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Intestinal γ/δ Receptor-Bearing T Lymphocytes in Celiac Disease and Inflammatory Bowel Diseases in Children. Constant Increase in Celiac Disease

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ABSTRACT. We studied the numbers of T-cell receptor α/β- and γ/δ-bearing lymphocytes in 27 jejunal specimens from 19 celiac patients, 27 rectal and colonic specimens from 14 ulcerative colitis patients and four patients with Crohn's disease, and 24 control specimens. MAb and a three-layer peroxidase staining method were used. Only low numbers of γ/δ+ cells were seen in normal jejunum and rectum of controls, as well as in the specimens of patients with inflammatory bowel diseases. In the lamina propria of celiac patients, the mean number of γ/δ+ cells was significantly higher than in the controls before treatment, during gluten-free diet, and after the gluten challenge. Within the jejunal epithelium, the number of γ/δ+ cells was elevated before and during gluten elimination and after the challenge test. The absolute number of intraepithelial γ/δ+ cells remained constant during gluten elimination and provocation. We infer that the constantly elevated population of γ/δ+ T cells in the epithelium of celiac patients may play an important role in the pathogenesis of celiac disease. (*Pediatr Res* 28: 579–581, 1990)

Abbreviations

IEL, intraepithelial lymphocytes
CD, cluster of differentiation
TCR, T-cell receptor
GFD, gluten-free diet

The intestinal IEL are a cell population whose significance in the immune defenses is presently unknown (1). The majority of IEL are CD3+ T cells, more than 90% of which express the surface CD8 antigen, with very few showing the CD4 antigen in normal human jejunal mucosa (2, 3). In patients with celiac disease, a considerable proportion of jejunal CD3+ IEL express neither the CD8 nor the CD4 antigen (2–4), suggesting that they might bear TCR γ/δ. In the normal human intestine, less than 10% of lymphocytes bear γ/δ TCR (5–8), as is the case in peripheral blood (9). In the intestine of patients with active celiac disease, the population of TCR γ/δ-bearing lymphocytes seems to be distinctly increased, particularly in the epithelium (6, 10).

We measured the total number of TCR-γ/δ+ cells and α/β+ lymphocytes in the normal jejunum and in the jejunum of patients with celiac disease. Particularly in the epithelium, but also in the lamina propria of celiacs, there was a constantly increased number of γ/δ+ cells regardless of dietary treatment and jejunal morphology. This finding is the only permanent abnormality described thus far in the jejunal mucosa of patients with celiac disease.

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MATERIALS AND METHODS

Patients. Twenty-five jejunal specimens from 19 pediatric patients with celiac disease were used in this study; nine were taken before treatment at a mean age of 5.3 y, eight were taken during GFD (12.1 y), and eight after a gluten challenge resulting in villous atrophy (12.2 y). From six patients, the specimens both during gluten elimination and after the gluten challenge were available. As controls, we studied 13 histologically normal jejunal specimens taken from children at a mean age of 8.2 y as a part of the evaluation of their asymptomatic growth failure. As a disease control, a jejunal biopsy specimen showing total villous atrophy from a 16-mo-old patient with multiple food allergies was studied. This patient had never had gluten-containing foods. A detailed study of lymphocytes bearing surface antigens CD3, CD4, and CD8 in the lamina propria and epithelium of the jejunum of these patients has been reported earlier (3).

Rectal biopsy specimens of 14 children (mean age 11.2 y) with ulcerative colitis were taken before any treatment and part of them were used for our study. From seven of the patients, a specimen from the sigmoid colon taken during colonoscopy was also available. Four rectal and two colonic specimens from four patients with Crohn's disease were also studied. All specimens showed inflammatory changes. As controls, we used parts of specimens taken from 10 neurologic patients (11).

Our study protocol was reviewed and approved by the Ethical Committee of Children's Hospital, University of Helsinki.

Tissue processing. The biopsy specimens were freshly embedded in OCT compound and stored at −70°C. Five-μm cryostat serial sections were fixed in acetone for 10 min, then in chloroform for 30 min, and washed three times in Tris buffer pH 7.4.

Immunohistochemical staining. After removing the buffer, sections were covered with the diluted MAb in Tris buffer for 16 h. Endogenous peroxidase was blocked by incubation in 0.5% peroxidase for 30 min. To stain MAb, a Vectastain Elite ABC kit (PK-6102, Vectro Laboratories, Burlinghame, CA) was used according to the instructions of the manufacturer.

Positively stained cells were counted with a light microscope using 900× magnification (3, 11).

MAb. MAb TCRdelta1 (T cell Sciences Inc., Cambridge, MA), recognizing constant region of δ-chain of TCR (12, 13) and all γ/δ+ cells (5, 14), and antibody betaF1, reacting with virtually all α/β TCR molecules (14), were used in dilution 1:100. MAb Leu4 (anti-CD3, Beckton-Dickinson, Mountain View, CA) was used in dilution 1:400.

Statistical analysis. The numbers of cells in the study specimens were compared with those of the controls using t test. A paired *t* test was used to compare cell numbers of celiac patients before and after the gluten challenge.

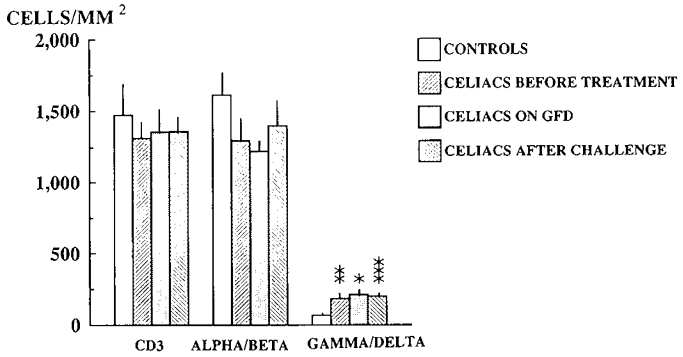

Fig. 1. Mean numbers of lamina propria lymphocytes/mm^2 expressing surface antigens CD3, α/β, and γ/δ for controls, patients with celiac disease before treatment, during a GFD, and after a gluten challenge. One SEM *vertical line* and the significance of the difference to the control are shown (*$p < 0.05$, $p < 0.01$, $p < 0.001$).

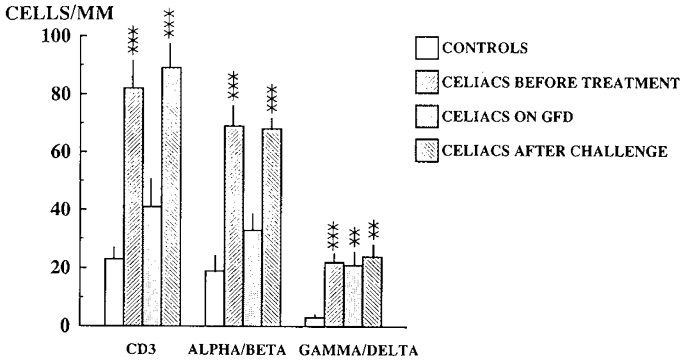

Fig. 2. Mean numbers of lymphocytes/mm of the epithelial expressing surface antigens CD3, α/β, and γ/δ. *Symbols* as in Figure 1.

RESULTS

TCR γ/δ-bearing lymphocytes in the lamina propria of jejunum. In the morphologically normal intestine of the controls, the great majority of T cells expressed α/β TCR; γ/δ+ cells were scarce and their mean proportion of the CD3+ cells was 5.5% (Fig. 1). The density and proportion of γ/δ+ cells was significantly higher in the lamina propria of the jejunal specimens taken from celiac patients before treatment (15% of CD3+ cells), during GFD (13%), and after the gluten challenge (14%) (Fig. 1) than in the controls; their density was not affected by gluten challenge or gluten elimination.

TCR γ/δ-bearing lymphocytes in jejunal epithelium. The mean number of γ/δ+ cells was 3.5 cells/mm in the surface epithelium of the control children with a normal jejunum (Fig. 2). In the specimen of the patient with total villous atrophy and food allergy, the number of γ/δ+ cells was 1.6/mm of surface epithelium.

In celiacs, the numbers of γ/δ+ cells were increased in the surface epithelium before treatment, during the GFD, and after the gluten challenge as compared with controls (Figs. 2 and 3). In the surface epithelium, γ/δ+ cells composed 32% of CD3+ cells before the treatment, 62% during the GFD, and 29% after the gluten challenge. In the jejunal specimens of all patients, the mean number of γ/δ+ cells was unchanged at different phases of the treatment of celiac disease in the epithelium (Fig. 2). This could be further corroborated in the six celiac patients followed through gluten challenge test (Fig. 4).

TCR γ/δ-bearing lymphocytes in rectum and colon. The numbers of γ/δ+ cells in the rectal and colonic lamina propria of patients with inflammatory bowel disease did not differ from those in the controls (Table 1). Within the epithelium, no γ/δ+ cells were seen in the majority of specimens, regardless of the diagnostic group (Table 1).

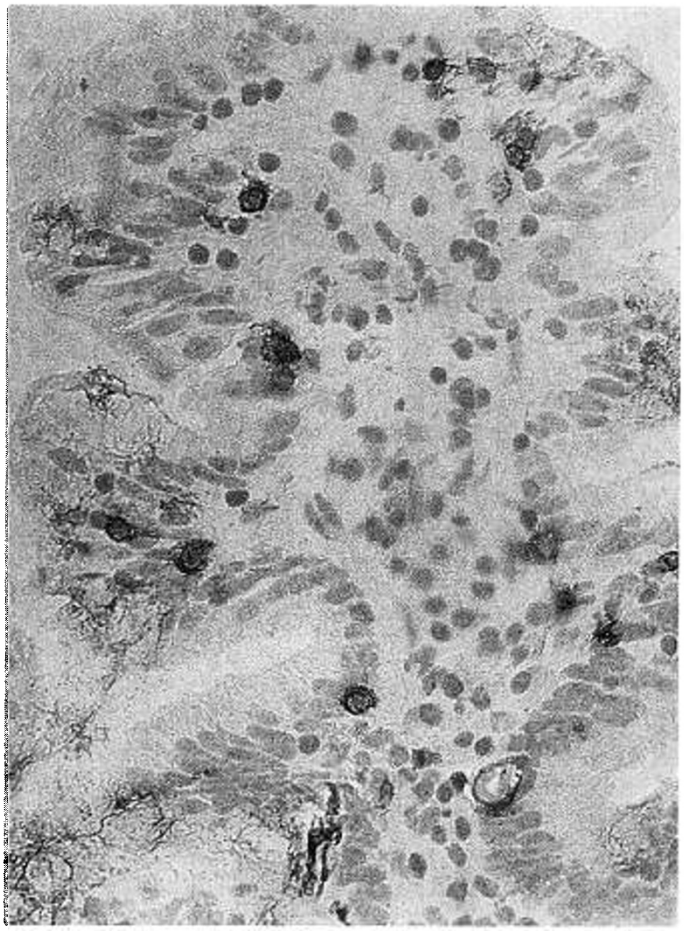

Fig. 3. Jejunal biopsy specimen of a patient with celiac disease taken during GFD and stained with antibody TCRdelta1. In a slender villus, there are several γ/δ+ cells intraepithelially and a few in the lamina propria; magnification ×320.

DISCUSSION

Our findings confirm the earlier observations of low numbers of TCR γ/δ+ cells both intraepithelially and in the lamina propria of normal human intestine (5–7), and increased numbers in active celiac disease (6, 10). Our main findings are the constantly elevated numbers of both intraepithelial and lamina propria γ/δ TCR+ cells in celiacs, regardless of dietary treatment and jejunal morphology. This increase was not seen in five patients with tropical sprue (6), nor in a case with total villous atrophy and food allergy in our study.

In striking contrast to the constant increase of γ/δ+ cells in the jejunal mucosa of celiac patients, the inflamed specimens from patients with inflammatory bowel diseases did not differ from the controls in this respect. This suggests that the observed increase in γ/δ+ cells is not linked with a tendency to inflammation in general, but may be specific of celiac disease.

The primary event in celiac disease is most likely the increased rate of destruction of epithelial cells, when the patient is eating foods containing gluten. IEL may be effectors in this process. TCR γ/δ-bearing cells can exert non-major histocompatibility complex-restricted cytolytic activity against tumor cells (15–16), and also toward IgG Fc receptor-bearing target cells (17) when first activated with MAb to CD3. It may be speculated that if γ/δ+ cells are stimulated by fractions of gluten or intraluminal microbial antigens, they may become cytotoxic to the epithelial cells possibly altered by adhering gluten fractions or fixed Ig. On the other hand, the increase of γ/δ+ cells may be associated with the human leukocyte antigen D region linked genetic immune abnormality shared by celiac disease and dermatitis herpetiformis

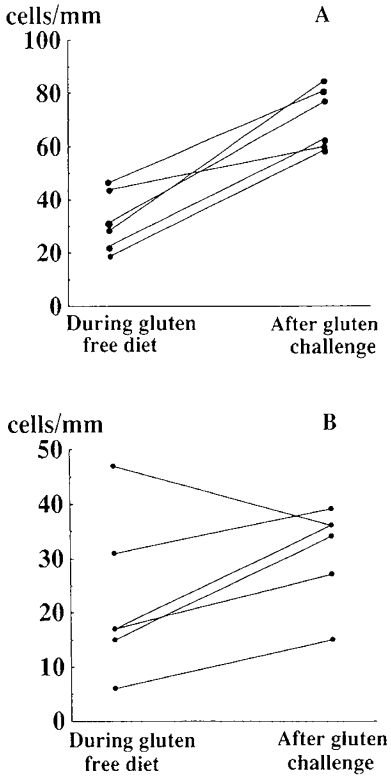

Fig. 4. *A*, Numbers of α/β+ cells (•, cells/mm of epithelium) in the surface epithelium of jejunal specimens from celiac patients during a GFD and after a gluten challenge. *B*, Numbers of γ/δ+ cells (•, cells/mm of epithelium) in the surface epithelium of jejunal specimens from the same patients as in *A*.

Table 1. *Number of rectal and colonic biopsy specimens with γ/δ+ cells of patients with ulcerative colitis and Crohn's disease and controls**

Group	Number of specimens	Lamina propria (cells/mm²)	Surface epithelium
Ulcerative colitis			
Rectum	14	11 (33, 7.8)	6
Colon	7	6 (31, 6.6)	4
Crohn's disease			
Rectum and colon	6	6 (35, 4.9)	4
Controls			
Rectum	10	7 (34, 11)	2

* In the positive specimens, the cell density for lamina propria was >10/mm²; for surface epithelium, >0.5/mm. For lamina propria, the mean and SEM for the cell density are given in parentheses.

(18), in which case the γ/δ+ cells might play no role in the pathogenesis of villous atrophy of celiac disease. This is advocated by the absence of γ/δ+ cells in other forms of diseases with villous atrophy, as shown in patients with tropical sprue (6) and in the food allergy case of this study, and by their abundance in morphologically normal jejunal specimens of patients with dermatitis herpetiformis on a normal gluten-containing diet (19). The measurement of γ/δ+ cells may also prove a valuable diagnostic tool in differentiation of celiac disease from other forms of villous atrophy; however, more experience is needed. The permanence of the increase may allow the identification of a celiac patient also when the biopsy is taken on a GFD and other morphologic signs are normalized.

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REFERENCES

1. Dobbins WO 1986 Human intestinal intraepithelial lymphocytes. Gut 27:972–985
2. Jenkins D, Goddall A, Scott BB 1986 T lymphocyte populations in normal and coeliac mucosa defined by monoclonal antibodies. Gut 27:1330–1337
3. Verkasalo M, Arato A, Savilahti E, Tainio V-M 1990 Effect of diet and age on jejunal and circulating lymphocyte subsets in children with coeliac disease. Gut 31:422–425
4. Spencer JG, MacDonald TT, Diss TC, Walker-Smith JA, Ciclitira PJ, Isaacson PG 1989 Changes in intraepithelial lymphocyte subpopulations in coeliac disease and enteropathy associated T cell lymphoma (malignant histiocytosis of the intestine). Gut 30:339–346
5. Groh V, Porcell S, Fabbi M, Lanier LL, Picker LJ, Anderson T, Warnke RA, Bhan AK, Strominger JL, Brenner MB 1989 Human lymphocytes bearing T cell receptor gamma/delta are phenotypically diverse and evenly distributed throughout the lymphoid system. J Exp Med 169:1277–1294
6. Spencer J, Isaacson PG, Diss TC, MacDonald TT 1989 Expression of disulfide-linked and non-disulfide-linked forms of the T cell receptor gamma/delta heterodimer in human intestinal intraepithelial lymphocytes. Eur J Immunol 19:1335–1358
7. Brandtzaeg P, Bosnes V, Halstensen TS, Scott H, Sollid LM, Valnes KN 1989 T lymphocytes in human gut epithelium preferentially express the alpha/beta antigen receptor and are often CD45/UCHL1-positive. Scand J Immunol 30:123–128
8. Bucy RP, Chen C-LH, Cooper MD 1989 Tissue localization and CD8 accessory molecule expression of Tgammadelta cells in humans. J Immunol 141:3045–3049
9. Brenner MB, Strominger JL, Krangel MS 1988 The gamma/delta receptor. Adv Immunol 43:133–192
10. Halstensen TS, Scott H, Brandtzaeg T 1989 Intraepithelial T cells of Tc-Rgamma/delta+CD8– and Vdelta1/Jdelta1+ phenotypes are increased in coeliac disease. Scand J Immunol 30:665–672
11. Arato A, Savilahti E, Tainio V, Klemola T 1989 Immunohistochemical study of lymphoplasmacytic infiltrate and epithelial HLA-DR expression in the rectal and colonic mucosae of children with ulcerative colitis. J Pediatr Gastroenterol Nutr 8:172–180
12. Band H, Hochstenbach F, McLean J, Hata S, Krangel MS, Brenner MB 1987 Immunochemical proof that a novel rearranging gene encodes the T cell receptor delta subunit. Science 238:682–684
13. Tribel F, Hercend T 1989 Subpopulations of human peripheral T gamma delta lymphocytes. Immunol Today 10:186–188
14. Faure F, Jitsukawa S, Triebel F, Hercend T 1988 Characterization of human peripheral lymphocytes expressing the CD3-gamma/delta complex with anti-receptor monoclonal antibodies. J Immunol 141:3357–3360
15. Borst J, van de Griend RJ, van Oostveen JW, Ang S-L, Melief J, Seidman JG, Bolhuis RLH 1987 A T-cell receptor gamma/CD3 complex found on cloned functional lymphocytes. Nature 325:683–688
16. Ciccione E, Ferrini S, Bottino C, Viale O, Prigione I, Pataleo G, Tambussi G, Moretta A, Moretta L 1988 Monoclonal antibody specific for a common determinant of the human T cell receptor gamma/delta directly activates CD3+ WT31-lymphocytes to express their functional program(s). J Exp Med 168:1–11
17. van de Griend RJ, Tax WJM, van Krimpen BA, Vreudgenhil J, Ronteltap CPM, Bolhius RLH 1987 Lysis of tumor cells by CD3+8⁻16+ T cell receptor alphabeta-clones, regulated via CD3 and CD16 activation sites, recombinant interleukin 2, and interferon beta. J Immunol 138:1627–1633
18. Hall RP 1987 The pathogenesis of dermatitis herpetiformis: recent advances. J Am Acad Dermatol 16:1129–1144
19. Savilahti E, Reunala T, Arato A, Verkasalo M 1990 Increase of gamma/delta T-cell receptor bearing lymphocytes in the jejunum of patients with dermatitis herpetiformis. In: Mulder C, Mearin L (eds) The Proceedings of the Dicke Symposium, 22 May 1990, Leiden, The Netherlands