Acta Derm Venereol (Stockh) 1989; Suppl 144: 88–92

IgG Subclass Antibodies to Dietary Antigens in Atopic Dermatitis

S. HUSBY,[2] F. SCHULTZ LARSEN[1] and S.-E. SVEHAG[2]

[1]*Department of Dermatology, Odense University Hospital and* [2]*Institute of Medical Microbiology, Odense University, DK-5000 Odense C, Denmark*

The role of IgG subclasses and IgG subclass antibodies in atopic disease is controversial. Serum IgG and IgG subclass (IgG1-4) antibodies to the two dietary antigens ovalbumin (OA) and β-lactoglobulin (BLG) were measured with ELISA-methods in 16 patients with mild or moderate atopic dermatitis (AD) and healthy controls. The IgG antibodies were measured in 31 patients with previous AD and controls. The IgG subclass antibodies to OA and BLG showed predominance of IgG4 and IgG1 for both patients and controls. The levels of IgG and IgG subclass antibodies to OA did not differ between the groups, but the levels of IgG and IgG4 anti-BLG antibody were higher in patients with active AD than in controls. The antibody levels did not correlate with severity of disease or with a history of food allergy/intolerance. IgG4 antibodies to dietary antigens may be elevated in AD, but the diagnostic significance of IgG subclass antibody measurement is limited.

Steffen Husby, M.D., Institute of Medical Microbiology, J. B. Winsløwsvej 19, DK-5000 Odense C, Denmark.

Interest in the IgG subclass antibodies to dietary antigens comes from the putative significance of IgG antibodies as "short term anaphylactic IgG antibodies" (1, 2) or as allergy modifying "blocking antibodies" (3). The present paper summarizes our results on IgG and IgG subclass antibodies to dietary antigens in patients with atopic dermatitis (4, 5). A brief description of the properties of the IgG subclasses, in particular IgG4, is given. The methods used are presented together with the results, and possible implications are discussed.

BACKGROUND

The four IgG subclasses are physicochemically characterized by differences in their polypeptide heavy chains, in particular in the so-called hinge region (6). Functionally, the IgG1 and IgG3 subclasses show a strong ability to activate the complement system and of binding to cellular receptors, whereas the IgG2 and IgG4 subclasses show weak activities in these respects (7, 8). As an exception, IgG4 exhibits binding to receptors on human basophils and may induce histamine release (9, 10). The latter finding, however, was not confirmed, as IgG4-antigen complexes failed to induce histamine-release from human leukocytes (11). A comparison of the properties of IgE and IgG4 is given in Table I. A shift from IgG1 to IgG4 of antivenom antibodies was observed in healthy bee-keepers who were repetitively stung (12) suggesting that IgG4 antibodies occur as a normal consequence of chronic antigen exposure.

It is well known that IgE levels are increased in atopic dermatitis (13). Serum levels of IgG4 were observed to be increased in adult patients with asthma or atopic dermatitis (14, 15, 16). In a study of children with AD raised IgG4 levels were found only in patients with concurrent asthma (17). The presence of IgG4 antibodies to grass pollen, house dust mite and food antigens were observed in asthma patients (18, 19). However, other studies showed that IgG4 antibodies to dietary antigens may occur in a considerable proportion of healthy subjects (20), and semiquantitatively determined levels of IgG4 antibodies to cow's milk did not relate to clinical cow's milk allergy in children (21). Thus, the role of IgG4 antibodies in atopic eczema is at present controversial.

PATIENTS

The patients studied by us form part of a previously published genetic study of atopic dermatitis in the general population (22). The patients (Table II) comprised 10 subjects with mild and 6 patients with moderate atopic dermatitis (AD), and 31 patients with a history of AD (only tested for total IgG antibodies). Three of the patients, two with a history of AD and one with moderate AD had experienced worsening of their eczema after the ingestion of food, but none reacted after the intake of milk or egg. Serum samples from the AD-patients were tested in parallel with a similar number of age- and sex-matched controls.

METHODS

IgG antibodies to ovalbumin (OA) from hen's egg and beta-lactoglobulin (BLG) from cow's milk were measured by en-

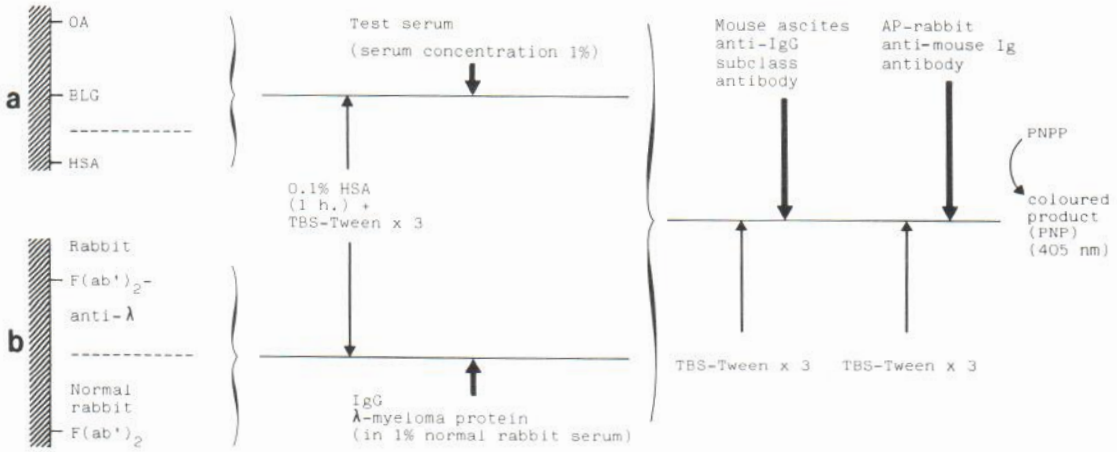

Fig. 1. Schematic drawing of the assay system for the determination of IgG subclass antibodies to dietary antigens, (*a*) test sera, (*b*) standards. From J Immunol Methods 1985; 83: 321, with the permission of the publishers (Elsevier).

zyme linked immunoassays (ELISA), as described previously (4). The assays were performed in microplates and included a biotin-avidin amplification step. The results were expressed quantitatively in mU/ml by referral to serial dilutions of a reference high-titered human serum.

For the measurement of IgG subclass antibodies to dietary antigens we employed ELISAs also developed by us (20). The principle for the IgG subclass antibody assays are shown in Fig. 1. Serum antibodies were bound to antigen (OA or BLG) on the solid phase, followed by the incubation with monoclonal anti-IgG subclass antibodies. Alkaline phosphatase-labelled rabbit anti-mouse Ig antibody was added and after further washings and the incubation with substrate the resulting colour reaction was determined in a photometer. To obtain an estimation of the antibody concentration the antibody binding (photometric measurement) was referred to a

Table I. *Biological properties of IgE and IgG4*

	IgE	IgG4
Serum concentration	0.1 μg/ml	100 μg/ml
Passage across placenta	–	+
Basophil/mast cell binding	+	+
Mediator release with		
(*a*) Specific anti-IgG4	–	+
(*b*) Specific antigen	+	–
Complement fixation	–	–
Genetic variants	+	+

Table II. *The characteristics of patients and controls*

	Mild atopic dermatitis ($n=10$)	Moderate atopic dermatitis ($n=6$)	Controls ($n=16$)
Respiratory atopy	1	3	0
Disease extent (0–16)			
Median	3	5	0
Range	2–3	5–10	0
IgE (μg/ml)			
Median	65	97	19
90% percentile	1 080	1 440	82

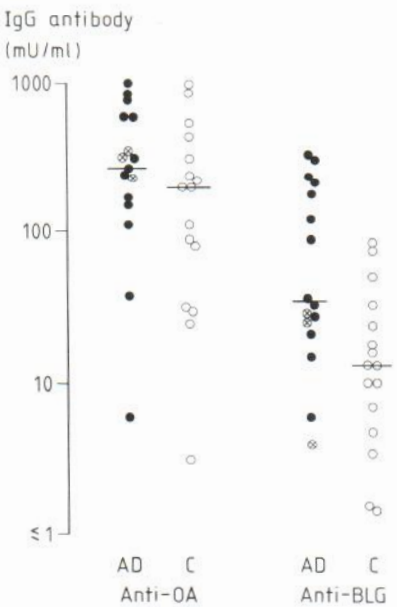

Fig. 2. Serum IgG antibodies to OA and BLG in patients with mild or moderate AD (●) and in controls (○). The logarithmic ordinate scale is expressed in arbitrary units. AD-patients with concomitant asthma/rhinitis are denoted as (⊗). Bars denote median values. From Allergy 1986; 41: 379, with the permission of the publishers (Munksgaard).

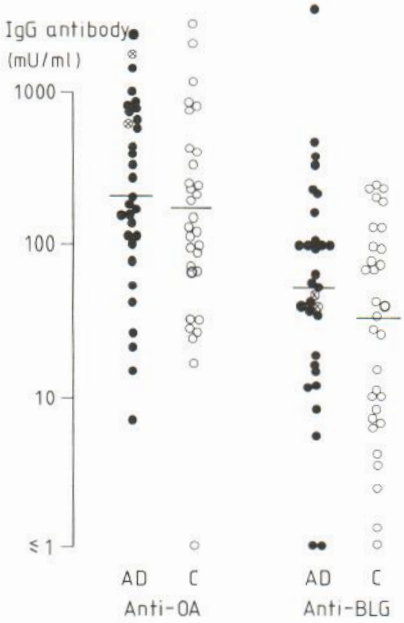

Fig. 3. Serum IgG antibodies to OA and BLG in patients with a history of AD (●) and in the corresponding controls (○). AD-patients with asthma/rhinitis (⊗). Bars indicate median values. From Allergy 1986; 41: 379, with the permission of the publishers (Munksgaard).

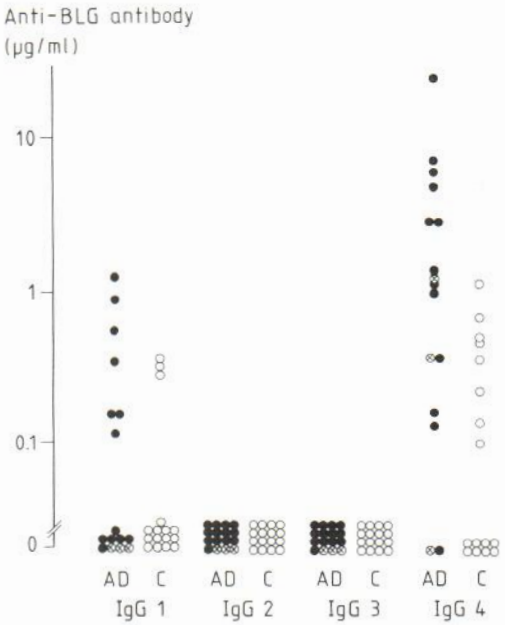

Fig. 5. IgG subclass antibodies to BLG in patients with AD (●) and in controls (○). AD-patients with concomitant asthma/rhinitis (⊗). From Allergy 1986; 41: 386, with the permission of the publishers (Munksgaard).

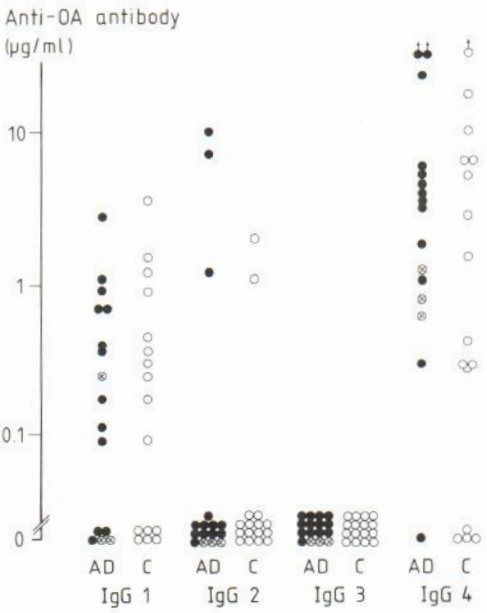

Fig. 4. IgG subclass antibodies to OA in patients with AD (●) and in controls (○). AD-patients with concomitant asthma/rhinitis are denoted by (⊗). From Allergy 1986; 41: 386, with the permission of the publishers (Munksgaard).

standard curve with established IgG subclass myeloma protein.

The statistical evaluations were made with the nonparametrical Mann-Whitney U-test as a two-tailed test.

RESULTS

The determination of IgG antibodies to OA and BLG showed detectable antibodies in the large majority of both patients with active AD and controls (Fig. 2) and the patients with previous AD and their controls (Fig. 3). The levels of IgG anti-OA antibodies did not differ between the groups, whereas the levels of IgG anti-BLG antibodies were significantly higher ($p < 0.05$) in patients with active disease than in their corresponding controls (Fig. 2). However, the anti-BLG antibody levels were not statistically different between the patients with active and the patients with previous AD.

The IgG subclasses of antibodies to OA were measurable in a high proportion of both patients and controls in IgG1 and IgG4, at comparable levels (Fig. 4). Also, a few serum samples contained antibody of the IgG2 subclass. Antibodies to BLG were observed only in IgG1 and IgG4 (Fig. 5). The IgG4 anti-BLG antibodies were significantly higher ($p < 0.001$) in AD-patients (median 1.1. µg/ml, range 0–24.0 µg/ml) than in controls (median 0.05 µg/ml, range 0–1.1 µg/ml).

DISCUSSION

From the present studies we may conclude that IgG antibodies to OA and BLG are produced in the majority of AD patients and in normals as well. This antibody activity is localized mainly in the IgG4 subclass, although an absolute subclass restriction is not present. The levels of IgG4 anti-BLG antibodies were significantly higher in atopic dermatitis patients than in controls. However, a considerable overlap was observed between the patients and the controls, so the determination of IgG4 anti-BLG antibodies seems of limited diagnostic value.

Our results of antibody measurements, which comprise all four subclasses, are partially in concordance with other studies of IgG4 antibodies alone. Merrett et al. (16) observed in AD patients high levels of IgG4 antibodies to a number of foods, including egg white, milk, codfish and peanut. Shakib et al. (23) found no difference in IgG4 antibodies to purified milk and egg antigens between sera from AD patients and normals, but the majority of both patients and controls had undetectable levels of antibody to e.g. BLG. Rowntree et al. (24) measured IgG4 antibodies to OA and BLG in adult AD-patients and observed the frequent occurrence of IgG4 anti-OA antibody as a significant proportion of total IgG anti-OA antibody. Few patients and controls had IG4 anti-BLG antibody, with no difference between patients and controls. In a prospective study (24) of children from atopic families the proportion of IgG4 to OA and BLG increased up to 5 years of age. Higher IgG4 anti-OA antibody levels were observed in 3-year-old children with positive prick test or IgE antibody (RAST) to OA, but no significance-testing was performed on these data.

Only three of the patients in our study were suspected of clinical food allergy as evaluated from their history and skin prick tests (data not shown). These patients did not show particularly high IgG4 antibody levels. However, we did not perform regular food diet and provocation tests in this study, leaving open the theoretical possibility that unrevealed milk or egg allergy could influence the antibody levels. Studies on IgG subclass antibodies specifically in relation to milk allergy are in progress. Furthermore, studies of patients with severe AD may show more clear results than the present, population-based patient material.

Genetic factors may influence both the antibody levels and the disease AD (22). In healthy twin subjects we demonstrated about one third of genetic dependence of IgG anti-OA and anti-BLG antibody levels (25). The levels of IgG4 antibodies were in a recent report related to Gm allotypic markers (26). However, we did not find any relation between the levels of antibodies to OA and BLG and the HLA-A, B, C antigens or the Gm and Km allotypes (Husby et al., unpublished). As to AD, no association was observed between the disease and the HLA-A, B or C antigens and several other genetic markers including the Gm and Km allotypes (27).

The biological and clinical significance of IgG4-antibodies is at present unclear, as noted above. Clearly, more research is needed to distinguish the physiological function of IgG4 from its putative role in atopy as a reaginic antibody or a "blocking" antibody. Further characterization of the IgG4 receptor on human basophils and mast cells is awaited.

REFERENCES

1. Parish WE. Short-term anaphylactic IgG antibodies in human sera. Lancet 1970; ii: 591.
2. Parish WE. Detection of reaginic and short-term sensitizing anaphylactid or anaphylactoid antibodies to milk in sera of allergic and normal persons. Clin Allergy 1971; 1: 368.
3. Giessen M v d, Homan WL, Kernebeek G v, Aalberse RC, Dieges PH. Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. Int Arch Allergy Appl Immun 1976; 50: 625.
4. Husby S, Schultz Larsen F, Hyltoft Petersen P, Svehag S-E. Humoral immunity to dietary antigens in atopic dermatitis. I. Biotin-avidin amplified ELISA-analysis for serum IgG antibodies. Allergy 1986; 41: 379.
5. Husby S, Schultz Larsen F, Ahlstedt S, Svehag S-E. Humoral immunity to dietary antigens in atopic dermatitis. II. Analysis of IgE and IgG subclass antibodies. Allergy 1986; 41: 386.
6. Burton DR. Immunoglobulin G: functional sites. Mol Immunol 1985; 22: 161.
7. Spiegelberg HL. Biological activities of immunoglobulins of different classes and subclasses. Adv Immunol 1974; 19: 259.
8. Unkeless JC, Fliit H, O'Hellman JS. Structural aspect and heterogeneity of immunoglobuline Fc-receptors. Adv Immunol 1981; 31: 247.
9. Nakagawa T, Takaishi T, Sakamoto Y, Miyamoto T, Skvaril F. Flow-cytometric analysis of human basophil degranulation. II. Degranulation induced by anti-IgE, anti-IgG4 and the calcium ionophore A23187. Clin Allergy 1981; 11: 21.
10. Fagan DL, Slaughter CA, Capra JD, Sullivan TJ. Monoclonal antibodies to immunoglobulin G4 induce histamine release from human basophils in vitro. J Allergy Clin Immunol 1982; 70: 399.
11. Toorenenbergen AW v, Aalberse RC. Allergen-specific IgE and IgG4 antibody levels in sera and the capacity of these sera to sensitize basophil leukocytes in vitro. Clin Allergy 1982; 12: 451.

12. Aalberse RC, v d Gaag R, v Leuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. J Immunol 1984; 130: 122.
13. Rajka G. Atopic dermatitis. London: WB Saunders, 1975.
14. Shakib F, McLaughlan P, Stanworth DR, Smith E, Fairburn E. Elevated serum IgE and IgG4 in patients with atopic dermatitis. Br J Dermatol 1977; 97: 59.
15. Gwynn CM, Morrison Smith J, Leon Leon G, Stanworth DR. IgE and IgG4 subclass in atopic families. Clin Allergy 1979; 9: 119–123.
16. Merrett J, Barnetson R StC, Burr ML, Merrett TG. Total and specific IgG4 antibody levels in atopic eczema. Clin Exp Immunol 1984; 56: 645.
17. Wütrich B, Benz A, Skvaril F. IgE and IgG4 levels in children with atopic dermatitis. Dermatologica 1983; 166: 229.
18. Bruynzeel PBL, Berrens L. IgE and IgG antibodies in specific human allergies. Int Arch Allergy Appl Immunol 1979; 58: 344.
19. Gwynn CM, Almousawi T, Stanworth DR. Clinical associations with serum allergen-specific IgG4 antibodies. Clin Allergy 1982; 12: 459.
20. Husby S, Jensenius JC, Svehag S-E. ELISA—Quantification of IgG-subclass antibodies to dietary antigens. J Immunol Methods 1985; 83: 321.
21. Björksten B, Ahlstedt S, Björksten F et al. Immunoglobulin E and immunoglobulin G4 antibodies to cow's milk in children with cow's milk allergy. Allergy 1983; 38: 119.
22. Schultz Larsen F. Atopic dermatitis. Etiological studies based on a twin population. Thesis, Odense University, 1985.
23. Shakib F, Brown HM, Stanworth DR. Relevance of milk- and egg-specific IgG4 in atopic eczema. Int Arch Allergy Appl Immunol 1984; 75: 107.
24. Rowntree S, Platts-Mills TAE, Cogswell JJ, Mitchell EB. A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): Comparison between IgG and IgG4 antibodies to food and inhalant antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. J Allergy Clin Immunol 1987; 80: 622.
25. Husby S, Schultz Larsen F, Hyltoft Petersen P. Genetic influence on the serum levels of naturally occurring human IgG antibodies to dietary antigens. Quantitative assessment from a twin study. J Immunogenetics 1987; 14: 131.
27. Schultz Larsen F, Grunnet N. Genetic investigations in atopic dermatitis. Tissue Antigens 1987; 29: 1.