

CLINICAL RESEARCH

α Gliadin antibody levels: a serological test for coeliac disease

CLIONA O'FARRELLY, J KELLY, W HEKKENS, B BRADLEY, A THOMPSON,
C FEIGHERY, D G WEIR

Abstract

The diagnostic value in coeliac disease of circulating antibodies to casein, crude gliadin, and α gliadin was assessed using an adaption of the enzyme linked immunosorbent assay system. α Gliadin was the only antigen which consistently separated 26 patients with untreated coeliac disease from 26 normal controls and 13 patients with chronic inflammatory bowel disease. The mean assay index for the 26 patients was 3.1 (SD 1.2) compared with 1.05 (0.5) for the normal controls and 1.1 (0.6) for patients with chronic inflammatory bowel disease. The α gliadin antibody levels of six patients with coeliac disease who had maintained a gluten free diet for at least two years were not significantly higher than normal (1.0 (0.4)). The validity of the test was determined in 90 consecutive patients who were being investigated for the presence of coeliac disease. Levels of α gliadin antibody were raised in 36 out of 44 patients found to have histologically proved coeliac disease and in six out of 46 subjects whose jejunal mucosa was normal. Serial α gliadin concentrations were measured in 12 patients with coeliac disease who had repeat jejunal biopsies performed six months after starting a gluten free diet. The levels of antibody fell in seven of the eight patients whose jejunal mucosa improved on maintaining the

diet. They remained raised in four patients who did not adhere to the diet and whose mucosa did not improve.

Although a test measuring α gliadin antibodies is unlikely to replace jejunal biopsy in the diagnosis of coeliac disease it may be useful in screening for the disease among outpatients.

Introduction

Patients with coeliac disease have been shown to have circulating antibodies to wheat proteins.¹⁻⁶ The diagnostic and pathogenic importance of this finding diminished, however, when similar antibodies were found in patients with other gastrointestinal disorders²⁻⁶ and when circulating antibodies to other dietary antigens were found in patients with coeliac disease.^{2-3,7} Antibodies to dietary proteins have also been detected in normal individuals.^{5,8} These reports must be interpreted in the context of current concepts which suggest that food protein antigens are readily absorbed across the normal gastrointestinal mucosa and are cleared from the circulation after immune complex formation with specific antibody.⁹ Enhanced antigen absorption occurs when the mucosa is damaged, together with increased antibody production.¹⁰

Previous reports have, however, suggested that in patients with coeliac disease there is an increased immunological reactivity to α gliadin, a purified wheat protein.^{11,12} This sensitivity appears to be specific for coeliac disease^{11,13} and may be of diagnostic use. In this study we specifically investigated the humoral response to α gliadin and contrasted the results with sensitivity to crude gliadin and a milk protein, casein. We used the enzyme linked immunosorbent assay which detects specific antibody levels and has a sensitivity and reproducibility comparable with those of radioimmunoassay.¹⁴

We consistently observed an enhanced humoral response to α gliadin in untreated patients with coeliac disease but not in other subjects tested. Accordingly, we decided to evaluate the measurement of α gliadin antibody levels in routine screening for coeliac disease. We also examined the association between the α gliadin antibody levels and the histological response to treatment with a gluten free diet.

Departments of Immunology and Clinical Medicine, Trinity College, and St James's Hospital, Dublin, Ireland

CLIONA O'FARRELLY, PHD, research fellow
J KELLY, BA, research assistant
B BRADLEY, MB, MRCPI, medical registrar
A THOMPSON, MB, MRCPI, medical registrar
C FEIGHERY, MB, MRCPI, consultant immunologist
D G WEIR, MD, FRCPI, regius professor of medicine

Physiology Department, University Hospital, Leiden, The Netherlands

W HEKKENS, PHD, senior lecturer

Correspondence to: Professor D G Weir, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Ireland.

Patients and methods

ENZYME LINKED IMMUNOSORBENT ASSAY

Plastic cups (Removastrip Greiner) were coated with 2 µg antigen/ml in carbonate buffer pH 9.6 by incubating them overnight at 4°C. After the cups had been washed four times with buffer of Tween and phosphate buffered saline, serum diluted 1/1000 with Tween-

phosphate buffered saline was added. The cups were incubated for three hours at room temperature. The washing was repeated, and antihuman immunoglobulin (IgG) conjugated to horse radish peroxidase (Cappel Laboratories) diluted 1/1000 with Tween-phosphate buffered saline was added. The cups were incubated for a further three hours at room temperature. After they had been washed again orthophenylene diamine, the substrate, dissolved in a citrate phosphate buffer, was added to the cups. The cups were incubated at room temperature for 30 minutes. The colour reaction was then stopped by adding 2 mol sulphuric acid/l. The colour intensity was determined with a Dynatech minireader and the results expressed as enzyme linked immunosorbent assay indices (EI), which were derived as follows:

$$EI = \frac{\text{mean (of 3) optical density readings of test}}{\text{mean (of 3) optical density readings of background}}$$

ANTIGENS

Gliadin was prepared from Scout 66 wheat flour by extraction with 60% (v/v) ethanol.⁵

α Gliadin was prepared from crude gliadin by ion exchange chromatography according to a modification of the technique used by Kendall *et al.*¹⁵

Casein was obtained commercially from Sigma.

PATIENTS AND STUDIES

Dietary protein antibody levels—The antibody response to α gliadin, and casein was assessed in four groups of subjects. Sera were obtained from 26 untreated patients with coeliac disease as defined by a characteristic jejunal lesion and a subsequent satisfactory clinical and histological response to dietary gluten withdrawal. As α gliadin antibody concentrations do not vary with age or sex in normal adults (unpublished observations), control groups comprised 26 healthy medical students and laboratory staff and 13 patients with chronic inflammatory bowel disease, nine of whom had Crohn's disease and four ulcerative colitis. A further group included six treated patients with coeliac disease who had maintained a strict gluten free diet for at least two years and whose jejunal mucosa showed a return to virtually normal.

Diagnostic value of raised α gliadin antibody levels—Ninety consecutive patients being investigated for coeliac disease had their serum α gliadin antibody levels measured and jejunal biopsy specimens taken simultaneously and examined independently. An enzyme linked immunosorbent assay index of greater than twice the mean of the normal control group represented a positive response.

Response to dietary gluten withdrawal—The α gliadin antibody response to a gluten free diet was examined in 12 untreated patients with coeliac disease. Serum samples were obtained at one, three, and six months after they had started a gluten free diet, and repeat jejunal biopsies were performed after six months.

STATISTICS

Analysis of variance was used to test whether observed differences in antibody levels in the four groups in the initial study could be attributed to chance or whether they indicated actual differences among the corresponding population means. The χ² test was used to assess the association between positive antibody results and abnormal jejunal mucosa in the 90 patients being investigated for coeliac disease. The paired *t* test was used to examine the effect of a gluten free diet on α gliadin antibody levels.

Results

Dietary protein antibody levels—The table shows levels of antibodies of α gliadin, gliadin, and casein in the 26 untreated patients with coeliac disease and the three control groups. When α gliadin was used as an antigen there was a highly significant difference between the patients with untreated coeliac disease and those in the other three groups (fig 1; *p* < 0.001). There were no significant differences between any of the groups with gliadin or casein.

Diagnostic value of α gliadin antibody levels—To assess the discriminatory value of α gliadin antibody levels in 90 patients referred

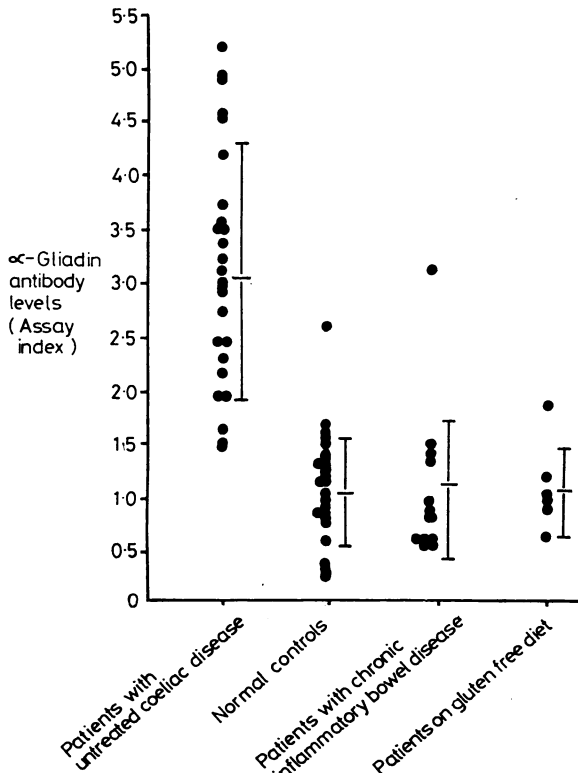


FIG 1—Levels of antibodies to α gliadin in untreated patients with coeliac disease, normal controls, patients with chronic inflammatory bowel disease, and patients with coeliac disease treated with gluten-free diet. Results are expressed as enzyme linked immunosorbent assay indices. Means and standard deviations are indicated by vertical lines.

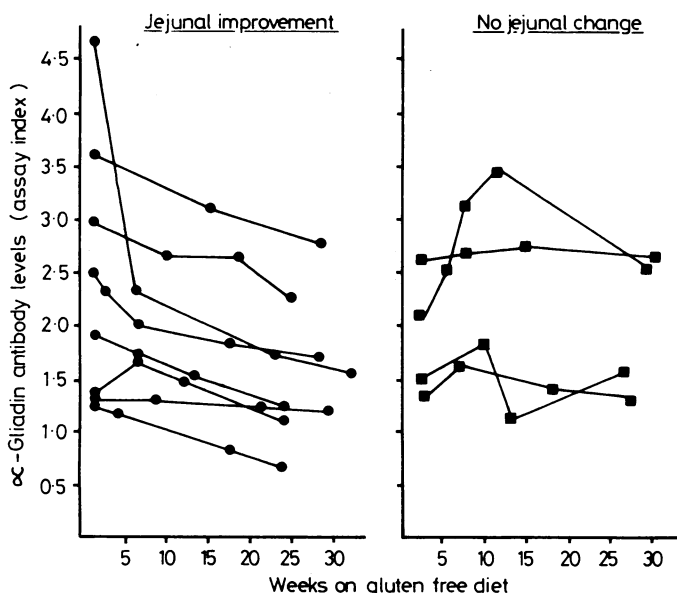


FIG 2—Variation of α gliadin antibody levels over time in patients who maintained a gluten free diet and showed jejunal improvement and in patients who failed to keep to the diet and showed no jejunal response. Results are expressed as enzyme linked immunosorbent assay indices.

Levels of antibodies to three dietary antigens in patients with untreated coeliac disease and three control groups. Results are mean (SD) enzyme linked immunosorbent assay indices

| | α Gliadin | Gliadin | Casein |
|--|------------------|------------|------------|
| Patients with untreated coeliac disease | 3.1 (1.2) | 0.8 (0.3) | 0.9 (0.75) |
| Normal controls | 1.05 (0.5) | 0.6 (0.3) | 0.5 (0.4) |
| Patients with chronic inflammatory bowel disease | 1.1 (0.6) | 0.7 (0.25) | 0.5 (0.45) |
| Patients on gluten free diet | 1.0 (0.4) | 0.6 (0.2) | 0.5 (0.3) |

for investigation for coeliac disease we used an index value of 2.1 or over as indicating a positive result in the antibody test. This value was twice that of the mean for the 26 normal controls. The antibody test was positive in 36 of the 44 patients who were shown to have histologically proved coeliac disease but in only six of the 46 patients whose jejunal mucosa was normal ($p < 0.001$).

Response of α gliadin antibody levels to dietary gluten withdrawal—In the eight patients who adhered to the diet and whose jejunal mucosa improved there was a mean fall in α gliadin antibody levels from 2.09 (SD 0.89) to 1.33 (0.5) after six months ($t = 2.58$; $p < 0.05$). In seven of these eight patients the levels fell (fig 2). In contrast, there was no significant difference between mean α gliadin antibody levels before and after six months' treatment in the four patients who failed to adhere to their diet and whose mucosa remained abnormal (mean 1.88 (0.12) *v* 1.95 (0.32)).

Discussion

The results of this investigation show that levels of IgG antibody to α gliadin, a purified wheat protein, are significantly raised in patients with untreated coeliac disease when compared with values in other individuals. α Gliadin has been shown both *in vivo*^{15, 16} and *in vitro*¹⁷ to be more toxic to untreated coeliac jejunal tissue than either crude wheat preparations or other gliadin fractions. A recent investigation has shown that the α gliadins are more antigenic to patients with coeliac disease than other wheat proteins,¹⁸ while other work has indicated that immunological sensitivity to α gliadin is specific to coeliac disease.^{11, 13} Our findings confirm the specificity of the humoral response to this wheat protein.

Crude gliadin has often been the antigen of choice in previous studies.^{8, 19, 20} Stern *et al* found IgG anti-gliadin antibodies in all of the 30 children with untreated coeliac disease whom they studied.⁸ They also found these antibodies in 52% of other individuals with gastrointestinal disease, although the titres were lower than in the coeliac group. Savilahti *et al* found IgG anti-gliadin antibodies in all of 20 untreated children with untreated coeliac disease but emphasised that this finding was specific for children under 2 years of age.¹⁹ Unsworth *et al* found IgA gliadin antibodies in six out of seven children with coeliac disease taking normal diets.²⁰ The findings of studies on adult patients are conflicting. Using an immunofluorescent technique, Eterman and Feltkamp showed that half their adult patients with coeliac disease on normal diets had raised gliadin antibody levels,⁶ and Kieffer *et al*, using the mixed reverse solid phase passive antiglobulin haemadsorption technique, found that 11 out of 12 untreated adults with coeliac disease had titres of gliadin antibodies higher than those of normal subjects.²¹ Half the patients with other gastrointestinal disorders, however, also had raised titres of gliadin antibodies, although in most cases the titres were not as high as those found in the coeliac group.²¹

In this study we have shown that the use of α gliadin as antigen in the enzyme linked immunosorbent assay provided a test which clearly discriminated between adults with coeliac disease and those without. A highly significant association ($p < 0.001$) was found between positive α gliadin antibody responses and the presence of abnormal jejunal biopsy findings and between negative antibody responses and normal jejunal mucosa. The sensitivity of the test was 82% and the specificity 87%. The assay is simple to perform. Unlike the immuno-

fluorescent tests, it is objective and does not require the technical expertise demanded by the mixed reverse solid phase passive antiglobulin haemadsorption technique. Although this test is unlikely to replace jejunal biopsy in the diagnosis of coeliac disease, it should be of use in screening for the disease in outpatient populations—an important consideration in view of the high incidence of coeliac disease in Great Britain²² and Ireland.²³

In 12 patients the response to gluten free diet was monitored by serial antibody estimations. Eight showed histological and clinical improvement after six months, indicating strict adherence to a gluten free diet, and in seven the α gliadin antibody levels fell. Four patients showed no histological or clinical response owing to inadequate compliance with the diet, and their α gliadin antibody levels showed no overall change. Six patients who had adhered strictly to a gluten free diet for over two years had antibody levels which were not significantly higher than those of the normal control population, suggesting that strict adherence to a diet does eventually produce normal α gliadin antibody levels. The change in α gliadin antibody levels in most patients, however, was gradual, indicating that a repeat jejunal biopsy remains the best method for showing a positive response to treatment.

There was no evidence from this study that the level of antibody was associated with the clinical severity, extent, or duration of the untreated lesion. The absence of any such relationship might result from the variable degree of immunoregulatory abnormality that occurs in coeliac disease.²⁴ α Gliadin has been shown to generate suppression in the cells of some patients with coeliac disease.²⁵ These cells may be responsible for the lower α gliadin levels seen in some patients with coeliac disease.

We thank Dr G McDonald for carrying out the histological studies and the Medical Research Council of Ireland and the Nutrition Fund of the Provost's Appeal, Trinity College, Dublin, for financial support.

References

- Immonen P. Precipitins to cows' milk and wheat protein in gastrointestinal disorders. *Acta Paediatr Scand* 1965;159, suppl:104.
- Ferguson A, Carswell F. Precipitins to dietary proteins in serum and upper intestinal secretions of coeliac children. *Br Med J* 1972;ii:75-7.
- Kenrick KG, Walker-Smith JA. Immunoglobulins and dietary protein antibodies in childhood coeliac disease. *Gut* 1970;11:635-40.
- Mietens C. Immunological aspects of coeliac disease. *Paediatr Paedol* 1973;8:68-79.
- Eterman KP, Hekkens WTJM, Pena AS, Lems-van Kan PH, Feltkamp TEW. Wheat grains: a substrate for the detection of gluten antibodies in serum of gluten-sensitive patients. *J Immunol Methods* 1977;14:85-92.
- Eterman KP, Feltkamp TEW. Antibodies to gluten and reticulin in gastrointestinal disease. *Clin Exp Immunol* 1978;31:92-9.
- Kivel R, Kearns DH, Liebowitz D. Significance of antibodies to dietary proteins in the serum of patients with non-tropical sprue. *N Engl J Med* 1964;271:769-72.
- Stern M, Fischer K, Gruttner R. Immunofluorescent serum gliadin antibodies in children with coeliac disease and various malabsorptive disorders. *Eur J Pediatr* 1979;130:155-64.
- Paganelli R, Levinsky RJ, Brostoff J, Wraith DG. Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption. *Lancet* 1979;ii:1270-2.
- Walker-Smith JA. Gastrointestinal allergy. *Practitioner* 1978;220:562-73.
- Haeney MR, Asquith D. Inhibition of leucocyte migration by alpha gliadin in patients with gastrointestinal disease: its specificity with respect to coeliac disease and alpha gliadin. In: McNicholl B, McCarthy CF, Fottrell PF, eds. *Perspectives in coeliac disease*. Lancaster: MTP Press, 1978:229.
- O'Farrelly C, Hekkens WTJM, Feighery C, Weir DG. The specificity of wheat protein reactivity in coeliac disease. *Scand J Gastroenterol* (in press).
- O'Farrelly C, Feighery C, Grealley J, Weir DG. Cellular response to alpha gliadin in untreated coeliac disease. *Gut* 1982;23:83-7.
- Anonymous. ELISA—replacement for radioimmunoassay? *Lancet* 1976;ii:406-7.
- Kendall MJ, Cox PS, Schneider R, Hawkins CF. Gluten subfractions in coeliac disease. *Lancet* 1972;ii:1065-7.

- ¹⁶ Hekkens WTJM, Haex AF, Willighagen RGJ. Some aspects of gliadin fractionation and testing by a histochemical method. In: Booth CC, Dowling RH, eds. *Coeliac disease*. London: Churchill Livingstone, 1970:11.
- ¹⁷ Howdle PD, Ciclitira PJ, Simpson FG, Losowsky MS. Are all gliadins toxic in coeliac disease? *Gut* 1981;**22**:A874.
- ¹⁸ Kieffer M, Frazier PJ, Daniels NWR, Coombs RRA. Wheat gliadin fractions and other cereal antigens reactive with antibodies in the sera of coeliac patients. *Clin Exp Immunol* 1982;**50**:651-60.
- ¹⁹ Savilahti E, Perkkio M, Kalimo K, Viander M, Vainio E, Reunala T. IgA anti-gliadin antibodies: a marker of mucosal damage in childhood coeliac disease. *Lancet* 1983;**i**:320-2.
- ²⁰ Unsworth DJ, Kieffer M, Holborow EJ, Coombs RRA, Walker-Smith JA. IgA anti-gliadin antibodies in coeliac disease. *Clin Exp Immunol* 1981;**46**:286-93.
- ²¹ Kieffer M, Frazier PJ, Daniels WWR, Ciclitira P, Coombs RRA. Serum antibodies (measured by MrsPAH) to alcohol soluble gliadins in adult coeliac patients. *J Immunol Methods* 1981;**42**:129-36.
- ²² Logan RF, Tucker G, Rifkind EA, Heading RC, Ferguson A. Changes in clinical features of coeliac disease in adults in Edinburgh and the Lothians 1960-79. *Br Med J* 1983;**286**:95-7.
- ²³ Stevens FM, Watt MD, Baker S, Egan-Mitchell B, McNicholl B, McCarthy CF. HLA antigens in coeliac patients and controls in the West of Ireland. In: McNicholl B, McCarthy CF, Fottrell PF, eds. *Perspectives in coeliac disease*. Lancaster: MTP Press, 1978:137.
- ²⁴ O'Farrelly C, McKeever U, Weir DG, Feighery CF. Increased suppressor cell activity in coeliac disease. *Irish J Med Sci* (in press).
- ²⁵ O'Farrelly C, McKeever U, Feighery C, Whelan CA, Weir DG. Alpha gliadin sensitive suppression in coeliac disease. *Gut* 1982;**23**:A884.

(Accepted 12 April 1983)

Seminal fluid excretion of cytomegalovirus related to immunosuppression in homosexual men

ROBERT J BIGGAR, H KERZEL ANDERSEN, PETER EBBESEN, MADS MELBYE, JAMES J GOEDERT, DEAN L MANN, DOUGLAS M STRONG

Abstract

Seminal fluid samples from 84 Danish homosexual men were successfully cultured to determine the prevalence of cytomegalovirus excretion. Ten (15%) out of 66 men positive for the antibody were found to be excreting the virus. Although the proportion excreting was inversely related to age ($p < 0.01$), three men aged over 30 and with many years of homosexual experience excreted the virus. In addition, a 50 year old man with Kaposi's sarcoma excreted the virus. A further study of the ratio of T cell helpers to suppressors in the men aged over 30 and a series of age matched non-excreting homosexual control or heterosexual men showed that those excreting cytomegalovirus in their seminal fluid had statistically lower ratios (all < 0.77) than the controls ($p < 0.05$).

Excretion of cytomegalovirus may be related to re-emergence of latent infection in immunosuppressed homosexual men.

Introduction

Homosexual men have a high prevalence of antibodies against cytomegalovirus. In a recent study in San Francisco 94% of 139 homosexual men had antibodies against cytomegalovirus compared with 54% of 70 heterosexual men.¹ Data from Denmark show a similar difference in the prevalence of antibodies against cytomegalovirus between homosexual and heterosexual men.^{1a}

Venereal transmission of cytomegalovirus may occur between heterosexuals^{2,3} and might explain the high prevalence of antibodies to cytomegalovirus in homosexual men. Cytomegalovirus is excreted in many body fluids, including seminal fluid.^{4,5} The frequency of isolation of the virus depends on the age and habits of the men under study. One per cent of men attending infertility clinics were found to be excreting cytomegalovirus in their seminal fluid,^{6,7} compared with four out of 64 (6%) of sexually active male university students and men attending venereal disease clinics.⁶ Eight attempts to isolate cytomegalovirus from the urine and blood of three of these four men all yielded negative results.⁶ Thus seminal fluid may be the only body fluid in which excretion of cytomegalovirus can be shown.

No studies have focused on seminal fluid excretion of cytomegalovirus in homosexual men. Among 190 homosexual men in San Francisco, however, 7% were excreting the virus in their urine compared with none of 101 similarly aged heterosexual men.¹ Studies of cytomegalovirus in seminal fluid have included a few homosexual men—for example, two out of five seminal fluid samples positive for cytomegalovirus in one study were donated by homosexual men, but no information was given about how many homosexual men were studied.⁸ In another study of 389 Canadian men nine were known to be homosexual, and three of these were found to be excreting cytomegalovirus in the seminal fluid.⁷

We obtained seminal fluid samples from 101 homosexual Danish men in an effort to establish a more reliable estimate of the prevalence of excretion of cytomegalovirus in this fluid, as it is the most probable source of exposure in homosexual men. In addition, we determined the ratio of T cell helpers to suppressors in selected men to examine the relation between viral excretion and immunosuppression.

Division of Cancer Cause and Prevention, National Institutes of Health, Bethesda, Maryland 20205, USA

ROBERT J BIGGAR, MD
JAMES J GOEDERT, MD

Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark

H KERZEL ANDERSEN, MD

Institute of Cancer Research, Aarhus, Denmark

PETER EBBESEN, MD
MADS MELBYE, medical student

Division of Cancer Biology and Diagnosis, National Institutes of Health

DEAN L MANN, MD

Department of Surgery, Uniformed Services University of Health Sciences, Bethesda, Maryland

DOUGLAS M STRONG, PHD

Correspondence and requests for reprints to: Dr R J Biggar, Landow Building 3C08, Environmental Epidemiology Branch, Division of Cancer Cause and Prevention, National Institutes of Health, Bethesda, Maryland 20205, USA.