

Allergen specific IgG Antibodies in Nasal Secretion During Allergen Specific Immunotherapy

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요 약

INTRODUCTION

들쭉 알레르겐으로 면역요법을 받고 있는 들쭉화분 알레르기성 비염환자 9례의 비점액 및 혈청내 들쭉 특이 IgG(Rw-IgG), IgG1(Rw-IgG1) 과 IgG4(Rw-IgG4) 를 단일클론항체와 fluorogenic substate 를 이용한 간접 효소면역측정법에 의하여 정량적으로 측정하였다. 이들 환자에서 평균 Rw-IgG 치는 면역치료 6개월후에 알레르겐의 용량 증가와 더불어 비점액과 혈청내 모두에서 의미있게 증가 되었다 ($p < 0.01$). 면역치료 첫 12개월 동안에 환자들중 4례는 비점액과 혈청 모두에서 알레르겐 면역치료 기간이 경과할 수록 점진적인 Rw-IgG 치의 증가를 보인 반면, 1례에서는 혈청내에서만 증가하는 양상을 보였다. 한편, 비점액내 Rw-IgG 치와 혈청내 Rw-IgG 치는 비교적 유의한 상관성이 있었으나($r=0.69$), 비점액내 Rw-IgG1 은 미량이지는 하지만 본 효소면역 측정법의 측정 하한치 수준의 Rw-IgG4치 보다 다소 높은 경향을 나타내었다.

결론적으로 들쭉알레르겐 면역치료를 따라 증기되는 혈청내 들쭉알레르겐 특이 IgG 및 IgG아형항체들과는 다르게 비점액내 들쭉알레르겐 특이 IgG 및 IgG아형항체들은 아마도 국소적으로 형성되고, 또한 국소적 면역반응으로 면역치료의 효과를 판정하기에는 더많은 연구가 필요할 것으로 사료된다.

In grass pollen-allergic patients, the successful allergen specific immunotherapy often results in an increase of allergen-specific IgG antibodies in serum and nasal secretions, and there is a close association between the specific IgG antibody level in the serum and their clinical effects^{1~6}). The antibody response is mainly restricted to IgG1 and IgG^{7,8}), and the IgG4 antibody has been proposed to be the blocking antibodies in serum of patients undergoing allergen immunotherapy^{9~15}). However, the origin and the blocking activity of the IgG antibodies in nasal secretions are still controversial, although there are several reports that the antibody is either synthesized locally^{16,17}) or predominantly reached nasal secretion by passive diffusion of serum-derived¹⁸), and may have either blocking effect in the secretion^{16,19,20}), or not^{16,17}).

This is a preliminary report attempting to measure the absolute amount of short ragweed-specific-IgG (Rw-IgG), and IgG subclasses (Rw-IgG and Rw-IgG4) in serum and nasal secretions of allergic rhinitis patients undergoing parenteral immunotherapy with short ragweed pollen extract to define possible origin and blocking activity of Rw-IgG

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and IgG subclass antibodies in nasal secretions.

MATERIAL AND MEHODS

Patients:

Nine patients with allergic rhinitis to short ragweed pollen were diagnosed by the history and skin testing. In some of them the diagnosis was confirmed by histamine releasing test, RAST and/or nasal provocation at the Allergy Center of Johns Hopkins University. The patients were currently undergoing parenteral immunotherapy with short ragweed pollen extract up to 12 months, as described previously⁵⁾. Serum and nasal washes were obtained from the patients and kept at 70°C until use.

Fractionation of nasal washes:

Nasal secretions were collected by nasal lavage with 15 ml of normal saline. Subsequently all samples were centrifuged and concentrated by using the Immersible-CX ultrafilters (Millipore, USA).

ELISA:

Indirect ELISAs were performed by using mouse monoclonal anti-human IgG subclass antibodies and fluorogenic substrate, described as previously²¹⁾. Briefly, wells of microtiter plates (Dynatec, Alexandria, VA) were coated 150 μ l/well of 200ng Amb a I/ml of ragweed extract in 0.1M acetate buffer (pH 6.5) and incubated overnight at 4°C. Proper dilutions of serum were made with PBS-Tween 20, pH 7.5, and 100 μ l of tjese serum and the concentrated nasal secretions were added to the wells and incubated overnight at 4°C. The standard reference serum pools were prepared from estimation by a multiple einear equation²¹⁾. Negative serum was prepared by pooling serum the from the laboratory personnel with no history of allergy. Adding and incubations with the second antibody

mouse monoclonal anti-human IgG (Lot No. 3598R, clone 6064) and IgG antibodies (Lot No. 3150R, clone HP 6012 for IgG1; Lot No 111185, HP 6025, Unipath, UK for IgG4), the third antibody alkaline-phosphate conjugated goat anti-mouse IgG (Lot No. 50-15-02, Tago, USA), and fluorogenic substrate (Sigma, USA) were performed in the same manner, respectively.

RESULTS

Allergen specific IgG antibody changes during allergen immunotherapy:

The changes of Rw IgG antibody responses in serum and nasal secretions during allergen immunotherapy were generally discernible ($p < 0.01$, Table 1). The mean values of Rw-IgG antibody in serum of the patients were significantly increased until twelve months of the specific immunotherapy. In contrast, Rw IgG antibodies in nasal secretions were small amount and somewhat increased throughout the period of the immunotherapy. More interestingly, Rw IgG antibodides in both serum and nasal secretions of four patients undergoing RWE immunotherapy were slowly increased till the

Table 1. The Changes of Allergen Specific IgG Antibodies in Serum and in Nasal Washes of Allergic Rhinitis Patients Undergoing Allergen Specific Immunotherapy

	Before treatment (N=9)	Duration of immunotherapy		
		3 months (N=5)	6 months (N=4)	12 months (N=5)
	Rw-IgG (ng/mL)	Rw-IgG (ng/mL)	Rw-IgG (ng/mL)	Rw-IgG (ng/mL)
Serum	389.4 $\pm$ 132.5*	860.5 $\pm$ 310.4	2805.2 $\pm$ 1130.3	2023.4 $\pm$ 808.0
Nasal	3.9 $\pm$ 1.5	2.4 $\pm$ 1.0	7.0 $\pm$ 3.8	5.0 $\pm$ 2.1

* Mean $\pm$ S.E.

** Significant difference both in serum and nasal IgG antibodies ($p < 0.01$), when the IgG levels before treatment were compared to those after the 6th month of immunotherapy.

sixth month of immunotherapy and then made plateaus in both serum and nasal secretions except one patient (Fig. 1).

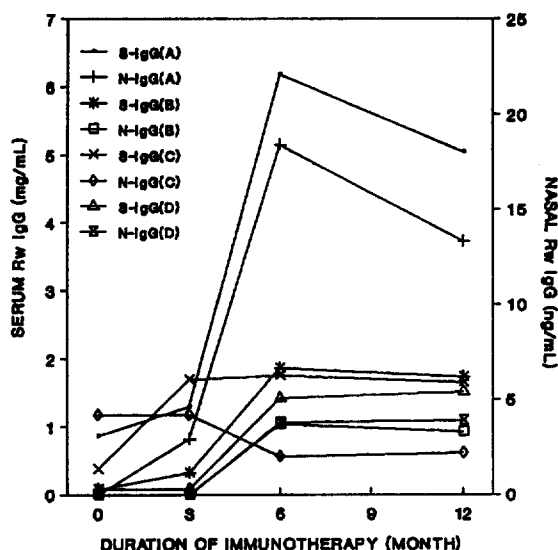


Fig 1. The ragweed specific IgG antibodies in both serum and nasal secretions of four typical allergic rhinitis patients undergoing short ragweed extract (RwE) immunotherapy were slowly increased till the sixth month of the immunotherapy and then made plateaus in both serum and nasal secretions except one patient.

Relationship between the accumulative doses of allergen and IgG antibody responses:

A profile of Rw IgG antibody responses in serum of patients undergoing immunotherapy was showing a positive correlation between the accumulative dose of ragweed extract administered during immunotherapy and Rw IgG antibody level ($Y=1099.6X+561.4$, $r=0.540$, Fig. 2). On the other hand, the specific IgG responses in nasal secretions were much more variable and were not persistently related to accumulative dose of Rw antigen (Fig. 3).

Relationship between allergen specific serum IgG and nasal IgG:

The levels of Rw IgG in nasal washes correlated

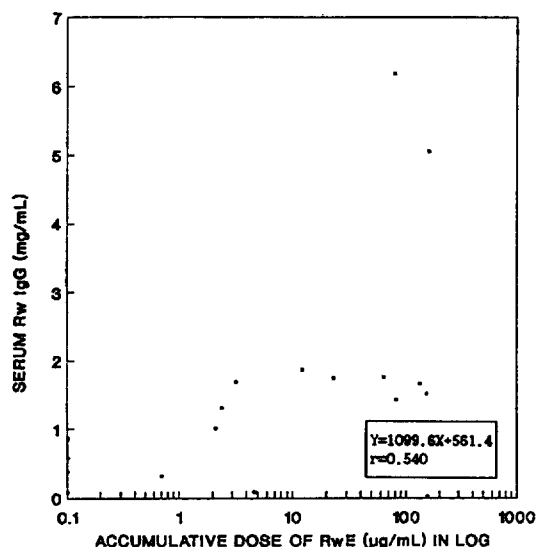


Fig 2. A profile of Rw IgG antibody responses in serum of patients undergoing immunotherapy is showing a positive correlation between the accumulative dose of ragweed extract administered during immunotherapy and Rw IgG antibody level ($Y=1099.6X+561.4$, $r=0.540$).

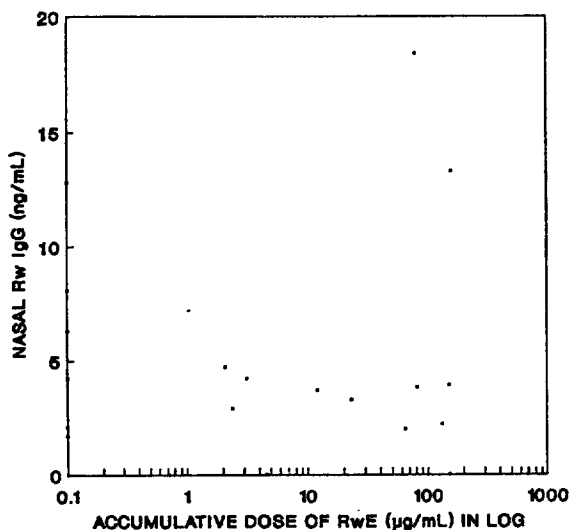


Fig 3. The allergen specific IgG responses in nasal secretions were much more variable and were not persistently related to accumulative dose of Rw antigen.

significantly with Rw IgG antibody titers in fifteen serum samples, but those of the others were outlier ($r=0.69$, $p=0.1$, Fig. 4).

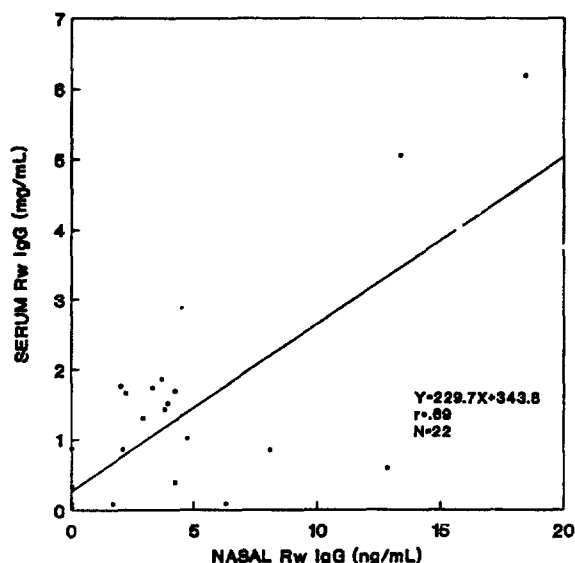


Fig. 4. The levels of Rw IgG in nasal washes correlated significantly with Rw IgG antibody titers in fifteen serum samples, but those of the others were outlier ($r=0.69$, $p=0.1$)

Allergen specific-IgG4 antibodies in nasal washes :

Rw-IgG1 and IgG4 antibodies in serum and nasal secretions at 12 month of allergen immunotherapy were randomly determined. In nasal secretions, IgG1 antibody levels were appreciably higher than IgG4 antibody levels in both serum and nasal washes (Table 2).

DISCUSSION

Efforts in laboratories have long been directed toward understanding the clinical responses and descriptive changes of the laboratory findings following successful allergen specific immunotherapy in patients with IgE antibody-mediated allergy to

Table 2. The Allergen Specific IgG and IgG Subclasses in Serum and Nasal Secretion of Patients with Allergic Rhinitis at 12th Month of Allergen Immunotherapy

Patient No.	Serum (ng/mL)			Nasal Secretion (ng/mL)		
	IgG	IgG1	IgG4	IgG	IgG1	IgG4
2	174	139.6	7.8	3.2	2.5	<0.05
11	5048	4049.0	922.2	13.3	3.2	0.13
12	ND*	ND	ND	1.3	1.1	<0.05
18	1732	1389.2	316.4	3.3	1.2	0.09
23	ND	ND	ND	1.3	1.2	0.14
29	ND	ND	ND	2.2	1.6	0.62
32	ND	ND	ND	4.0	3.8	0.20
36	ND	ND	ND	2.8	2.0	0.82
41	1510	1211.2	275.9	3.9	2.8	0.11

* Not determined

inhalant allergens. The parenteral immunotherapy first results in a marked rise of allergen-specific IgG antibodies and suppression of allergen specific IgE antibody production as well in nasal secretions^{16,22)}, as in serum^{5,23-26)}. The supposed explanation for the knowledge was partly supported by the result of experiment that 46 to 69% of nasal IgG cells were Jchain-positive immunocytes probably belonged to the secretory immune system²⁷⁾.

Concerning the change of Rw IgG antibodies in serum and nasal secretions during immunotherapy, serum IgG antibodies were significantly increased after six months of immunotherapy with increasing doses of Rw extract ($p<0.01$, Table 1), as reported previously⁵⁾. In contrast, Rw IgG antibodies in nasal secretions were minimally increased throughout the period of the immunotherapy. Interestingly Rw IgG antibodies in both serum and nasal secretions of four typical patients undergoing RwE immunotherapy were slowly increased till the sixth month of immunotherapy and then made plateaus in both serum and nasal secretions except one patient (Fig.1). Platts-Mills et al¹⁶⁾ also re-

ported that defined rise of IgG antibody in nasal secretion could not have been observed after immunotherapy with the doses of grass pollen extract. However, a profile of Rw IgG antibody responses in serum of patients undergoing immunotherapy is showing a positive correlation between the accumulative dose of ragweed extract administered during immunotherapy and Rw IgG antibody level ($Y = 1099.6X + 561.4$, $r = 0.540$, Fig. 2). On the other hand, the specific IgG responses in nasal secretions were much more variable and were not persistently related to accumulative dose of Rw antigen (Fig. 3). And the minor increases of allergen specific IgG and IgA antibodies in nasal secretion could not be explanation for being persistently related with incomplete relief of allergic rhinitis symptoms²⁸⁾. Furthermore, concerning the IgG subclass antibody responses during allergen immunotherapy Holt et al²⁹⁾ showed interesting results that after repeated inhalation of aerosols containing ragweed antigen, subsequent parenteral challenge of the animal revealed that aerosol exposure had selectively suppressed responsiveness to this antigen in the IgE antibody class, without affecting corresponding IgG responses²⁹⁾. And Deuschl et al²⁸⁾ were able to find modest increase in IgG antibodies in secretions after parenteral immunotherapy with grass pollen extract.

The allergen specific IgG subclass antibody responses might be mainly restricted to IgG1 and IgG4^{7,8)}, and the IgG4 antibody proposed to be the blocking antibodies⁹⁻¹⁵⁾, after allergen specific immunotherapy. However, the nature of antigen plays a role in determining antibody subclass³⁰⁾ and the recent reports emphasized the importance of IgG1 antibodies instead of IgG4^{7,31)}. In another point of view among the four IgG subclass IgG-producing cells, IgG1 largely dominates (60-70%) both in mucosa and tonsils³²⁾. In this study, IgG1 antibody levels were appreciably higher than IgG4

antibody levels in both serum and nasal washes (Table 2) and that should be partly supported by our previous unpublished data of Rw IgG subclasses in serum of Rw allergic patients³³⁾.

To define possible site of allergen-specific IgG and IgG subclass antibody produced predominantly, the crucial review was performed. Platts-Mills et al¹⁶⁾ previously suggested that no relationship between IgG antibody titer in serum and nasal secretions of patients with allergic rhinitis, and the IgG antibody in nasal secretion is likely produced locally¹⁶⁾. The levels of Rw IgG in nasal washes correlated significantly with Rw IgG antibody titers in fifteen serum samples, but those of the others were not ($r = 0.96$, $p = 0.1$, Fig. 4).

In conclusion, allergen-specific IgG and IgG Subclass antibodies in nasal secretion might be synthesized locally and appear to be not enough to play a crucial role in blocking activities to clinical symptoms.

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References

- 1) Lichtenstein LM, Norman PS, Winkler WL, Osler AG : In vitro studies of human ragweed allergy : changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 45 : 126, 1976
- 2) Lichtenstein LM, Norman PS, Winkler WL : Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. *Am J Med* 44 : 514, 1968
- 3) Yunginger JW, Gleich GJ : Seasonal changes in IgE antibodies and their reall-

- tionship to IgG antibodies during immunotherapy for ragweed hay fever. *J Clin Invest* 52 : 1268, 1973
- 4) Gleich GJ, Zimmerman EM, Hendersson IL, Yunginger JW : Effect of immunotherapy on IgE and IgG antibodies to ragweed antigens : a six-year prospective study. *J Allergy Clin Immunol* 70 : 261, 1982
 - 5) Creticos PS, van Metre TE, Mardiney MR, Rosenberg GL, Adkinson Jr NF : Dose response IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 73 : 94, 1984
 - 6) Hedlin G, Silber G, Schieken L, Naclerio R, Proud D, Eggleston P, Adkinson Jr NF : Attenuation of allergen sensitivity early in the course of ragweed immunotherapy. *J Allergy Clin Immunol* 84 : 390-399, 1989
 - 7) Djurup R, Osterballe O : IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. *Allergy* 39 : 433-441, 1984
 - 8) Djurup R : The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 40 : 469-473, 1985
 - 9) Devey ME, Wilson DV, Wheeler AW : The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 6 : 227-236, 1976
 - 10) Giessen M van der, Homan Wl, Kernebeek G van, Aalberse RC, Diges PH : Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Archs Allergy Appl Immunol* 50 : 625-640, 1976
 - 11) Nakagawa T, Takaishi T, Sakamoto Y, Ito K, Miyamoto T, Skvaril F : IgG 4 antibodies in patients with house dust mite-sensitive bronchial asthma : relationship with antigen-specific immunotherapy. *Int Archs Allergy Appl Immunol* 71 : 122-125, 1983
 - 12) Ortolani C, Pastorello E, Moss RB, Hsu YP, Resticcia M, Joppo G, Miadoma A, Cornelli U, Halpern G, Zanussi C : Grass pollen immunotherapy : a single-year double blind, placebo-controlled study in patients with grass pollen-induced asthma and rhinitis. *J All Clin Immunol* 73 : 283-290, 1984
 - 13) Aalberse RC, Dieges PH, Knul-Bretlova V, Vooren P, Aalbers M, Leeuwen J van : IgG4 as blocking antibody. *Clin Rev Allergy* 1 : 289, 1983
 - 14) Aalberse RC, Van der Zee JS : IgG 4 : Immunochemical aspects. *Proceedings of the XII International Congress of Allergology and Clinical Immunology*, Washington, D.C., 1985, p. 58
 - 15) Nakagawa T, Kozeki H, Katakiri J et al : Changes of house dust mite-specific IgE, IgG and IgG subclass antibodies during immunotherapy in patients with perennial rhinitis. *Int Arch Allergy Appl Immunol* 82 : 95, 1987
 - 16) Platts-Mills TAE, Von Maur RK, Ishizaka K, Norman PS, Lichtenstein LM : IgA and IgG anti-ragweed in nasal secretions. *J Clin Invest* 57 : 1041-1050, 1976
 - 17) Platts-Mills TAE : Local production of IgA, IgA, and IgE antibodies in grass pollen hay fever. *J Immunol* 122 : 2218-2225, 1979
 - 18) Gurgenedze GV, Baraban EI, Gamkre-

- lidze AG : Local humoral immunity in patients with pollen allergy. *Allergol Immunopathol* 18 : 315-319, 1990
- 19) Taylor G, Shivalkar PR : Local nasal sensitization in allergic rhinitis. *Clin Allergy* 2 : 125-136, 1972
- 20) Metha SB, Smith JM : Nasal hyposensitization and hay fever. *Clin Allergy* 5 : 279-284, 1975
- 21) Lee HB, Adkinson NF : Quantitative measurement of ragweed specific IgG subclass antibodies in human serum. *J Kor Soc Allergol* 10 : 64-70, 1990
- 22) Obtulowicz K, Wroblewska I, Radwan J, Miecznik B : Serum and nasal discharge immunoglobulins A, M, G and E in the pollinosis. *Polski Tygodnik Lekarski* 16 : 12-13, 1985
- 23) Lichtenstein LM, Norman PS, Winkler WL : A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. *Ann Intern Med* 75 : 663-671d, 1971
- 24) Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BN : IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 52 : 472-482, 1973
- 25) Lieberman P, Patterson R : Immunotherapy for atopic disease. *Adv Intern Med* 19 : 391-411, 1974
- 26) Rocklin RE : Clinical and immunological aspects of allergen-specific immunotherapy in patients with seasonal allergic rhinitis and/or allergic asthma. *J Allergy Clin Immunol* 72 : 323-334, 1983
- 27) Brandtzaeg P, Korsrud FR : Significance of J chain profiles in human tissues. *Clin Exp Immunol* 58 : 709-718, 1984
- 28) Deuschl H, Johansson SGO, Fagerberg E : IgE, IgG and IgA antibodies in serum and nasal secretion during parenteral hyposensitization. *Clin Allergy* 7 : 315-324, 1977
- 29) Holt PG, Sedgwick JD : Suppression of IgE responses following inhalation of antigen. *Immunol Today* 8 : 14-15, 1987
- 30) Rkubin RL, Tang FL, Chan EKL, Pollard KM, Tsay G, Tan EM : IgG subclasses of autoantibodies in systemic lupus erythematosus, Sjögren's syndrome and drug induced autoimmunity. *J Immunol* 137 : 2528, 1986
- 31) Malling JH, Djurup R : Diagnosis and immunotherapy of mould allergy. VI. IgG subclass response and relation to the clinical efficacy of immunotherapy with *Cladosporium*. *Allergy* 42 : 60-70, 1988
- 32) Brandtzaeg P, Kett K, Rognum TO, Soderstrom R, Hanson LA : The distribution of mucosal IgA and IgG subclass-producing immunocytes and alterations in various disorders. *Monogr Allergy* 20 : 179-194, 1986
- 33) Lee HB, Adkinson NF : Unpublished data