

A Brief Report: IgG and IgE Antibody Responses of Children and Adults Following Polymerized Tree Immunotherapy

LESLIE C. GRAMMER, M.D., MARTHA A. SHAUGHNESSY, B.S.,
LOUI SILVESTRI, Ph.D.¹ and ROY PATTERSON, M.D.

ABSTRACT

The IgG and IgE antibody responses to elm and oak tree pollen were analyzed by enzyme-linked immunosorbent assay (ELISA) in 18 children and 12 adults with tree pollinosis. Children and adults had similar initial ELISA titers. After receiving approximately 50,000 protein nitrogen units (PNU) of individually polymerized tree pollen (IPT) allergens in 12 weekly injections, children and adults had similar IgG responses to elm and oak and similar IgE responses to elm. Adults had a slight increase in IgE to oak that was statistically significant when compared with the increase in children ($p = 0.02$). In general, then, both children and adults have similar changes in IgG and IgE titers to tree pollens after IPT immunotherapy, demonstrating retained immunogenicity of IPT.

INTRODUCTION

NUMEROUS STUDIES have been reported comparisons of serologic responses in children and adults. After vaccination with *Haemophilus influenzae* type b capsule⁽¹⁾ and after influenza vaccination,⁽²⁻⁶⁾ antibody responses are significantly greater in adults as compared to children. The difference in response may be that adults tend to be primed for an anamnestic response because they have been exposed previously to similar antigens whereas children tend not to be primed.⁽⁶⁾ After natural infection with influenza, there have been conflicting reports as to whether adults have higher or similar influenza titers as compared to children.^(3,7) In contrast, it has been reported that children actually develop higher titers than adults during primary immunization

Section of Allergy-Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

¹Biosearch Inc., Pembroke Pines, Florida.

with tetanus and diphtheria toxoids.⁽⁸⁾ The differences in the serological responsiveness observed in the two different age groups may be related to the relative dose of antigen per kilogram of body weight,^(9,10) and many other factors such as age and prior exposure.

Immunotherapy has been studied in both children and adults, and its efficacy has been reported.^(11,12) Polymerized allergens have been studied and shown to be immunogenic in adults,⁽¹³⁾ but they have not been studied in children. The purpose of this study was twofold; we studied the immunogenicity of individually polymerized tree pollens (IPT)⁽¹⁴⁾ in children and also compared specific IgG and IgE titers in children and adults.

MATERIALS AND METHODS

Patients

At two sites, 12 adults and 18 children who had symptoms of sneezing, rhinorrhea, nasal congestion, and eye pruritus correlating with the tree pollen season and positive skin tests to tree allergens were recruited. Of the 12 adults, 11 were prick positive to oak and 9 to elm; of the 18 children, 14 were prick positive to oak and 12 to elm.

The patients were otherwise healthy as determined by history, physical examination, and clinical laboratory tests. No patient had received immunotherapy for at least 5 years prior to the initiation of immunotherapy injections. This protocol was approved by the Institutional Review Board (IRB) at each institution.

IPT Immunotherapy

Polymerized oak pollen and polymerized elm pollen were both prepared at Allergy Laboratories of Ohio (ALO). A detailed description of the preparation of IPT has been published previously.⁽¹⁴⁾ General safety and sterility requirements of the Food and Drug Administration, Office of Biologics Research and Review, and the determination of protein nitrogen units (PNU) per milliliter were performed on IPT by ALO. The preparations were also characterized by analytic gel filtration chromatography, and allergenicity was determined by intradermal end point titration on at least 3 sensitive individuals.⁽¹⁵⁾

The immunotherapy course consisted of a total of approximately 50,000 PNU administered over 12 weekly visits. The children were treated at Duke University, while the adults were treated at Northwestern University.

Enzyme-linked immunosorbent assay (ELISA)

A sample of blood was obtained from each patient prior to the initial injection and subsequent to the last injection. Serum was separated from the cells and was stored frozen (-20°C) until the immunoassays were performed. All assays were performed on at least two separate occasions. Pretreatment and posttreatment sera for each patient were assayed together.

For detection of IgG to each of the tree pollens, Immulon microELISA plates (Greiner and Son, Nurtingen, West Germany) were coated with each of the following reagents in sequence according to the methods of Voller *et al.*⁽¹⁶⁾ and Sepulveda *et al.*⁽¹⁷⁾: oak (lot no. A3022201) or elm (lot no. A3022102) extract (ALO) diluted to 150 μg of protein per milliliter, appropriate dilutions of patient's serum, rabbit antihuman IgG (Calbiochem-Behring, La Jolla, CA), goat antirabbit IgG alkaline phosphatase conjugated (Sigma Chemical Co., St. Louis, MO), and p-nitrophenyl phosphate. Appropriate washes were performed between the reagent additions. Optical density was read at 405 nm on an Artek automated ELISA reader (Farmingdale, NY). A positive titer was the highest dilution of patient serum resulting in an optical density more than twice that of the mean of the control sera.

POLYMERIZED TREE IMMUNOTHERAPY

For the detection of IgE, an identical sequence of reagents was added to the wells, except that rabbit antihuman IgE epsilon-specific (Calbiochem-Behring) was added instead of rabbit antihuman IgG. Control sera for each assay were obtained from nonatopic individuals of the same age group.

Statistical analyses

Statistical analyses were performed using Epistat software on an IBM PC XT. Comparisons of categorical data between groups were performed by chi square analysis. Comparisons of titers or changes in titers between groups were performed by Student's *t* test on log-transformed values, while comparisons of changes of titers within a group were performed by paired *t* test on log-transformed values. Skin test end point data were analyzed using the Mann-Whitney rank sum test.

RESULTS

ELISA

There were no significant differences between children and adults in terms of the $\log_{10}$ of their pretreatment ELISA titers (Table 1). The mean logs of the pretreatment IgE titers to elm in children and adults were 0.8 and 0.9, respectively ($p = 0.19$), while the mean log of the IgE titers to oak was 0.8 and for both children and adults ($p = 0.44$). The mean logs of the pretreatment IgG titers to elm in children and adults were 2.1 and 2.0, respectively ($p = 0.33$), while the mean logs of the IgG titers to oak were 2.2 and 2.1, respectively ($p = 0.41$).

The changes with immunotherapy in log titer of IgG against each tree pollen are presented in Fig. 1, while the changes in log titers to IgE are presented in Fig. 2. There were statistically significant increases in log IgG titer to both trees for children and adults, respectively (elm $p = 0.008$ and $p = 0.05$; oak $p = 0.006$ and $p = 0.007$). There were no significant differences between children and adults in the increase in IgG titer to elm ($p = 0.67$) or oak ($p = 0.62$).

There were similar very slight increases in IgE titer to elm both in adults ($p = 0.12$) and children ($p = 0.05$). There was no significant difference between children and adults in the increase in IgE titer to elm ($p = 0.37$). There was no increase in IgE titer to oak in children ($p = 0.48$) and a twofold increase in adults ($p = 0.02$); there was a statistically significant difference between children and adults in the change in oak IgE titer ($p = 0.02$).

Skin tests

There were no significant differences between children and adults in terms of their intradermal end point titers to oak and elm.

TABLE 1. PRETREATMENT CHARACTERISTICS OF PATIENTS

	<i>Children</i>	<i>Adults</i>	<i>Comparison</i>
Number	18	12	
Mean pre treatment ELISA titer (log)			
IgE elm	0.8	0.9	$p = 0.19$
oak	0.8	0.8	$p = 0.44$
IgG elm	2.1	2.0	$p = 0.33$
oak	2.2	2.1	$p = 0.41$

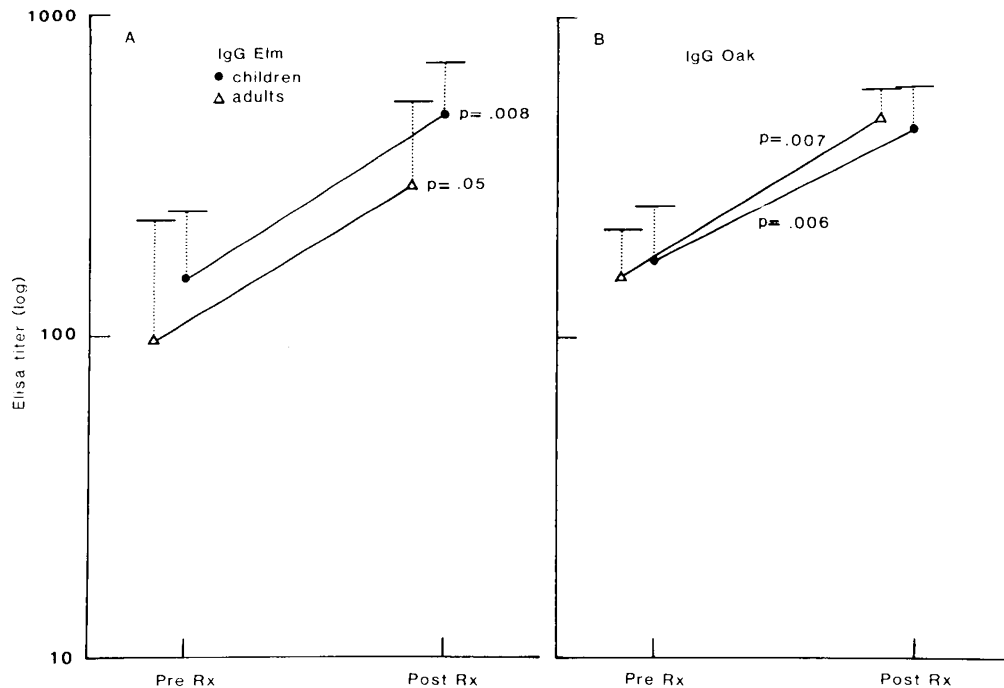


Fig. 1. a. IgG titers to elm by ELISA in children (●) and adults (Δ) before and after treatment with IPT. The vertical bars are SEM. There is no significant difference between the two groups with respect to change in titer with treatment ($p = 0.67$). b. IgG titers to oak by ELISA in children (●) and adults (Δ) before and after treatment with IPT. The vertical bars are SEM. There is no significant difference between the two groups with respect to change in titer with treatment ($p = 0.62$).

DISCUSSION

In this report, we described the development of a significant rise in IgG response after immunotherapy with oak and elm IPT in both adults and children. Preimmunotherapy levels of IgG were similar in children and adults. The levels after IPT were similar to those observed after immunotherapy with conventional, monomeric tree extracts.⁽¹⁸⁾ We also describe a slight rise in IgE to elm in children and adults after the elm IPT, a slight rise to oak in adults, and the absence of change in IgE titer in children after oak IPT. While there is a statistically significant difference between children and adults in the change of IgE titer to oak, that difference is mathematically small and thus we do not attribute any clinical importance to it. It should be noted that age is very important for choosing appropriate negative controls for this assay. The similarity of response in adults and children in our study is not unexpected as all of the children in this study were 5 or older. Studies that have described a pronounced difference between adults and children, have studied children younger than 5.

Polymerized allergens have been extensively studied in adults. Immunotherapy with polymerized ragweed^(19,20) and immunotherapy with individually polymerized grasses⁽²¹⁾ have been demonstrated to be immunogenic, safe, and result in lower symptom-medication scores. In addition, polymerized allergens are less allergenic on a weight basis than monomeric allergens.^(22,23) This is the first report of immunotherapy with polymerized allergens in children. We focused on the immune response in children and found that IPT is immunogenic in children just as it is in adults.⁽¹⁸⁾

In summary, this study demonstrates that the IgG and IgE immune responses to IPT immunotherapy are similar in children and adults. In addition, the responses are similar to those generated when patients are treated with conventional tree immunotherapy extracts.

POLYMERIZED TREE IMMUNOTHERAPY

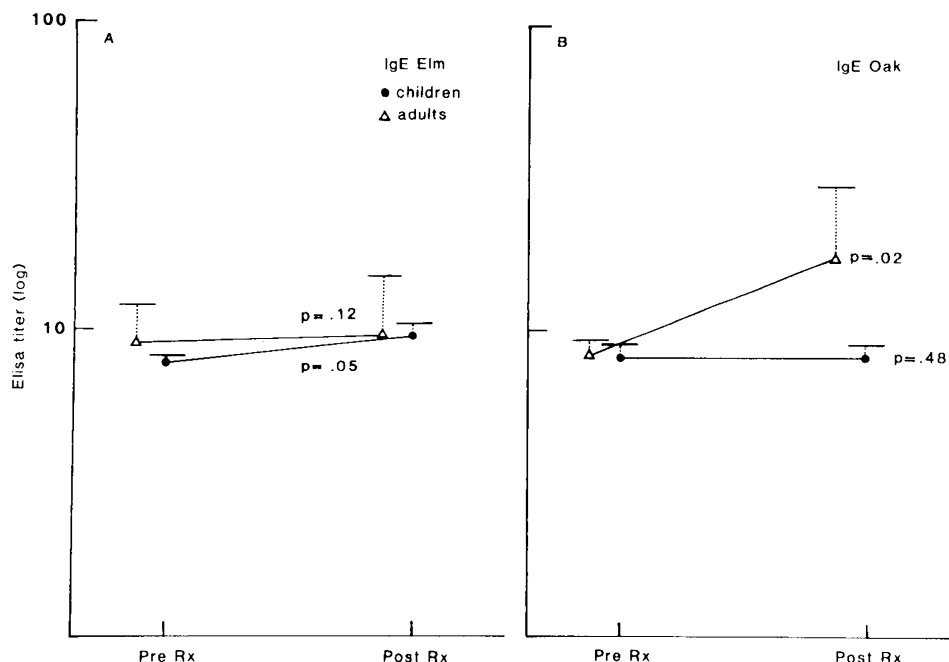


Fig. 2 a. IgE titers to elm by ELISA in children (●) and adults (Δ) before and after treatment with IPT. The vertical bars are SEM. There is no significant difference between the two groups with respect to change in titer with treatment ($p = 0.37$). b. IgE titers to oak by ELISA in children (●) and adults (Δ) before and after treatment with IPT. The vertical bars are SEM. There is a significant difference between the two groups with respect to change in titer with treatment ($p = 0.02$).

ACKNOWLEDGMENT

This work was supported by USPHS Grant AI 11403, the Ernest S. Bazley Trust, and Key Pharmaceuticals, Inc.

REFERENCES

1. Pichichero ME, Insel RA. Mucosal antibody response to parenteral vaccination with *Haemophilus influenzae* type b capsule. *J Allergy Clin Immunol* 1983;72:481-486.
2. Boyer KM, Cherry JD, Noble GR. IgM and IgG antibody responses following immunization of children and adults with influenza A/NJ/76 vaccines. In: International Symposium on Influenza Immunization, Develop Biol Standard. Basel: S. Karger, 1977.
3. Mancini G, Donatelli G, Ruiz A, Rozera C. Detection of antibodies to strain-specific and cross reactive antigenic determinants on the hemagglutinin of influenza viruses A/Texas/1/77 and A/Bangkok/1/79 in human sera. *Arch Virol* 1984;79:117-121.
4. Parkman PD, Galasso GJ, Top FH, Noble GR. Summary of clinical trials of influenza vaccines. *J Infect Dis* 1976;134:100-107.
5. Wright PF, Dolin R, LaMontagne JR. Summary of clinical trials of influenza vaccine II. *J Infect Dis* 1976;134:633-638.
6. Fazekas de St. Groth S, Webster RG. Disquisitions on original antigenic sin. I. Evidence in man. *J Exp Med* 1966;124:347-361.
7. Ruben FL, Bachmayer H, Fireman P. Parameters of humoral and cellular immunity to influenza A/USSR/90/77 (H1N1) virus in various age groups. *J Infect Dis* 1980;142:699-703.

8. Myers MG, Beckman CW, Vosdingh RA, Hawkins WA. Primary immunization with tetanus and diphtheria toxoids. *J Am Med Assoc* 1982;248:2478-2480.
9. Edsall G, Altman JS, Gaspar AJ. Combined tetanus-diphtheria immunization of adults: Uses of small doses of diphtheria toxoid. *Am J Public Health* 1954;44:1537-1545.
10. MacLennan R, Levine L, Newell KW. The early primary immune response to absorbed tetanus toxoid in man. *Bull WHO* 1973;49:615-626.
11. VanMetre TE, Adkinson NF. Immunotherapy for aeroallergen disease. In: E Middleton, CE Reed, EF Ellis, NF Adkinson, JW Yunginger, eds. *Allergy Principles and Practice*. St. Louis: C.V. Mosby, 1988.
12. Norman PS, Lichtenstein LM. Allergic rhinitis. In: M Samter, ed. *Immunologic Disease*. Boston: Little, Brown, and Company, 1978.
13. Patterson R. Allergen immunotherapy with modified allergens. *J Allergy Clin Immunol* 1981;68:85-90.
14. Patterson R, Suszko IM, Grammer LC. Polymerization of individual species of tree pollen allergens. *Int Arch Allergy Appl Immunol* 1984;73:1-4.
15. Grammer LC, Silvestri L, Suszko IM, Shaughnessy MA, Patterson R. Evaluation and standardization of polymerized ragweed extracts by chromatography, radioimmunoassay inhibition, and end point cutaneous titration. *J Allergy Clin Immunol* 1983;72:160-167.
16. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine: Theory and practice. *Bull WHO* 1976;53:55-65.
17. Sepulveda R, Longbottom JL, Pepys J. Enzyme-linked immunosorbent assay (ELISA) for IgG and IgE antibodies to protein and polysaccharide antigens of *Aspergillus fumigatus*. *Clin Allergy* 1979;9:359-371.
18. Grammer LC, Shaughnessy MA, Finkle SM, Shaughnessy JJ, Patterson R. Safety and immunogenicity of immunotherapy with polymerized tree, grass, and ragweed in patients with multiple inhalant sensitivities. *J Allergy Clin Immunol* 1986;77:53-58.
19. Grammer LC, Zeiss CR, Suszko IM, Shaughnessy MA, Patterson R. A double-blind placebo controlled trial of polymerized whole ragweed for immunotherapy of ragweed allergy. *J Allergy Clin Immunol* 1982;69:494-499.
20. Grammer LC, Shaughnessy MA, Bernhard MI, Finkle SM, Pyle HR, Silvestri L, Patterson R. The safety and activity of polymerized ragweed: A double-blind, placebo-controlled trial in 81 patients with ragweed rhinitis. *J Allergy Clin Immunol* 1987;80:177-183.
21. Grammer LC, Shaughnessy MA, Suszko IM, Shaughnessy JJ, Patterson R. A double-blind histamine placebo-controlled trial of polymerized whole grass for immunotherapy of grass allergy. *J Allergy Clin Immunol* 1983;72:448-453.
22. Patterson R, Suszko IM, Zeiss CR, Pruzansky JJ, Bacal E. Comparison of immune reactivity to polyvalent monomeric and polymeric ragweed antigens. *J Allergy Clin Immunol* 1978;61:28-35.
23. Hendrix SG, Patterson R, Zeiss CR, Suszko IM. Further studies on the safety of polymerized antigens for immunotherapy. *J Allergy Clin Immunol* 1981;67:124-128.

Address reprint requests to:
 Leslie C. Grammer, M.D.
 303 E. Chicago Avenue
 Chicago, IL 60611