

Specific IgG subclass antibodies, IgE and IgG S-TS antibodies to wheat gluten fraction B in patients with coeliac disease

P. M. RAWCLIFFE*, D. P. JEWELL* and J. A. FAUX†

*Gastroenterology Unit, Radcliffe Infirmary, Oxford and †Virology Department, John Radcliffe Hospital, Oxford, U.K.

(Received 2 August 1984; accepted for publication 10 August 1984)

Summary

Antibodies were measured in the sera of fifteen patients with untreated coeliac disease and twenty-eight patients with inflammatory bowel disease. Increased levels of specific IgG, IgG₁, IgG₂, and IgG₄ antibody to wheat gluten fraction B, measured by an enzyme-linked immunosorbent assay, were shown in the coeliac disease group, but not in the inflammatory bowel disease group. No specific IgE antibody to fraction B was detected but 33% of the patients with coeliac disease had specific short-term sensitizing (anaphylactic) IgG antibody activity (IgG S-TS) to fraction B. There was no correlation between the IgG₂ or IgG₄ specific antibody and the presence of IgG S-TS activity.

Introduction

Serum antibodies to wheat gluten have been frequently observed in the serum of patients with coeliac disease (Kenrick & Walker-Smith, 1970; Ferguson & Carswell, 1972; Kieffer *et al.*, 1981; Unsworth *et al.*, 1981). The significance of these antibodies in the pathogenesis of coeliac disease is still uncertain because antibodies to other food substances have been found in coeliac patients (Taylor, Truelove & Wright, 1964), and antibodies to gluten and these other foods have been demonstrated in patients with other gastro-intestinal disorders. This could suggest that antibody production may simply be due to increased antigen stimulation from increased absorption through a damaged intestinal mucosa.

Combined ultra-filtration and gel filtration of a peptic-tryptic digest of wheat gluten have been used to isolate toxic and non-toxic fractions (Dissanayake *et al.*, 1974; Anand, 1977). These fractions have been found to induce a late (3–8 hr) Arthus skin reaction in coeliac patients (Anand, Truelove & Offord, 1977) and late (3–4 hr) reactions in the small intestine on oral challenge (Anand *et al.*, 1981). These observations, and the finding of circulating immune complex in active coeliac disease (Doe, Booth & Brown, 1973; Jewell, 1979) and a fall in complement levels following *in*

vivo gluten challenge (Doe, Henry & Booth, 1975) have led to the suggestion that coeliac disease may represent an Arthus-type reaction in the small intestinal mucosa (Anand *et al.*, 1981).

The present study investigates the possibility that anaphylactic antibody, either IgE or short-term sensitizing IgG (IgG S-TS) (Parish, 1971; Goodwin, 1983) to a small toxic fraction of wheat gluten may provide a mechanism by which tissue-damaging immune complexes could be deposited in the intestinal mucosa. In addition total IgG and IgG subclass antibodies have been measured in these patients and in a group of patients with inflammatory bowel disease (IBD). An enzyme-linked immunosorbent assay (ELISA) has been used to detect specific IgG subclass antibodies to a fraction of a peptic-tryptic digest of wheat gluten, fraction B, which is known to cause histological damage to the small intestinal mucosa of patients with treated coeliac disease (Dissanayake *et al.*, 1974; Rawcliffe, unpublished observations).

Materials and methods

Patients

Sera were obtained, before treatment was instituted, from fifteen adult patients with coeliac disease, diagnosed on accepted clinical and histological criteria (Booth, 1975). Control sera were obtained from twenty-eight patients with active IBD—ulcerative colitis (20) and Crohn's disease (8)—and from healthy laboratory workers (17).

Antigens

Frazer's fraction III (a water-soluble fraction of a peptic-tryptic digest of wheat gluten; Frazer *et al.*, 1959) was prepared from gluten made from a single variety of wheat, Scout 66. Fractions A, B and C were made by ultra-filtration of fraction III using Amicon UM10 and XM100a membranes (Amicon Ltd, Stonehouse, U.K.) (Dissanayake *et al.*, 1974).

Egg albumin (grade V) was obtained from Sigma Chemical Co. Ltd, Poole, U.K., β -lactoglobulin from BDH Chemicals Ltd, Atherstone, U.K. and α -lactalbumin from Miles Laboratories Ltd, Slough, U.K.

Serological procedures

Radioallergosorbent test (RAST). RAST was carried out essentially as described by Wide, Bennich and Johansson (1967) using an allergosorbent prepared from gluten fraction B according to criteria described by Goodwin and How (1976). In some tests, sera (50 μ l) were absorbed prior to RAST by addition of protein-A Sepharose (Pharmacia Ltd, Milton Keynes, U.K.), 15 mg ^{125} I-labelled anti-IgE was also obtained from Pharmacia.

Enzyme-linked immunosorbent assay (ELISA). IgG antibodies to wheat gluten, egg albumin, β -lactoglobulin and α -lactalbumin were detected by the micro-ELISA test (Voller, Bidwell & Bartlett, 1976). Optimal concentrations of egg albumin (50 μ g/ml), β -lactoglobulin (50 μ g/ml) and α -lactalbumin (50 μ g/ml) were used to coat round-bottomed plastic wells (Removawells, M124A; Dynatech Laboratories Ltd, Billingshurst, U.K.). Optimal concentrations of wheat gluten fractions were determined in preliminary experiments using known positive sera. Where gluten fractions had more than one optimum concentration, both concentrations were used. All sera were

examined at a dilution of 1:100 and antibody was detected using an alkaline phosphatase-labelled anti-IgG preparation (Miles). IgG subclass antibodies to fraction B were detected by a modified ELISA technique (Scholes & Goodwin, unpublished observations) using polyclonal anti-IgG subclass sera (Seward Laboratory, London, U.K.; Royal Netherlands Blood Transfusion Service, PO Box 9190, 1006 AD Amsterdam, The Netherlands; and Nordic Immunological Laboratories Ltd, Maidenhead, U.K.) and/or monoclonal antibody preparations (Seward). Antibody bound to antigen in the plastic wells was detected using the relevant antiserum, and an alkaline phosphatase-labelled second antibody (anti-rabbit IgG, anti-sheep IgG, anti-pig IgG or anti-mouse IgG; Miles). Antigen concentrations were those used in the direct assay for IgG antibody.

In all ELISA tests, bound enzyme activity was measured by addition of p-nitrophenyl phosphate (Sigma) and the results expressed as the optical density at 405 nm of the hydrolysed substrate after correction for background activity in wells lacking test sera. The level of antibody was considered to be positive if the corrected optical density at 405 nm was greater than the mean plus two standard deviations of the value for normal healthy controls. Known positive and negative sera were included in each assay.

Monkey PCA for IgG S-TS antibody. IgG S-TS antibodies were detected by passive cutaneous anaphylaxis (PCA) tests in Cynomolgus monkeys (*Macaca cynomolgus fascicularis*) as described by Parish (1971). Sites on the thorax and abdomen were injected with heated (56°C, 2 hr) sera. Two hours later, 2 ml 5% Evans Blue dye was injected intravenously. Gluten fraction B (500 µg/ml) was injected intradermally into each site previously prepared by the injection of serum, and also into untreated skin. 20–30 min after challenge the sites were assessed for size and intensity of blueing. Reactions of at least 5 mm diameter were considered positive. Experiments were carried out blind, in two recipient monkeys for each serum, using undiluted sera.

Total serum IgE and IgG subclass determinations. Total IgE concentrations were measured by PRIST (Pharmacia). Total IgG₁₋₄ concentrations were measured by single radial immuno-diffusion (Mancini, Carbonara & Heremans, 1965; Scholes & Goodwin, unpublished observations) using Seward antisera. Data were analysed using the Student's *t*-test.

Results

IgG antibodies to wheat gluten

IgG antibodies to Frazer's fraction III and fractions obtained by ultra-filtration are shown in Table 1. Up to 80% of the patients with coeliac disease have IgG antibodies to one or more of these fractions, but only one patient with Crohn's disease and three patients with ulcerative colitis had antibodies to gluten fraction B. This fraction (polypeptides of around 8000 molecular weight) is the smallest fraction listed here known to be toxic (Dissanayake *et al.*, 1974) which is why it has been used in all further tests. In Table 2 it can be seen that IgG antibody to gluten fraction B is found in all four IgG subclasses. The highest incidence, twelve patients (80%), was found in the IgG₁ subclass, but eleven patients (73%) also have both IgG₂ and IgG₄ subclass antibodies to fraction B.

IgG S-TS antibody to gluten fraction B was detected in five (33%) of the coeliac patients, and in none of the healthy individuals. Four of these five patients had specific

Table 1. Incidence of IgG antibodies to wheat gluten fractions

Patient group (n)	Wheat gluten fraction			
	III	A	B	C
Coeliac disease (15)	5 (33%)	3 (20%)	8 (53%)	12 (80%)
Ulcerative colitis (20)	ND	ND	3 (15%)	ND
Crohn's disease (8)	ND	ND	1 (12%)	ND
Healthy controls (17)	ND	ND	0	ND

ND = not done.

Table 2. Correlation between IgG subclass antibodies and IgG S-TS antibodies to wheat gluten fraction B in coeliac patients

Patient	IgG ₁	IgG ₂	IgG ₃	IgG ₄	IgG S-TS	IgE
1	+	+	+	+	—	—
2	+	+	—	+	—	—
3	—	—	ND	—	—	—
4	+	+	+	+	—	—
5	+	—	—	—	—	—
6	+	+	+	+	+	—
7	+	+	+	+	—	—
8	+	—	—	+	—	—
9	+	+	+	+	—	—
10	+	+	—	+	+	—
11	—	+	—	+	+	—
12	+	+	—	+	+	—
13	+	+	—	—	—	—
14	+	+	+	+	—	—
15	—	—	—	—	+	—
Total	12 (80%)	11 (73%)	11 (73%)	6 (40%)	5 (33%)	0

* Detected using polyclonal antibody only.

ND = not done.

IgG₂ and IgG₄ antibody to fraction B, but seven of the other patients with specific IgG₂ and IgG₄ antibody did not have specific IgG S-TS antibody to fraction B. None of the patients had detectable specific IgE antibody to fraction B. No evidence was obtained for the presence of gluten-specific IgE antibody in the sera of the coeliac patients before or after absorption with protein A (to remove IgG₁, IgG₂ and IgG₄ antibody).

IgG antibodies to other dietary antigens

A high incidence of IgG antibodies to egg and cow's milk antigens was shown in the patients with coeliac disease (Table 3), but IgG antibodies to the dietary antigens were not detected in either the ulcerative colitis or the Crohn's disease group, except for five (25%) of the patients with ulcerative colitis having IgG antibody to egg albumin and two IBD patients having antibodies to lactalbumin.

Table 3. Incidence of IgG antibodies to egg albumin, β -lactoglobulin, and α -lactalbumin

Patient group (n)	Egg albumin	β -lactoglobulin	α -lactalbumin
Coeliac disease (15)	8 (53%)	11 (73%)	7 (47%)
Ulcerative colitis (20)	5 (25%)	0	1 (5%)
Crohn's disease (8)	0	0	1 (12%)

Table 4. Total IgE, IgG and IgG subclasses in the patient groups studied (mean $\pm$ s.d.)

Patient group (n)	IgG	IgG ₁	IgG ₂	IgG ₃	IgG ₄	IgE (i.u./ml)
Coeliac disease (13)	11.21 $\pm$ 3.75	7.91 $\pm$ 2.35*	3.16 $\pm$ 0.72‡	0.68 $\pm$ 0.31	0.35 $\pm$ 0.14	45.0 $\pm$ 65.8
Ulcerative colitis (20)	13.04 $\pm$ 4.49†	6.74 $\pm$ 2.35	3.64 $\pm$ 0.74‡	0.65 $\pm$ 0.38	0.57 $\pm$ 0.52	22.1 $\pm$ 26.2
Crohn's disease (8)	13.42 $\pm$ 3.68†	6.59 $\pm$ 2.96	3.31 $\pm$ 0.87‡	0.59 $\pm$ 0.30	0.58 $\pm$ 0.43	54.8 $\pm$ 66.9
Healthy controls (17)	9.28 $\pm$ 2.80	6.29 $\pm$ 1.47	1.90 $\pm$ 0.79	0.56 $\pm$ 0.25	0.36 $\pm$ 0.36	27.1 $\pm$ 21.4

Significantly different from controls:

* = $P < 0.05$

† = $P < 0.01$

‡ = $P < 0.001$

Total serum IgG, IgG subclass and IgE levels

The total IgG, IgG subclass and IgE levels for the patient and control groups studied are shown in Table 4. All three patient groups had significantly greater amounts of IgG₂ antibody than the control group; whereas the coeliac disease patients had a significantly higher level of IgG₁ than the other three groups. There was no significant difference in the IgE, IgG₃ or IgG₄ concentrations.

Discussion

Specific antibodies to gluten (or gliadin) and other dietary antigens have been measured in coeliac disease by a variety of techniques (Taylor *et al.*, 1961; Ferguson & Carswell, 1972; Haeney *et al.*, 1982; Kieffer *et al.*, 1981; Unsworth *et al.*, 1981) and they tend to fall towards normal levels with treatment. Antibodies to dietary antigens may be of the IgG or IgA class and have also been found, but in lower titres, in patients with other gastro-intestinal diseases and in healthy subjects. These findings have frequently been interpreted as indicating that anti-gluten antibodies are of little fundamental importance in coeliac disease, but are merely the result of the increased permeability of a damaged intestine (Bjarnason & Peters, 1984).

In the present study the micro-ELISA test has been used to detect IgG antibodies to fractions of wheat gluten in the sera of patients with coeliac disease, Crohn's disease, ulcerative colitis and healthy people. Using this procedure, antibodies to gluten fraction B, cow's milk and egg albumin antigens have been shown to be present frequently in the sera of coeliacs, but not of the IBD group. Haeney *et al.* (1982) measuring antibodies to soya protein by this technique, found them to be present in a group of coeliac patients but not in patients with inflammatory bowel disease. The

reason for this apparent difference between the two groups of patients has not been established. It might be expected that in both cases, where the intestine is damaged, circulating antibodies to dietary antigens would be formed. Since ELISA however, detects principally high affinity antibodies (Butler *et al.*, 1978; Lehtonen & Eerola, 1982) it is possible that the site of antigen exposure in the gut determines the affinity of the antibody produced, and that the antibodies produced by the upper intestinal route might be of greater affinity. Such a difference could also be a reflection of a genetically determined difference in the immune response repertoire: coeliac disease is closely associated with HLA-B8 and Dw3 (reviewed, Peña, 1981), antigens mapping to a region of the genome lying close to genes which are probably involved in determining the nature of the immune response. No such association exists for Crohn's disease or ulcerative colitis.

Further evidence of different immune responsiveness between coeliac and IBD patients is suggested in the different patterns of serum total IgG and IgG subclass observed. Previous studies (Shakib & Stanworth, 1980) have suggested that patients with coeliac disease may have increased levels of IgG₃ and IgG₄, which may in part reflect the increased incidence of a history of asthma reported in this condition (Hodgson *et al.*, 1976). The present studies have shown an increase in total IgG₁ levels in the coeliac patients but the increase in IgG₂ was shown in all three groups compared with the healthy controls. No significant difference was shown in the IgG₃ and IgG₄ groups, but we were clearly using patients who were not atopic; none had asthma and IgE levels were in the normal range.

The apparent immunogenic differences observed between the toxic and non-toxic gluten fractions are discussed elsewhere (Goodwin & Rawcliffe, 1983) and for this study the smallest molecular weight fraction of fraction III known to be toxic was chosen.

IgG antibody to wheat gluten fraction B in the coeliac patients belonged to all four IgG subclasses as detected by the micro-ELISA test. In addition to IgG₁ which was the most frequently detected antibody class, there was also a high incidence of IgG₂ and IgG₄ antibodies detected using the polyclonal or monoclonal antiglobulin reagents. The specific IgG₄ antibody was not accompanied, however, by a significant increase in the total level of IgG₄ reported by other authors (Shakib & Stanworth, 1980). The biological significance of IgG₄ antibodies remains circumstantial (Goodwin, 1983). However, IgG₄ is an important antibody in atopic disorders (Stanworth, 1983), and following desensitization may act as a blocking antibody (Aalberse *et al.*, 1983).

In this study, there was some correlation between IgG₄ antibody as detected by micro-ELISA and the presence of IgG S-TS antibody, as only one of the five serum samples in which IgG S-TS was detected contained no detectable IgG₄ antibody. However, in seven of the sera with detectable IgG₄ antibody, no IgG S-TS antibody was found. These observed correlations, and the similar observations made for IgG₂ antibody, provide no evidence for the identity of IgG S-TS.

Specific IgE is the antibody class normally associated with increased vascular permeability, but we were unable to find evidence for gluten-specific IgE antibody in the coeliac group, and this is consistent with the findings of Baldo and Wrigley (1978). In the absence of IgE antibody it has been suggested that IgG S-TS might provide a local increase in vascular permeability at sites of antigen contact, allowing antigen and IgG antibody to form complexes (Parish, 1971; Brighton, 1980). Such a mechanism including IgG S-TS and specific IgG antibody to gluten acting in the gut could result in

an Arthus-type reaction, consistent with the histological findings and the timing of reactions after gluten challenge (Anand *et al.*, 1981). If this is the case, then it might be expected that IgG S-TS would not be present to other dietary antigens where IgG antibody can be detected by ELISA and other techniques, and this is at present being investigated.

References

- AALBERSE, R.C., DIEGES, P.H., KNUL-BRETLOVA, V., VOOREN, P., AALBERS, M. & VAN LEEUWEN, J. (1983) IgG₄ as a blocking antibody. *Clinical Reviews in Allergy*, **1**, 289.
- ANAND, B.S. (1977) *Pathogenesis of Coeliac Disease*. PhD thesis, University of Oxford.
- ANAND, B.S., TRUELOVE, S.C. & OFFORD, R.E. (1977) Skin test for coeliac disease using a subfraction of gluten. *Lancet*, **i**, 118.
- ANAND, B.S., PIRIS, J., JERROME, D.W., OFFORD, R.E. & TRUELOVE, S.C. (1981) The timing of histological damage following a single challenge with gluten in treated coeliac disease. *Quarterly Journal of Medicine*, **50**, 83.
- BALDO, B.A. & WRIGLEY, C.W. (1978) IgE antibodies to wheat flour components. Studies with sera from subjects with bakers' asthma or coeliac condition. *Clinical Allergy*, **8**, 109.
- BOOTH, C.C. (1975) Definition of adult coeliac disease. In: *Coeliac Disease, Proceedings of the Second International Coeliac Symposium* (ed W.Th.J.M. Hekken and A.S. Peña), pp. 17–22. Stenfort Kroese, Leiden.
- BJARNASON, I. & PETERS, T.J. (1984) *In vitro* determination of small intestinal permeability: demonstration of a persistent defect in patients with coeliac disease. *Gut*, **25**, 145.
- BRIGHTON, W.D. (1980) Frequency of occurrence of IgG (S-TS). *Clinical Allergy*, **10**, 97.
- BUTLER, J.E., FELDBUSH, T.L., MCGIVERN, P.L. & STEWART, N. (1978) The enzyme-linked immunosorbent assay (ELISA): a measure of antibody concentration of affinity? *Immunochemistry*, **15**, 131.
- DISSANAYAKE, A.S., JERROME, D.W., OFFORD, R.E., TRUELOVE, S.C. & WHITEHEAD, R. (1974) Identifying toxic fractions of wheat gluten and their effect on the jejunal mucosa in coeliac disease. *Gut*, **15**, 931.
- DOE, W.F., BOOTH, C.C. & BROWN, D.L. (1973) Evidence for complement-binding immune complexes in adult coeliac disease, Crohn's disease and ulcerative colitis. *Lancet*, **i**, 402.
- DOE, W.F., HENRY, K. & BOOTH C.C. (1975) Complement in coeliac disease. In: *Coeliac Disease, Proceedings of the Second International Coeliac Symposium* (ed W.Th.J.M. Hekken and A.S. Peña), pp. 189–194. Stenfort Kroese, Leiden.
- FERGUSON, A. & CARSWELL, F. (1972) Precipitins to dietary proteins in serum and upper intestinal secretions of coeliac children. *British Medical Journal*, **1**, 75.
- FRAZER, A.C., FLETCHER, R.F., ROSS, C.A.C., SHAW, B., SAMMONS, H.G. & SCHNEIDER, R. (1959) Gluten-induced enteropathy: the effect of partially digested gluten. *Lancet*, **ii**, 252.
- GOODWIN, B.F.J. (1983) Nonreaginic anaphylactic antibodies in man. *Clinical Reviews in Allergy*, **1**, 249.
- GOODWIN, B.F.J. & HOW, M.J. (1976) RAST using crude and purified anti-IgE. *Clinical Allergy*, **6**, 441.
- GOODWIN, B.F.J. & RAWCLIFFE, P.M. (1983) Food allergies associated with cereal products. *Food Chemistry*, **11**, 321.
- HAENEY, M.R., GOODWIN, B.F.J., BARRATT, M.E.J., MIKE, N. & ASQUITH, P. (1982) Soya protein antibodies in man: their occurrence and possible relevance in coeliac disease. *Journal of Clinical Pathology*, **35**, 319.
- HODGSON, H.J.F., DAVIES, R.J., GENT, A.E. & HODSON, M.E. (1976) Atopic disorders and adult coeliac disease. *Lancet*, **i**, 115.
- JEWELL, D.P. (1979) Unpublished observations quoted in: *Clinical Gastrointestinal Immunology* (ed D.P.Jewell and H.C.Thomas), p. 108, Blackwell Scientific Publications, Oxford.
- KENRICK, K.G. & WALKER-SMITH J.A. (1970) Immunoglobulins and dietary protein antibodies in childhood coeliac disease. *Gut*, **11**, 635.
- KIEFFER, M., FRAZIER, P.J., DANIELS, N.W.R., CICLITIRA, P.J. & COOMBS, R.R.A. (1981) Serum antibodies (measured by MRS PAH) to alcohol-soluble gliadins in adult coeliac patients. *Journal of Immunological Methods*, **42**, 129.
- LEHTONEN, O.-P. & EEROLA, E. (1982) The effect of different antibody affinities on ELISA absorbance and titer. *Journal of Immunological Methods*, **54**, 233.
- MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

- PARISH, W.E. (1971) Detection of reaginic and short-term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons. *Clinical Allergy*, **1**, 369.
- PEÑA, A.S. (1981) Genetics of coeliac disease. In: *Topics in Gastroenterology*, 9 (ed D.P. Jewell and E. Lee), pp. 69–81. Blackwell Scientific Publications, Oxford.
- SHAKIB, F. & STANWORTH, D.R. (1980) Human IgG subclasses in health and disease. *Ricerca in Clinica e in Laboratorio*, **10**, 561.
- STANWORTH, D.R. (1983) IgG4 in atopics and their families. In: *XIth International Congress of Allergology and Clinical Immunology: Proceedings of Invited Symposia* (ed J.W. Kerr and M.A. Ganderton), pp. 337–340. Macmillan, London.
- TAYLOR, K.B., THOMSON, D.L., TRUELOVE, S.C. & WRIGHT, R. (1961) An immunological study of coeliac disease and idiopathic steatorrhoea: serological reactions to gluten and milk proteins. *British Medical Journal*, **2**, 1727.
- TAYLOR, K.B., TRUELOVE, S.C. & WRIGHT, R. (1964) Serologic reactions to gluten and cow's milk proteins in gastrointestinal disease. *Gastroenterology*, **46**, 99.
- UNSWORTH, D.J., MANUEL, P.D., WALKER-SMITH, J.A., CAMPBELL, C.A., JOHNSON, G.D. & HOLBOROW, E.J. (1981) New immunofluorescent blood test for gluten sensitivity. *Archives of Disease in Childhood*, **56**, 864.
- VOLLER, A., BIDWELL, D. & BARTLETT, A. (1976) Microplate enzyme immunoassays for the immunodiagnosis of virus infections. In: *Manual of Clinical Immunology* (ed N.R. Rose and H. Friedman), pp. 506–512. American Society for Microbiology, Washington, D.C.
- WIDE, L., BENNIC, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.