The effect of specific immunotherapy on T-cell receptor repertoire in patients with allergy to house-dust mite

**Background:** The precise mechanism of specific immunotherapy (SIT), long used for treating allergic diseases, remains undefined. SIT was shown to act by modifying the immune response of T lymphocytes to antigens. We examined the effect of SIT on the expression and use V-alpha, -beta, -gamma and -delta chains of T-cell receptors (TCR) in patients allergic to house-dust mite.

**Methods:** Peripheral venous blood was taken for lymphocyte TCR analysis from 10 house-dust mite (HDM) allergic adults before initiating SIT and 6 months after initiating the treatment. Twelve similarly allergic patients without SIT served as controls. TCR chains were identified by fluorescence-activated cell sorter (FACS) using the following monoclonal antibodies: CD3, CD14, CD8, pan alpha-beta, pan gamma-delta, V-alpha2, V-alpha12.1, V-beta5a, V-beta5b, V-beta5c, V-beta8a, V-beta8b, V-beta3.1, V-beta13, V-beta12, V-beta6.7, V-delta1, V-delta2, V-gamma9, and V-gamma4.

**Results:** Analyzed before and 6 months after SIT initiation, lymphocyte TCR showed significantly increased V-beta5b, V-beta12 and V-alpha12.1 values compared to controls (without significant changes in other markers).

**Conclusions:** SIT caused selective expansion of certain V-beta- and V-alpha-expressing T cells in patients allergic to HDM. Our results support the notion that the effect of SIT in patients with allergic rhinitis may be achieved by modifying the T lymphocyte response through the modulation of TCR usage.

Immunization of susceptible individuals with a specific allergen to inhibit the immune response directed towards that allergen, but leaving the remainder of the immune system intact, is the basis of specific immunotherapy (SIT). Although SIT has been used for nearly a century and has been proven to be highly effective in the treatment of IgE-mediated diseases, such as allergic rhinitis and asthma, its exact mechanism has not yet been elucidated (1–4).

Immunotherapy involves the subcutaneous administration of increasing doses of allergen. Several studies have demonstrated that it induces a number of immunological changes that may contribute to its efficacy, among them a decrease in allergen-specific IgE, an increase in allergen-specific IgG blocking antibodies, reduced basophil reactivity, the generation of allergen-specific suppressor T cells and the deviation of the Th2 response in favor of Th1 (3, 4).

T cells play a pivotal role in initiating and orchestrating allergic diseases through the production of cytokines that cause allergic inflammation and promote IgE production (5). Allergen-specific activation of T cells occurs when T-cell receptors (TCR) bind peptide fragments of the allergen bound to the major histocompatibility complex molecules. A TCR is a heterodimer composed of two glycoprotein chains (alpha and beta, or gamma and delta). Each chain consists of a variable region and a constant region that undergo somatic rearrangement during T cell development, resulting in an enormous number of different TCR (6–8). In spite of its diversity, the V-alpha, V-beta, V-gamma and V-delta segments can be grouped into families based upon similarities of the nucleotide sequences, and their protein products can be identified by family-specific monoclonal antibodies (9). The formation of the TCR repertoire in an individual is thought to be determined by both the genetic background and the response to environmental and self-antigens (6, 10, 11).

In some autoimmune diseases, there is an oligoclonal expansion of T cells that express a limited number of TCR-variable gene products, emphasizing the importance of the TCR repertoire in the pathogenesis of these diseases (12–14).

Recent data have suggested that the diversity of the TCR V-alpha or V-beta usage of the antigen-specific T cells appears to be limited in several allergic conditions as well (15–25).
Renz et al. demonstrated that specific TCR-V subsets mediate the allergic response to certain antigens, and that transfer of specific TCR-V subsets from sensitized mice to naive mice resulted in allergic sensitization in the naive recipients (15). Selective expansion of T cells expressing a particular TCR-chain variable gene segment was demonstrated in lymphocytes of humans allergic to isocyanates (16), peanuts (17,18), pollens (19–21), cats (20), and house-dust mite (HDM) (22–24).

If allergen-specific T cells with a limited use of TCR-V gene products play a role in the allergic response, the possibility arises that SIT may act upon the TCR repertoire. Although numerous studies had examined the effects of SIT on the numbers and subtypes of lymphocytes, markers of T cell activation and cytokine profile, none of them addressed the issue of the influence of SIT on the TCR repertoire of peripheral T cells (2–4).

The aim of the present study was to evaluate the effect of SIT on the TCR repertoire. For this purpose, we examined the expression of various markers of TCR in subjects with house-dust mite (HDM) allergic rhinitis before and after 6 months of SIT.

### Material and methods

Ten HDM monoallergic patients receiving SIT were studied. They all had histories of perennial allergic rhinitis of at least 2 years' duration, with positive immediate skin test reactivity solely to HDM allergens (Dermatophagoides pteronyssinus or Dermatophagoides farinae). Immunotherapy was performed according to a standard conventional immunotherapy protocol. The subjects were given subcutaneous injections of aqueous extracts of *D. farinae*/*D. pteronyssinus* (Bayer, USA). The initial doses of 10 activity units (AU) were increased once or twice weekly until the highest tolerated dose (0.5 ml of 500 AU/ml) was reached, usually after approximately 3 months. Subjects subsequently received injections of this dose once every 2–4 weeks for the following 6 months. A peripheral venous blood sample had been taken for lymphocyte analysis before SIT was initiated and again after 6 months of treatment had elapsed.

Twelve subjects matched for duration of perennial allergic rhinitis and skin-test reactivity who denied SIT served as the control group. Peripheral venous blood samples for lymphocyte analysis were taken twice from them within 6-month intervals.

All the SIT and control patients were monitored for symptoms and follow-up period and had neither local nor systemic complications throughout the study period. All the SIT patients reported an improvement in symptoms and a decrease in drug consumption for their nasal symptoms. In the control group the drug consumption remained unchanged and only four patients reported an improvement in symptoms.

The 10 treated subjects included seven men and three women aged 19–38 years (mean 25 ± 5.4). The control group included five men and five woman aged 19–36 (mean 24.6 ± 5.6). All subjects completed the treatment and follow-up period and had neither local nor systemic complications throughout the study period. All the SIT patients reported an improvement in symptoms and a decrease in drug consumption for their nasal symptoms. In the control group the drug consumption remained unchanged and only four patients reported an improvement in symptoms.

### Results

T cell subset and TCR-V expression analysis by flow cytomtery

Venous blood was drawn from each subject (2 ml), and 100 µl of whole blood was incubated in FACS tubes with saturating concentrations of each one of the monoclonal antibodies; the rest was used for carrying out a complete blood count.

Lymphocyte subsets and TCR-V expression were identified by a panel of 20 monoclonal antibodies against T-cell subsets and TCR-V family products: CD3, CD4, CD8, pan alpha-beta, pan-gamma-delta, V-alpha2, V-alpha12.1, V-beta5a, V-beta5b, V-beta5c, V-beta8a, V-beta8b, V-beta3.1, V-beta13, V-beta12, V-beta6.7, V-gamma9, V-delta1, V-gamma4, and V-delta2 (Becton-Dickinson and T cell Diagnostics, Woburn, MA). The monoclonal antibodies were directly conjugated to either phycoerythrin (RD1) or fluorescein isothiocyanate (FITC). Conjugated immunoglobulins of matched isotypes were used as negative control.

Flow cytometric analysis was performed on a dual FACS 440 equipped with an Ar⁺ and Kr laser (Becton-Dickinson Immuno-cytometry System). At least 10 000 events for each sample were collected. Data were collected and analyzed using the Consort Vax and Disp4 and Disp2D programs (Becton-Dickinson). The information was collected on a logarithmic scale.

An electronic gate was set on the lymphocytes on the forward and side-scatter plot. Lymphocytes subsets or TCR-V expression was then analyzed by detection of fluorescein isothiocyanate (FITC) or RD1 staining, and expressed as a percentage of the total number of lymphocytes.

### Statistical analysis

Statistical analyzes of the effects of SIT on cell numbers and percentages, T-cell subsets, and usage of variable chains, were done using a two-tailed paired t-test to compare data from the same subject before and after SIT, and between the SIT and control groups. Differences were not considered significant unless P < 0.05.
showed a modest but significant increase after 6 months of SIT (62.1% at baseline vs 69.3 after 6 months of SIT, $P = 0.035$). No other significant changes in lymphocytes counts and subsets were found in either group during the study period.

No significant changes were observed in the TCR-V expression of the 15 TCR-V markers in the control group during the study period. Significant increases in the expression of three of these markers (V-alpha12.1, V-beta12 and V-beta5b) were, however, observed in the SIT group after 6 months of treatment (Fig. 1).

**Discussion**

We designed the current study with the aim of expanding our understanding of the mechanism by which SIT alleviates symptoms in allergic diseases. Our results showed a significant increase in the expression of the TCR V-beta5b, V-beta12 and V-alpha12.1 chains in the peripheral blood of subjects with HDM perennial allergic rhinitis after 6 months of SIT. These changes were not seen in matched allergic control subjects who did not receive the immunotherapy. These data indicate that SIT changes the selective expansion of certain T cells in patients with allergy to HDM, implicating a crucial role of TCR in the pathogenesis of type I hypersensitivity and in the mechanism of SIT.

T cells constantly sample their environment using their TCR that interact with specific peptides bound to MHC molecules (5–7). Recently accumulated data suggest that the nature of the T cell receptor signaling provided by the allergen peptide ligand is the main factor which determines the profile of allergen-specific Th cells, and evidence of a linkage between the development of allergic diseases (including allergic rhinitis) and the TCR genotype and phenotype has been reported (24–30).

Once the TCR have been generated by random recombinations in the thymus during early ontogenesis of the immune system, they are further selected by interactions with antigen-presenting cells (APC) to generate a rather skewed repertoire that is considerably stable over time (6,7,31).

Several studies have demonstrated restricted TCR use in individuals with various allergic diseases and that these T cell subsets are allergen specific and can exhibit Th2 functional activities in terms of cytokine production (16–24). The TCR repertoire, however, is not static and can be further changed by antigen exposure in predisposed individuals (20,22,25,26).

Although we did not examine the influence of SIT and the changes in TCR use on IgE or T cell phenotypes, numerous studies have shown that SIT causes a shift in the balance of T-cell subsets away from Th2-type in favor of a Th1-type T-lymphocyte response (32–34). Although the mechanism of this phenomenon remains to be delineated, there is evidence to suggest that SIT can cause its effect by producing antigen-specific CD4+ suppressor T lymphocytes (4,5,32–34).

Our observation that certain TCR-V expressions were regulated by SIT may suggest that the repeated allergen administration during SIT caused the proliferation of suppressor T cells that have an oligoclonal TCR-V usage.

How SIT causes a change in the TCR repertoire is still unclear. Once shaped by selection in the thymus, the T cell repertoire is subject to further modification in the peripheral lymphoid organs. During the life of an individual, the
relative prevalence of the various TCR-V changes considerably—some are reduced from the peripheral pool while others become markedly more frequent (6,7).

It is possible that the changes in TCR usage after antigen stimulation are due to preferential export from the thymus or that they represent differential homeostatic expansion and maintenance in the periphery. Since TCR selection is strongly influenced by the relative contribution of self and foreign peptides, it is likely to be changed over time, especially when there is repetitive exposure to an antigen, as in the form of allergen SIT (6,7). Our results may therefore suggest that SIT causes its effect by antigenic stimulation that gradually changes the TCR repertoire into being more anergic or suppressive with respect to the allergen.

Although different groups have reported dominant expression of different TCR V-gene products, we believe that the differences are due to the different methods, the different type of allergy, the different genetic background and the different reagents available to the investigators.

Although self-peptide-MHC molecules are known to be crucial in the generation of a T-cell repertoire with a wide spectrum of specificities, only recently have data emerged that implicate TCR interaction with self-MHC in the survival and expansion of peripheral T cells (6). It is unclear if the different results in TCR usage in different studies are due to variable usage of self-peptide–MHC molecules by the tested individuals.

If indeed allergen-specific T cells with a limited use of TCR V-gene products play a role in the allergic response, then the possibility exists that selective manipulation of the TCR usage may be an effective strategy for specific immunotherapy directed against specific TCR-V chains.

Jarman et al. (35) and O’Hehir et al. (36) showed that T-cell responses to Der p in HDM allergy could be inhibited by either using specific T cell epitopes containing peptide, or by using superantigens that bind to specific chains of the TCR.

In BALB/c mice, the majority of ovalbumin-responsive T lymphocytes express the V-beta8.1+ and V-beta8.2+ T-cell receptor. Hofstra et al. recently showed that treatment of mice with antibodies to V-beta8 inhibited the ovalbumin-induced allergic response, suggesting that modulating the TCR usage can prevent allergic reaction, even without using a specific peptide directed toward a specific TCR allergen epitope (37).

Our study is a preliminary one and was limited by both the number of patients and the number of monoclonal antibodies available for conducting this investigation. Nevertheless, our findings support the previous experimental and epidemiological studies that indicate specific TCR-V to be important in allergic diseases, as well as the notion that the effect of SIT in patients with allergic rhinitis may be achieved by modifying the T-lymphocyte response through the modulation of TCR usage.

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References


