IgA and IgG reticulin antibodies in coeliac and non-coeliac patients

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SUMMARY Using indirect immunofluorescence, comparisons were made of patterns of staining of five tissue substrates with 28 R1 reticulin antibody-positive coeliac sera, and 23 R1 reticulin antibody-positive non-coeliac sera. IgA and IgG fluorescein conjugates were used separately. IgA antibodies were seen in 22 coeliac patients (78%) compared with three non-coeliacs (13%). Concomitant sinusoidal fluorescence (RS pattern) was seen more frequently with the non-coeliac (60-9%) than with the coeliac sera (10-7%). Such differences may help to distinguish those patients who should have a jejunal biopsy when using the reticulin antibody as a screening test for coeliac disease.

The reticulin antibody occurs in 36-75% of patients with coeliac disease and in patients with dermatitis herpetiformis (Seah et al., 1971; Alp and Wright, 1971). Prevalence of this antibody is particularly high among childhood coeliacs, being found in 67-93% of cases (Brown et al., 1973; Seah et al., 1973). The reticulin antibody has proved useful as a screening test for subclinical coeliac disease (Berrill et al., 1975; Stevens et al., 1975; Wilson et al., 1976). However, it may also be present in the sera of patients with Crohn’s disease (Alp and Wright, 1971) as well as in patients with a variety of other conditions and in apparently healthy individuals (Rizzetto and Doniach, 1974; Stevens et al., 1975).

Seah et al. (1971) described three patterns of reticulin antibody fluorescence on rat tissue substrates with coeliac sera. The pattern seen most commonly was a diffuse staining between kidney tubules and around Bowman’s capsule, liver sinusoidal fluorescence, and staining in the adventitia of blood vessels. Rizzetto and Doniach (1974) subsequently described five patterns of reticulin antibody fluorescence. They designated the original pattern of Seah (1971) as R1 reticulin antibody, emphasising perportal staining on liver tissue. R1 positive sera also show strong fluorescence around central veins. The R2 pattern included perivascular staining, with fluorescence around the portal tracts of the liver without extension into the lobule, and without kidney peritubular or Bowman’s capsule staining. The third type, the RS antibody, was a diffuse sinusoidal fluorescence combined with a variety of appearances in other organs. This occurred commonly in combination with an R1 type antibody. The other two types of reticulin antibodies described by Rizzetto and Doniach (1974) were the Kupffer cell antibody and sinusoidal adherent cell antibody.

It has been our impression that only the R1 type of reticulin antibody shows a significant association with coeliac disease. However, even this antibody may occur in the sera of non-coeliac patients. We have therefore undertaken to examine for further differences in staining pattern, immunoglobulin class, and concomitant fluorescence between R1 reticulin antibodies occurring in coeliac and in non-coeliac patients, using five tissue substrates and IgG and IgA fluorescein conjugates separately. An IgM conjugate was not used, as a pilot study failed to show any IgM fluorescence in a batch of reticulin antibody-positive sera.

Methods

Selection of sera

The sera of 28 coeliac patients with a reticulin antibody of the R1 type as defined by the presence of kidney peritubular and/or liver central vein connective tissue immunofluorescence were randomly selected from stored samples collected over four years.

The clinical details of the patients included in the study were reviewed; jejunal villous atrophy and a
Table  Distribution of IgG and IgA fluorescence patterns on rat kidney and liver tissue with coeliac and non-coeliac reticulin antibody positive sera

<table>
<thead>
<tr>
<th></th>
<th>Kidney</th>
<th>Liver</th>
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<tr>
<td></td>
<td>With IgA reticulin antibodies (%)</td>
<td>Peritubular and periglomerular fluorescence</td>
</tr>
<tr>
<td>Total patients (no.)</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Coeliac</td>
<td>23</td>
<td>3</td>
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good clinical response to a gluten-free diet had been demonstrated in all patients. The age range was 10 months to 62 years (mean age 29.2 years); seven of the patients being under the age of 15 years. Histological improvement was seen in nine of 10 patients who had a repeat jejunal biopsy. One patient, aged 62 years, still had a moderate degree of villous atrophy 12 months after starting her diet, but had made a marked clinical response. In 25 patients, the serum had been taken before starting a gluten-free diet; the remaining three were on a gluten-free diet.

Twenty-three control sera were randomly selected from non-coeliac reticulin antibody-positive samples with the R1 pattern which had been tested for a routine autoimmune profile during the past four years. None of the controls had any clinical or biochemical evidence of coeliac disease. Their ages ranged from 12 years to 66 years (mean age 38.4 years) and two were aged less than 15 years. Thirteen had a jejunal biopsy which showed normal histology.

In some of the subjects, more than one type of reticulin antibody was present, the combination of R1 with RS being more frequently seen among the non-coeliac patients.

**IMMUNOFLOUORESCENCE METHODS**

Sera were coded and stored at -20°C until tested. Immunofluorescence studies were carried out on cryostat sections of rat kidney, rat liver, rat salivary gland, mouse stomach, and mouse jejunum, using serum diluted 1 in 10. Fluorescein conjugated antihuman immunoglobulin and monovalent anti-IgG and anti-IgA anti-sera were obtained from Wellcome Reagents Ltd., and used at their optimal dilution. Sections were observed by fluorescent incident light microscopy, using a Zeiss Universal microscope equipped with a HBO 200 light source. Fluorescence patterns were read blind by one of us (R.L.). All moderate to strong reactions were photographed using Kodak 2475 Recording film.

**Results**

The distribution of IgA and IgG fluorescence patterns with coeliac and non-coeliac sera on rat liver and kidney sections is shown in the Table. There were no significant differences in patterns of fluorescence observed with the coeliac and non-coeliac sera on rat stomach, rat salivary gland, and mouse jejunum. IgA antibodies of the R1 type occurred significantly more frequently among the coeliacs (22) than non-coeliacs (three) (p = <0.001).

The pattern of fluorescence seen most frequently with the coeliacs was peritubular and Bowman's capsule staining. Twenty-six had an IgA and/or IgG antibody with this pattern, whereas this pattern was seen in only 12 out of 23 of the non-coeliac patients. In the liver the distribution of central vein fluorescence was similar among the coeliacs (24) and non-coeliacs (21), but sinusoidal fluorescence occurred with a significantly greater frequency among the non-coeliac patients (14) than the coeliacs (three) (p = <0.001).

**Discussion**

The results confirm a significant association between IgA R1 reticulin antibodies and coeliac disease, whereas antibodies of this immunoglobulin class are unusual in patients who are reticulin antibody-positive and have no clinical evidence of coeliac disease. Of the three coeliacs who had an IgA reticulin antibody, one patient, a schizophrenic, had a normal jejunal biopsy. The remaining two patients were both suffering from liver disease, one drug induced and the other alcoholic. Neither had a jejunal biopsy. Likewise, a jejunal biopsy was not felt to be indicated in a further eight control patients with a variety of disease states, some serious, not
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IgA and IgG reticulin antibodies in coeliac and non-coeliac patients. However, in both studies only small numbers of reticulin antibody-positive non-coeliacs were included, so the comparable prevalence of IgA antibodies in non-coeliacs was not established. In Magalhaes et al.'s study there was a significant reduction in the IgA reticulin antibodies in patients on a gluten-free diet. In the current study IgA reticulin antibodies were present in all three patients on a gluten-free diet. We were also able to examine further sera in three other coeliac patients with IgA reticulin antibodies after starting a gluten-free diet, and in all patients there was a reduction in titre of the antibodies, although they did not completely disappear.

MaWhinney and Love (1975) have shown that coeliac patients also secrete reticulin antibodies of IgA class into the jejunal juice. It seems likely, therefore, that the serum IgA reticulin antibodies found in coeliac disease are derived from IgA plasma cells in the gut mucosa. This would be consistent with another study by MaWhinney and Love (1975) in which they demonstrated an enhanced IgA antibody response to oral polio virus vaccine among coeliac patients.

It would appear that the fluorescence pattern that discriminated best between coeliac and non-coeliac patients was peritubular and Bowman's capsule staining in the rat kidney. This pattern was also frequently seen among coeliacs in the study by Williamson et al. (1976), who attributed it to endothelial basement membrane fluorescence. Liver sinusoidal fluorescence was seen commonly among the non-coeliacs, but occurred infrequently in the coeliacs.

On the basis of this study, we would recommend that, in patients in whom a reticulin antibody is found in the serum on routine testing for autoantibodies, in the absence of any other evidence of coeliac disease, the serum should be retested using an IgA conjugate. If this should prove positive, there is strong evidence for an underlying coeliac disorder and jejunal biopsy should be undertaken.

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References


