

Sub-class of IgG in allergic disease

I. IgG sub-class antibodies in immediate and non-immediate food allergy

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Summary

Previous studies have suggested that, apart from IgE-mediated reactions, some of the symptoms of food allergy may be caused by IgG antibodies to food proteins. This study was carried out to see if there were any distinctive features of the IgG sub-class antibody response to dietary antigens which occurs in food allergic patients. IgG sub-class antibodies were measured using a quantitative enzyme-linked immuno-sorbent assay (ELISA) to wheat gliadin, ovalbumin and bovine casein in twenty patients who had coeliac disease and in twenty-eight egg allergic patients. These were compared with twenty-one atopic dermatitis patients who did not have food allergy and twenty-six healthy control subjects. Coeliac disease patients tended to have raised IgG antibody levels (especially IgG1) to all three antigens but these overlapped considerably with that seen in egg allergic and atopic dermatitis patients.

Coeliacs who avoided gluten had anti-gliadin antibody levels which did not differ from those seen in healthy subjects but nevertheless had raised anti-ovalbumin and casein-specific antibodies. The IgG antibody was largely restricted to IgG1 and IgG4 sub-class although the relative amount of each varied with the antigen. Although gliadin-specific antibodies were mainly IgG1, ovalbumin-specific antibodies were mainly IgG4. The increased antibody levels to all three antigens in coeliacs were caused by a raised IgG1 response, IgG4 antibodies were usually normal. Egg allergic patients also had raised IgG1 but not IgG4 antibodies to ovalbumin.

These data show that the response to different dietary antigens can vary with the

antigen. The fact that IgG1 and not IgG4 antibodies were raised to all three antigens in patients with coeliac disease suggests that they are a secondary consequence of the disease, perhaps reflecting increased transport of antigens across a damaged gut mucosa rather than a specific immunopathological reaction. However, the observation that antibodies to gliadin, and not ovalbumin or casein, fell following gluten avoidance shows that the response to gliadin, at least, is dependent upon continued exposure to antigen.

Introduction

Differences between human IgG sub-classes were first described by Dray in 1960 [1] and confirmed by Terry & Fahey [2] and Grey & Kunkel [3] in 1964 using myeloma proteins. Subsequent studies have since shown them to differ in their ability to fix complement [4] and bind to basophils [5] or macrophages [6]. These functional differences between IgG sub-classes strongly suggest that they play a specific role in the immune system.

The relationship between IgG sub-class and immunity was first investigated in studies of patients with subclass deficiencies [7,8]. IgG2 deficiency was associated with chronic and recurrent infections [9], while combined IgG2 and IgG4 deficiencies were found in patients with IgA deficiency [10] or with ataxia-telangiectasia [11,12]. A genetic link with the levels of IgG sub-class antibody to grass pollen was demonstrated in studies of Gm allotypes in adults [13,14] and children [15].

Restriction of antibody responses to individual IgG sub-classes have been described to carbohydrate antigens [16] and to streptococcal antigens [17], using radiolabelled antigen to detect antibody activity in precipitated IgG sub-class protein. The possibility that sub-class restriction of antibodies might depend on the antigen is suggested by the finding that antibodies to *Staphylococcus aureus* M-associated protein are restricted to IgG3 and do not show the IgG2 response which is evoked by the whole organism [18]. This was further demonstrated by the inability of these antibodies to bind to protein A [17] and by their association with raised total IgG3 levels. IgG antibodies to viruses are commonly found in the IgG3 sub-class [19] but may also be found in IgG1 [20–22]. Published studies of Rh anti-D antibodies have included contradictory claims about IgG1/IgG3 restriction [23,24], possibly because of technical differences in the assays caused by the concentration of anti-Rh serum used to coat the red cells used in the assay [25]. Antibodies to bovine factor VIII in haemophiliacs are reported to be predominantly IgG4 [26,27].

In all of these studies, accurate quantification of IgG sub-class antibodies has been difficult due to the characteristics of the individual IgG sub-class antibodies and the nature of the available sub-class-specific antisera. The introduction of mouse monoclonal sub-class-specific antisera (McAb) [28] has, however, made it possible to develop more sensitive techniques [29].

The IgG sub-class antibody response to foods was studied in patients who had a clearly defined food hypersensitivity—either coeliac disease or immediate-type egg allergy. The foods studied were wheat gliadin, to which coeliac disease patients are known to react, ovalbumin, the major egg allergen, and bovine casein, a food antigen which was not thought to be involved in either disease. The pattern of IgG sub-class antibody to these foods in patients with food allergy was compared with atopic dermatitis patients and healthy control subjects.

Subjects and methods

Subjects

Twenty adult patients who had coeliac disease were studied. Ten had been on a gluten-free diet for at least 6 months (treated) and ten had not (untreated). In all cases the diagnosis of coeliac disease was made using criteria described elsewhere [30], including classical jejunal biopsy changes. Egg allergy was defined by a clear history of repeated oral [31], urticarial or eczematous reactions to ingested egg plus a positive skin-prick test (> 3 mm weal) to whole egg and twenty-eight such adult patients were studied, some of whom also had atopic dermatitis. Our two groups of control subjects were twenty-one adult patients who had atopic dermatitis but no history of food allergy and a negative skin-prick test, radio-allergo-sorbent test (RAST) and twenty-six adult healthy departmental staff who were not regarded as being allergic to foods using the same criteria, although some were atopic. Blood samples were taken by venepuncture and all serum samples stored at -20°C until used. In some cases we ran out of serum (two patients) and so the corresponding data is missing from some figures.

Reagents

Immulon 1 microtitre plates (M 129/A) and immuno - 1 (Nunc) were purchased from Dynatech Ltd, U.K. and Gibco Ltd, U.K., respectively. Horse serum was purchased from Sera Lab Ltd U.K. and p-nitrophenyl phosphate was purchased from Sigma Ltd, U.K. All other reagents were purchased from BDH Ltd, U.K.

Antigens and antisera

Bovine casein and ovalbumin were purchased from Sigma Ltd, U.K. in the purest form available. Wheat gliadin was prepared as described previously [30]. McAb to human IgG and IgG sub-classes 1-4 were a kind gift from Dr J. Lowe, (University of Birmingham, U.K.) and subsequently purchased from Unipath Ltd, U.K. (formerly Seward). The clones used were 8a4 (IgG), NL16 (IgG1), GOM 2 (IgG2), ZG4 (IgG3), RJ4 (IgG4). Affinity purified alkaline phosphatase-conjugated rabbit anti-mouse IgG (AP anti-MIG) was purchased from Sigma Ltd, U.K. and stored at 4°C until used.

ELISA

IgG and IgG sub-class antibodies were measured by ELISA as described elsewhere [29]. Briefly, antigen (Casein $1\text{ }\mu\text{g/ml}$, ovalbumin $1\text{ }\mu\text{g/ml}$, gliadin $10\text{ }\mu\text{g/ml}$) was bound to microtiter plates (Nunc for casein and ovalbumin, Dynatech for gliadin) in pH 9.6 carbonate/bicarbonate buffer or 70% ethanol for gliadin. All incubation volumes were $100\text{ }\mu\text{l}$ at 4°C . Between each step the plates were washed three times with phosphate buffered saline (PBS), pH 7.4, 0.1 M containing 0.05% Tween 20. Test serum was then incubated with the antigen-coated plate at 1/200-1/10,000 dilution in assay diluent (PBS + 0.5% horse serum + 0.5% Tween 20) for 2 h followed by monoclonal anti-IgG or IgG1-4 at 1/1000 dilution for 1 h and AP-anti MIG at 1/500 dilution for 1 hr. The substrate ($100\text{ }\mu\text{l}$), 1 mg/ml p-nitrophenyl phosphate in pH 9.8 diethanolamine buffer was added and the reaction stopped after 1.5 h at 37°C by addition of $50\text{ }\mu\text{l}$ of 3 M NaOH. Optical density was measured in a Titretek Multiskan (Flow, U.K.) at 405 nm. Results were expressed as arbitrary units/ml by reference to a high titre internal

standard serum as described elsewhere [29]. Interassay variation was low, coefficient of variation for casein was 11%, for ovalbumin 2.3%, and for gliadin 5.8%.

Statistical tests

The relationship between IgG1 and IgG4 antibody was tested by linear regression. Comparison between groups was by the Mann-Whitney *U*-test where the results were not normally distributed and by Student's *t*-test where they were. All tests were performed on a BBC model B computer (Acorn Ltd, U.K.) using BSTAT+ statistics programme (Finersoft (c) 1984).

Results

IgG antibodies to gliadin, ovalbumin and casein

The concentration of IgG antibodies to gliadin, ovalbumin and casein was measured by ELISA in patients with coeliac disease or egg allergy and compared with atopic dermatitis patients and healthy control subjects (Fig. 1). There was considerable overlap between the IgG antibody levels in the different groups and a high concentration of IgG antibody was not diagnostic of disease. Patients who had coeliac disease had raised IgG antibody levels to all three dietary antigens tested, compared with healthy control subjects (gliadin $P < 0.001$, ovalbumin < 0.01 , casein < 0.01 , Mann-Whitney *U*-test). Such differences as there were between the levels of total IgG antibody to these antigens in egg allergic or atopic dermatitis patients, and healthy control subjects did not reach statistical significance.

Untreated coeliac disease patients had higher levels of IgG antibodies to gliadin compared with those who had been treated with a gluten-free diet (treated) ($P < 0.001$ Student's *t*-test). Indeed, treated coeliacs had IgG anti-gliadin antibody levels that were indistinguishable from healthy control subjects. In contrast, there was no significant difference between the amount of ovalbumin or casein-specific IgG antibody in treated compared with untreated coeliacs.

IgG sub-class distribution

IgG sub-class antibodies to all three antigens were measured by ELISA. The relative amounts of each were determined by reference to a common standard curve of total IgG antibody. This was determined using a 'pan-IgG' reactive McAb to measure antibody in all four IgG sub-classes. Dilutions of pool of human sera which contained a high titre of IgG antibody were used to construct a reference curve and the amount of IgG antibody in each sub-class was expressed as arbitrary units per ml by reference to it. Extensive studies [29] have shown that this system of quantification provides remarkably good agreement with what would be predicted (shown by the solid line) when the antibody units for the individual sub-classes were added together (gliadin $r = 0.95$, ovalbumin $r = 0.76$, casein $r = 0.84$). A typical plot is shown in Fig. 2.

For all three antigens studied, the dominant sub-classes were 1 and 4 (Figs 3–5). IgG2 and IgG3 antibodies were usually at low or undetectable levels and represented a small percentage of total IgG antibody (Figs 6–8). The levels of IgG2 and IgG3 antibody to ovalbumin were below the limit of detection of the assay. All coeliac disease patients had raised levels of IgG1 antibodies to gliadin ($P < 0.001$), ovalbumin ($P < 0.001$) or casein ($P < 0.001$) Mann-Whitney *U*-test) compared with healthy control subjects. Levels of IgG4 antibodies to gliadin and ovalbumin did not differ

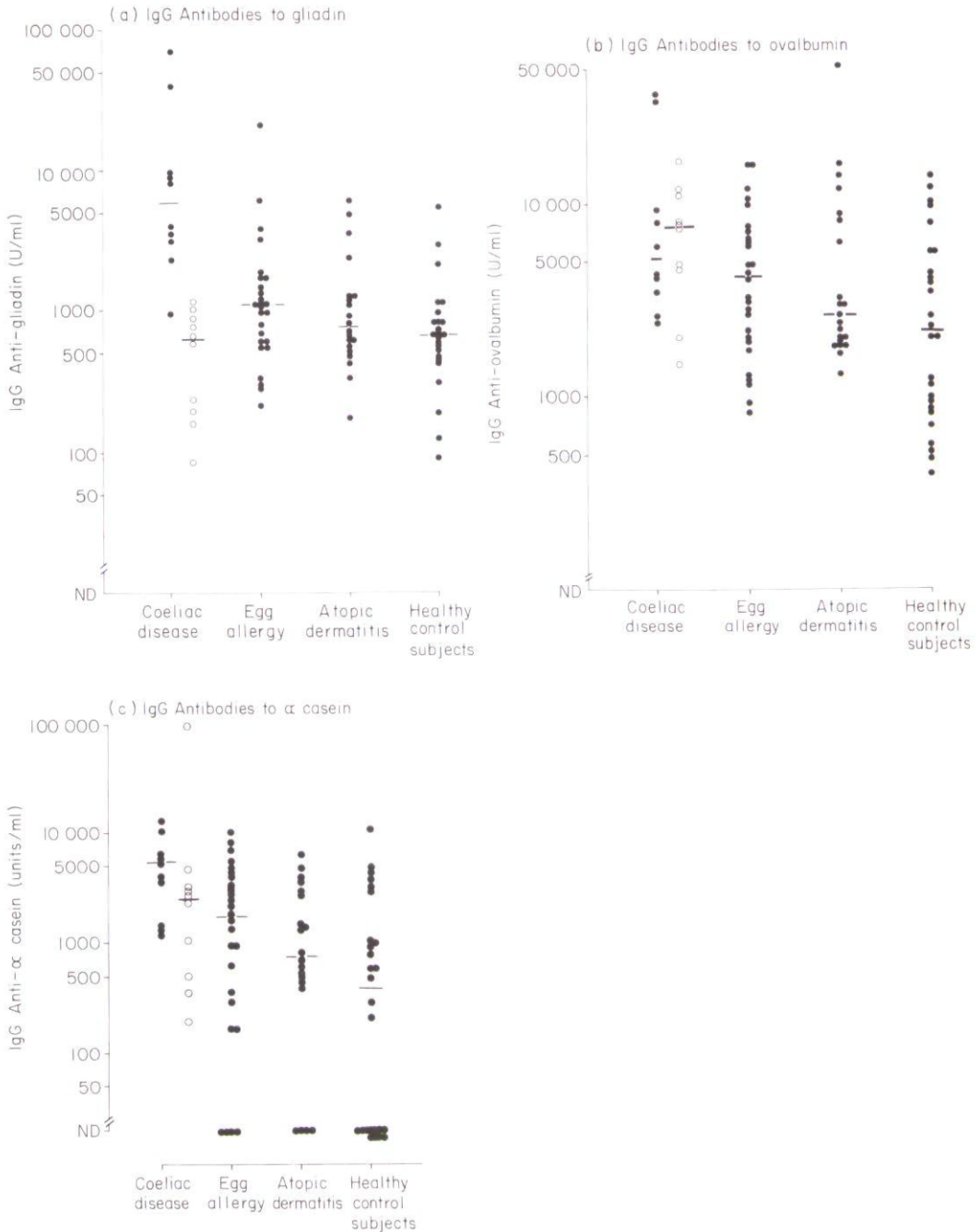


Fig. 1. IgG antibody to wheat gliadin (a), ovalbumin (b), and bovine casein (c) in patients with treated (○) or untreated (●) coeliac disease, in egg allergic (●) and atopic dermatitis patients (●) and in healthy control subjects (●). Median values (—). The level of IgG antibody was raised in coeliacs but not in egg allergic or atopic dermatitis patients. Untreated coeliacs had higher anti-gliadin antibodies than treated coeliacs but similar levels of anti-ovalbumin and casein-specific antibodies.

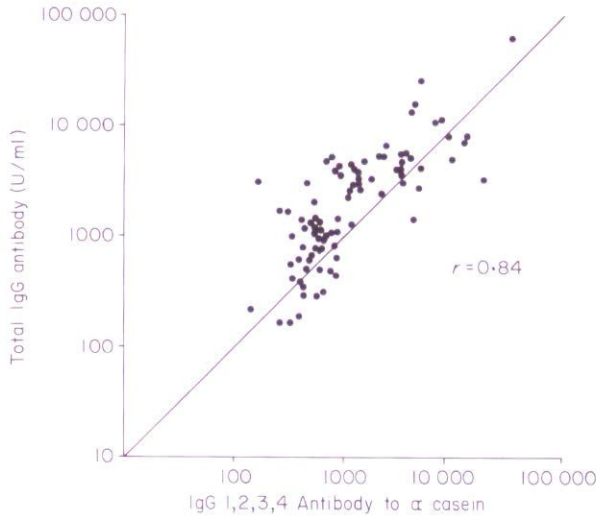


Fig. 2. The relationship between the total amount of IgG anti-casein antibody measured using the 'pan-IgG' reactive McAb and the summation of IgG sub-class antibodies in subjects in whom antibodies were detected.

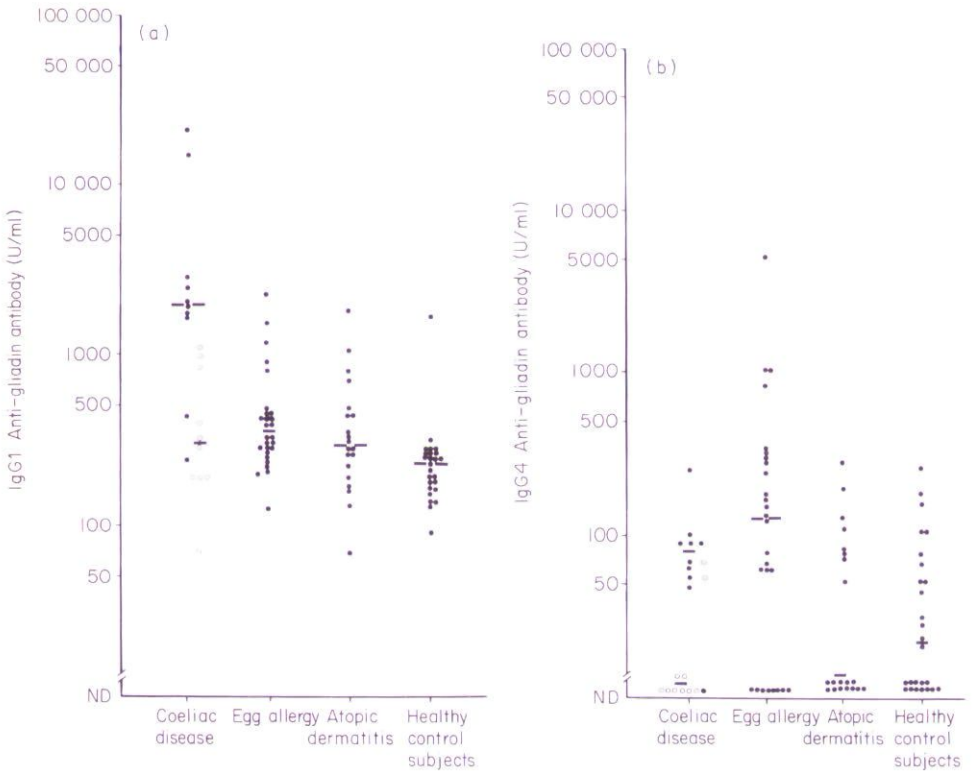


Fig. 3. The IgG1 (a) and IgG4 (b) antibody sub-class response to wheat gliadin in patients with coeliac disease, egg allergy, atopic dermatitis and healthy control subjects (O) = treated coeliacs, — = median.

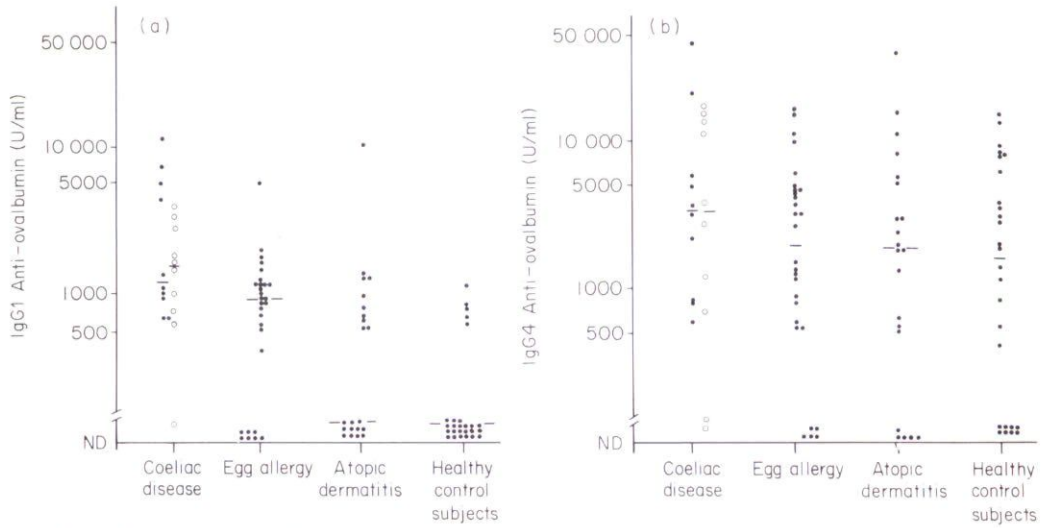


Fig. 4. The IgG1 (a) and IgG4 (b) subclass antibody response to ovalbumin in patients with coeliac disease, egg allergy, atopic dermatitis and in healthy control subjects (O) = treated coeliacs, — = median.

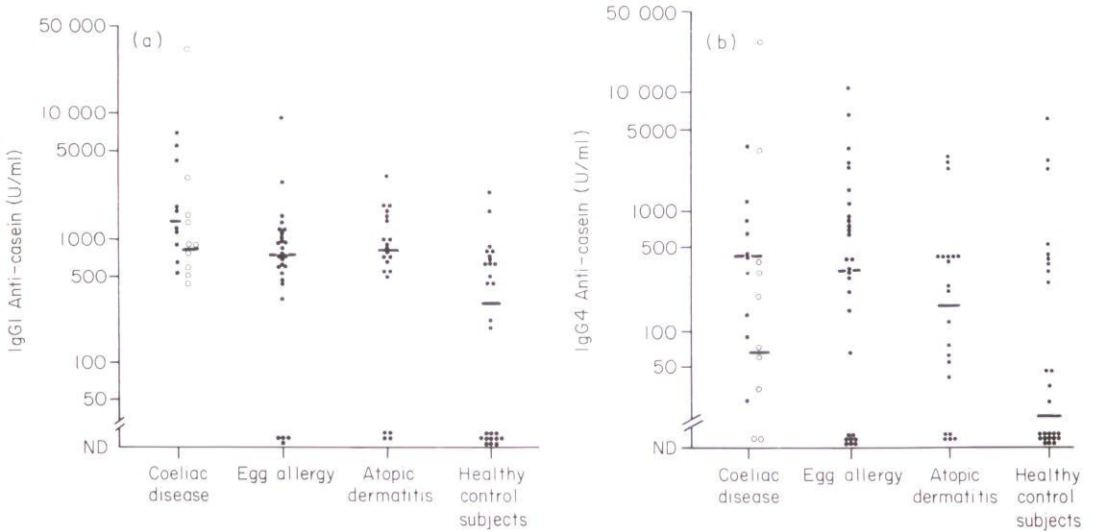


Fig. 5. The IgG1 (a) and IgG4 (b) sub-class antibody response to bovine casein in patients with coeliac disease, egg allergy, atopic dermatitis and in healthy control subjects (O) = treated coeliacs, — = median.

significantly between coeliac subjects and controls, but IgG4 antibody to casein appeared to be slightly raised in coeliacs ($P < 0.05$ Mann-Whitney U -test).

When the remaining three groups of subjects were considered, it was clear that egg allergic patients also had raised IgG1 antibody levels of ovalbumin compared with healthy control subjects ($P < 0.01$, Mann-Whitney U -test). IgG4 antibody levels to ovalbumin were higher than IgG1 antibodies but these did not differ significantly from controls in any of the groups studied. A solitary, unexplained finding in egg allergic

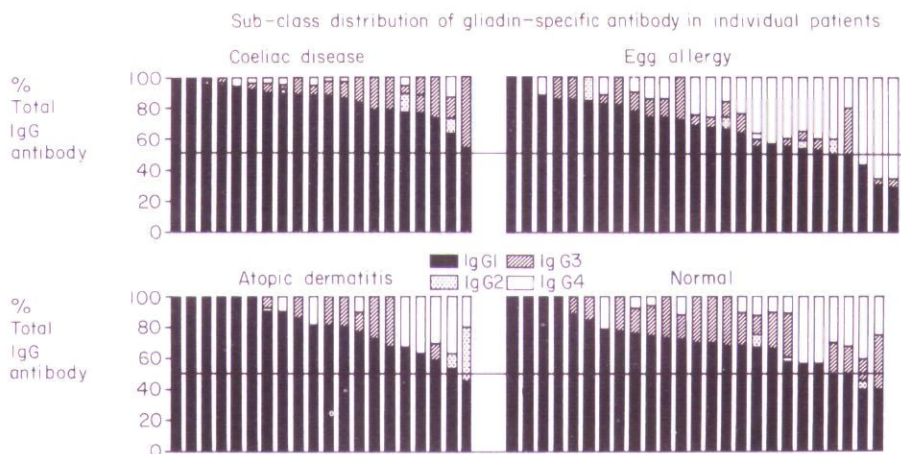


Fig. 6. The individual sub-class antibody profile for wheat gliadin-specific IgG antibodies in patients with detectable IgG sub-class antibody, expressed as the percentage of each subject's total IgG antibody.

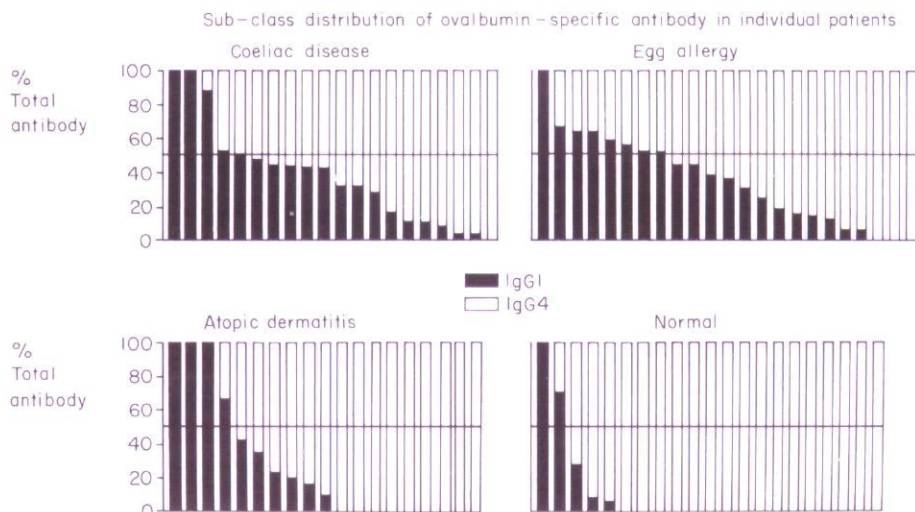


Fig. 7. The individual subclass antibody profile for ovalbumin specific IgG antibodies in patients with detectable IgG sub-class antibody expressed as the percentage of each subject's total IgG antibody.

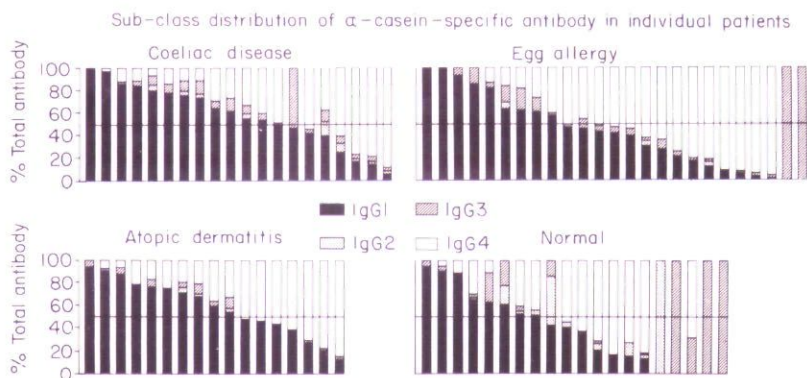


Fig. 8. The individual sub-class antibody profile for casein specific IgG antibodies in patients with detectable IgG subclass antibody expressed as the percentage of each subject's total IgG antibody.

individuals was that they had a significantly higher level of IgG4 antibody to gliadin ($P < 0.002$).

When the relative proportions of IgG1, IgG2, IgG3 and IgG4 antibodies were compared for each individual in all four groups, the relatively low levels of IgG2 and IgG3 antibody to gliadin was again evident (Fig. 6) but also the significantly higher IgG1 response to this antigen than IgG4 ($P < 0.01$ for individuals with atopic dermatitis, < 0.001 for the remaining groups' paired Student's *t*-test). The IgG2 and IgG3 responses to ovalbumin were undetectable (Fig. 7) and here the IgG4 response was significantly greater than the IgG1 response ($P < 0.05$ for coeliac patients, < 0.01 for the remaining groups). A more variable pattern was seen for antibody to casein and there were no significant differences between the IgG1 and IgG4 response in any group.

Discussion

It was the aim of this study to investigate the role of IgG sub-class in food allergy. IgG antibodies to common foods are frequently found early in life in healthy subjects [32,33] and their presence cannot, therefore, be taken to indicate an abnormal reaction to foods. The pattern of the IgG sub-class response has not, however, been adequately examined in well defined groups of patients. We have, therefore, studied conditions with clear-cut diagnostic criteria which are widely accepted as showing an abnormal immunological response to a food: coeliac disease with its associated abnormal response to wheat gliadin, and immediate-type egg allergy, an IgE-mediated condition. The IgG sub-class response to wheat gliadin, bovine casein and the major egg allergen, ovalbumin, was measured in these individuals and compared with that of patients who had atopic dermatitis but no known food allergy, and with healthy departmental staff.

As we have noted elsewhere [34], patients with coeliac disease have raised levels of IgG antibodies to dietary antigens. In this study we have found that patients with untreated coeliac disease but not egg allergy or atopic dermatitis have raised antibody levels to all three food antigens compared with healthy control subjects. We also found unexpectedly high levels of IgG antibody in some of the control subjects. These were selected on the basis of a negative history of food intolerance, skin-prick test and RAST with common foods but included atopic individuals. Coeliac disease patients who avoided wheat gliadin (treated) had lower levels of anti-gliadin antibodies compared with newly diagnosed (untreated) patients, although their anti-ovalbumin and bovine casein levels did not differ significantly from those of untreated patients. Wheat avoidance (and the absence of antigenic stimulation) may thus have been a significant factor in determining the level of IgG antibody. Antibodies to ovalbumin and casein, which were *not* excluded from the diet, did not fall. In this context it may be significant that our egg-allergic patients (who had learned to avoid egg) did not have raised levels of IgG antibody to ovalbumin.

The circumstances which determine the sub-class of IgG antibody produced are still unknown but the possibility that sub-class restriction is related to a particular antigen has already been referred to in the IgG2 and IgG3 response to Streptococci [17,18] and the IgG1 and IgG3 response to viruses [19–22]. Wheat gliadin produced a predominantly IgG1 antibody response in all four groups studied, while antibodies to ovalbumin were mainly IgG4. Bovine casein produced similar amounts of IgG1 and IgG4 antibody. Very little IgG2 and IgG3 antibody was detected for any of the antigens.

The increased levels of IgG antibody demonstrated against all three antigens in

coeliac patients were almost entirely in the IgG1 fraction, even for ovalbumin (which induces a predominantly IgG4 response). It seems possible that the increased levels of IgG1 antibodies in these patients, as in those with egg allergy, might reflect mucosal changes associated with an increased absorption of food antigens, but the question of whether such antibodies have a pathogenic role in the diseases described remains unanswered. Whereas repeated immunization with such antigens results in a switch from IgG1 antibodies to IgG4 antibodies [35,36], it was the level of both IgG1 and IgG4 antibody to gluten that fell in coeliac patients in a gluten-free diet. Nevertheless, the frequency with which healthy individuals possess IgG1 antibodies to dietary antigens does not suggest that they have a pathogenic role, and neither the amount of antigen absorbed nor the quantity of antibody seems sufficient to provoke immune complex disease [37]. As for the IgG4 antibody response this was so similar in the different groups that it could hardly be regarded as contributing to either of the diseases we have studied.

In conclusion, that the magnitude of the immune response to dietary antigens may be related to factors such as the amount of antigen absorbed; the sub-class of antibody produced is influenced by the type of antigen, the timing and the route of immunization; and the increased IgG antibody levels in coeliac patients may reflect increased absorption, especially since gluten avoidance is followed by a selective reduction in antibody to gluten but not to ovalbumin or casein.

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