Motility and Allergy

L. Bueno

Neurogastroenterology Unit, INRA, Toulouse, France

Introduction

Food allergy occurs in 6–8% of children and 1–2% of adults and is permanently increasing throughout the world [1, 2]. Most of the adverse reactions to food are immune-mediated reactions, and food antigens may cause IgE and non-IgE immune responses. Of the numerous symptoms of food allergy, at least in the early stages, gastrointestinal disorders, from food protein-induced enterocolitis to constipation, are of paramount importance and are often associated with proctitis. Celiac disease is a specific food protein-induced enteropathy, but eosinophilic gastroenteritis and esophagitis are combined IgE and T-cell-mediated disorders observed in food allergy. Most of these gut inflammatory responses are associated with diarrhea resulting from both secretory and motility disorders and constipation is only observed in IgE sensitization to cow's milk [3]. Experimental data suggest that a type-1 IgE and a mast cell-dependent hypersensitivity response, particularly for motility disorders, mediate the majority of this acute food antigen-induced allergic reaction in the gastrointestinal tract.

Colic is frequently associated with food hypersensitivity and is linked to abdominal distension, bloating and flatus resulting from gastrointestinal motor abnormalities [4]. Diarrhea and vomiting following the ingestion of food containing oral antigens are the most common symptoms of food allergy in children sensitized to various types of food antigens, suggesting the paramount importance of gastrointestinal motor disturbances in the genesis of these symptoms.

Experimental Approach

The era of experimental models for allergic enteropathy began in 1963 when Mota [5] demonstrated the ability to sensitize rats by the simultaneous
injection of antigen and *Bordetella pertussis* vaccine. Successful induction of this sensitization with dietary proteins in animals has been shown to require strict attention to the type and dose of antigen, the strain and age of animals, the need for adjuvant and route of immunization. In both rats and guinea pigs, the major antigens investigated to date have been cow's milk antigens and egg albumin. To avoid issues of tolerance, desensitization and blocking antibody, the animal is raised on a diet devoid of the antigen to be studied. In both species, immunologic responses to egg albumin are more predictable than cow's milk with regard to IgE antibody production [6]. Higher responder strains of animals are chosen, especially the Hooded-Lister rat and the Hartley guinea pig. In general, low doses of antigen favor IgE as opposed to IgG antibody production. The role of adjuvants, such as *B. pertussis* and aluminum hydroxide gel, is well established for IgE antibody elaboration in the rat and mouse. In contrast, the guinea pig achieves sensitization to egg albumin in the absence of adjuvant [7]. Immunization is generally performed by the parenteral administration of antigen to maximize the consistency of the response. Nonetheless, sensitization by oral, intratracheal and intradermal injection has been achieved with consistent gastrointestinal responses to oral challenge.

**In vitro Data**

Immediate hypersensitivity (type-I) reactions of the gastrointestinal tract have been characterized primarily in the small intestine. Isolated longitudinal segments of jejunal smooth muscle obtained from rats previously infected with nematode parasites contract after the addition of antigen prepared from worms, whereas issues obtained from naive rats are unresponsive [8]. This antigen-induced contraction is mediated by the release of 5-hydroxytryptamine (5-HT) from mast cells as evidenced by the ability of mast cell stabilizers, such as doxantrazole, and desensitization of the muscle to 5-HT to inhibit this response. Antigen in the form of ovalbumin administered to previously sensitized animals also contract isolated jejunal strips. However, differences exist in vitro in the response of various regions of the gastrointestinal tract. Indeed histamine, a major mediator released from mast cells, both contracts and relaxes the rat fundic strip, whereas histamine only contracts the longitudinal ileal smooth muscle. The response to 5-HT, a mediator found in the mast cells of some species, also varies among different regions of the gut. In the guinea pig ileum, 5-HT produces a contraction depending directly on the release of acetylcholine, whereas in the colon addition of 5-HT produces several responses, contraction, relaxation or both [9]. In sensitized guinea pigs, the in vitro addition of ovalbumin to isolated colonic segments of the circular layer produce a biphasic response. The initial response consists of a rapid contraction followed by a late response, which is a more sustained but smaller increase in tone and phasic activity [10]. Mepyramine inhibits the initial response
while the leukotriene antagonist, WY4852, and the mast cell stabilizer, doxantrazole, both inhibit the late response.

**Motility Effects of Challenge in Sensitized Animals**

The potential of food protein-induced anaphylaxis to alter gastrointestinal motility has been extensively explored in the Hooded-Lister rat and guinea pig.

In the small intestine, the anaphylactic response to challenge, whatever the route of sensitization, is characterized by IgE antibody-mediated mast cell degranulation and the release of preformed and newly generated mediators.

Numerous articles have described the changes in gastric, intestinal and colonic motility and transit following oral challenge in sensitized rats. Both gastric and intestinal slow waves are altered corresponding to a reduction in frequency; these effects being locally mediated as demonstrated by challenging isolated segments [11, 12]. In fasted rats, the intestinal motor activity is characterized by migrating motor complexes (MMCs) that are suppressed for several hours after a meal. Fargeas et al. [13] demonstrated that antigen challenge in Hooded-Lister rats sensitized to egg albumin also disrupts the MMC pattern replaced by a ‘fed’-type pattern for 2–3 h (fig. 1), and these effects differ from those of two mast cell degranulators, compound 48/80 and BrX-537A, with an initial strong motility inhibition followed by a progressive recovery. Similar data were obtained by Scott et al. [12] in association with diarrhea. However, the involvement of afferent vagal fibers in the genesis of these disorders found by Fargeas et al. [13] were not confirmed by simple vagotomy, also suppressing the efferent vagal fibers and suggesting the involvement of both local and central components in the genesis of small intestine motor alterations.

Antigenic challenge also affects colonic motility in rats sensitized to ovalbumin, however these effects are biphasic corresponding to an early short inhibition that may be attributed in part to mast cell degranulation and stimulation of motility, also blocked by the mast cell stabilizer, doxantrazole [14].

**Role of Mast Cell Mediators**

Mast cell mediators that are released under challenge stimulate the contraction of circular and longitudinal smooth muscle activity in vitro and altered myoelectric and motor activity in vivo. Mast cell degranulation induced by two mast cell degranulators (compound 48/80 and BrX-537A) on duodenal and jejunal myoelectric activity abolishes the intestinal spiking activity of the duodeno-jejunum with progressive recovery; BrX-537A being less active. These effects are antagonized by previous administration of selective 5-HT antagonists. Indeed, methysergide (a 5-HT1 antagonist) reduces by about 80% both the duodenal and jejunal inhibition of spiking activity with early recovery of a normal pattern. Ondansetron (5-HT3 antagonist) and ICS 205–930 (5-TH3/5-HT4 antagonist) respectively shorten and suppress the
inhibition of intestinal spiking activity with early restoration of intestinal motility in both the duodenum and jejunum. These data suggest that at least in rats: (i) the degranulation of peritoneal mast cells induces alterations in intestinal myoelectric activity through the release of 5-HT, and (ii) these effects are mainly mediated through both 5-HT1 and 5-HT3 receptors [15].

Similarly, BrX-537A inhibits colonic motility in a biphasic manner. The immediate strong inhibition of colonic motility lasting 30–40 min was inhibited by the 5-HT3 antagonist, granisetron, suggesting a local effect of 5-HT released from mast cells. In contrast the late inhibition lasting 3–4 h is partly suppressed by the 5-HT1 receptor antagonist, methysergide, and the H1 antagonist, chorpheniramine [16]. This late phase of inhibition has been shown to involve afferent nerves and particularly vagal afferent nerves since it is reduced by systemic capsaicin treatment and blocked by perivagal capsaicin [17]. More recent data also suggest that tryptase release by mast cell degranulation may participate in the inhibition of colonic motility following mast cell degranulation [18].

**Fig. 1.** Mediators and afferent nerves involved in motility disorders associated with oral challenge (ovalbumin) in sensitized rats (From Castex et al.1995).
Mediators of Challenge-Induced Motility Disturbances

The in vitro data obtained from isolated intestinal and colonic strips have suggested that mediators released by mast cell degranulation such as histamine and leukotrienes are partly responsible for the acute contractile response of ileal and colonic longitudinal layers [8, 10].

From in vivo investigations in 1988, Scott et al. [19] described the correlation between IgE titers and the intensity of diarrhea and intestinal myoelectric alterations following challenge in sensitized animals. The involvement of cholinergic motoneurons in the effects of oral antigen challenge on jejunal MMCs was established by blockade of the effects with atropine [13]. Regarding mast cell degranulation, these effects are also suppressed after non-selective destruction of afferent nerves by capsaicin. The role of substance P (SP) in these effects was identified using selective neurokinin-1 receptor antagonists that are able to block the effects of the challenge with egg albumin in sensitized rats [13]. Therefore, it was suggested that mast cell degranulation releases substances able to activate afferents fibers which in turn release SP, and SP may activate cholinergic motor neurons to produce these modifications in intestinal motility.

The role of vagal afferent fibers and 5-HT3 receptors in the effects of oral challenge was confirmed by showing that challenge activates c-Fos expression particularly in the nucleus tractus solitarius (table 1), a brain structure mainly receiving inputs from the vagus [16, 17]. In contrast, Scott et al. [12] did not observe these changes, but in these experiments the challenge was limited to isolated intestinal segments suggesting that passage through the whole gut is necessary to activate vagal afferent fibers. Previous treatment with the 5-HT3 antagonist, granisetron, just prior to challenge prevents both motility disorders and increases c-Fos expression at the nucleus tractus solitarius level suggesting that the motility disorders induced by antigenic challenge involve activation of vagal 5-HT3 receptor and are mediated through the central nervous system (fig. 1, 2). These results are in agreement with previous data.

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NTS = Nucleus tractus solitarius; LPB = lateral parabrachial nucleus; PVN = hypothalamic paraventricular nucleus. From Castex et al. [17].
showing the involvement of capsaicin-sensitive fibers in jejunal secretory response to oral challenge [20]. The fact that ondansetron, a 5-HT3 antagonist, was also active in preventing the duration of MMC disruption following challenge when injected intracerebroventricularly at a 10 times lower dose than those active by the systemic route and without affecting increased brain c-Fos expression, suggests that in addition to a peripheral component, 5-HT3 receptors are also involved at the CNS level in triggering intestinal motor disorders [17].

As for the effects of mast cell degranulators, methysergide and indomethacin reduce the effects of antigenic oral challenge but not chlorpheniramine suggesting that, in addition to 5-HT3, 5-HT1 and prostaglandins are involved in the motility disorders related to challenge but not histamine directly [13].

Colonic motility and transit are also affected by oral antigenic challenge in egg albumin-sensitized rats, these effects corresponding to a colonic inhibition associated with diarrhea [13, 21]. Mast cell involvement is suggested by a significant reduction in the number of granulated mucosal mast cells in sensitized tissues after Ag challenge and in the magnitude of the Ag-induced contractile response in the presence of mast cell stabilizers. This antigen-induced response is independently inhibited by both cyclooxygenase and lipoxygenase enzyme inhibitors and by leukotriene D4 and platelet-activating factor receptor antagonists. The Ag-induced response is resistant to histamine
and the 5-HT antagonists, atropine and tetrodotoxin. These results suggest that the food protein-induced contraction of colonic longitudinal smooth muscle in the sensitized rat is due to IgE-mediated mast cell activation with the subsequent production and release of membrane-derived mediators that, in vitro, act directly on the smooth muscle. However, different results were obtained in another species, the guinea pig.

**Species Differences**

In the guinea pig, sensitization to β-lactoglobulin is easily obtained by oral gavage or spontaneous ad libitum drinking of milk. In sensitized guinea pigs, in contrast to the effects of oral challenge in rats, oral challenge with β-lactoglobulin is associated with a strong stimulation of colonic motility giving rise to diarrhea and increased permeability to macromolecules [22]. We also established that this antigenic challenge shortens the duration of the colonic mean retention time by about 50% (fig. 3). Moreover, in this species we established that most of the digestive effects, including motility hyperkinesia and increased paracellular permeability, not only involved mast cell degranulation but also the release of cytokines such as IL-1β and prostaglandins [23]. Indeed, all these effects were abolished after treatment with the

*Fig. 3.* IL-1β, anaphylaxis and colonic transit in β-lactoglobulin-sensitized guinea pigs. *p < 0.05 from control. From Theodorou et al. [22, 23].
recombinant IL1 receptor antagonist, indomethacin, and a neurokinin-1 receptor antagonist, (SR140333, and the 5-HT3 receptor antagonist, ondansetron (fig. 3). These last data are in agreement with the participation of the brain, cytokines and SP in these motor alterations.

Studies of intestinal motility in calves given antigenic soya protein or sucrose have shown intestinal and colonic motor disturbances linked to diarrhea. Disorders arising from feeding antigenic soya protein were distinct from abnormal motility induced by indigestible carbohydrate. These motility disorders resemble those observed in sensitized guinea pigs and are partly suppressed by chromoglycate, a mast cell stabilizer [24].

Conclusions

Motility disorders observed in food allergy affect the whole digestive tract. The experimental data suggest that they are present in sensitized animals and are exacerbated during challenge. They mostly depend upon the release of mediators from resident gut mast cells with an immediate local component involving 5-HT and histamine and cholinergic stimulation. However most of these effects involve afferent nerves; the brain-gut axis plays an important role in the vagal afferent fibers activated by the local release of mediators and the final release of neurokines such as SP. Despite some species differences in the nature of the motility disorders, their major purpose, in addition to a gut secretory response, is to eliminate the antigen from the gastrointestinal tract.

References

Motility and Allergy


Discussion

Dr. Benninga: Looking at your last slide in which it is proposed that mast cell degranulation leads to motility disturbances, it would be expected that most of the time infants with allergy have diarrhea. But yesterday we also discussed that a minority of these children also have constipation. With your model could you prove that it is just an observation and not a pathophysiological mechanism that causes the constipation?

Dr. Bueno: I cannot answer directly. In our model we were sensitizing animals and triggering a clear-cut anaphylactic reaction when challenging them with antigen, giving rise to inhibition of the motility by a centrally mediated mechanism, and this inhibition may promote constipation. However, in vitro, local challenge of colonic strips from sensitized animals stimulates motility. But in vivo, this effect is associated with water and ionic secretion and may initiate diarrhea. This can explain that, in terms of fecal output, both diarrhea and constipation may be observed. Moreover, depending on the degree of sensitization, we can observe more or less selective degranulation of mast cells and this factor has also to be considered.

Dr. Heymans: If mast cells are playing such an important part, is there any place for chromoglycate in trying to influence the effects on motility? We have performed studies in infants with proven food allergy and looked for gut permeability, and we have shown that if you provide them with chromoglycate before re-challenge you can influence some of the changes in permeability [1]. Would you think that you can also change motility?
Dr. Bueno: Yes, of course. Clearly the involvement of mast cells in both the secretory reaction to challenge and the motility disorder is demonstrated and mast cell stabilizers may prevent both. In animal models, we use doxantrazole but we can speculate that the same results may be obtained with chromoglycate.

Mr. Benyacoub: Can you clarify how substance P, which promotes a pro-inflammatory response, can antagonize gut motility that is in fact initiated by inflammatory signals?

Dr. Bueno: During inflammation, inflammatory mediators and also substances released by mast cells may sensitize afferent neurons which are releasing substance P responsible for the motor disorders. This was demonstrated by the fact that substance P antagonists suppressed most of the motility disorders triggered by local inflammation or mast cell degranulation. This sensitization of afferent nerves by products released from mast cells and immunocytes is responsible for gut hypersensitivity to distension as observed in inflammatory bowel syndrome (IBS). Recently, an increase in nerve terminals close to the mast cells has been described in IBS patients.

Dr. Taminiau: In pediatrics we see only a little bit of IBS, but the majority have what we call chronic abdominal pain, and everything that might be a stress disorder. So how is stress related and is it possible to investigate it in your model and separate IBS from chronic abdominal pain? Is there a pathophysiological basis which we don't have?

Dr. Bueno: A number of factors can produce sensitization or degranulation of mast cells. Stress is able to cause mast cells to degranulate under various stimuli associated with a lowered threshold, generating pain under mechanical stimuli like intestinal contractions. Moreover, in animals sensitized to food allergen, we also observe a sensitization for the development of hypersensitivity to mechanical stimuli. When mast cells are sensitized, just the same pressure applied to the wall is able to produce degranulation without any additional factors. Therefore we can speculate that chronic abdominal pain may be linked to the sensitization of mast cells to degranulate under normal mechanical or chemical stimuli occurring within the gut and then triggering pain.

Dr. Benninga: In children with chronic abdominal pain we recognize functional abdominal pain and irritable bowel syndrome. We know from our own rectal barostat studies that children with IBS have rectal hypersensitivity whereas children with functional abdominal pain do not [2]. Could you suggest that mast cell degranulation plays a role in IBS children but not in children with functional abdominal pain? Is it that easy or is it too easy to conclude?

Dr. Bueno: As I explained earlier, we can hypothesize that in visceral pain, the sensitization of mast cells is not associated with stimulation of the immune system strong enough to produce motility or secretory disorders. For example, neonatal stress is associated with long-term sensitization of mast cells to degranulate and gut hypersensitivity, but no change in intestinal and colonic transit occurs despite the presence of an increased number of lymphocytes and neutrophils.

Dr. H. Hoekstra: If this suggestion is true then it might be worthwhile to test the newly developed anti-IgE in this condition.

Dr. Bueno: Yes, you are right, but not all mast cell degranulations are linked to IgE activation. A number of neuropeptides, as well as chemical or mechanical stimuli, are able to trigger selective degranulation. Recently, it appears that mast cells may selectively deliver one or the other type of granule containing different substances. There is no continuous overflow from mast cells delivering sensitizing molecules to nerve terminals.

Dr. Baerlocher: In humans we also know what is called the pseudo-allergic situation, meaning reaction to food colorings or others. Where would you put these in your scheme?
Dr. Bueno: It has been demonstrated in rats that mast cells may be conditioned to degranulate with a simple auditory conditioning stimulus. Consequently, it is realistic to believe that this can occur in humans and that may be very important in some allergic-like reactions to food. We have to integrate these important data into the pathophysiology of allergic disease.

Dr. Taminiau: But is there any human equivalent of allergy as compared to sensitization in your animal models? Is there any allergic study in humans?

Dr. Bueno: In humans, several studies have demonstrated that in uninformed patients allergic to a specific food, only a third of them produce local allergic reactions to this food when infused intraluminally.

Dr. Schmitz: Just for the pleasure of making a comment, I congratulate you for this very elegant lecture, and particularly for the slide which shows that stress is able to degranulate mast cells through CRF. I think here you have given a physiological answer to processes that we have always seen as purely psychological, and it is interesting to understand the biological mechanism through which psychological processes can affect the gut. My question is how important is this factor compared to the others? My comment relates to what was said yesterday that allergy is a growing condition in the modern world, particularly in developed countries. Could it be that the stressing life we are all living could be a factor of increased allergy? Can we speculate on that?

Dr. Bueno: Stress, as many other factors, has an effect on epithelial cells for the lung as well as the digestive tract. Stress increases tight junction permeability and consequently the uptake of allergens. Consequently, we can speculate that stress may favor the development of asthma and food allergy.

Dr. Taminiau: What extra slides do you have to answer questions we didn't ask. Can you give us the answer and then we will guess what the question should be.

Dr. Bueno: I take this opportunity to extend the data related to the mechanisms by which stress affects gut paracellular permeability, the uptake of allergens and toxins, and subsequently hyperalgesia. Stress activates mast cells promoting the production of cytokines by T lymphocytes and particularly interferon-\gamma responsible for the contraction of the epithelial cell cytoskeleton through an MLCK-dependent mechanism. This contraction of the cytoskeleton opens the tight junction. Such opening of the tight junction by stress is followed by a paracellular bacterial translocation and activation of the local immune system.

Dr. Taminiau: The slides?

Dr. Bueno: This slide shows you that a number of mediators released by mast cells are able to activate or to sensitize sensory nerve terminals. Among them, serotonin and tryptase directly activate receptors located on the nerve terminal to induce nociceptive signals.

Dr. Steenhout: Would you also have some explanation to make a relation between food allergy and migraine?

Dr. Bueno: Yes. Calcitonin gene-related peptide (CGRP) is a neuropeptide contained and released from afferent nerve terminals. CGRP has vasodilatatory effects and is considered to play a role in migraine. Therefore, we can speculate that under chronic activation of the sensory nerve, there is a high level of circulating CGRP suspected of triggering migraine.

Dr. Bee Wah Lee: Can I ask whether there is systemic inflammation when events in the gut result in mast cell degranulation? We have patients who have very severe abdominal symptoms and in whom food allergy is a possible cause, but they have absolutely no signs or hematologic evidence of any systemic inflammation. That is, they have severe gut symptoms but no signs of inflammation.

Dr. Bueno: No skin or other manifestations?

Dr. Bee Wah Lee: No.
Dr. Bueno: That probably depends upon the degree and localization of the activated mast cells. We can speculate that if the sensitization is only limited to the mucosal mast cells within the gut, then the presence of allergens will only activate these mast cells because they are strongly resident mast cells. When more mucosal cells are sensitized, mast cell degranulation may concern mast cells that have migrated or are present in other organs such as the lungs.

Dr. Sinaasappel: With your talk and also the earlier talk, we didn't pay attention to the influence of the narrow endocrine system and mediators at the cellular level on electrolyte and water transport, and there is a close connection between these two. Probably at this time it is not possible to give an answer to that, but it reminds me that in allergic diseases and also in other conditions that raise in stimulants of the intestinal tract that there is also an influence on water and electrolyte transport.

Dr. Bueno: We only have some preliminary data about the chloride transporter that can be modulated by granulate cyclase C presentation of enterocyte which can be activated in some stressful situations. So chloride and water secretions are affected by stress. Many mediators released by mast cells or during stress also affect secretions by the epithelial cells. Indeed, 5-hydroxytryptamine is an important mediator of these effects, and also cytokines from the activated immune system stimulate enterocyte secretions.

Dr. Fritsché: Looking at your slides, is it possible that stress has an influence on the IgE-mediated reactivity?

Dr. Bueno: Stress is able to modulate the sensitization to mast cells, and so perhaps also the synthesis of receptors for IgE. No experiment has demonstrated that stress may directly enhance the synthesis of IgE. However, as already mentioned, stress may affect the degree of the possible uptake of allergens and consequently the production of IgE or the reactivity of mast cells to IgE by sensitizing them to degranulate.

Dr. Taminiau: Thank you very much for your excellent discussion and presentation. I can highly recommend reading the articles in Gut on neonatal maternal deprivation and the changes in colonic epithelial barrier and immunity changes in the long term [3], and also in Gastroenterology [4] in which he explains the studies he was talking about.

References