Increase of lymphocytes bearing the γ/δ T cell receptor in the jejunum of patients with dermatitis herpetiformis

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Abstract

The densities of T cells and of cells bearing the T cell receptors γ/δ and α/β and the surface antigens CD4 and CD8 in jejunal specimens from 21 patients with dermatitis herpetiformis were compared with those in specimens from 13 untreated adults with coeliac disease and 13 control subjects. In the lamina propria of the jejunum the median density of γ/δ + cells was significantly ($p < 0.001$) greater in untreated patients with dermatitis herpetiformis than in control subjects (114 v 36 cells/mm²) and similar to that found in the patients with coeliac disease (115 cells/mm²). The difference in γ/δ + cell density between patients with dermatitis herpetiformis and control subjects was much greater in the surface epithelium of the jejunum: the median density for 14 untreated patients with dermatitis herpetiformis was 39 cells/mm, for seven patients with dermatitis herpetiformis on a gluten free diet 34 cells/mm, and for control subjects 2 cells/mm; the coeliac patients had the same density as the patients with dermatitis herpetiformis (45 cells/mm). The higher density of cells bearing the α/β T cell receptor in the epithelium (median 77 cells/mm) of untreated patients with dermatitis herpetiformis was associated with a gluten containing diet; in specimens taken from patients with dermatitis herpetiformis on a gluten free diet the median density was similar to that in the control subjects (44 v 39 cells/mm). The increase in the number of lymphocytes bearing the T cell receptor γ/δ , particularly in the epithelium of the jejunum, seems to be a constant marker for these closely related diseases, whereas the density of α/β + T cells is dependent on the diet.

The recently recognised T cell type bearing γ/δ chains in the antigen receptor together with the CD3 complex^{1–3} has not been assigned a specific role in human lymphoid tissue. The proportion of T cells that carries the γ/δ T cell receptor is low in peripheral blood and in the thymus – less than 10%^{3,4} – and is similar in the normal human jejunum.^{4,7} Patients with coeliac disease have a significant increase in the absolute and relative counts of γ/δ T cell receptor bearing cells in the jejunal epithelium^{5,9} and, according to our previous study,⁹ also in the lamina propria of the jejunum. Whether these cells play a part in the pathogenesis of coeliac disease is not known.¹⁰

A close relation exists between coeliac disease and dermatitis herpetiformis: both diseases are closely linked with DQw2 expression in the HLA DR region,¹¹ and they segregate to the same

families.¹² Although most patients with dermatitis herpetiformis show few or no gastrointestinal symptoms, most have jejunal damage similar to that observed in coeliac disease.^{13,14} This prompted us to study the density of γ/δ T cell receptor bearing cells in the jejunum of patients with dermatitis herpetiformis and to characterise the T cell infiltrate in the jejunal epithelium and lamina propria of patients before dietary treatment and after a strict gluten free diet.

We found a similar increase in the γ/δ T cell receptor bearing cells in the jejunum of patients with dermatitis herpetiformis and in patients with coeliac disease. Neither adherence to a gluten free diet for a long time nor the morphology of the jejunum had any influence on the density of γ/δ T cell receptor + cells.

Methods

PATIENTS

Jejunal biopsy specimens from 21 patients with dermatitis herpetiformis were available for the study. The diagnosis of dermatitis herpetiformis was based on the presence of the typical skin lesion and the demonstration by immunofluorescent study of a biopsy specimen of granular IgA deposits in the unaffected skin.^{13,15} Fourteen specimens were taken from patients whose mean age was 40 years (range 22–74 years) before any dietary treatment, and seven from patients with a mean age of 39 years (range 15–60 years) who were on a gluten free diet. The latter had been on the gluten free diet for periods ranging from one to 15 years; complete elimination of food containing wheat, rye, barley, and oats was advised. Comparison groups included 13 newly diagnosed adult patients with coeliac disease (aged 15 to 52 years, mean age 32 years) and 13 adult patients aged 17 to 57 years with histologically normal jejunal specimens. All the specimens from the coeliac patients showed total or subtotal villous atrophy. The biopsy specimens from normal jejunum (the control subjects) had a villous/crypt ratio greater than three and their intraepithelial lymphocyte count was less than 30/100 epithelial cells.

BIOPSY SPECIMENS

The jejunal biopsy specimens were divided into two parts and one part was processed by routine histological methods. The height of the villi and the depth of the crypts were measured.¹⁵ Another part of each fresh biopsy specimen was embedded in OCT for 30 minutes, and washed three times in Tris buffer, pH 7.4.

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IMMUNOHISTOCHEMICAL STAINING

The buffer was removed and the sections were covered with a dilution of monoclonal antibodies in Tris buffer for 16 hours. Endogenous peroxidase was blocked by incubation in 0.5% peroxide for 30 minutes. A Vectastain Elite ABC kit (PK-6102, Vectro Laboratories, Burlingame, CA) was used to show the binding sites of the monoclonal antibodies in accordance with the manufacturer's instructions.

The numbers of stained cells were counted with a light microscope through a calibrated graticule at $\times 900$ magnification.¹⁶

MONOCLONAL ANTIBODIES

Monoclonal antibody TCR δ 1 (T cell Sciences, Cambridge, MA), recognising a constant region of the δ -chain of T cell receptor^{17,18} and all γ/δ + cells¹⁹ and antibody β F1 (T cell Sciences) reacting with virtually all α/β T cell receptor molecules¹⁹ were used at a dilution of 1:100. Monoclonal antibodies anti-Leu4 (anti-CD3, Beckton-Dickinson, Mountain View, CA), T4 (anti-CD4, Coulter Immunology, Hialeah FL), and OKT8 (anti-CD8, Ortho Diagnostic System, Raritan, NJ) were used at a dilution 1:400.

STATISTICAL ANALYSIS

Differences between the densities of stained cells in the specimens from untreated patients with dermatitis herpetiformis and those from patients with dermatitis herpetiformis on a gluten free diet and between these groups and the two comparison groups (subjects with normal jejunum and untreated patients with coeliac disease) were calculated by the two tailed Mann-Whitney U test.

Results

MORPHOLOGY OF THE JEJUNUM

Of the specimens taken from 14 patients with dermatitis herpetiformis on a normal, gluten containing diet, seven showed subtotal villous atrophy with villous-to-crypt (V/C ratios of <1 , and five severe partial villous atrophy (V/H ratios of 1.0–1.99). In two the villous structure was normal. Six of the seven biopsy specimens from patients on a gluten free diet had normal villous structures (V/H ratios of >3.0); one showed partial villous atrophy.

T CELLS IN THE JEJUNAL LAMINA PROPRIA

The specimens from the 14 patients with dermatitis herpetiformis on a normal gluten containing diet contained significantly more γ/δ + cells in the lamina propria than those from control subjects, the median density of γ/δ + cells being similar to that found in the patients with coeliac disease (Fig 1). The patients with dermatitis herpetiformis on a gluten free diet also had higher densities of γ/δ + cells. The densities of α/β + and CD3+ of patients with dermatitis herpetiformis did not differ from those in the patients in the comparison groups. In all but one patient the density of CD4+ T cells was higher

than that of CD8+ cells (Fig 2). The densities of these cells in the lamina propria of patients with dermatitis herpetiformis were similar to those observed in the comparison groups (Fig 2).

T CELLS IN THE JEJUNAL EPITHELIUM

In the jejunal surface epithelium of specimens taken from patients with dermatitis herpetiformis who were on a gluten containing diet the densities of γ/δ + cells were greater than in the control subjects, but similar to those for the coeliac patients (Fig 3). The two specimens with normal villous structure taken from patients on a gluten containing diet had γ/δ + cell densities of the same order as the specimens with villous atrophy. In the specimens from patients with dermatitis herpetiformis taken while they were on a gluten free diet, the median density of γ/δ + cells remained unchanged; their median density was close to that of the α/β + cells (Fig 3).

The specimens from untreated patients with dermatitis herpetiformis showed higher densities of α/β + and CD8+ cells than those of the controls but similar densities to those of the coeliac patients (Figs 3 and 4). In specimens taken from patients on a gluten free diet the densities of α/β + and CD8+ were lower than in the specimens from the untreated patients with dermatitis herpetiformis and the coeliac patients but similar to those of the control subjects. The densities of CD4+ cells in the jejunal surface epithelium were low. In the patients with dermatitis herpetiformis they also seemed to change with the diet.

T CELLS IN THE JEJUNAL CRYPT EPITHELIUM

In the crypt epithelium the densities of T cells were lower, about 1/4 of that in the surface epithelium (Fig 5). The densities of CD3+ CD8+, and α/β + cells in the patients with dermatitis herpetiformis depended on the diet (Figs 4 and 5), but the density of γ/δ + cells was unaffected by a gluten free diet. The median percentage of CD3+ cells was highest (52%) in specimens taken while patients were on a gluten free diet.

Discussion

Our new finding is the significant increase in γ/δ + cells in the jejunum of patients with dermatitis herpetiformis. Both in the lamina propria and in the epithelium of jejunal specimens of patients with dermatitis herpetiformis the density was of the same order as in patients with coeliac disease. The epithelium contained 10 times as many γ/δ + cells as were seen in the control specimens, while in the lamina propria the density of γ/δ + cells was about three times that seen in control subjects. In all the patients with dermatitis herpetiformis the density of γ/δ + cells in the epithelium was closely similar irrespective of the diet or the morphology of the jejunum. In the patients following a gluten free diet, these cells were nearly as frequent as α/β + cells. The findings are similar to those reported earlier for patients with coeliac disease.^{5,8,9}

The densities of CD3+, CD8+, CD4+, and α/β + cells in the specimens of patients with

Figure 1: Densities of T cells (CD3+) and cells bearing T cell receptors γ/δ and α/β in the lamina propria of the jejunum of patients with untreated dermatitis herpetiformis, treated patients with dermatitis herpetiformis on a gluten free diet, control subjects with a normal jejunum, and untreated patients with coeliac disease. Horizontal bars indicate median densities. The cell densities in the two untreated patients with dermatitis herpetiformis with normal jejunal structure are indicated with ■ and similarly ▲ indicates the treated patient with partial villous atrophy. Comparisons between the two groups of patients with dermatitis herpetiformis, and between these two groups and (i) controls with a normal jejunum (ii) untreated patients with coeliac disease showed the significant differences.

Figure 2: Densities of CD4+ and CD8+ cells and the ratios of these densities in the lamina propria of the jejunum in patients, grouped as in Figure 1.

Figure 3: Densities of T cells (CD3+) and of cells bearing T cell receptors γ/δ and α/β in the jejunal surface epithelium in patients. Symbols as in Figure 1.

Figure 4: Densities of CD8+ cells in the surface and crypt epithelium and densities of CD4+ cells in the surface epithelium of the jejunum in patients. Symbols as in Figure 1.

dermatitis herpetiformis were also closely similar to those found in patients with coeliac disease. In the lamina propria there was a preponderance of CD4+ cells and the densities did not differ from those in control subjects.^{16 20 21} In contrast to the findings of Jenkins *et al*²² we did not see more T cells or CD8+ cells in the specimens from the patients with dermatitis herpetiformis than in specimens from the patients with coeliac disease. In the jejunal epithelium of patients with dermatitis herpetiformis the numbers of α/β + and CD8+ are significantly raised when the patients are on a normal diet, as in coeliac patients,^{16 20} and fall to the level of the control subjects when a gluten free diet is followed. In the jejunal epithelium of the patients with dermatitis herpetiformis CD4+ cells are not numerous, their density being only a small percentage of the number of CD3+ cells. The densities of T cells, α/β T cell receptor bearing cells, and CD8+ cells in the crypts changed much the same way as they did in the surface epithelium. Patients with dermatitis herpetiformis and coeliac disease had significantly higher counts of these cells in the crypts when taking food containing gluten in contrast to the finding of normal counts in an earlier study.²¹ Our present finding thus strongly suggests that there is an active increase of α/β T cell receptor bearing cells in the epithelium of patients with dermatitis herpetiformis and coeliac disease on gluten containing diets. Thus we disagree with the view that the increased density in the surface is merely a reflection of crowding of the T cells.²³ It has been suggested that γ/δ + cells may have

a special function in protecting mucosal surfaces by eliminating infected or otherwise damaged epithelial cells.²⁴ The same type of cytolytic activity of γ/δ -T cell receptor bearing cells which they exert against tumour cells^{25 26} might play a part in the pathogenesis of the jejunal damage always seen in coeliac disease and in most patients with dermatitis herpetiformis.¹³⁻¹⁵ Dermatitis herpetiformis and coeliac disease are associated with the same HLA antigens.^{11 27} These or other related restricting surface antigens may show a high affinity for the digested fractions of gluten and, possibly with specific antibodies, may cause the surface epithelial cells to become targets for cytotoxicity.

Our present results, however, suggest that γ/δ T cell receptor bearing cells are not involved in the elimination of epithelial cells. The two specimens which maintained their villous structure despite the patients being on gluten containing diets had numbers of γ/δ + cells as high as those with severe villous atrophy. This clearly indicates that the genetic make up of an individual, the presence of gluten in the diet, and high numbers of γ/δ + cells in the epithelium do not inevitably lead to villous atrophy. We have recently described the father of a patient with coeliac disease, a man with normal villous structure and a large number of γ/δ + cells in his jejunum, who two years after this finding developed villous atrophy and probably also has coeliac disease.²⁸

Elimination of gluten reverses all the morphological, enzymatic, and immunological alterations in the jejunum of patients with

Figure 5: Densities of T cells (CD3+) and of cells bearing T cell receptors γ/δ and α/β in the jejunal crypt epithelium in patients. Symbols as in Figure 1.

dermatitis herpetiformis and coeliac disease, but leaves the density of γ/δ + cells in the epithelium unchanged and remarkably high.^{8,9} In other diseases that lead to villous atrophy, such as tropical sprue,⁵ intractable diarrhoea in infancy,²⁹ and severe intestinal food allergy,⁹ the number of γ/δ + cells has been found to be normal. The presence of increased numbers of γ/δ -T cell receptor cells may be due to the inherited immune abnormality common to coeliac disease and dermatitis herpetiformis, which is expressed as similar HLA D associations.^{11,27} This increase may be the precursor or a prerequisite for the development of an intestinal lesion, but the lesion is only manifested in the presence of further environmental agents. In the present study we found no differences in the densities of T cells and their subgroups which would explain the differences in the clinical manifestations of dermatitis herpetiformis and coeliac disease.

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