

Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon- γ production in CD4⁺ T cells

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Background: Allergen immunotherapy results in a number of changes in clinical, inflammatory, and immunologic parameters. However, the basis for the specificity of this form of therapy is unknown, especially in the context of changes in T- and B-lymphocyte function after desensitization to specific allergens.

Objective: This study was designed to determine the immunologic consequences of rush immunotherapy.

Methods: We studied 10 patients who had positive skin test responses to the house dust mite *Dermatophagoides pteronyssinus* (Dpt) and cat dander extract. Each received rush immunotherapy to mite, but not cat dander, over a 2- to 4-week period until maintenance was achieved. Patients were evaluated before and when maintenance was achieved for skin test and nasal reactivity to mite and cat dander; antibody levels to the allergen were monitored, as were lymphocyte proliferative responses and cytokine production.

Results: Rush immunotherapy to house dust mite resulted in a significant reduction in skin and nasal reactivity to mite allergen, but not to cat allergen, in 10 of 10 patients. This was accompanied by a rise in serum anti-Dpt IgE, whereas anti-cat IgE was not altered (7 of 7 patients). In seven of seven patients there was an increase in anti-Dpt IgG₄ levels. T-cell proliferative responses to mite antigen were suppressed, and numbers of CD8⁺ T cells increased in frequency. There was a marked increase in interferon- γ production, particularly by CD4⁺ T cells in 10 of 10 patients. The correlation between the increases in interferon- γ production and the changes in cutaneous reactivity was highly significant.

Conclusion: We show that rush immunotherapy is immunologically specific in eliciting changes in T- and B-cell responses to the desensitization antigen. The specificity and potential benefit of immunotherapy may be linked to the increase in interferon- γ production by allergen-activated CD4⁺ T lymphocytes. (*J Allergy Clin Immunol* 1997;99:530-8.)

Key words: Rush immunotherapy, interferon- γ , T cells, B cells, specificity

Abbreviations used

AU:	Allergen units
Dpt:	<i>Dermatophagoides pteronyssinus</i>
IFN- γ :	Interferon- γ
PBMCs:	Peripheral blood mononuclear cells
RIT:	Rush immunotherapy

Although allergen immunotherapy has been shown to cause changes in numerous clinical, inflammatory, and immunologic parameters, little is known about the functional relevance of such changes. A variety of immunologic changes have been observed, including a rise in so-called serum IgG "blocking antibodies"¹⁻⁸; an early rise in serum IgE antibodies, followed by suppression of the usual rise seen after seasonal exposure to allergen and eventually succeeded by a slow decline to below pretreatment levels⁹; reduction of in vitro lymphocyte responsiveness to allergen¹⁰; an inhibition of the seasonal increase of eosinophilic cationic protein¹¹; a decrease in histamine releasing factor production¹²; a suppression of ocular, nasal, cutaneous, and bronchial reactivity to allergen¹³⁻¹⁵; a decrease in nasal mucosal mast cells¹⁶; and a decrease in eosinophil influx into the bronchi after seasonal pollen exposure.¹⁷ Any mechanism of action that is invoked needs to account for the clinical and immunologic specificity of allergic desensitization. Paradoxically, the rise in allergen-specific IgE in the serum, early in the course of immunotherapy, occurs at a time when cutaneous reactivity is diminished¹⁸; blocking antibodies, except perhaps in venom immunotherapy, do not appear to account for the early benefits of desensitization.¹⁹

If such changes in B-cell function cannot account for the early allergen-specific effects of immunotherapy, then changes in T-cell function may explain the specificity of the immunotherapy response. Indeed, studies have shown that allergen-specific T-cell proliferation is diminished after allergic desensitization.¹⁰ One hypothesis is that allergen-specific T cells are functionally altered during desensitization from a T-helper (T_{H2}) to a T_{H1}-type phenotype. There is evidence in a murine model of desensitization to ovalbumin that the induction of interferon- γ (IFN- γ) is capable of suppressing IgE production.²⁰ In human beings, there are reports of increased

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IFN- γ ²¹ and decreased IL-4²² production after allergen immunotherapy.

METHODS

Subjects

Patients with asthma (6 boys and 4 girls) who fulfilled inclusion and exclusion criteria with positive skin test responses to *Dermatophagoides pteronyssinus* (Dpt) and cat were enrolled in this study. All patients were receiving β -agonists and 200 to 800 mg of inhaled corticosteroids a day. Four patients were receiving theophylline preparations, and seven were receiving oral prednisone. Patients receiving more than 10 mg of prednisone every other day were excluded from the study. Also excluded were patients whose steroid requirements had altered in the 2 weeks before and during the rush immunotherapy (RIT) period. The mean age was 13.4 years (range, 10 to 16 years). No patient was taking any long-acting antihistamine for at least 3 months before the initiation of RIT. Short-acting antihistamines were stopped at least 4 days before skin testing and nasal challenges. Institutional review board approval and informed consent were obtained from all patients and one of their respective parents.

Protocol

Patients received RIT to Dpt according to the schedule previously described¹³ in our pediatric intensive care unit, until maintenance was achieved (5000 allergen units [AU]/day). Allergen extracts were obtained from ALK (Milford, Conn.). Dpt extract was provided in a 50% glycerine solution at a concentration of 10,000 AU/ml and stored at 4° C. Cat extract was lyophilized. Patients achieved maintenance within a period of 14 to 28 days, and in general, received a similar cumulative allergen dose. Tests were carried out 1 or 2 days before the initiation of RIT and 1 or 2 days after maintenance was achieved. Patients receiving systemic steroids every other day were assessed just before taking their steroid medication (i.e., 48 hours after the last dose). All testing was carried out in a blinded fashion, and no serious reactions were observed.

End-point titration testing

End-point titration for skin prick testing was carried out for Dpt with the following dilutions: 1, 3, 10, 30, 100, 300, 1000, 3000, and 10,000 AU/ml. For cat dander, the concentrations were half those of mite in bioequivalent allergy units per milliliter. Patients had not taken antihistamine medication for at least 1 week before testing. A positive reaction was defined as a wheal greater than or equal to 4 mm and greater than or equal to the histamine control. The end point was determined as being the smallest dilution that produced a positive cutaneous response. The change in end-point titration before and after RIT was expressed as change in log reactivity.

Nasal provocation

The method used for nasal challenge was that described by Bousquet et al.²³ and shown to have a significant correlation with symptom scores during inhaled allergen exposure.¹⁹ Nasal challenges with the same allergen extract as that used for skin testing were administered at room temperature, and successive concentrations were given every 20 minutes. Allergen challenges were delivered by using a plastic, hand-held atomizer and were performed by having the patient inspire to total lung capacity with head held level; while breath was being held, two

activations of the atomizer were instilled into the right nostril (0.135 ml). Challenges began with 1 AU of the selected allergen extract and increased successively by 0.5 log increments every 20 minutes until a positive symptom score was obtained. Symptom scores were obtained for nasal blockage, pruritus, sneezing, rhinorrhea, and conjunctivitis with a maximum possible score of 10. Symptom scores were recorded 15 minutes after each challenge; a symptom score of greater than 5 was considered positive, and the challenge was terminated. Nasal peak flow indices were determined both at the start and termination of the challenge procedure. A positive response was defined by a decline in the nasal peak flow index of more than 25%. Nasal challenge with Dpt was done through the right nostril and with cat allergen through the left nostril on 2 successive days. Allergen challenges were only considered positive if there were significant changes in both the symptom score and nasal peak flow.

Measurement of immunoglobulins to Dpt and cat

Serum was obtained before and after RIT, and samples were analyzed for allergen-specific IgE by using the Pharmacia CAP system. Measurement of the following immunoglobulin isotypes specific for Dpt and cat was performed by ELISA: IgG₁, IgG₂, IgG₃, IgG₄, IgA, and IgM. Briefly, 96-well plates with cat or house dust mite allergen were incubated overnight at 4° C. Plates were washed with phosphate-buffered saline-Tween and blocked with 1% bovine serum albumin for 1 hour at 37° C. Fifty lambda of serum was added at various dilutions in 1% bovine serum albumin and incubated for 1 hour at 37° C. After washing the plates, horseradish peroxidase-linked goat anti-human isotype antibodies were added for 1 hour at 37° C. The plates were washed, and dimethoxybenzidine was added. The reaction was terminated with 6N H₂SO₄. Plates were read at 540 nm.

Lymphocyte proliferation assay

Human peripheral blood mononuclear cells (PBMCs) were isolated from the patients by Ficoll-Hypaque gradient centrifugation (Pharmacia, Piscataway, N.J.) and were incubated in 96-well flat bottom plates at 10⁶ cells/ml in 200 μ l/well. The following glycerine-free allergens were used: cat extract in increasing concentrations of 1, 10, 100, 1000, 10,000, and 50,000 AU/ml and Dpt extract with the same concentrations. Proliferative responses were assayed by tritiated thymidine incorporation after 7 days' culture after addition of 1 mCi 6 hours before cell harvesting.

Serum cytokine levels

Aliquots of the frozen serum samples were assayed for IL-2, IL-4, and IFN- γ content by using solid-phase enzyme immunoassay kits from Genzyme Corp. (Cambridge, Mass.).

Flow cytometric analysis of PBMCs for intracellular cytokine production

Intracellular staining for cytokines was carried out as previously described.²⁴ Briefly, PBMCs were activated for 4 hours with phorbol myristate acetate-ionomycin and 1 mmol/L monensin. Cells were fixed in 4% paraformaldehyde and made permeable with saponin. After blocking with 10% AB serum, cells were incubated with a monoclonal mouse anti-cytokine antibody at 1 mg/ml in 0.1% saponin. Twenty minutes later, cells were washed twice in Hanks' balanced salt solution and incubated for an additional 20 minutes with a fluorescein isothiocyanate- or phycoerythrin-labeled polyclonal goat anti-

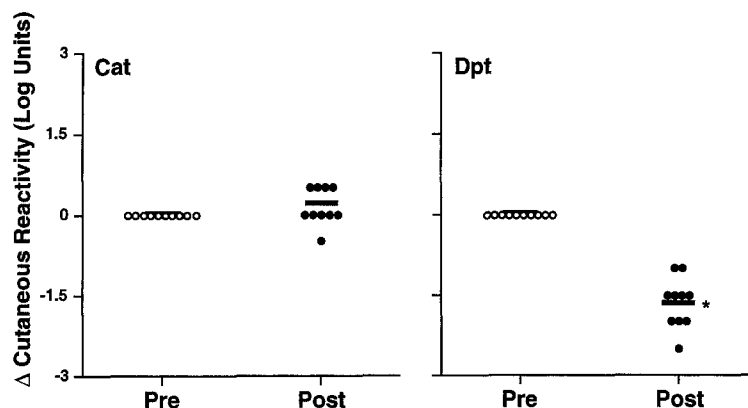


FIG. 1. RIT decreases allergen-specific cutaneous reactivity. Ten subjects underwent skin testing with Dpt and cat extracts in increments of 1/2 log unit (range, 1 to 10,000 AU/ml). Subjects were tested before RIT and after maintenance doses were reached. Expressed along the y axis is change in log concentration of extract after RIT that elicited a positive skin test response. * $p < 0.01$ compared with pretreatment levels.

mouse isotype-specific antibody and an anti-surface marker antibody (anti-CD3, anti-CD4, and anti-CD8). Cells were analyzed by flow cytometry, and the frequency of cytokine-producing cells in different subpopulations was calculated.

Statistics

Because this was a prospective study in which each subject acted as his or her own control, paired Student's *t* tests were used to compare changes in clinical and immunologic parameters before and after RIT. Significance was defined at $p < 0.05$, by using a two-tailed test. To further analyze whether associations exist between the changes that occur in various parameters during RIT, data were recorded separately for each patient and logarithmically transformed, and then multiple Spearman correlation analyses were done.

RESULTS

RIT to Dpt reduces skin prick test end-point titration threshold to Dpt allergen but not to cat allergen

End-point skin prick test titration to Dpt and cat extracts was carried out before and after RIT. The threshold for reactivity to Dpt and cat was between 30 and 300 AU/ml before RIT. There was a marked reduction in cutaneous reactivity to Dpt after RIT, with a reduction of 1 log or greater occurring in all 10 patients (Fig. 1). Two patients completely lost cutaneous reactivity to Dpt after RIT, with no response at 10,000 AU/ml. End-point titration to cat did not change after RIT.

Nasal reactivity to Dpt, but not to cat, decreases after RIT to Dpt

Nasal challenges to Dpt and cat extracts were carried out in four patients. Challenges were carried out on 2 consecutive days before and after RIT. A positive allergic response was obtained if the patient had both nasal symptoms (5 of 10) and nasal obstruction as measured by a fall in the nasal peak flow index after allergen challenge of more than 25%. Positive responses oc-

curred at an allergen concentration within 1 log unit of the cutaneous end-point titration threshold. After RIT to Dpt, all four patients showed a decline in nasal reactivity to Dpt. An increase of at least 1 log in allergen concentration was required to produce similar nasal symptoms and nasal obstruction (Fig. 2). No change in nasal reactivity to cat was observed after RIT.

RIT to Dpt causes an early rise in anti-Dpt IgE but not in anti-cat IgE

Allergen-specific immunoglobulins were measured in the serum before and after RIT in seven patients. All patients showed a rise in anti-Dpt IgE after RIT to Dpt (Fig. 3). Anti-cat IgE in the serum was unchanged after RIT. There was also an inconsistent rise in anti-Dpt IgG (3 of 7 patients), which was not statistically significant. There was no consistent change after RIT in other allergen-specific immunoglobulin isotypes in the serum (IgM, IgA) for Dpt or cat.

Because a variable increase was noted in anti-Dpt IgG, we determined the effects of RIT on immunoglobulin subclass composition (Fig. 4). A small increase in IgG₁ that did not achieve statistical significance was observed. Anti-Dpt IgG₂ and IgG₃ antibodies did not vary after RIT. In contrast, RIT was associated with a large and consistent (7 of 7 patients) increase in anti-Dpt IgG₄, which was statistically significant. No changes were observed in IgG subclass responses to cat antigen. There was no association between the increase in anti-Dpt IgG₄ and decrease in cutaneous reactivity that occurred after RIT.

RIT to Dpt is associated with a decrease in lymphocyte proliferation to Dpt but not to cat

Lymphocyte proliferative responses to Dpt and cat were measured in four patients. The responses varied from 12,000 to 18,000 cpm with stimulation indices ranging from 3.0 to 5.0 (compared with medium). RIT caused an ablation of the proliferative responses to Dpt

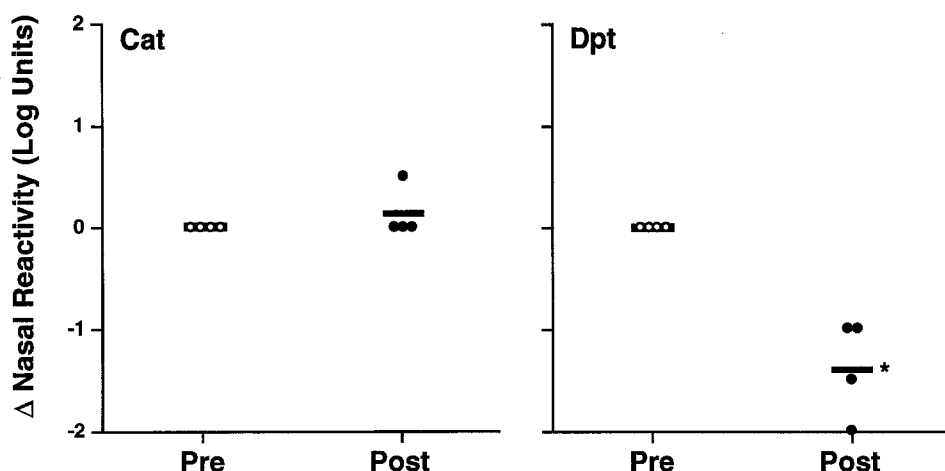


FIG. 2. RIT decreases allergen-specific nasal reactivity. Four subjects had nasal challenges to Dpt and cat extracts with the same concentrations of extract and at the same time points as for skin testing. Expressed along the y axis is change in log concentration of extract required to cause a positive response to nasal challenge after RIT. * $p < 0.01$ compared with pretreatment levels.

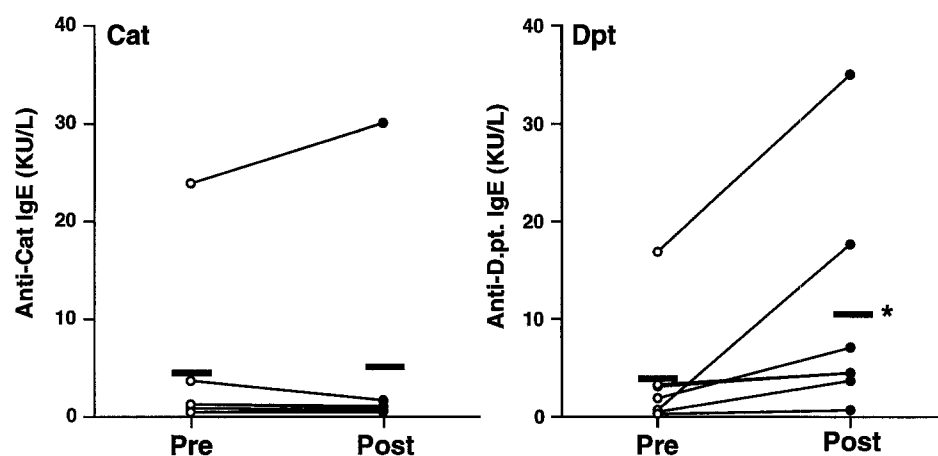


FIG. 3. RIT causes an increase in allergen-specific IgE in serum. Determination of allergen-specific IgE in serum was by RAST. Expressed along the y axis are serum RAST values for cat and Dpt (in kilounits per milliliter). * $p \leq 0.01$ compared with pretreatment levels.

in all four patients with a decrease in the stimulation index to 1.0 (Fig. 5). Even high concentrations of up to 100,000 AU/ml did not induce proliferation after RIT. There was no change in the proliferative response to cat antigen.

RIT to Dpt is associated with a decrease in the CD4:CD8 ratio

The frequencies of CD4⁺ and CD8⁺ T cells were analyzed before and after RIT by flow cytometry. In eight of 10 patients, there was a small increase in the percentage of CD8⁺ cells after RIT (Fig. 6). There was a decrease in the percentage of CD4⁺ T cells in nine of 10 patients, and in all 10 patients, a decrease in the CD4:CD8 ratio. No association was seen between the

change in CD4:CD8 ratios and decreases in cutaneous reactivity that followed RIT.

RIT to Dpt causes selective increases in IFN- γ levels in the serum but does not alter levels of IL-2 and IL-4

Levels of IFN- γ , IL-4, and IL-2 in the serum were measured before and after RIT. All patients showed an increase in serum IFN- γ levels from 194 ± 5 pg/ml before RIT to 633 ± 205 pg/ml after RIT (Fig. 7, A). Serum levels of IL-2 and IL-4 did not change after RIT (data not shown). A correlation was demonstrated between the increase in serum IFN- γ levels and the decrease in cutaneous reactivity to Dpt that followed RIT ($r = 0.97$, $p < 0.0002$).

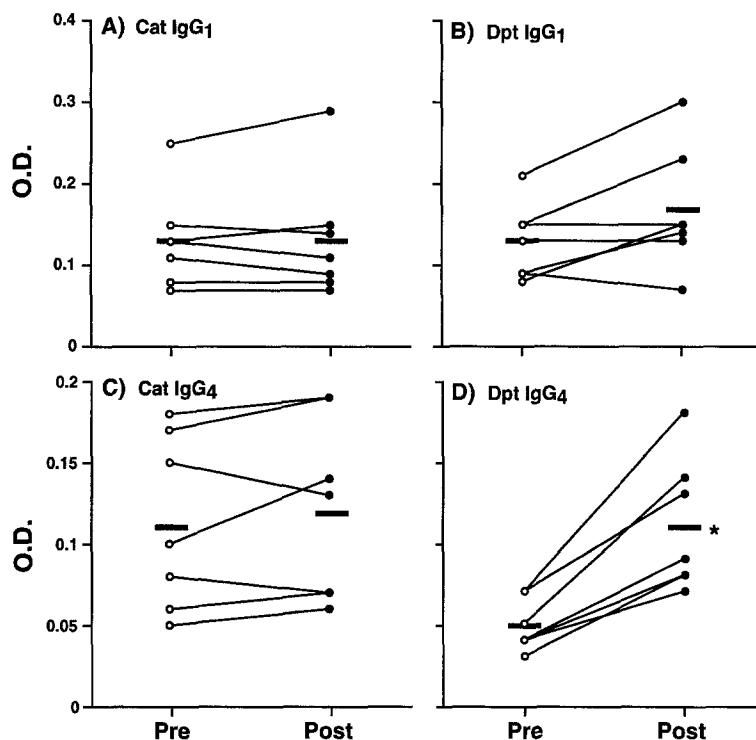


FIG. 4. RIT causes an allergen-specific increase in IgG₄. Determination of antibody levels to cat and Dpt was done by ELISA. Shown are serum concentrations of anti-cat IgG₁ (A), anti-Dpt IgG₁ (B), anti-cat IgG₄ (C), and anti-Dpt IgG₄ (D), expressed in units of optical density (O.D.). * $p < 0.01$ compared with pretreatment levels.

RIT to Dpt is associated with an increase in the frequency of IFN- γ -producing T cells

Surface staining for CD3 and intracellular staining for IFN- γ , IL-2, and IL-4 were carried out on activated PBMCs. RIT in all 10 patients caused a marked increase in the percentage of IFN- γ -producing CD3 cells (Fig. 7, B). The mean percentage of IFN- γ -producing T cells before RIT was 4.0%, and this increased to 9.4% after RIT ($p < 0.001$). There was no change in the percentage of IL-2-producing or IL-4-producing T cells after RIT.

Because CD8⁺ T cells produce more IFN- γ than CD4⁺ cells, the increase in the percentage of IFN- γ -producing T cells after RIT could have resulted from a shift in the CD4:CD8 ratio caused by RIT. To address this possibility, we analyzed the percentage of IFN- γ -producing cells in PBMCs stained with anti-CD4 or anti-CD8 antibody and were thus able to determine the frequency of IFN- γ -producing cells in the CD4⁺ and CD8⁺ T-cell subsets before and after RIT (Fig. 8). The mean frequency of IFN- γ -producing CD4⁺ T cells before RIT was 1.8%, whereas after RIT, the frequency had substantially increased to 8.2%. The mean frequency of IFN- γ -producing cells in the CD8⁺ population was 10% before RIT, and this did not change significantly with RIT. Therefore RIT caused a significant increase in the frequency of IFN- γ cells that occurred selectively in the CD4⁺ population. Because

CD4⁺ cells comprised the majority of the T cells in PBMCs, the increase in IFN- γ production by T cells is accounted for largely by the increased frequency of IFN- γ -producing CD4⁺ T cells and to a lesser extent by the small increase in the CD8⁺ T cell population. There was a positive correlation between the increased capacity of CD4⁺ T cells to produce IFN- γ and the rise in serum IFN- γ levels that followed RIT ($r = 0.92$, $p < 0.0025$). The change in frequency of IFN- γ -producing CD4⁺ T cells was also correlated with the changes in cutaneous reactivity to Dpt after RIT ($r = 0.82$, $p = 0.0031$).

DISCUSSION

We investigated changes in T-cell and B-cell function associated with RIT to delineate the mechanisms underlying its potential effectiveness and demonstrated that RIT is both clinically and immunologically specific for the administered allergen. After conventional allergen immunotherapy, there is an early decrease in end-organ responsiveness to allergen, which is paradoxically associated with a rise in allergen-specific IgE in the serum.¹⁸ Similarly, after RIT we demonstrated a decrease in cutaneous and nasal responses to Dpt in conjunction with increased anti-Dpt IgE levels in the serum. This was an allergen-specific effect because neither end-organ responsiveness to cat (the negative control allergen) nor

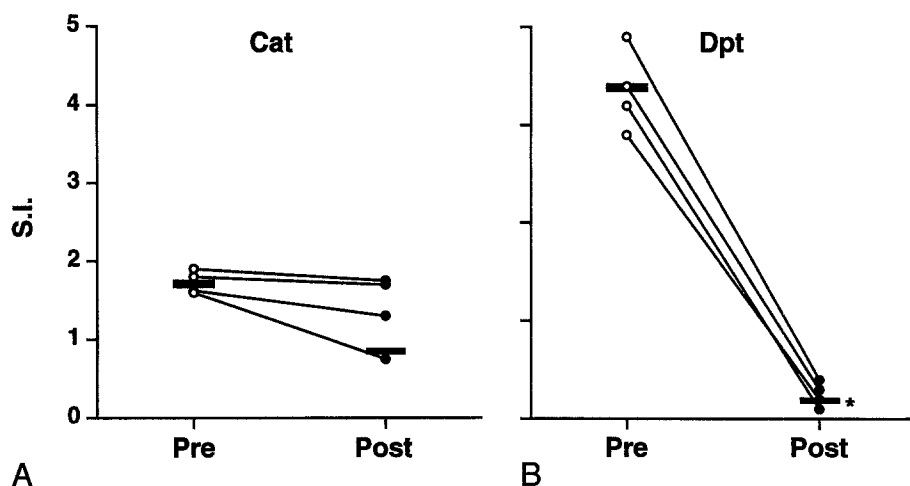


FIG. 5. RIT causes an allergen-specific decrease in lymphocyte proliferation. Lymphocyte proliferative responses to cat and Dpt allergens were measured in four patients before and after RIT. Expressed along the y axis is stimulation index (S.I.) for cat (A) and Dpt stimulation (B). Background response for the four patients was 3544 ± 496 cpm.

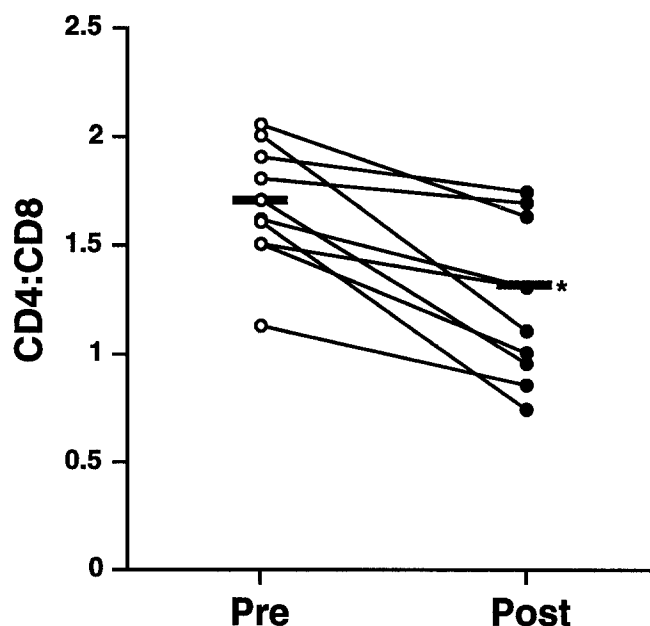


FIG. 6. RIT causes a decrease in the CD4:CD8 ratio. Lymphocyte subset analysis was done on PBMCs from 10 patients before and after maintenance doses of RIT were reached. Expressed along the y axis is the CD4:CD8 ratio for each patient. * $p < 0.05$ compared with pretreatment levels.

serum anti-cat IgE titers were altered by RIT. It is unlikely that blocking antibodies could account for this effect because there was no significant increase in anti-Dpt IgG, other than a consistent increase in specific IgG₄, and there was no correlation between the increase in IgG₄ and the decrease in cutaneous reactivity to Dpt. These results for RIT with Dpt do differ from the changes seen after conventional immunotherapy. Normally, conventional immunotherapy is followed early on by a rise in IgG₁ to the allergen with a gradual decline over time in this isotype and a slow rise in allergen-

specific IgG₄.⁹ We demonstrate here that RIT may cause a rapid increase in anti-Dpt IgG₄ levels. Our findings confirm those of a previous study that demonstrated high IgG₄ to house dust mite 4 to 8 weeks after RIT.²⁵

We were able to demonstrate induction of allergen-specific T-cell unresponsiveness after RIT. Although it is possible that this immunologically specific inhibition underlies the clinical specificity of RIT, it is not clear what mechanism is responsible for maintaining this lack of response by T cells to allergen. The induction of allergen-specific T suppressor cells could explain the

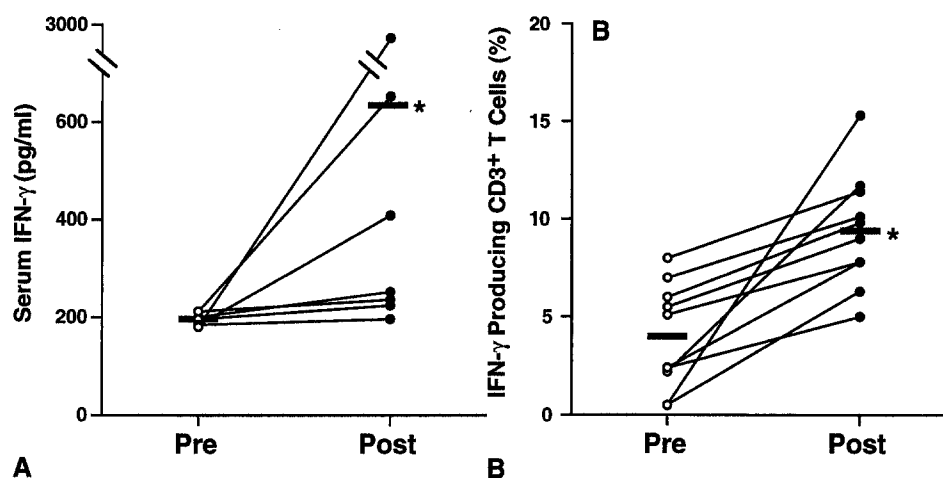


FIG. 7. RIT causes an increase in serum IFN- γ levels and in frequency of IFN- γ -producing T cells. IFN- γ was measured in serum by ELISA and by intracellular staining in activated CD3⁺ T cells. Shown are serum concentrations of IFN- γ for seven patients (A) and percentages of CD3⁺ T cells producing IFN- γ (B) before and after maintenance dose of RIT was reached. * $p < 0.05$ compared with pretreatment values.

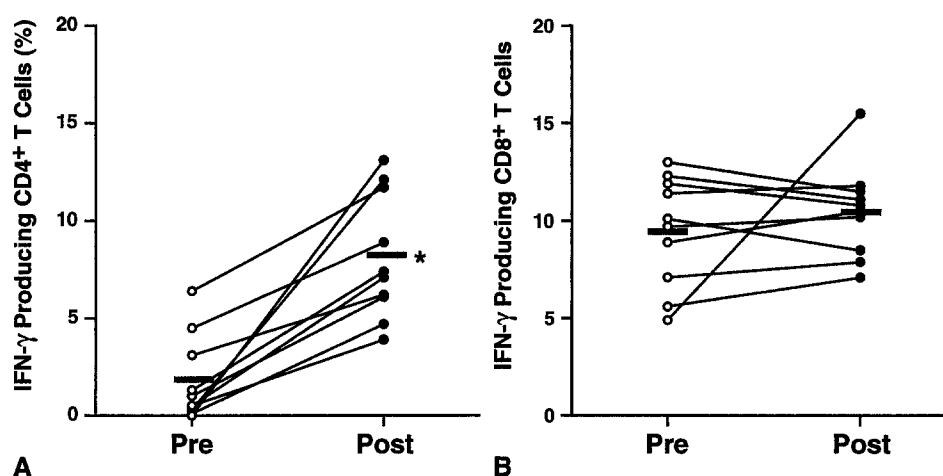


FIG. 8. RIT causes a selective increase in IFN- γ production by the CD4⁺ T-cell population. The percentage of IFN- γ -producing cells was analyzed in different T-cell subsets. Expressed is percentage of IFN- γ -producing CD4⁺ T cells (A) and CD8⁺ T cells (B) before and after maintenance dose of RIT was reached. * $p < 0.01$ compared with pretreatment values.

lack of proliferation in response to house dust mite after RIT.¹⁰ Immunotherapy has been shown to result in the expansion of allergen-specific CD8 suppressor cells, which inhibit allergen-specific IgE production and allergen-specific T-cell proliferation in vitro.²⁶ There is in vitro evidence illustrating that anergy can be induced in specific T-cell clones exposed to supraoptimal concentrations of peptide and that this state of anergy can be reversed by the addition of IL-2.²⁷ Yet another possibility is that the induction of IFN- γ by RIT inhibits the proliferation of T_{H2}-like allergen-specific T cells. IFN- γ selectively stimulates the proliferation and differentiation of T_{H1} clones and inhibits the proliferation of T_{H2} clones.²⁸ House dust mite-specific T-cell clones derived from patients with allergy have been shown to have a T_{H2} cytokine profile,²⁹ and it is possible that the increase in

IFN- γ production in vivo after RIT prevents the proliferation of these allergen-specific T_{H2} cells. These possibilities do not readily account for the demonstrated immunologic specificity of immunotherapy. If the induction of systemic IFN- γ prevents the proliferation of T_{H2} cells, proliferative responses to cat and other allergens should be similarly suppressed. However, if IFN- γ is only able to act in vivo on lymphocytes that have been activated by antigen, specificity may be maintained. There are data from both in vitro and in vivo systems that support this possibility.^{30, 31} Therefore IFN- γ may only affect B and T cells that have been activated and increased the expression of the IFN- γ receptor. Consequently, IFN- γ induced by RIT would only act on house dust mite-reactive T cells that have been activated and upregulated IFN- γ receptors or their cell surface after

antigen exposure. These cells do not proliferate in response to allergen in vitro after in vivo exposure to IFN- γ , whereas cat-specific T cells, which have not been activated in vivo, are unaffected by IFN- γ .

There was a small shift in the CD4:CD8 ratio with an increase in the population of CD8⁺ T cells after RIT. It is known that atopic patients have a decreased percentage of CD8⁺ T cells in the peripheral blood compared with normal subjects.³² Previous evidence has suggested that conventional allergic desensitization causes an increase in the percentage of suppressor T cells.³³ In this study we observed a consistent increase in the CD8⁺ T-cell population after RIT. Though small, this increase is unlikely to represent the clonal expansion of CD8 suppressor cells. This shift in population may be due to the increase in serum IFN- γ levels, as reported in patients with hyper-IgE syndrome treated with IFN- γ .³⁴

Immunotherapy may modulate cytokine production. Grass pollen immunotherapy increases cutaneous messenger RNA for IFN- γ and IL-2 during the late-phase response.²¹ This study showed no effect of immunotherapy on T_{H2}-type cytokine production (IL-4) in contrast with another study that showed decreased IL-4 production after long-term immunotherapy.²² Jutel et al.³⁵ have shown that RIT to bee venom is accompanied by decreased allergen-specific IL-4 production after 7-day culture of PBMCs. In this study serum cytokine levels and mitogen-induced cytokine production were measured but did not reveal changes in antigen-specific IL-4 production. We did, however, find a large increase in serum IFN- γ levels after RIT. In parallel, intracellular staining revealed the increased frequency of activated T cells producing IFN- γ after RIT. This increased capacity was attributable largely to increased production within the CD4⁺ T-cell population. In contrast, the production of other cytokines was not altered. The induction of IFN- γ by RIT is thus a potentially important, early event resulting in the inhibition of allergic responses. The significant inverse correlation between IFN- γ production and cutaneous reactivity to Dpt strongly suggests that this cytokine plays an important role in the clinical response to RIT. Hayglass et al.²⁰ have shown, in the mouse, that the induction of IFN- γ is necessary for successful immunotherapy. Mice injected with anti-IFN- γ antibody did not respond to immunotherapy and had persistent IgE responses. There are numerous ways that IFN- γ could modulate immediate hypersensitivity responses. IFN- γ prevents IgE production by antagonizing the effect of IL-4 on activated B cells.³⁶ It can prevent the expansion of activated T_{H2}-like CD4⁺ T cells. It also has inhibitory activity on mast cell differentiation and degranulation,³⁷⁻⁴⁰ as well as effects on eosinophil recruitment and degranulation.^{41, 42} In an in vivo murine model of allergic sensitization, IFN- γ treatment has been shown to inhibit the development of allergen-specific IgE, to decrease immediate cutaneous reactivity to allergen, and to normalize airway function.⁴³

The mechanism that underlies the increase in IFN- γ

production by RIT is not defined. It has been postulated that the route of antigen exposure, rather than the specific properties of the allergen, may determine the nature of cytokine responses that develop in allergen-specific T cells.⁴⁴ Normal exposure to aeroallergens occurs by inhalation, whereas during immunotherapy allergen is injected subcutaneously. The amount of allergen administered during RIT is much greater than the amount inhaled, and the differences in local concentrations of antigen may alter the nature of the interaction between antigen-presenting cells and T cells.⁴⁵ Different concentrations of antigen presented by antigen-presenting cells may alter the relative production of IL-4 and IFN- γ by T cells.⁴⁶ The use of repeated high allergen concentrations during RIT may lead to the shift in the T-cell response toward IFN- γ production. There is evidence that the type of antigen-presenting cell may influence cytokine production by T cells.⁴⁶

We have demonstrated that RIT is clinically and immunologically specific. This specificity cannot be explained in terms of B-cell activity alone but more likely is due to allergen-specific changes in T-cell function. We found significant changes in the composition of T cells with an increase in the CD8⁺ population and a change in the CD4⁺ population toward a T_{H1} (IFN- γ -producing) phenotype, as well as increased levels of IFN- γ in the serum. The specificity of RIT may be explained by the restriction of action of IFN- γ to antigen-activated T lymphocytes. It appears that RIT is able to correct the deficiency of IFN- γ production observed in the CD4⁺ population of atopic individuals, and this may contribute to the clinical improvement seen after RIT.

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