



Cow milk is not responsible for most gastrointestinal immune-like syndromes—evidence from a population-based study^{1–3}

Laura Paajanen, Riitta Korpela, Tuula Tuure, Jarno Honkanen, Irma Järvelä, Jorma Ilonen, Mikael Knip, Outi Vaarala, and Jorma Kokkonen

ABSTRACT

Background: Gastrointestinal hypersensitivity to cow milk (CM) may be more common among school-aged children and young adults than previously thought.

Objective: The objective was to study various gastrointestinal complaints and the immunologic mechanisms associated with food-related, especially CM-related, gastrointestinal disorders in young adults.

Design: Of 827 subjects aged 16–21 y who completed a questionnaire on food-related gastrointestinal symptoms, 49 symptomatic subjects agreed to a clinical examination, including an interview, blood tests, a lactose-maldigestion test, a blinded CM challenge and, in severely symptomatic subjects ($n = 12$), an endoscopic examination. Twenty-nine subjects served as controls.

Results: Approximately 10% of the subjects reported having major gastrointestinal symptoms, mainly food-related ($n = 70$ of 86), during the preceding year. Specific organic disease was found in 2 symptomatic subjects: 1 case of celiac disease and 1 of colitis. The result of the lactose-maldigestion test was positive in 16 of the remaining 47 symptomatic subjects, but only 4 carried the C/C_{-13910} genotype for adult-type hypolactasia. The symptomatic subjects had restricted their consumption of certain foods, particularly CM. However, in a blinded challenge, CM-induced symptoms were rare. The symptomatic subjects had higher plasma soluble intercellular adhesion molecule 1 ($P = 0.007$) and lower granzyme A ($P = 0.001$) concentrations than did the control subjects. Duodenal biopsy samples tended to have higher intraepithelial CD3⁺ cell counts ($P = 0.065$) and a higher expression of transforming growth factor β ($P = 0.073$) and interleukin 12p35 messenger RNA ($P = 0.075$) than did the control subjects.

Conclusions: In an unselected cohort of young adults, 8% reported food-related gastrointestinal symptoms. The finding of immunologic activity implied the existence of a food-related gastrointestinal syndrome but not one induced by CM. *Am J Clin Nutr* 2005;82: 1327–35.

KEY WORDS Cow milk hypersensitivity, cytokines, endoscopy, gastrointestinal symptoms

INTRODUCTION

Subjects with gastrointestinal complaints are one of the major groups of patients seeking medical advice and are a diagnostic challenge to clinicians. The elucidation of the etiologic factors underlying these symptoms has remained a controversial issue.

Definite organic pathology [celiac disease, microscopic colitis, gastroesophageal reflux with or without esophagitis, lactose intolerance, *Helicobacter pylori* infection, chronic inflammation, or irritable bowel syndrome (IBS)] may be identified to explain the symptoms. Some cases, however, remain undiagnosed. The subjects have symptoms such as recurrent abdominal pains, regurgitation, chronic nonspecific diarrhea, nonulcer dyspepsia, dyschezia, and functional constipation.

The role of food in vague gastrointestinal complaints has been a subject of dispute. Many patients with IBS feel that food, especially that rich in carbohydrates and fat, triggers their symptoms (1–3). Patients often blame milk and dairy products, but very little evidence has accumulated to support the role of lactose or cow milk protein intolerance as the cause of these symptoms (4–6). However, in school-aged children with obscure gastrointestinal discomfort, we have recently observed evidence of a delayed-type gastrointestinal cow milk allergy, cow milk sensitive enteropathy, manifested by abdominal pain, diarrhea, constipation, or hematochezia (7–9). Peltö et al (10) calculated that as many as 3–6% of young adults may have cow milk protein allergy characterized by gastrointestinal symptoms. In gastrointestinal food allergy and hypersensitivity, the aberrant immune responses of the intestine are essential to the disease process (11, 12). Once the process has started, an influx of luminal antigens continuously challenges the mucosal immune response, which leads to the activation of a wide variety of immunologic mediators, such as cytokines, and adhesion molecules, such as soluble

¹ From the Foundation for Nutrition Research, Helsinki, Finland (LP and RK); the Department of Allergy (LP) and the Laboratory of Molecular Genetics (IJ), Helsinki University Central Hospital, Helsinki, Finland; Valio Ltd, Research and Development, Helsinki, Finland (RK and TT); the Hospital for Children and Adolescents (MK) and the Institute of Biomedicine, Department of Pharmacology (RK), University of Helsinki, Helsinki, Finland; Viral Diseases and Immunology, National Public Health Institute, Helsinki, Finland (JH and OV); the Department of Virology, University of Turku, Turku, Finland (JI); the Department of Pediatrics, Tampere University Hospital, Tampere, Finland (MK); and the Department of Pediatrics, Oulu University Central Hospital, Oulu, Finland (JK).

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³ Address reprint requests to L Paajanen, Foundation for Nutrition Research, PO Box 30, FIN-00039 Valio, Helsinki, Finland. E-mail: laura.paajanen@helsinki.fi.

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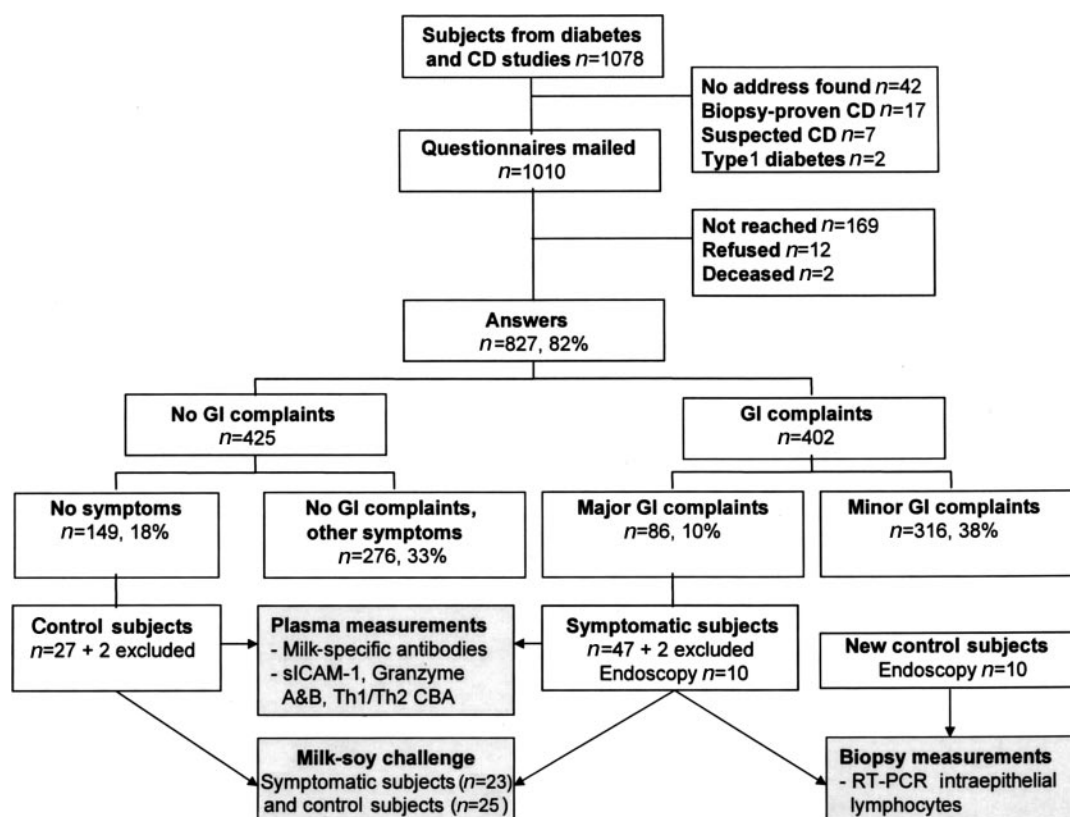


FIGURE 1. Flow chart of the study design. CBA, cytometric bead array; CD, celiac disease; GI, gastrointestinal; RT-PCR, reverse transcriptase–polymerase chain reaction; sICAM-1, soluble intercellular adhesion molecule 1; Th1 and Th2, T helper cell 1 and 2, respectively.

intercellular adhesion molecules (sICAMs). The regulatory cytokines transforming growth factor β (TGF- β) and interleukin (IL) 10 control these inflammatory responses and immunologic homeostasis. As a sign of an intestinal inflammatory process, the accumulation of intraepithelial CD3⁺, $\alpha\beta$ ⁺, and $\gamma\delta$ ⁺ T cells is seen in food-induced hypersensitivity states, especially in celiac disease and to a lesser extent in delayed-type food allergy and food-sensitive enteropathy (8, 12–16).

To investigate the occurrence of food-induced gastrointestinal symptoms, self-reported symptoms related to lactose and cow milk, and intestinal immune responses possibly triggered by cow milk protein, we carried out a population-based survey in young adults and investigated the study subjects as thoroughly as was clinically relevant.

SUBJECTS AND METHODS

Subjects and study design

The study group was drawn from a population-based cohort of children living in northern Finland, who were initially recruited in 1994 for a study of risk factors for type 1 diabetes ($n = 3652$; median age: 12 y; range: 7–16 y) (17). All of these subjects were also screened for celiac disease and examined for the presence of human leukocyte antigen (HLA) DQ2 molecule encoding genes (18). Those who had been drawn from 4 rural communities and were 16–21 y of age were included in the present study in 2002, which amounted to a total of 1078 young adults. Of these subjects, 26 were excluded because of biopsy-proven celiac disease ($n = 17$), suspected celiac disease on the basis of elevated serum

immunologic (Ig) A–class antibodies to tissue transglutaminase (tTG) ($n = 7$; 3 refused biopsy confirmation and 4 were considered to be healthy) or type 1 diabetes ($n = 2$) (17, 18). The address of 42 subjects was unknown; therefore, postal questionnaires on gastrointestinal symptoms and foods related to these symptoms were mailed to 1010 young adults (Figure 1). In all, 827 subjects (82%; $n = 367$ males) completed the form and constituted the final study group.

In the questionnaire, 86 subjects (10%; $n = 11$ males) reported severe abdominal complaints (severe pain, persistent pain or pain combined with abnormal defecation functions); 49 of these subjects agreed to take part in a clinical examination and were examined by a pediatric gastroenterologist. None of the symptomatic subjects had any previous diagnosis of a gastrointestinal disease, and none regularly used medication for gastrointestinal symptoms. An endoscopic examination was performed on those who had persistent and clinically significant symptoms and who agreed to the examination ($n = 12$). Celiac disease was diagnosed in one subject, and ulcerative colitis was diagnosed in another subject; both subjects were excluded from further analyses. Thus, the clinical examination was conducted in 47 symptomatic subjects (3 males and 44 females; median age: 18.7 y; range: 16–21 y.). Ten symptomatic subjects had elevated serum IgE concentrations (>110 kU/L), and 27 (57.4%) reported symptoms of one or more allergic disease during the previous year: 17 subjects (36.2%) reported atopic dermatitis, 16 (34.0%) allergic rhinitis, 16 (34.0%) allergic eye symptoms, and 5 (10.6%) asthma. The numbers include both self-made diagnoses and diagnoses made by a physician, except for asthma, which was only



accepted if diagnosed by a physician. The mean ($\pm$ SD) body mass indexes (BMI; in kg/m^2) of the symptomatic girls and boys were 21.3 ± 2.1 and 20.9 ± 2.2 , respectively.

Of those interviewed, 149 subjects (18%; $n = 91$ males) had no history of gastrointestinal or allergic complaints and consumed milk daily; 29 of these subjects (13 males) were willing to participate as healthy control subjects. Two control subjects had elevated serum IgE concentrations and were excluded; thus, the final control group consisted of 27 subjects ($n = 12$ males and 15 females; median age: 17.5 y, range: 16–21 y). The mean ($\pm$ SD) BMIs of the control girls and boys were 22.1 ± 4.4 and 23.8 ± 4.4 , respectively.

The clinical examination comprised an interview; a food-use survey; a lactose-maldigestion test; a genetic test of the *C/T*₋₁₃₉₁₀ variant associated with adult-type hypolactasia; a blinded milk protein tolerance test; the measurement of antibodies to *Helicobacter pylori*, tissue transglutaminase and milk proteins; and the quantification of the plasma concentrations of sICAM-1, granzymes A and B, and, in subjects with severe symptoms, the measurement of mucosal mRNA expression in the following cytokines: interferon γ (IFN- γ), tumor necrosis factor α , IL-2, IL-4, IL-5, and IL-10.

Additional control subjects ($n = 2$ males and 8 females; median age: 16 y; range: 14–18 y) were enrolled outside the original study population from among patients of Oulu University Hospital, to serve as control subjects for the endoscopic examination and the measurement of mucosal mRNA expression and intra-epithelial T cell densities. The endoscopy control subjects were studied for prolonged gastrointestinal symptoms but had normal endoscopic findings and remained without any definite diagnosis. The study protocol was approved by the Ethics Committee of Oulu University Hospital. Informed written consent was obtained from all subjects.

Questionnaire and interview

In the structured questionnaire, the subjects ($n = 827$) were asked about the intensity and frequency of various gastrointestinal symptoms, whether or not the symptoms were related to specific foods, atopic history, lactose intolerance, chronic diseases, and the use of milk and dairy products and foods often related to gastrointestinal symptoms. The questionnaire was pre-tested for clarity in 30 volunteers living in northern Finland who belonged to the same age group as the study subjects.

In the clinical examination, the 47 symptomatic subjects and the 27 control subjects were interviewed about gastrointestinal and atopic symptoms and the use of milk and dairy products at the time of the study and during childhood. They also completed a food-usage frequency form, concentrating on dairy product consumption and calcium intake, modified from a form validated by S Cheng and A Lyytikäinen (Calex Study, Jyväskylä, Finland, unpublished observations, 1998). The parents of the symptomatic subjects and of the control subjects completed a semistructured form, also used in a former study (19), including questions regarding the infancy of the study children, such as duration of breastfeeding and whether food hypersensitivities, excessive crying, vomiting, regurgitation, or any other gastrointestinal symptoms or allergic symptoms were manifested. Any gastrointestinal and allergic diseases of the parents were also inquired about.

Lactose tolerance tests

Lactose tolerance in the symptomatic subjects was determined with the alcohol-galactose test. The patients ingested 50 g lactose plus 150 mg alcohol/kg body weight after an overnight fast. Blood galactose was measured after 40 min (Galac; Roche Diagnostics, Basel, Switzerland) with a method validated by Iso-koski et al (20) that was modified according to Pelto et al (21). A serum galactose concentration <0.2 mmol/L indicated hypolactasia. Lactose absorption in 7 subjects, whose religious beliefs forbade the consumption of alcohol, was determined after an overnight fast by serial (0, 20, 40, and 60 min) glucose measurements after a 50-g lactose load (Glucose HK Liquid; Roche Diagnostics). A difference of ≤ 1.1 mmol/L between the lowest and the highest measurements indicated hypolactasia. The subjects reported gastrointestinal symptoms in a structured questionnaire during the 24 h after the test. Both the symptomatic subjects and the control subjects were genotyped for the *C/C*₋₁₃₉₁₀ variant of lactase persistence or nonpersistence (adult-type hypolactasia) (22–24).

Milk-protein tolerance test

The tolerance of cow milk protein was tested in a double-blind, placebo-controlled, crossover test in symptomatic subjects who agreed to participate (starting: $n = 30$; completing: $n = 23$). Healthy control subjects (starting: $n = 27$; completing: $n = 25$) were included to investigate normal gastrointestinal symptoms during the study drink challenges. The subjects ingested, in random order, one of the following beverages (500 mL/d) for 21 d: 1) a chocolate soy drink (Carlshamn Mejeri Ltd, Karlshamn, Sweden) and 2) the same soy drink fortified with a liquid low-lactose, low-fat milk protein concentrate (Valio Ltd, Helsinki, Finland), which were packed in identical 250-mL ready-to-drink packs. The milk protein content of the milk challenge corresponded to the use of 460 mL milk/d. A 7-d washout period preceded both challenges. The symptomatic subjects consumed a milk-free diet throughout both the test and the washout periods, were carefully instructed and followed by a nutritionist (LP), and were interviewed on the first and last days of both drink challenges. Milk-free substitutes were supplied to the subjects to help them maintain their diet. The healthy control subjects were instructed to follow their normal milk-containing diet and were interviewed once during both challenges. All subjects kept a daily diary of the intensity of various gastrointestinal symptoms (theoretical maximal sum of symptoms: 140 points/wk) and of study drink consumption.

Assays for antibodies, plasma cytokines, and HLA DQ2-encoding genes

Serum IgA-class antibodies to tTG were measured by enzyme-linked immunosorbent assay (Celikey IgA; Pharmacia Diagnostics, Freiburg, Germany), and a serum concentration >5 U/mL was considered positive.

Serum IgG-class antibodies to *Helicobacter pylori* were measured by an enzyme immunoassay method (Pyloriset EIA-G III; Orion Diagnostica, Espoo, Finland), and a serum titer >20 U/mL was considered positive.

The presence of casein- and β -lactoglobulin-specific IgG and IgA in plasma was measured with an enzyme-linked immunosorbent assay according to a method described earlier (25). The

plasma concentrations (pg/mL) of sICAM-1 (HyCult Biotechnology, Uden, Netherlands) and granzymes A and B (Sanquin, Amsterdam, Netherlands) were measured with commercial kits according to the manufacturer's instructions.

A Cytometric Bead Array Human Th1/Th2 Kit (BD Biosciences, San Diego, CA) was used to measure plasma concentrations (pg/mL) of IFN- γ , tumor necrosis factor α , IL-2, IL-4, IL-5, and IL-10 according to the manufacturer's instructions. In a previous study (18), the subjects were analyzed for HLA DQ alleles to examine susceptibility to celiac disease and other autoimmune diseases, and these data were used in the present study.

Endoscopic examinations and samples

The subjects with persistent gastrointestinal symptoms ($n = 2$ males and 10 females) were studied by endoscopy: both upper and lower endoscopies were performed on 7 subjects, an upper endoscopy was performed on 4 subjects, and a lower endoscopy was performed on 1 subject. Endoscopies were carried out before the milk-tolerance test was conducted. Of the 10 control subjects, 4 were studied by both lower and upper endoscopy, 4 by upper endoscopy, and 2 by lower endoscopy. Gastro-duodenoscopies were performed with an Olympus CIF-IT140 (Tokyo, Japan), and colonoscopies were performed with an Olympus GF Q1401; both procedures were performed under general anesthesia.

Biopsy samples for real-time quantitative reverse transcriptase-polymerase chain reaction measurements were taken from the bulb of the duodenum (upper endoscopy) and from the terminal ileum (lower endoscopy) from sites where local disease alterations were seen. The mRNA expression of IFN- γ , TGF- β , IL-6, IL-12p35, and IL-18 mRNA was measured as described previously (26) with the following modifications: the sample size in the complement DNA synthesis was 20 μ L, and predesigned and tested fluorogenic FAM-labeled TaqMan Gene Expression primers/probes (Applied Biosystems, Foster City, CA) were used to measure transcription levels of the selected genes. The mRNA expression of the cytokines was analyzed by a comparative threshold method in which the relative amount of the sample is calculated in comparison with the amount measured with a calibrator; both of these values were normalized to an endogenous control (18S) (27). To obtain whole numbers for the plots, the relative numbers were multiplied by 100, except for IL-18, which was not multiplied.

Biopsy samples were taken for routine histologic examination from ≥ 4 separate areas during the upper endoscopy and from another 4 separate areas during the lower endoscopy. Additional samples were taken for immunohistochemical stainings, and the densities of intraepithelial T cells (CD3 $^{+}$, $\alpha\beta^{+}$, and $\gamma\delta^{+}$) were calculated as described previously (14). During the endoscopic examination, special interest was paid to the assessment of lymphoid nodularity on the mucosa (28).

Statistics

The independent-samples t test was used to test the significance of the differences between the groups. Dichotomous variables were tested with Pearson's chi-square test. The 95% CIs for the prevalence of gastrointestinal disorders were tested with the normal distribution approximation or, in the case of small sample sizes ($n < 50$), with the exact method based on binomial distribution. In the case of skewed data (sICAM-1, granzyme A, and granzyme B), a logarithmic transformation (ln) was used, and the

TABLE 1

Number and percentage of subjects with gastrointestinal complaints (and 95% CIs) during the previous year, as reported by 827 young adults

Gastrointestinal complaints	No. of subjects	Percentage (95% CI) ¹
		%
Abdominal pain	402	48.6 (45.2, 52.0)
Single or monthly bouts	332	
Weekly bouts	30	
Persistent	40	
Abnormal defecation frequency weekly	152	18.3 (15.7, 21.0)
< 3 defecations/wk	81	
> 3 defecations/d	71	
Abnormal formation of stools weekly ²	273	33.0 (29.8, 36.2)
Hard stools	196	
Loose stools	177	

¹ The 95% CIs were tested with normal distribution approximation.

² One hundred of the 273 subjects reported both hard and loose stools weekly.

results are presented as medians. A P value < 0.05 was considered significant. Data were analyzed by using SPSS, version 11.0 (SPSS Inc, Chicago, IL).

RESULTS

Gastrointestinal symptoms

In the questionnaire, 86 of 827 young adults (10.4%; 95% CI: 8%, 13%) reported major gastrointestinal complaints during the previous year (Figure 1). Specific gastrointestinal symptoms are presented in **Table 1**. Forty-three subjects (5.2%; 95% CI: 3.8%, 6.9%) fulfilled the Rome II criteria for IBS (29). The criteria for diarrhea-predominant IBS were met by 9 subjects (1.1%; 95% CI: 0.5%, 2.1%) and the criteria for constipation-predominant IBS were met by 6 (0.7%; 95% CI: 0.3%, 1.6%). Four subjects (0.5%) reported having been treated for *H. pylori* infection, 2 (0.2%) for celiac disease, and 1 (0.1%) for inflammatory bowel disease.

Gastrointestinal symptoms were far more frequent in females than in males: the prevalence of major symptoms was 16.4% in females compared with only 3.0% in males ($P < 0.001$). Similarly, 93.6% (44 of 47) of the symptomatic subjects who participated in the clinical examinations were female; 55.5% (15 of 27) of the control subjects were female. Paternal abdominal pains were more common among the symptomatic subjects who were clinically examined than among the control subjects (8 compared with 0 subjects; $\chi^2 = 4.6$, $P = 0.031$), and no other risk factors for gastrointestinal complaints were found in the parental questionnaire.

Food use and tolerance

On the questionnaire, 109 of the 827 subjects (13.2%; 95% CI: 10.9%, 15.5%) reported that they did not drink milk; this characteristic was more common in the females (86 of 458; 18.8%) than in the males (23 of 369; 6.2%; $P < 0.001$) and in the group with major gastrointestinal symptoms (31 of 86; 36.0%) than in all other subjects (78 of 741; 10.5%; $P < 0.001$). Milk-related complaints are presented in **Table 2**.

The subjects with major gastrointestinal complaints reported more food-related symptoms than did the other subjects. The



TABLE 2

Number and percentage of subjects with milk-related complaints (and 95% CIs) during the previous year, as reported by 827 young adults

Milk-related complaints	No. of subjects	Percentage (95% CI) ¹
		%
Symptoms related to regular milk ²	198	23.9 (21.0, 26.9)
Abdominal pain	185	
Loose stools	65	
Constipation	21	
Atopic symptoms	9	
Symptoms related to low-lactose milk ³	49	5.9 (4.4, 7.7)
Abdominal pain	45	
Loose stools	15	
Constipation	2	
Atopic symptoms	4	
Lactose intolerance	108	13.1 (10.8, 15.4)
Self-diagnosed	66	
Physician-diagnosed	42	
Milk allergy	28	3.4 (2.3, 4.9)
Self-diagnosed	22	
Physician-diagnosed	6	

¹ 95% CIs were tested with normal distribution approximation or the exact method based on binomial distribution.

² Seventy subjects reported more than one symptom.

³ Fourteen subjects reported more than one symptom.

percentage of subjects who reported symptoms from various foods in this group ($n = 86$) compared with all other subjects ($n = 741$), respectively, was as follows ($P < 0.001$ for all): milk (69% compared with 19%); low-lactose milk (23% compared with 4%); newly baked bread or buns (40% compared with 12%); xylitol-containing sweets commonly used in Finland (40% compared with 13%); vegetables with indigestible carbohydrates, ie, peas, beans, cabbage, and onion (29% compared with 14%); and apples, carrots, and nuts, all of which cross-react with birch pollen (24% compared with 10%).

The 47 symptomatic subjects who were clinically examined restricted their diet more so than did the 27 control subjects. The symptomatic subjects consumed the following foods less frequently: milk ($P < 0.001$), hot chocolate ($P < 0.001$), ice cream ($P = 0.001$), cheese spread ($P = 0.049$), bread ($P = 0.015$), savory pastries ($P = 0.029$), sweet pastries ($P = 0.045$), some fruit (grapes, $P = 0.005$; kiwi, $P = 0.003$), mashed potatoes ($P = 0.042$), sausages ($P = 0.009$), and soft drinks ($P < 0.001$). The only food that the symptomatic subjects consumed more often was juice ($P = 0.001$). None of the symptomatic subjects who were clinically examined reported eliminating all dietary cow milk proteins, either at the time of the interview or during their earlier life. However, avoidance of milk or dairy products was common, because only 14 subjects (29%) consumed such products without any restrictions.

Lactose intolerance

On the questionnaire, 108 subjects (13.1%) reported lactose intolerance (Table 2). In the clinical examination, the lactose-maldigestion test result was positive in 16 of the 47 symptomatic subjects (34.0%; Table 3). However, only 4 subjects (8.5%) had the C/C_{-13910} genotype associated with adult-type hypolactasia, and the rest of the subjects carried the C/T_{-13910} genotype of lactase persistence. Of 27 symptom-free control subjects, 1

TABLE 3

Gastrointestinal disorders diagnosed in symptomatic subjects and control subjects during clinical examination

	No. of symptomatic subjects ($n = 47$) ¹	No. of control subjects ($n = 27$) ²
Cow milk-related symptoms	17	—
Lactose maldigestion ³	16	—
Hypolactasia-associated genotype C/C_{-13910} ⁴	4	1
Cow milk protein hypersensitivity	1	—
<i>Helicobacter pylori</i> infection	3	3
Functional gastrointestinal disorders	28	—
Irritable bowel syndrome, normolactasia	6	—
Functional constipation	7	—
Unspecified functional bowel disorder	13	—
Unspecified functional abdominal pain	2	—

¹ One subject with celiac disease and one with ulcerative colitis were excluded.

² Two subjects with high serum immunoglobulin E concentrations were excluded.

³ Seven subjects who tested positive with the lactose-maldigestion test had irritable bowel syndrome and one had *H. pylori* infection.

⁴ All 4 symptomatic subjects had tested positive with the lactose-maldigestion test.

(3.7%) had the C/C_{-13910} genotype of adult-type hypolactasia, 15 (55.5%) had the C/T_{-13910} genotype, and 11 (40.7%) had the T/T_{-13910} genotype of lactase persistence.

Milk-specific antibodies

The symptomatic subjects had lower concentrations of casein-specific IgG than did the control subjects ($n = 47$ compared with $n = 27$; 0.91 compared with 1.20; mean difference: 0.28; 95% CI: 0.01, 0.57; $P = 0.043$). The difference was greatest between those symptomatic subjects who did not drink milk and the control subjects ($n = 31$ compared with $n = 27$; 0.83 compared with 1.20; mean difference: 0.37; 95% CI: 0.05, 0.68; $P = 0.023$).

Milk-protein tolerance test

In the blinded cow milk protein challenge, there was no difference in the symptoms yielded by cow milk and the placebo soy drink (mean difference: -0.34 ; 95% CI: -7.1 , 6.4 ; $P = 0.920$). Of the 23 symptomatic subjects who completed the challenge, 16 experienced intense gastrointestinal symptoms during both the milk and the placebo soy challenges, 5 experienced more symptoms (>10 points/wk) during the 3-wk low-lactase milk challenge, and 9 experienced more symptoms during the 3-wk placebo soy challenge; no difference was observed between the challenges in 9 subjects. Only 2 subjects, one with lactose intolerance, had severe and permanent symptoms during the milk challenge. Of the 25 nonsymptomatic control subjects who completed the challenge, 1 experienced some gastrointestinal symptoms during the milk challenge and another during the soy challenge.

HLA alleles

The *HLA-DQA1*05-DQB1*02* allele combination encoding the DQ2 molecule, associated with celiac disease and a group of autoimmune diseases, was slightly more frequent in the group with major gastrointestinal complaints than in the nonsymptomatic group (26% compared with 15%; $\chi^2 = 3.6$, $P = 0.057$). The respective frequency was 17.7% in the group with minor gastrointestinal symptoms and was 23.9% in the group with other gastrointestinal symptoms (see Figure 1 for group classification).

Endoscopic findings

Of the 12 subjects studied by endoscopic examination ($n = 11$ gastroscopies, $n = 8$ colonoscopies), 2 had a definite disease confirmed by histology: 1 had elevated antibodies against tTG and celiac disease, and the other had ulcerative colitis. Both subjects were treated accordingly and excluded from further analyses. Only unspecific findings were seen from the remaining 9 gastroduodenoscopies and 7 colonoscopies. None of the subjects showed lymphoid nodules in the bulb of the duodenum or distally in the descending duodenum.

The symptomatic subjects showed a tendency toward a slight elevation in intraepithelial CD3⁺ T cell counts in the duodenal samples compared with the control subjects ($n = 8$ compared with $n = 8$; 25.6 compared with 17.1; mean difference: 8.5; 95% CI: -0.6, 17.6; $P = 0.065$).

Immunologic findings

The 47 symptomatic subjects had higher plasma concentrations of sICAM-1 (median: 68 500 compared with 58 200 pg/mL; $P = 0.008$) and lower plasma concentrations of granzyme A (19.4 compared with 37.6 pg/mL; $P = 0.001$) than did the 27 control subjects (Figure 2). No significant differences in plasma cytokine concentrations were observed between the groups. The symptomatic subjects tended to express slightly more TGF- β mRNA ($n = 8$ compared with $n = 8$; mean: 3.3 compared with 1.0; mean difference: 2.3; 95% CI: -0.2, 4.9; $P = 0.073$) and IL-12p35 mRNA (26.5 compared with 13.6; mean difference: 12.9; 95% CI: -1.5, 27.2; $P = 0.075$) in the duodenal biopsy samples than did the control subjects who underwent endoscopy. No significant differences were found in the ileal samples.

DISCUSSION

We previously described, in school-aged children, an intestinal hypersensitivity to cow milk protein associated with mucosal lymphoid nodular hyperplasia and a mild elevation in intraepithelial $\gamma\delta^+$ T cells. The main aim of the present survey was to evaluate the occurrence of similar hypersensitivity in young adults with gastrointestinal complaints. However, we were not able to find even one such case. In a blinded challenge, no difference in the symptoms experienced after consumption of cow milk and the placebo soy drink was discerned. Although cow milk did not trigger gastrointestinal symptoms, our results suggest that inflammatory mechanisms are involved in food-related gastrointestinal complaints, because, compared with the control subjects, the symptomatic cases as a group showed higher concentrations of circulating sICAM-1 and a tendency toward the up-regulation of TGF- β and IL-12p35 mRNA expression in the mucosal biopsy samples.

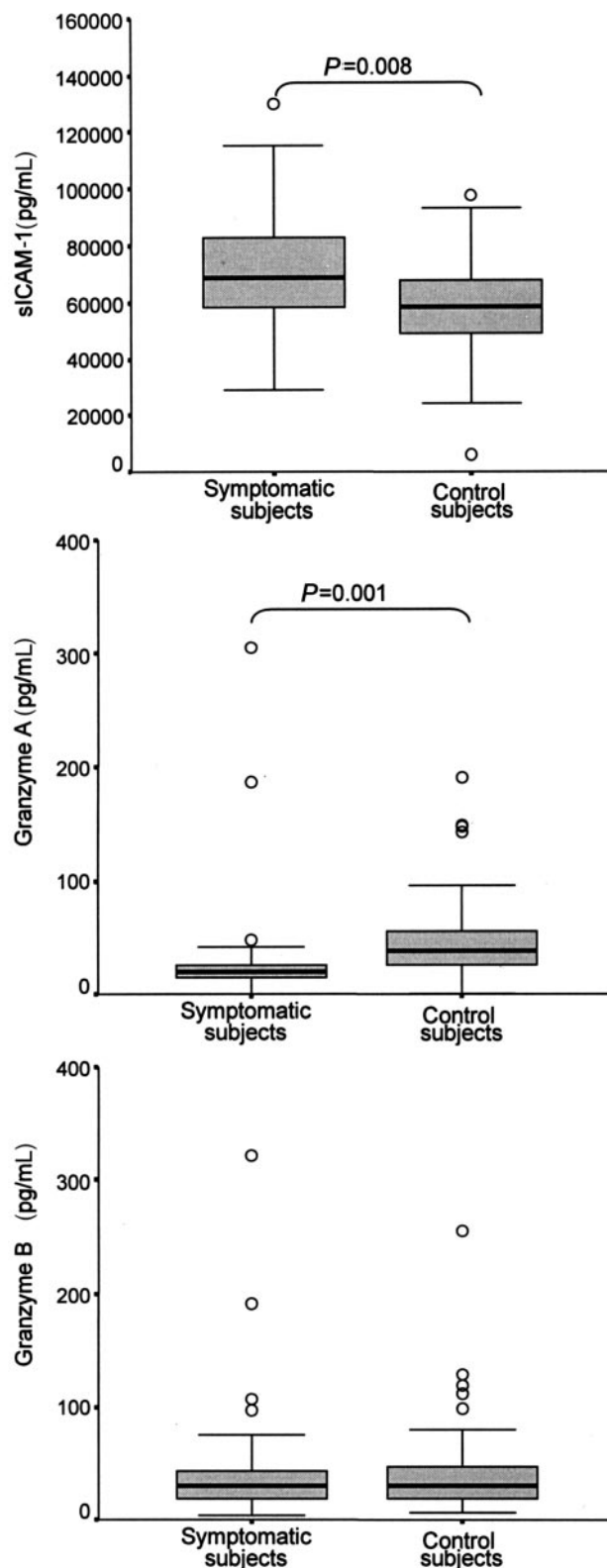


FIGURE 2. Plasma concentrations of soluble intercellular adhesion molecule 1 (sICAM-1), granzyme A, and granzyme B in 47 subjects with gastrointestinal symptoms and 27 control subjects. The gray boxes indicate the lower and upper quartiles with medians, the vertical lines represent minimum and maximum values, and the outliers (>1.5 box length from the end of the box) are indicated by circles. In the statistical analysis, data were logarithmically transformed, and an independent-samples t test was used. Figure is drawn with nontransformed numbers.



Almost 50% of the subjects reported having had some abdominal complaints during the previous year, but in most cases the symptoms appeared infrequently and did not disturb their normal life. In accordance with earlier studies on children (8, 19), 10% of the young adults in the present study reported intensive and major gastrointestinal complaints. The frequency of gastrointestinal symptoms subjectively related to cow milk consumption was surprisingly high, almost 25% of all the subjects. Lactose intolerance was present in 13% of the study subjects, and 3% claimed to have milk allergy; both the lactose intolerance and the allergy were, in most cases, self-diagnosed. Pelto et al (10) calculated that the frequency of cow milk protein hypersensitivity is 3–6% among young adults in Finland, whereas the prevalence of lactose intolerance was estimated to be $\approx$ 6%. Our negative results from the blinded milk protein challenge and the hypolactasia gene test do not support these frequencies. In the placebo-controlled low-lactose milk challenge, milk protein was found to cause pronounced symptoms in only 2 of the 23 subjects who completed the challenge, and 4 of the 47 symptomatic subjects had the *C/C*₋₁₃₉₁₀ genotype associated with low lactase activity (24). On the basis of these observations, there seems to be a marked discrepancy between self-observed food-related reactions and reliably diagnosed hypersensitivity or intolerance states as demonstrated by many earlier investigators (4–6). The avoidance of milk and dairy products by symptomatic subjects, as evidenced by decreased concentrations of cow milk-specific antibodies, often leads to impaired nutrient intake (30).

Approximately 17% of Finns have hypolactasia, but only 12% are symptomatic (31). On the questionnaire, we observed a similar prevalence of lactose intolerance (13%), although the lactose-maldigestion test was positive more often in the symptomatic study subjects (33%). However, only 4 of the 47 (8.5%) symptomatic subjects carried the genetic variant *C/C*₋₁₃₉₁₀ of the *LCT* gene as a definite marker of low genetic lactase activity (22–24). One nonsymptomatic control subject also had this genotype. Our finding supports the view that lactose-maldigestion tests show a discrepancy with *LCT*-gene tests when the *C/C*₋₁₃₉₁₀ genotype of the *LCT* gene is used as a diagnostic marker of lactose intolerance. The diagnosis of hypolactasia based on lactose-maldigestion tests may be unreliable (32), and some of the positive lactose-maldigestion tests may give false-positive results because of the poor absorption of lactose in the human intestine (33). It is also possible that secondary lactose intolerance may have a significant role in food-related gastrointestinal complaints. Interestingly, the *T/T*₋₁₃₉₁₀ genotype associated with high lactase activity (22–24) was only detected in the healthy control subjects.

Celiac disease was thoroughly diagnosed in this cohort ($n = 1078$) because the healthy subjects were also screened (18). One newly diagnosed case and 2 cases reported on the questionnaire amounted to a total of 20 patients plus 3 tTG antibody-positive cases who refused confirmation by biopsy. Thus the frequency of celiac disease was surprisingly high (1.9–2.1%). The true figure might be even higher, because we rescreened only the symptomatic cases. The latest estimations from other studies range from one case in 100 individuals to one case in 500 individuals (18, 34, 35). The occurrence of IBS was lower (5.2%) than that reported in adults in epidemiologic studies (15–20%) (36). However, according to our phone calls to initial nonresponders, the percentage of replies was highest in symptomatic young adults. In the present study, gastrointestinal symptoms were far more common

in females than in males, as also reported earlier in functional gastrointestinal diseases such as IBS (5, 37, 38). This finding may have had some effect on the results, because the sex distribution was close to even in the control group.

In the present study, the symptomatic subjects had higher circulating concentrations of sICAM-1 and tended to express more TGF- β and IL-12p35 mRNA in the duodenal biopsy samples than did the control subjects. TGF- β is an inhibitory cytokine recognized as a key regulator of immunologic homeostasis and inflammatory responses and is associated with the development of oral tolerance (39). It inhibits T cell proliferation and is also a product of the mucosal regulatory T cell population (40). IL-12 is a monocyte-derived cytokine that enhances IFN- γ up-regulation. sICAM-1, induced by IFN- γ , is important for eosinophil and neutrophil adhesion, and a high concentration of plasma sICAM-1 has been observed as a marker of persistent airway inflammation in asthmatic patients (41). Exposure to cow milk during infancy also induces circulating sICAM-1, which reflects an inflammatory response to orally ingested foreign proteins (42). Consequently, we postulate from these measurements that our subjects with gastrointestinal symptoms had either a special or an abnormal skewing of the immune responses or inflammation of the intestinal mucosa. In support of this conclusion, the symptomatic patients tended to have higher counts of intraepithelial CD3⁺ T cells in the duodenal mucosa than did the control subjects. A massive accumulation of intraepithelial CD3⁺, $\alpha\beta$ ⁺, and $\gamma\delta$ ⁺ T cells is typical of celiac disease and is also seen to a lesser extent in delayed-type food allergy and food-sensitive enteropathy (8, 12–16).

The symptomatic subjects had decreased plasma concentrations of granzyme A, whereas the concentrations of granzyme B were not significantly different from those seen in the control subjects. In a recent study we found significantly increased circulating concentrations of granzyme B in children with cow milk-sensitive enteropathy manifested by intestinal lymphoid nodular hyperplasia and up-regulated counts of $\gamma\delta$ ⁺ T cells; the concentrations were even higher than in celiac disease cases (43). Thus, the present symptomatic young adults also differed from patients with cow milk-sensitive enteropathy in relation to circulating concentrations of granzyme B. Nor did any of the symptomatic young adults have the massive intestinal lymphoid excess that we earlier described in school-aged children with cow milk-sensitive enteropathy (8, 28). The decreased plasma concentrations of granzyme A in the symptomatic individuals was an unexpected finding. Granzyme A is found in the cytotoxic cells, the intraepithelial lymphocytes, and the T regulatory cells (44, 45). High concentrations of soluble granzyme A are reported in viral infections (46) and chronic inflammations such as rheumatoid arthritis (47, 48). To our knowledge, low concentrations of granzyme A have not been reported in disease states; thus, the reason for our finding remains unknown.

In conclusion, we observed that 10% of young Finnish adults reported major gastrointestinal complaints, 24% reported cow milk-induced gastrointestinal symptoms, and 13% did not drink any milk as such. In a blind challenge, cow milk protein-induced symptoms were, however, rare and were similar to those observed after the consumption of a placebo soy drink. Yet, we found markers of a skewed immune response of the gastrointestinal mucosa in the symptomatic subjects. We conclude that food-related gastrointestinal symptoms in young adults are caused by unspecific and unknown traits of altered mucosal

immune response rather than by cow milk, as is often suspected by the patient. We suggest that this new entity, ie, intestinal immune-mediated disorder, may be a self-perpetuating disease with fluctuations in symptoms. An autoimmune characteristic of the syndrome, at least in a subgroup of the affected subjects, cannot be ruled out. This hypothesis is supported by the observation that the *HLA DQ*02* allele predisposing to autoimmunity was almost twice as common in the symptomatic subjects as in the rest of the subjects. 

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LP, RK, TT, OV, and JK designed the study, collected the data, and drafted the manuscript. OV and JH were responsible for the immunologic laboratory measurements. IJ was responsible for the lactase genotyping. JI was responsible for the HLA genotyping. The study population was a cohort derived from a previous study that was designed and implemented by MK as the principal investigator. All of the authors contributed to the formulation of the final manuscript. None of the authors had any conflict of interest.

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