HLA-B8 and cell-mediated immunity to gluten

F G SIMPSON, A W BULLEN, D A F ROBERTSON,
AND M S LOSOWSKY

From the Department of Medicine, St. James's University Hospital, Leeds

SUMMARY The leucocyte migration inhibition (LMI) test was used as an indicator of cell mediated immunity to gluten fraction III in 30 healthy controls and 58 patients with adult coeliac disease and the results related to HLA status and duration of treatment with a gluten-free diet. HLA-B8 controls showed significantly lower leucocyte migration indices, indicating greater immune response, than non-HLA B8 controls. Untreated coeliacs showed no difference from HLA-B8 controls. There was no difference between results from HLA-B8 and non-HLA-B8 coeliacs. Leucocyte migration was even lower in coeliacs early in treatment but rose after treatment for over one year. These results may reflect an immune response gene for gluten in linkage disequilibrium with HLA-B8. The increased immune response to gluten as measured in this test cannot be the sole factor in the aetiology of coeliac disease. Furthermore, it is necessary to re-evaluate earlier results of cell-mediated immunity in coeliac disease with reference to HLA status of the controls.

About 80% of patients with coeliac disease have the HLA-B8 antigen compared with about 20% of the normal population.^{1 2}

Using the leucocyte migration inhibition (LMI) test, increased cell mediated immunity to gluten in coeliac disease compared with controls has been demonstrated³⁻⁶ and this is more frequent in patients taking a gluten free diet.^{4 5} It has been suggested that these findings are specific for coeliac disease⁵ and may be helpful as a diagnostic test.^{3 6}

There is some evidence, however, that possession of HLA-B8 itself confers a generalised increase in humoral immune responsiveness⁷ and it has been demonstrated that HLA-B8 individuals with a variety of conditions have higher titres of anti-gluten antibodies in serum than those lacking HLA-B8.⁸ As an additional point of relevance to the mechanism of coeliac disease, it has been suggested that lymphocytes from healthy HLA-B8 controls show transformation with gluten.⁹

We have, therefore, re-examined the LMI test in normal controls and patients with coeliac disease and related the results to HLA status and to the effects of a gluten free diet.

Methods

PATIENTS AND CONTROLS

Fifty-eight patients with adult coeliac disease were studied. All had typical jejunal biopsy abnormalities with biopsy-proven improvement on gluten withdrawal. There were two families where one parent and one child were both affected and one where three siblings had the disease. Twenty-six patients were studied while on a normal diet, 25 had been taking a gluten free diet for between three months and one year, and 32 had been on a gluten free diet for over one year at the time of study. The 30 controls were healthy hospital personnel.

LEUCOCYTE MIGRATION INHIBITION TEST

This test was performed using a capillary tube method as previously described.⁴ The antigen used was gluten fraction III prepared from BDH gluten according to the method of Frazer *et al.*¹⁰ and was used at a concentration of 1 mg/ml. Migration areas were measured by planimetry on traced projected images of the migration chambers and migration index calculated as mean area of migration in the presence of antigen divided by mean area of migration of control chambers.

HLA TYPING

HLA typing for A and B loci was performed by the standard NIH microlymphocytotoxicity test¹¹ using a bank of 60 sera.

Results

There were no differences between area of migration of control cultures in the different groups nor evidence of variability of migration index with time in controls or patients, except when dietary status was altered. One HLA-B8 control was tested five times over the period of the study and the coefficient of variation of these results was 9%.

Leucocyte migration indices in the various groups divided according to the presence or absence of HLA-B8 are shown in Fig. 1. In normal controls who have HLA-B8 there is a significantly lower migration index (mean 0.88, SD 0.08), indicating greater immunity compared with those lacking HLA-B8 (mean 0.98, SD 0.10, $P < 0.02$). Untreated coeliacs, whether HLA-B8 or not, showed no difference from HLA-B8 controls (HLA-B8: mean 0.88, SD 0.12; non-HLA-B8 mean: 0.91, SD 0.15). Coeliacs early in treatment (three months to one year) showed depressed migration compared with untreated patients (HLA-B8: mean 0.79, SD 0.06; non-HLA-B8: mean 0.76, SD 0.05, $P < 0.025$), which improved after treatment for over one year (HLA-B8: mean 0.88, SD 0.09; non-HLA-B8: mean 0.88, SD 0.06). It is

Fig. 1 Leucocyte migration indices to gluten fraction III in controls, untreated coeliacs, and coeliacs treated with a gluten free diet for between three months and one year ('early') and over one year ('late').

Fig. 2 Leucocyte migration indices to gluten fraction III in six patients studied sequentially at different stages of treatment.

noteworthy that HLA-B8 and non-HLA-B8 coeliacs gave similar results at all stages.

Six patients were studied untreated and after treatment for both less than and over one year. Results are shown in Fig. 2 and show a significant fall in migration index early in treatment ($P < 0.0125$) with a later rise ($P < 0.025$).

Discussion

One possible explanation for the high prevalence of HLA-B8 in coeliac disease is that possession of HLA-B8 is associated with an increased immune response to gluten. Immune response (Ir) genes which modulate humoral and cellular immune responses to specific antigens have been described in several species and are commonly linked to genes of the major histocompatibility systems.¹²⁻¹⁵ In man the position is less clear, as the antigens used for testing are often complex and presumably bear multiple determinants. However, HLA status has been shown to affect immune response to several antigens in man¹⁴⁻²³ and genetic analysis in one study has suggested that a single immune response gene is involved despite the complex nature of the antigen.²³ Our results and previous work^{8,9} are consistent with there being an Ir gene for gluten in linkage disequilibrium with HLA-B8. Family studies are being undertaken to further investigate this hypothesis.

Production of leucocyte migration inhibitory factor (LIF) by peripheral blood lymphocytes of coeliacs⁵ and by jejunal mucosa from untreated coeliacs cultured with gluten^{24, 25} has been cited as evidence for cell-mediated immunity to gluten being involved in the production of damage to jejunal mucosa seen in coeliac disease. Our results support the suggestion that cellular responses to gluten are important in the pathogenesis of the mucosal lesion as HLA-B8 and non-HLA-B8 coeliacs show similar responses in the LMI test. However, the observation that many HLA-B8 normal individuals also possess the proposed Ir gene for gluten suggests that possession of the gene may be necessary but is not sufficient for the development of coeliac disease, a position similar to that seen in HLA linked immune responses to ragweed pollen and the development of clinical hay fever.¹⁶

We have confirmed earlier findings^{4, 5} that LIF production in the peripheral blood of coeliacs is increased when a gluten free diet is started and also observed that this falls again if treatment is continued for more than one year. The initial fall may represent release into the blood of gluten-reactive lymphocytes which were 'trapped' in the gut on the gluten-containing diet.⁵ Antigen specific trappings of lymphocytes in the gut has been demonstrated in rats sensitised to cholera toxoid.²⁶ The later decrease in LIF production presumably represents a decline in the number of gluten-reactive cells after prolonged lack of antigenic stimulation.

Some workers have suggested that the leucocyte migration inhibition test may be useful in the diagnosis of coeliac disease.^{3, 6} Our results suggest that much of the difference shown between coeliacs and normal subjects is related to the HLA status of the control group, which, if unselected, would be expected to be composed largely of non HLA-B8 individuals. Previous work needs to be re-evaluated with reference to HLA status of controls before the LMI test in any form can be considered as a diagnostic aid.

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