

LYMPHOID NODULAR HYPERPLASIA AND COW'S MILK HYPERSENSITIVITY IN CHILDREN WITH CHRONIC CONSTIPATION

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Objective To investigate the incidence of cow's milk allergy as evidenced by milk challenge and the findings of endoscopic and immunohistochemical examinations in children with chronic and refractory constipation.

Study design Thirty-five study subjects (mean age, 8.3 ± 3.3 years; range, 3-15 years; 17 girls) and 15 control subjects (mean age, 11.7 ± 3.2 years; range, 2-15 years; 9 girls) were studied by colonoscopy and a 4-week cow's milk elimination and challenge.

Results Lymphoid nodular hyperplasia was the most prominent endoscopic finding in half of the subjects (46%), mostly occurring patchily in the transverse colon. Histologic findings other than lymphoid accumulation and mildly increased density of eosinophils were few. During the milk elimination and with supportive medication, 83% of subjects remitted. Constipation and/or other gastrointestinal or skin symptoms relapsed only in one third (34%) during the cow's milk challenge, these having significantly higher densities of intraepithelial $\gamma\delta^+$ T cells ($P < .001$) in the biopsy samples of the terminal ileum as compared with the control subjects.

Conclusions We were able to find formal evidence for the presence of cow's milk allergy in children with chronic constipation. (*J Pediatr* 2004;145:606-11)

During the past few years, lymphonodular hyperplasia (LNH), presenting as patchy or segmental on various parts the mucosa of the gastrointestinal tract and/or increased densities of intraepithelial $\gamma\delta^+$ T cells, has been described as being associated with cow's milk allergy (CMA) in school-aged children as well as with other food hypersensitivities such as celiac disease.¹⁻⁴ Both of these phenomena are considered an indication of enhanced local immune responses against food antigens in a certain proportion of these children.^{2,3-5} LNH in the terminal ileum (TI) is a common finding in pediatric colonoscopies, with a mild degree being considered as a normal or age-related phenomenon.^{5,6} Lymphoid aggregates are also apparent in the colon in most patients when viewing carefully well-cleaned mucosa, but clearly protruding nodules, referred to as LNH, are considered pathologic. In some patients, an association with food hypersensitivity has been observed.^{5,7,8}

Chronic constipation is a severe clinical problem affecting 1% to 3% of school-aged children.⁹

Iacono et al^{10,11} reported an association between chronic constipation and milk hypersensitivity in children by using a delicate, blinded, crossover study. Indeed, children with a response to milk elimination had a higher frequency of coexistent allergic manifestations and inflammation of the rectal mucosa on biopsy than children with no response. Daher et al¹² described a small series of older children with the same association. The pediatric literature also contains case reports.¹³⁻¹⁵ We hypothesized that even chronic constipation refractory to a variety of treatments in school-aged children might be related to CMA or associated with signs of local immunologic activation in the intestinal mucosa.

In this study, we sought to investigate the incidence of CMA in children with chronic constipation referred to a tertiary level hospital. For the diagnosis of CMA, we used long elimination and challenge tests. In addition, evaluation of the presence of LNH on the mucosa of the colon and TI and determination of densities of $\gamma\delta^+$ T cells were used as a markers of a locally upregulated immune response.

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CMA	Cow's milk allergy	TI	Terminal ileum
LNH	Lymphonodular hyperplasia		

METHODS

Subjects

During 1999 to 2002, a colonoscopy was performed on 35 of 158 subjects treated for chronic constipation at the Department of Paediatrics, Oulu University Hospital (mean age, 8.3 ± 3.3 years; range, 3–15 years; 17 girls). The group was formed by including the children with more than 1 year of intermittent, continuous treatment of the symptom without permanent success; the children and their parents were asked to participate in a clinical study to investigate whether children might have changes similar to those we had described earlier in children with CMA. The decision to participate was made by the children and their parents. The study group was compared with 15 children (mean age, 11.7 ± 3.2 years; range, 2–15 years; 9 girls) investigated for variable gastrointestinal symptoms but remaining in the long follow-up without any specific diagnosis. As the main symptom, 11 had long-term abdominal pains and 4 had growth restriction or anorexia.

Study Protocol

Constipation in this study was defined as passing hard stools, with a frequency of fewer than 3 per week. In our clinical practice, all endoscopies were performed under general anesthesia in an operating room. The aim in each case was to reach the TI, which was successful in all but 3 cases. If a patient had any upper gastrointestinal complaints, a gastroduodenoscopy was scheduled for the same session. Accordingly, the foregut was also studied in 19 study subjects and control subjects (38%).

After an endoscopic examination, all subjects were scheduled for a 4 + 4-week cow's milk elimination and challenge test. The parents completed a semistructured questionnaire about well-being during infancy and possible atopic and gastrointestinal symptoms. The serum concentrations of IgA (reference values, 0.52–2.57 g/L) and IgE (reference values, <160 kU/L) were determined.

Association with Milk

Before the examinations, all subjects were on a milk-containing diet. The subjects were counseled by a trained nurse to give information on the recommended fiber-rich diet and medical treatment with lactulose and sodium picosulfate. If the subject responded to the treatment protocol, milk was used as a challenge during the next 4 weeks, starting from minimal amounts and increasing the milk-containing product to the equivalent of half a liter of skim milk per day if the symptoms did not manifest earlier. The parents kept written notes during the protocol.

An increment of the bowel movements to at least 3 per week was regarded as a remission and response to the elimination treatment. Decreasing frequency of bowel movements into 2 or fewer per week and/or appearance of abdominal pains or exacerbation of dermatitis was considered a positive result in the milk challenge and was recorded as a relapse.

Endoscopic Examinations and Histology

The colonoscopies were performed with an Olympus GF-Q1401 (Olympus, Japan). During the examination, at least 8 mucosal samples were drawn for a microscopic examination (TI, cecum, transverse colon, and rectum; two from each region). The biopsy specimens were furnished in a visual control and from the sites where local disease alterations such as LNH were seen on each region. Moreover, an extra sample was taken from the TI for immunohistochemical studies. Upper intestinal endoscopies with biopsy specimens from the duodenum, the antrum of the stomach, and esophagus were performed with an Olympus GIF-XQ140. All biopsy specimens were reevaluated by one pathologist blinded to the clinical and endoscopic data and according to the established criteria.¹⁶ In addition, the presence of lymphoid nodules with or without germinal centers was separately assessed. The number of eosinophilic granulocytes was counted in lamina propria by using a graticule in a minimum of 3 fields and expressed cells/mm². For statistical analysis, the cases were divided into those with a low number of eosinophils (<80 cells/mm²) and those with a high number (≥ 80 cells/mm²). Mononuclear inflammatory cell density in the lamina propria was graded from sparse (0) to a high-density score (4) by using a modified visual analog scale.¹⁷

Endoscopic Assessment of LNH

In this study, the assessment of lymphoid nodularity on the mucosa of the gastrointestinal tract was based on an endoscopic evaluation after filling the area to be inspected with air. In the bulb of duodenum and colon, only a cluster of lymphoid nodules ($n > 10$ nodules/visible field) was considered significant. Four sections in the colon and rectum were evaluated separately: (1) the rectum, (2) the sigmoid and descending colon, (3) the transverse colon (triangular appearance), and (4) the cecum and ascending colon.

In the TI, the presence of LNH was inspected in the area of approximately 10 to 15 cm, which could be seen during the procedure. The following description was used to grade the prominence of LNH: 0 = no or sparsely dispersed lymphoid follicles; grade 1 = mild, lymphoid follicles dispersed all through the walls; and grade 2 = severe, TI massed with lymphoid tissue, the valve protruding toward the cecum.

Immunohistochemical Staining

The extra biopsy samples from the TI were embedded in an optimal cutting temperature compound (Sakura Finetek Europe BW, Zoeterwoude, The Netherlands), frozen in liquid nitrogen, and stored at -70°C until analysis. The procedure for counting CD3⁺ T cells and $\alpha\beta$ and $\gamma\delta$ TCR-bearing intraepithelial lymphocytes has been described in detail in the earlier reports by the same group.¹ Mucosal staining for HLA-DR expression was investigated by using mAb HLA-DR (Becton Dickinson, San Jose, Calif) in a dilution of 1:500. Staining was separately evaluated in 5 compartments of the mucosa, that is, epithelial cells, the intercellular space, the basement membrane, the lamina

propria, and crypts.^{1,2} HLA-DR genotyping was performed as described previously.²

Statistical Methods

The data collected from the history notes and clinical findings were analyzed by using the SPSS package. The Kruskal-Wallis test, χ^2 test, and Student *t* test were used to estimate the significance of the differences between the subgroups.

Ethical Aspects

In our clinical practice, constipation is not an official or generally accepted indication to do a colonoscopic examination in children. Therefore, based on evidence from earlier literature suggesting an association of milk hypersensitivity and constipation, we asked the children and their parents to participate in the investigation to enhance the medical knowledge on this matter. The subjects were informed about the study and signed a written consent. The study protocol was accepted by the Ethics Committee of Oulu University Hospital.

RESULTS

Clinical Findings

All subjects had been treated by various therapies including a fiber-rich diet, guidance in toileting habits, lactulose-increment, and repeated enemas at home and in hospitals for 1 to 10 years (mean, 4.7 ± 3.1 years) for various periods. Medical disorders such as Hirschsprung disease had been excluded in all subjects by a barium enema or a biopsy. At the time of the endoscopic examination, barium enema radiographic examination was performed on 16 subjects, revealing a dilated rectum with fecalomas in 13 subjects and normal lower colon caliber but with hard stools higher up in the colon in 3 subjects.

The study subjects had been nursed 5.5 months in infancy on average, as compared with the 7.8 months for the control subjects. According to the questionnaire completed by the parents, 40% of the subjects had occasional abdominal pains, of which in 10% had been associated with ingestion of a particular foodstuff.

Nine study subjects (25%) and two control subjects (13%) had been diagnosed with food allergies at infancy, as reported by the parents. According to the clinical data, asthma had been diagnosed at some age in 2 study subjects (5%) and in none of the control subjects; atopic dermatitis had been diagnosed in 9 study subjects (25%) and 3 control subjects (20%); and allergic rhinitis had been diagnosed in 3 study subjects (8%) and no control subjects. As a whole, 12 study subjects (34%) and 3 control subjects (20%) had a history of atopic manifestations.

Response to Milk Elimination and Challenge

During the milk elimination and with supportive medication, 29 (83%) remitted, 7 passing stools every day

and the rest 2 to 3 times per week at average; 6 (17%) study subjects showed no clear response, all having hard stools and fewer than 3 motions per week. In 12 (34%) children, the symptoms that remitted during the elimination period relapsed during the following 4-week period and were designated as having milk hypersensitivity. Three subjects finished the challenge before the intended 4-week period, 2 for exacerbation of dermatitis and no alteration in the bowel frequency and 1 for abdominal pains and constipation. Of the other 9 subjects completing the challenge, 3 reported only constipation by definition and 6 reported abdominal pains and constipation.

Endoscopic Findings

Lymphonodular hyperplasia was the most prominent endoscopic finding in the colonoscopic examinations, being far more common among the study subjects than the control subjects (Table I). It was diagnosed in half (46%), with the transverse colon being the most common site, followed by the cecum (41%). In all, 20 subjects (57%) presented the lesion in some segment of the colon. In most patients, it was found to be patchy, and only in 8 subjects (23%) was it spread all through the colon. Only one of the control subjects (7%) showed LNH in the transverse colon, whereas the other segments were normal. Of 12 subjects reacting during the challenge, 8 (75%) had LNH at any segment of the colon, whereas in the other two groups the figures were 53% and 50%.

Lymphoid mass or nodular LNH in the terminal ileum was diagnosed in 26 of 32 subjects (81%) in whom this end point was reached. In 14 subjects (54%), the lesion was classified as mild and in 12 subjects (46%) as severe. In the control group only 3 (20%) patients had mild LNH of the TI. Overall, LNH in any area of the colon and in TI was significantly more frequent among the study subjects than among the control subjects.

An upper gastrointestinal endoscopy performed on 10 study subjects and 9 control subjects revealed esophagitis in 5 (3 vs 2), gastritis in another 5 (3 vs 2), LNH of the bulb of the duodenum in 6 study subjects and in none of the control subjects ($P = .06$), and duodenitis in 1 control subject.

Histologic Findings

Lymphoid nodules with germinal centers were seen in all levels examined, but only in the terminal ileum was the difference between study subjects and control subjects significant ($P < .05$) (Table II). The high density of lamina propria eosinophils was also significantly more common in the TI ($P < .001$) and transverse colon of the study subjects than the control subjects. The density of mononuclear cells in the lamina propria at various levels was similar between study subjects and control subjects. Melanosis, that is, pigmentation in the lamina propria, was seen in some study subjects and control subjects at the rectum and transverse colon but particularly at the cecum and TI. We found only mild correlation between LNH as an endoscopic finding and microscopically visible lymphoid follicles in TI ($r = 0.292$, $P = .047$) but in none of the colon segments ($r = 0.03$ – 0.23).

Table I. The presence of lymphoid nodular hyperplasia (LNH) as an endoscopic finding at various levels in 35 subjects examined for refractory chronic constipation

	Study subjects n = 35	Control subjects n = 15	χ^2	P value [‡]
Rectum	9	0	4.7	.043
Sigmoid colon	12	0	6.8	.01
Transverse colon	16	1	7.1	.009
Cecum*	14	0	8.6	.002
Colon, anywhere	20	1	10.9	.001
Terminal ileum [†]	26	3	16.2	<.001

*Level reached in 34 study subjects and all controls.

†Level reached in 32 study subjects and all controls.

‡ χ^2 tests.

Immunohistochemical Findings

The study subjects with chronic constipation who remitted during the milk elimination and especially those reacting during the milk challenge had significantly higher densities of intraepithelial $\gamma\delta^+$ T cells ($P < .001$) in the biopsy samples of the TI than the control subjects (Figure and Table III). Accordingly, their $\gamma\delta^+/\text{CD}3^+$ ratio was higher ($P < .001$). However, the density of $\text{CD}3^+$ as well as the $\gamma\delta^+$ subset were similar in both groups. We did not find any difference in the upregulation of DR in the mucosal samples. An equal number of control subjects (9, 26% vs 3, 20%) were positive for DQ2 alleles (data not shown). There were no subjects with IgA deficiency, and the serum IgA concentrations were equal between the main groups (1.08 vs 1.16) and within a normal range.

DISCUSSION

Our findings suggest that among the children with chronic and refractory constipation, there are those who actually have cow's milk hypersensitivity with characteristic endoscopic and histologic findings and elevated densities of $\gamma\delta^+$ T cells.³ This was also strongly supported by the subsidence of constipation and other gastrointestinal or other symptoms during the milk elimination and the relapse of original symptoms during the challenge. The observed association is in line with the previous literature and especially with the findings of Iacono et al,^{10,11} who showed the same connection in small children. However, because our patient series was collected in a tertiary center and therefore was enriched with cases with severe and long-term problems of defecation, the result should be considered preliminary.

This multidisciplinary study allowed us to correlate findings characteristic to milk hypersensitivity from the endoscopic and immunohistochemical examinations and the results of a long elimination and challenge test in evaluating school-aged children with chronic constipation. The main conclusion was based on all these examinations, by which such

Table II. Histologic features in 35 children examined for chronic constipation and in 15 control subjects

	Study subjects	Control subjects	χ^2	P value*
Rectum	n = 35	n = 15		
Lymphatic follicles	8	5	0.513	NS [†]
Germinal centers	8	2	0.666	NS
Eosinophils $\geq 80/\text{mm}^2$	7	1	1.477	NS
Mononuclear cells	13	2	3.039	NS
Melanosis	2	1	0.011	NS
Transverse colon	n = 35	n = 15		
Lymphatic follicles	19	4	2.964	NS
Germinal centers	11	4	0.066	NS
Eosinophils $\geq 80/\text{mm}^2$	23	5	4.160	.041
Mononuclear cells	20	7	0.314	NS
Melanosis	5	4	1.251	NS
Cecum	n = 34	n = 15		
Lymphatic follicles	10	3	0.401	NS
Germinal centers	11	6	0.344	NS
Eosinophils $\geq 80/\text{mm}^2$	26	9	1.020	NS
Mononuclear cells	29	12	0.058	NS
Melanosis	14	4	0.81	NS
Terminal ileum	n = 32	n = 15		
Lymphatic follicles	27	9	1.521	NS
Germinal centers	23	5	4.468	<.05
Eosinophils $\geq 80/\text{mm}^2$	32	7	12.26	<.001
Mononuclear cells	24	11	0.113	NS
Pigmentation	12	3	1.020	NS

†NS = Not significant.

were found imprecise as diagnostic tools. So far, in a clinical practice, a trial with milk elimination may be recommended even for school-aged patients with long-term constipation refractory to standard therapy.

In individual patients, we found only a fair correlation between the results of endoscopic and histologic studies. The number of histologically detected lymphoid follicles or germinal centers did not show a significant correlation with the endoscopic observation of LNH. This might be related to a sampling error caused by small size of the lymphoid follicles, which might affect both their access during biopsy and their occurrence in the histologic sections. In addition, the endoscopic viewing of the lymphoid follicles is related to their size, which was not currently assessed by histology. Nevertheless, by correlating histology and endoscopy, we were able to confirm that the mucosal nodules interpreted as LNH really are lymphoid tissue and not mucosal elevations of other origin. In our experience, an endoscopic assessment appears to be the best tool for their evaluation.

In subjects with constipation, LNH was present in 74% of subjects and was most prominent in the TI and transverse colon. In the bulb of the duodenum, the figure was 60% among 10 subjects with gastroduodenoscopy performed. As reported earlier, the distribution of the mucosal alterations in subjects with milk hypersensitivity has a patchy or segmental

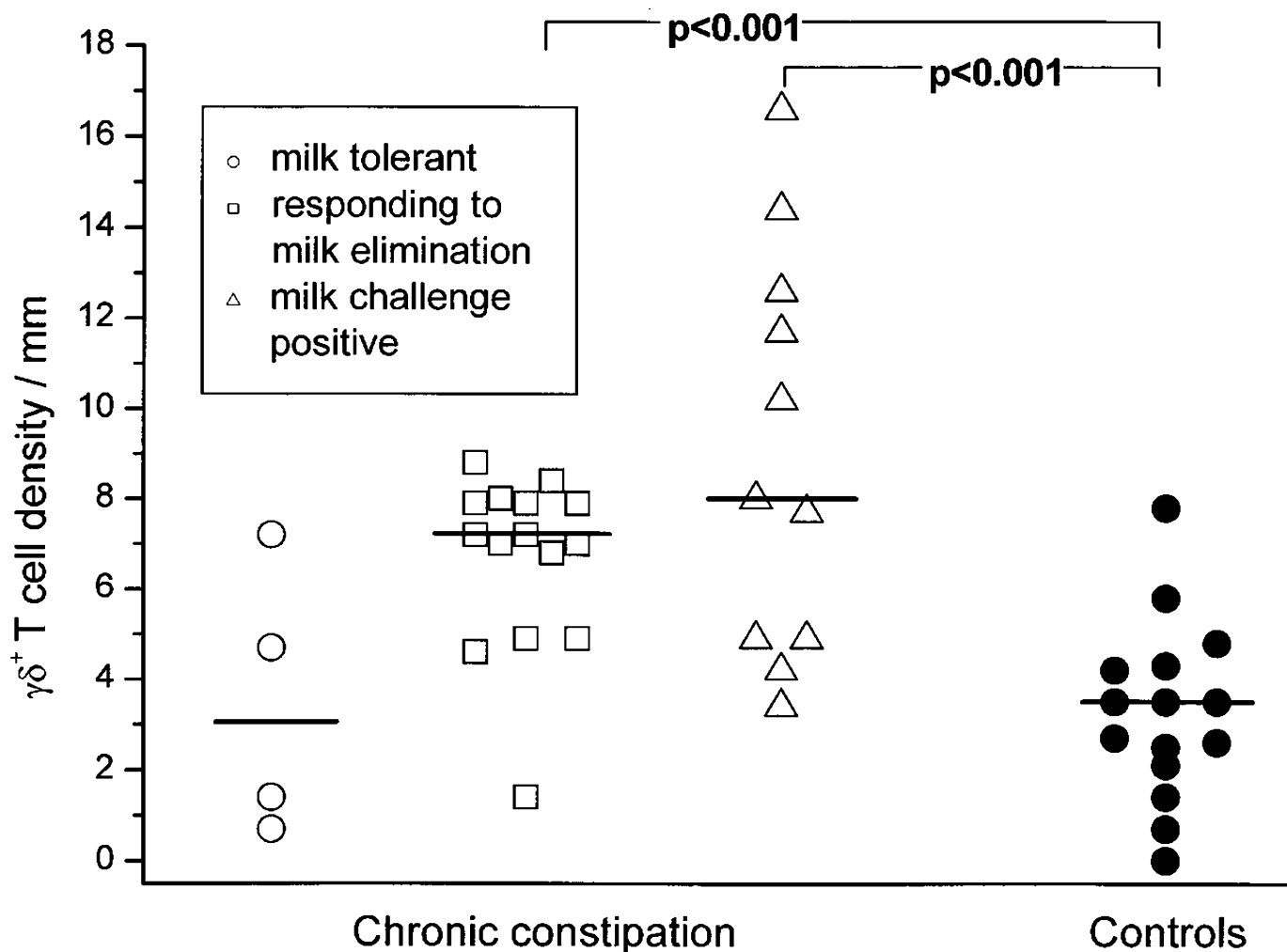


Figure. Density of $\gamma\delta^+$ T cells in mucosal samples of terminal ileum in children with chronic constipation and control subjects. Subjects with constipation are divided to those not responding to milk elimination, those responding to an elimination but not a challenge, and to those responding both to an elimination and a challenge.

occurrence and may be found at any location between the stomach and the anus and to any extent. Finding the lymphoid accumulation and related immune response highest in the lower gastrointestinal tract also has been observed by other investigators.^{18,19} It may be considered as expected because the hypersensitivity reaction occurs locally and food antigens are digested along the downstream of the gastrointestinal tract so as to be small enough to activate the cell-mediated immune response. However, it is puzzling why the subjects of this study presented with constipation, whereas the patients in our earlier series with the same findings mostly had diarrhea. Our suggestion is that the skewing of the underlying cytokine societies may be more indicative of the symptom than the endoscopic, histologic, or immunohistochemical alterations.²⁰ In the current study, we found an increased density of intraepithelial $\gamma\delta^+$ T cells in the biopsy samples from the TI. Moreover, the subjects reacting in a milk challenge tended to have higher densities than those recovering during the milk elimination but not responding during a 4-week challenge. In earlier series in patients with milk hypersensitivity, we have

Table III. Mean ($\pm$ SD) densities of intraepithelial CD3⁺, $\alpha\beta^+$, and $\gamma\delta^+$ T cells in mucosal samples drawn from the terminal ileum in children with chronic constipation and control subjects

	Chronic constipation	Control subjects
CD3 ⁺	26.4 $\pm$ 7.2	23.7 $\pm$ 10.6
$\alpha\beta^+$	18.6 $\pm$ 6.8	19.3 $\pm$ 8.8
$\gamma\delta^+$	7.1 $\pm$ 3.6*	3.3 $\pm$ 2.0
$\gamma\delta^+$ /CD3 ⁺	27.4 $\pm$ 12.3*	12.7 $\pm$ 4.9

* $P < .001$, study subjects compared with control subjects.

demonstrated that the rise in $\gamma\delta^+$ T cells is dependent on the exposition of the antigen.¹⁻³ However, we there was so much overlap in the density that even this measure would not work as a diagnostic tool to separate those getting help from a milk-free diet.

Half of our patients responding clinically during the milk-free diet but not in the milk challenge showed the same

endoscopic or immunohistochemical findings as the challenge-positive ones. One explanation of this problem may be the amount of ingested cow's milk. We had difficulty in getting the children to use milk and dairy products up to the recommended amount when, after long and numerous treatment trials, they had finally remitted with their symptoms. The other potential explanation is that the process of development of milk-related enteropathy may be slow and the upregulation of the immune response may require some other enhancers, such as virus infections. Finally, there is a possibility that the observed endoscopic and immunohistochemical findings do not necessarily have any pathogenetic role in constipation but are an expression of an unrelated process.

In conclusion, this report shows an association between milk hypersensitivity and chronic constipation. However, because our material is selected, population-based studies with a high-quality setup are urgently needed to confirm this association. In a diagnostic workup in a child with suspected milk-related constipation, a careful clinical assessment should be completed with a trial of milk elimination and a long challenge. Endoscopic examinations with biopsies and immunohistochemical studies may help to establish the diagnosis and exclude other conditions but currently should probably be limited in the most difficult cases.

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