Increase of duodenal and ileal mucosal cytotoxic lymphocytes in juvenile idiopathic arthritis

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ABSTRACT

Objective

Intestinal gamma/delta- intraepithelial lymphocytes (IEL) have increased in children with juvenile idiopathic arthritis (JIA). To further characterise intestinal immune activation in these children, we have quantitated cytotoxic lymphocytes in intestinal mucosa.

Methods

We studied 23 children with JIA suffering from gastrointestinal symptoms with gastroduodenoscopy and colonoscopy. The control children (n=20) had GI-symptoms but eventually shown not to have any significant gastrointestinal disease. Granzyme A (GrA) and Granzyme B (GrB) expressing lymphocytes in the epithelium and lamina propria were counted in immunostained sections of ileal and duodenal biopsies.

Results

The number of GrB expressing IELs was increased in duodenal mucosa in patients with JIA. In the ileum the number of both GrB and GrA positive IELs was similarly increased. No significant differences in the counts of the lamina propria GrA or GrB expressing cells were observed. Granzyme expression was not associated with the duration or with the severity of the disease, or with medication.

Conclusions

These observations suggest that lymphocyte cytotoxicity is abnormally increased in the intestinal mucosa in JIA. Since a similar pattern of activation has been seen in food allergy and celiac disease, we speculate that some luminal, possibly a nutritional factor may be involved in JIA as well. Further studies are needed to see whether cytotoxic activation plays any role in the pathogenesis of JIA.

Key words

Granzyme, gamma/delta lymphocyte, lymphoid hyperplasia, inflammatory bowel disease, allergy, juvenile idiopathic arthritis.

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Introduction

There is increasing evidence that gastrointestinal mucosal immune system may be involved in the pathogenesis of rheumatic diseases (1). In children with juvenile idiopathic arthritis (JIA) there are signs of abnormalities of gastrointestinal mucosa such as evidence of increased mucosal permeability (2) and mucosal inflammatory changes in seronegative spondyloarthropathy (3). The pathogenesis of these abnormalities is not known, however, and they do not seem to be consequences of medication (2, 3). Recently we reported that children with JIA or connective tissue diseases, suffering from gastrointestinal symptoms, show mild structural changes in the intestinal mucosa, suggesting activation of the mucosal immune system. These changes included the excessive formation of lymphoid nodules in intestinal mucosa (lymphonodular hyperplasia, LNH) and the increased number of intraepithelial lymphocytes (IELs), the gamma/delta receptor-bearing subset in particular (4).

The exact physiological functions of IELs are not known, but they are currently considered to participate in immune protection, the surveillance of the epithelium and the induction and maintenance of oral tolerance, and have roles in both innate and acquired immunity (5). The important effector function of IELs is their cytotoxicity. The subpopulations of both CD3+ and CD3- IELs have cytotoxic granules, containing granzymes and perforin, and the activation is associated with their increased expression (6). The proportion of cells expressing GrA in the normal intestine is about 30-60% of the number of CD3 expressing cells, and that for GrB 2-15% (7, 8). The numbers of GrA or GrB expressing cells are increased in conditions with intestinal immune activation including coeliac disease and the delayed type of milk allergy (8,9). Activated cytotoxic lymphocytes can destroy their target cells either by the granzyme mediated mechanisms or Fas/FasL pathways (6). In coeliac disease, cytotoxic activity is one mechanism leading to enterocyte apoptosis and villous atrophy (9). Interestingly enterocyte apoptosis may induce an increase of intestinal permeability (10). Based on our observation of the increase of intestinal IELs in children with JIA (4), we were interested in the signs of functional aberration of the IELs in these conditions. Furthermore, there are no previous observations of abnormal intestinal lymphocyte cytotoxicity in any group of rheumatic diseases. Therefore, we have quantified here the numbers of intestinal cytotoxic lymphocytes expressing granzymes A and B in children with JIA, suffering from gastrointestinal symptoms. We found, that the increased expression of GrA and B is present in intestinal mucosa.

Patients and methods

Patients and controls

In total 23 (mean age 10.1 years, range 2–16, 15 girls) out of 178 children with JIA, followed up at the Paediatric Rheumatology OPD of Oulu University Hospital, Oulu, Finland, in 1999–2004 had GI symptoms and were studied with endoscopy as described previously (4). The control group included 20 children (mean 11.2 years, range 5–14, 10 girls) examined for various gastrointestinal symptoms but assessed as having no significant GI disorders, including food intolerance or rheumatic disease in follow-up. The same subjects with representative tissue samples available, as in previously described study (4), were included. Indication for endoscopy included abdominal pain (patients/controls; 13/12), diarrhea (3/5), constipation (2/1), melena (4/2), vomiting (1/0). Of the 65 endoscopic examinations (40 for 23 patients and 25 for 20 controls), 33 were gastroduodenoscopies (19 patients, 14 controls) and 32 colonoscopies (21 patients, 11 controls). Thus for most patients both gastroduodenoscopy and ileocolonoscopy had been performed. Milk elimination challenge test was performed if there was any suspicion of milk-sensitive enteropathy in the anamnesis.

Clinical features and the medication of JIA

The patients had been mean 6.6 (range 0.7–15.8) years old at the onset of JIA. The mean duration of JIA before endoscopy was 4.3 (range 0.2–15.8) years.

The diagnosis of JIA was based on ILAR (International League of Associations for Rheumatology) classification (11). Seven children had oligoarthritis, two extended oligoarthritis, nine polyarthritis, two systemic arthritis and three enthesitis related arthritis.

The use of anti-rheumatic medication at the time of the endoscopy was the following: no medication 1/23; only NSAIDs 7/23; disease-modifying anti-rheumatic drugs (DMARDs; methotrexate, sulphasalazine, oxiclorin) 7/23; DMARD + prednisolon 7/23; DMARD +TNF-α modulator 2/23. The degree of activity of the disease was analysed by the same physician (PV) with an anchored horizontal 10 cm visual analogue scale (VAS) (12), and the clinical remission on medication was defined as six continuous months of inactive disease (13). At the time of endoscopy the activity of the rheumatic disease was varying including features like the number of active joints (range 0–12), sedimentation rate (range 2–92). There were eleven (11/23) patients in remission on medication at the time of endoscopy. Of the 23 patients 16 (70%) were HLA-B27-negative and 7 (30%) were HLA-B27-positive which is close to the antigen frequency of the Oulu Paediatric Rheumatology OPD population (27%) and twice as common as in the Finnish population (14.4%) (14).

Endoscopic samples for histological and immunohistochemical staining and the quantification of granzyme positive lymphocytes

Gastroduodenoscopies were performed with an Olympus GIF-XQ 140 and the colonoscopies with an Olympus GF-Q1401. Biopsies were taken for routine histology as described previously (4). Samples from duodenal bulb and terminal ileum were taken for granzyme A and B immunohistochemistry, fixed in 10% neutral buffered formalin, processed in paraffin, and sectioned at 4-5μm. The sections were heated by microwaves in Tris-EDTA (pH 9) with 850 W for 2 min and with 300 W for 15 min. The mouse monoclonal antibodies against GrA (CLB, Clone CLB-GA6) and GrB (CLB, Clone CLB-GB27; both from Sanquin, Amsterdam, The Netherlands) were used with dilution 1:200 and bound antibodies were detected by using EnVision™ detection system kit (Dako, Copenhagen, Denmark), as described previously (8).

The cell counting of granzyme A or B positive lymphocytes was performed with a light microscopy and a 100x objective with oil immersion by two observers blinded for all clinical information including the subject group. One investigator (MA), counted the cells in the epithelium and the other (LA) in the lamina propria. Epithelial cell counting was performed in every optimal cut-half villus, where it could be observed for its whole length. We divided the villus into thirds (tip, middle and basal) to obtain information of the granzyme expression in different parts of the villus (8). We calculated the ratio of positive cells and all epithelial cells in each villus and each third of the villus (8). The number of positive cells in the lamina propria was counted separately in the villuses and in the lamina propria of crypt zone. The approximate area of the lamina propria in villus was measured by taking the height and width of the villus, and the areal density of positive cells (cells/mm^2) in the lamina propria was calculated.

Intraobserver agreement was good with the correlation coefficient being 0.872. (Spearman). Similarly, the interobserver reproducibility of the control assessor and main assessor was good with the correlation coefficient being 0.843.

Statistics

The collected data was analysed with the SPSS 16.0 package (SPSS Inc., Chicago, Illinois, USA). A 95-percent confidence interval was used whenever appropriate to estimate the standard error, and the Fisher's exact test, Spearman's correlation test, Pearson Chi-square and Mann-Whitney U-test were used to estimate the significance of the difference between the groups, depending on the type of variable and number of cases in each subgroup. To obtain information on the granzyme expression in different parts of the villus (tip, middle and basal) Wilcoxon rank test was used.

Non-parametric tests were used based on the skewed distribution of the most continuous variables in either group.

Ethical considerations

Written parental consent for a superfluous biopsy sample, with a signature after a verbal explanation of the study plan, was obtained from the parents of all children. The protocol was approved by the Ethics Committee for Clinical Science of the Oulu University Hospital.

Results

Granzyme A or B expressing cells in epithelium and lamina propria

GrA and GrB were expressed in the cytoplasm of mononuclear cells with the morphology of lymphocytes (Fig. 1). The positive cells were located in both the epithelium and the lamina propria (Fig. 1). The counts of granzyme expressing cells in the villous epithelium are shown in Table I. The number of cases in each analysis varied according to the availability of representative sections.

For GrA, the counts of intraepithelial positive cells (Table I) and lamina propria cells (Table II) in the duodenum did not differ significantly between the patients and the controls. GrA showed a tendency toward a higher expression in the basal lamina propria in the patients than in the controls (p=0.060; Table II). In the ileum the patients showed significantly higher density of GrA expressing cells in the villus epithelium (Table I), and most evidently in the villus tip region (0.15; 0.06–0.36 *vs*. 0.05; 0.00–0,14, p= 0.023), while in the ileal lamina propria there was no difference between the groups (Table II).

GrB cell counts of the whole duodenal villus epithelium were higher in the JIA patients than in the controls, but the difference was not significant. In the duodenal villus tip region, however, the numbers of GrB expressing were significantly increased in the patients (0.027; 0.00–0.08) as compared with the controls (0.01; 0.00–0.03; p=0.026; Table I; Fig. 2). Similarly in the ileum, the patients showed higher density of GrB expressing cells than the controls in the villus epithelium (Table I), and especially in the villous tip (0.04; 0.00–0.23 *vs*. 0.00; 0.00, p<0.003) region but also in the middle (0.03; 0.00–0.18 *vs*. 0.01; 0.00–0.02, p=0.032) and lower

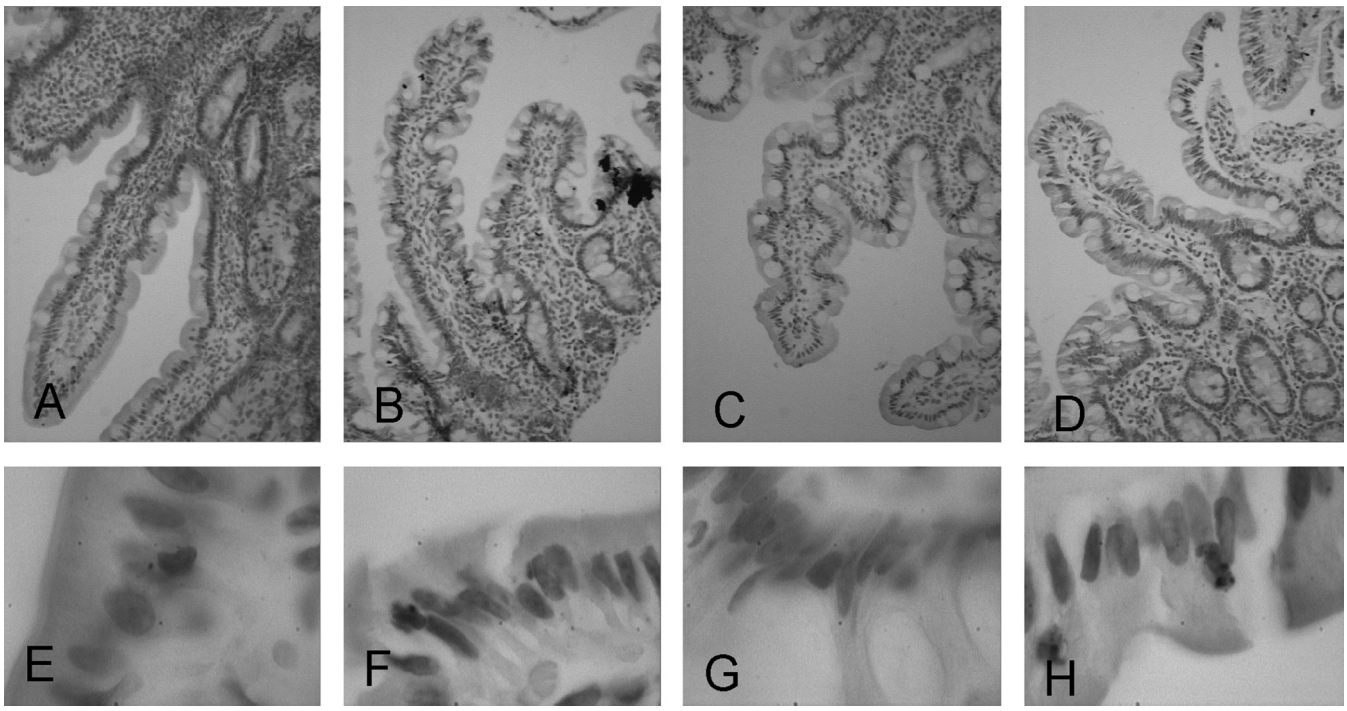

Fig. 1. Duodenal biopsies stained for granzymes A and B (GrA, GrB) in a control case (GrA: A, E; GrB: C, G) and in a subject with JIA (GrA: B, F; GrB: D, H). Granzyme expressing cells present in both the epithelium and in the lamina propria.

third (0.03; 0.00–0.06 *vs*. 0.00; 0.00–0.02, p=0.012) of the villus. In the lamina propria there was no evidence of differences in the expression of GrB in duodenum or ileum (Table II).

Based on normal duodenal villous structure, no cases of coeliac disease were found in the JIA group. There were 2 patients with JIA (8.7%) responding to the milk elimination challenge tests, and diagnosed for cow's milk allergy. The granzyme counts in these patients did not differ significantly from other patients with JIA. After omitting these patients from the JIA group, significant increases of the GrA and GrB counts were still present.

Granzyme expression in the upper, middle and lower thirds of the villi

In the control group there was no significant differences in the numbers of granzyme expressing cells between the upper, middle and lower thirds of the villi in either the duodenum (n=13) or in the ileum. In the duodenal mucosa of the patients with JIA (n=20) the number of GrA expressing cells was higher in the tip region of the villus (uppermost third) than in the middle (p=0.044) or basal (p=0.001) thirds, and also higher in the middle third than in the basal third (p=0.022, Wilcoxon ranks test). In the ileum similar difference in the GrA counts was seen in the patients with JIA (n=13) between the tip and the basal third of the villus (p=0.033).

Clinical features of JIA and mucosal granzyme expression

There was no association between the numbers of granzyme positive cells and endoscopical features such as endoscopical gastritis, duodenitis, duodenal ulceration or duodenal LNH. However, in JIA patients the finding of LNH of the terminal ileum was associated with a higher number of GrA expressing cells in villous lamina propria (250; 107–563; n=8 *vs*. 92; 91–93; n=2, p=0.044, Mann-Whitney U-test) and basal lamina propria in ileum (429; 234–593; n=8 *vs*. 158; 134–180; n=2, p=0.030) than in the patients without LNH.

There was no association between the medication and the GrA or GrB levels in intestinal mucosa (patients with NSAID *vs*. DMARD *vs*. DMARD + prednisolon *vs*. DMARD + TNF-α modulator, Mann-Whitney U-test) in JIA. The duration of the disease before gastroscopy did not show any correlation with the granzyme counts of duodenal mucosa. The duration of the disease before ileoconoloscopy did not correlate with the

Table I. Counts of granzyme A (GrA) and granzyme B (GrB) expressing cells (positive cells/1 epithelial cell) in the villus epithelium of duodenal and ileal mucosa in the JIA patients and in the controls. Median and range (in parentheses) are shown. Significant differences between the patients with JIA and the controls are indicated. Mann-Whitney U-test.

	Duodenum			Ileum		
	JIA	Controls	p	JIA	Controls	p
GrA	n=20 0.13 (0.00–0.30)	n=13 0.15 (0.00–0.36)	0.46	n=13 0.12 (0.05–0.42)	n=7 0.05 (0.00–0.11)	0.036
GrB	n=19 0.023 (0.00–0.07)	n=13 0.014 (0.00–0.03)	0.14	n=13 0.03(0.00–0.15)	n=7 0.00 (0.00–0.01)	0.002

Table II. Counts of granzyme A and granzyme B expressing cells (positive cells/mm^2) in the lamina propria of duodenal and ileal mucosa in the JIA patients and in the controls. The cells were separately counted in the villus lamina propria and in the lamina propria of basal mucosa. Median and range (in parentheses) are shown. Mann-Whitney U-test.

	Duodenum			Ileum		
	JIA	Controls	*p*	JIA	Controls	*p*
GrA	n=15	n=12		n=13	n=6	
Villus	57.7 (0.0–207.8)	49.1 (12.7–185.0)	0.25	190.5 (90.9–563.8)	120.6 (11.1–287.0)	0.30
Basal mucosa	227.6 (20.9–495.2)	125.6 (43.4–269.8)	0.06	415.2 (134.7–593.8)	224.9 (40.0–456.6)	0.14
GrB	n=14	n=13		n=13	n=10	
Villus	47.5 (18.4–105.5)	69.2 (10.7–110.6)	0.35	84.5 (25.1–240.0)	106.7 (47.7–247.3)	0.45
Basal mucosa	113.5 (54.7–346.7)	90.0 (29.3–373.3)	0.56	148.6 (41.1–682.9)	131.7 (11.3–352.0)	0.92

GrA or GrB expression in intraepithelial lymphocytes, but showed a negative correlation with the numbers of GrA expressing cells in both the lamina propria of the villus (-0.627, p=0.039, n=11) and the basal mucosa (-0.632, p=0.021, n=13). The overall granzyme counts in villus epithelium or lamina propria showed no correlation with the clinical parameters (sedimentation rate, active joints, remission, VAS). There was no significant difference between the granzyme counts of HLA-B27 positive and negative patients. After the omission of the HLA-B27 positive patients significant increases of the GrA and GrB counts were still mainly present in the patients in the villus epithelium of the duodenum and ileum.

Discussion

Our results indicate that the number of intestinal intraepithelial lymphocytes, expressing granzyme A or B, has increased in the gastrointestinal mucosa in children with JIA, suffering from GI symptoms. The difference for granzyme A was significant in the ileal mucosa and that for granzyme B in both duodenal and ileal mucosa. No previous reference values for granzyme expressing cells in the ileal mucosa are available, but the increase of the intraepithelial granzyme expressing cells in the duodenal mucosa was somewhat less than reported previously for the delayed type of milk allergy, and much less than in coeliac disease (8). Our observations favour the idea that cytotoxic lymphocytes are activated in the intestinal mucosa of children with JIA with gastrointestinal symptoms. However, more studies are needed to see whether this activation has any significance in the pathogenesis of JIA.

The mechanisms of the observed increase of the granzyme expression in the duodenal and ileal mucosa remain speculative at present. The duration of the disease or medication did not show any association with the counts of granzyme expressing cells in the epithelium. High doses of immunosuppressive medication are known to downregulate cytotoxic lymphocytes in the synovia (15) and similar effect could be plausible in the intestinal mucosa as well, favouring the idea that in the intestine, intensive treatment is not the cause of the observed increase. However, since there was only one JIA patient without any concurrent treatment, and the number of patients in different treatment groups was low, more studies are needed to conclusively exclude any connection between treatment and intestinal granzyme expression.

Intestinal mucosa is the largest immune organ of human body and constantly exposed to environmental antigens, like nutritional and microbial antigens. Here we show that children with JIA show immunological features similar to cow's milk sensitive enteropathy and celiac disease, such as increased granzyme levels in mucosa (8, 16). This suggests a possibility that in JIA the increase of cytotoxic lymphocytes might be related with luminal factors. The

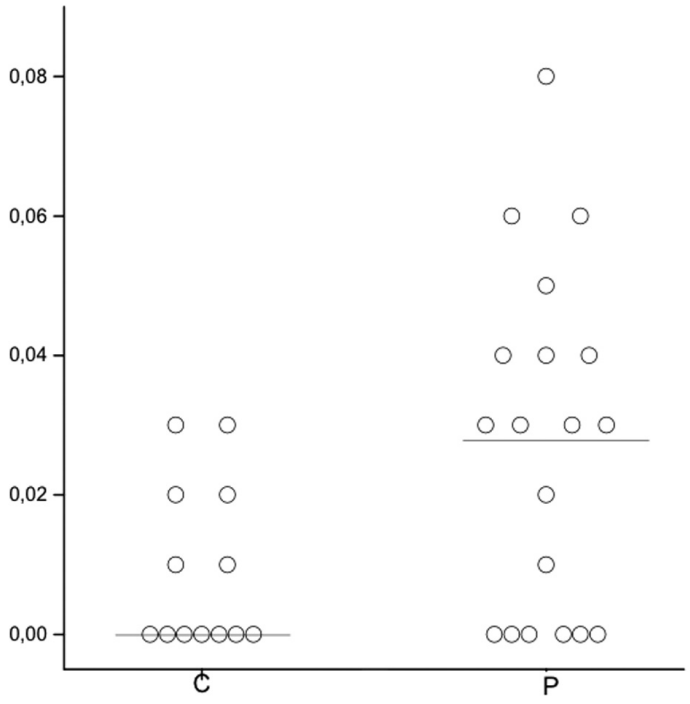

Fig. 2. Scatterplot showing counts of granzyme B expressing intraepithelial cells (cells/one epithelial cell) in the uppermost third of villi in duodenal mucosa. The counts in are significantly higher (p=0.026; Mann-Whitney) higher in the patient group (P) than in the controls (C). Horizontal line indicates median.

magnitude of the increase of granzyme expressing cells in the duodenal epithelium is close to that in cow's milk sensitive enteropathy, and clearly less than in coeliac disease (8,16). There was no association with HLA-B27 positivity, a factor which may be involved both in the modification of mucosal response to luminal factors and in the pathogenesis JIA. Interestingly, villous tip region seems to be the most sensitive indicator of the possible effect of the luminal factors as shown in the current study and in the previous studies (8, 17, 18).

Abnormal intestinal permeability, not related with treatment, has been reported in children with JIA (2) and the permeability abnormalities have been suggested to play a role in the pathogenesis of inflammatory arthropathies (19). Although the relationship between intestinal permeability and the number of cytotoxic cells is unknown at present, it is plausible that cytotoxicity might affect epithelial barrier function (20, 21). The previous studies have shown correlation between the numbers of intestinal intraepithelial lymphocytes and permeability (22). The mechanism involved might be the increased apoptosis of epithelial cells related with cytotoxic activation (10) or other mechanism altering epithelial integrity. In the future, it should be studied if granzyme mediated mechanisms in small intestinal mucosa would explain the observed permeability abnormalities in JIA (2).

In addition to the potential links with intestinal permeability, there are other possible mechanisms linking abnormal cytotoxicity in the intestinal mucosa with the pathogenesis of arthritis. These include the formation of autoantigens, the induction of proinflammatory cascades and the possible effects of gut originating granzymes. Autoantibodies, directed specifically against the granzyme B-induced, cleaved form of autoantigen, have been detected in adult Sjögren's syndrome sera (23). Proinflammatory cascades can be triggered by extracellular GrA, which can convert IL-1β to its mature form IL-1 (24). IL-1 is an important mediator of the inflammatory response, playing an important role in the development of pathological conditions leading to chronic inflammation (25, 26). Finally the granzyme activation is involved in the pathogenesis of the joint destruction in arthritis (15, 27, 28) and the serum concentrations of granzymes correlate with the prognosis of arthritis (29). It is of interest that granzyme mediated immune activation in intestinum may induce the release of granzymes or their fragments to the circulation (16). However, it is not known whether these substances in the circulation are enzymatically active.

In summary, our analysis shows evidence for abnormal lymphocyte cytotoxic activation in the duodenal and ileal mucosa in children with JIA. The clinical significance remains speculative, but the cytotoxic activation could not be explained by the treatment or the long duration of the disease. There is similar reaction in food allergy and in celiac disease, which favours the idea that some luminal, possibly nutritional factors, may be involved in the pathogenesis of intestinal cytotoxic activation in our patients. Further studies are needed to characterise the factors inducing this cytotoxic activation in the intestinal mucosa of JIA patients, and to find out the role of this activation in the pathogenesis of JIA. Finally, in case the intestinal immune activation would act as a factor triggering JIA, it would be important to find ways to down regulate this activation by medical or dietary treatment.

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References

1. HVATUM M, KANERUD L, HALLGREN R, BRANDTZAEG P: The gut-joint axis: cross reactive food antibodies in rheumatoid arthritis. *Gut* 2006; 55: 1240-7.
2. PICCO P, GATTORNO M, MARCHESE N *et al.*: Increased gut permeability in juvenile chronic arthritides. A multivariate analysis of the diagnostic parameters. *Clin Exp Rheumatol* 2000; 18: 773-8.
3. CONTI F, BORRELLI O, ANANIA C *et al.*: Chronic intestinal inflammation and seronegative spondyloarthropathy in children. *Dig Liver Dis* 2005; 37: 761-7.
4. KOKKONEN J, ARVONEN M, VAHASALO P, KARTTUNEN TJ: Intestinal immune activation in juvenile idiopathic arthritis and connective tissue disease. *Scand J Rheumatol* 2007; 36: 386.
5. CHEROUTRE H: IELs: enforcing law and order in the court of the intestinal epithelium. *Immunol Rev* 2005; 206: 114-31.
6. RUSSELL JH, LEY TJ: Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol* 2002; 20: 323-70.
7. CHOTT A, GERDES D, SPOONER A *et al.*: Intraepithelial lymphocytes in normal human intestine do not express proteins associated with cytolytic function. *Am J Pathol* 1997; 151: 435-42.
8. AUGUSTIN MT, KOKKONEN J, KARTTUNEN TJ: Duodenal cytotoxic lymphocytes in cow's milk protein sensitive enteropathy and coeliac disease. *Scand J Gastroenterol* 2005; 40: 1398-406.
9. CICCOCIOPPO R, DI SABATINO A, PARRONI R *et al.*: Cytolytic mechanisms of intraepithelial lymphocytes in coeliac disease (CoD). *Clin Exp Immunol* 2000; 120: 235-40.
10. ABREU MT, PALLADINO AA, ARNOLD ET, KWON RS, MCROBERTS JA: Modulation of barrier function during Fas-mediated apoptosis in human intestinal epithelial cells. *Gastroenterology* 2000; 119: 1524-36.
11. PETTY RE, SOUTHWOOD TR, MANNERS P *et al.*: International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004; 31: 390-2.
12. FALCONE A, CASSONE R, ROSSI E, PISTORIO A, MARTINI A, RAVELLI A: Inter-observer agreement of the physician's global assessment of disease activity in children with juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2005; 23: 113-6.
13. WALLACE CA, RUPERTO N, GIANNINI E, CHILDHOOD ARTHRITIS AND RHEUMATOLOGY RESEARCH ALLIANCE, Pediatric Rheumatology International Trials Organization, Pediatric Rheumatology Collaborative Study Group. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. *J Rheumatol* 2004; 31: 2290-4.
14. SIREN MK, SARENEVA H, LOKKI ML, KOSKIMIES S: Unique HLA antigen frequencies in the Finnish population. *Tissue Antigens* 1996; 48: 703-7.
15. GRIFFITHS GM, ALPERT S, LAMBERT E, MCGUIRE J, WEISSMAN IL: Perforin and granzyme A expression identifying cytolytic lymphocytes in rheumatoid arthritis. *Proc Natl Acad Sci USA* 1992; 89: 549-53.
16. AUGUSTIN MT, KOKKONEN J, KARTTUNEN R, KARTTUNEN TJ: Serum granzymes and CD30 are increased in children's milk protein sensitive enteropathy and celiac disease. *J Allergy Clin Immunol* 2005; 115: 157-62.
17. JARVINEN TT, COLLIN P, RASMUSSEN M *et al.*: Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. *Scand J Gastroenterol* 2004; 39: 428-33.
18. BIAGI F, LUINETTI O, CAMPANELLA J *et al.*:

Intraepithelial lymphocytes in the villous tip: do they indicate potential coeliac disease? *J Clin Pathol* 2004; 57: 835-9.

19. HOLDEN W, ORCHARD T, WORDSWORTH P: Enteropathic arthritis. *Rheum Dis Clin North Am* 2003; 29: 513-30, viii.
20. SHIBAHARA T, MIYAZAKI K, SATO D *et al*.: Alteration of intestinal epithelial function by intraepithelial lymphocyte homing. *J Gastroenterol* 2005; 40: 878-86.
21. DALTON JE, CRUICKSHANK SM, EGAN CE *et al*.: Intraepithelial gammadelta+ lymphocytes maintain the integrity of intestinal epithelial tight junctions in response to infection. *Gastroenterology* 2006; 131: 818-29.
22. VOGELSANG H, OBERHUBER G, WYATT J: Lymphocytic gastritis and gastric permeability in patients with celiac disease. *Gastroenterology* 1996; 111: 73-7.
23. HUANG M, IDA H, KAMACHI M *et al*.: Detection of apoptosis-specific autoantibodies directed against granzyme B-induced cleavage fragments of the SS-B (La) autoantigen in sera from patients with primary Sjögren's syndrome. *Clin Exp Immunol* 2005; 142: 148-54.
24. IRMLER M, HERTIG S, MACDONALD HR *et al*.: Granzyme A is an interleukin 1 beta-converting enzyme. *J Exp Med* 1995; 181: 1917-22.
25. PASCUAL V, ALLANTAZ F, ARCE E, PUNARO M, BANCHEREAU J: Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J Exp Med* 2005; 201: 1479-86.
26. MADSON KL, MOORE TL, LAWRENCE JM 3RD, OSBORN TG: Cytokine levels in serum and synovial fluid of patients with juvenile rheumatoid arthritis. *J Rheumatol* 1994; 21: 2359-63.
27. KRAAN MC, HARINGMAN JJ, WEEDON H *et al*.: T cells, fibroblast-like synoviocytes, and granzyme B+ cytotoxic cells are associated with joint damage in patients with recent onset rheumatoid arthritis. *Ann Rheum Dis* 2004; 63: 483-8.
28. BUZZA MS, ZAMURS L, SUN J *et al*.: Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. *J Biol Chem* 2005; 280: 23549-58.
29. GOLDBACH-MANSKY R, SUSON S, WESLEY R, HACK CE, EL-GABALAWY HS, TAK PP: Raised granzyme B levels are associated with erosions in patients with early rheumatoid factor positive rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 15-721.