

Serum and Tissue CD23, IL-15, and FasL in Cow's-Milk Protein-sensitive Enteropathy and in Coeliac Disease

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ABSTRACT

Objectives: The aim of the study was to explore pathogenesis and find new serum markers for cow's-milk-sensitive enteropathy (CMSE) and coeliac disease (CD). We assessed the intestinal expression and serum concentration of CD23, IL-15, and FasL. We hypothesised that the serum levels of CD23, a protein expressed in the lymphoid follicles, would be associated with lymphonodular hyperplasia (LNH), a feature characteristic of CMSE. We also presumed that interleukin (IL)-15 and FasL, functionally connected with proliferation and apoptosis of the intraepithelial lymphocytes (IELs), would relate with the increased numbers of IELs present in both CMSE and CD.

Methods: Twenty-three children with CMSE, 20 with untreated CD, and 14 controls were studied for CD3, α/β - and γ/δ -expressing IELs, and for duodenal and ileal expression of CD23, FasL, and IL-15 by immunohistochemistry, and for serum concentration of sCD23, sFasL, and sIL-15 by enzyme-linked immunosorbent assay.

Results: There was a trend for increase in sCD23 serum levels in untreated CMSE and in CD ($P=0.074$; $P=0.077$). CD23 was expressed in the mucosal germinal centres, but sCD23 was not related to presence of LNH. In CMSE, there was a trend for increase in serum sFasL ($P=0.07$) and high levels associated with LNH ($P=0.025$) and correlated with the IEL numbers ($P<0.05$). Mucosal high endothelial venules adjacent to lymphoid follicles showed an intensive FasL expression.

Conclusions: Serum sCD23 shows a trend of increment in CMSE and CD, and in the latter, sCD23 level may provide information about the severity of villous atrophy. In CMSE, high serum sFasL indicates both LNH and an increase of IELs, suggesting importance of FasL-mediated mechanisms in the pathogenesis of these features characteristic of CMSE. Further studies are necessary to evaluate whether intensive FasL expression in mucosal high endothelial venules presents a regulatory element in mucosal immunity.

Key Words: CD23, children, CMSE, coeliac disease, enteropathy, FasL, IEL, IL-15, milk allergy, serum

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Cow's-milk-sensitive enteropathy (CMSE) is a non-immunoglobulin E (IgE)-related form of food allergy in children (1,2). The symptoms are variable and include recurrent abdominal

pain, diarrhoea or constipation, blood in the stool, anaemia, and even failure to thrive (2,4–6). Little is known about the pathogenesis and the mechanisms of the characteristically slow responses in elimination and challenge tests (1,2). Increase in intestinal intraepithelial lymphocytes (IELs) with cytotoxic activation (6) and intestinal lymphonodular hyperplasia (LNH) are the characteristic features of CMSE (2,4,6,8,9). Increase in intraepithelial cytotoxic lymphocytes is also seen in coeliac disease (CD), in which the increase is associated with a villous atrophy (6).

Diagnosis of CMSE is often difficult. Skin prick tests and measurements of allergen-specific IgE antibodies usually produce negative results (2). LNH, present in 40% to 75% of cases, and a slight increase in IELs (2,4,6,8,9) are characteristic but not specific findings of this entity, and require discovery by endoscopy. The mild increment in the intraepithelial T lymphocytes in CMSE has low diagnostic specificity and sensitivity because of an overlap with healthy subjects and with latent CD (2,9). In many cases, the whole set of investigations, including tedious elimination challenge tests and clinical, endoscopic, and histological examinations, are needed to confirm the diagnosis.

Immunopathogenesis of CMSE is largely unknown. T_H1 -prone interferon- γ is elevated together with T_H2 cytokine interleukin-6 (IL-6) (10,11). Recent studies, in addition to our own, have shown that the functional aberration of the mucosal immune system in CMSE has features in common with CD, including increment of the duodenal intraepithelial gamma delta ($\gamma\delta$) T-cell receptor bearing T lymphocytes (3,9), and cytotoxic lymphocytes expressing T-cell-restricted intracellular antigen type 1; (12), granzymes A and B, and perforin (7). As evidence of actual cytotoxic activation in CMSE, we have shown elevated serum concentrations of granzymes A and B in both CD and CMSE (13). In contrast to CD, the lymphocyte cytotoxicity in CMSE seems not to target the enterocytes because no evidence of increased enterocyte apoptosis was detected. Instead, evidence for an increased apoptosis rate of IELs has been observed in CMSE (14).

In the present study, we have aimed to assess immunopathogenetic mechanisms of CMSE and CD and explored potential new noninvasive markers for the intestinal immune activation in these conditions. We have focused on markers related with the appearance of characteristic features of CMSE, such as LNH and the increase of IELs. For this purpose, we have assessed serum levels of CD23, IL-15, and FasL. The rationale for selecting these markers was based on their roles in allergic reactions and in germinal centre activation (CD23), their potential role in the activation of IELs (IL-15), and the apoptosis of IELs (FasL).

METHODS

Patients and Controls

The present study consisted of an unselected series of 57 children with a mean age of 10.2 years (range 4–15 years;

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22 boys) referred to the Oulu University Hospital, Finland, for paediatric gastroenterological consultation because of refractory gastrointestinal (GI) complaints. The study population is the same as observed earlier for assessment of serum granzymes, CD30, and soluble Fas concentrations (13).

Of the patients (mean age 9.2 years, range 4–15; 11 boys), 23 were finally diagnosed as having CMSE. They had been referred for various symptoms, the dominant symptom being abdominal pain in 11 patients, and diarrhoea or loose mucous stools in 12 patients, 4 of which had blood in stools. The severity of symptoms was a clinical indication that endoscopic assessment should be included in the diagnostic workup. Diagnosis of CMSE was based on an open elimination and challenge test as described previously (7). Abdominal pain, diarrhoea or loose mucous stools, and exacerbation of dermatitis were each considered a positive result. In cases with atopic symptoms, possible IgE-mediated food allergy was assessed by determination of milk-specific IgE concentration (RAST test), all of the 8 remaining negative. Of the patients with CMSE, 18 (mean age 9.3 years, range 4–15; 9 boys) were studied before the definite diagnosis was assessed, whereas 5 (mean age 9 years, range 6–14; 2 boys) were on the elimination diet during the examinations (treated CMSE).

The other group consisted of 20 patients with CD (mean age 10.5, range 5–15 years; 5 boys) diagnosed according to the acknowledged criteria (13). Only those with subtotal or total villous atrophy with crypt hyperplasia in the duodenal sample were included. Referral symptoms were diarrhoea or loose mucous stools in 12, abdominal pain in 5, growth retardation in 2, and recurrent vomiting in 1 case. Four of the patients with CD showed patchy villous atrophy in the upper duodenal samples and were considered to have mild CD. Colonoscopy had been performed in 10 children with CD as indicated by clinical symptoms and by the lack of a definite diagnosis of malabsorption at the time of planning the set of clinical investigations.

The control group consisted of 14 subjects (mean age 11.4 years, range 6–15; 6 boys) referred and examined for exclusion of any definite GI diseases. Four cases with both recurrent abdominal pain and diarrhoea and 2 cases with abdominal pain were tested with a milk elimination and challenge test similar to the CMSE group; their results remained negative. The occasional abdominal pain of the other 5 control cases indicated no need for an elimination and challenge test, and the symptoms subsided during a long follow-up of at least 6 months with a milk-containing diet. None of the 3 cases with eating disorders had any abdominal symptoms, which would have indicated the need to perform elimination and challenge tests. After an adequate examination and follow-up, it was concluded that none of the 14 control subjects had a manifest GI disease and/or that the symptom had remitted.

The ethics committee for clinical science of Oulu University Hospital approved the study protocol. Informed, written consent was obtained from all of the parents of the study children.

Determination of Serum Concentration of sCD23, sFasL, and sIL-15

Serum samples were evaluated by the enzyme-linked immunosorbent assay method for sFasL (Medical and Biological Laboratories, Nagoya, Japan), sCD23 (R&D Quantikine, Minneapolis, MN), and sIL-15 (R&D Quantikine). All of the samples were analysed simultaneously following the manufacturers' instructions.

Endoscopic Examinations and Samples

Mucosal biopsy samples from the proximal duodenum were taken during an endoscopic examination performed under general

anaesthesia, as is the normal procedure at our hospital, using an Olympus GIF-XQ 140 gastroscope (Olympus, Tokyo, Japan). Biopsy samples were drawn for routine histology from at least 4 separate areas. Additional specimens were obtained from the sites where local disease alterations were seen.

The assessment of lymphoid nodular hyperplasia on the duodenal, ileal, and colorectal mucosa was based on an endoscopic evaluation after filling the area to be inspected with air (2,15,16). Only a cluster of lymphoid nodules ($n > 10$ nodules) was considered significant.

Immunohistochemistry

Ileal and duodenal (descending part) samples taken during gastroscopy or ileocolonoscopy were stained using the DAKO EnVision (DAKO, Glostrup, Denmark) detection system kit with 3,3'-diaminobenzidine as a chromogen for CD23 (Clone 1B12; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK), CD30 (Novocastra), Fas (Santa Cruz Biotechnology, Santa Cruz, CA), FasL (Pharmingen, San Diego, CA), and IL-15 (R&D Systems, Minneapolis, MN), which were consequently analysed. Fresh-frozen duodenal samples were studied for the densities of CD3, α/β , and γ/δ IELs, as described previously (2).

We analysed the expression of CD23, FasL, and IL-15 separately in the epithelium and lamina propria (LP). The extent of expression in the epithelium was assessed using a 6-point scale: 0 = negative; 1 = 1% to 20%; 2 = 21% to 40%; 3 = 41% to 60%; 4 = 61% to 80%; 5 = 81% to 100%. The intensity of epithelial staining was evaluated using a 4-point scale: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong staining. The proportion of IL-15- and FasL-expressing cells among the mononuclear inflammatory cells in the villous LP was evaluated by counting positive and negative cells in 1 to 2 villi by using $\times 400$ magnification. Positive cells in the intercryptal LP were similarly counted. To obtain an estimate of the relative areal density of the positive cells, we first assessed the density of mononuclear inflammatory cells in the LP by using a visual analogue scale (3). Based on the proportion of cells expressing IL-15 or FasL, their relative areal density in the LP was calculated. The mean proportion of villous and intercryptal LP was used in these calculations.

Statistical Methods

The data were analysed using SPSS (version 18, SPSS Inc, Chicago, IL). Because of the skewed, abnormal distribution of serum concentrations and cell densities in most of the patient groups, the nonparametric Mann-Whitney *U* test and the Kruskal-Wallis test were used to estimate the significance of differences between the groups. Results are expressed as medians. A *P* value of < 0.05 was considered significant. Scatterplot illustrations were made with Origin Pro 8 (OriginLab Corp, Northampton, MA).

RESULTS

Endoscopic Findings and the Numbers of Duodenal IELs

All 57 subjects had been examined with gastroduodenoscopy and 20 of them were examined with colonoscopy extending to the terminal ileum. None of the patients with CMSE or controls had villous atrophy or crypt hyperplasia, which was seen in all of the CD cases. LNH was seen in the gastroscopy or colonoscopy in 78% (14/18) of untreated patients with CMSE, 20% (4/20) of patients

with CD, 21% (3/14) of controls, and 80% (4/5) of patients with treated CMSE. Presence of LNH in any location of the GI tract was significantly more common in CMSE (treated and untreated combined) than in the controls ($P=0.001$), but no such difference between CD and CTRL emerged ($P=0.919$). Details concerning describing the presence of LNH in different anatomical locations and significant differences between the study groups are summarised in Figure 1.

As we have already reported, the untreated patients with CMSE had significantly higher densities of CD3, $\alpha\beta$ - and $\gamma\delta$ -positive T cells in duodenal samples than in the controls (6). In all of the CMSE cases on a milk-free diet, however, the densities were comparable with the controls. In subjects with CD, all of these measures were higher than in any other group (6). There were no differences in the serum concentrations of IgA or IgE between the groups (data not shown).

Serum Concentration and Mucosal Expression of CD23, FasL, and IL-15

Serum concentrations of sCD23, sFasL, and IL-15 as determined by enzyme-linked immunosorbent assay are summarised in Figure 2. There was a trend of increased sCD23 concentration in CMSE (median 105.0 $\mu\text{g/mL}$; $P=0.064$; Mann-Whitney) and in CD (106.5 $\mu\text{g/mL}$; $P=0.077$) as compared with controls (69.0 $\mu\text{g/mL}$) and some trend for increase in sFasL in treated CMSE ($P=0.087$), but no differences in sIL-15 concentrations emerged.

Mucosal expression of CD23, FasL, and IL-15 was assessed with immunohistochemical staining of the duodenal and ileal biopsies (Figs. 3 and 4; Tables 1 and 2). CD23 expression was seen in the germinal centre area of lymphoid follicles, which were occasionally seen in duodenal and ileal biopsies but not in other cells of the LP or in the epithelium (Fig. 3A). FasL expression was seen in epithelial cells (Fig. 3C), including Paneth cells, in the crypt bases (Fig. 4) and in scattered mononuclear cells in the LP (Fig. 3D; Table 1). Interestingly, endothelial cells of the high endothelial venules (HEVs) present close to mucosal germinal centres showed a constant expression of FasL (Fig. 4). Intensity or the extent of FasL expression in the epithelium did not show any differences between the groups (data not shown). The proportion of cells expressing FasL in the LP did not differ between the groups (Table 1), but relative areal density of FasL-expressing cells in the LP (combined villous and cryptal LP) was higher in patients with CD than in controls ($P=0.017$) or untreated CMSE ($P=0.009$), as seen in Table 2.

IL-15 was seen in all of the villous epithelial cells and in most cryptal epithelial cells. About half of the cells in the LP expressed IL-15 and its density was similar in both villous and cryptal LP (Fig. 3F; Table 1). There were no differences between the groups in epithelial expression of IL-15 (data not shown) or in the proportion of positive cells in the LP (Table 1). A comparison of the estimated areal cell densities of IL-15-expressing cells in the LP (combined villous and cryptal LP) showed significantly increased cell densities in CD ($P=0.006$) but not in CMSE ($P=0.771$) compared with controls (Table 2).

There was no correlation between the serum levels and mucosal proportional expression of CD23 and IL-15. Serum sFasL showed a negative correlation with the intensity of FasL expression in the lower half of duodenal villus epithelium ($n=44$; $c=-0.378$; $P=0.011$; Spearman), and a similar negative correlation was evident in the duodenal crypt epithelium in CD ($n=17$; $c=-0.503$; $P=0.039$; Spearman). There was no correlation between estimated areal cell densities of FasL or IL-15 with serum levels of sFasL and sIL-15.

Relation of Serum Levels of sCD23, sFasL, and sIL-15 and the IEL Counts

Because the increase in the numbers of intraepithelial CD3, $\alpha\beta$ - and $\gamma\delta$ -expressing lymphocytes is characteristic of both CMSE and CD, we were interested to see whether the serum concentrations or mucosal expression of CD23, FasL, or IL-15 would show any correlation with the counts of IELs. Serum sFasL did not show an association with IEL counts in CD, but in CMSE (untreated) the numbers of CD3 IELs, $\alpha\beta$ -expressing IELs, and $\gamma\delta$ -expressing IELs showed a significant positive correlation with sFasL (Fig. 5).

Relation of LNH and Serum Concentrations of sCD23, FasL, and sIL-15

LNH is the characteristic endoscopic feature of CMSE (2,4,6,8,9). Accordingly, we were interested to see whether the occurrence of LNH has any association with the serum concentrations of CD23, FasL, or IL-15. Only in untreated CMSE, was sFasL higher in patients with LNH than in those without (Fig. 6; $P=0.025$; Mann-Whitney). In contrast, serum sCD23 or sCD15 concentrations did not relate to LNH (data not shown).

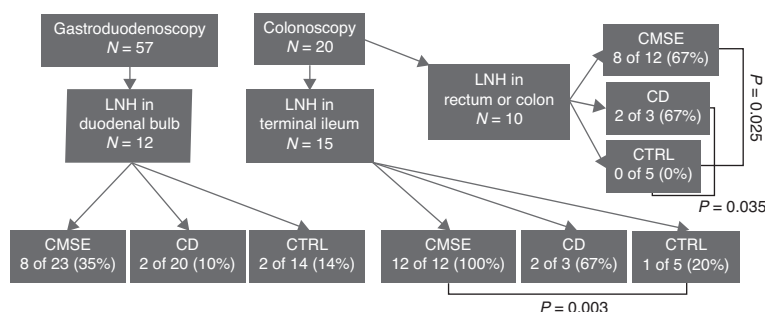


FIGURE 1. Flowchart summary showing presence of endoscopically visible lymphonodular hyperplasia (LNH) in gastroduodenoscopy or ileocolonoscopy in different groups of subjects including controls (CTRL), cow's-milk-sensitive enteropathy (CMSE), and coeliac disease (CD). Significant ($P<0.005$) statistical differences (χ^2 test or Fisher test) are indicated.

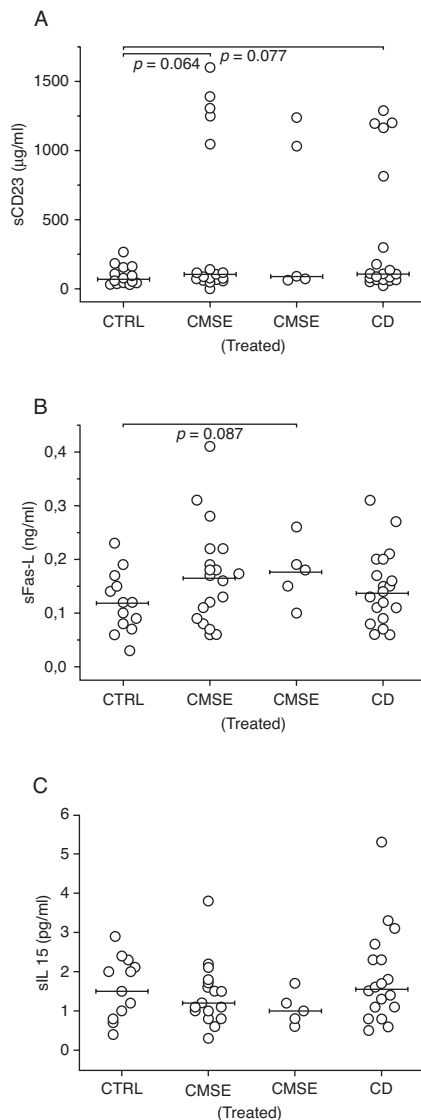


FIGURE 2. Scatterplot showing distribution of (A) serum sCD23 (microgram/milliliter), (B) sFasL (nanogram/millilitre), and (C) sIL-15 (picograms/millilitre) concentrations in controls (CTRL), cow's-milk-sensitive enteropathy (CMSE; untreated and treated), and coeliac disease (CD). Medians (horizontal lines) and significant differences are indicated (Mann-Whitney). IL = interleukin.

Relation of Villus and Crypt Height and Serum Concentrations of sCD23, sFasL, and sIL-15

Because villous atrophy is the hallmark of CD, we were interested to see whether measured serum levels would correlate with the height of villus or crypt in the duodenum. sCD23 concentration showed a trend of negative correlation with villus height ($n = 48$; $c = -0.256$; $P = 0.079$; Spearman), and a similar correlation was evident within the CD group ($n = 19$; $c = -0.472$; $P = 0.041$; Spearman), but no significant correlation with the crypt height was observed. The positive predictive value of sCD23

(threshold 100 μg/mL) for the presence of villous atrophy is 42.3%. In contrast, sFasL and sIL-15 concentrations did not correlate with villus or crypt measurements.

DISCUSSION

We have analysed both intestinal mucosal expression and serum levels of CD23, FasL, and IL-15 to find new diagnostic markers in CMSE and CD and to explore the pathogenetic mechanisms. There was a trend of an increase in serum sCD23 concentration in CMSE and CD ($P = 0.074$; $P = 0.077$). There was also a similar trend of an increase in serum sFasL concentration in CMSE ($P = 0.07$), in which high sFasL concentrations also associated with LNH and correlated with the numbers γ/δ -expressing IELs.

CD23, also known as Fc ϵ -RII, is the "low-affinity" receptor for IgE. Although we found some evidence of a trend for increase in serum sCD23 concentration in CMSE and CD, variation of serum sCD23 levels was considerable in both CMSE and CD, with several subjects showing concentrations comparable to those in the control group (Fig. 2). Previously, an increase in serum levels of sCD23 has been reported in rheumatoid arthritis and in immediate types of allergies (17–19), but there are no studies of sCD23 in CMSE. In contrast to our result, decreased concentration of sCD23 has been reported in CD (17). The reasons for these somewhat discrepant findings in CD remain unknown, but may involve methodological differences in the assay methods, age of the subjects (20), and severity of the disease. Interestingly, serum sCD23 concentration showed a significant negative correlation ($c = -0.472$) with the height of villi in CD. The potential value of sCD23 determination in the noninvasive assessment of the severity of villous atrophy in CD should be assessed on a larger scale.

CD23 is expressed in several types of cells including B lymphocytes, monocytes, macrophages, and dendritic reticulum cells of the lymphatic tissue. Expression in the intestinal epithelium has been reported as well (21). CD23 has a role in the intraepithelial processing of food allergens during their transepithelial transport by opening the "protected" transport in the enterocytes. Increased epithelial expression of CD23 has been reported in inflammatory bowel disease and in children with food intolerance to cow's-milk proteins (21). In contrast to previous observations (21), we did not detect any immunohistochemical expression of the protein in the epithelium in either duodenal or ileal mucosa. Reasons for this discrepancy remain speculative and may be related to tissue processing, optimisation of the immunostaining, or the antibodies used. One potential explanation is based on the fact that human CD23 occurs in 2 isoforms, CD23a and CD23b. Of these isoforms, CD23a is constitutively expressed in the epithelial cells (19) and CD23b is inducible. There is, however, a controversy relating to the expression patterns of these 2 isoforms (19,22). Immunoreaction for CD23 was always present in the germinal centres (Fig. 3A), where dendritic reticulum cells show a constant expression (23,24), and have recently been shown to be a dynamic population with ample apoptosis (24). Because the presence of germinal centres in mucosa correlates with endoscopic LNH, a feature clearly connected with CMSE (4–6,8,15,25), the presence and extent of LNH would have been a plausible explanation for the increase of sCD23; however, our finding that sCD23 concentrations in patients with CMSE were not related to the absence or presence of LNH favours the idea that increase in sCD23 does not originate from the abundant mucosal lymphatic tissue. We conclude that more studies are needed to see whether the determination of serum concentration of sCD23 may be used as an additional noninvasive test for CD and to dissect the immunological mechanism and the cellular sources of serum increase.

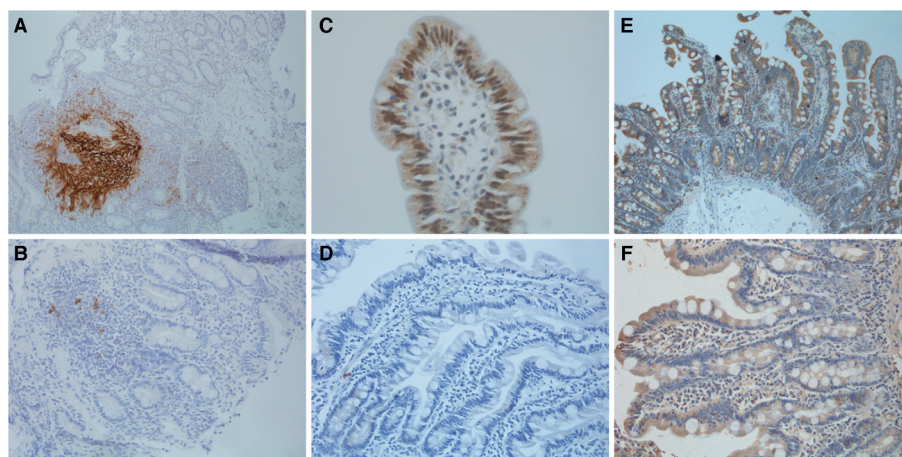


FIGURE 3. CD23 (A, original magnification $\times 100$), CD30 (B, original magnification $\times 100$), FasL (C, original magnification $\times 400$ and D, original magnification $\times 200$), Fas (E, original magnification $\times 100$), and IL-15 (F, original magnification $\times 200$) expression in intestinal mucosa. CD23 (A) and CD30 (B) are both almost exclusively seen inside the lymphoid follicles. FasL (C and D) is uncommonly seen in lamina propria cells (D) and occasionally in epithelial cells (C). Such epithelial expression is seen in some individual villi predominantly in the upper third of villi. Fas (E) and IL-15 (F) are both seen in all of the epithelial cells and in about half of the lamina propria cells.

We found evidence of a trend ($P = 0.07$) in increased soluble FasL in nontreated CMSE but no evidence for changes in CD. Epithelial expression of FasL did not differ between the groups or show correlation with the serum values; however, areal density of FasL-expressing cells in the duodenal LP was elevated in CD ($P = 0.017$, Table 2) but not in CMSE ($P = 0.699$, Table 2). Membrane-bound FasL activates apoptotic cascade in Fas-expressing cells and causes target cells to undergo apoptosis (24,26,27). The function of the soluble form of FasL is still unclear. At high doses in presensitized cells, sFasL induces apoptosis, but in lower concentrations, it downregulates apoptotic activity of the membrane-bound FasL (28). Serum sFasL is increased in several types of leukaemia, colon cancer, autoimmune diseases, and viral infections. In autoimmune diseases, such as connective tissue diseases, Sjögren disease, and ulcerative colitis (29), the concentrations correlate with disease activity. There have been no studies focused on serum sFasL in CMSE or CD (30).

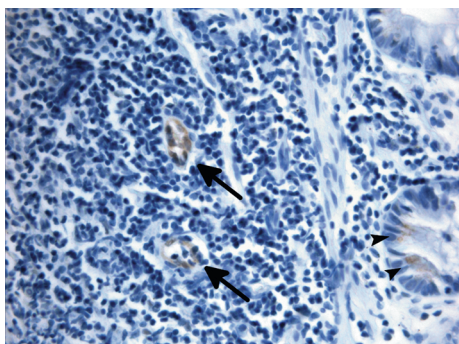


FIGURE 4. FasL (original magnification $\times 400$) expression in crypt lamina propria close to a germinal centre in ileal mucosa. FasL expression is seen in endothelial cells of venules with a high endothelium (arrows) and in Paneth cells of the crypt base (arrowheads).

Although the increase in serum FasL in CMSE did not reach statistical significance ($P = 0.07$), the observed significant correlations with the numbers of CD3⁺ IELs in the duodenal mucosa, including $\gamma\delta$ -T-cell receptor-bearing IEL counts and with the presence and severity of LNH in CMSE, favour the idea that these characteristic features of CMSE contribute to serum sFasL concentration. We have previously provided evidence that in CMSE, the apoptosis rate of IELs is increased (14), and considering the role of sFasL in apoptosis, positive correlation of the sFasL and the IEL counts may be a manifestation of an attempt to regulate the IEL population. Similarly, association with LNH and high sFasL levels could be related to the presence of ample mucosal lymphatic tissue with germinal centres, where Fas/FasL-mediated apoptotic cell death is common (24).

We found FasL expression in the endothelial cells of the HEVs, which are present in the vicinity of mucosal lymphoid follicles (Fig. 4). Such expression has not been described previously in the intestinal mucosa, and the physiological role is not known; however, this expression pattern is similar to that reported by us in lymph node HEVs (24,31). We have suggested that FasL expression in the HEVs of the lymph nodes has a role in immunoregulation by inducing apoptosis of activated, and therefore Fas-expressing, lymphocytes entering lymph node parenchyma through the HEVs (24,31). Whether FasL expression has any immunoregulatory role in the mucosal lymphoid tissue is presently not known. Besides providing 1 additional potential explanation for the association of sFasL increase and LNH in CMSE in the present study, this finding may have implications in the pathogenesis of inflammatory conditions of GI mucosa, in which abnormalities of Fas/FasL-mediated apoptosis may be involved (32–34).

IL-15 is an activator for IELs and has a significant role in the pathogenesis of CD by inducing accumulation of $\gamma\delta$ lymphocytes and the cytotoxic activation of IELs, finally leading to enterocyte apoptosis and villous atrophy (35–37). Here, we show that serum levels of IL-15 do not differ from controls in CMSE or CD, but the areal density of IL-15-expressing cells in the duodenal mucosa is increased in CD ($P = 0.006$, Table 2) but not in CMSE ($P = 0.771$,

TABLE 1. Proportions (%) of cells in LP expressing FasL or IL-15

	Controls	CMSE	Treated CMSE	Coeliac disease
FasL				
Villous LP (%)	0.0 (2.5)	0.0 (12.5)	0.0 (0.0)	0.0 (9.1)
Cryptal LP (%)	1.6 (7.7)	3.5 (10.5)	4.8 (7.4)	4.4 (11.5)
IL-15				
Villous LP (%)	49.5 (32.6)	48.8 (52.7)	45.8 (33.1)	53.9 (46.4)
Cryptal LP (%)	43.3 (40.4)	47.8 (38.6)	51.1 (30.2)	45.3 (62.7)

Median and range are indicated. Counts were separately performed in villous and deep (cryptal) LP. There were no significant differences between the groups. CMSE = cow's-milk-sensitive enteropathy; IL = interleukin; LP = lamina propria.

TABLE 2. Areal density of LP cells expressing FasL or IL-15 in duodenal mucosa

	Controls	CMSE	Treated CMSE	Coeliac disease
FasL	1.9 (8.0)	1.6 (1.1)	2.4 (4.0)	4.1 (24.0)*
IL-15	0.49 (0.84)	0.49 (0.62)	0.48 (0.32)	1.18 (1.63)*

Areal density of cells was determined as described in Methods. Median and range are indicated. Asterisk shows significant difference compared with controls. CMSE = cow's-milk-sensitive enteropathy; IL = interleukin; LP = lamina propria.

Table 2). This finding suggests that IL-15 does not have a similarly important role in the accumulation of IELs in CMSE as in CD (35–37). No studies about serum levels of IL-15 in CD have been published. Because the serum concentration of IL-15 is increased in several autoimmune diseases such as type 1 diabetes mellitus (38), multiple sclerosis (39), and rheumatoid arthritis (40), the lack of increase in serum levels in CD is rather unexpected. Reasons for the lack of increase of IL-15 in CD remain speculative; however, because degradation of IL-15 has been observed within 1 year of storage (41), the short half-life of this protein could be one explanation for the observation.

In conclusion, our results indicate that although serum sCD23 concentration is increased in some cases of CMSE and CD, there is no overall significant difference in the sCD23 concentration between the groups. Serum concentrations of IL-15 were similar in all of the groups, but the number of IL-15-expressing cells in duodenal LP was elevated in CD. Elevated levels of sFasL in CMSE seem to be associated with the presence of LNH and the increase in IELs. LNH visibility during colonoscopy is a major sign of CMSE. Specific expression of FasL in the endothelium of mucosal high endothelial vessels has not been described and is suggested to be a mechanism in mucosal immunoregulation.

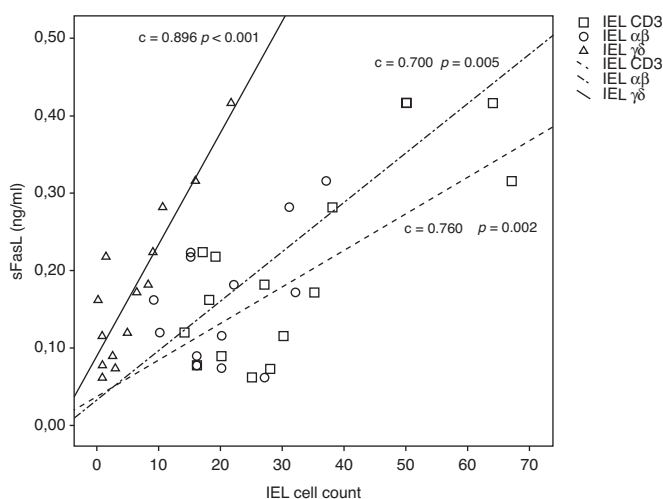


FIGURE 5. Correlation of serum sFasL concentration and the numbers of CD3, α/β - and γ/δ -expressing intraepithelial lymphocytes (IELs) in children with cow's-milk-sensitive enteropathy. Significance of correlation was assessed with a nonparametric Spearman test.

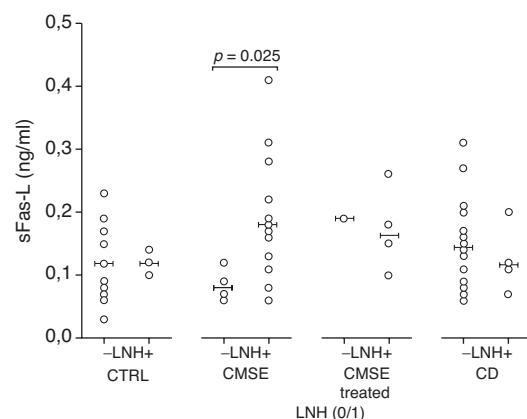


FIGURE 6. Association of soluble FasL concentration and the presence of the observed endoscopical lymphonodular hyperplasia (LNH) in the small or large intestine in controls (CTRL), untreated cow's-milk-sensitive enteropathy (CMSE), treated CMSE, and coeliac disease (CD). Horizontal line indicates median. Significant difference in the CMSE group is shown (Mann-Whitney).

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