HCl-induced and ATP-dependent upregulation of TRPV1 receptor expression and cytokine production by human esophageal epithelial cells

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Several studies using animal models and human tissue samples suggest that proinflammatory cytokine production may underlie the development of erosive esophagitis (EE) in gastroesophageal reflux disease (GERD) (8, 11, 15, 22, 56). The secretion of these mediators is believed to precede the infiltration of neutrophils and eosinophils into the mucosa and submucosa (4, 18, 21, 50), ultimately leading to tissue damage and ulcerations. This viewpoint is supported by endoscopically obtained esophageal biopsies from patients with EE showing infiltration of immune cells. Esophageal biopsies primarily contain epithelial cells, the major component of the esophageal mucosa, suggesting that chemoattractants may be present in and derived from epithelial cells. Enhanced expression of chemokines such as IL-8, a potent chemoattractant for neutrophils, has been detected in esophageal biopsy samples of EE patients, with IL-8 levels being associated with the endoscopic severity of EE (22). Thus production of chemokines by esophageal epithelial cells may be an important step in initiating the inflammatory process. However, the specific mediators responsible for infiltration of immune cells (4, 18) in EE have not been defined.

Given the nature of the inflammatory infiltrate in GERD, possible candidates for chemoattractants released by epithelial cells are IL-5, IL-8, regulated upon activation, normal T cell (MIP-1α), and macrophage inflammatory protein 1α (MCP-1, or CCL-2), and macrophage inflammatory protein 1β (MIP-1β, or CCL-3). These act primarily as chemotactic factors for eosinophils, monocytes, and other immune cells (29, 57). Platelet-activating factor (PAF) is a potent chemoattractant for eosinophils and selectively induces the migration of eosinophils over that of neutrophils (24, 49, 55).

We previously showed that the esophageal mucosa contains acid-sensing transient receptor potential cation channel, subfamily vanilloid member 1 (TRPV1) receptors and, when exposed to HCl, releases substance P, calcitonin gene-related peptide, PAF, and IL-8 (32). We further showed that HCl-induced activation of TRPV1 causes ATP release from esophageal epithelial cells, which, in turn, causes release of calcitonin gene-related peptide and substance P from esophageal submucosal neurons and activation of acetyl-CoA:1-O-alkyl-sn-glycero-3-phosphocholine acetyltransferase (lyso-PAF AT), the enzyme responsible for the production of platelet-activating factor (PAF). This viewpoint is supported by endoscopically obtained esophageal biopsies from patients with EE showing infiltration of immune cells. Esophageal biopsies primarily contain epithelial cells, the major component of the esophageal mucosa, suggesting that chemoattractants may be present in and derived from epithelial cells. Enhanced expression of chemokines such as IL-8, a potent chemoattractant for neutrophils, has been detected in esophageal biopsy samples of EE patients, with IL-8 levels being associated with the endoscopic severity of EE (22). Thus production of chemokines by esophageal epithelial cells may be an important step in initiating the inflammatory process. However, the specific mediators responsible for infiltration of immune cells (4, 18) in EE have not been defined.

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Given the nature of the inflammatory infiltrate in GERD, possible candidates for chemoattractants released by epithelial cells are IL-5, IL-8, regulated upon activation, normal T cell expressed and presumably secreted (RANTES, or CCL-5), eosinophil protein-1α, and monocyte chemoattractant protein-1 (MCP-1, or CCL-2), and macrophage inflammatory protein 1β (MIP-1β, or CCL-3). These act primarily as chemotactic factors for eosinophils, monocytes, and other immune cells (29, 57). Platelet-activating factor (PAF) is a potent chemoattractant for eosinophils and selectively induces the migration of eosinophils over that of neutrophils (24, 49, 55).

We previously showed that the esophageal mucosa contains acid-sensing transient receptor potential cation channel, subfamily vanilloid member 1 (TRPV1) receptors and, when exposed to HCl, releases substance P, calcitonin gene-related peptide, PAF, and IL-8 (32). We further showed that HCl-induced activation of TRPV1 causes ATP release from esophageal epithelial cells, which, in turn, causes release of calcitonin gene-related peptide and substance P from esophageal submucosal neurons and activation of acetyl-CoA:1-O-alkyl-sn-glycero-3-phosphocholine acetyltransferase (lyso-PAF AT), the enzyme responsible for the production of PAF in epithelial cells (33). Repeated application of HCl or ATP causes upregulation of lyso-PAF AT in epithelial cells (33). These data point to ATP as a critical inducer for release of inflammatory mediators, such as PAF, and possibly for cytokines or chemoattractants, such as IL-8.

In the present investigation, we performed a systematic screening of possible chemoattractants or cytokines released by TRPV1 activation in the human esophageal epithelial cell line...
HCl (2 N) was added to BEBM to bring the solution to pH 5.0. Neutrophils, and monocytes, as well as secretion of multiple cytokines, with preference for ageal biopsies from patients with EE. Taken together, these HET-1A cells were similar to those observed in human esophageal epithelium. The HCl-induced cytokine increases observed in cytes. The cytokine increase was inhibited by an ATP receptor antagonist. The HCl-induced cytokine increases observed in HET-1A cells were similar to those observed in human esoph-ageal epithelial morphology, stain positively for cytokeratins, and re-

HET-1A cells were exposed to BEBM, pH 5, with 1.2 mM Ca2+ for 12-min on seven occasions over 48 h. Cells were exposed for 12 min to acidified medium at 8:30 AM, 11:30 AM, and 2:30 PM each on days 1 and 2. On day 3, final exposure took place at 8:30 AM for 12 min. At 1 h after readdition of nonacidified culture medium, the cells were harvested. Gene (mRNA) expression was determined by real-time PCR and protein expression by multiplex ELISA cytokine assay (Eve Technologies, Calgary, AB, Canada). To confirm involvement of vanilloid receptors in acid-induced mRNA changes, cells were pretreated with the TRPV1 receptor antagonist iodoesiniferatoxin (IRTX; Sigma-Aldrich, St. Louis, MO) at 3 × 10−6 M or 10−6 M JNJ-17203212 (Tocris Bioscience, Minneapolis, MN) for 20 min before each exposure to acid. To confirm involvement of the TRPV1 receptor in these acid-induced changes, we used the selective TRPV1 agonist capsaicin (10−3 M; Sigma-Aldrich) with the same repeated exposure protocol and measured mRNA for lyso-PAF AT, IL-8, and eotaxin-1 for comparison with acid-induced changes.

To confirm involvement of ATP in these acid-induced changes, we used the nonselective purinergic receptor antagonist suramin (10−6 M; Sigma-Aldrich) for 20 min before each exposure to acid or direct exposure to the ATP analog ATP-S (10−6 M; Sigma-Aldrich) with the same repeated exposure protocol and measured mRNA for lyso-

PAF AT, IL-8, eotaxins, MCP-1, and MIP-1α for comparison with acid-induced changes.

**Table 1. Primers**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplipcon, bp</th>
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<tr>
<td></td>
<td>Antisense 5′-GCTTAATAAGGACACCGTATGAA-3′</td>
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<td>IL-8</td>
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<td>112</td>
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<tr>
<td></td>
<td>Antisense 5′-AACCTCTCCGACCCAGATTG-3′</td>
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<td>Antisense 5′-TGGTTTGCAGAGGCTGCAAT-3′</td>
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<td></td>
<td>Antisense 5′-ATTTTCCCTCTTGATGACG-3′</td>
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Lyso-PAF AT, acetyl-CoA:1-O-alkyl-sn-glycero-3-phosphocholine acetyl-transferase; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1α; RANTES, regulated upon activation, normal T cell expressed and secreted; hEot, human eotaxin.

HET-1A and examined the role of ATP in mediating the release of these mediators, as well as their upregulation by repeated TRPV1 stimulation. After repeated acid exposure mimicking reflux episodes in GERD patients (2, 43, 47), HET-1A cells upregulate mRNA and protein for the TRPV1 receptor itself and increase expression for lyso-PAF AT, as well as selected cytokines and chemokines known to function as chemoattractants for neutrophils, eosinophils, and monocytes. The cytokine increase was inhibited by an ATP receptor antagonist. The HCl-induced cytokine increases observed in HET-1A cells were similar to those observed in human esophageal biopsies from patients with EE. Taken together, these data indicate that acid-induced changes in the esophageal mucosa may begin with epithelial cells responding to acid by releasing ATP. ATP, in turn, induces upregulation of TRPV1, as well as secretion of multiple cytokines, with preference for mediators that promote epithelial infiltration by eosinophils, neutrophils, and monocytes.

**METHODS**

**HET-1A cell culture.** Human esophageal squamous (HET-1A) cells (American Type Culture Collection, Manassas, VA) were cultured at 37°C in a 5% CO2-humidified atmosphere in bronchial epithelial cell medium (BEGM BulletKit, Lonza, Walkersville, MD) containing basal medium (BEBM) plus additives (BEGM SingleQuots, Lonza) in wells precoated with a mixture of 0.01 mg/ml fibronectin and 0.03 mg/ml Vitrogen 100 (Cohesion, Palo Alto, CA). HET-1A cells were originally obtained from normal human esophageal autopsies. They have been shown to retain main nontumorigenic (51). Experimental procedure. HET-1A cells were exposed to BEBM, pH 5, with 1.2 mM Ca2+ for 12-min on seven occasions over 48 h. HCl (2 N) was added to BEBM to bring the solution to pH 5.0.
achieved with Western Lightning ECL agent (Perkin Elmer, Waltham, MA). Molecular weight was estimated by comparison of sample bands with a prestained molecular weight marker (Bio-Rad, Melville, NY).

**Multiplex ELISA.** The cell culture medium (from control and acid-treated HET-1A cells) was collected and sent to Eve Technologies (Calgary, AB, Canada) for multiplex ELISA 65 cytokine assay, including eotaxin-1, -2, and -3, IL-8, MCP-1, MIP-1α, and RANTES. The complete panel of cytokines is shown in Table 2.

**Human data.** A total of 25 consecutive patients (mean age 63 yr, range 47–76 yr) attending the outpatient units of Campus Bio Medico University of Rome for recurrent typical GERD symptoms (heartburn and/or acid regurgitation) lasting >6 mo and with evidence of EE at endoscopy (grade A in 10 patients, grade B in 4 patients, and grade C in 1 patient, according to the Los Angeles classification) were invited to take part in the study. Exclusion criteria were as follows: presence of Barrett’s esophagus, peptic ulcer disease, history of gastrointestinal (GI) cancer, and GI tract surgery (with the exception of appendectomy). Patients on proton pump inhibitors, H2 antagonists, or prokinetic drugs underwent a 3-wk pharmacological washout before upper endoscopy. Seventeen asymptomatic hospital staff volunteers with no history of GERD (2, 43, 47), HET-1A cells were exposed seven times over 48 h to pH 5 for 2–16 min. TRPV1, IL-8, and eotaxin-1 mRNA increased on incubation with acidified medium, with a significantly higher mRNA expression with longer exposures (Fig. 2). For instance, when the cells were subjected to seven 2-min exposures to pH 5, their mRNA levels did not increase with respect to nonexposed cells. The maximum mRNA increase occurred for repeated 12-min exposures. Increasing the duration of the exposure to 16 min did not measurably increase mRNA for TRPV1, IL-8, or eotaxin-1. The maximally effective 12-min exposure was therefore used for subsequent experiments. This acid exposure protocol induced <2% cell death over the treatment period as measured by Trypan blue exclusion, with HET-1A monolayers remaining intact when examined microscopically. We previously demonstrated that in vitro exposure of esophageal mucosa to a more acidic pH, for example, pH 4, causes significant cell death (11), most likely because the preparation is not buffered by continuous blood perfusion, as occurs in vivo.

Figure 3 shows that the selected acid exposure protocol increases TRPV1 mRNA and protein, similar to the TRPV1 increase previously reported for EE patients (18).

**HCl-induced inflammatory mediators and cytokines.** Consistent with previously reported data (33), the selected acid exposure protocol caused an increase in lyso-PAF AT mRNA and protein (Fig. 4), likely reflected by increased PAF production in response to HCl-induced stimulation (32). A similar increase in lyso-PAF AT mRNA was noted in esophageal biopsies from EE patients. Figure 5 shows a similar increase in IL-8 mRNA and protein in HET-1A cells and increased IL-8 mRNA in esophageal biopsies from EE patients, consistent with data previously demonstrated in rabbit mucosa (32).

A multiplex ELISA for 65 cytokines (Table 2) was used to explore the changed cytokines or chemokines on protein level in response to repeated HCl exposure.

**RESULTS**

**TRPV1 receptors in esophageal epithelium.** To verify the presence of acid-sensing receptors on HET-1A cells, we measured mRNA expression for TRPV1–TRPV6 (Fig. 1). Expression was higher for TRPV1 than TRPV2–TRPV6 mRNA ($P < 0.05$ by ANOVA), suggesting that TRPV1 is the dominant TRPV subfamily member transducing the effects of H⁺ in epithelial cells.

To simulate the recurrent epithelial exposure to acid in GERD (2, 43, 47), HET-1A cells were exposed seven times over 48 h to pH 5 for 2–16 min. TRPV1, IL-8, and eotaxin-1 mRNA increased on incubation with acidified medium, with a significantly higher mRNA expression with longer exposures (Fig. 2). For instance, when the cells were subjected to seven 2-min exposures to pH 5, their mRNA levels did not increase with respect to nonexposed cells. The maximum mRNA increase occurred for repeated 12-min exposures. Increasing the duration of the exposure to 16 min did not measurably increase mRNA for TRPV1, IL-8, or eotaxin-1. The maximally effective 12-min exposure was therefore used for subsequent experiments. This acid exposure protocol induced <2% cell death over the treatment period as measured by Trypan blue exclusion, with HET-1A monolayers remaining intact when examined microscopically. We previously demonstrated that in vitro exposure of esophageal mucosa to a more acidic pH, for example, pH 4, causes significant cell death (11), most likely because the preparation is not buffered by continuous blood perfusion, as occurs in vivo.

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A multiplex ELISA for 65 cytokines (Table 2) was used to explore the changed cytokines or chemokines on protein level in response to repeated HCl exposure.
HCl also caused an increase in mRNA for eotaxin-1, -2, and -3 (Fig. 6–8) that was associated with a corresponding increase in protein secretion. A similar increase in mRNA was observed in esophageal biopsies from EE patients.

A comparable HCl-induced increase in MCP-1 and MIP-1α mRNA and protein expression in HET-1A cells is shown in Figs. 9 and 10. A similar mRNA increase was observed in esophageal biopsies from EE patients.

Role of TRPV1 and ATP in upregulation of inflammatory mediators/cytokines. To investigate the mechanisms underlying the HCl-induced mediator secretion by HET-1A cells, we performed experiments exploring the role of TRPV1 and examining a possible role of ATP in this process. mRNA expression for lyso-PAF AT, IL-8, eotaxin-1, -2, and -3, MCP-1, and MIP-1α was significantly greater in HCl-exposed than control HET-1A cells, as previously shown (Fig. 11; \( P < 0.05 \)). Preincubation with the TRPV1 receptor antagonists IRTX and JNJ-17203212 inhibited the increase in mRNA for lyso-PAF AT, IL-8, eotaxin 1, -2, and -3, MCP-1, and MIP-1α (\( P < 0.05 \)). The values after TRPV1 blockade were not significantly different from control values, supporting a role of TRPV1 in mediating the HCl-induced mRNA increase. Similarly, preincubation with the nonselective ATP antagonist suramin inhibited the increase in mRNA for lyso-PAF AT, IL-8, eotaxin-1, -2, and -3, MCP-1, and MIP-1α (\( P < 0.05 \)). The values after suramin exposure were not significantly different from control values, supporting a role of ATP in mediating the HCl-induced, TRPV1-mediated mRNA increase.

To confirm the involvement of the TRPV1 receptor and ATP in the HCl-induced changes, using the same repeated-exposure protocol used for HCl, we treated HET-1A cells with the selective TRPV1 agonist capsaicin and measured mRNA for lyso-PAF AT, IL-8, and eotaxin-1 (Fig. 12). Repeated capsaicin exposure increased lyso-PAF AT, IL-8, and eotaxin-1 mRNA to levels comparable to those induced by HCl exposure. As expected, the capsaicin-induced mRNA increase was...
significantly \((P < 0.05)\) reduced by the TRPV1 antagonists IRTX, and JNJ-17203212, similar to antagonism for the HCl-mediated response. The capsaicin-induced mRNA increase was also blocked by the ATP antagonist suramin \((P < 0.05)\), indicating a role of ATP in TRPV1-induced mRNA upregulation.

Direct and repeated exposure of the epithelial cells to the nonhydrolyzable ATP analog ATP\(\gamma\)S using the above protocol reproduced the HCl- and TRPV1-induced upregulation of lyso-PAF, IL-8, eotaxin-1, -2, and -3, MCP-1, and MIP-1\(\alpha\), further supporting a role of ATP in the upregulation (Fig. 13).

It has been suggested that IRTX may act as a TRPV1 agonist at \(10^{-7}–10^{-6}\) M, evoking a hypothermic response similar to that evoked by capsaicin. To exclude this possibility in our system, HET-1A cells were exposed for 5 min to \(10^{-7}–10^{-5}\) M IRTX. In addition, cells were exposed for 5 min to HCl (pH 5.0) as positive control. ATP released by HET-1A cells in response to these stimuli was measured using the ATP light luminescence ATP detection assay system (Perkin Elmer). The medium was used to measure ATP according to the manufacturer’s instructions.

Figure 14 indicates that whereas HCl induced significant release of ATP from epithelial cells, IRTX did not induce ATP release from these cells, excluding the possibility that the inhibitory role of IRTX in this system may be due to receptor desensitization.

**DISCUSSION**

While in many chronic inflammatory processes the initiating event of inflammation remains elusive, in esophagitis a likely trigger is the effect of the gastroesophageal refluxate, containing HCl, enzymes such as pepsin, and sometimes bile acids, on the esophageal epithelium. We have focused our investigations on the mechanisms of HCl-induced inflammation in the esophageal mucosa. We previously reported that circular muscle contraction is not affected by exposure to low pH but is significantly reduced when the muscle is exposed to mucosa-derived mediators after preincubation of the mucosa with HCl (11). However, the relevance of HCl-induced esophageal mucosa-derived mediators might extend beyond influencing muscle contraction and may directly contribute to recruitment of the immune cell infiltrate seen in GERD and subsequent inflammatory damage. Epithelial cells constitute the first barrier encountered by acid reflux. Production of inflammatory mediators and cytokines by these cells may therefore be the first step in the inflammatory process, contributing to induction of esophagitis.

The human esophageal epithelial cell line HET-1A was originally obtained from normal human esophageal autopsy tissue. It retains epithelial morphology and cytokeratin expression and has remained nontumorigenic (51). HET-1A cells have been used to examine signaling pathways and transcriptive studies.
tional regulation of cytokine expression (44), to characterize the role of fibroblast growth factor in normal esophageal epithelium and in eosinophilic esophagitis (39), and to examine expression of mucin genes in the esophageal mucosa (54). It is a relevant and well-established tool to examine esophageal epithelial cell response to bile acids (30, 40, 41) and acid-induced activation of TRPV1 (34).

We previously showed that HCl-induced activation of TRPV1 causes release of ATP in HET-1A cells, which in turn activates lyso-PAF AT, inducing production of the inflammatory mediator PAF, and that repeated exposure to low pH or ATP enhances the expression of lyso-PAF AT mRNA and protein (33). Exposure of esophageal epithelium to HCl also causes production and release of the cytokine IL-8 at concentrations sufficient to promote directed migration of leukocytes (32).

To extend the above-described findings, we undertook a systematic investigation of cytokines and chemokines released by esophageal epithelial cells in response to HCl-induced activation of TRPV1 receptors. We used the selective TRPV1 agonist capsaicin to confirm the role of TRPV1 in mediator secretion and confirmed that the regulation is mediated by TRPV1-induced ATP release.

To simulate recurrent exposure to acid, as clinically observed in GERD patients, HET-1A cells were exposed to acidified medium multiple times over 48 h. The selected protocol (seven 12-min exposures to pH 5 over 48 h) caused the maximal increase in mRNA for TRPV1, IL-8, and eotaxin-1, which was not increased by extending the duration of low-pH exposure to 16 min. Thus 12-min HCl exposure may achieve maximal effects for release of inflammatory mediators/cytokines without damaging the cells and closely reproduces mRNA changes observed in human biopsies from EE patients (Figs. 4–10).

HCl-induced upregulation of TRPV1. HET-1A cells contain mRNA for several vanilloid receptors, with substantially higher mRNA levels of TRPV1 than TRPV2–TRPV6. We show that the selected HCl exposure protocol caused mRNA and protein upregulation of TRPV1. This is in agreement with the previously found TRPV1 upregulation in esophageal biopsies from EE patients compared with controls (18).

HCl-induced upregulation of lyso-PAF AT. In cat (9, 12), rabbit (32), and human (9, 10) esophageal mucosa, exposure to acid results in formation of PAF. PAF is an important chemoattractant and activator of immune cells (32), particularly eosinophils (55). In this study, we confirmed that HET-1A cells repeatedly exposed to acidified medium increase lyso-PAF AT mRNA and protein, presumably reflecting increased synthesis of PAF, as previously demonstrated (33). A similar increase in lyso-PAF AT mRNA was observed in esophageal biopsies from EE patients.

HCl-induced upregulation of other chemoattractants in epithelial cells mediated by TRPV1 receptors. We recently showed that acid exposure of the esophageal mucosa causes release of IL-8 in concentrations sufficient to stimulate directed peripheral blood leukocyte migration in an experimental model.
of acid-induced inflammation (32), confirming the role of acid-induced release of this cytokine in immune cell recruitment. We now demonstrate that repeated acid exposure increases IL-8 mRNA and protein in HET-1A cells. The increase in IL-8 mRNA in HET-1A cells is similar to the increase in IL-8 previously demonstrated in rabbit mucosa (32) and similar to the increase in esophageal biopsies from EE patients. These results are consistent with the findings of Souza et al. (50) in several esophageal epithelial cell lines.

We next performed a screening approach using a multiplex ELISA for 65 cytokines demonstrating upregulation of selected mediators that induce infiltration of eosinophils, neutrophils, and monocytes. These include IL-8, a CXC chemokine with potent chemotactic activity for neutrophils; eotaxin-1, -2, and -3, CC chemokines that promote the recruitment of inflammatory cells, particularly eosinophils (45); and MCP-1 and MIP-1α, which are involved primarily in recruitment of monocytes and macrophages (3, 38). These findings were confirmed at the mRNA level using quantitative PCR.

There are three members of the eotaxin family, eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26); their genes are located in different chromosomal positions, but all act on the same chemokine receptor, CCR3, which is highly expressed on eosinophils (1, 25, 26, 42). Thus eotaxins promote eosinophil recruitment and activation, enhanced adhesion, superoxide generation, and degranulation (17, 23, 57). An increase in eotaxin levels was also observed in esophageal biopsies from EE patients and is consistent with recruitment of eosinophils in esophageal inflammation during acid reflux injury (6, 31, 35, 36).

In addition, the data show an increase in MCP-1 and MIP-1α mRNA and protein expression after acid exposure. A similar mRNA increase was found in esophageal biopsies from EE patients. MCP-1 and MIP-1α are involved primarily in recruitment of monocytes and macrophages (3, 38). The presence of mononuclear cells in the esophageal lamina propria, but not in the epithelium, is indicative of EE (16, 20, 27). Production of these chemoattractants by esophageal epithelial cells after acid exposure may promote monocyte recruitment, even if the presence of monocytes is difficult to assess in esophageal biopsies of EE patients, because few biopsies are sufficiently deep to contain a significant amount of lamina propria (27).

The TRPV1 antagonists IRTX and JNJ-17203212 significantly inhibited HCl-induced increases in mRNA levels for the investigated mediators, supporting a role of TRPV1. IRTX is a classic TRPV1 antagonist, but it may also have other effects (14): it may act as an agonist at high doses (48). In the current study, however, IRTX completely inhibited the effect of the selective TRPV1 agonist capsaicin (Fig. 14). In any case, the more recently developed antagonist JNJ-17203212 (52), a second-generation TRPV1 antagonist (5), was used for comparison and had exactly the same effect as IRTX. The critical involvement of TRPV1 in HCl-induced mRNA upregulation of lyso-PAF AT and se-
Selected cytokines is confirmed by the finding of a comparable mRNA upregulation by the selective TRPV1 agonist capsaicin.

Similarly, the ATP antagonist suramin significantly inhibited HCl- or capsaicin-induced increases in mRNA levels for the cytokines examined, supporting a role of ATP in the upregulation. For all cytokines, there were no significant differences in mRNA levels between control values and values after exposure to HCl. Repeated direct exposure to ATP over 48 h results in an increase in mRNA for lyso-PAF AT and all relevant cytokines. Interaction between TRPV1 and ATP has been demonstrated in several experimental preparations, with TRPV1 inducing ATP release (33, 46) and ATP, in turn, potentiating TRPV1-mediated signaling (28, 53).

These results using HET-1A cells are consistent with data obtained in esophageal biopsies from EE patients, in which mRNA for lyso-PAF AT, IL-8, eotaxin-1 and -2, MIP-1α, and MCP-1 were not significantly different from those induced by HCl exposure and significantly greater (*P < 0.001) than control values. As expected, pretreatment with suramin (10−4 M) significantly reduced capsaicin-mediated mRNA increase (#P < 0.001), confirming that the antagonists properly inhibit TRPV1 activation. Inhibition by the nonselective ATP antagonist suramin (Sur, 10−4 M) supports a role of ATP in mediating mRNA upregulation.
MCP-1 was significantly elevated compared with biopsies from healthy controls.

The current study shows that a highly selective spectrum of mediators is induced by exposure of human esophageal epithelial cells to low pH, as occurs in patients with GERD. Among the significantly elevated cytokines, there was a remarkable dominance for mediators that induce infiltration of eosinophils, neutrophils, and monocytes. This suggests that acid exposure is not an indiscriminate inducer of mediator release that leads to nonspecific recruitment of inflammatory cells. HCl can be considered a highly selective inducer of epithelial cell-released mediators recruiting restricted leukocyte subsets involved in acute inflammatory responses (neutrophils and monocytes) and allergic inflammation (eotaxins). This observation may help clarify, at least in part, the poorly understood clinical overlap between GERD and eosinophilic esophagitis: in fact, esophageal eosinophils at levels consistent with eosinophilic esophagitis can be found in up to 30% of patients with reflux esophagitis (13). In addition, our findings may also help explain why suppression of acid production can be beneficial in eosinophilic esophagitis and GERD patients (19, 37). Both diseases likely begin at the epithelial level, and the acidic refluxate may be seen as a regulator of a highly selective set of mediators by esophageal epithelial cells that determine the fate of the inflammatory process.

In summary, this study suggests that exposure to acid activates TRPV1 receptors in esophageal epithelial cells, causing release of ATP. ATP then causes upregulation of the TRPV1 receptors, as well as PAF, IL-8, eotaxins, MCP-1, and MIP-1α, which may contribute to inflammation and injury of the esophageal mucosa. Parallel findings in HET-1A cells treated with this HCl-exposure protocol and esophageal biopsies from EE patients provide a simple and powerful experimental model to examine inflammation-related changes in the esophageal epithelium.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.M., A.A., M.P.G., and D.L. performed the experiments; J.M. and P.B. analyzed the data; J.M., A.A., M.C., F.R., C.F., W.C., J.B., P.B., and K.M.H. interpreted the results of the experiments; J.M., A.A., M.P.G., M.C., F.R., C.F., W.C., J.B., P.B., and K.M.H. edited and revised the manuscript; J.M., A.A., M.P.G., C.F., W.C., J.B., P.B., and K.M.H. approved the final version of the manuscript; M.C., F.R., C.F., J.B., P.B., and K.M.H. are responsible for conception and design of the research; P.B. and K.M.H. prepared the figures; P.B. and K.M.H. drafted the manuscript.
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