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Mast Cell–Nerve Interactions in Children With Functional Dyspepsia

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ABSTRACT

Background and Aims: Functional dyspepsia in childhood is commonly triggered by food allergen in sensitised individuals. We investigated the topography of eosinophils and mast cells in gastric antral lamina propria, the interaction of mast cell products with mucosal nerve fibres, and changes in gastric antral muscle slow wave activity in children with atopy and non–atopy-related functional dyspepsia.

Patients and Methods: Open label study of gastric mucosal cow's milk challenge in 10 atopic and 6 nonatopic children (ages 2–12 years) investigated consecutively with gastroscopy for functional dyspepsia. Simultaneous surface electrogastrography and milk challenge were undertaken and laser scanning fluorescence microscopy used to examine the association of mast cell tryptase with mucosal nerves in the gastric mucosa before and after challenge.

Results: Eosinophils and mast cells within the lamina propria were increased in number in children with atopic functional dyspepsia and degranulated rapidly after cow's milk challenge in the atopic group. For degranulating eosinophils, median = 13.0% (interquartile range = 3.7–31.0) premilk versus 32.0% (12.0–42.0) after milk biopsies ($P < 0.05$); for degranulating mast cells, 5.35% (2.7–10.9) premilk biopsies versus 18.75% (12.9–22.1) after milk biopsies ($P < 0.05$). No such differences were seen in nonatopic patients. Mast cells were closely associated with mucosal nerve fibres and released tryptase, which colocalised with proteinase-activated receptors on mucosal nerve fibres. The gastric antral slow wave became abnormal within 2 minutes of antigen challenge in atopics with an increase in dominant frequency instability coefficient ($P < 0.005$), decrease in 3 cycles per minute myoelectrical activity ($P < 0.01$), and increase in bradygastria ($P < 0.01$).

Conclusions: Early-onset neuroimmune interactions induced by cow's milk in the gastric mucosa of atopic children are associated with rapid disturbance of gastric myoelectrical activity and dyspeptic symptoms. ***JPGN 47:472–480, 2008.*** **Key Words:** Dyspepsia—Mast cells—Neuroimmune interaction. © 2008 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

Functional dyspepsia as defined by the Rome II criteria refers to pain or discomfort in the upper abdomen in association with fullness, early satiety, bloating, belching, nausea, retching, or vomiting (1). Disturbed gastric myoelectrical activity is a frequent finding in patients experiencing functional dyspepsia, and it is likely that a number of different mechanisms are implicated in these dysrhythmias (2). In patients with functional dyspepsia who are atopic, it has been suggested that allergic reactions to food proteins may be causative in the genesis of their symptoms (3). In contrast, in nonatopic dyspeptic patients the cause of symptoms is less clearly defined, although mast cell–nerve interactions have been implicated. The mechanisms by which food allergic reactions cause vomiting and abdominal pain have been poorly investigated in humans. It is established that mast cells and eosinophils are involved in immediate hypersensitivity responses in atopic individuals and that mast cells are involved in the pathophysiology of functional gastrointestinal disorders of the hindgut (4,5). There is increasing evidence that mast cell–nerve interactions are critical for the initiation of the disturbance of muscle electrical activity (6). Animal studies have demonstrated that activated mast cells release mediators that increase excitability of enteric neurons leading to abnormal gut sensory and motor function (7,8). In this study, we investigated the topography of mast cells and eosinophils in the gastric antral lamina propria, the interaction of mast cell products with mucosal nerve fibres, and their capacity to cause an immediate change in gastric antral muscle

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slow wave activity in children with atopy and non–atopy-related functional dyspepsia. We demonstrate that allergen-induced changes in gastric myoelectrical activity are associated with degranulation of gastric antral lamina propria mast cells and association of released mast cell tryptase with proteinase-activated receptors on gastric mucosal nerve fibres.

PATIENTS AND METHODS

Patients

The study group comprised 10 atopic children undergoing investigation at Great Ormond Street Hospital for Children with allergen-provoked symptoms fulfilling the Rome II criteria for functional dyspepsia. In all cases, cow's milk protein was suspected of inducing dyspeptic symptoms as suggested by a clinical improvement in symptoms on allergen withdrawal. Children with a personal history of a severe immediate (type 1) allergic reaction to allergen contact/ingestion were excluded from the study. The ''atopic'' group comprised 6 males and 4 females ages 2 to 10 years. The nonatopic dyspeptic (''disease control'') group comprised 6 children (4 male, 2 female) ages 2 to 12 years who had similar dyspeptic symptoms but who had not responded clinically to dietary cow's milk exclusion and had no personal history of atopy. Total immunoglobulin (Ig) E, allergen-specific IgE, total immunoglobulins, and IgG subclasses were measured in all subjects.

The study was approved by the ethics committee of the institution and written informed consent was obtained from the parents of the children studied.

Endoscopic Cow's Milk Challenge With Simultaneous Electrogastrography

All of the children underwent routine diagnostic upper gastrointestinal endoscopy and mucosal biopsy using a paediatric videoendoscope (GIF XP-240; Olympus, Melville, NY). During the procedure, a biopsy-controlled challenge of the gastric antrum with cow's milk was undertaken. Gastric myoelectrical activity was recorded before, during, and after the endoscopic cow's milk challenge. Gastric antral mucosal biopsies were taken before and 10 minutes after the application via the endoscope biopsy channel of 10 mL of a whole-protein cow's milk–based infant formula (SMA Gold; SMA, Maidenhead, UK) with the patient nursed in the right lateral position to keep the milk applied to the gastric antrum. All of the procedures were performed under conscious sedation with midazolam (0.1–0.2 mg/kg) and pethidine (1.0 mg/kg) according to in-house clinical guidelines for endoscopy sedation. Sedation protocols were identical in both groups to minimise any influence of sedation-induced changes in gastric myoelectrical activity. Endoscopies were performed by either of 2 experienced endoscopists (P.J.M., K.J.L.) so that total intubation times were comparable in all of the patients.

Gastric myoelectric activity was recorded by surface electrogastrography (EGG). Potential differences were measured at the abdominal surface by placing 2 recording silver-silver chloride bipolar surface electrodes on the skin of the epigastrium and a reference electrode on the skin in the left iliac fossa. Skin impedance was reduced by applying a skin preparation paste (Omni Prep; Weaver, Aurora, CO). One electrode was positioned over the gastric antrum, after sonographic localisation of the antrum. The second electrode was positioned on the abdomen to the left of the epigastrium, just below the lower rib margin and above the level of the first electrode.

The EGG was recorded for 1 hour before, during the endoscopic milk challenge, and for 1 hour afterward. The electrodes were connected to a 96-kb portable battery-operated recorder (Synectics, Stockholm, Sweden). All of the recordings were made with a sampling frequency of 4 Hz and high and low bandpass filters were set at 1.8 and 16 cycles per minute (cpm). The signals were digitised and downloaded onto a personal computer. The digitised signals were subjected to running frequency spectral analysis using fast Fourier transformation (Synectics Multigram, Stockholm, Sweden). Using this technique, power spectra were computed every 64 seconds from the preceding 256 seconds of the EGG real-time signal. A Hanning window was applied to the data signal to reduce leakage. This procedure generated a series of overlapping spectra, which were then displayed graphically as a time series in the form of a pseudo 3-dimensional plot. This method made both frequency and time analysis possible. The following parameters were measured: dominant frequency (the frequency at which peak power occurs with the normal frequency range defined as 2.0–4.0 cpm) and percentage of normal gastric myoelectrical rhythm and percentage of dysrhythmias (ie, the percentage of time during which normal myoelectrical frequency and dysrhythmias were observed, respectively). Bradygastria was defined as the dominant power lying within the 0.5–2.0 cpm range and tachygastria when the dominant power was in the 4.0–9.0 cpm range. A dysrhythmic episode had to be recorded for at least 2 minutes, with the normal signal simultaneously absent. A measure of the instability of the myoelectrical frequency was provided by the dominant frequency instability coefficient (DFIC) before and after the milk challenge; DFIC is a measure of the dominant frequency change over the EGG recording period. EGG analysis was undertaken by a single investigator (O.B.) experienced in these methods working remotely from the institution in which the recordings were made. The raw EGG signal was visually inspected to verify the presence of motion artifacts during the period of recording before and after milk challenge. This investigator was unaware of whether the child was from the atopic or nonatopic group at the time of analysis. Periods that contained motion artifacts were disregarded for the purposes of the computer analysis. In no case was more than 4% of the total recording disregarded. The postprandial/preprandial power ratio was not included in the study because of the possibility that other factors (eg, gastric volume) may have influenced the parameter.

Histology and Immunocytochemistry

The endoscopic gastric antral biopsies were either fixed in 10% phosphate buffered formalin or snap-frozen. Formalin-fixed biopsies were processed into paraffin wax sections (4-μm thick), whereas whole snap-frozen biopsies were fixed in acetone at −20°C for 20 minutes, then washed in phosphate buffered saline containing 1% bovine serum albumin. Paraffin wax sections were stained with haematoxylin and eosin. Specific antibodies against eosinophil cationic protein, mast

TABLE 1. *Antibodies used in immunocytochemical methods*

Antibodies	Provenance	Dilution	Time
First layer			
IgE (polyclonal)	Dako	1:50 (section)	1 h at room temperature
Mast cell tryptase (monoclonal)	Dako	1:300 (section)	1 h at room temperature
Mast cell tryptase (monoclonal)	Dako	1:500 (whole)	Overnight at 4°C
PGP 9.5 (polyclonal)	UltraClone	1:1200 (whole)	4 h at room temperature
PAR-2 (polyclonal)	Gift from Dr Kapas, London	1:500 (whole)	6 h at room temperature
Second layer			
Alexa 568 antimouse	Molecular Probes	1:200	2 h at room temperature
Alexa 568 antirabbit	Molecular Probes	1:200	2 h at room temperature
Alexa 488 antimouse	Molecular Probes	1:200	2 h at room temperature

Ig = immunoglobulin; PGP = protein gene product; PAR = proteinase activated receptor.

cell tryptase (MCT), IgE, protein gene product (PGP 9.5), and proteinase activated receptor-2 (PAR-2) were used to demonstrate eosinophils, mast cells, IgE, and mucosal nerve fibres, respectively, in whole biopsies.

Gastric biopsies, either slide mounted 4-μm sections or whole biopsies, were incubated in phosphate-buffered saline with primary monoclonal or polyclonal antibodies against MCT, IgE, PGP 9.5, or PAR-2. Working dilutions and times are shown in Table 1. Slides/whole biopsies were then washed and incubated with secondary antibodies, anti-mouse or anti-rat as appropriate, and conjugated to green or red Alexa fluorochrome (Molecular Probes, Eugene, OR). The stained tissue was examined using a laser scanning confocal microscope and digital images captured (TCS NT; Leica UK, Milton Keynes, UK). Total immunocytes, total eosinophils, degranulating eosinophils, total mast cells, and degranulating mast cells were counted in pre- and postmilk challenge biopsies and expressed quantitatively as the percentage of immunocytes within the lamina propria/10 high-power fields. Numbers of degranulating eosinophils/mast cells were expressed as a percentage of the total number of each cell type.

Statistical Analysis

The Student unpaired *t* test was used to compare groups of parametric data including the EGG parameters (DFIC, percentage of bradygastria, and percentage of tachygastria) in the 2 patient groups. A Mann-Whitney *U* test was used to compare the cell counts in pre- and postchallenge biopsies.

RESULTS

Patients

The clinical symptoms of the patients and the results of the systemic immune function tests are shown in Table 2.

Gastric Antral Mast Cells Are Closely Apposed to Mucosal Nerves

Eosinophils and mast cells were readily identifiable in gastric mucosal biopsies from both groups of children. The mast cells present in the lamina propria were mostly around the crypts. Intact cells were easily recognised when stained for MCT by their round shape with a central black hole created by the presence of the nucleus (Fig. 1). The double immunostaining for MCT and PGP 9.5 showed that the mast cells were nearly always in close apposition to the mucosal nerve fibres and that the nerves were wrapped around the crypts as well as running beneath the surface epithelial cells. The close apposition of nerves and mast cells was present in both pre- and postchallenge biopsies; however, numbers of mast cells within 5 μm of mucosal nerves were higher in the atopic dyspeptic biopsies (atopic median [range] = 11 [5–20]; nonatopic = 4 [2–8]). Double staining for MCT (red) and IgE (green) showed that the majority of the mast cells colocalised IgE in the atopic subjects but not in the controls (Fig. 2). The lamina propria of atopic individuals but not controls also contained cells expressing IgE but negative for MCT, which morphologically had the appearance of plasma cells (Fig. 2). Mast cell degranulation was characterised by a change in the appearance of mast cells, with free tryptase granules spreading out from the cell silhouette.

Gastric Antral Mast Cell Degranulation Increases Following Cow's Milk Challenge in Atopic Subjects

Numbers of eosinophils and mast cells and the proportion that were degranulating as a percentage of the total number were counted in the lamina propria. A significant increase in numbers of eosinophils and mast cells was apparent in the gastric mucosal biopsies after cow's milk challenge in the atopic patients compared with nonatopic patients. Eosinophil percentage (median and interquartile range) for atopic premilk was 4.4% (3.7–5.6) versus postmilk 8.4% (5.5–9.7), $P < 0.05$; for nonatopic, premilk was 1.0% (0.7–1.3) versus postmilk 1.2% (0.8–1.6), P = not significant. Mast cell percentage for atopic premilk was 1.9% (1.1–3.7) versus postmilk 7.9% (4.3–13.0), $P < 0.05$; for controls premilk was 2.6% (2.1–3.7) versus postmilk 4.0% (2.9–4.4), P = not significant (Fig. 3).

TABLE 2. *Summary of the 2 patient groups including cow's milk–induced symptoms and atopy-related systemic immune function tests*

Patient no.	Age, y	Atopic family history	Atopic symptoms	GI symptoms	Peripheral blood eosinophil count	Total IgE, kU/L	Total immunoglobulins	IgE-specific RAST
Atopic								
1	6	+	−	Failure to thrive, vomiting	↓	165	N	N
2	3	+	+	Vomiting, diarrhoea	N	72.8 kU/L	N	N
3	9	+		Abdominal pain, food refusal, vomiting, diarrhoea	N	134	N	N
4	6	+	−	Vomiting, early satiety, nausea	↓	N	N	N
5	5	−	+	Abdominal pain, vomiting, nausea, early satiety, constipation	N	N	N	N
6	3	+	+	Regurgitation, vomiting, retching, constipation, diarrhoea	↓	85	N	N
7	9	+	+	Vomiting, abdominal pain	N	57	N	N
8	7	+	+	Vomiting, abdominal pain	N	108	N	Positive CM
9	10	+	+	Vomiting, early satiety	N	360	N	N
10	2	+	+	Vomiting, food refusal, failure to thrive	N	85	↓ IgA, ↓ IgM	N
Nonatopic								
1	2	−	−	Vomiting, failure to thrive	N	N	N	N
2	4	−	−	Vomiting, abdominal pain	N	N	N	N
3	12	−	−	Abdominal pain, nausea,	N	N	N	N
4	7	−	−	Abdominal pain, vomiting	N	N	N	N
5	8	−	−	Abdominal pain, retching, nausea	N	N	N	N
6	11	−	−	Abdominal pain	N	N	N	N

GI = gastrointestinal; Ig = immunoglobulin; RAST = radioallergosorbent test; N = normal/negative, CM = cow's milk.

The proportion of degranulating cells (both eosinophils and mast cells) increased markedly in the atopic patient's postmilk biopsies (degranulating eosinophils median [interquartile range)] = 13.0% [3.7–31.0] premilk vs 32.0% [12.0–42.0] postmilk biopsies, $P < 0.05$; degranulating mast cells 5.35% [2.7–10.9] premilk biopsies vs 18.75% [12.9–22.1] postmilk biopsies, $P < 0.05$). In the nonatopic dyspeptic group, there was no significant increase in degranulating mast cells (premilk 14.5% [15.0–23.0] vs postmilk 17.7% [14.0–24.0]) or eosinophils (premilk 8.6% [8.4–8.8] vs postmilk 9.4% [9.1–9.5]) (Fig. 3). The absolute numbers (density) of degranulating mucosal mast cells were lower in the nonatopic group (data not shown); hence, the higher percentage of degranulating mast cells prechallenge in the nonatopic group, which at first appears paradoxical, represents a smaller absolute number.

Gastric Antral Mast Cell Tryptase Associates With PAR-2 Receptors on Mucosal Nerves

Nearly all of the tryptase granules released from the mast cells remained in close contact with the nerve fibres (Fig. 4). In some places, tryptase granules colocalised (in yellow in Fig. 4) with the nerve fibres. Double immunostaining mucosal nerve fibres for PGP 9.5 and PAR-2 showed that a population of nerve fibres possessed PAR-2 receptors. When nerve fibres were immunostained for

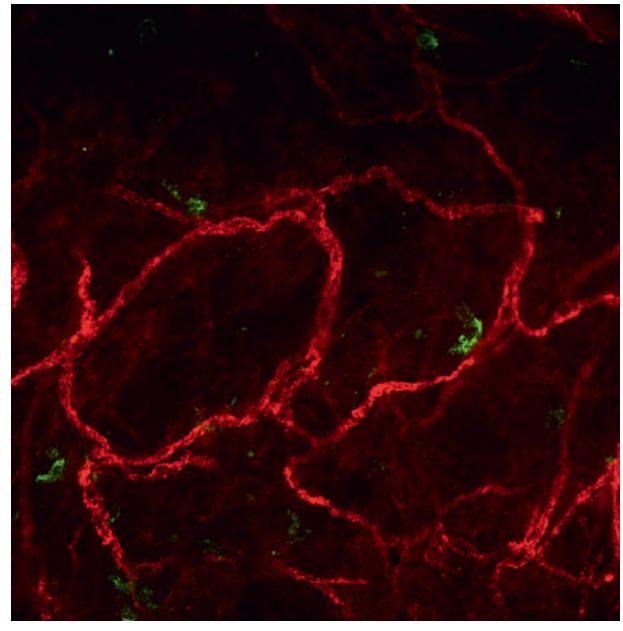

FIG. 1. Gastric antral mucosal biopsy from an atopic subject stained for mast cell tryptase (green) and protein gene product 9.5 (red) showing mast cells present in the lamina propria mostly around the crypts. Intact nondegranulating cells are recognised by their discrete round shape with a central black hole created by the presence of the nucleus. Original magnification ×100.

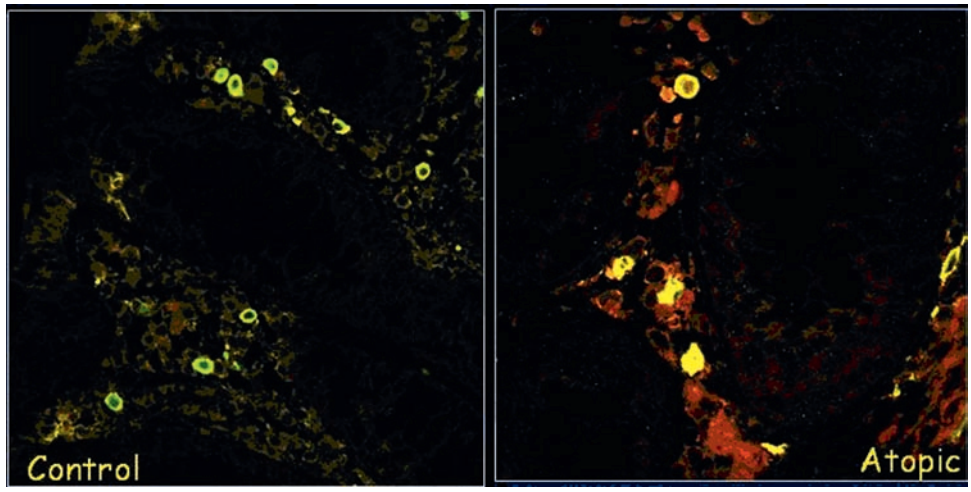

FIG. 2. Double immunostaining of gastric antral mucosal biopsy from nonatopic and atopic subjects stained for mast cell tryptase (green) and immunoglobulin (Ig)E (red) showing colocalisation of tryptase and IgE (yellow) in atopic subjects. Original magnification ×100.

PAR-2 and MCT, tryptase granules could be seen to colocalise with them, suggesting that tryptase released from mast cells was closely associated with mucosal nerve fibre PAR-2 receptors (Fig. 5).

Electrogastrographic Abnormalities Are Induced by Cow's Milk Challenge

In each of the atopic patients, cow's milk challenge was followed by a degree of EGG disorganisation. Within 2 minutes of the challenge, there were signs of disruption of the normal 3 cpm myoelectric activity; this was not seen in any of the nonatopic group (Fig. 6). The percentage of the DFIC increased significantly from 18.10 ± 12.28 (mean ± standard deviation [SD]) prechallenge to 47.2 ± 22.4 postchallenge in the atopic group ($P < 0.005$), but changed little in the nonatopic group (27.2 ± 11.15 prechallenge vs 22.5 ± 12.24 afterward, $P =$ not significant). The percentage of 3 cpm gastric myoelectrical activity diminished postchallenge in atopy-related dyspepsia but not in nonatopic dyspeptics (Table 3). The percentage of bradygastria also increased

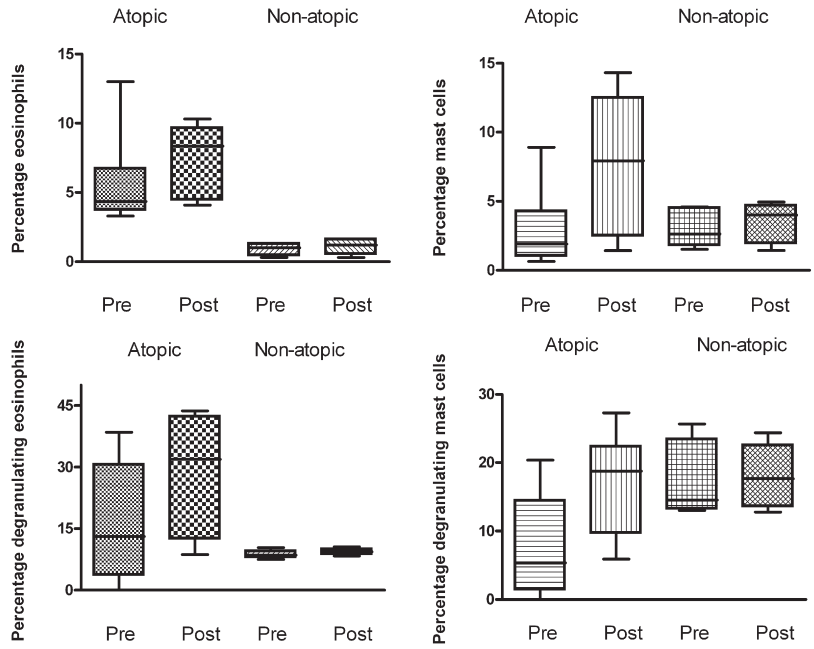

FIG. 3. Percentage of (A) eosinophils and (C) mast cells over total lamina propria immunocytes/10 high-power fields in nonatopic and atopic gastric antral biopsies before and after cow's milk challenge. B and D depict the percentage of each cell type that is degranulating.

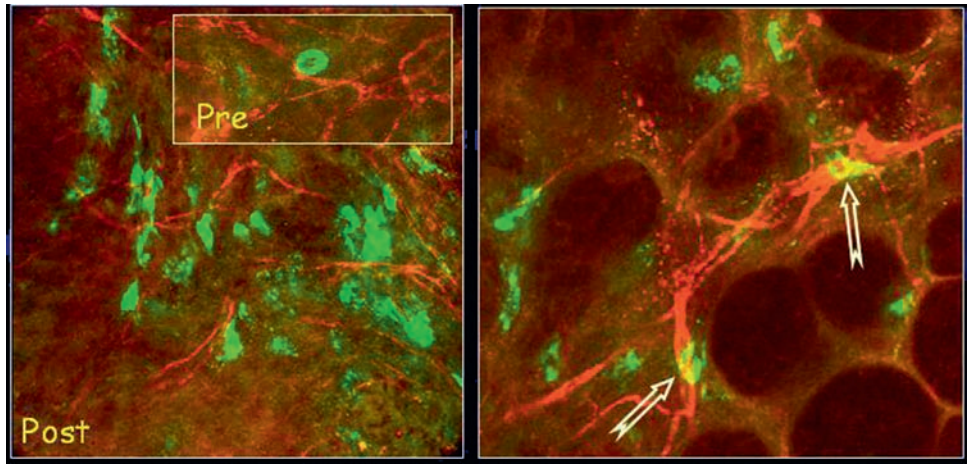

FIG. 4. Left and Inset, Double immunostaining of gastric antral mucosal biopsy from an atopic subject for mast cell tryptase (green) and protein gene product 9.5 (red) showing mast cell degranulation in close proximity to mucosal nerves. Original magnification ×100. Inset, Appearance of mast cell before degranulation. Right, Double immunostaining of gastric antral mucosal biopsy from an atopic subject for mast cell tryptase (green) and protein gene product 9.5 (red) showing tryptase granules colocalising (yellow) with the nerve fibres. Original magnification ×100.

significantly in the atopic group when compared with nonatopics (Table 3).

DISCUSSION

This study demonstrates that cow's milk challenge of the gastric mucosa of children with atopy-related functional dyspepsia induces rapid degranulation of mast cells and eosinophils within 10 minutes of the mucosal application of milk allergen. Activated mucosal mast cells are closely related to mucosal nerves, and mast cell tryptase released from degranulating mast cells is seen to associate with PAR-2 receptors, which colocalise with mucosal nerve fibres. In the same timeframe as these morphological changes occurred, there was a rapid (within 2 minutes) induction of electrogastrographic myoelectrical abnormalities, which are similar to those previously described in functional dyspepsia (2,9). These findings support the notion that mast cell products, notably tryptase, are important factors in the disturbed sensorimotor function of the atopy-related functional dyspeptic state in childhood.

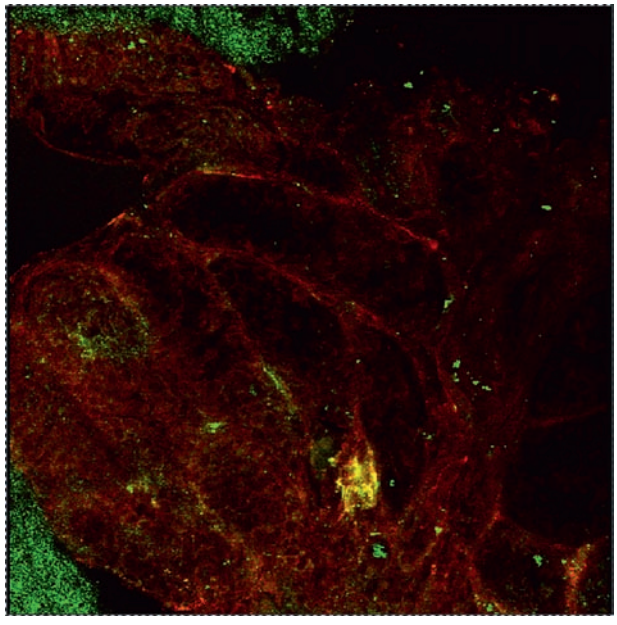

FIG. 5. Double immunostaining of a gastric antral mucosal biopsy from an atopic subject showing mast cell tryptase granules (green) colocalising (yellow) with proteinase activated receptor-2 positivity (red) in the region of mucosal nerves. Original magnification ×100.

Gastric dysrhythmia and subnormal increments in EGG power ratio have been previously reported in atopic children with symptoms of foregut dysmotility (3); however, the mechanism of these changes and their intimate relationship to allergen challenge have not been explored previously in humans. Studies of rodents undergoing gastric luminal challenge with antigen to which they previously have been sensitised have demonstrated IgE-mediated degranulation of mucosal mast cells that causes delayed gastric emptying through a decrease in both the number and amplitude of gastric antral contractions (10,11).

We also have shown that children with symptoms of functional dyspepsia who have an atopic background have higher counts of eosinophils and mast cells within the gastric mucosa than nonatopic dyspeptics, and they have higher numbers of mast cells within 5 μm of mucosal nerves. Also, a higher proportion of mast cells are activated following cow's milk challenge. Whilst there was no evidence of a sex-specific increase in mast cells as described in adults with irritable bowel syndrome (4), it was apparent that basal (unprovoked) levels of mast

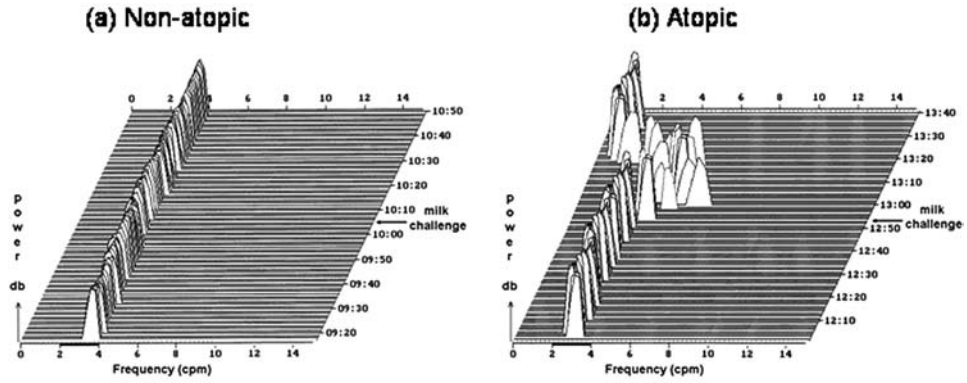

FIG. 6. Pseudo 3-dimensional running spectral analysis plots of surface electrogastrography recordings from (a) a nonatopic and (b) an atopic subject showing the rapid (within 2 minutes) effect of milk provocation in the atopic individual.

cell degranulation were higher in the nonatopic dyspeptic group than in the atopic group. This was probably related to the fact that the children were fasted, and hence not exposed to allergen, for typically 8 to 12 hours before endoscopy. IgE is attached to ε receptor I on the mast cell surface and, in type I hypersensitivity, bridging of these receptors by specific antigens will trigger mast cell degranulation. Mast cells secrete a variety of molecules including vasoactive, nociceptive, and proinflammatory mediators. Histamine and tryptase are quantitatively major inflammatory mediators released during mast cell degranulation.

A role for histamine released from mucosal mast cells during type I hypersensitivity reactions has been appreciated for many years (12). Histamine acts either directly on gastrointestinal smooth muscle (11,13) or indirectly through the activation of enteric neurons (14,15). A close spatial relation between mast cells and mucosal nerve fibres also has become apparent (6). Histaminergic H_1 receptor stimulation of mesenteric afferent nerve fibres in the rat and guinea pig jejunum, but not guinea pig stomach, can lead to activation of vagal afferents (7,15,16). Anaphylactoid reactions within the intestine of rodent models activates neurons within the nucleus tractus solitarius of the brainstem via vagal afferents (17,18). Histamine and other mast cell mediators also increase neuronal excitability and sensitize sensory neurons, which in turn reciprocally activate mast cells further through release of neurotransmitters or neuropeptides including neurotensin, substance P, and acetylcholine (19–21).

Histamine alone, however, does not account for all of the neuronal excitation evoked by mast cell degranulation; mast cell tryptase also is involved in this phenomenon (20,22–24). Mast cell tryptase, which accounts for up to 25% of mast cell total protein in humans (25), is one of a number of proteinases that signal through PARs (26–28). Tryptase cleaves within the extracellular N-terminal tails of PARs to expose tethered ligand domains that bind and activate the cleaved receptors. Tryptase selectively activates PAR-2, which is highly expressed in the gastrointestinal tract upon epithelial cells, myocytes, and enteric neurons (29,30). We have demonstrated that PAR-2 is closely associated with mucosal nerves in the gastric antral mucosa of children and that mast cell tryptase colocalises with PAR-2 immunoreactivity after mast cell degranulation. In the mouse gastric fundus motor responses to PAR activation are biphasic with relaxation dominating (31). Contractile responses to PAR agonists are desensitised by neurokinin receptor antagonists, suggesting that the contractile element of the biphasic response is neuronally mediated. The

TABLE 3. *Summary of electrogastrographic data from atopic and nonatopic (control) dyspeptic groups*

	Atopic pre	Atopic post	Atopic pre vs post, *P*	Control pre	Control post	Control pre vs post	Atopic vs control post, *P*
DF	3.05 (2.3–3.3)	2.9 (1–5.6)	NS	2.8 (1.6–3.5)	3.1 (2.3–3.5)	NS	NS
DFIC	18.1 (5–36)	47.2 (11–81)	< 0.005	23.5 (5–45)	22.5 (8–39)	NS	0.01
% 3 cpm	88.6 (66.7–100)	64.3 (43.5–92.6)	< 0.01	86.2 (54.5–100)	90.9 (81.5–100)	NS	< 0.005
% Brady	9.0 (0–33.3)	28.5 (7.4–56.5)	< 0.01	11.5 (0–36.6)	6.5 (0–13.6)	NS	< 0.005
% Tachy	2.4 (0–11.1)	6.8 (0–40)	NS	2.7 (0–9.1)	2.5 (0–7.4)	NS	NS

NS = not significant; DF = dominant frequency; DFIC = dominant frequency instability coefficient; % 3 cpm = percentage of time with dominant 2–4 cycles per minute gastric myoelectrical activity; % Brady = percentage of time with dominant 0.5–2.0 cycles per minute gastric myoelectrical activity; % tachy = percentage of time with dominant 4.0–9.0 cycles per minute gastric myoelectrical activity.

Data shown as mean with 95% confidence interval (CI) unless otherwise indicated. Groups were compared using the Student *t* test.

methodology used in this study did not include measurement of gastric accommodation in the children studied, although the universal finding of early satiety in the atopic group provides indirect evidence in support of the notion that gastric receptive function is impaired in children with atopy-related functional dyspepsia.

PAR-2 agonists may signal to spinal afferent neurons and cause persistent neurogenic inflammation and hyperalgesia (28,32–34). PAR-2 activation also provokes long-lasting visceral hypersensitivity and hyperexcitability of enteric neurons, suggesting an important role for PAR-2 activation in visceral hypersensitivity states including atopy-related functional dyspepsia (8,35,36). Formal assessment of foregut sensory function was not undertaken in this study.

This study has demonstrated many parallels between the neuroimmunology of atopy-related functional dyspepsia in childhood and irritable bowel syndrome in adults. In both conditions, there may be increased numbers of mast cells within the intestinal lamina propria (5) and evidence of mast cell activation in close proximity to mucosal nerves (4). It is of interest in this study that nonatopic dyspeptics had a higher percentage of degranulating mast cells evident before milk challenge than the atopic dyspeptic group. The stimulus for symptoms in the nonatopic dyspeptics is unknown, although it is possible that such stimuli (eg, stress) may contribute to the higher levels of basal mast cell degranulation in these individuals (37). The surprise finding remains unexplained.

The functional consequences of mast cell–nerve signalling on gastrointestinal motility are well described in the rodent small and large intestine (21,38), and we provide here the first human data suggesting that analogous mechanisms are of importance in the human foregut. We were unable to assess the involvement of extrinsic neuronal pathways, which have been implicated in rodents (39), in the genesis of the electrogastrographic abnormalities, although the increased prevalence of nausea following cow's milk challenge in similarly affected children provides clinical evidence that this is the case.

In summary, this study shows that in cow's milk–allergic atopic patients with functional dyspepsia mast cells coated with IgE are activated by food allergen. Within minutes of allergen contact with the gastric mucosa, mast cells and eosinophils degranulate. Released mast cell tryptase is in close apposition to PAR-2 receptors colocalised with mucosal nerve fibres. In the same time frame, control of gastric myoelectric activity is lost and patients develop symptoms of gastric dysmotility.

REFERENCES

1. Rasquin-Weber A, Hyman PE, Cucchiara S, et al. Childhood functional gastrointestinal disorders. *Gut* 1999;45 (Suppl 2): II60–8.
2. Riezzo G, Chiloiro M, Guerra V, et al. Comparison of gastric electrical activity and gastric emptying in healthy and dyspeptic children. *Dig Dis Sci* 2000;45:517–24.
3. Ravelli AM, Tobanelli P, Volpi S, et al. Vomiting and gastric motility in infants with cow's milk allergy. *J Pediatr Gastroenterol Nutr* 2001;32:59–64.
4. Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004;126:693–702.
5. O'Sullivan M, Clayton N, Breslin NP, et al. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil* 2000;12: 449–57.
6. Wood JD, Alpers DH, Andrews PL. Fundamentals of neurogastroenterology. *Gut* 1999;45 (Suppl 2):II6–16.
7. Liu S, Hu HZ, Gao N, et al. Neuroimmune interactions in guinea pig stomach and small intestine. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G154–64.
8. Reed DE, Barajas-Lopez C, Cottrell G, et al. Mast cell tryptase and proteinase-activated receptor 2 induce hyperexcitability of guinea-pig submucosal neurons. *J Physiol* 2003;547 (Pt 2):531–42.
9. Cucchiara S, Riezzo G, Minella R, et al. Electrogastrography in non-ulcer dyspepsia. *Arch Dis Child* 1992;67:613–7.
10. Catto-Smith AG, Patrick MK, Scott RB, et al. Gastric response to mucosal IgE-mediated reactions. *Am J Physiol Gastrointest Liver Physiol* 1989;257:G704–8.
11. Catto-Smith AG, Tan D, Gall DG, et al. Rat gastric motor response to food protein-induced anaphylaxis. *Gastroenterology* 1994;106: 1505–13.
12. Serafin WE, Austen KF. Mediators of immediate hypersensitivity reactions. *N Engl J Med* 1987;317:30–4.
13. Perdue MH, Chung M, Gall DG. Effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984;86:391–7.
14. Cooke HJ, Nemeth PR, Wood JD. Histamine action on guinea pig ileal mucosa. *Am J Physiol* 1984;246 (4 Pt 1):G372–7.
15. Kreis ME, Haupt W, Kirkup AJ, et al. Histamine sensitivity of mesenteric afferent nerves in the rat jejunum. *Am J Physiol* 1998;275 (4 Pt 1):G675–80.
16. Hillsley K, Kirkup AJ, Grundy D. Direct and indirect actions of 5-hydroxytryptamine on the discharge of mesenteric afferent fibres innervating the rat jejunum. *J Physiol* 1998;506 (Pt 2):551–61.
17. Castex N, Fioramonti J, Fargeas MJ, et al. C-fos expression in specific rat brain nuclei after intestinal anaphylaxis: involvement of 5-HT3 receptors and vagal afferent fibers. *Brain Res* 1995;688: 149–60.
18. Kreis ME, Muller M, Zittel TT, et al. Mediators of neuronal activation in the rat brainstem following intestinal anaphylaxis. *Neurosci Lett* 2000;289:45–8.
19. Bauer O, Razin E. Mast cell–nerve interactions. *News Physiol Sci* 2000;15:213–8.
20. Frieling T, Cooke HJ, Wood JD. Neuroimmune communication in the submucous plexus of guinea pig colon after sensitization to milk antigen. *Am J Physiol* 1994;267 (6 Pt 1):G1087–93.
21. Wood JD. Enteric neuroimmunophysiology and pathophysiology. *Gastroenterology* 2004;127:635–57.
22. Weinreich D, Undem BJ, Taylor G, et al. Antigen-induced long-term potentiation of nicotinic synaptic transmission in the superior cervical ganglion of the guinea pig. *J Neurophysiol* 1995;73:2004–16.
23. Weinreich D, Undem BJ, Leal-Cardoso JH. Functional effects of mast cell activation in sympathetic ganglia. *Ann NY Acad Sci* 1992;664:293–308.
24. Weinreich D, Undem BJ. Immunological regulation of synaptic transmission in isolated guinea pig autonomic ganglia. *J Clin Invest* 1987;79:1529–32.
25. Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells. Purification and characterization. *J Biol Chem* 1981;256:11939–43.

26. Dery O, Corvera CU, Steinhoff M, et al. Proteinase-activated receptors: novel mechanisms of signaling by serine proteases. *Am J Physiol* 1998;274 (6 Pt 1):C1429–52.
27. Cocks TM, Moffatt JD. Protease-activated receptors: sentries for inflammation? *Trends Pharmacol Sci* 2000;21:103–8.
28. Vergnolle N, Bunnett NW, Sharkey KA, et al. Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat Med* 2001;7:821–6.
29. Corvera CU, Dery O, McConalogue K, et al. Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. *J Clin Invest* 1997;100:1383–93.
30. Corvera CU, Dery O, McConalogue K, et al. Thrombin and mast cell tryptase regulate guinea-pig myenteric neurons through proteinase-activated receptors-1 and -2. *J Physiol* 1999;517 (Pt 3): 741–56.
31. Cocks TM, Sozzi V, Moffatt JD, et al. Protease-activated receptors mediate apamin-sensitive relaxation of mouse and guinea pig gastrointestinal smooth muscle. *Gastroenterology* 1999;116:586–92.
32. Cenac N, Garcia-Villar R, Ferrier L, et al. Proteinase-activated receptor-2-induced colonic inflammation in mice: possible involvement of afferent neurons, nitric oxide, and paracellular permeability. *J Immunol* 2003;170:4296–300.
33. Cenac N, Coelho AM, Nguyen C, et al. Induction of intestinal inflammation in mouse by activation of proteinase-activated receptor-2. *Am J Pathol* 2002;161:1903–15.
34. Steinhoff M, Vergnolle N, Young SH, et al. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. *Nat Med* 2000;6:151–8.
35. Coelho AM, Vergnolle N, Guiard B, et al. Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. *Gastroenterology* 2002;122: 1035–47.
36. Kirkup AJ, Jiang W, Bunnett NW, et al. Stimulation of proteinase-activated receptor 2 excites jejunal afferent nerves in anaesthetised rats. *J Physiol* 2003;552 (Pt 2):589–601.
37. Santos J, Saperas E, Nogueiras C, et al. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* 1998;114:640–8.
38. Saavedra Y, Vergara P. Hypersensitivity to ovalbumin induces chronic intestinal dysmotility and increases the number of intestinal mast cells. *Neurogastroenterol Motil* 2005;17:112–22.
39. Scott RB, Tan DT, Miampamba M, et al. Anaphylaxis-induced alterations in intestinal motility: role of extrinsic neural pathways. *Am J Physiol* 1998;275 (4 Pt 1):G812–21.