

Altered allergen binding capacities of Amb a 1-specific IgE and IgG4 from ragweed-sensitive patients receiving immunotherapy

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Background: The mechanisms for the effectiveness of allergen immunotherapy (IT) are not well understood. The binding potential for immunoglobulins is a function of both antibody concentration and affinity (K_A).

Purpose: The purpose was to perform a cross-sectional preliminary study to investigate any differences in allergen-specific antibody affinity and concentration following ragweed immunotherapy by introducing a new concept of antibody binding capacity ($[Ig] \times K_A$).

Methods: The binding capacity of allergen-specific IgE and IgG4 was determined for ragweed-allergic individuals undergoing ragweed immunotherapy and compared with the capacity of ragweed-specific IgE and IgG4 for allergic individuals not receiving immunotherapy.

Results: The mean binding capacity for IgG4 after long-term immunotherapy was 1.6 log units higher ($P < .0001$) than for individuals not receiving IT. The binding capacity for allergen-specific IgE was 1.2 log units lower following long-term immunotherapy ($P < .0001$) compared with individuals not receiving ragweed IT.

Conclusions: We hypothesize that a primary effect of immunotherapy is to increase IgG4 binding capacity and concomitantly decrease IgE binding capacity.

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INTRODUCTION

The clinical effectiveness of desensitization by allergen immunotherapy (IT) is well established, but the mechanisms responsible are not well understood.¹ It appears that the threshold for the allergen-induced reaction is raised and the concentrations of major effector molecules, IgE and IgG4, are changed. These changes, however, often do not correlate well with the clinical

benefits.² The ultimate clinical picture is, in part, related to the binding capacity between the allergen and the antibody.

Allergen-specific immunoglobulin (Ig) concentration is not an adequate measure for Ig binding potential. This potential is a function of both affinity and concentration. By introducing binding capacity (affinity $\times$ concentration) as a variable, we can test if the binding potentials of IgE and IgG4 are affected by IT.

Newly developed methods allow us to determine the affinity distributions for the heterogeneous responses of IgE and IgG4 to purified allergens.³ To determine the effect of IT on the response potential of Amb a 1-specific IgE and IgG4, we compared two groups of ragweed sensitive donors receiving and not receiving IT. In this report we show that, although both affinity and concentration are influenced, the prin-

cipal effect is on the induced response capacities of IgE and IgG4.

METHODS

Serum was obtained with informed consent from ragweed-sensitive patients receiving treatments in the Allergy Clinic of the Fairview-University Medical Center. A total of 15 ragweed-sensitive donors (9 men and 6 women, average age = 30) who did not receive IT for any allergen and 15 ragweed-sensitive donors (8 men and 7 women, average age = 38) who did receive IT for ragweed were recruited. All IT donors received the same standardized protocol, were receiving maintenance level doses (median 8.2 μ g of RW antigen E), and serum was obtained after at least 2 years following the start of immunotherapy treatment. Serum concentrations of Amb a 1-specific IgE and IgG4 (in IU/mL) were determined as previously described.³ We also determined the binding affinities between the free allergen (Ag) and IgE and Ag and IgG4.³

RESULTS

Immunotherapy appeared, as expected,⁴ to increase the average concentration of Amb a 1-specific IgG4 from 54.0 IU/mL (range 32–150) to 101 IU/mL (range 64–320) and to decrease the specific IgE level from 12.01 (range 0.02–131) to 1.04 (range 0.01–6.11). The differences in average levels are not statistically significant due to a high S.D.

In contrast, there were significant differences in the binding affinities of IgE and IgG4 due to IT, which decreased the affinities of IgE and increased the affinities of IgG4. These

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effects are best demonstrated by binding capacities, which define the binding potential for Ig's. The capacities were defined by:

$$CAP = \sum_i [Ig]_i \times N[K]_i \times K_i$$

where $[Ig]_i$ is the antibody concentration, $N[K]_i$ is the relative contribution of each reaction, and K_i is the affinity constant of the i th reaction. We used a log transformation to express the calculated capacities.

Figure 1A shows the IgG4 binding capacities (dimensionless units on log scale) for donors with and without IT. For donors who did not receive IT, the mean IgG4 binding capacity was -2.3 (SD = 0.62) while for donors who did receive IT, the mean IgG4 capacity was -0.68 (SD = 0.42), a 1.6 log unit increase. Comparison of these mean values by Student's t test was highly significant ($P < .0001$).

Figure 1B shows the IgE binding capacities for donors with and without IT. For donors who did not receive IT, the mean IgE binding capacity was -2.2 (SD = 0.54) while for donors who did receive IT, the mean IgE binding capacity was -3.4 (SD = 0.62), a 1.2 log unit decrease. Comparison of these means by t test was also highly significant ($P < .0001$). The line is a fit of the capacities for 73 individuals from a previous study (Submitted) that demonstrates the capacities for a larger group of allergic individuals not receiving IT (average = log -2.2).

CONCLUSION

A major interest in allergy is to determine the mechanisms responsible for the effectiveness of allergen injection in alleviating allergic symptoms. An understanding of these mechanisms will shed some light on the mechanisms important in the allergic immune response. This study was performed as a preliminary cross-sectional study to investigate any differences in antibody capacity for individuals receiving ragweed immunotherapy.

Immunotherapy increased the Amb a 1-specific IgG4/IgE ratio from 4.50 before to 97.1 after IT therapy con-

firmed previous reports of IgG/IgE ratios following IT.⁵ The mean concentrations of IgE and IgG4, however, did not differ significantly. We hypothesize that a primary effect of IT is to increase IgG4 binding capacity and concomitantly decrease IgE binding capacity.

We report a significant difference in the antibody immune response that occurs in a group receiving immunotherapy. Although it may not be the primary effect, the significant alterations in antibody capacity may be a measurable reflection of the state of the allergic response. Prospective studies of larger donor populations with pre-IT and post-IT measurements are underway to confirm these findings. In addition, prospective studies are neces-

sary to correlate these findings with clinical effectiveness.

The alteration in affinity, which occurred in a group receiving IT, is a dramatic measurable variable of the allergen-specific immune response that may be an indicator of the state of the immune response. It is hoped that these results may stimulate other studies or be taken into consideration when trying to investigate the mechanisms and molecules involved in the allergic immune response.

The affinity of the response is based on the molecular interaction between the antibody combining site and the antigenic epitope on the antigen molecule. The specificity of antigen recognition by an antibody is due to the structural diversity of the variable do-

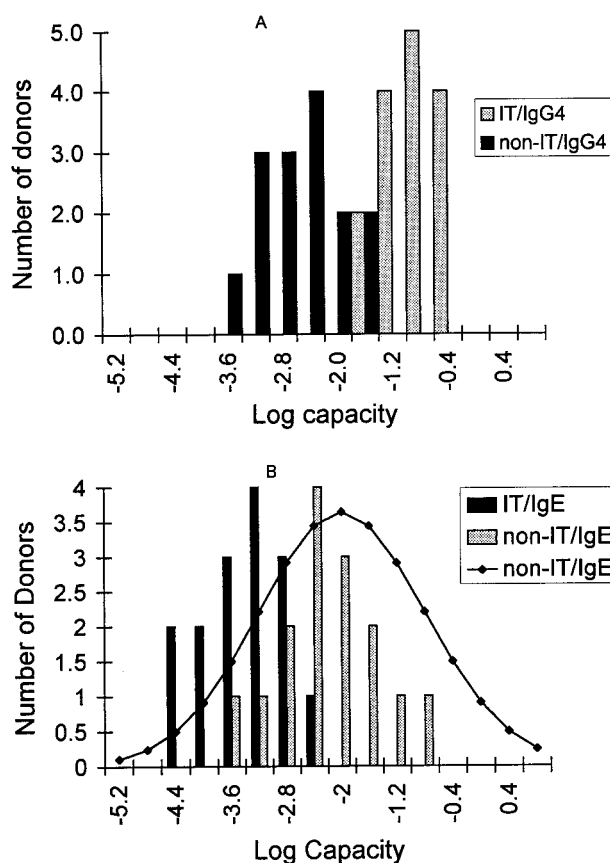


Figure 1. Frequency histograms for binding capacities of (A) Amb a 1-specific IgG4 for 15 individuals not on IT (dark colored bars) and 15 individuals receiving ragweed IT (light colored bars). (B) Amb a 1-specific IgE capacities for 15 individuals receiving IT (light colored bars), 15 individuals not on IT (dark colored bars), and 73 individuals not on IT (line).

main. After recognition by the primary repertoire, antibodies are selected by binding to the antigen. Affinity maturation occurs after multiple exposures to the antigen and leads to the selection of higher affinity antibodies. Although there are exceptions where low affinity antibody producing clones can persist⁶ and a predominantly high affinity response can switch to a low affinity response.⁷ Genetic and non-genetic factors can affect antibody affinity.⁸ The time, dose, and route of administration of an exposure can lead to different outcomes of an immune response. In our experiments we compare the effect of low and high allergen concentration and different routes of administration in normal and atopic populations. Low allergen (natural exposure) favors high affinity clones but for the IgE isotype. High allergen exposure (IT) results in maturation of the IgG4 response; however, unexpectedly, IgE affinities become attenuated. These findings are consistent with the presence of a limiting variable for which liganded B-cell clones have to compete. Possibilities include the presence of suppressor T cells⁹ or inhibition of T-helper cells, which normally precedes high affinity antibody production.¹⁰

The affinity of a response is related to the effectiveness of a response.¹¹ A decrease in the IgE affinity and an increase in the IgG4 affinity may enhance the competitive binding of IgG4 specific for the allergen, therefore diminishing the pathologic effect of the atopic state.

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