NEUROIMMUNOPHYSIOLOGY OF THE GASTROINTESTINAL MUCOSA: IMPLICATIONS FOR INFLAMMATORY DISEASES

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The major functions of the gastrointestinal tract are under complex regulatory control. These functions include the absorption and secretion of nutrients, water and electrolytes, and also the mechanical movement of intraluminal contents from the mouth to the anus. Up until approximately a decade ago, the major systems controlling the gastrointestinal tract were considered to be the enteric nervous system (ENS) and the endocrine system (1). The ENS is comprised of extrinsic components such as the sympathetic and parasympathetic nerves, and intrinsic components which consist of the submucosal and myenteric plexuses as well as peptide-secreting enteroendocrine cells interspersed within the gastrointestinal epithelium. The endocrine system regulates through the secretion of blood-borne hormones which affect distant cells or organs. Examples include the hormones gastrin or secretin which regulate gastric HCl or pancreatic secretion, and aldosterone which is released from the adrenal gland and acts on the colon to increase sodium and water absorption. Intestinal function is also altered by pathological stimuli, ie., bacterial enterotoxins such as cholera toxin or *E.coli* ST toxin which stimulate an outpouring of water and electrolytes from the infected gut (2). This is an example of regulation subverted by a disease process.

In the last five years, a new regulatory system has been discovered and extensively studied - the immune system (3,4). It is now clear that the elements of the immune system which either transiently or permanently reside in the lamina propria (consisting of lymphocytes, phagocytes [neutrophils, eosinophils, and macrophages] and mast cells) have close anatomical and physiological interactions with the neural elements of the ENS and with the intestinal mesenchymal cells (subepithelial intestinal myofibroblasts). In so doing, they aid in the regulation of intestinal water and electrolyte transport as well as smooth muscle contraction in both health and disease.

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IMMUNE SYSTEM REGULATORY ELEMENTS

Intestinal lymphocytes play an important role in controlling the immune cells in the lamina propria. However, the best studied effector cells are the mast cells and phagocytes, whose secretory products directly alter either intestinal epithelial cell or smooth muscle function.

Mast Cells. Mast cells are heterogeneous cells that have different morphologic, histochemical and functional characteristics depending on where they reside in the body (5). Thus, connective tissue mast cells in the skin or peritoneum are quite different from pulmonary mast cells or mucosal mast cells of the gut. When compared to the connective tissue mast cell, for example, the mucosal mast cell proliferates in response to the T cell cytokine secretion of interleukin 3 (IL 3), contains chondroitin sulfate rather than heparin as the major proteoglycan, and secretes less histamine and more leukotrienes than prostaglandins.

Mast cells can be activated by cross-linking IgE receptors on the cell membranes (Figure 1). This is the classic anaphylactic reaction, so well understood in the upper airways as part of the pathophysiology of allergic rhinitis, where a genetically susceptible individual's plasma cells secrete anti-IgE in response to some specific allergen, e.g., ragweed pollen. The secreted IgE occupies receptors on mast cells. When the individual comes in contact again with the ragweed pollen, there is an antigen-antibody reaction between the pollen and IgE. This causes clumping of mast cell receptors resulting in activation and degranulation of the cells. The process of degranulation releases the preformed allergic mediators such as adenosine, serotonin and histamine, and also newly synthesized lipid mediators such as leukotriene B_4, C_4, and prostaglandin D_2 (6). These secreted mediators can then affect either the epithelium or the smooth muscle in the upper airways. This same process can occur in the gut, and is called intestinal anaphylaxis.

Phagocytes. Normally present in the lamina propria of the intestine are resident phagocytes of macrophage, eosinophil, and neutrophil lineage (3,7). Interleukins released by the intestinal epithelium, particularly the chemokine IL 8, cause a chemotactic movement of neutrophils into the lamina propria in response to disease states such as invasion of the epithelium by microorganisms (8). Thus, the normal resident population of phagocytes can be greatly expanded with disease. Phagocytes are activated and degranulated during the act of phagocytosis or when specific receptors on the phagocyte cell membrane are occupied with other immune cell products. These cell prod-

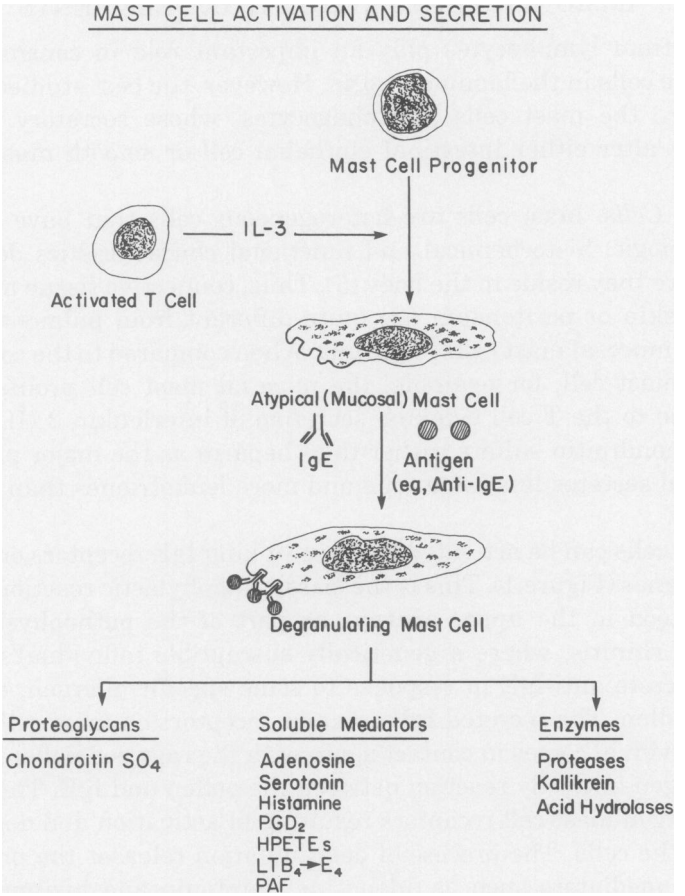


FIG. 1. Schematic representation of the origin stimulation and secretory products released by degranulating mucosal mast cells. (Reproduced from Ref. 3, with permission).

ucts are, for example, leukotriene B_4, platelet activating factor (PAF), complement fragments (C3a and C5a), tripeptides secreted by gram-negative bacteria such as formylmethionylleucylphenylalanine (FMLP) or lipopolysacchride (LPS) which is a component of bacterial cell walls (9) (Figure 2). When activated, phagocytes release a host of immune mediators including prostaglandins of various types (particularly PGE_2), leukotrienes, PAF, interleukins, reactive oxygen species such as superoxide anion ($O_2^{\cdot-}$) hydrogen peroxide (H_2O_2) and hypochlorous acids (HOCl), as well as proteases capable of attacking the basement membrane underlying the epithelium (10).

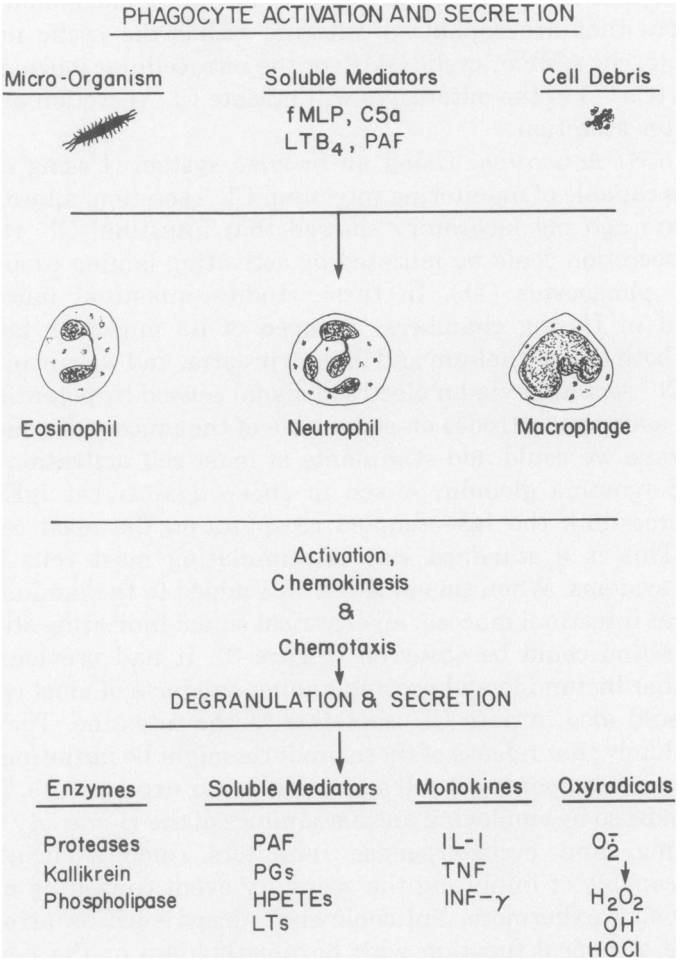


FIG. 2. Schematic representation of the stimulants and products of phagocyte activation and degranulation. (Reproduced from Ref. 3, with permission).

IMMUNE SYSTEM REGULATION OF INTESTINAL ELECTROLYTE TRANSPORT

The fluidity of bowel contents is regulated by the absorption and secretion of electrolytes and water (1,2). The absorption of sodium (Na^+) from luminal fluid is the driving force for the movement of water from the intestinal lumen to the blood and lymph. Conversely, the active secretion of chloride (Cl^-) by the intestinal epithelium is the driving force for the movement of water from the blood into the lumen. Thus, the liquidity of bowel contents results from a balance between

the absorptive and secretory processes. Neural, hormonal and immune mediators that are capable of altering either the cyclic nucleotide content (cyclic AMP or cyclic GMP) or the intracellular ionized calcium content (Ca^{++}) of the enterocyte will initiate Cl^- secretion and therefore water secretion.

Mast Cell Activation. Using an *in vitro* system (Ussing chamber) which is capable of monitoring intestinal Cl^- secretion, approximately five years ago my laboratory showed that intestinal Cl^- (therefore water) secretion could be initiated by activating lamina propria mast cells or phagocytes (11). In these studies intestinal mucosa was mounted in Ussing chambers, stripped of its muscular layers but leaving both the epithelium and lamina propria, and was monitored for active Cl^- secretion via an electrical signal sensed by potential difference measuring electrodes on either side of the mucosa. To such intestinal tissue we could add stimulants of mast cell activation such as anti-IgE (gamma globulin raised in sheep against rat IgE), which would cross-link the IgE-occupied receptors on the mast cell membrane. This is a standard way of stimulating mast cells in other biologic systems. When this anti-IgE was added to the lamina propria side of rat intestinal mucosa, an electrical signal indicating stimulated Cl^- secretion could be observed (Figure 3). It had previously been shown that histamine and prostaglandins, products of mast cell secretion, would also initiate Cl^- secretion in the intestine. Therefore it seemed likely that release of these products might be initiating the Cl^- secretory event upon mast cell activation in our experiments. This was shown to be so by employing antihistaminics of the H_1 variety (diphenhydramine) and cyclooxygenase inhibitors (indomethacin) which proved capable of inhibiting the secretory event caused by mast cell activation. Furthermore, anticholinergic drugs such as atropine, or blockade of neural function with hexamethonium or the neurotoxin tetrodotoxin, would also inhibit the mast cell-mediated secretory event. These latter studies indicated that the mediators released from the activated mast cells were stimulating the ENS in addition to directly stimulating the gut epithelium.

Phagocyte Activation. Neutrophils, but not macrophages or mast cells, express receptors for FMLP. Therefore, this tripeptide is a convenient agent for specifically stimulating the neutrophil. When rabbit colon is mounted in the Ussing chamber and FMLP added to the lamina propria, a short circuit current response results indicating active Cl^- secretion (11) (Figure 4). This secretory event is entirely inhibited by indomethacin pretreatment indicating that prostaglandins are important mediators released from the neutrophil which are

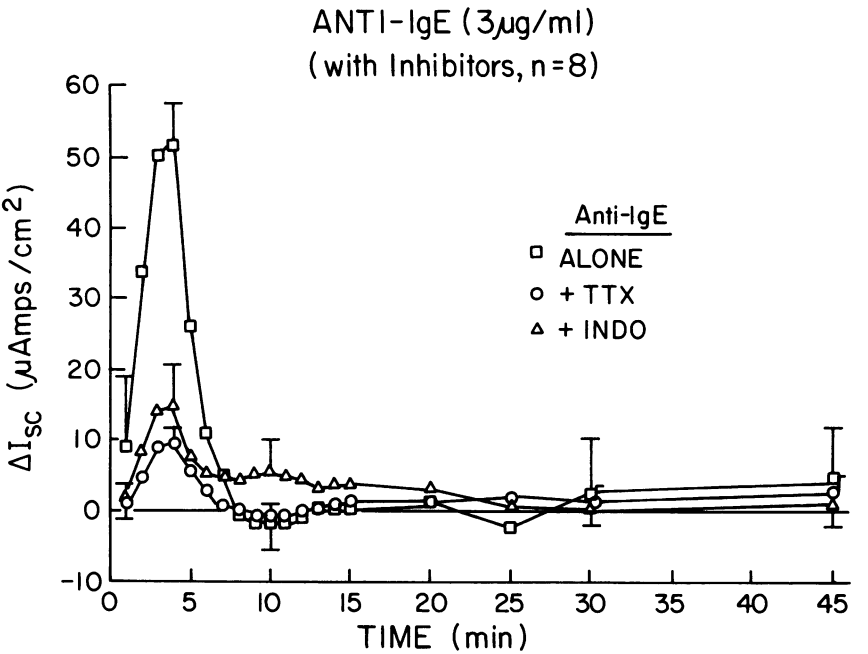


FIG. 3. Chloride secretion by rat colon mounted in Ussing chambers is measured by the short circuit current (ΔI_{sc}). This secretory response to anti-IgE is inhibited by 80% by pretreating the tissue with either the neural inhibitor tetrodotoxin (TTX), or the prostaglandin synthesis inhibitor indomethacin (INDO).

stimulating Cl^- secretion by the epithelium. Furthermore, the response is also blocked by atropine, hexamethonium and tetrodotoxin, indicating that the ENS has also been stimulated by products released from the neutrophil.

The overall scheme of immune system control of intestinal electrolyte transport, as demonstrated by my laboratory (3,4,10) and other investigators (12–14) over the last 5–8 years, can be summarized in Figure 5. Activation of phagocytes or mast cells through disease processes (phagocytosis or intestinal anaphylaxis), or by specific chemical mediators such as FMLP or anti-IgE, causes activation and degranulation of phagocytes or mast cells with the resulting secretion of both presynthesized and newly synthesized inflammatory mediators. These inflammatory mediators may act directly on the epithelium to inhibit Na^+ absorption and to stimulate Cl^- secretion, or they may act on the ENS which releases neurotransmitters such as acetylcholine which have similar effects on the epithelium. Other investigators have shown that these same events result in the stimulation of intestinal smooth

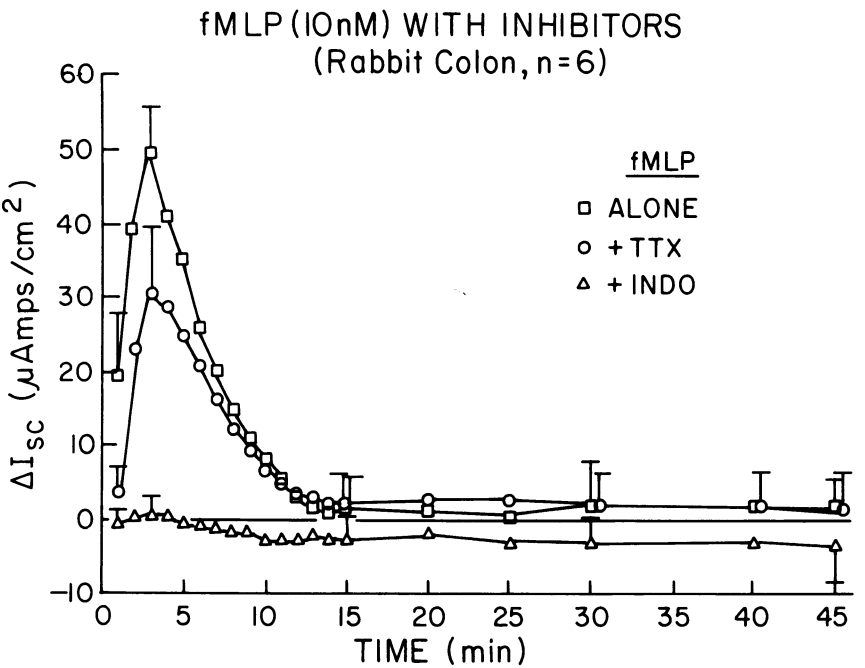


FIG. 4. Chloride secretion by rabbit colon in the Ussing chamber is inhibited partially by pretreatment with tetrodotoxin (TTX), and is almost completely inhibited by pretreatment with indomethacin (INDO).

muscle causing profound changes in gut motility [see summary in (15)]. These responses of the epithelium, intestinal smooth muscle upon activation of the immune system, together with the involvement of the ENS and mesenchymal cells such as myofibroblasts, constitute a new field of regulatory physiology termed *neuroimmunophysiology* (3,4,15,16).

ROLE OF NEUROIMMUNE SYSTEM IN INTESTINAL DISEASE

The involvement of the neuroimmune system in disease processes can be demonstrated by studying specific models of human disease, e.g., nematode infestation or experimental salmonellosis. When rats are infected with nematodes, such as *Nippostrongylus brasiliensis* or *Trichinella spiralis*, there is a proliferation of mast cells in the lamina propria. Interestingly, these mast cells align themselves with the neural fibers of the ENS and nearly 90% of all mast cells are either touching or within 2 μm of the neuron (17). When the infestation has

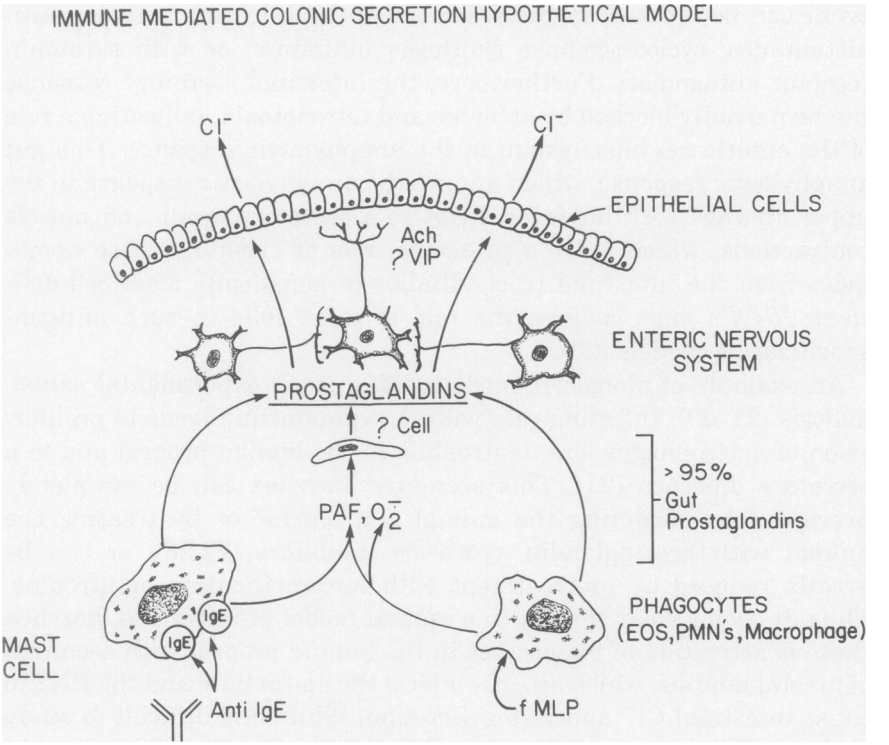


FIG. 5. Hypothetical model for immune-mediated colonic secretion. Activation of mast cells by anti-IgE or activation of phagocytes by FMLP releases inflammatory mediators such as platelet activating factor (PAF), reactive oxygen species (O_2^-) and prostaglandins which either directly stimulate the epithelial cells to secrete Cl^- or, alternatively, activate the enteric nervous system (ENS) which releases neurotransmitters such as acetylcholine (Ach) or vasoactive intestinal polypeptide (VIP) which may stimulate the epithelium to secrete Cl^- Secretion by the intestinal epithelium is accompanied by water secretion. The ?cell between the mast cell/phagocyte and the enteric nervous system (ENS) is the intestinal myofibroblast which responds to inflammatory mediators with release of prostaglandins, thus augmenting the secretory signal (see text).

run its course, and worms have left the body, the intestine of such previously-infected animals can be mounted in the Ussing chamber. In separate Ussing chambers the intestines of uninfected litter mates can be mounted as controls (18,19). If ground-up worm antigen is added to the lamina propria of the control animals who are immunologically naive, no Cl^- secretory event occurs. Conversely, when added to the previously-infected animals, the worm antigen cross-links IgE-occupied receptors on the membranes of the resident mast cells and a significant Cl^- secretory event can be measured. This Cl^- secretory

event can be blocked by pretreatment of the intestine with H_1 antihistaminics, cyclooxygenase synthesis inhibitors, or with serotonin receptor antagonists. Furthermore, the intestinal secretory response can be partially blocked by atropine and tetrodotoxin, indicating a role of the enteric nervous system in the anaphylactic response. This gut anaphylactic response, which mimics the anaphylactic response in the upper airways with hayfever, leads to a secretory event and muscle contractions, which serve a protective role of expelling these nematodes from the intestinal tract. Studies in genetically mast cell-deficient (W/W^v) mice confirm the role of mast cells in such antigen-sensitization models (20).

An example of phagocyte-mediated disease is experimental salmonellosis (21–23). Infecting rats with S. typhimurium leads to proliferation of macrophages and neutrophils in the lamina propria and to a secretory diarrhea (21). This secretory diarrhea can be completely prevented by rendering the animal neutropenic or by treating the animal with prostaglandin synthesis inhibitors (22,23), or can be greatly reduced by pretreatment with hexamethonium or atropine. Thus, it seems clear that in this animal model of infectious diarrhea there is activation of phagocytes in the lamina propria with secretion of prostaglandins, which directly affects the epithelium and the ENS to cause intestinal Cl^- and water secretion. While it is difficult to study such conditions in man, it seems fair to presume that these animal models are reasonable representations of natural human disease.

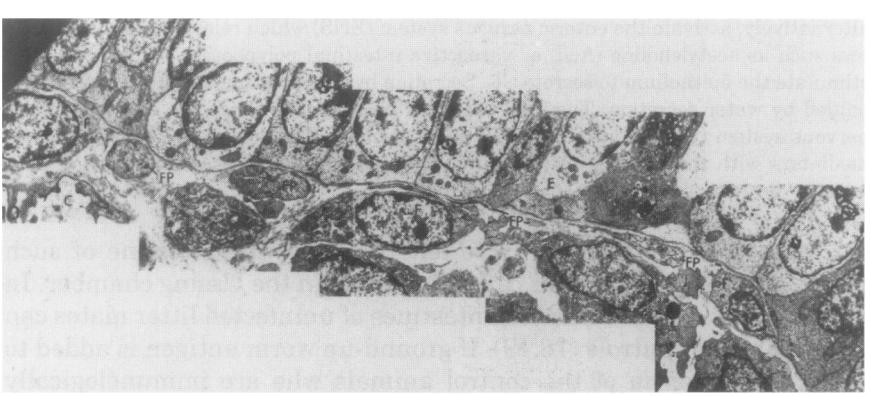
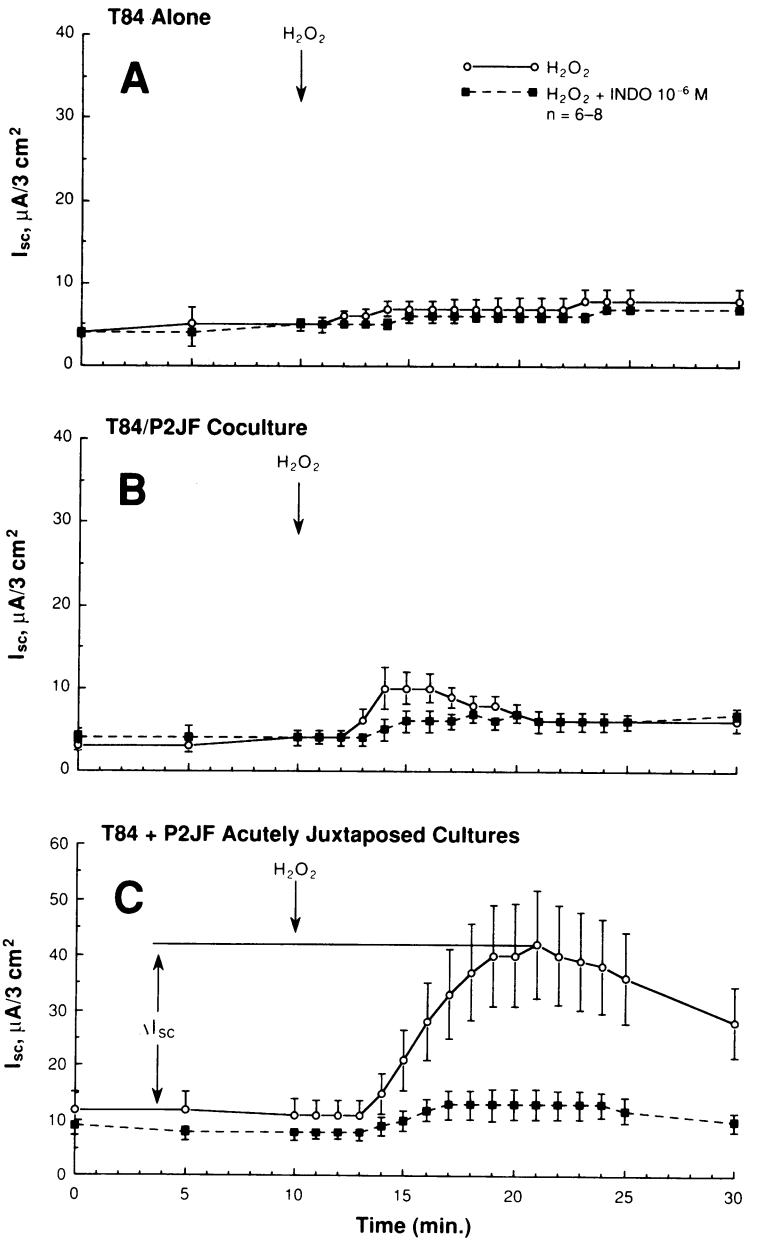


FIG. 6. Electron micrograph of the intestinal epithelium (labeled E) and the underlying myofibroblastic sheath (labeled FP). The epithelial cells are separated from the interconnecting network of myofibroblasts by the basal lamina. The close anatomic relationship between these two cell types is ideal for paracrine interactions. (Reproduced from Ref. 34, with permission).


T84 Alone
A
H_2O_2
H_2O_2
H_2O_2 + INDO 10^{-6} M
n = 6–8
I_{sc}, μA/3 cm^2
T84/P2JF Coculture
B
H_2O_2
I_{sc}, μA/3 cm^2
T84 + P2JF Acutely Juxtaposed Cultures
C
H_2O_2
ΔI_{sc}
I_{sc}, μA/3 cm^2
Time (min.)

INTESTINAL MYOFIBROBLASTS

In both the mast cell and phagocyte models of neuroimmunophysiology, prostaglandin E_2 and prostacyclin PGI_2 are released in large quantities (11). Degranulation of either phagocytes or mast cells alone cannot account for the amounts of PGE_2 or PGI_2 measured. Thus, we postulated that there must be an amplification step in the process of immune-mediated Cl^- secretion. Upon viewing intestinal mucosa histology, we noticed that an interconnecting meshwork of cells called the intestinal fibroblastic sheath lies just under the epithelial basement membrane (24,25). This sheath extends throughout the intestinal tract in a subepithelial location in both the small intestine and the colon (Figure 6). Recently, this fibroblastic sheath has been studied by several investigators and found to have smooth muscle characteristics; therefore they are called myofibroblasts (26). Studies suggest that myofibroblasts in the intestine are analogous to myofibroblasts in other organs such as the glomerular mesangial cell of the kidney, the pericytes which line blood vessels throughout the body, the pulmonary interstitial fibroblasts and the collagen-synthesizing Ito cell of the liver (27). In epithelial locations these cells are arranged in a network with gap junctions and macular adherens junctions serving to connect these cells functionally and electrically. These cells have been shown to express receptors for histamine, bradykinin, acetylcholine, and IL 1, and to respond to these ligands with prostaglandin secretion. When exposed in culture to stimulants that increase intracellular cyclic AMP content, the myofibroblasts assume a stellate morphology (27). This stellate morphology is similar to that seen *in situ* in the gut. We postulated that the myofibroblasts were the cells responsible for amplifying the prostaglandin secretion seen in the gut after mast cell or phagocyte activation. It seemed likely that the secreted prostaglandins could then act both directly on the epithelium to augment Cl^- secretion or could stimulate enteric nerves to induce Cl^- secretion.

FIG. 7. T84 cells cultured on permeable supports do not respond to H_2O_2 with significant Cl^- secretion (panel A). When T84 cells are co-cultured with pig myofibroblasts (P2JF) and then stimulated with H_2O_2, there is a significant Cl^- secretory response which is inhibited by pretreatment of the co-culture with indomethacin (INDO) (panel B). If T84 cells and P2JF cells are separately cultured, acutely juxtaposed, and then stimulated with H_2O_2, there is a significantly augmented Cl^- secretory response which is almost entirely inhibited by pretreatment with indomethacin. These data indicate that T84 cells alone barely respond to H_2O_2 with significant Cl^- secretion. However, when myofibroblasts are present, H_2O_2 releases prostaglandins from the myofibroblasts, which subsequently synergizes with H_2O_2 to cause impressive Cl^- secretion by the T84 cells. (Reproduced from Ref. 29, with permission).

In order to prove this hypothesis, we developed cell culture systems for testing the possibility that fibroblasts might augment the Cl^- secretory response of epithelial cells (28,29). The T84 colon carcinoma epithelial cell line is unique in that it is a well-differentiated epithelial cell line which retains the properties of the native colonic epithelium. When grown to confluence on permeable membranes, it forms tight junctions and also contains a normal complement of secretory receptors on the basolateral cell membranes. Thus, T84 cell cultures can be mounted in modified Ussing chambers and stimulated to secrete Cl^- much in the same way as does the native intestine. Intestinal myofibroblasts have been isolated and grown from human intestine (18 CO) or from piglet intestines (P2JF). When these intestinal myofibroblasts are grown in culture, they can be shown to respond to various stimulants with prostaglandin secretion. For example, acetylcholine, histamine, serotonin, and H_2O_2 are but a few of the secretagogues capable of stimulating prostaglandin E_2 secretion by these intestinal myofibroblasts (27–29).

In order to determine whether myofibroblasts, by secreting prostaglandins could augment the Cl^-secretory response of epithelial cells to agonists, we separately grew T84 epithelial cells and myofibroblasts on permeable supports. By determining the response of the T84 cells alone to a given agonist such as histamine or serotonin, one can measure Cl^- secretion due to receptors on the T84 cells for that agonist (Figure 7A). There was an augmented Cl^- secretory response when T84 cells were co-cultured (Figure 7B) or separately cultured and acutely juxaposed (Figure 7C) with 18 CO myofibroblasts or P2JF myofibroblasts, and then stimulated with the same secretory agonist. The heightened Cl^- secretory response is due to both the direct stimulation of the T84 cells by the agonist (in Fig. 7 the agonist was H_2O_2) combined with the prostaglandins which were synthesized and secreted by the intestinal fibroblasts in response to this agonist (29). If this juxtaposed culture system is pre-treated with indomethacin to prevent prostaglandin synthesis and release, the T84 cells secrete Cl^- at the same rate as they would if the fibroblasts were not present. Thus, with these acutely juxtaposed and separately cultured cell lines we have shown an amplification process that would account for what is seen in the native intestine.

Lastly, recent studies have demonstrated that cytokines such as IL 1 will augment prostaglandin production by these intestinal myofibroblasts. There are two isoforms of cyclooxygenase present in many cells; COX-1 and COX-2 (30). COX-1 is the normally-present, constitutive form of cyclooxygenase and it accounts for the low level, constant

prostaglandin secretion by these cells. In contrast, COX-2 is induced through gene expression by inflammatory mediators such as IL 1. These two forms of cyclooxygenase are under intense investigation by the pharmaceutical industry hoping to find specific inhibitors for COX-1 or COX-2 that have therapeutic anti-inflammatory effects with none of the side-effects attributable to non-steroidal anti-inflammatory drugs (NSAIDS) (31,32). In our own 18 CO cell cultures, we have shown the induction of COX-2 expression. We are currently investigating the implications of this genetic response (33).

SUMMARY

In conclusion, studies of the neuroimmunophysiology of the intestinal mucosa of the past 5–8 years have demonstrated an important role for the immune system in modulating water and electrolyte transport as well as intestinal motility in the gut. Activation of mast cells and phagocytes leads to heightened Cl^- and water secretion, as well as changes in intestinal motility which lead to diarrheal states. These diarrheal responses are self-protective; they rid the intestine of offending microorganisms and antigens. Our investigation of this response has uncovered a new immune accessory cell Cz, the intestinal myofibroblast. This cell seems to play an important role in amplifying the immune signal. This cell is probably also important for the secretion of growth factors onto the epithelium and also the secretion of collagen which results in fibrosis under diseased states. These intestinal myofibroblasts are prolific prostaglandin producers, an important finding because prostaglandin synthesis inhibition has been shown to decrease the development of neoplasia in the gut. Thus, these intestinal myofibroblasts may have other important roles in addition to just modulating water and electrolyte secretion or gut motility. Our laboratory is now engaged in studying these intestinal myofibroblasts in some detail hoping to better understand the biology of these interesting cells.

ACKNOWLEDGMENTS

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DISCUSSION

Sessions, Chapel Hill: I was thrilled when I saw Don's title knowing of his special skills in ion chasing. The conjoint use of the term "inflammatory" excited me. I wonder, since I am interested in inflammatory bowel disease, if you would comment on the way this mechanism might function with the genetic as well as the nonspecific infection in ulcerative colitis and Crohn's Disease.

Powell: Basically, inflammatory bowel disease is the initiation of inflammation by some idiopathic mechanism. Clearly, the various pro-inflammatory and anti-inflammatory cytokines are involved as well as the normal flora. We know that from a host of new genetic models wherein one can use genetic knock-out of various cytokines. If you knock-out all of the anti-inflammatory cytokines, then you get intestinal inflammation that mimics the inflammation of inflammatory bowel disease. Furthermore, if you raise

those animals in a germ-free environment, you don't see that inflammation. So, some disregulation of the immune system combined with normal flora, is what probably accounts for inflammatory bowel disease. Once the inflammation takes place, then these events that I've described today are what drive the symptoms: the diarrhea, the cramping abdominal pain, and the changes in epithelium that lead to bleeding and ulceration.

Dupont, Houston: Your presentation helps us to understand more about the pathogenesis of bacterial diarrhea. I have a question about therapy. You have been interested in salicylates and their effect on the gut in decreasing secretion for a long time. Does your new concept help us understand better how Pepto-Bismol might work in treating diarrhea? Where do you think the future is in terms of strategies of anti-diarrheal therapy concerning mast cell activation in some of these other mechanisms?

Powell: Well, clearly, nonsteroidal anti-inflammatory drugs can be useful in decreasing the secretion of some of the mild inflammatory diarrheas. Indomethacin and salicylates, perhaps like that in Pepto-Bismol, all have some antisecretory effects. The problem you get into in intense inflammation is that there are other inflammatory mediators released besides prostaglandins such as platelet activating factor and oxygen radicals. In severe inflammation they can overwhelm or drive the secretory events apart from prostaglandins. In that case, it appears that salicylates and other prostaglandin-synthesis inhibitors are not very effective. I am not sure whether Pepto-Bismol is going to turn out to be an effective antisecretory medication for severe inflammatory diarrheas.

Now, about mast cells, one of the things that we have to remember is that the inflammatory response is a protective response. The secretory events and the muscle contractions that we see with infectious diarrheas are protective. They are aimed at ridding the body of these offending antigens and if one alters the inflammatory process too much, I am afraid we may move from a self-contained diarrhea to septicemia. So I am a little bit concerned about treating routine infectious diarrhea with intense anti-inflammatory medication. Now, in the case of inflammatory bowel disease where the process is out of control, that is a different situation. I think we have to be careful about some of these anti-inflammatory medications and treatment of routine infectious diarrheas.

Rochester, Charlottesville: May I ask you about the possible role of mast cells and other immune cells in food allergy?

Powell: Well, this is an area in which there hasn't been a lot of research until just recently. Prior to this, it has been in the realm of mysticism. Clearly there are food allergies that we know of, as in the case of infants with milk-protein allergy. I am convinced that there are food allergies in adults as well. The mechanisms discussed today probably apply, but we need more research to track mast cell degranulation in the normal human. With that kind of research, I think we then can devise appropriate therapies for some of these food allergies.

Flemming, Jacksonville: Octreotide administration has been shown to markedly decrease the very high output losses in the end-jejunostomy syndrome. Do you know if octreotide, among its many functions, affects the sodium-chloride exchange, and does that explain some of the marked improvement?

Powell: Octreotide is an analog of somatostatin in the gut. There are somatostatin receptors on enteric nerves and on some of the hormone-producing cells which probably modulate water and electrolyte absorption and secretion. Much of the time, octreotide is partially effective and may decrease secretion by 25%, or as much as 50%, which is a help. Sometimes Octreotide can be a Godsend, but in most of the severe diseases it never completely turns off the diarrhea. This is one of the problems with somatostatin therapy.

Clifton, Iowa City: Would you go back to inflammatory bowel disease and Crohn's specifically, and say a word about the granuloma, which is a little manufacturing plant for a whole lot of cytokines and prostaglandins, and how that might fit into the model.

Powell: Well, granulomas are conglomerations of macrophages and myoepithelial cells. What needs looking at very hard now is whether those myoepithelial cells might really be myofibroblasts from the fibroblastic sheath. These myofibroblasts have mobility and may well form the granuloma. Our current thought is that perhaps these granulomas, or more correctly, the myoepithelial cells are actually modified, activated myofibroblasts. You are correct; they are intense producers not only of prostaglandins, but of growth hormones and a host of other cytokines as well.

One of the recent findings that I think is of interest is that we now know that there is a clear connection between cyclooxygenase and neoplasia of the colon. A number of studies now show that people who have taken salicylates or aspirin for a long time have a decreased incidence of colon cancer. You can actually cause familial polyps to regress by treating patients with nonsteroidal anti-inflammatory drugs. So there is some kind of connection between intense cyclooxygenase stimulation and neoplasia which I think is going to be exciting to investigate over the next few years.