IgA class reticulin antibodies in relatives of patients with coeliac disease

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SUMMARY IgA class reticulin antibodies were not found in patients with Crohn's disease, ulcerative colitis, and hiatus hernia despite a significant incidence of IgG class reticulin antibodies. None of 56 normal healthy subjects was positive. In contrast, 13 (76%) of the sera from 17 patients with coeliac disease on normal diet were positive for IgA class antibodies as were 19 (20%) of 93 first degree relatives. Seventy-three relatives underwent jejunal biopsy. Grade III (flat) histology was found in 13 and, of these patients, 10 (77%) showed IgA class reticulin antibody in their serum. It is suggested that determination of IgA class reticulin antibodies was a useful test to determine which relative must be biopsied.

The incidence of coeliac disease in first-degree relatives of coeliac patients varies between 10 and 18% (Macdonald *et al.*, 1965; Robinson *et al.*, 1971; Mylotte *et al.*, 1972; Shipman *et al.*, 1973; Stokes *et al.*, 1976). In view of the complications of coeliac disease, such as malignancy, and the improvement in health brought about by a gluten-free diet, the detection of any affected relatives is desirable. At present, jejunal biopsy is the only acceptable method of establishing the diagnosis, but, for obvious reasons, it is not a satisfactory screening test. The need for a simpler test for diagnosis is apparent. In 1971, Seah *et al.* (1971a) described an antibody reacting with connective tissue components in the sera of patients with coeliac disease and in dermatitis herpetiformis. The reactivity appeared to be directed against reticulin.

With regard to the immunoglobulin class of reticulin antibodies the reports vary. Originally Seah *et al.* (1971a) using antihuman IgG and IgM antisera found the antibody to be IgG class. Subsequently, it has been reported to be IgG, IgA, or mixed IgA and IgG class with varying frequencies. The occurrence of IgM class reticulin antibody has not been reported (Seah *et al.*, 1971b; Alp and Wright, 1971; Brown *et al.*, 1973). Magalhaes *et al.* (1974), using monospecific IgA, IgG, and IgM antisera, studied patients with adult coeliac disease, a variety of other diseases, and normal subjects and have shown that the IgA class antibody activity was 'practically confined' to

sera from adult coeliac patients taking a normal diet.

Stevens *et al.* (1975) found an increased incidence of reticulin antibody in relatives of coeliac patients using polyvalent antihuman antisera. Out of 82 first degree relatives investigated by jejunal biopsy, 14 had coeliac disease of whom 12 were positive for the antibody, suggesting that this investigation might be a good screening test to detect coeliac disease in relatives. Brown *et al.* (1973) studying 48 coeliac children found a 67% incidence of reticulin antibody, with 15% in controls. In all the positive patients, antibody in the IgG class was found, and, in addition, in 11 of 48 coeliac patients and also in two of the 48 controls antibodies of the IgA class were also present.

In view of the apparent discrepancies concerning the reticulin antibodies, the purpose of this study was to investigate again relatives of coeliac patients, using monospecific anti-IgA and IgG antisera.

Methods

Ninety-three first-degree relatives (20 parents, 50 siblings, and 23 children) of coeliac patients have been studied, ranging in age from 13 to 62 years (mean age 37.4 years) and being 45 male and 48 female. Jejunal biopsy was obtained in 73 of them, using the Choudhury multiple biopsy capsule (Roy Choudhury *et al.*, 1964). Those who were not biopsied either had refused or were living elsewhere in Great Britain. Histological findings were classified according to that of Roy Choudhury *et al.* (1964), grade III being the classical flat biopsy of coeliac

Fig. 1 Rat renal cortex showing reactivity with the parietal layer of Bowman's capsule, together with some peritubular basement membrane staining. Serum from relatives of coeliac disease patient + FITC anti-IgA, $\times 300$.

Fig. 2 Rat renal medulla. This shows the typical peritubular basement membrane staining. Coeliac disease relative's serum + FITC anti-IgA, $\times 120$.

disease, grade II being moderately abnormal, grade I having minor non-specific abnormalities, and grade 0 normal.

Peripheral blood samples were obtained and the serum separated and stored at -20°C . For the estimation of the reticulin antibodies sera were diluted 1 in 5 using a standard indirect immunofluorescent technique (Seah *et al.*, 1971a) on frozen sections of rat kidney, liver, stomach, and salivary

gland. Commercially available rabbit fluorescent conjugated monospecific antihuman IgA (Wellcome Laboratories) was used at a 1 in 10 dilution. Monospecific sheep antihuman IgG (Department of Experimental Pathology, University of Birmingham) was conjugated with fluorescein and used as previously described (Williamson *et al.*, 1976). The reticulin antibody was considered to be positive if the characteristic pattern (Seah *et al.*, 1971a) was seen

Table Reticulin antibodies of IgA and IgG class

Group	No.	IgA (no.)	IgG (no.)	IgG + IgA (no.)	All positive reticulin antibodies (%)	Total IgA positives (%)
Control	56	0	1	0	1.8	0
Crohn's	19	0	2	0	10.5	0
Ulcerative colitis	22	0	0	0	0	0
Coeliac disease						
Normal diet	17	11	1	2	82.4	76.5
Gluten-free	7	0	0	1	14.3	14.3
Relatives (total)	93	13	10	6	31.2	20.4
Relatives (biopsied)	73	10	6	6	30.1	21.9
Grade 0	26	2	3	2	26.9	15.4
Grade I	30	0	3	1	13.3	3.3
Grade II	4	1	0	0	25.0	25.0
Grade III	13	7	0	3	76.9	76.9

staining the peritubular connective tissue and Bowman's capsule in the kidney (Figs 1 and 2), the sinusoids and portal tract in the liver, and between the gastric glands in the stomach. This antibody corresponds with the endothelial basement membrane antibody of Williamson *et al.* (1976) and to the R1 antibody of Rizzetto and Doniach (1973). Kidney sections were the best substrate organ and sometimes it was the only organ which was positive. The antibody was also sought in the sera of 17 coeliac patients before treatment, in seven coeliac patients on a gluten-free diet, 19 with Crohn's disease, 22 with ulcerative colitis, 19 with hiatus hernia known to be IgG positive, and 56 healthy subjects—doctors, nurses, and laboratory personnel—who were used as controls. All the serum samples examined were randomised without knowing the biopsy or other results of the subject. Positive and negative control sera were always used with each batch of tests. The HLA antigens were determined in each of the relatives using a modification of the micro-lymphocytotoxicity test of Terasaki and McClelland (1964).

Results

The Table shows the incidence of the reticulin antibody for both IgA and IgG class in 56 normal controls in 17 coeliac patients on a normal diet, seven on a gluten-free diet, 19 with Crohn's disease, 22 with ulcerative colitis, and in 93 relatives of coeliac patients. The same Table shows also the incidence of the antibody in 73 of the relatives who had had jejunal biopsy examination, grouped according to their biopsy grade. Eleven out of 17 coeliac patients were IgA class antibody positive, and one IgG and two mixed IgA and IgG—that is, 13 out of 17 were positive to IgA class antibody. From the seven coeliac patients on gluten-free diet, one was positive for both IgA and IgG class. The overall incidence of

the reticulin antibodies (IgA and/or IgG class) in the 93 relatives studied was 31.1% which was significantly greater than the 1.7% found in the normal population (one IgG class antibody from 56 normal sera, $P < 0.005$). IgA class was present in 19 relatives (20.4%) of whom 13 (14%) were IgA alone and six (7%) were both IgA and IgG. Ten (10.7%) were IgG only.

Of the 73 relatives subjected to biopsy (Table) 22 (30%) had IgA and/or IgG antibodies of whom 10 (14%) were IgA alone, six (8%) were IgG and IgA, and six (8%) were IgG alone. Thirteen subjects (18%) had grade III histology, compatible with coeliac disease. Ten of those 13 (77%) were IgA type reticulin antibody positive of whom three were IgG class positive also. Four subjects had grade II histology, one of whom had IgA class antibodies. Of the 30 who were grade I histologically, four had the IgG class and one of them had IgA as well. Of 26 with grade 0 biopsy, there were three IgG only positive, two with IgA class only, and two subjects with both. This represents a significantly increased incidence of the IgA class antibody in grade III histology compared with those relatives who had grade 0 and grade I ($P < 0.005$). No statistical differences were found when comparing the IgG class antibody. Of the five relatives with IgA class antibody positive and with non-specific histological findings, increased plasma cell counts in the lamina propria in the range found in treated coeliac subjects were present in three patients (IgA and IgG positive), in whom the epithelial lymphocytes were increased in one and at the upper limits of normal in a second. In the remaining two patients (IgA positive only) the counts were completely normal. Increased cell counts were noted in many of the relatives submitted to biopsy with non-specific histological findings and negative IgA reticulin antibodies, while the incidence of HLA-8 was also increased over that found in normal control subjects.

Discussion

The findings in this study are similar to, although somewhat greater than, those of Stevens *et al.* (1975), who, using polyvalent antihuman antiserum, found that the reticulin antibody was positive in 18% of the 121 relatives of the coeliac patients studied. In our study, of the relatives who underwent jejunal biopsy, 18% had histological findings compatible with coeliac disease, of whom 77% had IgA class antibody activity. In the known untreated coeliac subjects who were studied, the IgA class antibody was the predominant class. These results confirm the findings of Magalhaes *et al.* (1974). They are, however, different from those of Seah *et al.* (1971b) and Brown *et al.* (1973) who found that the IgG class predominated in children with coeliac disease. For this we have no obvious explanation, but differences in age may be important. Magalhaes *et al.* (1974) reported no IgA class reticulin antibody positives in a variety of diseases and in the normal control subjects they studied. In our control subjects, who included patients with Crohn's disease, ulcerative colitis, and hiatus hernia, no example of a positive IgA class reticulin antibody was encountered, though IgG class antibodies were present in a number.

According to our results, by performing the IgA class reticulin antibody test we could pick up 10 out of 13 asymptomatic relatives with coeliac disease and one with a moderately abnormal jejunal biopsy (grade II), but there were three (4%) false negatives (of grade III histology) and five (7%) false positive (grade 0 or I histology). In the five relatives with IgA class antibody positive and normal jejunal histology, cell counting of the epithelial and the lamina propria showed a significant increase in plasma cell counts in the lamina propria in three patients and of intraepithelial lymphocytes in one patient. Such patients must be regarded as potential coeliac patients and will be kept under review. There were no distinguishing clinical features in the three relatives with grade III jejunal histology who were reticulin antibody negative. One, who had no symptoms, refused a gluten-free diet, one showed an excellent response to a gluten-free diet, and the third had a poor response. All three had HLA antigens A1.B8.

In conclusion, our data support the idea that the IgA class anticonnective tissue antibody test could

be a useful screening test in relatives of coeliac patients to help to decide who must have a jejunal biopsy.

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