

Timothy-Specific IgG Antibody Levels Vary with the Pollen Seasons

S. L. NORDVALL, P. H. LARSSON and S. G. O. JOHANSSON

Dept. of Paediatrics, St Göran's Children's Hospital and Dept. of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden

Serum samples were collected from eight grass pollen hypersensitive children during a 4-year period. The sera were assayed for contents of timothy-specific IgE antibodies by RAST. Timothy-specific IgG and IgA antibodies were quantified by a refined ELISA in which covalent binding of the antigen to the polystyrene solid phase had been performed. IgG antibodies were also assayed by a Sepharose-protein-A technique with radiolabelled timothy allergens as the antigen. It was possible to register clearcut seasonal variations with postseasonally boosted antibody levels not only of timothy-specific IgE but also of IgG antibody. Both IgG1 and IgG4 antibodies specific for timothy showed seasonal variations of a similar degree. It was not possible to register seasonal variations of the same magnitude of timothy-specific IgA antibodies.

Key words: ELISA; IgG-antibodies; surface-grafting; timothy.

Accepted for publication 5 June 1986

Pollens from wind-pollinating herbs appear seasonally in the atmosphere. In the Scandinavian countries the major causes of pollenosis are pollens from the deciduous trees, which are predominant in the spring time (March-June), and grass pollens, which appear in the summer (June-August). The intermittent yearly exposure to pollens causes the characteristic symptoms of hay fever, which are known to be well correlated to the pollen load (7). The seasonal allergen boost is also known to cause an elevation of the allergen-specific serum IgE level which after some time subsides. Thus seasonal variations in the pollen-specific IgE levels are caused (2).

With sensitive techniques it has been possible to show that not only allergen-specific IgE antibodies are raised in sera of atopics but also allergen-specific IgG and IgA antibodies (15). Due to the high levels of serum IgG, the accurate determination, especially of low IgG antibody

concentrations, involves considerable problems caused by non-specific background binding. Thus, with many techniques, allergen-specific IgG antibody levels in sera of non-hyposensitized patients are registered within or only barely above background. Nevertheless, allergen-specific IgG-antibody concentrations may very well be just as high as the corresponding IgE antibody concentrations or even higher (15).

In the present work we have applied two highly sensitive assay techniques for detection of low concentrations of timothy-specific IgG antibodies: an enzyme-linked immuno-sorbent assay (ELISA) with recent modifications for improvement of sensitivity (10) and a Sepharose protein-A method (6). Sera from 4 consecutive years drawn from eight children with grass pollen hay fever were analysed for contents of timothy-specific IgG, IgA and IgE antibody and the results were related to the occurrence of pollen season.

MATERIAL AND METHODS

Patients

Eight children aged 7–16 years at the start of the study and judged to be grass pollen allergics by a typical history, positive RAST tests, positive skin prick, and conjunctival provocation tests, were included in the study. Blood was drawn annually on several occasions, before and after the grass pollen season during a 4-year period. The sera were kept frozen -18°C before analyses. During the 4 years the patients were given ordinary treatment with antihistamines and disodium cromoglycate, none were treated with corticosteroids.

Antibody analyses

Timothy-specific serum IgE antibodies were assayed by RAST, (8, 18) using the commercially available Phadebas RAST kits (Pharmacia Diagnostics AB, Uppsala, Sweden). Allergen-coated paper discs were incubated with undiluted patient serum for 3 h, washed 3 times (in approximately 2.5 ml of saline containing 0.1% Tween 20), aspirated dry and after that incubated with 100 μl of ^{125}I -labelled D_e2-specific rabbit anti-IgE (1) (50,000–70,000 cpm/test tube) overnight. After washes (see above) the count rates were determined in a gamma scintillation counter. The counts were related to those of a reference serum of the Phadebas RAST reference system and expressed in PRU/ml.

ELISA was principally run as previously described by others (5) but with several important modifications for the improvement of the sensitivity of the assay. The antigen used was a refined preparation of timothy pollen (Spectralgen) kindly provided by Pharmacia Diagnostics AB, Uppsala, Sweden. The antigen was covalently bound to polystyrene microtitre plates according to a recent method of Larsson et al. (10). The method was based on surface grafting of reactive monomers to a polymeric substrate using polystyrene microtitre plates (Nunc Immunoplate II, Nunc, Denmark). The free radical mediated reaction was initiated and

sustained by a 3 Mrad delivery, at 0.25 Mrad/h, from a ^{60}Co gamma-source. All monomeric solutions were kept free of oxygen throughout the grafting reaction.

Two kinds of surface-modified microtitre plates were used for covalent solid phase binding. The ELISA assays for total timothy-specific IgA and IgA antibody concentrations were run on acrylamide (Bio-Rad, Richmond, USA) grafted plates, which were activated with 1% aqueous glutaraldehyde and buffered to pH 7.6 at 37°C overnight. The plates were then extensively washed with distilled water in four 5-min cycles. The antigen was dissolved in a 0.1 M sodium phosphate buffer, pH 7.6, 100 μl of which was added to each well at the concentration 20 $\mu\text{l}/\text{ml}$. The binding step was allowed to continue overnight at $+4^{\circ}\text{C}$. Inactivation of remaining aminogroup reactive sites was performed by addition of 100 μl 1 M ethanolamine, pH 8 to each well. Elimination of noncovalently bound material was done by three alternate washings with a 0.1 M sodium acetate buffer, pH 4 and a 0.1 M borate buffer, pH 8.

The ELISA assays for timothy-specific IgG1 and IgG4 were performed on crotonic acid (purum, Fluka AG, Busch, Switzerland) grafted microtitre plates. The plates were activated with EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl, Bio-Rad, Richmond, USA). The carbodiimide was added in the volume of 100 $\mu\text{l}/\text{well}$ at a concentration of 10 mg/ml in 3 mM potassium phosphate buffer, pH 6.3. The plates were then incubated for 60 min at ambient temperature. After three washings with ice-cold redistilled water, 100 $\mu\text{l}/\text{well}$ of the antigen was added at a concentration of 20 $\mu\text{l}/\text{ml}$ in the pH 6.3 buffer. The binding reaction was allowed to continue overnight at 4°C . Inactivation and washing cycles were performed as for the acrylamide-grafted plates. In the assay serum was diluted 1/100 in an assay buffer solution (50 mM phosphate buffer pH 7.4, 0.15 M NaCl, 0.2% BSA, 0.1% Tween 20 and 0.02% NaN₃). 100 μl of this dilution was incubated in each well for 3 h at room temp. after which three washings were performed (see above). The aforescribed assay buffer solu-

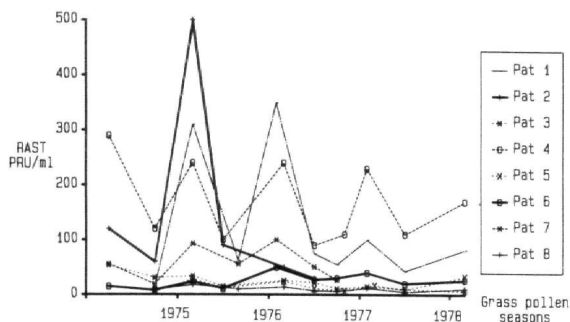


Fig. 1. Timothy-specific serum IgE antibody concentrations as measured by ELISA technique in eight atopic children during a 4-year period in relation to the pollen seasons.

tion was used also for the below described dilutions of sera and reagents. Bound IgG and IgA were detected with affinity purified anti-IgG and -IgA antisera, respectively, both at the concentration of 200 ng/ml. 100 μ l was added to each well and the incubations were performed at 37°C for 1 h. In a second step an SPDP-coupled conjugate of alkaline phosphatase and F(ab)' fragments of affinity purified sheep anti-rabbit gammaglobulin (MIAB, Uppsala, Sweden) was used, 100 μ l at the dilution 1/200.

IgG subclasses were detected using mouse monoclonals to the different IgG subclasses in the form of ascitic fluid (Seward Laboratory, London, England). Preliminary experiments indicated significant presence of only IgG1 and IgG4 antibodies, which was also in agreement with findings of others (4). Thus interest was focused on these two subclasses. 100 μ l serum diluted 1/10 was incubated overnight and after washings 100 μ l of the monoclonals was added, each at the dilution 1/2000. In the next step 100 μ l of a rabbit anti-mouse antiserum diluted 1/2000 was used, the binding of which was traced using the sheep anti-rabbit conjugate as described above.

The antisera used, with exception of the mouse monoclonals, were affinity-purified and obtained from MIAB, Uppsala, Sweden. All sera were run in duplicates and the intraassay variation for IgG, IgA, IgG1 and IgG4 were 7%, 8%, 9% and 8% respectively. p-Nitrophenyl-phosphate was used as the substrate, and extinction at 405 nm was read with a KONTRON spectrophotometer. A reference curve was run in parallel on each plate using a

reference serum. All data were expressed in relation to this serum which was arbitrarily given the contents of 100 U antibody, irrespective of class and subclass assayed.

Timothy-specific IgG antibodies were also measured by a sandwich technique using Sepharose protein-A for adsorption of IgG from the serum samples (6). ^{125} I-labelled allergen was added and after washing the bound radioactivity was measured in a gamma scintillation counter. The uptake was expressed in parts/1000 of that of a reference serum. In this assay a partially purified timothy pollen preparation obtained from Pharmacia AB, Uppsala, Sweden (Batch 780404) was used.

RESULTS

The timothy-specific IgE antibody concentrations are shown in Fig. 1. Evident variations are seen with high levels after the grass pollen seasons. Later in the years the IgE antibody levels subside. The interindividual spread in timothy-specific IgE level is about 30-fold and the individuals tend to remain at a similar level of IgE antibody concentration over the years.

The ELISA results of timothy-specific IgG (Fig. 2) showed clearcut seasonal variations which were not as pronounced as for IgE antibodies. These variations were more prominent in the patients with high levels of timothy-specific IgE antibody compared to those with low levels, in which antibody variations could be lacking entirely. The seasonal boosting effect could be registered as seasonal variations of both timothy-specific IgG1 and IgG4 (Figs. 3

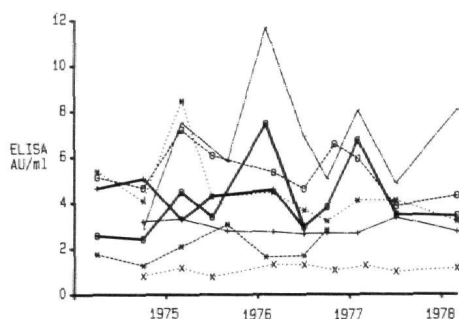


Fig. 2. Timothy-specific serum IgG antibody concentrations as measured by ELISA technique in eight atopic children during a 4-year period in relation to the pollen seasons. (Symbols see Fig. 1).

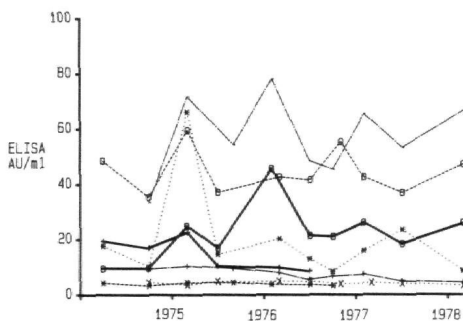


Fig. 3. Timothy-specific serum IgG1 antibody concentrations as measured with ELISA technique in eight atopic children during a 4-year period in relation to the pollen seasons. (Symbols see Fig. 1).

and 4). Since the levels of IgG1 and IgG4 were both assigned arbitrary values without mutual correspondence, they can not be accurately compared. However, these variations in antibody levels were of a similar magnitude.

The results of the Sepharose protein-A assay of IgG antibody showed seasonal variations of a similar degree (Fig. 5). These were, however, less obvious in some patients than those obtained with the ELISA technique.

The corresponding contents of timothy-specific serum IgA antibody showed considerable interindividual variations. Seasonal boost-

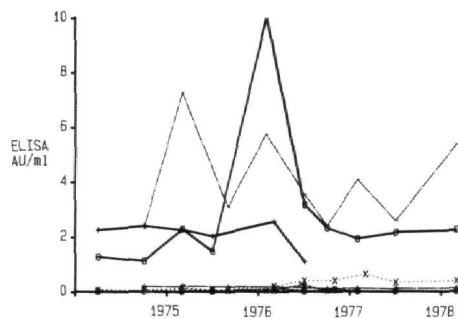


Fig. 4. Timothy-specific serum IgG4 antibody concentrations as measured with ELISA technique in eight atopic children during a 4-year period in relation to the pollen seasons. (Symbols see Fig. 1).

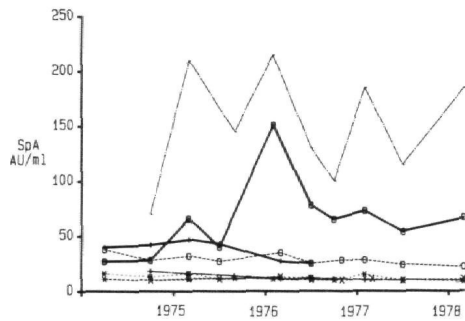


Fig. 5. Timothy-specific serum IgG antibody concentrations as measured with Sepharose protein-A technique in eight atopic children during a 4-year period in relation to the pollen seasons. (Symbols see Fig. 1).

ing effects were, however, not registered as consistently as in the case of timothy-specific IgG antibody (Fig. 6).

DISCUSSION

Atopic sensitization is characterized by the occurrence of IgE antibody synthesis. It is a typical feature that exposure in the respiratory mucosal membranes of only minute amounts of antigen, e.g. of pollen origin, is sufficient for atopic sensitization in the predisposed (11). This sensitization, however, involves not only

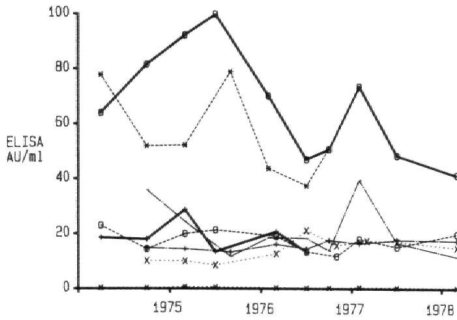


Fig. 6. Timothy-specific serum IgA antibody concentrations as measured with ELISA technique in eight atopic children during a 4-year period in relation to the pollen seasons. (Symbols see Fig. 1).

the synthesis of IgE antibody but also IgG and IgA antibody (15). The yearly seasonal pollen exposure causes the well-known variations of, e.g. the grass pollen specific IgE which is boosted postseasonally. The here presented results clearly show that timothy-specific IgG antibodies co-vary with IgE antibody of the same specificity and with the seasonal pollen exposure. This was true for IgG1 as well as IgG4 antibody which appeared to be boosted to a similar extent. Serum IgA antibody levels were not clearly boosted by the seasonal pollen load, even though there was some tendency.

Children are known to be more disposed to IgE response than adults, which is reflected in the peak level of total IgE in the early teen-ages (9). The children investigated here were of approximately this age group. Most probably, this age-related influence on the antibody response of respiratory mucosal antigen administration applies just as much for non-IgE as for IgE antibody. Concomitant decrease of mite and grass pollen-specific IgG and IgE antibody with age (16) favours this notion. It may thus be, that boostable IgG antibody responses by seasonal pollen exposure are more prominent in the young atopics. Pollen allergens are complex and timothy pollen is known to be composed of some 30 antigens, one third of which are known as allergens (17). The strongest IgE antibody responses are directed to two of these antigens,

which are considered as major allergens (17). IgG antibodies are evoked primarily to those two substances in children given immunotherapy with timothy pollen allergen extracts (14), and most probably the naturally occurring IgG antibody responses are directed primarily to these two antigens.

The present data, showing a covariation of IgG and IgE antibody levels caused by seasonal pollen exposure, fit with other data showing correlating IgG and IgE antibody levels in individual sera (3), concomitant fall of IgE and IgG antibody to allergens during aging (16), and the finding of IgG and IgE antibody responses to the same components of complex allergens (12, 13, 14). These results together show that IgE antibody responses in the atopic are an integrated part of a complex immune response to allergens.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (16X-105), the Swedish Asthma and Allergy Association and the Swedish Work Environment Fund.

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Address

Dr. Lennart Nordvall

Department of Paediatrics

St Göran's Children Hospital

Box 12500, S-112 81 Stockholm

Sweden

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