data were presented to indicate whether this was due to the mast cell deficiency of the *W/W^v* mice or reflected other genetically determined abnormalities. In this study, we compared ion transport parameters and responses to antigen in isolated segments of small intestine obtained from sensitized genetically mast cell-deficient *W/W^v* and congenic normal +/+ mice. We found that *W/W^v* mice did express detectable responses to antigen challenge, but that their response was only ~ 30% that of the congenic +/+ animals. In addition, absence of mast cells resulted in reduced responses to electrical transmural stimulation of intestinal nerves. Similar abnormalities were present in another mast cell-deficient mutant mouse, WCB6F$_1$-*Sl/Sl^d* (*Sl/Sl^d*), which has mast cell deficiency as a result of mutations and a mechanism distinct from those responsible for the mast cell deficiency of *W/W^v* mice (29, 30). Importantly, the reduced responses observed in *W/W^v* mice were normalized in *W/W^v* mice that had undergone reconstitution of their mast cell populations after injection of bone marrow cells from the congenic normal mice.

Methods

Animals. Mast cell-deficient (WB/ReJ-*W*/+ × C57BL/6J-*W^v*/+)F$_1$-*W/W^v* mice (28) and (WB/ReJ-*Sl*/+ × C57BL/6J-*Sl^d*/+)F$_1$-*Sl/Sl^d* mice (29, 30) and the congenic normal (+/+) mice (WBB6F$_1$-+/+ or WCB6F$_1$-+/+ mice, respectively) were obtained from Jackson Laboratories, Bar Harbor, Maine. They were housed in separate quarters under filter hoods and were allowed free access to food and water. Experiments were conducted when the mice were ~ 5–8 mo of age. Mast cells in *W/W^v* mice were reconstituted by intravenous injection with 2×10^7 bone marrow (BM) cells from +/+ congenic controls (28, 29) and studies were conducted 10 wk later. Such mice are designated +/+BM → *W/W^v* mice.

Sensitization. Mice were sensitized with 0.1 mg chicken egg albumin (ovalbumin OA, grade V; Sigma Chemical Co., St. Louis, MO) in 0.2 ml alum solution and 0.1 ml of *Bordetella pertussis* vaccine (2×10^9 organisms) injected intraperitoneally (14). The mice were studied 12–15 d after sensitization.

Ussing chambers. Mice were killed by cervical dislocation. Segments of mid small intestine were removed and placed in oxygenated 37° Krebs buffer (35). The segments were opened along the mesenteric border and cut into sheets ~ 2 cm in length. Up to five adjacent sheets from each animal were mounted in flux chambers that had been modified to contain Ag/AgCl stimulating electrodes on opposite sides of the tissue (35). The chamber opening was rectangular exposing 0.6 cm^2 of serosal surface area to Krebs buffer. Glucose (10 mM) was included in the serosal buffer and mannitol (10 mM) in the mucosal buffer. When chloride-free buffer was used, isethionate and acetate ions were substituted for chloride (36). Agar-salt bridges were used to monitor PD and to inject current. The tissues were short-circuited at zero volts using a W-P Instruments automatic voltage clamp (Narco Scientific, Downsview, Ontario, Canada). The short-circuit current (Isc) was recorded continuously. PD was recorded at 10-min intervals during the experiment. Conductance (G) was calculated using values of Isc and PD. Tissues were allowed to equilibrate for at least 15 min until the Isc was stable before any manipulation was undertaken.

Response to antigen. We measured the Isc increase after administration of OA, 100 μg/ml, on the serosal side of the tissue. We had determined in preliminary experiments that this concentration produced the maximal change. OA added to the luminal side also caused the Isc to increase, but this response was smaller and less consistent. Responses to serosal antigen challenge were calculated as the difference between the basal Isc and the early peak Isc within 5 min after challenge (phase I) and as the difference between the basal Isc and the sustained elevation of Isc that was measured 15 min after challenge (phase II).

Response to nerve stimulation. Electrical transmural stimulation was used to stimulate intestinal neurons in the preparation. Rectangular current pulses (10 Hz, 10 mA, 0.5 m) were passed across the tissue in a perpendicular direction for a total time of 5 s (35). Current was delivered from a Grass 88 stimulator via a current isolator of the optical type (Grass Instruments, Quincy, MA). The direction of the stimulating current was alternated in successive stimulations to avoid polarization of the electrodes. The response, the change in Isc between the basal Isc and the maximal Isc after stimulation, was measured after the current was turned off (35). Under these conditions maximal responses were produced that were reproducible.

Effect of inhibitors. Effects of the following inhibitors added to the serosal side of the chambers at least 15 min before OA or transmural stimulation on changes in Isc were examined: the H_1 anti-histamine, diphenhydramine, at 10^{-5} M (Sigma Chemical Co.); the 5-HT$_2$ serotonin antagonist, ketanserin, at 10^{-5} M (Janssen Pharmaceuticals, Belgium); the cyclooxygenase inhibitor, piroxicam, at 10^{-5} M (Squibb, Princeton, NJ); and the neurotoxin, tetrodotoxin, at 10^{-6} M (Sigma Chemical Co.). At these doses, we found in preliminary experiments that diphenhydramine blocked the Isc increases to histamine and tetrodotoxin blocked the Isc increases to transmural stimulation. Ketanserin and piroxicam were used at doses previously found to inhibit Isc responses to antigen or anti-IgE in preparations of rat intestine (13, 14, 37).

Histology. Segments adjacent to those taken for the chamber studies were removed, opened along the mesenteric border, flattened and stapled on a card and placed in fixative. Carnoy's fixative was used for tissues that were processed and stained with toluidine blue for the detection of mast cells. Sections for identification of nerves were fixed in 10% acetic acid/90% ethanol, sliced in 1-mm strips which were embedded in paraffin mucosal face down, and cut (3 μm) horizontally through the villi. Neuron-specific enolase (NSE) was detected in mucosal nerves by immunohistochemistry as described (16). The primary antiserum was rabbit anti-NSE, secondary was swine anti-rabbit immunoglobulin, tertiary was rabbit peroxidase-antiperoxidase complex. All antisera were obtained from Dako Corp., (Santa Barbara, CA).

Statistics. Statistical analyses were performed using analysis of variance and Student's paired or unpaired t test where appropriate.

Results

Basal parameters. As shown in Table I, no significant differences were apparent in the basal parameters of Isc and G for intestinal preparations from sensitized *W/W^v* mice, congenic normal +/+ mice, or +/+ bone marrow-reconstituted *W/W^v* (+/+BM → *W/W^v*) mice. During the course of a 90-min experiment, all preparations exhibited a slight decline in the Isc, but G remained constant.

Table I. Basal Parameters for Short-Circuit Current and Conductance

	Control (+/+)	W/W^v	+/+BM → W/W^v
n	43	26	14
Isc ($\mu A/cm^2$)	24.0±2.4	26.2±2.5	26.1±2.8
G (mS/cm^2)	23.9±1.5	18.8±1.6	19.1±1.8

Values represent the means±SEM; *n* indicates numbers of mice. Short-circuit current (*Isc*) and conductance (*G*) were recorded at equilibrium, ~ 15 min after beginning the experiment.

Responses to antigen in +/+ and W/W^v mice. In intestine from sensitized +/+ control mice, the Isc response to the specific antigen, OA, was biphasic. As shown in Fig. 1 *A*, the Isc began to rise within 50–70 s after addition of OA and reached a peak within 4 min (phase I). Thereafter, the Isc fell slightly, sometimes increasing to a second peak but always remaining elevated above the original baseline (phase II). There was no Isc change at all in response to challenge with an unrelated antigen, bovine serum albumin. Responses to OA were never present in unsensitized mice (data not shown). A response to OA was also apparent in intestine from W/W^v mast cell-deficient mice (Fig. 1 *B*) but the magnitude of the increase in Isc was significantly less, with phase I only ~ 30% of that in intestine from congenic normal (+/+) animals. The pattern and magnitude of the response to OA challenge in +/+BM → W/W^v mice (Fig. 1 *C*) was very similar to that observed in +/+ mice.

The effects of chloride-free buffer and of pharmacological agents on antigen-induced Isc responses in +/+ and W/W^v mice are shown in Table II. Chloride ions were necessary for the increase in Isc in +/+ mice; chloride-free buffer dramatically reduced both phase I and phase II of the response. In +/+ mice, the mast cell mediator antagonists, diphenhydramine and ketanserin, each produced significant inhibition of the first phase of the response, but neither affected phase II. By contrast, the cyclooxygenase inhibitor, piroxicam, significantly reduced both phase I phase II. Adding all three inhibitors in combination virtually eliminated both phases of the response. The neurotoxin, tetrodotoxin, caused a significant reduction in phase I of the response; phase II was not affected. The addition of tetrodotoxin to a mixture of the other three inhibitors did not reduce the response compared with using the three inhibitors without tetrodotoxin.

In W/W^v mice, elimination of exogenous chloride ions significantly reduced both phases of the response to OA, to levels similar to those in +/+ tissues bathed in chloride-free buffer (Table II). Piroxicam also significantly diminished both phases of the response in W/W^v intestine. However, in contrast to the results in +/+ intestine, diphenhydramine and ketanserin were without significant effect. An additional point illustrated in Table II, is that tetrodotoxin, by markedly reducing the intensity of phase I in +/+ intestine, eliminated the difference in the responses of +/+ and W/W^v tissues to OA challenge.

Responses to nerve stimulation in +/+ and W/W^v mice. In all tissues, transmural stimulation of nerves caused a rise in Isc which began within 2 s after the stimulating current was turned off, reached a peak at ~ 30–45 s, and returned to the original baseline within 3–5 min. In each tissue, the response was reproducible many times during the course of a 90-min experiment regardless of the direction of the stimulating current. As shown in Fig. 2 and Table III, the response in intestine from W/W^v mice was ~ 50% of that in intestine from congenic normal mice. The effects of inhibitors on these responses are shown in Table III. In +/+ mice, responses were abolished by tetrodotoxin (to 1.5±0.9 $\mu A/cm^2$) and reduced by the mast cell mediator antagonists, diphenhydramine and ketanserin; piroxicam had no effect. In W/W^v mice, only tetrodotoxin inhibited the response.

Because of the reduced responses to transmural stimulation in W/W^v mice, we counted NSE-stained nerve profiles in at least four well-cut cross-sectioned villi in each of six W/W^v mice and six +/+ controls. No obvious morphological or quantitative differences were observed (6.8±0.5 nerves per villus cross-section in +/+ mice versus 6.9±0.9 in W/W^v mice).

Responses in +/+ bone marrow-reconstituted W/W^v mice. The success of +/+ bone marrow reconstitution was established by documenting that the anemia of the W/W^v recipients was corrected, and by examination of histological sections of small intestine to confirm that mast cells were present. In fact, intestine from +/+BM → W/W^v mice contained more mast cells than intestine from +/+ mice, whereas no mast cells at all were detectable in intestine from W/W^v mice which did not receive bone marrow transplantation. Baseline parameters in intestine from mast cell-reconstituted W/W^v mice are shown in Table I. Responses to either OA or transmural stimulation were completely restored by bone marrow reconstitution (Fig. 2). A tracing showing the Isc response to OA challenge in one mast cell-reconstituted W/W^v mouse compared with one W/W^v and one +/+ mouse is shown in Fig. 1. Note the similarity between the response in the reconstituted mouse (Fig. 1 *C*) and that of the +/+ mouse (Fig. 1 *A*).

Responses in mast cell-deficient Sl/Sl^d mice. To determine if the defects in ion transport in the W/W^v mice were due to their mast cell deficiency rather than to some other consequence of their *W* mutations, we also examined responses in mast cell-deficient Sl/Sl^d mice. These mice have mutations at the *Sl* locus on chromosome 10 which are distinct from those of W/W^v mice at the *W* locus on chromosome 5 (29, 30). Sl/Sl^d mice have mast cell deficiency because of a defect in a microen-

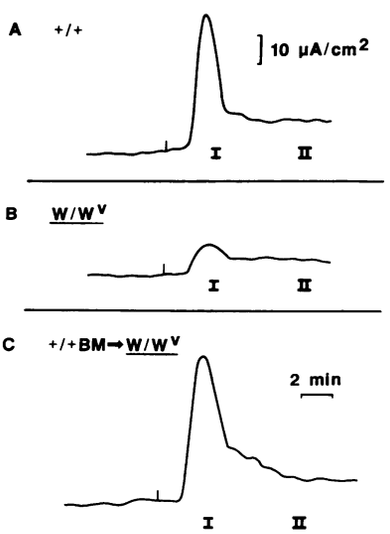

Figure 1. Representative tracings of Isc from a single intestinal preparation in each mouse group showing the pattern of the response to antigen (100 μg/ml OA), added at the vertical mark. (*A*) Response in intestine from sensitized +/+ congenic control mouse. (*B*) Response in intestine from sensitized W/W^v mast cell-deficient mouse. (*C*) Response in intestine from sensitized W/W^v mouse which had been reconstituted with mast cells by injection of bone marrow cells derived from congenic +/+ mice. Studies were conducted ~ 10 wk after intravenous injection of 2×10^7 cells.

Table II. Effect of Cl^--free Buffer and Various Pharmacological Agents on Short-Circuit Current (Isc) Responses to Antigen

	Δ Isc ($\mu A/cm^2$)			
	Control (+/+)		*W/W°*	
	Phase I	Phase II	Phase I	Phase II
none	43.1±4.2	11.0±2.5	11.9±2.0	9.0±1.8
Cl-free	8.7±2.4‡	3.9±2.8*	6.9±2.3*	1.4±1.7*
DPH	27.0±4.4*	10.2±2.3	11.9±2.2	7.5±3.0
KET	20.5±4.5*	10.2±2.3	11.5±3.1	7.5±2.3
PIR	24.9±2.7*	4.8±3.0*	3.4±2.0*	2.3±1.2*
DPH + KET + PIR	5.6±2.4‡	−0.3±0.8‡	2.6±3.0*	−0.5±1.5*
TTX	12.2±3.5‡	9.5±1.5	8.4±1.6	7.5±1.9
DPH + KET + PIR + TTX	4.4±1.0‡	0.6±0.6‡	0.3±0.5‡	0.4±0.5‡

Values represent the means±SEM. At least four tissues were obtained from each sensitized mouse; one tissue was untreated (none) and each of the others was subjected to a different treatment (preincubated with an inhibitor for at least 15 min before addition of OA [100 μg/ml] or incubated in chloride-free buffer instead of Krebs buffer). Diphenhydramine (*DPH*), ketanserin (*KET*), and piroxicam (*PIR*) were used at 10^{-5} M; tetrodotoxin (*TTX*) was used at 10^{-6} M. The phase I response was the maximal change in Isc within the first 5 min after addition of OA; the phase II response was the sustained elevation in Isc measured at 15 min after addition of OA. n = 18 for controls; 5–11 for each treatment; * $P < 0.05$, ‡ $P < 0.01$ compared with untreated "none" tissues.

vironmental factor, perhaps the yet unidentified ligand for *c-kit*, required for normal mast cell maturation (30). Therefore, the mast cell deficiency of *Sl/Sl^d* mice cannot be repaired by adoptive transfer of congenic +/+ bone marrow cells (reviewed in reference 29 and 30). In Table IV, we show that *Sl/Sl^d* mice exhibited abnormalities in ion transport which were virtually identical to those of *W/W^v* mice (compare with Fig. 2). Phase I of the antigen-induced response and the response to transmural stimulation were significantly reduced (but not completely eliminated) in *Sl/Sl^d* mice compared with the values in their +/+ congenic controls, whereas phase II of the antigen-induced responses were virtually identical in *Sl/Sl^d* and +/+ mice.

Discussion

Our studies provide convincing evidence that mast cells play an important role in altering intestinal ion transport during anaphylactic reactions to antigens. However, our results also indicate that mast cells are not the only cells involved. Finally, our findings support the concept that interactions between mast cells and nerves can contribute to the regulation of ion transport in the gut.

In intestine from control +/+ mice, antigen produced a biphasic increase in Isc, a response similar to that obtained in previous studies using rats sensitized to OA or in rats infected with *Trichinella spiralis* (11, 13, 17). The specificity of the response in OA-sensitized mice was demonstrated by the complete lack of reaction to an unrelated antigen, bovine serum albumin. Both phases of the response to OA were inhibited in chloride-free buffer. This finding was expected since net chloride secretion has been shown to be the driving force for accumulation of water and electrolytes in the lumen of rats during intestinal anaphylaxis (17, 18). Other studies have demonstrated inhibition of antigen-induced Isc increases by furosemide, an inhibitor of chloride secretion and by diphenyl-2-carboxyamine, a chloride channel blocker (12, 14, 19).

Mast cells mediate allergic reactions through the secretion of mediators triggered by antigen-dependent cross-bridging of IgE-antibodies bound to the cell surface (4). The mediators then act on target cells to cause alterations in physiology. In rat intestine, evidence has been provided for the involvement of histamine, serotonin, and prostaglandins in antigen-induced transport changes (13, 14). The changes are thought to occur by the effects of mast cell mediators acting directly on the epithelium and/or indirectly via stimulated intestinal nerves (13–15). In this study, phase I of the response in normal mice was significantly reduced by antagonists of two mast cell mediators, histamine and serotonin. The neurotoxin tetrodotoxin also inhibited the early phase of the response. None of these agents affected the second phase of the response. However, the cyclooxygenase inhibitor piroxicam significantly decreased phase I and abolished phase II. This result suggests a role for prostaglandins or thromboxanes in both phases, and indicates that phase II may be entirely dependent on cyclooxygenase products of arachidonic acid metabolism. Although some mast

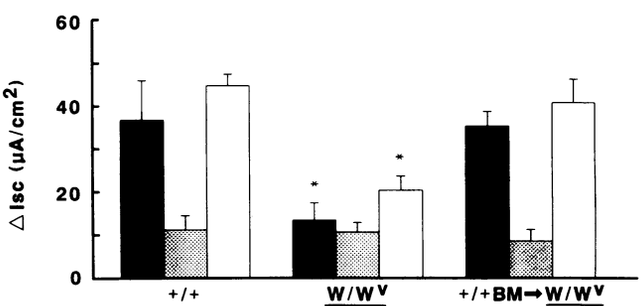

Figure 2. Effect of mast cells on short-circuit current (*Isc*) responses to antigen and transmural stimulation. Values represent the means±SEM; n = 43 for +/+, 26 for *W/W^v*, and 14 for bone marrow-reconstituted *W/W^v* (+/+BM → *W/W^v*) mice. The phase I antigen response (OA-I) (■) was the maximal change in Isc within the first 5 min after addition of OA; the phase II antigen response (OA-II) (■) was the sustained elevation in Isc measured at 15 min after addition of OA. The transmural stimulation (*TS*) (□) response was the difference between the peak Isc after transmural stimulation and the baseline value. *$P < 0.05$ compared with +/+ controls.

Table III. Effect of Various Pharmacological Agents on Short-Circuit Current (Isc) Responses to Transmural Stimulation

	Δ Isc ($\mu A/cm^2$)	
	Control (+/+)	W/W^v
none	67.8±4.7	41.6±3.6
DPH	52.4±4.0*	39.2±5.7
KET	38.8±5.0*	39.0±5.7
PIR	58.2±9.9	34.6±4.1
TTX	1.5±0.9‡	5.3±2.1‡

Values represent the means±SEM. At least four tissues were obtained from each sensitized mouse; one tissue was untreated (*none*) and each of the others was subjected to a different treatment (preincubated with an inhibitor for at least 15 min before transmural stimulation). Diphenhydramine (*DPH*), ketanserin (*KET*), and piroxicam (*PIR*) were used at 10^{-5} M; tetrodotoxin (TTX) was used at 10^{-6} M. The response was calculated as the difference between the peak Isc after transmural stimulation and the baseline value. $n = 16$ for untreated; 5–9 for each treatment; * $P < 0.05$, ‡ $P < 0.01$ compared with untreated tissues.

cells can themselves release prostaglandins upon appropriate stimulation (6, 38), the ability of mouse intestinal mast cells to produce cylooxygenase products has not been determined. Moreover, certain mast cell mediators and neurotransmitters are known to induce the generation of prostaglandins from other sources (39). While the cells responsible for such prostaglandin production have not yet been clearly identified, recent evidence implicates fibroblasts (40). In our studies, piroxicam treatment of W/W^v intestine resulted in inhibition of the antigen-induced increase in Isc, suggesting that mast cells are presumeably not the only source of these arachidonic acid metabolites in normal mice.

In sensitized W/W^v mast cell-deficient mice, the initial (phase I) response to antigen was reduced to ~ 30% of that in the congenic normal mice. Surprisingly, the response to transmural stimulation of enteric nerves was also inhibited. Absence of mast cells could account for the reduced response to antigen, but the latter abnormality is more difficult to explain. Three possibilities exist: (*a*) a separate epithelial abnormality affecting ion transport exists in W/W^v mice, (*b*) transmural stimulation affects mast cells either directly or via release of neurotransmitters from enteric nerves, or (*c*) innervation of the mucosa is deficient in W/W^v mice. Our morphological investigations show that the number of mucosal nerves per villus cross-section was not different in W/W^v and +/+ mice. While this finding suggests that a gross neuroanatomical defect in enteric nerves is unlikely in W/W^v mice, it does not eliminate the possibility that W/W^v mice exhibit abnormalities in intestinal nerve function.

However, our bone marrow transplantation studies indicate that the ion transport abnormalities in W/W^v mice cannot reflect solely an epithelial or neural defect. Although W/W^v mice do have abnormalities in addition to the absence of mast cells (lack of hair pigment, anaemia, and sterility) (28–30), all of these abnormalities appear to reflect defects in a single gene encoding the *c-kit* tyrosine kinase growth factor receptor on certain precursor cells of the abnormal lineages (30–33). Injection of normal bone marrow precursors from congenic +/+ mice has been shown to result in reconstitution of both mast cell and erythrocyte populations in W/W^v animals (28–30). Therefore, we used this technique to determine if the reduced responses to antigen and transmural stimulation were correctable by replacing mast cells. Both responses were entirely repaired after reconstitution. This suggests that mast cells (and/or other bone marrow-derived cells resident in the gut) represent an essential component of each response. Additional support for this hypothesis comes from the inhibitor studies demonstrating that responses to either antigen or transmural stimulation were inhibited by mast cell mediator antagonists in +/+ but not W/W^v mice. Finally, we showed that a second mutant mouse profoundly deficient in mast cells, the Sl/Sl^d, exhibited reduced responses to either antigen or transmural stimulation which were virtually identical to the impaired responses to these stimuli seen in W/W^v mice. Because the Sl/Sl^d mouse lacks mutations at the *W* locus, the defects in intestinal ion transport expressed by Sl/Sl^d mice cannot represent a consequence of the *W* mutations which is independent of their ability to produce mast cell deficiency.

Mast cell/nerve interactions have been demonstrated previously in morphological and functional studies (16, 41–50). Stead et al. (16) showed an intimate relationship between mucosal mast cells and mucosal substance P containing nerves in normal and nematode-infected rats. Studies in mast cell-deficient and mast cell-reconstituted mice indicate that virtually all of the alterations of vascular permeability, fibrin deposition, and granulocyte infiltration associated with the intradermal injection of substance P were dependent on mast cell activation (47). In rat intestine, field stimulation of enteric nerves caused reduced granularity of mast cells (48). Finally, conditioning studies showed that mucosal mast cells were activated by signals from the central nervous system in response to an audiovisual cue following learned associations of antigen with the cue (50).

Given the large body of evidence pointing to the existence of important interactions between nerves and mast cells, it is not surprising that stimulation of intestinal nerves in the ab-

Table IV. Basal Parameters and Short-Circuit Current (Isc) Responses in Mast Cell-deficient Sl/Sl^d and Congenic Normal (+/+) Mice

	Control (+/+)	Sl/Sl^d
Basal Values		
Isc ($\mu A/cm^2$)	28.1±2.9	34.3±2.0
G (mS/cm^2)	21.9±1.2	17.3±1.1
Isc responses ($\mu A/cm^2$)		
Antigen (phase I)	30.7±5.5	7.8±1.4*
Antigen (phase II)	7.3±1.8	7.2±1.6
Transmural stimulation	44.2±4.3	25.4±3.2*

Values represent the means±SEM (15 tissues from 5 +/+ mice, 25 tissues from 7 Sl/Sl^d mice). Short-circuit current (*Isc*) and conductance (*G*) were recorded at equilibrium, ~ 15 min after beginning the experiment. The phase I antigen response was the maximal change in Isc within the first 5 min after addition of OA; the phase II response was the sustained elevation in Isc measured at 15 min after addition of OA. The transmural stimulation response was the difference between the peak change in Isc after transmural stimulation and the baseline values. * $P < 0.05$ compared with +/+ controls.

sence of mast cells resulted in a reduced epithelial response. In addition, the anaphylactic response to OA was significantly inhibited by neural blockade with tetrodotoxin in +/+ but not in W/W^v mice. Our studies thus support the concept that mast cell/nerve communication occurs in both directions and that stimulation of one cell can result in activation of the other. On the other hand, the response to transmural stimulation was ~ 50% of normal in W/W^v and Sl/Sl^d mice, indicating that only a portion of this response is mediated by mast cells. Epithelial cells have been demonstrated to have receptors for some neurotransmitters that stimulate secretion, including acetylcholine and vasoactive intestinal polypeptide (reviewed in reference 51). Therefore, it is reasonable to postulate that in this model some (perhaps half) of the response to transmural stimulation is due to the direct action of neurotransmitters on the epithelium and the remainder is related to the activation of mast cells. Evidence for the involvement of both mast cell-dependent and mast cell-independent mechanisms in the vascular permeability response to electrical stimulation of nerves has also been proposed in the skin (52).

In the absence of any identifiable mast cells, W/W^v mouse gut was still capable of generating a small response to antigen. This strongly suggests that mast cells are not the only cells involved in mediating ion secretion during intestinal anaphylaxis. Our studies do not indicate the identity of the other cell(s). One possibility is the eosinophil, since there is evidence that these cells play a role in hypersensitivity reactions, and eosinophils are present in W/W^v mice (53). Moreover, Arizono et al. (49) recently showed close physical proximity of eosinophils to neural processes in the jejunal lamina propria of the rat. However, the response to antigen in W/W^v mice was not affected by tetrodotoxin, suggesting that interaction with nerves is not required for the function of the "non-mast cell" involved in anaphylactic responses in this mutant. Further studies are obviously necessary to investigate the identity of the additional component(s) of the antigen-mediated response in mast cell-deficient animals.

In summary, in two distinct mutant mice, mast cell deficiency was associated with a major reduction in net ion secretion in response to either antigen or electrical transmural stimulation of the intestine. Our results raise the possibility that mast cells may play an important role in the regulation of intestinal ion transport in other pathological situations besides anaphylaxis and perhaps even in homeostasis in the normal gut.

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