

Neuroimmune Interaction and Anorectal Motility in Children With Food Allergy-Related Chronic Constipation

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- OBJECTIVES:** Food allergy is thought to trigger functional constipation in children but the underlying mechanisms are still unknown. Mast cells (MCs) and their relationship with nerve fibers (NFs) in the rectal mucosa, as well as anorectal motility, were studied in children with refractory chronic constipation before and after an elimination diet for cow's milk, egg, and soy proteins.
- METHODS:** Thirty-three children (range: 1–10.8 years) underwent anorectal manometry and suction rectal biopsy before and after 8 weeks of oligoantigenic diet. MCs and NFs were identified immunohistochemically. Quantification of MCs (%MC/area) and MCs within 10 μ m of NFs (%MC-NF/area) was performed by computer-assisted analysis.
- RESULTS:** Eighteen children responded to the diet (R-group) and fifteen did not (the NR-group). At baseline there was a significant difference in anal resting pressure (ARP; mm Hg), percentage of relaxation (%R), and residual pressure (RP; mm Hg) of anal canal during rectal distension between the R-group (66 $\pm$ 4.1, 84.3 $\pm$ 2.8, 10.4 $\pm$ 2.3, respectively) and the NR-group (49 $\pm$ 5, 92.2 $\pm$ 1.7, 4.8 $\pm$ 1.7, respectively; P <0.05). After the diet, significant changes in ARP, RP, and %R were observed only in the R-group (44 $\pm$ 3.7, 93.7 $\pm$ 1.5, 3.8 $\pm$ 1.2, respectively; P <0.05). At baseline, the R-group showed an increase in %MC/area (8.3 $\pm$ 0.7) and %MC-NF/area (5.2 $\pm$ 2.6) with respect to the NR-group (5.1 $\pm$ 0.5 and 2.3 $\pm$ 0.4, respectively; P <0.05). After the diet, only the R-group showed a significant reduction of %MC/area and %MC-NF/area (4.4 $\pm$ 0.5 and 2.2 $\pm$ 0.4, respectively; P <0.001). Both ARP and RP significantly correlated with %MC/area and %MC-NF/area; %R showed a significant inverse correlation with both %MC/area and %MC-NF/area.
- CONCLUSIONS:** In children with food allergy-related chronic constipation, an increase in both rectal MC density and spatial interactions between MCs and NFs correlates with anal motor abnormalities. These variables are significantly affected by the diet.

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INTRODUCTION

Constipation, a common complaint in children, accounts for ~3% of all general pediatric outpatient visits and up to 25% of all cases seen in a pediatric gastroenterology tertiary center (1–3). In the majority of cases the underlying mechanisms

remain unknown and patients are labeled as having idiopathic or functional constipation.

A causal relationship between cow's milk protein allergy and chronic constipation has been described recently at least in a subgroup of patients with functional constipation unresponsive

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to stool softeners (4). The mechanisms by which food allergies are involved in the pathogenesis of chronic constipation have, however, received little attention.

Food allergies are commonly encountered conditions affecting up to 8% of children <10 years of age (5). Allergic reactions to food evoke immune inflammatory cell infiltration and activation at various gastrointestinal mucosal sites (5). In this context, mast cells (MCs) are regarded as key effector cells of both immediate and delayed-type hypersensitivity reactions. Upon activation, MCs release both preformed and newly generated active mediators, which can act as neurotransmitters (6). As MCs are in close proximity of enteric neurons and can influence nerve function with a variety of mediators, MC degranulation results in activation of neural reflexes leading to changes in gut motility (7,8).

Experimental and clinical data support a role for MCs in the pathophysiology of functional gastrointestinal disorders in adults (9–11). It is, however, still debated whether MCs contribute to the pathogenesis of chronic constipation related to food allergies.

The degree of MC infiltration in the rectal mucosa, the MC-to-mucosal innervation relationship, and anorectal motor activity were assessed in a group of children with constipation either related or unrelated to food allergy. The effect of an elimination diet for cow's milk, egg, and soy proteins on anorectal motility, MC topography, and neuroimmune interaction was also studied.

METHODS

Patients

All children with refractory chronic constipation attending the Pediatric Gastroenterology and Liver Unit of La Sapienza University of Rome between January 2005 and January 2006 were considered eligible for the study. Chronic constipation was defined as having a duration of ≥ 12 weeks and meeting at least two of the following criteria: (i) frequency of bowel movement <3 times per week; (ii) fecal incontinence more than once a week; (iii) passage of a large amount of stool every 7–30 days; (iv) presence of retentive posturing; (v) evidence of fecal impaction, defined as a palpable mass in left iliac fossa and/or a large amount of hard stool on rectal examination (12). The criteria for inclusion were as follows: (i) symptoms unresponsive to conventional treatments such as increased dietary fiber intake, lactulose (5 g per 10 kg body weight daily), polyethylene glycol 3350 (0.5–0.7 g/kg); (ii) being on regular diet. The exclusion criteria were: (i) history of abdominal surgery or major extraintestinal surgery; (ii) organic cause of chronic constipation; (iii) use of drugs affecting gastrointestinal motility at the time of the study; (iv) the use of nonsteroidal antiinflammatory drugs or other antiinflammatory drugs, including steroids and MC stabilizers. Celiac disease was excluded by using the tissue transglutaminase assay.

The study protocol was defined in accordance with the Helsinki Declaration and approved by the faculty's ethical committee. Written informed consent was obtained from the parents of all the children involved.

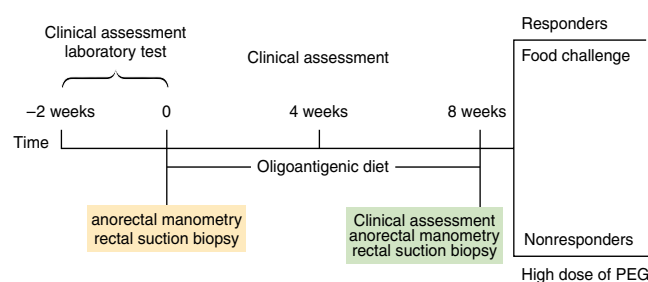


Figure 1. Flow chart of patient progress throughout the study.

Study design

The trial design is shown in **Figure 1**. After enrolment, all the patients underwent a workup including a detailed medical history, a careful physical examination, and laboratory and immunologic tests. Anorectal manometry and suction rectal biopsies for histology and immunohistochemistry were then performed. After baseline assessment, all the children were put on a restricted oligoantigenic diet lasting 6–8 weeks in the hypothesis of multiple food allergies. This diet excluded cow's milk, egg, and soy proteins. During the first week of the diet, parents were instructed to administer a daily rectal enema or orally polyethylene glycol 3350 (1.5 g/kg body weight) at home (13). During the following weeks, enemas alone were given whenever spontaneous defecation was delayed for more than 3 days. All other medications used for the treatment of constipation were banned during the treatment program. All the children underwent clinical assessment 4 weeks after the beginning of the trial and at the end, whereas measurement of anorectal motility and rectal biopsies were repeated only at the end of the diet course.

Double-blind, placebo-controlled challenges with cow's milk, egg, and soy were carried out on the children responding to the diet. The patients received the opaque white capsules with lyophilized food or placebo (sucrose) in randomized order with a "washout" period of two weeks. The patients were kept under close observation for 4–6 h after the administration of the oral challenge. If no clinical reactions were observed, the patients were discharged and the challenge continued on an open basis with fresh food for a period of at least 2 weeks. In the event of a clinical reaction occurring, the challenge was stopped, the child was examined by one of the investigators, and the food was excluded from the diet.

Clinical assessment

Clinical assessment of the patients was performed at baseline and at 4 and 8 weeks after starting the dietetic therapy. At baseline, the children and parents underwent a detailed case history, assessment of constipation and bowel habits, and physical examination. During the treatment period, children and parents were instructed to keep a diary recording frequency and stool characteristics, and symptom occurrence. At the follow-up, clinical progress, stool frequency and consistency, frequency of fecal incontinence, occurrence of abdominal

pain, and use of medication were collected from histories and diaries. Physical examinations were also carried out at each follow-up. The adequacy of the patient's compliance with the diet was also assessed at each scheduled visit.

Laboratory test

At baseline, all the children were scheduled for routine laboratory and immunologic tests. The laboratory tests included red- and white-cell counts, hemoglobin, C-reactive protein, albumin, and liver and kidney function tests. Total serum immunoglobulin E (IgE) was determined by the RIA method (Phadebas PRIST; Pharmacia Diagnostics, Uppsala, Sweden), as well as specific serum IgE, by using radioallergosorbent test (Phadebas RAST; Pharmacia Diagnostics) for a food group, consisting of whole cow's milk, casein, α -lactoalbumin, β -lactoglobulin, soy, egg, wheat, rice, and fish (14). The upper normal limit for serum IgE varied according to age-dependent cutoffs (for each age class the cutoff was equal to the mean value + 2s.d. observed in healthy children of the same age range). Specific IgE >0.35 kU/l were considered abnormal (14). Skin-prick tests (SPTs) were carried out with the following food allergens: casein, α -lactoalbumin, β -lactoglobulin, ovalbumin, rice, soya, wheat, and fish. The allergens were diluted in glycerin to a concentration of 1/100 (LOFARMA, Milan, Italy). All the subjects were also tested with negative and positive control solutions (saline and 0.1% histamine, respectively). The diameter of the weal was measured after 15 min and considered positive if the mean of the weal diameters was at least 2 mm greater than that induced by the control solution (14).

All the patients also underwent atopy patch test using the same extracts as used for SPTs. Finn chambers were used with a 12 mm aluminum cup applied to an area of skin on the children's backs (15). The occlusion time was 48 h and the results were read 30 min after the removal of the cups for the final evaluation of the test. The reactions were considered to be positive if erythema and clear infiltration occurred (erythema and slight infiltration = +, erythema and papules = ++, erythema and vesicles = +++).

Anorectal manometry and rectal biopsy

Anorectal manometry was performed by using a water-perfused catheter with four radially arranged side holes spaced 10 mm apart and a 3 cm × 5 cm balloon tied at the end of the probe (MUI Scientific, Mississauga, Ontario, Canada). The distance from the balloon base and the nearest side holes was 1 or 3 mm, depending on the age of the children. The side holes of the catheter were perfused at a rate of 0.5 ml/min with a pneumohydraulic perfusion pump (MUI Scientific; model PIP-4-8SS). Pressures were recorded by transducers placed at each perfusion line and connected to a multichannel polygraph recorder (Polygraph; Medtronic, Milan, Italy). The recorded signals were preamplified, digitized, and transmitted to a personal computer to display the recording and store the data by a commercially available computer program (Polygram NET; Medtronic).

Anal resting sphincter pressure was calculated as the mean of 3-min periods obtained on three separate occasions from each transducer. The recto-anal inhibitory reflex (RAIR) was elicited by rapidly inflating the rectal balloon with air in random order, starting each time at 0 ml and using distending volumes up to 60 ml (16). The RAIR was defined as normal when the rectal distension produced a relaxation of the anal sphincteric pressure of at least 5 mm Hg. The following parameters were evaluated: (1) mean anal resting pressure (ARP), (2) percentage of relaxation of the anal canal during the maximum distending volume (%R), a percentage anal relaxation of $\leq 20\%$ being defined as abnormal anal relaxation; (3) residual pressure of the anal canal during maximum distending volume (RP, mm Hg).

After the manometric study, rectal biopsies were performed at 2 and 5 cm from the pectinate line by a rectal suction biopsy tool (Model SBT-100; Medical Measurements, Hackensack, NJ). At least two specimens were taken from each patient.

Histology and immunohistochemistry

Biopsies were fixed in buffered 10% formalin and processed for either H&E histology or immunohistochemistry, as previously described (11,17). MCs were identified by using mouse monoclonal antibody directed against tryptase (1:2000 dilution; Dakopatts, Glostrup, Denmark), and nerve fibers (NFs) were immunolabeled using a rabbit polyclonal neuron-specific enolase (NSE, general neuronal marker; 1:500 dilution; Dakopatts) antibodies. Histological sections were evaluated by a pathologist unaware of the diagnosis so as to rule out mucosal inflammation or Hirschsprung's disease. Quantification of inflammatory cells to determine the area occupied by MCs over that occupied by lamina propria (%MC/area), and quantification of MCs in close vicinity ($< 10 \mu\text{m}$) of NFs (%MC-NF/area) were performed by previously validated methods (11,17).

Study end points and statistical analysis

Clinical remission was defined on the basis of frequency of bowel movement per week, frequency of fecal incontinence, and presence of abdominal pain. The 8-week oligoantigenic diet was considered successful if patients achieved ≥ 3 bowel movements per week, less than one episode of fecal incontinence per week, and the absence of abdominal pain while not receiving enemas. The final analysis was performed after all responsive patients had completed the food challenges. The children who had to be switched to a different therapy were excluded from the analysis together with those who were lost at follow-up or refused to repeat the anorectal manometry and biopsies. The baseline characteristics of the patients were evaluated by simple descriptive analysis. Simple and multiple linear regressions were applied to examine predictive factors for therapeutic success. The Fisher's exact test was used at baseline and after 8 weeks to analyze the differences in the clinical and immunology data between children who had clinical remission during the elimination period and those who did not improve. The Mann-Whitney *U*-test was applied to compare independent samples and the Wilcoxon signed rank test

Table 1. Demographics and baseline disease characteristics of patients who completed the trial

	Responders (R-group)	Nonresponders (the NR-group)	P value
No. of cases	18	15	
Age (years; median and range)	4 (1–10.8)	4 (2.5–9)	NS
M/F	10/8	9/6	NS
Disease duration (months; median and range)	24 (3–56)	24 (4–60)	NS
Family history of constipation (numbers and percentage)	9/18 (50)	8/15 (53)	NS
Family history of atopy (numbers and percentage)	11/18 (61)	5/15 (33)	NS
Personal history of atopy (numbers and percentage)	11/18 (61)	4/15 (27)	$P < 0.05$
<i>Previous treatment (numbers and percentage)</i>			
Lactulose	14/18 (78)	12/15 (80)	NS
Polyethylene glycol	7/18 (39)	5/15 (33)	NS
Rectal enema and/or suppositories	10/18 (55)	9/15 (60)	NS
Defecation frequency (per week; mean $\pm$ s.d.)	1.7 $\pm$ 0.8	1.6 $\pm$ 0.8	NS
Fecal incontinence frequency (per week; mean $\pm$ s.d.)	0.8 $\pm$ 0.9	0.7 $\pm$ 0.7	NS
Abdominal pain (numbers and percentage)	13/18 (72)	10/15 (67)	NS
Fecal mass (numbers and percentage)	13/18 (72)	11/15 (73)	NS
Anal fissure (numbers and percentage)	7/18 (39)	6/15 (40)	NS
Urinary symptoms (numbers and percentage)	10/18 (55)	10/15 (67)	NS
Failure to thrive (numbers and percentage)	4/18 (22)	1/15 (8)	NS
Regurgitation and/or vomiting (numbers and percentage)	4/18 (22)	5/15 (33)	NS
Early satiety (numbers and percentage)	5/18 (28)	4/15 (27)	NS
<i>Abnormal immunologic tests (numbers and percentage)</i>			
Total serum IgE antibodies	6/18 (33)	0/15 (0)	$P < 0.05$
Specific serum IgE (RAST)	3/18 (17)	0/15 (0)	NS
Skin-prick tests (SPTs)	11/18 (61)	3/15 (20)	$P < 0.05$
Atopy patch tests (APT _s)	5/18 (28)	2/15 (13)	NS
One or more positive tests	15/18 (83)	5/15 (33)	$P < 0.05$

IgE, immunoglobulin E.

for relative samples. Data were expressed as median value and ranges, as mean values $\pm$ s.d., and as mean values $\pm$ s.e. Different parameters were correlated with the Spearman's rank correlation coefficient. All the statistical tests were two tailed using 0.05 level of significance. The analysis was performed by running SPSS/PC+ (SPSS, Chicago, IL; version 11).

RESULTS

Patients

Of the 215 patients with functional constipation seen during the study period, 40 children were enrolled into the study. Seven of these were excluded from the trial because three were unable to carry on the scheduled diet and the other four refused to repeat anorectal manometry and rectal biop-

sies at the end of the study. Of the 33 patients who completed the trial, 18 showed clinical response to diet (responders: the R-group) and 15 did not (nonresponders: the NR-group). The challenge with the identified food allergens confirmed food allergy in all 18 responsive patients. Diagnostic food challenge showed the recurrence of constipation in all patients within 2 weeks after the beginning of the challenge (median 4 days; range 2–14 days): cow's milk challenge induced clinical relapse in 10 children; cow's milk and soy in 2; egg in 4; egg, soy, and cow's milk in the remaining 2 children.

Clinical assessment

As reported in **Table 1**, baseline demographic characteristics were similar in the two groups. No difference was found between the two groups for defecation and fecal incontinence

Table 2. Effects of elimination diet on clinical characteristics

	Responders (R-group)			Nonresponders (NR-group)		
	Pre-trial	Post-trial	P	Pre-trial	Post-trial	P
Defecation frequency (per week; mean±s.d.)	1.7±0.8	5±1.1*	P<0.001	1.6±0.8	2.3±0.4	P<0.05
Fecal incontinence frequency (per week; mean±s.d.)	0.8±0.9	0.2±0.4	P<0.05	0.7±0.7	0.4±0.5	NS
Abdominal pain (numbers and percentage)	13/18 (72)	2/18 (11)*	P<0.001	10/15 (67)	9/15 (53)	NS
Fecal mass (numbers and percentage)	13/18 (72)	3/18 (17) [§]	P<0.01	11/15 (73)	10/15 (67)	NS
Anal fissure (numbers and percentage)	7/18 (39)	0/18 (0)	P<0.01	6/15 (40)	2/15 (13)	NS
Urinary symptoms (numbers and percentage)	10/18 (55)	3/18 (17)	P<0.01	10/15 (67)	7/15 (47)	NS
Failure to thrive (numbers and percentage)	4/18 (22)	3/18 (17)	NS	1/15 (8)	1/15 (8)	NS
Regurgitation and vomiting (numbers and percentage)	4/18 (22)	2/18 (11)	NS	5/15 (33)	5/15 (33)	NS
Early satiety (numbers and percentage)	5/18 (28)	3/18 (17)	NS	4/15 (27)	5/15 (33)	NS
Use of enema during the trial (numbers and percentage)	10/18 (55)	2/18 (11)*	P<0.05	9/15 (60)	15/15 (100)	P<0.05

*P<0.001 between responders and nonresponders for the post-trial values. [§]P<0.01 responders and nonresponders for the post-trial values.

frequency, presence of abdominal pain, fecal mass, anal fissure, urinary symptoms, failure to thrive, dyspeptic symptoms, and type of pre-trial treatment. The two groups differed in terms of history of atopy ($P<0.05$) and the number of patients with high serum level of total IgE antibodies ($P<0.05$), with positive SPTs ($P<0.05$), and with positive results for one or more of the immunologic tests ($P<0.05$).

At the end of the trial, the R-group showed a significant increase in mean ($\pm$ s.d.) defecation frequency/week by comparison with the NR-group (5 ± 1.1 vs. 2.3 ± 0.4 ; $P<0.001$). As shown in **Table 2**, a significant decrease in mean fecal incontinence frequency/week and in the frequency of abdominal pain, fecal mass, anal fissure, and urinary symptoms was found at the end of the trial only in the R-group. Both groups showed no change in the frequency of dyspeptic symptoms. Although the frequency of abdominal pain and fecal mass were significantly lower in the R-group than the NR-group at the end of the trial, there was no significant difference between them with respect to frequency of anal fissure, urinary, and dyspeptic symptoms.

Anorectal manometry

Figure 2 shows examples of manometric tracings before and after the dietetic therapy. At baseline, the mean ($\pm$ s.e.) values of ARP were significantly higher in the R-group as compared to the NR-group ($P<0.05$; **Table 3**). At the end of the elimination period, ARP significantly decreased only in the R-group

($P<0.001$ vs. baseline; the NR-group: NS vs. baseline); no difference in the post-trial ARP values was found between the two groups (**Table 3**).

At baseline, eight children in the R-group and one in the NR-group had an abnormal anal relaxation (i.e., $\%R\leq80\%$; $P<0.05$). After elimination diet, only one child of the R-group showed an abnormal anal relaxation ($P<0.01$ vs. baseline) and none in the NR-group (NS vs. baseline, NS vs. the R-group). At baseline, mean ($\pm$ s.e.) $\%R$ was significantly lower in the R-group as compared to the NR-group ($P<0.05$; **Table 3**). A significant increase in $\%R$ at the end of the trial was found only in the R-group ($P<0.01$ vs. baseline; the NR-group: NS vs. baseline). The two groups showed no difference in post-trial $\%R$ values (**Table 3**).

Finally, the R-group showed significantly higher baseline RP mean ($\pm$ s.e.) values than the NR-group ($P<0.05$) (**Table 3**). At the end of the trial, RP had decreased significantly only in the R-group ($P<0.05$ vs. baseline; the NR-group: NS vs. baseline). No difference was found between the two groups in post-trial ARP (**Table 3**).

Histology and immunohistochemistry

Tissues were immunostained for tryptase and NSE to quantify MCs and investigate their spatial relationships with NFs in the colonic lamina propria. MCs were scattered throughout the mucosal lamina propria of both groups before and after

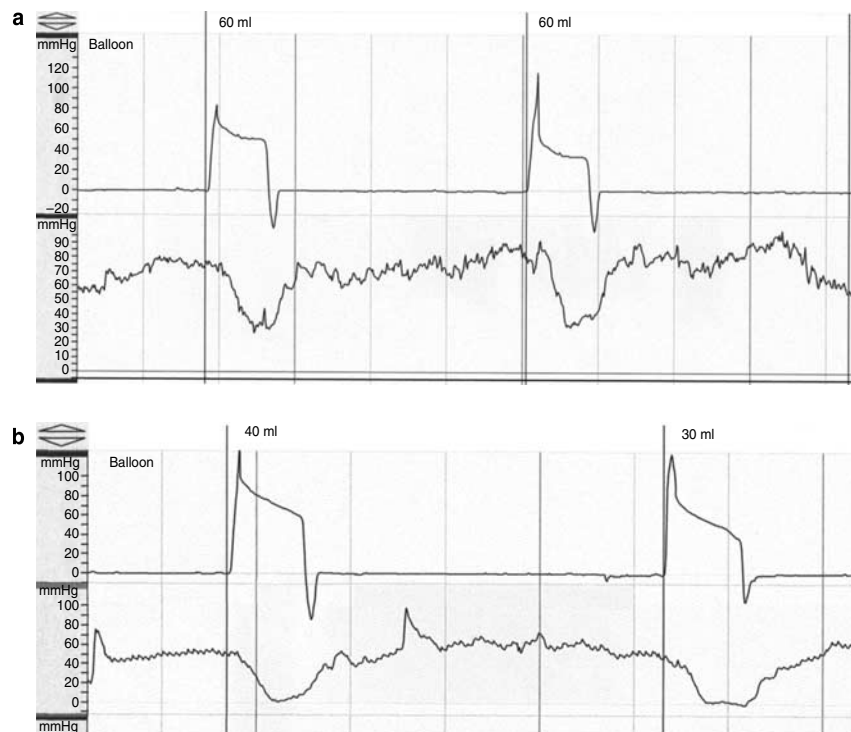


Figure 2. Manometric tracings in a patient with food allergy-related constipation: (a) before the elimination diet, showing high resting pressure at the level of the second channel as well as incomplete relaxation of the anal canal during balloon distension; (b) after the diet, showing decreased anal resting pressure and normal relaxation of the anal canal during balloon distension.

Table 3. Manometric and immunohistochemistry variables before and after oligoantigenic diet (values as mean $\pm$ s.e.)

Variables	Responders			Nonresponders		
	Pre-trial	Post-trial	<i>P</i> value	Pre-trial	Post-trial	<i>P</i> value
ARP (mm Hg)	66 $\pm$ 4.1*	44 $\pm$ 3.7 ^a	<i>P</i> <0.001	49 $\pm$ 5	44 $\pm$ 2	NS
% R (%)	84.3 $\pm$ 2.8*	93.7 $\pm$ 1.5 ^a	<i>P</i> <0.01	92.2 $\pm$ 1.7	95 $\pm$ 1.7	NS
RP (mm Hg)	10.4 $\pm$ 2.3*	3.8 $\pm$ 1.2 ^a	<i>P</i> <0.05	4.8 $\pm$ 1.7	2.5 $\pm$ 0.8	NS
% MC/area	8.3 $\pm$ 0.7*	4.4 $\pm$ 0.5 ^a	<i>P</i> <0.001	5.1 $\pm$ 0.5	4.8 $\pm$ 0.6	NS
% MC-NF/area	5.2 $\pm$ 2.6*	2.2 $\pm$ 0.4 ^a	<i>P</i> <0.001	2.3 $\pm$ 0.4	2.2 $\pm$ 0.3	NS

ARP, anal resting pressure; % R, percentage of relaxation of anal canal; RP, residual pressure of the anal canal; % MC/area, mean area of mucosa occupied by tryptase+ mast cells; % MC-NF/area, mean area of mucosa occupied by tryptase+ mast cells within 10 μ m of nerve fibers.

* *P*<0.05 between responders and nonresponders for the pre-trial values.

^aNS between responders and nonresponders for the post-trial values.

the diet (**Figure 3**). At baseline, mean ($\pm$ s.e.) %MC/area was significantly higher (62% greater) in the R-group than the NR-group (*P*<0.05; **Table 3**). At the end of the trial, %MC/area showed a significant decrease in the R-group (*P*<0.001 vs. baseline) but not in the NR-group (NS vs. baseline). No difference was found between the two groups at the end of the diet (**Table 3**).

The association of MCs with NFs was assessed by colocalization of tryptase and NSE in both groups of patients (**Figure 3**). At the beginning of the trial, mean ($\pm$ s.e.) %MC-

NF/area in the R-group was significantly greater (136%) than in the NR-group (*P*<0.05; **Table 3**). After the trial, %MC-NF/area showed a significant decrease in the R-group (*P*<0.001 vs. baseline) but not in the NR-group (NS vs. baseline). No difference was found between the two groups at the end of the diet (**Table 3**).

Regression analysis and correlation

Linear regression was used to identify predictors of clinical response. Factors including age at enrolment, gender,

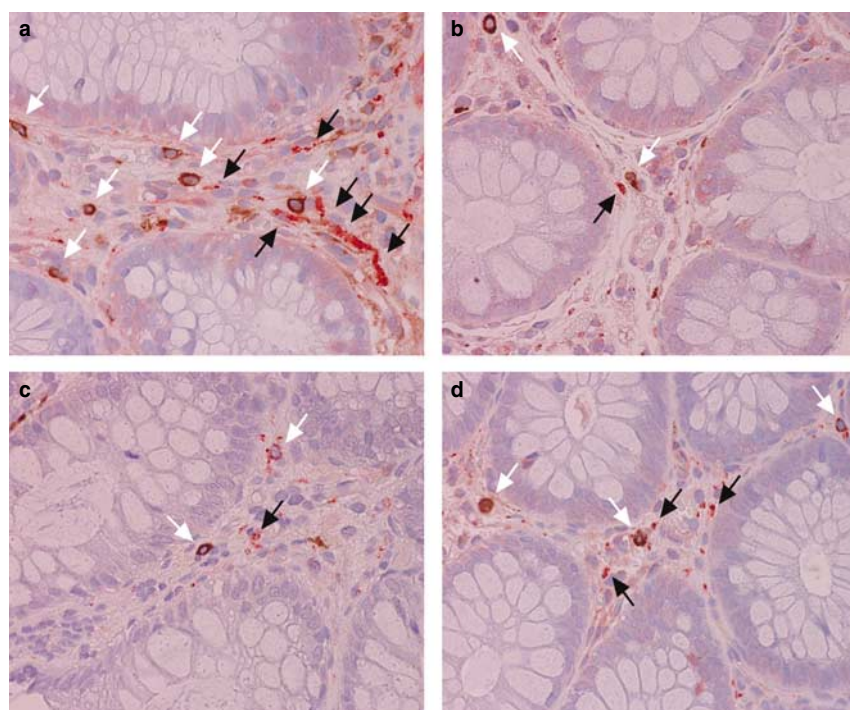


Figure 3. Mast cells (white arrows) and their interaction with nerve fibers (black arrows) in the rectal mucosa of children responsive (R-group; **a–b**) and nonresponsive (NR-group; **c–d**) to the oligoantigenic diet. Mast cells and nerve fibers were identified by immunohistochemistry for tryptase and neuron-specific enolase (NSE), respectively. The association of mast cells with nerve fibers was assessed by simultaneously localizing tryptase and NSE. At baseline, mast cell infiltration in the lamina propria and mast cell–nerve contacts were significantly higher in the R-group (**a**) than the NR-group (**c**). After the diet, no difference was observed between the R-group (**b**) and the NR-group (**d**) for both variables.

Table 4. Stepwise regression analysis of predictors of response to diet

	F ratio	Significance	R ² (partial)	R ² (cumulative)
Positivity of at least one immunological test	11.3	0.002	26.7%	26.7%
% mast cells/area	13.5	0.001	20.6%	47.3%

family history of constipation, and atopy as well as treatment months before enrolment made no significant contribution to diet effect. Personal history of atopy, the presence of one or more positive immunologic tests, and baseline values of ARP, %MC/area, and %MC-NF/area were, however, significantly related to achievement of clinical response ($r=0.4$, $P<0.05$; $r=0.51$, $P<0.01$; $r=0.43$, $P<0.05$; $r=0.52$, $P<0.01$; $r=0.57$, $P<0.001$, respectively). Multiple regression analysis showed that the presence of one or more positive immunologic tests and %MC/area were independent and significant predictors of clinical response ($r^2=0.47$; **Table 4**). Personal history of atopy was excluded from the model because of its correlation with positivity of tests ($r=0.41$, $P<0.05$).

Throughout the study a significant correlation was observed between %MC/area and both ARP and RP ($r=0.68$, $P<0.001$

and $r=0.64$, $P<0.001$) as well as a significant inverse correlation between %MC and %R ($r=-0.65$, $P<0.001$; **Figure 4a–c**). A significant correlation was also observed between %MC-NF/area and both ARP and RP ($r=0.62$, $P<0.001$ and $r=0.70$, $P<0.001$) as well as a significant inverse correlation between %MC-NF/area and %R ($r=-0.68$, $P<0.001$; **Figure 4d–f**).

DISCUSSION

This study provides evidence that children with chronic constipation related to food allergy show an increase both in the density of rectal mucosa MCs and in the number of MCs in close proximity to submucosal rectal nerve endings, which are correlated with abnormalities in anorectal motility. Moreover, the diet is effective for the same patients in reducing MC

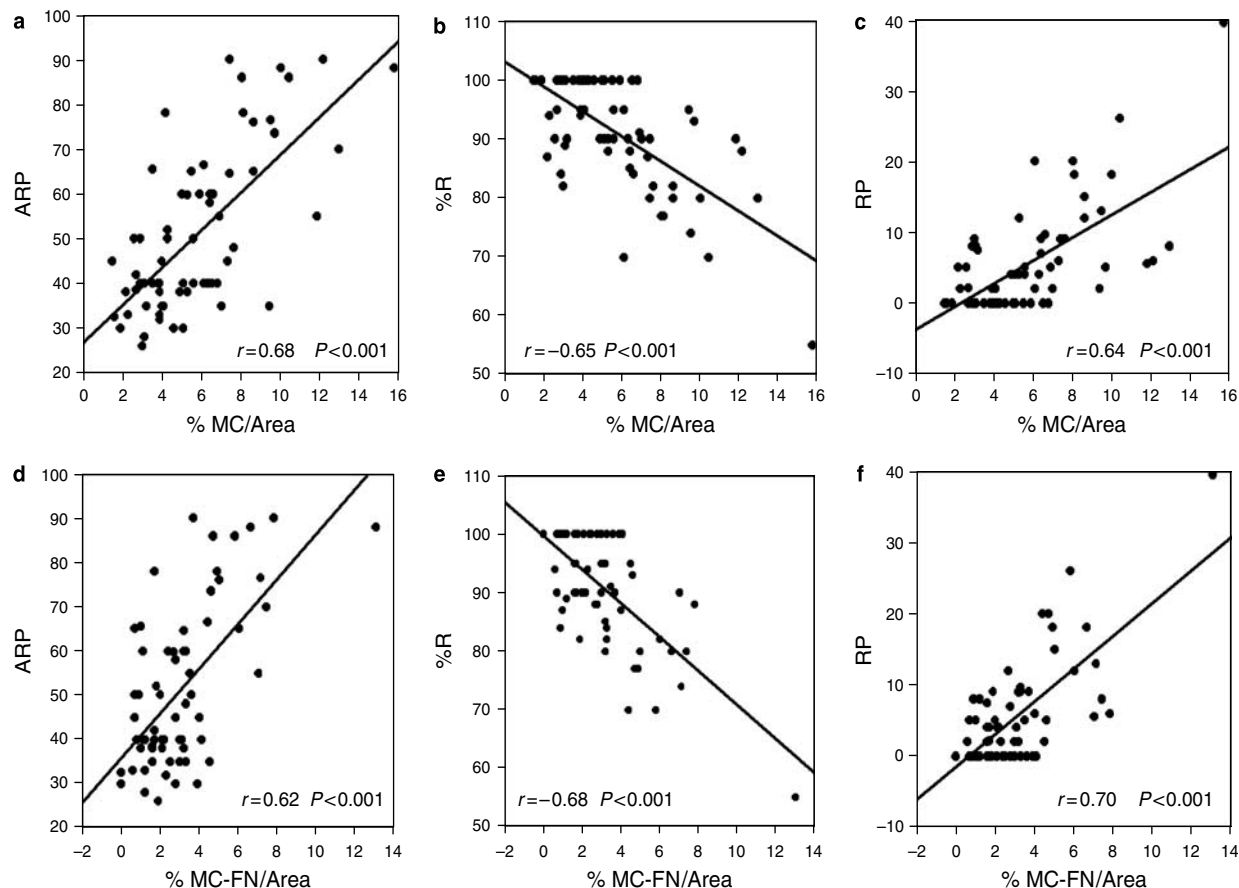


Figure 4. Correlation between anorectal resting pressure (ARP), percentage of relaxation (%R) and residual pressure (RP) and the mean area of lamina propria occupied by mast cells (%MC/area; **a, b, c**, respectively), and the number of mast cells located within 5 μ m of nerve fibers in the rectal mucosa (%MC-FN/area; **d, e, f**, respectively) of all patients who completed the trial.

mucosal infiltration, normalizing MC–nerve interactions, and improving motor abnormalities.

Although chronic constipation is a common condition in pediatrics, its treatment is far from being satisfactory. More than one third of patients are still symptomatic 5 years after diagnosis and constipation may persist in one-third into early adulthood (18).

Attention has been drawn in recent years to a relationship between cow's milk protein allergy and chronic constipation. In an open-label study, 21 out of 27 children with chronic refractory constipation responded to a cow's milk protein-free diet (19). It was later shown in a double-blind crossover study that clinical improvement occurred in 68% of 65 children with chronic constipation when cow's milk was replaced by soy milk, whereas none of the children on cow's milk showed any response. Furthermore, all responsive children relapsed on subsequent challenge with cow's milk (20). Similarly, other studies have suggested that food allergies may be the underlying cause at least in a subgroup of children with refractory chronic constipation (21,22). On the other hand, Loening-Baucke recently found a very low prevalence (2%) of food allergy in over 185

children less than 2 years of age with functional constipation (23). This study did not, however, undertake a systematic assessment of food allergy by elimination diet and challenge.

In our study, 18 of the 33 children who completed the trial responded to the diet, and food challenge caused recurrence of constipation within 2 weeks in all cases. These children could therefore be defined as affected by chronic constipation related to food allergy (5,24). The comparable demographic variables between responsive and unresponsive groups indicated that the difference in clinical response to the diet was not due to demographic confounding factors. This was also borne out by linear regression analysis, showing that age at enrolment, gender, family history of constipation and atopy, and treatment months before enrolment did not significantly contribute to the success of the elimination diet. A history of atopy and the presence of one or more positive immunologic tests were, however, significantly related to clinical response.

Food allergy, either due to IgE- or non-IgE-mediated mechanisms, is commonly thought to elicit gut mucosa inflammation, where different types of immune cells (i.e., MCs, eosinophils, and T and B lymphocytes) are present and scattered along

different sites of the gut (5). Although it has been shown in the last decade that MCs regulate gastrointestinal neuromuscular functions, their role in the pathogenesis of food allergy-related constipation has not been investigated in depth. Gastrointestinal MCs usually act either as effector cells secreting autocrine factors or facilitate the recruitment of other immunocompetent and inflammatory cells (i.e., eosinophils, lymphocytes, and neutrophils), which may in turn contribute to the persistence of allergic reactions (6). On activation, MCs release a variety of bioactive substances, including vasoactive, nociceptive, and proinflammatory mediators, most of which are capable of disturbing the enteric nerve function and muscle contractility (7,8). In adults with irritable bowel syndrome, the average numbers of mucosal MCs increase at different intestinal sites (9,10), and MCs in close proximity to mucosal nerve endings are more common than in controls (11). MCs have also been shown to release larger amounts of mediators such as histamine and tryptase, with spatial proximity to NFs being correlated with the severity of perceived abdominal pain in patients with irritable bowel syndrome (11).

In our study, the baseline mean area of rectal mucosa occupied by MCs was significantly higher in responsive than unresponsive children. Furthermore, a significant decrease occurred only in the responsive children. This supports the hypothesis that the increase in MCs infiltration detected was food allergen dependent. Interestingly enough, in responsive children at baseline MCs were located in closer vicinity to mucosal NFs by comparison with nonresponsive, whereas no difference was found between the two groups after the trial. This suggests that the close spatial relationship between MCs and submucosal nerve endings is of functional significance in affecting neuromuscular function. It is also of interest that both the increased density of rectal MCs and the close proximity of the latter to NFs were significantly related to achievement of clinical response.

It should be stressed that motor variables of the anal canal such as resting pressure, percentage of relaxation and residual pressure upon rectal distension were markedly deranged in children responsive to the diet but unaffected in nonresponsive children. Importance attaches to the fact that ARP and residual anal pressure following rectal distension correlated significantly both with density of rectal MC infiltration and with spatial vicinity of MCs to submucosal rectal NFs. The latter were inversely correlated with the percentage of anal relaxation upon rectal distension.

Our results support the experimental and clinical observations linking intestinal inflammation and derangement in motility of the gut. Neurally mediated gastrointestinal dysmotility has been shown to occur in animal models of immediate-type hypersensitivity reaction following antigen challenge (25–27). On the other hand, an increase in MCs has been found in adults both with constipation and with diarrhea-predominant irritable bowel syndrome (10,11). Previous reports in constipated children unresponsive to the traditional treatment have shown both abnormal intestine transit time (28)

and irregularities in anorectal motility such as increased ARP (29). These features tended to normalize or disappear on elimination diet. It should be noted that high resting anal pressure was also found in our patients with food-related constipation. It is known that ARP results from a combination of both internal and external anal sphincter (30). The tone of the internal anal sphincter, which is made of smooth muscle cells, is known to be modulated by several neurohumoral substances, such as nitric oxide, vasoactive intestinal polypeptide, substance P, prostanooids, serotonin, histamine, and angiotensin II. Human MCs are an additional source of renin and constitute a unique mobile extrarenal renin-angiotensin system (31). Furthermore, angiotensin II and its related peptides have been shown to be released locally at the level of the internal anal sphincter in animal models and to exert a paracrine action in the enteric nervous system enhancing the release of norepinephrine from sympathetic postganglionic axon (32,33). The latter is also able to activate α_1 -adrenoceptors at the smooth muscle cells, inducing IAS contraction, as well as α_2 -adrenoceptors inhibiting nonadrenergic noncholinergic-mediated RAIR-induced relaxation (34). It is tempting to suggest that in the case of food allergy-related constipation, an increase of MCs and their mediators may involve deranged anorectal motility with an increase in ARP and abnormal RAIR, as found in our patients.

In conclusion, the present data indicate that MCs may contribute to anorectal motor abnormalities in children with food allergy-related chronic constipation. The study suggests that an elimination diet can be recommended for children with refractory chronic constipation and especially those with a history of atopy and/or positivity for at least one of the commonly performed tests for food allergy. The mechanisms through which MCs may affect anorectal motility and how the diet can influence the relationship between MCs and anorectal motility will be examined in future studies.

CONFLICT OF INTEREST

Guarantor of the article: Salvatore Cucchiara, MD, PhD.

Specific author contributions: O.B. performed clinical assessments, anorectal motility studies, rectal biopsies and the writing of the article; G.D.N. and C.C. performed histology and immunohistochemistry studies; S.L. and T.F. performed allergic tests (skin-prick test and atopy patch test), M.P. performed statistical analysis; G.B., R.D.G., V.S., and S.C. were responsible for the study design, data analysis and the writing of the article.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ A causal relationship between cow's milk protein allergy and chronic constipation has been described recently at least in a subgroup of patients with functional constipation.
- ✓ Mast cells seem to play an important role in the pathophysiology of functional gastrointestinal disorders in adults.
- ✓ No data are available on the role of mast cells in the pathogenesis of chronic constipation related to food allergies.

WHAT IS NEW HERE

- ✓ Children with chronic constipation related to food allergy show an increase both in the density of rectal mucosa mast cells and in the number of mast cells in close proximity to submucosal rectal nerve endings.
- ✓ Mast cells may contribute to anorectal motor abnormalities in children with food allergy-related chronic constipation.
- ✓ Elimination diet should be considered for children with refractory chronic constipation and especially those with a history of atopy and/or positivity for at least one of the commonly performed tests for food allergy.

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