PAPERS AND ORIGINALS

Lymphocytic Infiltration of Epithelium in Diagnosis of Gluten-sensitive Enteropathy

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Summary

The macroscopic appearance of the mucosa of the small intestine and the lymphocytic infiltration of the epithelium were studied in 27 patients with dermatitis herpetiformis and in 11 control subjects. The mucosa was abnormal in appearance in 13 of the patients and normal in 14 patients and in all the controls. In 25 (93%) of the patients the intraepithelial lymphocyte count was significantly raised compared with the controls. The increased lymphocytic infiltration of the epithelium in the patients probably represented an underlying immunological reaction of the small intestine to gluten, since the infiltration lessened in five out of six patients after a year on a gluten-free diet and in all of four patients after three years on a gluten-free diet.

Increased lymphocytic infiltration of the epithelium of the small intestine seems a surer sign of gluten sensitivity than the macroscopic appearance of the mucosa, and a diagnosis of gluten-sensitive enteropathy may no longer be excluded when the mucosa appears normal. Further evidence of the significance of increased lymphocytic infiltration is that patients with normal-looking mucosa but with raised intraepithelial lymphocyte counts often had low serum folate levels.

Introduction

About two-thirds of patients with dermatitis herpetiformis have an enteropathy judged by macroscopic and histological features

of the mucosa of the small intestine (Marks *et al.*, 1966; Fraser *et al.*, 1967; Fry *et al.*, 1967). The enteropathy is similar to that of coeliac disease (Fry *et al.*, 1968, 1969; Shuster *et al.*, 1968; Marks and Whittle, 1969), which is usually diagnosed on the appearance of the intestinal mucosa (Booth *et al.*, 1962), and there is now mounting evidence of immunological abnormalities in both coeliac disease and dermatitis herpetiformis (Hobbs and Hepner, 1966; Crabbe, 1967; Douglas *et al.*, 1969; Soltoft, 1970; Seah *et al.*, 1971a, 1971b). Immunoglobulin deposits on the reticulin fibres of the skin have been found in dermatitis herpetiformis (Seah *et al.*, 1972), and lymphocytic infiltration of the lamina propria (Rubin *et al.*, 1960; Shiner and Drury, 1962) and of the intestinal epithelium (Ferguson and Murray, 1971) have been reported in coeliac disease.

We report here a study of the lymphocytic infiltration of the epithelium of the small intestine in a group of patients with dermatitis herpetiformis to see whether in those cases in which the mucosa was macroscopically normal there was abnormal infiltration and thus evidence of an immunological abnormality. The patients' folate status, as a sensitive indicator of functional disturbance of the mucosa of the upper small intestine, was also assessed.

Patients and Methods

Twenty-seven patients were studied. Dermatitis herpetiformis was diagnosed on the clinical and histological features of the rash, its clearance with dapsone therapy, and relapse on withdrawal of the drug.

An initial biopsy on mucosa from the small intestine of each patient was performed. Six of the 27 patients were put on a gluten-free diet for one year, and gluten was then reintroduced into the diet of five of them for a period of three months. At the end of the three months four of the five returned to a gluten-free diet for another three years. Further biopsies on mucosa from the small intestine were performed in the six patients at the end of their year on a gluten-free diet, in the five patients at the end of their three-month period on a diet containing gluten, and in the four patients at the end of their three-year period on a gluten-free diet.

The mucosa of the small intestine of 11 subjects who did not have dermatitis herpetiformis or coeliac disease was also studied.

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One specimen was obtained from a volunteer physician, four were obtained during partial gastrectomy for gastric ulcer, five were obtained from patients with eczema, and one was obtained from a patient with psoriasis.

The specimens were taken with the Crosby capsule from the duodenojejunal flexure under radiographic control in all patients. The macroscopic appearance was assessed with a dissecting microscope and the specimen photographed for record. It was then fixed in formol-saline and embedded in wax. Sections 7 μ m thick were cut and stained with haematoxylin and eosin.

The epithelial cells and intraepithelial lymphocytes were counted in parts of the specimens where the plane of section was vertical through the epithelium and the underlying basement membrane. In villous specimens counts were made on the sides and tips of the villi, and in convoluted and flat specimens the surface cells were counted. (Casual observation indicated that glandular epithelium was much less heavily infiltrated with lymphocytes than that on the sides of villi or on the surface of flat and convoluted specimens.) The number of lymphocytes among 1,000 cells were counted in each specimen and the results expressed as lymphocytes per 1,000 epithelial cells. Note was taken of whether the lymphocytes were in a basal position, adjacent to the basement membrane, or at a higher level among the epithelial cells. Lymphocyte nuclei were usually easily distinguished from those of epithelial cells by their smaller size, more rounded overall shape, and deeper staining. Nuclei or nuclear fragments that could not be positively identified were ignored, as were occasional intraepithelial eosinophils.

Serum folate levels were determined by microbiological assay with *Lactobacillus casei* as the test organism (Waters and Mollin, 1961). The normal range is 6.0-21.0 ng/ml. Levels less than 3.0 ng/ml are regarded as abnormal and those between 3.0 and 5.9 ng/ml as borderline.

Red cell folate levels were estimated according to the modification of the serum folate assay described by Hoffbrand *et al.* (1966). The normal range is 160-640 ng/ml packed red cells.

Results

MACROSCOPIC APPEARANCES

The macroscopic appearance of the mucosa of the small intestine was flat in six of the 27 patients with dermatitis herpetiformis, convoluted in seven, leaves only in seven, and fingers and leaves in seven (Fig. 1). The macroscopic appearance in the 11 control subjects was leaves only in two and fingers and leaves in nine.

The initial macroscopic appearance of the mucosa in the six patients who had serial biopsies was flat in one, convoluted in two, leaves only in two, and fingers and leaves in one (see Table). After one year on a gluten-free diet the mucosa in the three patients in whom it had been abnormal (flat or convoluted) became normal (leaves only). After the reintroduction of gluten to the diet the mucosa became abnormal again in two of these three patients. The mucosa in the three patients in whom it was

initially normal remained normal in two after one year on a gluten-free diet and on subsequent reintroduction of gluten, but in one patient (in whom the initial appearance was leaves only) the mucosa was convoluted after a year on gluten-free diet and remained so after the reintroduction of gluten.

FIG. 1—Macroscopic appearance of small-intestinal mucosa in relation to lymphocyte counts in control and dermatitis herpetiformis specimens. O = Macroscopic appearance of leaves only in control group.

The mucosa of two of the four patients who were studied at the end of three years on a gluten-free diet had initially been abnormal and one of these two reverted to normal. There was no change in the two patients whose mucosa had appeared normal before their three-year period on gluten-free diet.

LYMPHOCYTE COUNTS

The mean lymphocyte count per 1,000 epithelial cells (Fig. 1) was $451 \pm \text{S.E. } 70$ (range 313 to 627) in the six flat specimens (Fig. 2), $435 \pm \text{S.E. } 88$ (range 130 to 739) in the seven convoluted specimens, $502 \pm \text{S.E. } 44$ (range 408 to 727) in the leaves only

FIG. 2—Increased lymphocytic infiltration of epithelium in dermatitis herpetiformis. (Periodic-acid Schiff. $\times 400$.)

Macroscopic Appearance of Mucosa of Small Intestine and Lymphocytic Infiltration of Epithelium in Relation to Gluten Withdrawal and Normal Diet

Case No.	Appearance of Mucosa				Lymphocyte Count per 1,000 Epithelial Cells			
	A	B	C	D	A	B	C	D
1	Con.	L.	Flat	Con.	295	308	238	221
2	Con.	L.	Con.	L.	635	375	369	277
3	F.L.	L.	F.L.	F.L.	374	304	307	293
4	L.	L.	L.	F.L.	408	209	438	103
5	L.	Con.	Con.	—	434	353	355	—
6	Flat	L.	—	—	399	348	—	—

Con. = Convoluted. L. = Leaves only. F.L. = Fingers and leaves. A = Initial biopsy. B = After one year on gluten-free diet. C = Three months after reintroduction of gluten. D = After three years on gluten-free diet.

specimens, and $300 \pm$ S.E. 50 (range 89 to 422) in the fingers and leaves specimens.

In the 11 control specimens showing leaves only and fingers and leaves (Fig. 3) the mean count (Fig. 1) was $139 \pm$ S.E. 14 (range 65 to 235).

FIG. 3—Minimal lymphocytic infiltration of epithelium from volunteer physician. (Periodic-acid Schiff. $\times 400$.)

The counts for each of the four groups of patients were widely scattered but in the control group they were very similar. The mean counts for the flat, convoluted, leaves only, and fingers and leaves specimens were not significantly different. Though the counts for the fingers and leaves specimens tended to be lower than for the other groups the difference was not significant ($P > 0.10$). The mean count for the fingers and leaves specimens, however, was significantly greater in the patients than in the control group ($P < 0.001$). Counts of less than 200 were found in only two specimens from patients, one of which had a convoluted and one a fingers and leaves macroscopic appearance. In only one of the 11 control specimens was the count above 200.

Serial Lymphocyte Counts in Six Patients.—The lymphocyte count had fallen in five and risen in one of the six patients after

one year on a gluten-free diet (see Table). Nevertheless, in none did the count fall to below 200. The lymphocyte count rose in three and fell in two of the five patients in whom gluten was reintroduced and biopsy repeated three months later. There was a significant rise only in Case 4.

All four patients who were studied after three years on a gluten-free diet showed a fall in the lymphocyte count compared with their initial count. It was greatest in Case 4—from 408 to 103 (see Table).

SERUM AND RED CELL FOLATE LEVELS

Some serum folate levels of less than 3.0 ng/ml were found in all four groups of patients, but more often in patients with abnormal mucosa (Fig. 4). All the six patients with flat mucosa had low serum folate, and in five of them it was below 3.0 ng/ml. The red cell folate level was also subnormal in two of the six patients. The serum folate level was subnormal in six of the seven patients with a convoluted mucosa, and in five of them it was below 3 ng/ml. Three of the seven also had subnormal red cell folate levels. Five of the seven patients with a leaves only appearance of the mucosa had low serum levels, and in three of them it was below 3.0 ng/ml, but the red cell level was subnormal in only one. Four of the seven patients with fingers and leaves appearance had a low serum level but it was below 3.0 ng/ml in only one. All this group had normal red cell folate levels.

Discussion

There was a significant increase in the number of lymphocytes in the epithelium of the small intestine in 25 (93%) of the 27 patients with dermatitis herpetiformis. That the lymphocyte count was raised in 13 of the 14 macroscopically normal biopsy specimens is particularly interesting. Indeed, there was no significant difference in the degree of lymphocytic infiltration between specimens with a flat, convoluted, leaves only, or fingers and leaves appearance. In 61% of the 83 patients with dermatitis herpetiformis studied by four different groups of workers (Fraser *et al.*, 1967; Fry *et al.*, 1967; Marks *et al.*, 1968; Shuster *et al.*, 1968) the appearance of the mucosa of the small intestine was flat or convoluted. However, since the finding of circulating antireticulin antibody in patients with dermatitis herpetiformis (Seah *et al.*, 1971a, 1971b) Seah *et al.* (1972) have shown immunoglobulin deposits on the reticulin fibres of the upper dermis in all of 18 patients with dermatitis herpetiformis. This suggests that an immunological disorder is present in the skin in all patients with dermatitis herpetiformis, and if that is so some abnormality of the small intestine may also be present.

The report (Ferguson and Murray, 1971) that in untreated coeliac disease but not in other gastrointestinal diseases the lymphocytic infiltration of the small-intestinal epithelium is increased is probably evidence of an immunological abnormality at this site. Our finding of increased lymphocytic infiltration in 93% of the present patients strongly suggests that all patients with dermatitis herpetiformis have an immunological abnormality in the epithelium of the small intestine.

It seems that lymphocytic infiltration of the epithelium is a more sensitive marker than macroscopic appearance for detecting gluten sensitivity, and our study provides good evidence that lymphocytic infiltration is an indication of gluten sensitivity of the small intestine. Firstly, the skin condition of patients with dermatitis herpetiformis in whom the mucosa of the small intestine has the appearance of fingers and leaves or leaves only improves on a gluten-free diet and the serum folate level rises. When gluten is reintroduced the rash returns, larger doses of dapsone are needed, and the serum folate level falls (Fry *et al.*, 1969). Secondly, the present study shows that the lymphocytic infiltration lessens (irrespective of the original macroscopic appearance) when the patient takes a gluten-free diet, particularly

FIG. 4—Serum and red cell folate levels in relation to macroscopic appearance of mucosa. S.F. = Serum folate. R.C.F. = Red cell folate.

for a long period. Ferguson and Murray (1971) also reported that lymphocytic infiltration was less in patients with coeliac disease taking a gluten-free diet than in untreated patients.

Our findings together with those of Ferguson and Murray (1971) therefore raise the question of the criteria for diagnosing coeliac disease. Four out of seven of the present patients with a finger and leaf appearance of the mucosa and five of the seven with a leaves only appearance had subnormal serum folate levels. If these patients had not had a rash and had been found incidentally to have low folate levels they might have been labelled nutritional folate deficiency or temperate sprue (Cooke *et al.*, 1963). We suggest that some patients with folate deficiency and a normal macroscopic appearance of the small intestine may have a mild form of coeliac disease.

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Carcinoma of Large Bowel in the Young

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Summary

The incidence of large-bowel carcinoma in young people seems to be increasing. Twenty-one cases occurring between the ages of 13 and 25 are reviewed. Most cases were found to be in an advanced state of the disease. High grade malignant growths were found to be twice as common in the young as in adults. The mode of presentation is no different from that in adults and a plea is made for improving results of treatment by earlier diagnosis, which can be established by an increased awareness of the disease in children and young adults.

Introduction

Cancer of the large bowel is one of the most frequent malignant diseases in the adult, with its peak incidence in the sixth decade. Fortunately it is also one of the more favourable forms of malignancy in terms of operability and long-term survival. In children it is uncommon, but its occurrence in the young has generated much interest, especially with regard to its unfavourable prognosis.

The youngest patient was recorded by Ahfeld (1880), who reported a case in a premature infant. Since then many reports have been made (Sessions and Riddel, 1961; Recio and Bussey, 1965; O'Brien, 1967; Rosato *et al.*, 1969). It has also been noted that there is an increased incidence in young adult life as well as in childhood. Ehrlich and Hunter (1947) examined 813

tumours occurring in young men of military age and found no fewer than 342 cancers of the colon and rectum. In St. Mark's Hospital, out of 4,430 cases 1% occurred before the age of 30 (Recio and Bussey, 1965), while in the Vanderbilt Hospital the incidence below the age of 20 was 0.65% (Sessions *et al.*, 1965). In our own practice in the professorial surgical unit we too have encountered an increasing number of tumours, not only in young adults but also in children and adolescents.

Present Series

Twenty-one cases of large-bowel carcinoma in young patients have been collected since 1961, during which time the total cases in all age groups numbered 582. This represents 4% of the total number.

Age and Sex.—The average age of the patients in this series was 21.1 years (range 13-25 years). Most of the patients therefore were young adults, whereas in the past interest has been focused mainly on the occurrence of the disease in young children. The average age in our overall series of large-bowel carcinoma patients was 54 years. Among a total of 582 cases of large-bowel cancer there was a slight predominance of females (301) over males (281). Not only was there a reversal of the sex incidence among the young patients but a noticeable preponderance of 15 males to 6 females—a ratio of 2.5:1 (see Table).

Clinical Features.—Seventeen patients had melaena either in the form of tarry motions or frank blood. The next most common symptom was an alteration of bowel habit (14), followed by abdominal colic (7), presence of a mass (4), and tenesmus (4). In addition, two patients presented with the clinical picture of acute appendicitis. The duration of symptoms ranged from two days to 18 months, with an average of three months. The corresponding figure for our overall group of patients was four months.

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