Proinflammatory cytokines alter/reduce esophageal circular muscle contraction in experimental cat esophagitis

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Cytokines represent a complex group of secreted molecules involved in most aspects of inflammation in all organs and systems, including intestinal inflammation (46, 52). A number of properties are attributed to these proteins, which mediate stimulatory or inhibitory signals within one or multiple cell types. Cytokines are grouped according to common functional characteristics, and cytokines such as IL-1β, TNF-α, and IL-6 are classified as proinflammatory because of their roles in inflammation-mediated tissue damage.

The bulk of information on cytokines in the gastrointestinal tract is based on studies of human inflammatory bowel disease (IBD), in which levels of proinflammatory cytokines are almost invariably elevated in the affected tissue. High concentrations of IL-1 (IL-1α and IL-1β) are found in both Crohn’s disease and ulcerative colitis intestine (38, 58), where its local effects are largely determined by the relative concentration of its natural receptor antagonist (IL-1ra). IL-6 is also consistently elevated in IBD mucosa derived primarily from macrophages and epithelial cells (34, 53). In contrast to IL-1 and IL-6, protein and mRNA levels of TNF-α have been reported as both normal and elevated in IBD. High TNF-α concentrations are found in the stools of children with Crohn’s disease and ulcerative colitis (9), and production of TNF-α is high in cultures of ulcerative colitis mucosal mononuclear cells (47).

In addition to IBD, proinflammatory cytokines are also elevated in other gastrointestinal conditions characterized by chronic inflammatory processes of infectious or autoimmune etiology, such as Helicobacter pylori-induced gastritis and celiac disease (36, 43). However, very little is known about cytokine profiles in esophagitis. In a recent study, Fitzgerald et al. (21) found that, compared with noninflamed squamous esophageal epithelium, esophagitis tissue is characterized by significantly increased levels of IL-1β. Others found that mucosal biopsies from children and adults with reflux esophagitis released higher amounts of IL-6 than normal esophageal mucosa (18, 48). More importantly, it is unknown whether circular muscle cells produce proinflammatory cytokines in esophagitis and what effect they may have on esophageal contractility.

In the present investigation, we examined IL-1β, IL-6, and TNF-α as possible inflammatory mediators produced in experimental esophagitis in cats and compared motor disturbances induced by these cytokines to those observed in esophagitis specimens. The results show that IL-1β and IL-6, but not TNF-α, are present in the muscle layer of esophagitis and that they induce some of the changes in motor function that occur with esophagitis. We conclude that IL-1β and IL-6 may be responsible for some of the motor function changes observed in experimental esophagitis.

METHODS

Esophagitis model. Experimental procedures were approved by the Animal Welfare Committee of Rhode Island Hospital. Adult male cats weighing between 3.5 and 5.5 kg were used in this study. After an overnight fast, they were anesthetized with ketamine hydrochloride (10 mg/kg), and maintenance doses of ketamine (2.5–5 mg/kg) were administered as needed. To determine lower esophageal sphincter (LES) position, esophageal pressure was measured by a repeated-station pull-through technique, 1–2 mm at a time, with a multilumen catheter having three proximal openings 3 cm apart. A distal perfused smooth muscle; signal transduction; hydrogen peroxide

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Dentsleeve was used to monitor sphincter pressure after the location of the high-pressure zone was established with the perfused side openings, and the three proximal openings measured the amplitude of contraction in the esophageal body. With the sleeve placed across the LES, the most proximal opening, which was 9 cm proximal to the LES, was used to perfuse 0.1 N HCl. The animals were killed and the esophagus was perfused with 0.1 N HCl over three consecutive days, and each esophagus was tested on the fourth day. This protocol has been shown to produce inflammatory changes in the esophageal mucosa and concurrent reduction in the LES in vivo retesting pressure and in vitro spontaneous tone, whereas esophageal perfusion with distilled water had no effect on mucosal appearance or LES resting pressure (3, 25).

In a group of six animals, esophageal pressures were measured at 3 cm proximal to the LES during six successive swallows induced by introduction of 2 ml of water in the proximal esophagus. The pressure measurements were obtained on day 1, before beginning the acid perfusion, and on day 4, before the animals were killed.

**Preparation of esophageal smooth muscle strips and tissue squares.** Animals were initially anesthetized with ketamine (Aveco, Fort Dodge, IA), then euthanized with an overdose of phenobarbital (Scherger, Kennilworth, NJ). The chest and abdomen were opened with a midline incision exposing the esophagus and stomach. The esophagus and LES were isolated and excised as previously described (3, 7). The esophagus and stomach were removed together and pinned onto a wax block at their in vivo dimensions and orientation. The esophagus and stomach were opened along the lesser curvature. The high-pressure zone is characterized by a visible thickening of the circular muscle layer in correspondence with the squamocolumnar junction and immediately proximal to the sling fibers of the stomach. We have shown previously that a 5- to 8-mm band of tissue, coinciding with the thickened area, constitutes the LES and has distinct characteristics when examined in vivo, in the organ bath. The mucosa and submucosal connective tissue were removed by sharp dissection, and the esophagus was excised, beginning at 1 cm proximal to the thickened area and extending proximally to the smooth-striated muscle junction, which was visible to the naked eye. The 2-mm-circular muscle strips were mounted in separate 1-ml muscle chambers and equilibrated for 1 h with continuous perfusion of oxygenated physiological salt solution (PSS) as previously described in detail (5, 6, 10).

To prepare esophageal muscle for Western blot and enzyme immunoassay, the circular muscle layer was cut into 0.5-mm-thick slices with a Stadie-Riggs tissue slicer (Thomas Scientific Apparatus, Philadelphia, PA), and tissue squares were made by cutting twice with a 2-mm blade block, the second cut at a right angle to the first.

**Measurements of contraction.** The 2-mm-wide circular muscle strips were mounted in separate 1-ml muscle chambers as previously described in detail (4). They were initially stretched to 2.5 g to bring them near conditions of optimum force development and were equilibrated for 2 h while being perfused continuously with oxygenated PSS at 37°C. The PSS contained (in mM) 116.6 NaCl, 21.9 NaHCO3, 1.2 KH2PO4, 3.4 KCl, 2.5 CaCl2, 5.4 glucose, and 1.2 MgCl2. The solution was equilibrated with a gas mixture containing 95% O2 and 5% CO2 at 37°C, pH 7.4.

After equilibration, esophageal strips were stimulated with square-wave pulses of supramaximal voltage, 0.2 ms, 2-10 Hz, 10-s trains, delivered by a stimulator (Grass Instruments model S48) through platinum-wire electrodes placed longitudinally on either side of the strip. After electrical stimulation ended, the strips were equilibrated for 30 min, then cumulative dose responses were obtained for ACh (10-7–10-4 M).

To study the effect of selected cytokines on contraction in response to electrical field stimulation (EFS) and ACh, the strips were then incubated in an appropriate concentration of the cytokine for 2 h, and then contraction in response to EFS and ACh were obtained.

**Western blot.** Esophageal and LES circular muscle (100 mg) obtained from thin circular muscle slices, as described in Preparation of esophageal smooth muscle tissue squares, was homogenized in 2 ml PBS (Sigma, St. Louis, MO) (pH 7.4) containing (in mM) 0.1 phosphate buffer, 0.0027 KCl, and 0.137 NaCl. The suspension was centrifuged at 12,000 g for 20 min. The supernatant was frozen in liquid nitrogen for later use. The supernatant was mixed with SDS loading buffer (Sigma) containing 0.125 M Tris-HCl (pH 6.8), 20% glycerol, 10% mercaptoethanol, 0.004% bromophenol blue, and 4% SDS and was boiled for 5 min. Prestained molecular weight marker was prepared in the same manner. After being boiled, these supernatant samples were subjected to SDS-PAGE (90 V, 2 h) using 15% SDS gel. The separated proteins were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad, Melville, NY) at 100 V for 1 h. Transfer of proteins to the nitrocellulose membrane was confirmed with ponceau S staining reagent (Sigma). To block nonspecific binding, the nitrocellulose membrane was incubated in 5% donkey serum in PBS for 2 h followed by three rinses in serum-free buffer. Samples were incubated with anti-IL-1β (1:500, 2 h; R&D Systems, Minneapolis, MN), anti-IL-6 (1:1,000, overnight; R&D Systems), or anti-TNF-α (1:1,500, overnight; Santa Cruz Biotechnology, Santa Cruz, CA) by shaking followed by three washes with antibody-free PBS with 0.5% Tween 20. This was followed by a 60-min incubation in peroxidase-conjugated donkey anti-goat IgG (Jackson Immunoresearch, West Grove, PA). Detection was achieved with an enhanced chemiluminescence agent (Amersham, Arlington Heights, IL). Molecular weight was estimated by comparison of sample bands with prestained molecular weight marker (Amersham).

**Measurement of IL-β, IL-6, and TNF-α.** Esophageal and LES circular muscle (100 mg) was homogenized in 2 ml PBS (pH 7.4). Homogenization consisted of three 10- to 20-s bursts with a Tissue
The suspension was centrifuged at 2,000 g, 4°C, for 20 min. An aliquot of homogenate was taken for protein determination. The supernatant was frozen in liquid nitrogen for later use. The tissue concentrations of cytokines were measured by using enzyme immunoassay kits from Cayman Chemical (Ann Arbor, MI) for IL-6 and TNF-α and from R&D Systems for IL-1β.

ACh release. The release of ACh was measured by using a well-established technique in which ACh stores in a circular smooth muscle preparation are previously labeled with [3H]choline. (14). This technique has been used extensively to examine myenteric or submucosal plexus function of several species (33, 54, 55). Muscle strips were mounted in 1-ml muscle chambers as previously described (4, 5). Mounted strips were incubated for 1 h at 37°C in Krebs buffer containing 0.2 μM [3H]choline (80 Ci/mM; New England Nuclear, Boston, MA), 50 μM physostigmine, and 10 μM hemicholinium. The strips were washed by changing the solution every 3 min for 1 h. After 1 h, the basal tritium release approached a plateau level. After incubation in [3H]choline, the strips were washed three times with 1 ml Krebs containing 50 μM physostigmine and 10 μM hemicholinium alone, and the 3-ml sample was collected and used as basal release. To measure EFS-induced ACh release, strips were stimulated with the appropriate frequency for 0.2 ms. After 30 s, strips were washed three times with 1 ml Krebs containing 50 μM physostigmine and 10 μM hemicholinium alone, and the 3-ml sample was collected for radioactivity measurement. Strips were allowed to rest for 30 min before the next stimulation. Frequencies tested included 0.5, 1, 2, and 5 Hz. Under these experimental conditions, Collins (14) reported that 90% of the radioactivity in the superfusate was [3H]ACh as measured by HPLC.

Protein determination. The amount of protein present was determined by colorimetric assay (Bio-Rad) according to the method of Bradford (8).

Materials. The agents used were SDS from Bio-Rad (Hercules, CA), polyacrylamide from BDH Chemicals (Poole, England), acetic acid from Malinkrodt Specialty Chemicals (Paris, KY), IL-6 and TNF-α from Pierce Endogen (Rockford, IL), TNF-α antibody from Santa Cruz Biotechnology, and IL-6 antibody and IL-1β antibody from R&D Systems. ACh, physostigmine, hemicholinium, saponin, and other reagents were purchased from Sigma.

Statistical analysis. Data are expressed as means ± SE. Statistical differences between means were determined by Student’s t-test. Differences between multiple groups were tested by using ANOVA for repeated measures and checked for significance using Scheffe’s F-test.

RESULTS

In normal animals, the in vivo amplitude of esophageal peristaltic contraction during induced swallowing averaged 107 ± 6 mmHg. After repeated esophageal perfusion with HCl, the contraction was significantly decreased (4 ± 1 mmHg), reflecting reduced contraction of the circular muscle (P < 0.01, unpaired t-test). To investigate the mechanisms responsible for this decrease in pressure, circular muscle strips from normal and acid-perfused esophagi were tested in vitro.

Figure 1 shows that esophageal circular muscle contraction in response to EFS is largely mediated by ACh, as previously

Fig. 2. Frequency-response curves for normal control and IL-1β-incubated circular muscle strips. Amplitude of contraction in response to electrical (i.e., neural) stimulation was significantly reduced (1.2 ± 0.6 g) after acute esophagitis (AE) compared with normal specimens (5.0 ± 1.3 g; P < 0.05 by ANOVA). In contrast, ACh-induced contraction was not significantly affected, suggesting that esophageal circular muscle is not damaged by acid perfusion but that the release of excitatory neurotransmitters may be affected. Values are means ± SE.
reported (2), because the contraction is almost abolished by atropine. Figure 2 shows frequency-response curves for normal and esophagitis circular muscle strips. For normal specimens, the amplitude of contraction in response to electrical (i.e., neural) stimulation was greatest at 5 Hz and was 5.0 ± 1.3 g. EFS-induced contraction was significantly reduced (1.2 ± 0.6 g; n = 3, P < 0.05 by ANOVA) after acute esophagitis. In contrast, ACh-induced contraction was not significantly affected, suggesting that esophageal circular muscle is not damaged by acid perfusion but that the release of excitatory neurotransmitters may be affected. The maximum contraction was 4.0 ± 0.8 and 4.7 ± 0.5 g for control and esophagitis strips, respectively.

To confirm esophagitis-induced impairment of ACh release, we measured ACh release in response to EFS at frequencies of 5 Hz, which produced maximal contraction or lower (Fig. 3). 3[H]ACh release, measured in the supernatant, increased with frequency of EFS compared with levels in the absence of EFS (basal). The measurements confirmed that EFS-induced release of ACh was significantly reduced (P < 0.01, ANOVA) in muscle strips from esophagitis animals, resulting in 258 ± 17 counts/min (cpm)/g of tissue at 5 Hz compared with normal controls (621 ± 42 cpm/g tissue at 5 Hz).

**IL-1β-induced changes.** To examine whether IL-1β may contribute to changes in motor function associated with experimental esophagitis, we incubated normal circular muscle strips in IL-1β (100 U/ml) for 2 h and examined IL-1β-induced changes in contraction in response to EFS or ACh. Figure 4 shows frequency-response curves for normal controls and IL-1β-incubated muscle strips. The amplitude of contraction in response to electrical (i.e., neural) stimulation was significantly reduced (89 ± 7%; n = 5, P < 0.001 by ANOVA) after IL-1β treatment. At 5 Hz, contraction was 4.3 ± 1.0 g for normal and 1.5 ± 0.9 g for IL-1β-treated strips. In contrast, ACh-induced contraction was not significantly affected, suggesting that esophageal circular muscle is not affected by IL-1β but that the release of excitatory neurotransmitters may be inhibited.

We therefore measured ACh release in response to EFS (Fig. 5) from circular muscle incubated in IL-1β (100 U/ml, 2 h). 3[H]ACh release, measured in the supernatant, was significantly reduced (P < 0.01, ANOVA) in IL-1β-treated muscle strips, resulting in 218 ± 23 cpm/g tissue at 5 Hz, compared with 621 ± 42 cpm/g tissue at 5 Hz in normal controls. Values are means ± SE for 6–8 cats.
These data were confirmed by measurement of esophageal protein in normal esophagus showing that IL-1β/H9252/H11006 levels increased from 853 nM/mg protein to 2,017 nM/mg protein after induction of experimental esophagitis as measured by enzyme immunoassay (P < 0.05, unpaired t-test). Western blot did not reach statistical significance. Values are means ± SE.

To determine whether IL-1β is elevated in experimental esophagitis, the presence of IL-1β in the circular muscle layer was examined. Circular muscle tissue was prepared by slicing the circular muscle layer with a Stadie-Riggs slicer, as described in METHODS. The slices consisted largely (~90%) of circular muscle, although the presence of other cell types cannot be excluded. Rat IL-1β antibody was used to detect IL-1β levels by Western blot in the muscle. Figure 6 shows that IL-1β levels increased after experimental esophagitis. These data were confirmed by measurement of esophageal smooth muscle IL-1β by enzyme immunoassay. The figure shows that IL-1β levels increased from 853 ± 99 pg/mg protein in normal esophagus to 2,017 ± 672 pg/mg protein after induction of esophagitis. These data suggest that inflammation-induced motor dysfunction in esophagitis may result from elevated levels of IL-1β, which reduces the release of the endogenous excitatory neurotransmitter ACh.

IL-6-induced changes. Similarly, we examined IL-6-induced changes in contraction in response to EFS or ACh. Figure 7 shows frequency-response curves for normal controls and IL-6-incubated (1 ng/ml for 1 h) circular muscle strips. The amplitude of contraction in response to electrical (i.e., neural) stimulation was significantly reduced (58 ± 10%; n = 6 for control, P < 0.05 by ANOVA) after IL-6 treatment. At 5 Hz, contraction was 8.9 ± 2.08 g for normal and 2.4 ± 1.0 g for IL-6-treated strips. In contrast, ACh-induced contraction was not significantly affected (control, n = 7; IL-6, n = 8), suggesting that, similarly to IL-1β, esophageal circular muscle is not affected by IL-6 but that the release of excitatory neurotransmitters may be inhibited.

We therefore measured ACh release in response to EFS (Fig. 8) from circular muscle incubated in IL-6 (1 ng/ml, 2 h). [3H]ACh release, measured in the supernatant, was significantly reduced (P < 0.01, ANOVA) in IL-6-treated muscle strips, resulting in 218 ± 23 cpm/g tissue at 5 Hz compared with normal controls (621 ± 42 cpm/g tissue at 5 Hz).

To determine whether IL-6 is elevated in experimental esophagitis, the presence of IL-6 in the circular smooth muscle layer was examined by Western blot and enzyme immunoassay. Figure 9 shows that IL-6 levels in esophageal circular smooth muscle significantly increased after induction of experimental esophagitis as measured by enzyme immunoassay (P < 0.05, unpaired t-test). IL-6 levels in esophageal circular smooth muscle increased from 241 ± 72 to 659 ± 84 pg/mg protein after induction of experimental esophagitis. Western blot data did not reach statistical significance because of the large standard errors in the assay. The trend, however, was in the right direction and in agreement with the difference in IL-6 content as measured by immunoassay.

These data suggest that, similarly to IL-1β, IL-6 may contribute to inflammation-induced motor dysfunction in esophagitis. Elevated levels of IL-6 may contribute to reduced release of the endogenous excitatory neurotransmitter ACh in response to electrical (i.e., neural) stimulation, contributing to a reduction in neurally mediated contraction.

TNF-α. In contrast to IL-1β and IL-6, TNF-α (1 ng/ml, 1 h) did not significantly affect either EFS- or ACh-induced contraction of esophageal circular muscle strips (Fig. 10). At a high concentration (100 ng/ml, 1 h), TNF-α significantly reduced both EFS- and ACh-induced contraction, possibly by directly affecting myogenic contractile mechanisms (Fig. 11).

To determine whether these effects of TNF-α on esophageal circular muscle were pharmacological or physiological, we examined, by Western blot and enzyme immunoassay, whether TNF-α was present in the circular muscle layer and increased with experimental esophagitis. Figure 12 shows that TNF-α levels were barely detectable in normal muscle and did not
increase after experimental esophagitis. TNF-α levels were also measured by immunoassay, as shown in the figure. TNF-α levels in esophageal circular smooth muscle were low and did not increase after the induction of experimental esophagitis.

**DISCUSSION**

The role of cytokines as key mediators of inflammation is firmly established, and their blockade has led to the development of new forms of therapy for IBD (24, 41, 50, 51). IBD in animals or humans is associated with changes in motility that may reflect alterations in the function of enteric nerve and muscle (39), as demonstrated both in vivo and in vitro (57). Several animal models of intestinal inflammation have been developed, and abnormalities of intestinal motility can be detected in animals with chemically induced colitis. They include colitis induced by agents administered either directly by enema, such as acetic acid, formalin, or trinitrobenzene sulfonic acid, or by mouth, such as carrageenan or dextran sulfate sodium. In addition, a model of parasite-induced colitis, such as *Trichinella spiralis* (14, 15) has been well characterized. Experimental evidence increasingly suggests that IBD results from dysfunctional immunoregulation manifested by an inappropriate production of mucosal cytokines (29).

In this cat model of experimental esophagitis, we show that esophageal motor function is impaired both in vivo and in vitro. After induction of experimental esophagitis, the in vivo amplitude of the pressure excursion recorded during swallowing was drastically reduced compared with normal animals. Similarly, in vitro contraction of circular muscle strips in response to EFS, which is neurally mediated, was significantly reduced in animals with esophagitis. In contrast, contraction in response to the neurotransmitter ACh, which directly activates muscarinic receptors on the muscle cell membrane, was not affected, suggesting that muscle function is not directly affected by esophagitis-related inflammation. In contrast, the neurons mediating esophageal contraction may be affected by inflammation, and the observed inflammation-induced changes may reflect reduced release of the endogenous neurotransmitter ACh. This conclusion is supported by reduced in vitro release of ACh in response to electrical (i.e., neural) stimulation in experimental esophagitis.

**Cytokines and intestinal inflammation.** As previously mentioned, IL-1β, IL-6, and TNF-α are the major proinflammatory cytokines involved in the inflammatory cascade. Enhanced production of IL-1 has been demonstrated in animal models of intestinal inflammation (16, 40), in the colonic mucosa from ulcerative colitis patients (20, 28, 35), and in monocytes isolated from colonic mucosa of ulcerative colitis patients (38). Tissue levels of IL-1β correlate with disease activity, and the ratio of the endogenous IL-1 receptor antagonist IL-1ra to IL-1 shows the closest correlation with inflammation (12, 44). Administration of IL-1ra attenuates rabbit immune complex colitis (17), and reduction of IL-1ra levels by either gene knockout in mice (26) or by antibody neutralization in rabbits (19) increases the susceptibility to the induction of experimental colitis. These data support an important role of IL-1β in the pathogenesis of gut inflammation.

IL-6, a broad-spectrum cytokine with characteristics of an acute-phase reactant, is produced by immune cells, intestinal epithelial cells, and smooth muscle cells, and IL-6 production is profoundly stimulated by treatment with IL-1β (42). Circulating levels of IL-6 are high in patients with active Crohn’s disease.

![Fig. 11. Frequency-response curves for normal controls and TNF-α-incubated (100 ng/ml, 1 h) circular muscle strips. At a high concentration, TNF-α reduced both EFS- and ACh-induced contraction, possibly by directly affecting myogenic contractile mechanisms. Values are means ± SE.](image1)

![Fig. 12. TNF-α in the circular muscle smooth layer of the esophagus was examined by Western blot and by enzyme immunoassay. TNF-α levels were barely detectable in normal muscle (lanes 2–4) and did not increase after experimental esophagitis (lanes 6–7). Lane 1 shows a positive control for TNF-α. Data shown are for 3 normal cats and 3 esophagitis cats, with each lane representing a different cat. As measured by immunoassay, TNF-α levels in esophageal circular smooth muscle did not change after induction of experimental esophagitis. Immunoassay values are means ± SE for 3 cats.](image2)
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disease (37) and are consistently elevated in IBD tissues, where its source is primarily macrophages and epithelial cells (34). TNF-α is another pleiotropic cytokine with multiple and potent proinflammatory activity (45), and its blockade results in profound downregulatory effects on intestinal inflammation, particularly in Crohn’s disease (1). We therefore investigated a possible role of these proinflammatory cytokines in esophageitis-induced motor dysfunction.

Cytokines and esophageal motor dysfunction. Only a handful of reports have examined cytokine content in esophagitis mucosa or muscle. Recently, immunological determinants of esophageal response to gastroesophageal reflux have been examined to demonstrate that, in esophagitis, endoscopic and histopathological grades of inflammation correlate with mRNA expression of IL-1β, the chemokine IL-8, and IFN-γ. These cytokines are increased 3- to 10-fold compared with non-inflamed squamous or Barrett’s esophageal samples (21). IL-6 release by tissue fragments obtained from esophageal biopsies was determined in children with reflux esophagitis. Esophageal cells from biopsy tissue fragments synthesize and release in vitro a significantly higher amount of IL-6 than controls (18).

We found that the changes in muscle contraction induced by esophagitis were reproduced in vitro by incubating esophageal circular muscle strips in IL-1β or IL-6. Incubation of normal circular muscle strips in IL-1β or IL-6 resulted in reduced in vitro response to EFS but did not significantly affect the response to direct myogenic stimulation with ACh. Similarly to experimental esophagitis, the in vitro release of ACh in response to EFS was significantly reduced by either IL-1β or IL-6. In addition, IL-1β and IL-6 levels were elevated in the circular smooth muscle layer after the induction of experimental esophagitis, supporting the proposition that an esophagitis-induced increase in the levels of these cytokines in the esophageal circular muscle layer may contribute to the reduction of in vivo response to swallowing and in vitro response to EFS. Exogenous IL-6 caused similar effects to those of IL-1β and acid-induced esophagitis. IL-6 may be produced by macrophages and epithelial cells, and its production can be stimulated by IL-β. Collin and colleagues (31, 49, 56) have used cultured smooth muscle cells to show that IL-1β can stimulate IL-6 synthesis by muscle cells. Thus the effect of IL-1β could reflect, in part, stimulation of IL-6 production.

In contrast, TNF-α did not reproduce esophagitis-induced motor changes. At low pharmacological doses (1 ng/ml), TNF-α failed to affect contraction in response to either ACh or EFS, and at high doses (100 ng/ml), it reduced both. Finally, TNF-α levels were barely detectable in normal tissue and did not increase in esophagitis.

These data are consistent with recently reported findings in human esophagitis (48). Endoscopic mucosal biopsies obtained from the esophagus of adult patients with endoscopic evidence of esophagitis were organ-cultured overnight in a Transwell system, and the underneat was recovered for cytokine content. IL-6 and IL-8 were present in all biopsies, and patients with reflux esophagitis showed increased concentration of the proinflammatory cytokine IL-6 in the inflamed tissue compared with controls. IL-1β was detected in patients with more severe inflammation, whereas TNF-α was present sporadically and in low concentrations (48). These data support the view that inflammation-induced motor dysfunction in esophagitis results from elevated levels of IL-1β and IL-6, which reduce the release of the endogenous excitatory neurotransmitter ACh.

Increased release of substance P by the intestine in response to inflammation or to inflammatory cytokines has been previously reported (23, 30, 32). EFS-induced circular muscle contraction, however, is largely mediated by ACh, because contraction is almost abolished by atropine. We did not examine changes in release of other neurotransmitters, because diminished release of ACh is sufficient to account for the observed changes in esophagitis specimens.

These inflammation-induced changes are similar to those reported by Collins and colleagues (14, 15, 22) in T. spiralis-infected rodents. T. spiralis infection induced an acute inflammatory response in intestinal muscle of rats and mice, resulting in suppression of neurotransmitter release, such as ACh and norepinephrine, by enteric nerves. The proinflammatory cytokines IL-1β and TNF-α released from macrophages were reported as potential mediators of the functional alterations because 1) administration of exogenous IL-1β and TNF-α to normal control tissue mimicked the impairment of neurotransmitter release from longitudinal muscle-myenteric plaque observed during T. spiralis infection, 2) increased mRNA expression of these cytokines was present in the longitudinal muscle-myenteric plaque of infected animals, and 3) macrophage depletion prevented the suppression in 3[H]ACh release (22, 27, 39).

Unlike this well-explored model of inflammation, and in agreement with human esophagitis (48), our esophagitis cat model does not involve TNF-α but only IL-1β and IL-6. Recent preliminary data (13) suggest that, when exposed to acid, esophageal mucosa produces both IL-1β and IL-6, but only IL-6 is released to diffuse to the muscle layer, where it induces production of H2O2. H2O2, in turn, may induce production of IL-1β and other inflammatory mediators both in the muscle and mucosal layers. Thus, although initially the mucosal layer releases only IL-6, prolonged inflammation may result in the presence of IL-1β and other inflammatory mediators, both in the mucosa and muscle layer.

In summary, we conclude that proinflammatory cytokines play an important pathophysiological role in experimental esophagitis in the cat and that IL-1β and IL-6, but not TNF-α, are present in the circular muscle layer and may contribute to reduced esophageal contraction in this model by inhibiting release of ACh from myenteric neurons.

GRANTS

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