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Lymphocyte subpopulations in the peripheral blood of children with coeliac disease

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Circulating lymphocytes were enumerated in 28 children with coeliac disease and in 13 healthy controls by immunofluorescent staining using monoclonal antibodies for T surface phenotypic markers and polyvalent antisera for surface immunoglobulins (B cells). Proportion of peripheral T and B cells and the ratio of helper and suppressor T cells were not significantly different in coeliac children from those in controls. Authors conclude on the basis of the results that a significant impairment of circulating pool of lymphocytes in coeliac disease is improbable.

In patients with coeliac disease ingestion of gluten causes lymphocytic infiltration of the jejunal mucosa and intestinal epithelial damage with crypt hyperplastic villous atrophy [11]. The mechanism whereby gluten causes mucosal damage in coeliac disease is unknown, although there is abundant evidence of altered humoral and cell mediated immunity. Demonstration of cellular sensitisation to the different gluten fractions with the mononuclear cells of peripheral blood [2, 8] and of small intestinal mucosa [6] of coeliac patients suggest that cell mediated immunity is fundamental in the pathogenesis of this disorder. The presence of serum antibodies to gluten [3, 10] and the demonstration of antigliadin antibody production by cultured coeliac mucosa exposed to gluten demonstrate that there is also a humoral immune response [5], which may have a role in the pathogenesis of coeliac disease.

Many recent research efforts have been directed towards elucidation of the abnormality of lymphocyte regulation in coeliac disease. The introduction of monoclonal antibodies directed against phenotypic subclass markers on lymphocytes has given a great impetus to this research [12].

In patients with active coeliac disease the number of intraepithelial lymphocytes is increased [11] and the majority of these cells are suppressor T cells [9, 13, 17]. In the lamina propria the lymphocyte numbers and the proportion of the subsets have been reported to be comparable to those in healthy controls [7] or showing only a slight predominance of suppressor T cells [18].

In the peripheral blood of adult coeliacs the proportions of the different

lymphocyte subpopulations showed slight variation with dietary changes. In those patients the absolute number of T helper/inducer cells have been reported to be lower than in control subjects, thus bringing the helper/suppressor ratio to a relatively low value [16, 17, 19].

As no information is known about blood lymphocyte subpopulations in children with coeliac disease we have studied the T and B cell subpopulations in the peripheral blood of coeliac children before any treatment, during gluten free diet and after gluten challenge.

Materials and Methods

Patients

28 children with suspected or confirmed coeliac disease (13 girls, 15 boys, mean age at the biopsy before treatment: 7.3 years, range: 0.76—13 years have been studied). In all patients the jejunal biopsy specimens taken before dietary treatment showed severe villous atrophy. In 19 patients the diagnosis of coeliac disease was established by the criteria of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) [15]. The jejunal mucosa of all these patients became normal during a gluten free diet and relapsed after gluten challenge. In four children a significant improvement in jejunal morphology appeared on a gluten-free diet. The remaining five patients had only recently been put on a gluten free diet; the history with characteristic physical and laboratory findings, followed by clinical improvement on a gluten free diet, strongly suggest that these patients are also suffering from coeliac disease. The duration of the gluten free diet was on average 4.3 years (range: 1.0—11.7 years). The duration of gluten challenge (normal gluten-containing diet) was on average 4.6 months (range: 1.1—7.5 months).

Immunofluorescence determination of the subpopulation of lymphocytes was performed in 10 patients before dietary treatment. Blood sample was drawn for lymphocyte subclass determination from 17 coeliac children on a gluten free diet before the planned challenge, and from 16 after the gluten challenge. In 9 coeliacs immunohistological analysis of the circulatory lymphocytes were done both before and after gluten challenge.

Healthy controls

In 13 control patients a blood sample for lymphocyte subclass determination was drawn for this study during routine blood testing. Jejunal biopsy was taken from all of these patients because of poor weight gain or growth retardation. None of the controls had gastrointestinal symptoms; careful clinical and laboratory examination could not reveal any gastrointestinal disorders. In every case the histology of the proximal jejunum was normal.

Isolation of lymphocytes

1 ml of venous blood was drawn into an EDTA tube. The absolute number of lymphocytes was calculated from the total white blood cell count, measured by a cell counter, and from the differential cell count on May—Grünwald—Giemsa smears. Blood sample was centrifuged at 700 *g* for 5 min at room temperature. Lymphocytes were isolated from the buffy coat. The buffy coat was added to 4 ml phosphate-buffered-saline (PBS, pH 7.2, maintained at 0—4 °C). The cell suspension was underlain with 1.25 ml Ficoll-Isopaque density solution and then centrifuged for 30 min at 300 *g* at room temperature. The mononuclear cells were collected and washed

twice with PBS after centrifugation at 300 *g*. The cells were counted and the viability of the cells was demonstrated with trypan blue.

Antisera

Monoclonal antisera produced by mouse hybridomas to all mature T cells (OKT 3), helper/inducer T cells (OKT 4), suppressor/cytotoxic T cells (OKT 8) were obtained from Ortho Pharmaceutical Corporation. Antimouse IgG labelled with fluorescein isothiocyanate (FITC) was a product of Miles Yeda Ltd. B cells were identified by direct immunofluorescence using a conventional FITC-conjugated polyvalent antiserum to human immunoglobulins.

Immunofluorescence method

200 μl aliquots containing 5×10^5 cells in 5% newborn calf serum were incubated with 5μl of reconstituted monoclonal antiserum or FITC-conjugated antihuman immunoglobulin serum for 30 min at 0 °C. Cells were washed twice at room temperature. In order to stain the fixed monoclonal antisera, FITC-conjugated antimouse IgG was added to the respective tubes and the washings were repeated.

Cytocentrifuge slides were prepared and cells were counted with a Leitz fluorescence microscope with epi-illumination and phase contrast. Fluorescent cells were counted among 200 lymphocytes. The proportion of monocytes among the separated cells was counted with the aid of May—Grünwald—Giemsa staining.

Statistical analysis

Analysis of variance was used for comparisons between the patients at different stages of dietary treatment and the controls. The numbers from the 9 patients, at whom immunofluorescent analysis were done both before and after gluten challenge, were compared by the paired Student *t* test.

Results

Table I summarizes the results of our study. The leucocyte count and the absolute number of lymphocytes in coeliac children were normal. Total counts of circulating B and T cells were not significantly different in patients and controls. The absolute number of lymphocytes and lymphocyte subsets showed a little variation with regard to the dietary changes. Their number tended to be lower in patients during gluten free diet and after gluten challenge compared to the values of patients before treatment and controls, although the difference was not significant. The percentage of all lymphocytes was also lower in these groups of coeliac patients. The percentage of different lymphocyte subsets showed no variation with regard to the dietary changes in the coeliac patients. The helper/suppressor ratio (H/S, i.e. immunoregulatory index) did not differ in the different groups of patients from the control values.

The numbers of leucocyte counts and the absolute number of lymphotes, as well as, all studied subpopulation did not change with regard to diet in the 9 patients followed through both on gluten free diet and gluten challenge (Table II)

TABLE I

Subpopulations of peripheral blood lymphocytes in coeliac patients and controls
(All values are expressed as mean ± S.D.)

	Coeliac patients			
	Before treatment (n = 10)	During gluten free diet (n = 17)	After gluten challenge (n = 16)	Controls (n = 13)
Leucocyte count				
No+	6.87±2.62	5.98±1.59	7.03±3.42	7.21±2.60
Lymphocytes				
No	3.68±2.28	2.45±0.92	2.47±0.76	3.63±2.03
%[1]	52.8±18.7	40.9±9.1	35.0±12.0	49.3±17.0
Total T cells				
No	2.36±1.45	1.59±0.61	1.52±0.50	2.42±1.99
%[2]	64.1±4.1	61.4±6.1	61.4±6.1	66.9±6.5
Helper T cells				
No	1.46±0.90	0.79±0.38	0.88±0.33	1.43±1.47
%[2]	39.7±3.9	32.3±11.5	35.2±3.7	39.2±9.5
Suppressor T cells				
No	0.88±0.53	0.53±0.20	0.54±0.21	0.91±0.72
%[2]	24.0±4.2	22.0±4.5	21.9±5.9	25.0±7.3
H/S ratio	1.68±0.39	1.58±0.58	1.72±0.75	1.44±0.6
B Cells				
No	0.50±0.41	0.32±0.15	0.32±0.14	0.54±0.41
%[2]	13.5±6.36	13.1±5.0	13.0±4.4	14.8±8.0

+ Absolute number (G/l)
[1] Percentage of leucocytes
[2] Percentage of absolute number of lymphocytes

DISCUSSION

All of the monoclonal antibodies used in this study appear to be quite effective in identifying the alleged lymphocyte subpopulations.

The absolute number and the percentage of the different lymphocyte subsets, as well as, the ratio of helper/suppressor cells in our control patients corresponded to the values reported by Davies et al in healthy children of the same age-group [4].

Our present study shows that in the coeliac patients — regardless of the type of the diet — the percentage of OKT 4 and OKT 8 phenotyped T cell subpopulations which contain the helper and suppressor T cells, as well as, the B cell subpopulation were not impaired as compared to the controls.

TABLE II

Subpopulations of peripheral blood lymphocytes in 9 coeliac patients on gluten free diet and followed through gluten challenge (All values are expressed as mean ± S.D.)

	During gluten free diet	After gluten challenge
Leucocyte count		
No+	6.39±1.60	6.42±1.54
Lymphocytes		
No	2.55±0.79	2.44±0.65
%[1]	40.0±8.7	38.0±11.2
Total T cells		
No	1.67±0.53	1.49±0.49
%[2]	65.4±6.6	57.8±5.1
Helper T cells		
No	0.90±0.29	0.84±0.28
%[2]	35.2±10.7	34.3±3.5
Suppressor T cells		
No	0.53±0.12	0.52±0.22
%[2]	20.9±5.6	21.2±5.9
H/S ratio	1.72±0.44	1.81±0.52
B cells		
No	0.30±0.11	0.26±0.07
%[2]	11.7±5.4	10.8±5.3

+ Absolute number (G/l)
[1] Percentage of leucocytes
[2] Percentage of absolute number of lymphocytes

The absolute number of lymphocytes and the lymphocyte subsets in coeliac patients before dietary treatment was the same as in the controls, as noted earlier in adult coeliacs [16, 19] but in contrast to the result of Selby et al [17]. The latters found that the absolute number and the percentage of circulating lymphocytes and the OKT 4 positive helper T cells were reduced in untreated adult coeliacs compared to the values seen in control subjects.

The lower absolute number of lymphocyte and the lymphocyte subsets in the coeliac children on gluten free diet and gluten challenge can be explained by the different age of the studied groups. The age of our control patients and the coeliac patients before dietary treatment was almost the same, while the coeliacs during gluten free diet and after gluten challenge were on average more than four years older, when the number of lymphocytes in the peripheral blood is lower. This explanation is also proved by the fact that the decrease of absolute

number of cells belonging to different lymphocyte subpopulations was proportionate in these groups of coeliac patients and the H/S ratio was practically unchanged.

Our results do not show any significant change in the T—B cell balance and among the T lymphocyte subpopulations between patients and controls, which suggests that it is probably not a dramatic impairment of the circulating pool of lymphocytes in coeliac disease. This is in contrast with the finding in the small intestinal mucosa of coeliac patients, where there is a striking difference between the distribution of lymphocyte subpopulations compared to the controls [13, 17]. However, we have to keep in mind, that a study of cell surface markers does not readily disclose functional properties, such as the division kinetics, cell traffic speed or differentiation capacity of the cells [14]. Therefore functional study of the peripheral blood lymphocytes are also very important.

Recently published functional studies indicate that peripheral blood mononuclear cells from treated coeliac patients have an impaired suppressor activity when compared to healthy controls [16]. We observed a significant elevation in the natural killer cell activity in treated coeliac children after a single gluten challenge [1].

Finally we have to emphasize that characterization of lymphocyte subpopulations in affected tissues of coeliacs, i.e. in jejunal biopsy specimens is also very important.

References

1. Arató A, Kósnai I, Gergely P: Natural killer cell activity in celiac children on gluten-free diet and after a single gluten challenge. J Pediatr 44: 112, 1988
2. Bullen A, Losowsky MS: Cell mediated immunity to gluten fraction III in adult coeliac disease. Gut 19: 126, 1978
3. Csorba S, Szabolcs M, Kávai M, Jezerniczky J, Szabó B: Über die Antigenität der Gliadins und die Antikörper gegen Gliadin. Acta Paediatr Acad Sci Hung 16: 249, 1975
4. Davies EG, Levinsky RJ, Butler M, Thomas RM, Linch DC: Lymphocyte subpopulations in primary immunodeficiency disorders. Arch Dis Childh 58: 346, 1983
5. Falchuk ZM, Strober W: Gluten-sensitive enteropathy — Synthesis of anti-gliadin antibody in vitro. Gut 15: 947, 1974
6. Ferguson A, MacDonald TT, McClure JP, Holden RJ: Cell-mediated immunity to gliadin within the small intestinal mucosa in coeliac disease. Lancet 1: 985, 1975
7. Flores AF, Winter HS, Bhan AK: In situ characterization of T cell subpopulations in gluten sensitive enteropathy (GSE). Ped Res 16: 161, 1982
8. Guan R, Rawcliffe PM, Priddle JD, Jewell DP: Cellular hypersensitivity to gluten derived peptides in coeliac disease. Gut 28: 426, 1987
9. Jenkins D, Goodall A, Scott BB: T-lymphocyte populations in normal and coeliac intestinal mucosa defined by monoclonal antibodies. Gut 27: 1330, 1986
10. Juto P, Fredrikson B, Hernell O: Gliadin specific serum immunoglobulins A E, G and M in childhood: relation to small intestinal mucosal morphology. J. Pediatr. Gastroenterol Nutr 4: 723, 1985
11. Kuitunen P, Kósnai I, Savilahti E: Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. J Ped Gastroenterol Nutr 1: 525, 1982
12. Kung PC, Goldstein G, Reinherz EL, Schlossman SF: Monoclonal antibodies defining distinctive human T cell surface antigens. Science 206: 347, 1979
13. Malizia G, Trejdosiewic LK, Wood GM, Howdle PD, Janossy G, Losowsky MS: The microenvironment of coeliac disease: T cell phenotypes and expression of

the "T2" T blast antigen by small bowel lymphocytes. Clin Exp Immunol 60: 437, 1985
14. Marsh MN: Functional and structural aspects of the epithelial lymphocytes with implications for coeliac disease and tropical sprue. Scand J Gastroenterol 20: (Suppl. 114) 55, 1985
15. Meeuwisse G: Diagnostic criteria in coeliac disease. Acta Paediatr Scand 56: 461, 1970
16. Pignata C, Troncone R, Monaco G, Ciriaco M, Farris E, Carucinati G, Auricchio S: Impaired suppressor activity in children affected by coeliac disease. Gut 26: 285, 1985
17. Selby WS, Janossy G, Bofill, M, Jewell DP: Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. Clin Exp Immunol 52: 219, 1983
18. Tsibassu M, Geboes K, Eggermont E, Desmet V: Jejunal mucosa lymphoid cell subsets and the expression of major histocompatibility antigens in children. Eur J Pediatr 146: 251, 1987
19. Vetvicka T, Tlaskaslova-Hogenova H, Fric P, Brochier J: Subpopulations of circulating lymphocytes in adults with coeliac disease. Immunol Letter 13: 15, 1986

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