

Specific IgE and IgG antibody responses in children to timothy pollen components during immunotherapy

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Fourteen children with timothy grass pollinosis were given immunotherapy (IT) for 3 years with a purified and characterized timothy grass pollen preparation or a crude aqueous timothy pollen extract. Crossed radioimmunoelectrophoresis (CRIE) showed that 75% of the children under 11 years of age developed new specificities of IgE antibodies against timothy antigens, in contrast to older children, where no development of IgE antibodies against new timothy antigens could be detected. IgE antibodies were only detected against antigens formerly known as allergens. Timothy-specific IgG antibodies increased in most children during hyposensitization against the major allergens Ag 19 and Ag 24/25 and several other IgE-binding timothy antigens.

Key words: IgE; IgG; CIE/CRIE; timothy immunotherapy.

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It is well known that immunotherapy (IT) causes a rise in allergen-specific IgG antibodies with a possible protective effect. After an initial rise of allergen-specific IgE antibodies in the early phase of immunotherapy these antibodies tend to diminish slowly upon continued therapy (18). Pollen allergen extracts are complex mixtures of proteins, glycoproteins, peptides and different low molecular weight molecules (3, 8). The number of identified allergens in the pollen preparations vary in the range 5-20, of which grasses exhibit the highest number and trees the lowest (1, 3, 4, 9). In a previous study the effect of immunotherapy on specific IgG and IgE antibodies to birch pollen was studied in children with special reference to the possibility that immunotherapy causes sensitization ("new" IgE specificities) (12, 13). Results from that study indicated "new" IgE specificities in some children after IT but without any clinical significance. Crossed radioimmunoelectropho-

resis (CRIE) analysis of adult patients on hyposensitization with timothy grass extracts showed no development of allergy towards new timothy antigens (22, 23).

The purpose of the present investigation was to study whether IT with timothy grass pollen in children with grass pollen pollinosis induced new timothy grass pollen sensitivities. The changes in the specific IgE and IgG antibody patterns were monitored by CRIE.

MATERIAL AND METHODS

The study comprised 14 children, aged 6-16 years, with hay fever and/or asthma due to grass pollinosis, positive clinical history, positive RAST and positive skin prick test (SPT) (2). Blood was drawn before and after 3 years of IT. The sera were kept frozen (-20°C) prior to analysis.

Two different allergen preparations were used throughout the studies. One preparation

was a partly purified timothy allergen preparation containing all relevant allergens (2, 3). The low molecular weight material was removed by gel filtration on Sephadex G-75 and supplied as a freeze-dried material (Pharmacia AB, Uppsala, Sweden). The other preparation was a crude aqueous timothy pollen extract (1/10 w/v) in Coca's solution (Kabi Vitrum, Stockholm, Sweden).

A rush IT regimen was initiated under hospitalization and continued at an outpatient clinic with injections given at weekly to monthly intervals. Dosages were increased as much as possible judged by the local reactions. Detailed descriptions of the treatment regimens, allergen extracts used and clinical results have been published previously (2).

Antisera to timothy antigens were raised in six rabbits by bi-weekly subcutaneous injections

of 300 µg (dw) of a crude timothy extract. The immunization procedure was performed essentially as described by Harboe & Ingild (6).

Crossed immunoelectrophoresis (CIE) was carried out on 5 × 7 cm gel bond film (Pharmacia LKB Biotechnology) using 0.023 M Tris/Tricine Buffer (BioRAD) and 0.3 mM calcium-lactate, pH 8.6 in 1% Agarose (Agarose A, Pharmacia LKB Biotechnology) essentially as previously described (19). Samples of 5 µl of crude timothy pollen extract (10 mg/ml) were run in the first dimension electrophoresis at 10 V/cm, 15°C for 30 min. The second dimension electrophoresis was performed using 30 µl and 20 µl of rabbit anti-timothy serum/cm² in the anodic and cathodic antibody-containing compartments, respectively. The second dimension electrophoresis was run at 2 V/cm for 18 h at 15°C.

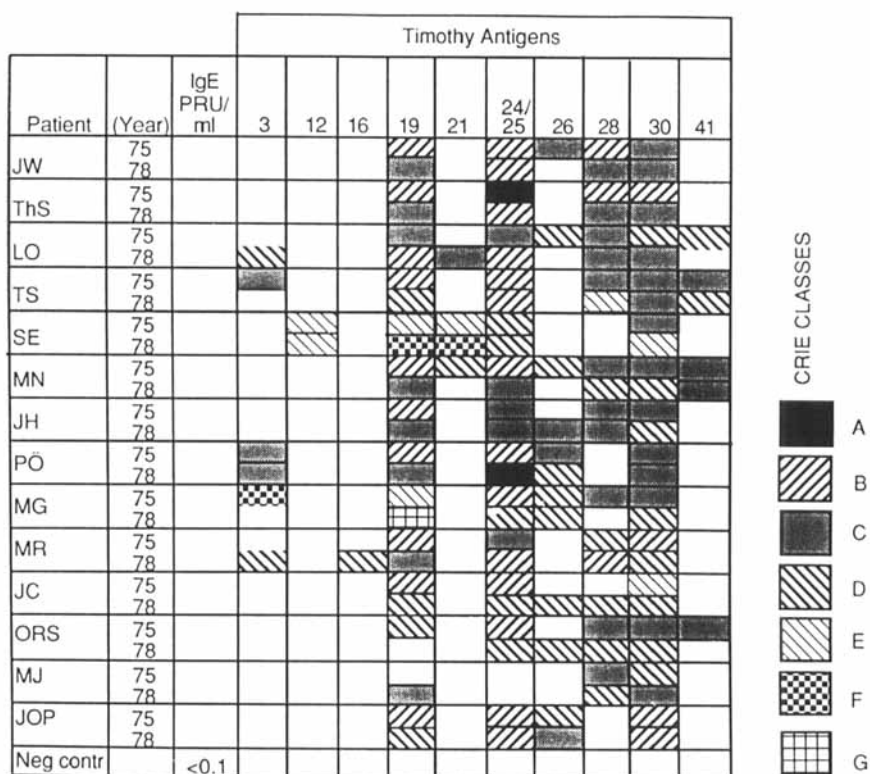


Fig. 1. Allergogram showing IgE-CRIE radiostaining of different precipitates in a CIE reference pattern of timothy grass before and after a 3-year treatment with partly purified timothy allergen preparations. The intensity of radiostaining is shown as hatched squares symbolizing the CRIE classes (A-G).

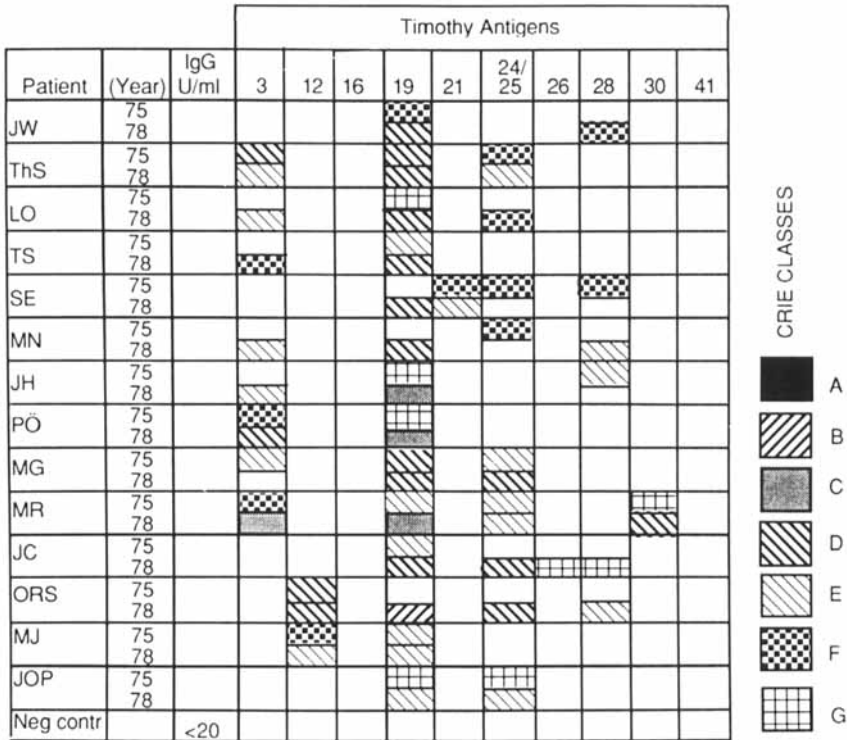


Fig. 2. Allergogram showing IgG-CRIE radiostaining of different precipitates in a CIE reference pattern of timothy grass prior to and after 3-year treatment with partly purified timothy allergen preparations. Specific IgG-binding to timothy antigens is shown as hatched squares symbolizing the CRIE classes (A-G).

IgE- and IgG-CRIE analyses were performed essentially as described previously (16, 19). CRIE classification was obtained by sequential exposure according to a previously described procedure (20).

RESULTS

The individual patients' IgE and IgG CRIE radiostaining patterns against individual timothy grass pollen antigens, before and after 3 years' IT, are presented in Figs. 1 and 2. The standard CIE reference pattern of timothy grass with arbitrary numbers assigned to the individual precipitates was used (4). Only data from distinct radiostained immunoprecipitates were evaluated, and due to difficulties in discrimination between antigens 24 and 25 they were read as 24/25. The clinically most important timothy allergens (Ag 19, Ag 24/25 and Ag 30)

showed strong IgE-CRIE radiostaining for almost all patients prior to treatment (Fig. 1). Most of the patients showed unchanged or slightly decreased radiostaining intensity for the three major allergens as a result of treatment. However, in exceptional cases there was a slight increase in IgE-binding capacity, demonstrated as increased radiostaining of the antigens representing the three major allergens.

Besides the clinically important allergens, visible radiostaining was detected for antigens 3, 12, 16, 21, 26, 28 and 41. Of these IgE-binding components a great number of the investigated patients showed relatively strong and frequent binding to Ag 26 and Ag 28, while few individuals showed radiostaining towards Ag 3. In 6/8 children under 11 years IgE specificities developed during the treatment period against Ags 3, 16, 19, 21, 26, 28, and 41. However, there was no difference due to the allergens used for im-

munotherapy as 50% of these children were treated with purified extract and 50% with crude extract. The CRIE-staining of these sensitivities was relatively strong (CRIE class C and D in the allergogram), indicating high IgE binding capacity. In total, we could detect 10 different timothy grass antigens which showed binding of specific IgE in the sera of the patients studied. All of these IgE binding components are previously identified as allergens in timothy grass.

The specificities of the IgG antibodies were very similar to those of the IgE antibodies, i.e. IgG antibodies to the two major allergens 19 and 24/25 were most frequently found (Fig. 2). Of these two antigens, antigen 19 appeared as the most important with respect to induction of specific IgG antibodies. IgG antibody responses to Ags 3 and 30 were also recorded. In the pretreatment sample specific IgG antibody binding occurred only rarely; in those cases mostly towards antigen 19. However, the degree of radiostaining corresponding to antigen 19 had increased markedly when the results of the pretreatment and post-treatment samples were compared.

DISCUSSION

The mode of antigen presentation is known to be of importance for IgE antibody synthesis in experimental allergy (14). Inhalation allergy occurs as a consequence of sensitization via the mucous membranes, and it is known that among more than 30 identified timothy pollen antigens, at least 16 may bind specific IgE antibodies in CRIE analysis (4, 9). In hyposensitization therapy high antigen dosages are given subcutaneously. There might be a risk for sensitization to other antigens than the 16 already known to be allergens. However, this did not appear to be the case in the present study. In none of the cases did new IgE specificities occur to antigens not previously known as allergens. Here our data are in good agreement with those of others (5, 7, 10, 11). In 75% of the children under 11 years, CRIE demonstrated that new IgE specificities developed towards at least seven different antigens. These data are in

good agreement with the recently data published by Möller et al. (12, 13). No such new IgE specificities were found in older children, which is in agreement with CRIE data from Østerballe et al. (22, 23) who studied adult patients during timothy grass pollen therapy. However, the detection of IgE sensitivities in post-treatment samples which were not detectable in the pretreatment samples cannot be ascribed to the therapy alone, since sensitization may also be due to the seasonal pollen exposures during the observation time. This latter explanation is very likely since there is a strong boost of antibody responses to pollen allergens as a consequence of seasonal pollen exposure (15). IgG antibody patterns showed agreement with the IgE antibody patterns shown in other allergen systems (14, 17, 21).

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