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CLASS AND SUBCLASS DISTRIBUTION OF SPECIFIC ANTIBODIES TO CODFISH ALLERGEN IN A PATIENT WITH ATOPIC ALLERGY

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Abstract. Using an indirect ELISA technique together with rabbit antisera specific for human immunoglobulin classes and IgG subclasses, an estimate was made of the contribution of immunoglobulin classes and subclasses to the overall antibody response against codfish allergen in serum from a patient allergic to codfish. Allergen-specific antibodies were found to be of immunoglobulin classes IgM, IgD and IgE and the subclasses IgG1, IgG3, and IgG4. No antibodies of IgA class or IgG2 subclass were present. High levels of IgG3 and IgD were found.

Key words: Atopic allergy; Codfish allergen; Allergen specific antibodies; Immunoglobulin classes and subslcasses

The role of antibodies other than IgE in atopic allergy is a field of growing interest and attention. The interest has concentrated on antibodies belonging to the IgG class, and short-term anaphylactic (11) as well as blocking IgG antibodies have been described (13, 16, 17).

Particular attention has been focused on antibodies of the IgG4 subclass. Elevated serum IgG4 concentrations have been reported in patients with atopic dermatitis (14) and in patients with cystic fibrosis, who have been reported to have a high incidence of immediate-type hypersensitivity reactions (15). A higher incidence of IgG4 was also detected in patients with allergic asthma to castor bean allergen (5), and elevated serum concentrations of IgE and IgG4 were found to correlate with the clinical picture in asthmatic patients (9). Van der Giessen et al. (19) reported that a relatively high proportion of grass pollen specific antibodies belonged to the IgG4 subclass, and an increase in IgE, IgG1 and IgG4 grass pollen-specific antibodies during hyposensitization of hay fever patients has been reported (6).

In the present study we used the indirect enzyme-linked immunosorbent assay (ELISA) to determine the class and subclass distribution of antibodies to the highly purified allergen fraction (DS22) from codfish in serum from a patient allergic to codfish.

MATERIALS AND METHODS

Patient. Serum was obtained from a 22-year-old male with a history of asthma, allergic rhinitis as well as atopic eczema of the severest type with frequent bacterial superinfections. He was allergic to codfish, grass pollen, house dust and animal dander, as determined by a positive history, positive skin prick tests and the presence of specific IgE antibodies measured by Phadebas® RAST kits (Pharmacia, Swedcn). Exposure to codfish allergen induced an attack of asthma. He was treated with topically applied steroid creams and an antihistaminic drug, but no internal steroids. The serum IgE level was raised to 1 540 U/ml (normal: 50–700), serum IgA to 4.4 g/l (normal: 0.5–3.3) and serum IgD to 0.3 g/l (normal: 0.005–0.1), while serum IgG and IgM levels were normal. ESR, haemoglobin and white cell counts were normal.

Antisera. Antiscra were raised in rabbits using conventional immunization procedures. The immunogens used were all highly purified myeloma proteins, some of them purified to homogeneity and characterized by amino acid sequencing.

Immunosorbent techniques. The sera were rendered class or subclass specific by absorption on a series of immunosorbent columns loaded with appropriate myeloma proteins. The myeloma proteins were either coupled to Sepharose 4B (Pharmacia) employing a modification of the CNBr activation method outlined by March et al. (10), or by carboiimide conjugation to AH-Sepharose 4B (Pharmacia). Usually the gels were substituted with protein in a concentration between 1 and 5 mg/ml packed gel. The antiserum against IgG1 was made specific for the Gm markers a, x and f (Glm 1, 2 and 3) by batch absorption with relevant Gm-typed human scra.

Enzyme linked immunosorbent assay (ELISA). The assay was performed in polystyrene tubes (N 1070, NUNC, Denmark) basically as described by Engvall and Perlmann (7). The tubes were coated with the purified codfish allergen fraction DS 22 (usually 100 ng/ml) dissolved in 0.05 M $NaHCO_3$, pH 9.6 with 0.02% NaN_3. After incubation at 4°C overnight, the tubes were emptied by suction and rinsed 3 times in washing buffer consisting of 0.9% saline, 0.02% azide and 0.05% Tween 20 (Sigma Chem. Co., Mo.). If not used immediately, the coated tubes were kept dry and

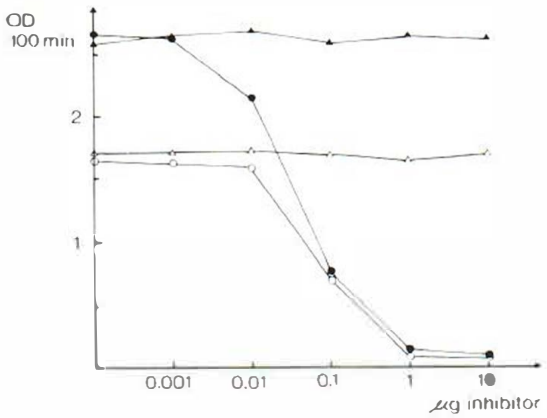

Fig. 1. Specificity test of antisera: Anti-μ antiserum against IgM coat inhibited with an IgM myeloma protein (○) and irrelevant IgG myeloma protein (△). anti-γ3 antiserum against IgG3 coat inhibited with an IgG3 myeloma protein (●) and an IgA myeloma protein (▲).

closed at 4°C. Then the tubes were incubated with a serum sample from the patient (usually 10–20 μl diluted to 1 ml in PBS with NaN_3 and Tween 20, and left for 2 hours at room temperature and 2 hours at 4°C. After washing, the tubes were incubated with saturating amounts of the specific antisera at room temperature. After 4 hours the tubes were emptied and rinsed 3 times with washing buffer before adding 1 ml alkaline phosphatase (ALP) conjugated swine anti-rabbit IgG (Orion Pharmaceutica, Helsinki, Finland) usually diluted 1:500 in PBS with azide and Tween 20. After a further 4 hours' incubation at room temperature the tubes were emptied and washed. Finally, they were filled with 1 ml of enzyme substrate, *p*-nitrophenyl phosphate (PNPP, Sigma Chem. Co., Mo.), 1 mg/ml in 1 M diethanolamine buffer, pH 9.8, containing 1 mM $MgCl_2$ and incubated usually at room temperature, or overnight at 4°C. The enzyme was inactivated by adding 0.1 ml 5 M NaOH and the optical density read at 400 nm. Two sets of blank controls were always included, one in which patient serum were omitted, and one where empty tubes were used instead of coated tubes. The optical density o these blanks was usually well beyond 0.1, and when occasionally it was higher, the whole series was rejected.

Specificity testing of antisera. After absorption the specificity of the antisera was examined by ELISA, employing tubes coated with different myeloma protein (usually 100 ng/ml).

RESULTS

All antisera were shown to be highly specific as they reacted with several myeloma proteins within the same class or subsclass but not across classes or subclasses.

The specificity and strength of the different antisera were further assessed by performing inhibition experiments with the ELISA technique. In these experiments the antisera were preincubated with varying amounts of the inhibiting myeloma proteins in glass tubes bevore being added to the coated polystyrene tubes for further processing as described above. Fig. 1 shows two typical examples of results of specificity tests on antisera. An IgM myeloma protein inhibited the anti-μ antiserum, while an IgG myeloma protein did not. The anti-γ^3 antiserum was inhibited with an IgG_3 myeloma protein, but not with an IgA myeloma protein. Fig. 2 shows the relative amounts of allergen-specific antibodies of various classes and subclasses. Serum from a healthy donor was tested as a reference and no antibodies against codfish were detected. It is seen that in the patient serum, no antibodies of IgA class or IgG_2 subclass were detected. Antibodies of IgG_1, IgG_3 and IgG_4 subclasses and IgM as well as IgD and IgE classes were present and especially the amount of IgD was found to be considerable.

DISCUSSION

The present investigation demonstrates the presence of DS22-specific antibodies of the classes IgM, IgD and IgE, and the subclasses IgG1, IgG3 and IgG4 in serum from a patient allergic to codfish. Antibodies of the IgA class and IgG2 subclass were not detected, although the antisera employed were shown by ELISA to react strongly against relevant myeloma proteins.

The DS22 allergen fraction is a well defined purified allergen fraction from codfish, containing the major allergen M. There are several reports on the identification and characterization of this preparation (1, 2). This allergen was selected because of its protein nature and the purity of the fraction, making coating to polystyrene tubes easy as well as reducing the possibility of non-specific interaction between coat and antisera or conjugate.

The role of the different immunoglobulin classes and subclasses in allergy is a still unsettled issue. While the role of IgE in immediate hypersensitivity reactions seems to be established, the possible role of other immunoglobulins in allergic patients has to be clarified. The IgG4 subclass has been reported to act as a blocking antibody (19, 20) capable of inhibiting reagin-mediated PCA in baboons (17). But a short-term anaphylactic antibody of the IgG class has also been demonstrated (11). Antibodies other than IgG4 and IgE may also be involved and

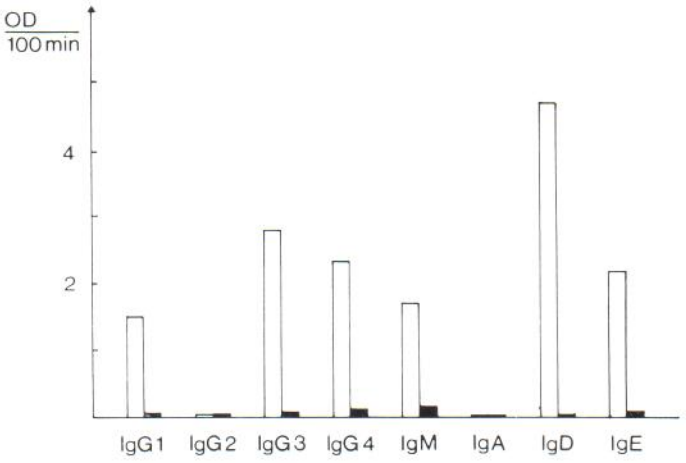

Fig. 2. Reactivity of patient serum (□) and normal serum (■) against DS22-coated tubes as measured by class- and subclass-specific antisera by indirect ELISA.

the presence of allergen-specific antibodies of the other IgG subclasses (i.e. IgG1, IgG2 and IgG3) in allergy have been reported (19). IgA antibodies to allergens have been demonstrated earlier (12). The patient in this study had an increased serum concentration of IgA to 4.4 g/l (normal 0.5–3.3 g/l but in spite of this no allergen-specific antibodies of the IgA class to the codfish allergen could be detected in his serum. This finding may be of special interest in view of the fact that IgA is the predominant immunoglobulin in mucous membranes. The serum concentration of IgD was also elevated, and large quantities of allergen-specific antibodies of the IgD class were detected.

The hypothesis has been presented that atopic individuals with elevated IgE and IgG4 subclass antibodies are likely to have dermatitis (8). Shakib et al. (14) found elevated serum IgE as well as IgG4 in patients with atopic dermatitis, and in the patient material presented by Gwynn et al. (9) 5 children with eczema, asthma and hay fever and 4 adults who had had atopic eczema as children, all had grossly increased concentrations of both IgE and IgG4.

While many patients with atopic dermatitis have very high serum IgE levels, and most patients somewhat elevated levels, some do however have normal IgE values (personal observations). Whether IgG4 serum concentrations are of significance for the presence of atopic dermatitis needs to be more closely investigated.

Bruynzel and Berrens (4) found that the sera of some patients with unequivocal allergies did not contain allergen-specific IgE nor IgG4 antibodies, or else contained solely IgG4 antibodies. Furthermore, in manifest allergy to guinea pig dander, IgG4 antibodies were found more frequently than IgE antibodies. To a lesser extent, the same phenomenon was observed in cat dander and grass pollen allergy.

Antibodies to different antigens may be restricted to IgG subclasses, as has been described for some antigens (19) and Thomas, Watkins & Asherson (18) presented evidence that in mouse, genes in the major histocompatibility complex can selectively control antibody classes. The production of antibodies to antigen is dependent on the nature of the antigen, the mode of exposition, and the immune responsiveness of the individual. Heredity probably plays a major role concerning the observed differences between immune responses of allergic patients and normals.

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DISCUSSION

Aas (Oslo). Q: An inverse correlation between total serum concentration of IgE and IgG when you follow different samples has been described. Have you looked at different samples from your patients with respect to these parameters? A: No, we have only investigated one blood drawing.