Mucosal Pathophysiology and Inflammatory Changes in the Late Phase of the Intestinal Allergic Reaction in the Rat

Ping-Chang Yang, M. Cecilia Berin, Linda Yu, and Mary H. Perdue

From the Intestinal Disease Research Program, McMaster University, Hamilton, Ontario, Canada

Relatively little information exists concerning the late phase of the allergic reaction in the gastrointestinal tract. Here, we characterized jejunal mucosal pathophysiology and inflammation after oral antigen challenge of sensitized rats, and examined the role of mast cells in events after challenge. Sprague-Dawley rats, mast cell-deficient (Ws/Ws), and +/+ control rats were sensitized to horseradish peroxidase, and challenged intragastrically with antigen 14 days later. Jejunal segments were obtained at 0.5 to 72 hours after challenge for functional assessment in Ussing chambers and for morphological assessment by light and electron microscopy. Intestine from sensitized Sprague-Dawley rats demonstrated enhanced ion secretion and permeability at all times after challenge. Electron microscopy revealed abnormal mitochondria within enterocytes and disruption of the epithelial basement membrane associated with influx into the mucosa of mast cells, eosinophils, neutrophils, and mononuclear cells. Many inflammatory cells appeared activated. In contrast, antigen-challenged Ws/Ws rats demonstrated no functional changes or inflammatory cell infiltrate. We conclude that oral antigen challenge of sensitized rats induces sustained epithelial dysfunction. Mast cells mediate both epithelial pathophysiology and recruitment of additional inflammatory cells that may contribute to persistent pathophysiology and symptoms. (*Am J Pathol* 2001, 158:681–690)

Several hours after an immediate hypersensitivity reaction, there is a subsequent phase of release of inflammatory mediators associated with histopathological changes and clinical symptoms.¹ This reaction, termed the late-phase reaction, has been well documented in nasal mucosa,² bronchial airways,³ and skin,⁴ but much less has been published regarding this phase of the allergic reaction and its significance in the intestinal tract.

A mixed cellular influx usually occurs in nasal cavity of allergic rhinitis patients during the late-phase reaction.

The cellular elements include eosinophils, neutrophils, and mononuclear cells. Mast cells are typically activated in a biphasic manner in airway tissue after specific antigen challenge.^{3–5} In the intestinal tract of humans, inflammatory changes after antigen challenge are difficult to study because of the invasive techniques needed to obtain tissue samples. However, rodent models of intestinal anaphylaxis/food allergy have been studied for many years and have provided much information regarding the early phase of the allergic reaction.⁶ Mast cells have been shown to be the key effector cells, releasing mediators that alter mucosal physiology. Mast cell-dependent neutrophil infiltration of the stomach wall has been documented after antigen ingestion in mice.⁷ Another study described an increase in eosinophils in the mucosa of the small intestine that was related to the chemokine, eotaxin.⁸ However, little is known about functional changes in the intestine that may be related to the influx and activation of inflammatory cells occurring throughout several days after the acute reaction.

A continuous single cell layer of epithelial cells lines the gastrointestinal tract. This epithelial lining acts as a barrier limiting the uptake of noxious material and antigens from the gut lumen. However, it is clear that a small quantity of intact protein antigen is transported across the epithelium. Sensitized rats demonstrate an increased rate and amount of antigen crossing the epithelium, initially via a transcellular pathway.⁹ Once in the lamina propria, antigen cross-links IgE antibodies bound to mast cells releasing mediators that act on epithelial receptors to induce ion secretion and enhance permeability of the paracellular pathway (both of which contribute to diarrhea).^{6,9} In segments of intestine studied in Ussing chambers, these events are indicated by increased values for short-circuit current (Isc) and conductance, respectively.^{9,10} Our previous studies have demonstrated that the secretory response begins within ~3 minutes after antigen challenge and continues for at least 2 hours.^{9–11} However, it is not clear if the epithelial dysfunction lasts for days or resolves within several hours.

Supported by the Canadian Institutes for Health Research.

Accepted for publication October 17, 2000.

Address reprint requests to Dr. Mary H. Perdue, HSC-3N5C, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada, L8N 3Z5. E-mail: perdue@mcmaster.ca.

The aim of this study was to determine the pattern of functional changes, specifically ion secretion and permeability that characterize the late-phase reaction in rat intestine, and to examine the role of mast cells, both in the pathophysiology and in the recruitment of inflammatory cells into the jejunal mucosa.

Materials and Methods

Animals

All experiments were approved by the Animal Care Committee at McMaster University. Pathogen-free, male Sprague-Dawley rats (Charles River Breeding Laboratories, St. Constant, Quebec, Canada), mean weight ~300 g, were housed in cages equipped with filter hoods. Rats were actively sensitized to a model protein antigen, horseradish peroxidase (HRP, type II; Sigma Chemical Co., St. Louis, MO)⁹ by injection of 1 mg of protein in 1 ml of alum and 50 ng of pertussis toxin as previously described.¹¹ Fourteen days after sensitization, the rats were challenged intragastrically with 0.5 ml of HRP (5×10^{-4} mol/L). The rats were sacrificed at 0.5, 8, 48, or 72 hours after challenge. Control groups included naive and sensitized unchallenged rats.

To determine the role of mast cells in the inflammatory and functional changes involved in the late-phase reaction, mast cell-deficient *Ws/Ws* rats and *+/+* littermates were used. These rats were maintained in a breeding colony at McMaster University (original colony developed by Y. Kitamura, Osaka, Japan). *Ws/Ws* rats have a 12-base deletion in the tyrosine kinase domain of the *c-kit* gene and by 10 weeks of age no mast cells can be detected in their intestine, whereas *+/+* rats have a normal number of mast cells.¹² Early in life, *Ws/Ws* rats also have anemia, but this is primarily corrected in rats older than 10 weeks of age. Rats were sensitized and challenged with HRP as above, and studies were performed 48 hours after challenge.

Ussing Chamber Studies

Rats were anesthetized with urethane and a 10-cm segment of intestinal tissue was excised from the jejunum (beginning 5 cm distal to the ligament of Treitz). The external muscle with adherent myenteric plexus was carefully stripped off. From each rat, four adjacent pieces of intestine were mounted in Ussing chambers (WPI Instruments, Narco Scientific, Mississauga, Ontario, Canada), exposing 0.6 cm² of serosa to Krebs buffer containing (in mmol/L): 115 NaCl, 8 KCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0 KHPO₄, 25 NaCO₃ (pH 7.35 $\times$ 0.02).⁹ The serosal buffer included 10 mmol/L of glucose as an energy source osmotically balanced with 10 mmol/L of mannitol on the luminal side. A circulating water bath maintained the buffer at 37°C. The electrical current crossing the tissue was measured in the voltage-clamp mode (zero volts potential difference (PD)) and expressed as short-circuit current (Isc, in μ A/cm²). At intervals, the circuit was opened to record the PD and conductance (in mS/

cm²) was calculated according to Ohm's law. Baseline measurements of Isc and conductance were recorded at equilibration, 15 minutes after mounting.

Permeability

The inert probe, ⁵¹Cr-ethylenediaminetetraacetic acid (EDTA) (6 μ Ci/ml) (Radiopharmacy, McMaster-Chedoke Hospital, Hamilton, Canada) was added to the luminal buffer of the Ussing chambers, balanced by an equivalent concentration of unlabeled Cr-EDTA in the serosal buffer. Four 1-ml samples were taken from the serosal buffer at 20-minute intervals after equilibrium (30 to 90 minutes), whereas duplicate 0.05-ml samples were obtained from the luminal buffer at the beginning and end of the experiment to calculate specific activity. The radioactivity of ⁵¹Cr-EDTA was measured in a γ counter.¹³ The transepithelial flux was calculated and expressed as nmol/cm²/hour.

Light Microscopy

Segments of jejunum were excised immediately after the rats were sacrificed. Tissues fixed in 4% formaldehyde were processed and stained with hematoxylin and eosin. The morphology of the epithelium, villi, and subepithelial layer were assessed, and the number of eosinophils, neutrophils, and mononuclear cells were counted in 10 random fields (magnification, $\times$ 200) for each rat (40/rat group). To determine mast cell numbers, tissues were fixed in Carnoy's fixative and stained with 0.5% toluidine blue. Mast cells were counted in 25 fields for each rat (100/rat group). Cell numbers were expressed per mm² of mucosa. All sections were coded to avoid observer bias.

Electron Microscopy

Segments of jejunal tissue were fixed in 2% glutaraldehyde/cacodylate buffer (pH 7.4) and processed for routine transmission electron microscopy. Tissues were cut to obtain longitudinal sections of epithelial cells. Ultrathin sections were placed on copper grids, stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope. Photomicrographs were prepared and evaluated using a computerized image analysis system. The total area of granules (area bounded by granule membrane) within 1,000-nm² regions of mast cells were measured; 20 photomicrographs were analyzed for each rat (80/rat group).

Other Measurements

Serum samples were obtained from rats at time of sacrifice, and the concentration of rat mast cell protease (RMCP II) was measured using an enzyme-linked immunosorbent assay kit (Mordun Animal Health Ltd., Glasgow, Scotland). IgE antibody titers in serum were measured by passive cutaneous anaphylaxis.

Figure 1. Functional parameters of jejunal mucosal tissues after sensitization/challenge. Baseline short-circuit current (Isc) (A) and conductance (G) values (B) of jejunal tissues obtained from Sprague-Dawley rats, either naive controls (open bars) or sensitized (hatched bars) unchallenged (0 hours) or at specific times after oral antigen challenge. The solid bars show values of tissues from sensitized Ws/Ws mast cell-deficient rats 48 hours after challenge. Bars indicate means $\pm$ SEM; values were obtained from four tissues/rat (16 per group). Statistically significant values ($P < 0.05$) are indicated as follows: a, different from control; b, different from 0 hours; c, different from 0.5 hours; d, different from 8 hours; e, different from 48 hours; f, different from 72 hours.

Statistical Methods

Data are expressed as mean $\pm$ SEM. Differences between groups were tested by analysis of variance, with post hoc analysis by Newman-Keuls test or Student's *t*-test when appropriate. *P* values less than 0.05 were accepted as significant.

Results

Mucosal Function

There was no difference in net active ion transport of jejunal tissues from naive *versus* sensitized unchallenged (time 0) Sprague-Dawley rats, as indicated by the similar values for baseline Isc in both groups (Figure 1A). However, intestine from sensitized rats challenged orally with HRP demonstrated a significant ($P < 0.001$) increase in baseline Isc at 0.5 hours after antigen challenge, and another small but significant rise at 48 hours. The Isc remained elevated for at least 72 hours after challenge (the last time point in our study). Previously, we showed that the antigen-induced Isc elevation is because of secretion of Cl^- ions.^{10,14}

Tissue conductance, indicating passive ionic permeability, was also not different in naive control *versus* sensitized unchallenged rats, but was significantly elevated in intestine from sensitized rats at 0.5 to 72 hours after antigen challenge (Figure 1B). There was no obvious phasic pattern to the conductance change.

Mucosal Structure (Light Microscopy)

The morphology of the jejunal mucosa was essentially normal in sensitized rats compared with controls (Figure

2, A and B). However, on closer observation increased cellularity was apparent (Figure 2B). This was confirmed by counting specific cell types at higher magnification (see below). The mucosa was noticeably altered after antigen challenge of sensitized rats. At 0.5 hours, some swelling of the villi was observed (not shown) and by 8 to 72 hours, there was obvious edema and abnormal villus architecture (Figure 2C). In some sections, the epithelial cells were observed to be detaching from the underlying tissue.

Inflammatory cells within the intestinal mucosa were quantified by light microscopy, and results are shown in Figure 3, A–D. The numbers of mast cells, eosinophils, neutrophils, and mononuclear cells were greater in sensitized/challenged rats compared with naive controls, although the pattern of change differed for the various cell types. Mast cell numbers were greater in the mucosa of sensitized rats compared with naive rats and demonstrated a further biphasic increase after antigen challenge, with peaks at 0.5 and 72 hours and lower values between 8 to 48 hours (Figure 3A). Similarly, the numbers of eosinophils, neutrophils, and mononuclear cells were already elevated in jejunal mucosa of sensitized rats compared with controls (Figure 3, B–D). The number of eosinophils dipped at 0.5 hours and peaked at 48 hours (Figure 3B). Neutrophil numbers were greatest at 0.5 hours (Figure 3C), although compared with other cell types, neutrophil numbers were relatively low. The most striking observation in tissues from challenged rats was a dramatic increase in the infiltration of mononuclear cells (Figure 3D). Thirty minutes after antigen challenge, the cell number had doubled, and by 48 hours it was ~ 10 -fold that in controls, reaching ~ 300 per mm^2 (*versus* a maximum of ~ 70 for mast cells, ~ 120 for eosinophils,

Figure 2. Light microscopy of jejunal mucosa after sensitization/challenge. Representative H&E-stained sections from Sprague-Dawley rats are shown: control rat (A), sensitized unchallenged rat (B), or sensitized rat (C) 48 hours after oral antigen challenge. Original magnifications, $\times 200$.

Figure 3. Numbers of inflammatory cells in jejunal mucosa after sensitization/challenge. Mast cells (A), eosinophils (B), neutrophils (C), or mononuclear cells (D) were counted in light microscopic sections of jejunum (tissues fixed and stained as indicated in Methods), and expressed as number of cells per mm^2 . Tissues were obtained from Sprague-Dawley rats, either naive controls (open bars) or sensitized (hatched bars) unchallenged (0 hours) or at specific times after oral antigen challenge. The solid bars show values in tissues from sensitized Ws/Ws mast cell-deficient rats 48 hours after challenge. Bars indicate means $\pm$ SEM; values were obtained for 25 fields per rat (100 per group) for mast cells, 10 fields per rat (40 per group) for the other cell types. Statistically significant values ($P < 0.05$) are indicated as follows: a, different from control; b, different from 0 hours; c, different from 0.5 hours; d, different from 8 hours; e, different from 48 hours; f, different from 72 hours.

and ~ 14 for neutrophils). At 72 hours, the number of mononuclear cells in the mucosa had decreased toward the baseline value but was still significantly greater than the control value.

Mucosal Structure (Electron Microscopy)

Changes were also evident at the ultrastructural level. Compared with enterocytes in control rats (Figure 4A), enterocytes in sensitized/challenged rats at 48 hours contained abnormal mitochondria (Figure 4B), many with autophagosomes (insert).¹⁵ The basement membrane was often disrupted (Figure 4C), frequently associated with immune cell infiltration into the epithelial layer (Figure 4, D and E). The majority of infiltrating cells were lymphocytes (Figure 4D) and eosinophils (Figure 4E). Large spaces were observed between invading cells and epithelial cells (Figure 4D). Electron microscopy also confirmed the exfoliation of epithelial cells (Figure 4F).

Figure 4. Electron microscopy of jejunal mucosa after sensitization/challenge. Representative sections from Sprague-Dawley rats are shown: control rat (**A**), and sensitized rats (**B–F**) 48 hours after oral antigen challenge. **A:** Normal epithelial cells in tissue from a control rat. **B:** Epithelial cells in tissue from a sensitized rat at 48 hours showing many abnormal mitochondria containing autophagosomes (arrow, also insert). **C:** Disrupted basement membrane (Bm) beneath the epithelium (Ep). **D:** Lymphocyte (Lc) penetration into the epithelial layer. **E:** Eosinophil (Eo) penetration into the epithelium. **F:** In some sections, there were large spaces between epithelial cells that seemed to be detaching from the basement membrane. Scale bars, 2 μ m.

Mast Cell and Eosinophil Activation

Mast cells and their granules were evaluated in electron photomicrographs of intestinal sections from naive *versus* sensitized/challenged rats (Figure 5, Table 1). In sensitized/challenged rats, signs of mast cell activation were obvious, including haloing (spaces) around the granule core and empty granules (Figure 5C), compared with mast cells in naive controls (Figure 5A). Some changes were apparent in sensitized rats even before challenge (Figure 5B), such as enlarged granules and granules with decreased density. There was a significant increase in the proportion of mast cells showing signs of activation (threefold) at time 0 and this increased with time (12-fold by 72 hours) (Table 1). In addition, activation of eosinophils was observed between 48 and 72 hours, as indicated by a decrease in the core density of their granules (Figure 4E and Figure 5D).

The concentration of RMCP II in serum was also used as an index of mucosal mast cell activation. Circulating

RMCP II was significantly elevated in sensitized *versus* control rats (Figure 6). After antigen challenge, a biphasic pattern of further increase occurred with peaks at 0.5 and 72 hours. This pattern mirrored that seen for mast cell accumulation in the mucosa.

Mucosal Function and Structure in Mast Cell-Deficient Rats

To determine the role of mast cells in the functional and structural changes observed after antigen challenge in sensitized rats, we conducted experiments using *Ws/Ws* mast cell-deficient rats and their *+/+* normal littermates. Rats were sacrificed 48 hours after oral antigen challenge, when abnormalities in epithelial physiology and changes in infiltrating mononuclear cells were maximal. Intestine from sensitized unchallenged *Ws/Ws* rats had similar baseline values for *Isc* and conductance at 48 hours after challenge compared to control rats (data not

Figure 5. Electron microscopy showing granules in mucosal mast cells and eosinophils. Representative sections from Sprague-Dawley rats are shown. **A:** Normal nonactivated mast cell in a section from a control rat. **B:** Mast cell from a sensitized unchallenged rat at higher magnification. **C:** Mast cell from a sensitized rat 48 hours after oral antigen challenge containing granules with halos (spaces between the granule membrane and core) and reduced density indicative of activation. **D:** Eosinophil granules with reduced core density indicative of activation, from rat 48 hours after challenge. Original magnification: 2 μm (**A**), 1 μm (**B** and **C**), 0.2 μm (**D**).

Table 1. Changes in Mucosal Mast Cell Granules after Sensitization/Challenge

Rat group	Granule size (nm ²)	Activated granules (% of total)
Control	260 ± 11	4.0 ± 0.3
Sensitized (0 hours)	310 ± 8*	13.9 ± 0.8*
Sensitized (0.5 hours)	340 ± 12*	28.7 ± 0.7 [†]
Sensitized (8.0 hours)	390 ± 16 [†]	36.2 ± 1.0 [†]
Sensitized (48 hours)	350 ± 13*	45.3 ± 1.9 [†]
Sensitized (72 hours)	320 ± 10*	49.6 ± 2.1 [†]

Electron microscopic photomicrographs were prepared of jejunal mucosa from Sprague-Dawley rats, naive controls, or sensitized unchallenged (0 hours) or at specific times after oral antigen challenge. Computerized image analysis was used to measure the total area of granules (area bounded by granule membrane) within 1,000-mm² regions inside mast cells. Granules with signs of activation included those with empty granules, clear zones around the granule core, and/or decreased granule density; the proportion (expressed as percent of cells with activated granules compared with the total evaluated) is shown. Values represent the mean ± SEM.

* $P < 0.05$, [†] $p < 0.01$ compared with values in naive control rats; 20 photomicrographs were evaluated per rat (80/rat group).

shown). However, in contrast to the significant elevation of these parameters after antigen challenge in sensitized +/+ rats, the Isc and conductance values were unaffected by antigen challenge in *Ws/Ws* rats (Figure 1, A and B). To further evaluate the role of mast cells in the antigen-induced permeability change, we measured the flux of ⁵¹Cr-EDTA across the tissues. The flux of this standard permeability probe was significantly increased (approximately fourfold) in tissues from sensitized/challenged +/+ rats compared with sensitized unchallenged rats, but no flux change occurred in tissues from sensitized *Ws/Ws* rats after antigen challenge (Figure 7).

Figure 6. Serum concentration of rat mast cell protease II (RMCP II) after sensitization/challenge. Serum was obtained from Sprague-Dawley rats, either naive controls (open bars) or sensitized (hatched bars) unchallenged (0 hours) or at specific times after oral antigen challenge. Serum from sensitized *Ws/Ws* mast cell-deficient rats 48 hours after challenge contained no detectable RMCP II. Bars indicate means ± SEM; *, $P < 0.05$; $n = 4$ rats per group. Statistically significant values ($P < 0.05$) are indicated as follows: a, different from control; b, different from 0 hours; c, different from 0.5 hours; d, different from 8 hours; e, different from 48 hours; f, different from 72 hours.

Figure 7. Epithelial permeability 48 hours after challenge. Jejunal tissues were obtained from +/+ or *Ws/Ws* rats, either controls or sensitized rats 48 hours after oral antigen challenge. ⁵¹Cr-EDTA was added to the luminal side of tissues in Ussing chambers. Buffers were sampled at 20-minute intervals after equilibration and mucosal-to-serosal flux values were calculated as the mean of at least two stable periods. **, $P < 0.01$ versus control value; values were obtained for four tissues per rat (16 per group).

No mast cells at all were visualized by light or electron microscopy in the mucosa of *Ws/Ws* rats, either before sensitization or after sensitization and antigen challenge (Figure 2A). In addition, no RMCP II was detectable in serum of *Ws/Ws* rats (Figure 6). With respect to other types of inflammatory cells in the mucosa, there were normal numbers of resident eosinophils, neutrophils, and mononuclear cells in the mucosa of naive rats (not shown), and no increases greater than control values at 48 hours after challenge (Figure 2, A–D). In addition, no epithelial damage was observed by electron microscopy. However, anti-HRP antibody production was normal in *Ws/Ws* rats (mean serum titer of 1:512 at 48 hours versus 1:256 in +/+ rats). Heat treatment of the serum abolished the passive cutaneous anaphylactic reaction, confirming that the antibodies were of the IgE isotype.

Discussion

The purpose of this study was to define the late phase of the allergic reaction in rat small intestine, specifically examining the role of mast cells in epithelial pathophysiology and allergic inflammation. Our results demonstrated significant functional and structural changes that lasted for several days after oral antigen challenge of sensitized rats. Epithelial dysfunction, including ion secretion and enhanced permeability, was evident for at least 72 hours associated with intestinal tissue edema and ultrastructural signs of damage. Inflammatory cells accumulated in the lamina propria and the epithelium, with a clear later phase of cell recruitment subsequent to the immediate hypersensitivity reaction. Our studies comparing findings in mast cell-deficient rats with those in normal rats revealed that mast cells were critical for both

the physiological abnormalities and the inflammatory events.

The principal roles of the epithelial lining of the gastrointestinal tract are the exclusion of luminal macromolecules from the body (barrier function) and the selective absorption/secretion of ions and nutrients (transport function). These functions are perturbed during food allergic reactions and intestinal inflammation. For example, *in vivo* studies in sensitized rats showed that perfusion of small intestine with a food antigen stimulates ion and fluid secretion resulting in diarrhea.^{16,17} The Ussing chamber technique provides an instant readout of the immediate hypersensitivity reaction because ion secretion is indicated online by a rise in *I*_{sc} that occurs within 2 to 3 minutes of antigen challenge of tissues from sensitized rodents.¹⁰ Enhanced permeability of the epithelium is also a consequence of intestinal anaphylaxis, and has been documented both *in vivo* and in tissues studied in the Ussing chamber where conductance and probe flux values increase.^{9,11,18} Therefore, here we used this apparatus to examine epithelial ion transport and permeability abnormalities during the late phase of the allergic reaction, recording electrical parameters at times from 0.5 to 72 hours after oral antigen challenge.

Baseline values for *I*_{sc} and conductance were similar in naive control and sensitized rats. However, oral antigen resulted in an ~50% increase in baseline *I*_{sc} and ~60% increase in conductance at 0.5 hours. There was an additional small but significant increase in *I*_{sc} at 48 hours. The secretory and permeability changes persisted for at least 3 days after a single antigen challenge with no signs of decrease toward normal values.

The epithelial pathophysiology was associated with ultrastructural abnormalities. Specifically, at 48 hours epithelial mitochondria appeared swollen with loss of cristae, and autophagosomes were present in many mitochondria. A reduction in ATP production from the mitochondria has previously been demonstrated in association with epithelial dysfunction.¹⁹ The basement membrane was frequently disrupted and eosinophils and lymphocytes were observed penetrating into the epithelial layer. Spaces appeared in these regions with actual sloughing of epithelial cells in some sections. It is not clear from our study if the invading inflammatory cells were responsible for the damage; however, the eosinophils were shown to contain hypodense granules typical of activated cells. Both eosinophils and T cells can release bioactive mediators/cytokines with the potential to induce tissue damage.^{20–23}

Infiltration of inflammatory cells into the mucosa was also documented by counting numbers of specific cells within a defined area of the lamina propria. Sensitization alone resulted in greater numbers of mast cells, eosinophils, neutrophils, and mononuclear cells in the intestinal mucosa. An increase in mucosal mast cells after sensitization has been reported previously in our rodent model of food allergy.¹¹ This finding is interesting because the model does not involve any local administration of antigen, suggesting that mast cells spontaneously accumulate at mucosal sites, as well as at other locations, after systemic sensitization. The mechanism directing mast

cell traffic to the gut is not clear. However, the gut is a large immune organ, containing resident T and B cells as well as mast cells and nerves, which are stimulated by sensitization to synthesize Th2 cytokines (eg, interleukin-3, interleukin-4, interleukin-5) and/or possibly other factors that attract and stimulate cell growth (ie, stem cell factor, nerve growth factor).^{22–25}

After antigen challenge, a biphasic pattern of increased mast cells was demonstrated, with peaks at 0.5 hours and 72 hours. This pattern correlated with release of RMCP II, suggesting that the number represents activated cells. However, this approach may not present the entire profile of events with respect to this cell type. Although we were using high-power light microscopy, and therefore were able to see partially degranulated cells, our numbers reflect only the number of mast cells with sufficient granule contents to stain. Experiments in progress (to be reported separately) indicate that several phenomena are occurring simultaneously: recruitment, maturation, activation, and resynthesis of granule contents after antigen-induced degranulation. Neutrophil numbers followed a similar but less obvious pattern to that of mast cells, with the first peak at 0.5 hours and a second wave of elevation at 48 to 72 hours. The number of eosinophils decreased after antigen exposure and then increased again with the greatest number being present at 48 hours. In contrast, mononuclear cells continued to accumulate in the mucosa, reaching a peak at 48 hours when the number was 10-fold greater compared with controls.

Our results are consistent with and expand findings from the few other studies reporting gut inflammatory changes in the late-phase allergic reaction. For example, several hours after antigen challenge of mice, infiltration into jejunal mucosa of mast cells, eosinophils, and intraepithelial lymphocytes was described.²⁶ Wershil and colleagues⁷ reported neutrophil and mononuclear cell infiltration in the stomach of mice after antigen challenge, with neutrophils peaking at 2 hours and mononuclear cells rising several fold at 12 to 24 hours. In another study, mast cell influx was observed at 1 hour, villus edema at 3 hours, eosinophil infiltration at 6 hours, and villus atrophy associated with increased numbers of lymphocytes at 24 hours,²⁷ although exact counts were not performed. Increased permeability was documented at 1, 6, and 24 hours with decreases between the peaks. No structural or functional analysis was performed at later times in those studies. In our investigation, the most severe morphological, ultrastructural, and physiological changes were documented at 48 to 72 hours, the time of greatest inflammatory cell accumulation within the tissues.

In other organ systems, the late-phase reaction involves similar cell types, although the pattern of accumulation may vary. In nasal mucosa, eosinophil accumulation begins within minutes after the immediate allergic reaction.^{2,3,28} These cells are followed by neutrophils, then by basophils, mast cells, and macrophages. In the skin, a nonmucosal site, early skin swelling is caused by released mast cell mediators, whereas the delayed reaction has peaks at 2 hours and 24 to 48 hours, the latter probably because of T cell activation.^{3,4,29} In asthma, the

late-phase response occurs 6 to 10 hours after allergen exposure and is characterized by infiltration of inflammatory cells in the airway mucosa, mainly eosinophils, but also other cell types (mast cells, lymphocytes, and macrophages).^{30,31}

Mast cells seemed to be critical for recruiting (and possibly activating) the other inflammatory cells because no inflammatory cell infiltration occurred in mast cell-deficient rats. *Ws/Ws* rats have a 12-base deletion in the tyrosine kinase domain of the *c-kit* gene that results in the lack or deficiency of melanocytes and erythrocytes as well as mast cells, although the anemia repairs in early life.¹² The ideal experiment to determine the role of mast cells in a particular response is to specifically reconstitute mast cell populations in deficient animals. Mast cells have been reconstituted in mast cell-deficient mice by injection of precursor cells derived from *+/+* bone marrow,³² but because of the heterogeneous genetic background of the *Ws/Ws* and *+/+* rats (*F*₂ generation of two inbred rat strains), reconstitution is not feasible in *Ws/Ws* rats because of cell rejection. Therefore, we cannot rule out other phenotypic abnormalities as contributors to the lack of observed responses to antigen challenge in *Ws/Ws* rats. However, *Ws/Ws* rats have normal intestinal epithelial responses to carbachol and forskolin (M. C. Berin and M. H. Perdue, unpublished data). In addition, these deficient rats have been used to determine the role of mast cells in mucosal changes induced by sensitization and the immediate hypersensitivity reaction in the gut and airways.^{33,34}

Mast cells synthesize and secrete a host of bioactive factors, many of which have chemotactic properties, such as tumor necrosis factor- α , interleukin-5, leukotriene B₄, platelet-activating factor, complement C5a, and various other cytokines/chemokines.³⁵ We found evidence for a degree of mast cell activation even before antigen challenge, with changes in granule size and density and an ~15% increase in the concentration of RMCP II in the circulation. In addition, the numbers of inflammatory cells in the mucosa were increased above control values. However, no physiological abnormalities were documented. In contrast, oral antigen administration resulted in more dramatic morphological and functional changes, including depletion of mast cell granules associated with epithelial pathology and pathophysiology. Release of different types of mast cell mediators after sensitization (eg, chemokines) *versus* antigen challenge (eg, typical mediators of immediate hypersensitivity such as histamine) might account for the disparate affects. In addition, cross-linking of the IgE receptor stimulates synthesis of additional mediators and cytokines/chemokines³⁵ that are likely to be involved in the late-phase reaction. This would account for the additional inflammatory cell recruitment that occurred after antigen challenge, with somewhat distinct patterns for each cell type. The most unique pattern was the 10-fold increase in numbers of mononuclear cells at 48 hours, which was relatively transient because the value was decreased to threefold 24 hours later. We are unable to explain this effect. However, lymphocytes are highly motile cells known for their trafficking characteristics.

Mast cells have been shown to be required for neutrophil accumulation in the stomach after local antigen challenge of sensitized mice, with tumor necrosis factor- α playing a major role.^{7,36} Thus, there is evidence for mast cell-dependent recruitment of inflammatory cells into the gastrointestinal tract. Data from both *in vivo* and *in vitro* systems suggest that mast cells may serve a gatekeeper function by regulating leukocyte migration at the level of microvascular endothelium that is a key step in initiating the late-phase reaction.^{37–40} In addition, tumor necrosis factor- α , RMCP II, histamine, and other mast cell mediators have been shown to induce pathophysiological changes in the gastrointestinal tract.^{6,18,41,42} The role of other inflammatory cells in the epithelial dysfunction during the late-phase reaction cannot be determined from this study, but the most severe pathophysiology coincided in time (at 48 hours) with the presence of the greatest numbers of cells in the mucosa. Studies in progress suggest that at these cells do contribute mediators that compound the injury.

In summary, our study has shown that both functional and morphological abnormalities persist after the immediate phase of the intestinal allergic reaction. During the late phase, epithelial dysfunction included on-going ion secretion and increased permeability. The mucosal barrier defect can result in greater nonspecific uptake of luminal antigens/microbial products with the potential to exacerbate the inflammatory response. Epithelial cell loss further contributed to the tissue damage. Our findings implicate mast cells as critical to the late-phase reaction, although influx of other inflammatory cells correlated with more extensive injury. Further studies will be required to determine the precise role of eosinophils, neutrophils, and T cells in the intestinal pathophysiology of the late-phase allergic reaction in the gut.

References

1. Dorsch W (Ed): Definitions and clinical symptoms. Late Phase Allergic Reactions. Boca Raton, CRC Press, 1990, pp 9–15
2. Pelikan Z: Late nasal response—its clinical characteristic features, and possible mechanisms. Late Phase Allergic Reactions. Boca Raton, CRC Press, 1990, pp 111–117
3. Pearlman DS: Pathophysiology of the inflammatory response. *J Allergy Clin Immunol* 1999, 104:S132–S137
4. Charlesworth EN, Hood AF, Soter NA, Kagey-Sobotka A, Norman PS, Lichtenstein LM: Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J Clin Invest* 1989, 83: 1519–1526
5. Denburg JA: Basophils and mast cells in airway inflammation and asthma. *Can Respir J* 1998, 5(Suppl A):41–44
6. Crowe SE, Perdue MH: Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992, 103:1075–1095
7. Wershil BK, Furuta GT, Wang Z-S, Galli SJ: Mast cell-dependent neutrophils and mononuclear cell recruitment in immunoglobulin E-induced gastric reactions in mice. *Gastroenterology* 1996, 110:1482–1490
8. Rothenberg ME, MacLean JA, Pearlman E, Luster AD, Leder P: Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophils. *J Exp Med* 1997, 185:785–790
9. Berin MC, Kiliaan AJ, Yang PC, Groot JA, Taminiau JAM, Perdue MH: Rapid transepithelial antigen transport in the rat jejunum: impact of sensitization and the immediate hypersensitivity reaction. *Gastroenterology* 1997, 113:856–864

10. Crowe SE, Sestini P, Perdue MH: Allergic reactions of rat jejunal mucosa. Ion transport responses to luminal antigen and inflammatory mediators. *Gastroenterology* 1990, 99:74–82
11. Kosecka U, Marshall JS, Crowe SE, Bienenstock J, Perdue MH: Pertussis toxin stimulates hypersensitivity and enhances nerve-mediated antigen uptake in rat intestine. *Am J Physiol* 1994, 267:G745–G753
12. Niwa Y, Kasugai T, Ohno K, Morimoto M, Yamazaki M, Dohmae K, Nishimune Y, Kondo K, Kitamura Y: Anemia and mast cell depletion in mutant rats that are homozygous at "white spotting (Ws)" locus. *Blood* 1991, 78:1936–1941
13. McKay DM, Croitoru K, Perdue MH: T cell-monocyte interactions regulate epithelial physiology in a coculture model of inflammation. *Am J Physiol* 1996, 270:C418–C428
14. Perdue MH, Gall DG: Intestinal anaphylaxis in the rat: jejunal response to in vitro antigen exposure. *Am J Physiol* 1986, 250:G427–G431
15. Stromhaug PE, Berg TO, Fengsrud M, Seglen PO: Purification and characterization of autophagosomes from rat hepatocytes. *Biochem J* 1998, 335:217–224
16. Perdue MH, Chung M, Gall DG: The effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984, 86:391–397
17. Scott RB, Diamant SC, Gall DG: Motility effects of intestinal anaphylaxis in the rat. *Am J Physiol* 1988, 255:G505–G511
18. Crowe SE, Perdue MH: Intestinal permeability in allergic rats: nerve involvement in antigen-induced changes. *Am J Physiol* 1993, 264:G617–G623
19. Madsen KL, Yanchar NL, Sigalet DL, Reigel T, Fedorak RN: FK506 increases permeability in rat intestine by inhibiting mitochondrial function. *Gastroenterology* 1995, 109:107–114
20. Seminario MC, Gleich GJ: The role of eosinophils in the pathogenesis of asthma. *Curr Opin Immunol* 1994, 6:860–864
21. Winterkamp S, Raihel M, Hahn EG: Secretion and tissue content of eosinophil cationic protein in Crohn's disease. *J Clin Gastroenterol* 2000, 30:170–175
22. Yssel H, Groux H: Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. *Int Arch Allergy Immunol* 2000, 121:10–18
23. Bellanti JA: Cytokines and allergic diseases: clinical aspects. *Allergy Asthma Proc* 1998, 19:337–341
24. Galli SJ, Hammel I: Mast cell and basophil development. *Curr Opin Hematol* 1994, 1:33–39
25. Arizono N, Kasugai T, Yamada M, Okada M, Morimoto M, Tei H, Newlands GF, Miller HR, Kitamura Y: Infection of *Nippostrongylus brasiliensis* induces development of mucosal-type but not connective tissue-type mast cells in genetically mast cell-deficient *Ws/Ws* rats. *Blood* 1993, 81:2572–2578
26. Ohtsuka Y, Yamashiro Y, Maeda M, Oguchi S, Shimizu T, Nagata S, Yagita H, Yabuta K, Okumura K: Food antigen activates intraepithelial and lamina propria lymphocytes in food-sensitive enteropathy in mice. *Pediatr Res* 1996, 39:862–866
27. Sakamoto Y, Ohtsuka T, Yoshida H, Ohto K, Onobori M, Matsumoto T, Iikura Y, Morohoshi T: Time course of changes in the intestinal permeability of food-sensitized rats after oral allergen challenge. *Pediatr Allergy Immunol* 1998, 9:20–24
28. Palczynski C, Walusiak J, Ruta U, Gorski P: Nasal provocation test in the diagnosis of natural rubber latex allergy. *Allergy* 2000, 55:34–41
29. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR, Kay AB: High-affinity immunoglobulin E receptor (Fc epsilon RI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *Immunology* 1998, 93:281–288
30. Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM, Bochner BS: Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoic production. *J Allergy Clin Immunol* 1994, 93:725–734
31. Pin I, Radford S, Kolendowicz R, Jannings B, Denburg JA, Hargreave FE, Dolovich J: Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. *Eur Respir J* 1993, 6:1249–1256
32. Perdue MH, Masson S, Wershil B, Galli S: 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. *J Clin Invest* 1991, 87:687–693
33. Berin MC, Kiliaan AJ, Yang PC, Groot J, Kitamura Y, Perdue MH: The influence of mast cells on pathways of antigen transport in rat intestine. *J Immunol* 1998, 161:2561–2566
34. Yang PC, Berin MC, Perdue MH: Enhanced antigen transport across rat tracheal epithelium induced by sensitization and mast cell activation. *J Immunol* 1999, 163:2769–2776
35. Williams CM, Galli SJ: The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000, 105:847–859
36. Furuta GT, Schmidt-Choudhury A, Wang MY, Wang ZS, Lu L, Furlano RI, Wershil BK: Mast cell-dependent tumor necrosis factor alpha production participates in allergic gastric inflammation in mice. *Gastroenterology* 1997, 113:1560–1569
37. Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF: Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991, 88:4220–4224
38. Bochner BS, Luscinskas FW, Gimbrone Jr MA, Newman W, Sterbinsky SA, Derse-Anthony CP, Klunk D, Schleimer RP: Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991, 173:1553–1557
39. Gonzalo JA, Lloyd CM, Kremer L, Finger E, Martinez-A C, Siegelman MH, Cybulsky M, Gutierrez-Ramos JC: Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J Clin Invest* 1996, 98:2332–2345
40. Thornhill MH, Wellicome SM, Mahiouz DL, Lanchbury JSS, Kyan-Aung U, Haskard DO: Tumor necrosis factor combines with IL-4 or IFN-gamma to selectively enhance endothelial cell adhesiveness for T cells. *J Immunol* 1991, 146:592–598
41. Stenton GR, Vliagoftis H, Befus AD: Role of intestinal mast cells in modulating gastrointestinal pathophysiology. *Ann Allergy Asthma Immunol* 1998, 81:1–11
42. Scudamore CL, Thornton EM, McMillan L, Newlands GEJ, Miller HRP: Release of the mucosal mast cell granule chymase rat mast cell protease II, during anaphylaxis is associated with rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995, 182:1871–1881