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Enhanced Antigen Transport Across Rat Tracheal Epithelium Induced by Sensitization and Mast Cell Activation

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Ag challenge to the apical surface of tracheal epithelium results in a rapid ion secretory response due to the activation of mast cells. The aim of this study was to examine the impact of sensitization and specific Ag challenge on the timing, route, and quantity of Ag transported across tracheal epithelium. After sensitization of rats to a model protein, HRP, tracheal tissues were excised and mounted in Ussing chambers. Tracheas from HRP-sensitized rats, but not naive or OVA-sensitized rats, responded to apical HRP challenge with a rise in short-circuit current (beginning at ~2 min). Photomicrographs of tissues fixed at 2 min showed that initial transepithelial HRP transport occurred via endosomes and was significantly enhanced in HRP-sensitized rats compared with both control groups. In addition, nonciliated cells, the proportion of which increased after sensitization, contained significantly more HRP than ciliated cells. The hypersensitivity response occurred only in HRP-sensitized and challenged rats and was associated with increased conductance of tracheal epithelium and overall flux of HRP across the tissue. This increased flux of Ag and elevated conductance was not observed in mast cell-deficient Ws/Ws rats. Photomicrographs of tissues fixed 90 min after challenge also showed HRP in the paracellular spaces between adjacent epithelial cells. We conclude that sensitization increases uptake of specific Ag initially via an endosomal transcellular pathway across tracheal epithelium and that, after the hypersensitivity reaction, mast cell-dependent recruitment of the paracellular pathway further augments Ag influx into airway tissue. The Journal of Immunology, 1999, 163: 2769–2776.

Allergic diseases are common in Western countries and are increasing in prevalence in most countries of the developed world (1). Although airway allergy has been studied for many years in humans and animal models, various aspects of its pathophysiology remain puzzling. The epithelium theoretically acts as a barrier restricting passage of macromolecules (2). However, immunologically intact Ags do cross the epithelium to access and activate effector cells in the tissue. In addition, transepithelial transport of proteins normally takes approximately 20 min (3); however, allergic symptoms may begin very rapidly after encounter with Ag (4). We have previously documented, in rat models of airway and intestinal hypersensitivity (5, 6), that a secretory response begins within minutes after Ag contact with the apical membrane of epithelial cells.

Recently, we compared transepithelial transport of a model protein Ag, HRP, in the intestine of sensitized vs control rats (7). We determined that the initial rate of protein transport across the epithelium was enhanced by sensitization such that HRP was already in the interstitial space by 2 min after luminal challenge in sensitized rats, whereas this finding was never apparent in naive control rats. More remarkable was the fact that the amount of Ag within epithelial cells and also in the lamina propria was significantly greater when the challenge protein was the one to which the rats had been sensitized. In addition, following the onset of the hypersensitivity reaction, there was evidence of increasing tissue conductance, and HRP was visualized in the paracellular spaces between adjacent epithelial cells. These findings suggest increased leakiness of the intercellular tight junctions. The initial phase of Ag transport (phase I) was subsequently shown to be mast cell independent, while the recruitment of the paracellular pathway (phase II) required the presence of mast cells (8). Since we have previously used a rat model of airways hypersensitivity to characterize tracheal epithelial ion secretion in response to Ag challenge (5), here we examined whether sensitization and the hypersensitivity reaction also augment Ag transport across airway epithelium. We used an in vitro approach to study Ag absorption directly across epithelial cells in the absence of confounding factors such as blood flow and changes in vascular permeability.

Materials and Methods

Animal model

Most experiments were conducted in pathogen-free male Sprague Dawley rats, (300–350 g; Charles River, St. Constant, Quebec, Canada). Additional experiments were performed in Ws/Ws mast cell-deficient rats (colony at McMaster University; original Ws/+ breeder rats provided by Dr. Y. Kita- mura, Osaka University Medical School, Japan). Ws/Ws rats have a 12-base deletion in the tyrosine kinase domain of the c-kit gene (9) that results in a lack of melanocytes, erythrocytes, and mast cells (10). Rats were housed in cages equipped with filter hoods. Rats were sensitized to HRP (type II; Sigma, St. Louis, MO) by s.c. injection of 1 mg protein in 1 ml alum, plus i.p. injection of 1 ml Bordetella pertussis vaccine (Connaught Laboratories, Mississauga, ON, Canada). To assess the effect of nonspecific sensitization, rats were injected with OVA (grade V; Sigma) following the same protocol. Naive control rats were injected with saline. All experiments were conducted with approval from the McMaster University Animal Care Committee.

Fourteen days after sensitization, the rats were anesthetized with urethane, and the trachea was removed and immediately immersed in warmed oxygenated Krebs buffer.

Ussing chamber experiments

The trachea was then slit open longitudinally and mounted in Ussing chambers (surface area 0.6 cm²). The tissue was bathed in 10 ml of oxygenated Krebs buffer (in mM: 115.0 NaCl, 8.0 KCl, 1.25 CaCl₂, 1.2 MgCl₂, 2.0
**Table 1. Basal electrophysiological parameters**

<table>
<thead>
<tr>
<th></th>
<th>Isc (μA/cm²)</th>
<th>G (mS/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.5 ± 2.1</td>
<td>20.3 ± 0.8</td>
</tr>
<tr>
<td>OVA</td>
<td>25.7 ± 1.2</td>
<td>21.2 ± 1.1</td>
</tr>
<tr>
<td>HRP</td>
<td>27.5 ± 2.3</td>
<td>19.5 ± 0.9</td>
</tr>
</tbody>
</table>

*Values for short-circuit current (Isc) and conductance (G) were recorded at equilibrium, 15 min after mounting tracheas in Ussing chambers. Values represent means ± SE; n = 3 rats in each group.*

**Statistics**

Differences between groups were tested by ANOVA, with post hoc analysis using Newman-Keuls or Student’s t tests where appropriate. The data were expressed as mean ± SE. A value of p < 0.05 was considered significant.

**Results**

The timing of the hypersensitivity reaction

HRP challenge evoked a rapid ion secretory response only in HRP-sensitized rats. There were no significant differences in basal electrophysiological parameters in tracheas from HRP-sensitized rats compared with OVA-sensitized or naive control rats (Table I). Tracheal tissues from HRP-sensitized rats responded to luminal HRP challenge with a rapid (beginning after ~2 min) increase in Isc (previously shown to be due to secretion of chloride ions (6)). The ΔIsc within 15 min was 23.9 ± 3.0 μA/cm² (mean ± SE; n = 3 rats). Tracheas from naive controls or OVA-sensitized rats (3 rats/group) did not respond at all to HRP challenge. Representative Isc tracings are shown in Fig. 1. In addition, mast cell-deficient Ws/Ws rats sensitized to HRP did not respond to HRP challenge.

Mast cells were activated after HRP challenge only in HRP-sensitized rats. The numbers of mast cells/1000 epithelial cells were counted in tracheal sections from all 3 rat groups. In tracheas from naive control rats, there were 11.0 ± 1.5 (mean ± SE) mast cells vs a significantly greater (p < 0.001) number, 21.0 ± 0.6, in the OVA-sensitized rats and 25.3 ± 2.6 in the HRP-sensitized rats. At 90 min after HRP challenge, mast cells in tracheas from naive controls and OVA-sensitized rats were mostly normal in appearance, containing electron-dense granules (Fig. 2, A and B). However, in HRP-sensitized rat tracheas, mast cells demonstrated changes typical of activation: 1) decreased granule density (82%) and 2) spaces between the granule core and its membrane (76%). A typical activated mast cell from this rat group is shown in Fig. 2C. In naive rats, very few cells demonstrated decreased granule density (3%), and none (0%) showed spaces around the granule core; the same was true in OVA-sensitized rats, where 4% had reduced density and again none showed spaces around the granule core. No mast cells were detectable in tracheas from sensitized Ws/Ws mast cell-deficient rats.

Transcellular Ag transport

HRP uptake occurred initially by transcytosis and was enhanced by specific sensitization. To determine whether Ag had crossed the epithelium within 2 min and to identify the route of the initial Ag transport, high power EM photomicrographs were evaluated of tissues fixed 2 min after HRP challenge. At this time, there were only a few HRP-containing endosomes in the apical region of epithelial cells of naive control rats and OVA-sensitized rats (Fig.
FIGURE 2. EM photomicrographs of mast cells in rat tracheal epithelium from control (A) and from OVA- (B), and HRP-sensitized (C) rats. Tracheas were fixed 90 min after addition of HRP to the luminal side. Mast cells from control and OVA groups showed no signs of activation whereas mast cells from the HRP group were activated, as indicated by a loss of granule density and clear zones surrounding the remaining matrix (bars = 2 μm). These photomicrographs are representative of 20 from each rat, three rats per group.

FIGURE 3. EM photomicrographs of epithelial cells in tracheas fixed 2 min (A and B) or 90 min (C and D) after addition of HRP to the luminal side. Arrows indicate endosomes containing HRP in cells from control rats (A and C) or HRP-sensitized rats (B and D) (bars = 2 μm). These photographs are representative of 20 from each rat, three rats per group.
membrane of an epithelial cell. To quantify HRP uptake, the area of HRP-containing endosomes was measured in epithelial cells of the 3 rat groups. In epithelial cells of HRP-sensitized rats, the area of HRP endosomes was significantly increased (p < 0.01) compared with results in both control groups (Fig. 5A). The value for HRP-sensitized rats was ~4-fold that for naive control rats; the result in OVA-sensitized rats was not significantly different from that in naive control rats. In mast cell-deficient Ws/Ws rats sensitized to HRP, the area of HRP endosomes was increased significantly (p < 0.01) compared with the value in naive rats, 1.21 ± 0.06 μm²/window vs 0.31 ± 0.04 μm²/window (n = 24). At 2 min, there was no HRP observed in the paracellular regions between epithelial cells in any of the groups.

**Transcytosis of HRP transport remained increased after the hypersensitivity reaction.** The area of endosomes in epithelial cells remained significantly increased (p < 0.001) 90 min after challenge in HRP-sensitized rats (Fig. 5B). The area of HRP-containing endosomes in OVA-sensitized rats was also significantly greater (p < 0.05) than in naive controls, but still well below the value in HRP-sensitized rats. Representative EM photomicrographs of tissues fixed 90 min after HRP challenge are shown in Fig. 3, C and D. In mast cell-deficient Ws/Ws rats sensitized to HRP, the area of HRP endosomes was no different at 90 min (1.29 ± 0.04 μm²/window) than at 2 min.

**Distribution of HRP endosomes within different epithelial cell types.** Nonciliated and ciliated cells in the surface layer were examined to determine their relative importance in transcytosis of HRP. In nonciliated cells, HRP endosomes were clearly distinguishable from secretory granules due to differences in electron density and texture. At 2 min after HRP addition, the area of HRP endosomes was ~5-fold greater in nonciliated cells compared with ciliated cells, both in control rats as well as in those sensitized to HRP (Fig. 6A). In both types of cells, the values increased significantly in HRP-sensitized rats compared with naive controls and OVA-sensitized rats. In ciliated cells, although the area of HRP endosomes increased to a greater extent (severalfold) following sensitization, it was still much less than in nonciliated cells (where the increase was ~2-fold). At 90 min, findings followed a similar pattern (note scale change), but, for nonciliated cells, the difference between HRP-sensitized rats and the other groups was greater (~4-fold) (Fig. 6B). Again, the area of HRP endosomes in ciliated cells was very small in controls but increased in HRP-sensitized rats compared with the other groups.

Scanning EM revealed that the ratio of nonciliated cells to ciliated cells in the surface layer of the tracheal epithelium had changed after sensitization (Fig. 7, A-C). The percentage of nonciliated cells increased significantly (p < 0.001) from 30.2 ±

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**FIGURE 4.** EM photomicrographs of epithelial cells in tracheas from HRP-sensitized rats fixed 2 min (A) or 90 min (B) after addition of HRP to the luminal buffer. A. The arrow indicates HRP that is in the process of being endocytosed by a tracheal epithelial cell. B. Full thickness view of tracheal epithelium showing HRP in paracellular regions (arrows) extending continuously from the level of the tight junctions to the basement membrane (bar = 2 μm).

However, in epithelium of HRP-sensitized rats, HRP-containing endosomes were more numerous and were located throughout epithelial cells (Fig. 3A). HRP was also identified in the lamina propria, occasionally within macrophages. Fig. 4A shows an example of HRP in the process of being endocytosed at the apical membrane of an epithelial cell. To quantify HRP uptake, the area of HRP-containing endosomes was measured in epithelial cells of the 3 rat groups. In epithelial cells of HRP-sensitized rats, the area of HRP endosomes was significantly increased (p < 0.01) compared with results in both control groups (Fig. 5A). The value for HRP-sensitized rats was ~4-fold that for naive control rats; the result in OVA-sensitized rats was not significantly different from that in naive control rats. In mast cell-deficient Ws/Ws rats sensitized to HRP, the area of HRP endosomes was increased significantly (p < 0.01) compared with the value in naive rats, 1.21 ± 0.06 μm²/window vs 0.31 ± 0.04 μm²/window (n = 24). At 2 min, there was no HRP observed in the paracellular regions between epithelial cells in any of the groups.

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**FIGURE 5.** Effect of sensitization on uptake of HRP into tracheal epithelial cells. The area of HRP-containing endosomes was measured by computerized image analysis in EM photomicrographs of tracheas from naive controls or from OVA-sensitized or HRP-sensitized rats. Tracheas were fixed 2 min (A) or 90 min (B) after addition of HRP to the luminal side. Values are expressed as means ± SE, and represent endosomal area (μm²) in 206-μm² windows; n = 20 photomicrographs (20 windows) per rat, three rats/group; *, p < 0.05 and **, p < 0.01 compared with values in control rats.
1.6% in naive controls to 79.9 ± 3.7% in HRP-sensitized rats and to 81.4 ± 4.2% in OVA-sensitized rats.

**Paracellular Ag transport**

**Overall HRP transport increased in association with enhanced tissue conductance subsequent to the hypersensitivity reaction.**

To determine the overall rate of transepithelial Ag transport, luminal to serosal HRP fluxes across tracheal tissues were measured over three 30-min periods for 90 min (Fig. 8). Compared with results in control rats, HRP flux was significantly greater in HRP-sensitized rats, and the difference increased with time (p < 0.05 in the first period, p < 0.001 in the third period). HRP-sensitized Ws/Ws mast cell-deficient rats had a flux rate of the same magnitude as the control groups. In addition, tissue conductance, a measure of the integrity of the tight junctions, began to rise postchallenge in HRP-sensitized rats, but not in OVA or saline rats, and was significantly elevated (p < 0.01) at 90 min (33.5 ± 1.2 mS/cm² vs 22.2 ± 1.0 mS/cm² and 20.8 ± 0.6 mS/cm², respectively; n = 3 rats in each group). In addition, there was no conductance change in tracheas from HRP-sensitized Ws/Ws rats following HRP challenge.

**HRP was located in the paracellular spaces subsequent to the hypersensitivity reaction.** EM photomicrographs prepared 90 min after HRP addition (well after the Isc change indicating the hypersensitivity reaction) showed HRP in the paracellular spaces between adjacent epithelial cells, but only in HRP-sensitized rats (Fig. 4B). Of paracellular spaces examined (spaces between 250 cells), HRP was present in 17%. The cell types bordering the spaces did not appear to influence the presence or absence of HRP. HRP was visualized both within spaces that were not dilated and those that were. HRP was never demonstrated in paracellular spaces between epithelial cells in tracheas from control or OVA-sensitized rats. HRP was also not observed in paracellular spaces in HRP-sensitized Ws/Ws rats.

**Discussion**

Extrinsic Ags are responsible for initiating pathophysiology in most cases of airway allergy. Allergic reactions have been well studied, both in humans and in animal models. The accepted mechanism involves a sequence of events beginning with inhalation of the Ag that must pass through the epithelial barrier to reach and activate effector mast cells, located in the epithelium below the level of the tight junctions and in the lamina propria. These cells then release a host of bioactive mediators responsible for functional changes that include secretion of fluid and mucus and contraction of smooth muscle.

Despite the vast literature in the field, there is little information related to events involved in Ag uptake at the epithelium. Neither of the two potential routes of transepithelial macromolecular transport accounts for the rapidity of the secretory response to luminal Ag that we have previously documented in sensitized rats. Under normal circumstances, the paracellular route excludes protein (12);
the transcellular route takes 20–30 min before any intact protein appears at the basal surface of the cell (3). In this study, we demonstrated that Ag challenge of specifically sensitized rats alters not only the timing and route of transepithelial Ag transport, but also dramatically enhances the quantity of intact Ag that penetrates through the epithelial barrier.

For these studies, we used HRP as an Ag, since the intact protein can be measured quantitatively by enzymatic assay, thus allowing us to accurately determine the flux of the whole molecule across tracheal tissue. (Because fragments of HRP may retain the ability to activate mast cells, our data may underestimate the amount of immunologically reactive Ag crossing the epithelium.) Since the reaction product of HRP can be visualized by EM, we were able to identify the transport pathway of this protein across the epithelium. When added to the luminal side of sensitized rat trachea in Ussing chambers, HRP produced a similar pattern of increase in Isc (magnitude and timing) as we have documented for other Ags (5), indicating that HRP is a suitable model Ag for these studies. The increase in Isc in HRP-challenged tracheas from HRP-sensitized rats began shortly after 2 min. Therefore, we fixed tracheal tissues for EM 2 min following luminal challenge to observe the initial pathway used for Ag uptake.

At 2 min after luminal HRP challenge, endosomes containing HRP were present mainly in the apical region of epithelial cells of tracheas from naive control rats and rats sensitized to OVA. However, HRP endosomes were seen throughout epithelial cells in tracheas from HRP-sensitized rats. There was a significant increase in the total size of HRP endosomes in epithelial cells from sensitized rats compared with the other two groups, but no morphological evidence of mast cell activation in any group. To confirm the apparent lack of requirement for activated mast cells in the enhanced uptake of Ag, additional studies were conducted in mast cell-deficient Ws/Ws rats. In these animals, sensitization to HRP again significantly stimulated epithelial uptake of HRP. However, the baseline values for HRP uptake were less in mast cell-deficient rats than control animals, implying a possible role for mast cells in epithelial Ag transport under certain conditions, a postulate requiring further investigation. Our results are similar to those we previously described for enhanced Ag transport across intestinal epithelial cells in specifically sensitized and challenged rats (7, 8), and suggest that sensitization to a particular Ag causes alterations in the normal uptake of that Ag at all mucosal surfaces. HRP was not present within the tight junctions or paracellular regions in any of the groups 2 min after HRP challenge, indicating that HRP transport to the lamina propria occurred via a very rapid transcellular route. The finding that HRP uptake was increased in HRP-sensitized, but not OVA-sensitized, rats at 2 min suggests that there may be recognition of Ag at the apical surface of the epithelium, potentially through an Ig-mediated uptake system. Receptors for Ig have been demonstrated on epithelial cells, including pIgR and FcRn for IgA and IgG, respectively (13, 14). In addition, immunization has been shown to affect protein transport in rat airways (15). Additional experiments are needed to confirm the involvement of Ig and to identify the isotype and receptor mediating the effects reported in our study.

At 90 min postchallenge, the area of HRP endosomes in epithelial cells of HRP-sensitized rats remained significantly ($p < 0.001$) increased compared with the other two experimental groups. At this time point, OVA-sensitized rats also had significantly increased total area of HRP endosomes compared with naive controls. These results suggest that transcellular transport of nonspecific macromolecules is influenced by sensitization alone in the absence of Ag. The regulation of Ag trafficking in epithelial cells is still very poorly understood, but recent preliminary evidence suggests that certain cytokines are capable of enhancing epithelial Ag uptake. Nasal epithelial cells cultured with IFN-$\gamma$ demonstrated greater and more rapid uptake of Ag than unstimulated cells (16). We have also shown enhanced endocytic uptake of HRP protein in intestinal epithelial cells treated with IL-4 (17), a cytokine elevated in allergic states (18, 19). Enhanced cytokine production in sensitized animals could explain, at least in part, the changes observed in transepithelial Ag transport in rats sensitized to an irrelevant Ag.

A comparison of the contribution of different epithelial cell types to endosomal Ag traffic provided interesting and unexpected findings. Ciliated cells took up very little HRP (almost none) in the control condition. Although the area of HRP endosomes in these cells increased severalfold following sensitization and challenge, the value per cell was still relatively low compared with nonciliated cells (20–25% at 2 min and $\approx 5\%$ at 90 min). In addition, scanning EM revealed a difference in the proportion of nonciliated cells in the epithelium of sensitized vs control rats. This was confirmed by counting numbers of ciliated and nonciliated cells in low power scanning electron photomicrographs. The proportion of nonciliated cells in the epithelium increased from $\approx 30\%$ of the total to $\approx 80\%$ after sensitization. Therefore, the contribution of these cells to overall Ag uptake became even greater after sensitization. The factor(s) responsible for altering the ratio of nonciliated:ciliated cells in sensitized rats was not identified in our study. Loss of cilia from epithelial cells has been described as a consequence of exposure to a number of different substances (20), including ozone (21) and rhinovirus infection (22), but to our knowledge it has not been reported in a hypersensitivity model. From our data it is not clear whether the decreased proportion of ciliated cells in sensitized rats was due to a loss of cilia from ciliated cells, or an increase in number of microvillous cells, perhaps due to abnormal proliferation/differentiation of epithelial stem cells. Further studies are needed to explore this issue.

At 90 min postchallenge, HRP was also apparent in the paracellular spaces and tight junctions in epithelium of HRP-sensitized and challenged rats; this observation was never recorded in naive
control or OVA-sensitized rats, even though very thorough analysis was conducted. This finding coincided in time with significantly increased conductance from baseline of tracheal tissues in HRP-sensitized and challenged rats. The morphological and electrophysiological data support the conclusion that opening of the tight junctions occurred after the hypersensitivity reaction to allow paracellular Ag transport. Overall HRP transport across tracheal epithelium determined by flux measurements over 90 min was greater during all three flux periods in HRP-sensitized rats compared with naive controls and OVA-sensitized rats. HRP flux across tracheal epithelium from Ws/Ws rats sensitized to HRP was of the same magnitude as control rats, indicating that this phase of Ag transport was mast cell dependent. Recruitment of the paracellular pathway after intestinal hypersensitivity is also dependent on the presence of mast cells (8). Unlike controls, where the flux stabilized with time, the magnitude of the rate of HRP transport continued to increase in HRP-sensitized/challenged rats. Overall, our results indicate that the enhanced transepithelial Ag transport observed over the 90-min flux period was likely due to transport via both paracellular and transcellular pathways.

Studies on the effect of Ag challenge or application of allergic mediators to airway epithelium in vivo have produced conflicting results, with reports of both increases (23, 24) and decreases (25, 26) in absorption of tracer proteins. Our experiments, performed in vitro, clearly demonstrate an increase in paracellular permeability after a single Ag challenge. Although we reported enhanced influx of Ags into the airway mucosa, it is also possible that opening of the tight junctions would also permit the outflow of extracellular fluid and Igs onto the epithelial surface. In vivo, factors such as blood flow and vascular permeability contribute to overall Ag absorption. In addition, chronic exposure to Ag may alter tracheal mucosal structure that undoubtedly would affect transepithelial protein transport, depending on the condition of the epithelium (i.e., whether undergoing damage or restitution).

At 90 min in tissues from HRP-sensitized rats, mast cell activation was confirmed in several ways: mast cell granules were decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear decreased in density and many granules demonstrated clear. Mast cells contain a number of mediators that have been shown to have effects on epithelial barrier function. Mast cells in rat tracheal epithelium are predominately of the “mucosal” type and contain the specific protease, rat mast cell protease II, that acts on collagen as a substrate. Infusion of rat mast cell protease II i.v. has been shown to increase epithelial permeability to proteins in the intestine (27); it is possible that similar effects would be observed in respiratory epithelium. Histamine has been demonstrated to increase tracheal permeability to HRP in vivo (28), although in vitro studies using primary cultures of bronchial epithelial cultures indicate that histamine has no direct effect on epithelial permeability (29). Mast cells could also potentially affect tight junctions indirectly through the activation of nerves. Mast cells have been shown to activate nerves when cocultured in vitro (30), and mast cell degranulation also induces vagal sensory neuron excitation (31). Mast cells and nerves act as a functional unit to regulate intestinal epithelial ion secretion (32). Cholinergic stimulation of intestinal epithelium causes an increase in tight junction permeability, such that large protein tracers can leak through the paracellular pathway (33). Clearly, there is potential for mast cells to regulate the integrity of the epithelial tight junction directly or indirectly through nerve activation. Mast cells are also capable of releasing a number of cytokines after stimulation with IgE, including IFN-γ (34), TNF-α (35), and IL-4 (36). These cytokines have been demonstrated to decrease the resistance of cultured monolayers of polarized epithelial cells (37–39). In summary, transepithelial Ag transport across airway epithelium in specifically sensitized rats occurred in two phases. Initially, Ag was taken up through a transcellular pathway. Sensitization increased both the amount of specific Ag taken up and the rate at which it appeared in the lamina propria. Subsequent to activation of mast cells and the hypersensitivity reaction, a large increase in protein flux was observed coincident with increased conductance and morphological evidence of paracellular protein transport. These results suggest that, in an allergic individual, small amounts of Ag are initially preferentially transported across the airway epithelium, subsequently activating subepithelial mast cells, resulting in a nonspecific barrier defect that amplifies the hypersensitivity reaction. Our findings from this study suggest that epithelial events involved in Ag uptake may be novel targets for allergic therapy. Acknowledgments

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