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Human Serum Antibodies Reactive with Dietary Proteins

IgG Subclass Distribution

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Abstract. The isotype distribution of human IgG antibodies reactive with common dietary proteins has been evaluated in sera from adult patients with the irritable bowel syndrome and with bronchial asthma using a solid-phase immunoassay (ELISA). In both these medical disorders, serum antibodies reactive with ovalbumin or gliadin were restricted predominantly to the IgG4 isotype; however, IgG antibodies reactive with bovine milk antigens, notably casein, were often restricted to both the IgG2 and IgG4 isotypes. A similar serum IgG antibody isotype distribution for these dietary protein antigens was also demonstrated in IgG antibody-positive healthy adults. These data amplify the view that production of antibodies of the IgG4 isotype may reflect a normal immune response to dietary protein antigens presented at mucosal surfaces.

Introduction

There is continuing interest in the IgG isotype of human serum antibodies reactive with bacterial, viral and environmental (inhalant and dietary) antigens [1] following initial reports of IgG4 antibodies reactive with milk and egg proteins, although the other three IgG subclasses were not evaluated [2, 3].

Although found also in some healthy adults [4], a significantly increased prevalence of IgG antibodies reactive with common dietary protein antigens (namely wheat gliadin, bovine milk or chicken ovalbumin) has been demonstrated by enzyme-linked solid-phase immunoassay (ELISA) in various clinical disorders including atopic eczema [5], coeliac disease [6], dermatitis herpetiformis [7] and chronic urticaria [8]. These antibodies have been shown to be often, although not exclusively, restricted to the IgG4 isotype [6–8].

We have also demonstrated IgG antibodies reactive with dietary proteins at significantly increased prevalence in adult patients with the irritable bowel syndrome (IBS) [9] and bronchial asthma [10]. In the present study the isotype distribution of these IgG antibodies has been evaluated in parallel with IgG antibody-positive sera from healthy adults.

Materials and Methods

Sera. Sera from adult patients with the IBS (30 positive from a total of 57, i.e. 30/57), bronchial asthma (20/52), as well as from a healthy blood donor population (76/232), had been shown previously by ELISA to contain IgG antibodies reactive with wheat gliadin, ovalbumin or bovine milk [9, 10].

Antigens. Gliadin, ovalbumin, bovine casein, α-lactalbumin, and β-lactoglobulin were obtained from Sigma Chemical Co. (Poole, UK). Fresh cow's milk was used as previously described [4].

Antisera. Peroxidase-conjugated rabbit antibodies to human Ig, peroxidase-conjugated swine anti-rabbit Ig and peroxidase-conjugated rabbit anti-mouse Ig, were obtained from Dako Ltd. (High Wycombe, UK). Murine monoclonal antibodies specific for human IgG1 (clone NL16), IgG2 (GOMI), IgG3 (2G4) and IgG4 (RJ4) were obtained as ascitic fluid from Oxoid Ltd., Bedford.

ELISA. The IgG antibody ELISA has been described in detail previously using the dietary protein antigens at 0.5 μg protein/ml for antigen coating [4–10]. Human sera were tested at 1:200 dilution and the peroxidase-conjugated anti-human Ig at 1:1,000 dilution. Antibody binding was expressed as a binding index [4–10] calculated as follows:

$$\text{Binding index} = \frac{A_{492\,nm}\,(\text{test serum}) - A_{492\,nm}\,(\text{background})}{A_{492\,nm}\,(\text{reference negative}) - A_{492\,nm}\,(\text{background})}$$

The reference negative for each assay plate was the mean $A_{492\,nm}$ value for 6 normal adult sera shown previously to express low binding activity for the various dietary proteins included in this study.

The background was the $A_{492\,nm}$ value for the assay with omission of test human sera. Sera with binding indices >3.0 were regarded as positive for antibody reactive with the corresponding dietary antigen [4–10]. Sera were assayed on duplicate microtitre plates. Positive and negative controls also included hyperimmune and preimmune rabbit sera.

The IgG subclass distribution in IgG antibody-positive sera was also determined by ELISA [6] with test human sera diluted 1:20 (IgG2, IgG3 and IgG4) or 1:400 (IgG1). Human IgG subclass-specific monoclonal antibodies were used at 1:100 dilution and the peroxidase-conjugated rabbit anti-mouse Ig at 1:1,000 dilution. Optimal test human serum dilutions were determined by comparison with the IgG subclass distribution of serum anti-CMV antibodies determined by ELISA [6, 11] as previously described: anti-CMV antibody was restricted largely to IgG1 and IgG3, but not to IgG4 [6].

Results

Specificity of Antibodies Reactive with Bovine Milk

Sera from healthy adults and asthma patients, shown to contain antibodies reactive with bovine milk, were evaluated for antibody binding to three separate major bovine milk protein antigens, casein, α-lactalbumin and β-lactoglobulin (fig. 1). Whereas all sera showed positive binding reactions against casein, only occasional individual sera were also positive against α-lactalbumin or β-lactoglobulin. This pattern was no different between healthy adult sera and asthma patients. Bovine casein and/or milk was used in subsequent experiments.

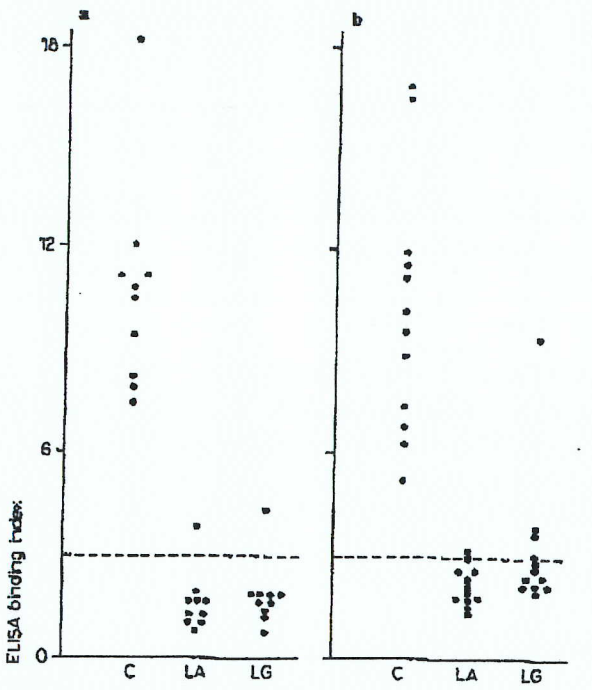

Fig. 1. ELISA binding reactions of sera containing antibodies reactive with bovine milk proteins. C = Casein; LA = α-lactalbumin; LG = β-lactaglobulin. ELISA-positive sera reactive with bovine milk were obtained from healthy adults (a) and asthma patients (b).

IgG Isotype of Antibodies Reactive with Dietary Antigens in IBS and Asthma Patients

The IgG isotype distribution of serum antibodies reactive with dietary antigens was evaluated in IgG antibody-positive sera from IBS patients (table I) and asthma patients (table II). Antibodies reactive with ovalbumin were restricted almost exclusively to the IgG4 subclass, as were more than 50% of antibodies reactive with gliadin in both IBS and asthma patients. In contrast, however, antibodies reactive with either milk or casein were distributed largely between both the IgG2 and IgG4 subclasses in both IBS and asthma sera. IgG1 antibodies reactive with these dietary antigens were uncommon although, under these same assay conditions, IgG1 anti-CMV antibody activity was readily detectable in antibody-positive sera (see Materials and Methods). There were no significant differences in the IgG isotype distribution between IBS or asthma sera.

IgG Isotype of Antibodies Reactive with Dietary Antigens in Healthy Adults

IgG antibody-positive sera from a healthy blood donor population were evaluated similarly for the subclass distribution of antibodies reactive with ovalbumin and gliadin, as well as with bovine milk and casein (table III). Antibodies reactive with ovalbumin, and less so for gliadin, were largely restricted to the IgG4 subclass (100% for ovalbumin, 54% for gliadin). Antibodies reactive with both milk and casein were distributed between both the Ig2 and Ig4 subclasses, with some examples also in the IgG3 subclass. IgG1 antibodies reactive with either milk or casein were detected at low frequency. Nonetheless, the IgG isotype restriction for these IgG antibody-positive sera from healthy adults was similar overall to that in both IBS and asthma patients; that is to IgG4 for antibodies to ovalbumin and gliadin, and to IgG2 and IgG4 for antibodies to bovine milk or casein.

Table I. Subclass distribution of IgG antibodies reactive with dietary protein antigens in patients with the irritable bowel syndrome

	n[1]	Predominant IgG subclass[2]			
		IgG3	IgG1	IgG2	IgG4
Chicken ovalbumin	20	0	0	0	18 (90)
Wheat gliadin	12	0	1 (8)	1 (8)	6 (50)
Bovine casein	21	3 (14)	4 (24)	13 (62)	14 (75)

Values in parentheses are percentages.
[1] IgG ELISA-positive sera.
[2] ELISA binding index > 3.0; some sera had antibody activity in more than one subclass.

Table II. Subclass distribution of IgG antibodies reactive with dietary protein antigens in patients with asthma

	n[1]	Predominant IgG subclass[2]			
		IgG3	IgG1	IgG2	IgG4
Chicken ovalbumin	17	0	0	0	16 (94)
Wheat gliadin	15	0	1 (7)	4 (27)	9 (60)
Bovine milk	20	2 (10)	2 (10)	13 (65)	15 (75)

Values in parentheses are percentages.
[1] IgG ELISA-positive sera.
[2] ELISA binding index > 3.0; some sera had antibody activity in more than one subclass.

Table III. Subclass distribution of IgG antibodies reactive with dietary protein antigens in healthy adult sera

	n[1]	Predominant IgG subclass[2]			
		IgG3	IgG1	IgG2	IgG4
Chicken ovalbumin	40	1 (3)	3 (8)	0	40 (100)
Wheat gliadin	26	0	6 (23)	3 (12)	14 (54)
Bovine milk	53	28 (53)	12 (23)	43 (81)	50 (94)
Bovinme casein	53	19 (36)	1 (2)	40 (75)	30 (57)

Values in parentheses are percentages.
[1] IgG ELISA-positive sera.
[2] ELISA binding index > 3.0; some sera had antibody activity in more than one subclass.

Discussion

This study has evaluated the IgG isotype of serum antibodies reactive with common dietary proteins in adult IBS patients and adult asthma patients, as well as healthy individuals. Antibody activity of restricted isotype was most prevalent within the IgG4 subclass (notably for ovalbumin), although some activity within the other IgG subclasses was apparent, notably for IgG2; IgG1 antibody activity was not dominant. We have made similar observations in adults with atopic eczema [6], chronic urticaria [8], coeliac disease [6] and dermatitis herpetiformis [7]. Taken together with observations in healthy individuals, this suggests these IgG antibodies, which are commonly restricted to IgG4 and/or IgG2, may be part of a *normal* immune response to environmental (dietary and inhalant) proteins which could be exaggerated in certain medical conditions.

Several groups have reported on the IgG isotype distribution of serum antibodies reactive with dietary protein antigens in ELISA for patients with atopic eczema, coeliac disease and gluten-sensitive enteropathy [6, 12–14]. Difficulties in interpretation may arise from differences in antigen preparations, age profile of subjects, as well as choice of control assay procedures and human sera. Nonetheless, there is broad agreement that IgG antibodies reactive with ovalbumin are almost always exclusively restricted to IgG4. Antigliadin antibodies have been variously reported as IgG4 [6], IgG1 and IgG4 [13], or IgG1 and IgG3 [14]; this latter study was untertaken in children where serum levels of total IgG2 and IgG4 may be low or absent [15], as indeed they can be in certain adult gluten-sensitive enteropathy patients [6]. Serum antibodies from a variety of medical disorders which are reactive with milk proteins have been shown to be largely of both the IgG2 and IgG4 subclasses, as previously noted [6], and were predominantly reactive with bovine casein. A restriction to the IgG4 isotype for anti-casein antibodies has also been reported recently [12]; other studies, however, have detected dominant antibody activity also in the IgG1 subclass against both bovine casein and wheat gliadin [13, 14].

IgG1 represents approximately 70% of the normal total serum IgG pool, whilst IgG4 is the least represented isotype [15]. Under certain conditions, solid-phase assays for specific antibodies can overestimate the contribution of IgG1. Therefore, we firstly evaluated assay conditions for determining the IgG isotype

Table IV. Predominant IgG isotype of human serum antibodies reactive with dietary protein antigens

	Ovalbumin	Gliadin	Casein
Atopic eczema and asthma	IgG4	IgG4	IgG2 and IgG4
Chronic urticaria and IBS	IgG4	IgG4	IgG2 and IgG4
Coeliac disease and dermatitis herpetiformis	IgG4	–[1]	IgG2 and IgG4
Healthy adults	IgG4	IgG4	IgG2 and IgG4

[1] ‚No predominant IgG subclass restriction.

distribution for anti-CMV antibodies using CMV-antibody positive and negative sera. The predominant anti-CMV activity was confirmed to be restricted to IgG1 as well as IgG3, with no IgG4 activity as shown previously [16]. This would appear to corroborate the technical reliability of our observations identifying IgG4 antibodies reactive with dietary antigens, as well as the sparse occurrence of the corresponding IgG1 antibodies. An 'internal' control for IgG2 was obtained since only casein antibodies, but neither anti-gliadin nor anti-ovalbumin, were found within the IgG2 subclass.

The restriction of anti-milk and anti-casein antibodies to both IgG2 and IgG4 is of interest. It is possible that, on the solid-phase in ELISA, casein forms micelles [as in fresh milk, see 17] expressing repeating glycosylated epitopes analogous to those of bacterial polysaccharides. Bovine casein micelles are spherical particles containing α-, β- and κ-casein molecules, but with κ-casein dominating the surface and with more than half the total κ-casein in the glycosylated form [18, 19]. Human serum antibodies reactive with carbohydrate and polysaccharide antigens are commonly restricted to the IgG2 isotype [20].

We have now demonstrated a significantly increased prevalence of serum IgG antibodies, usually of the IgG4 isotype, reactive with dietary antigens in adults with atopic eczema [6] and asthma, with chronic urticaria [8] and the IBS and with coeliac disease and dermatitis herpetiformis [6, 7]: this is summarised, together with the present results, in table IV using clinical groupings based on atopy, chronic hypersensitivity or gluten-sensitive enteropathy (GSE). A common feature of those medical disorders listed in table IV is immunopathological events at both skin and mucosal surfaces of the gut and respiratory tract. Although it is presumed that immunological sensitisation to dietary proteins could occur at these sites, there is as yet no compelling evidence that these IgG antibodies have a primary immunopathological role. However, taking into account the genetic order of Ig heavy chain genes, the less pronounced restriction of anti-gliadin antibodies to the IgG4 isotype in GSE could reflect a defect in downstream switching of Ig heavy chain genes, notably Cγ4 [6]. In support of this concept, we have now detected a total serum IgG4 deficiency (≤0.05 g/l) in 16% (12/74) GSE patients, compared with 6% (6/100) healthy adults [R.M.R. Barnes, unpubl. observations]. IgG4 antibody responses are thought to be more dependent on T cell help than the upstream Ig isotype (IgG3 and IgG1) [21, 22], and abnormalities of immunoregulatory T cells in coeliac disease have been documented [23].

In conclusion, the data presented here amplify the view that production of IgG4 isotype antibodies may reflect a normal immune response to dietary protein antigens presented at mucosal surfaces.

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