

T cell receptor-V β repertoire in allergen-specific sensitization and increased airway responsiveness

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An animal model system was developed to study the mechanisms resulting in allergic sensitization. Local allergen exposure via the airways and the lung stimulated an allergen-specific immunoglobulin E (IgE) response that was paralleled by the development of increased airway responsiveness (AR). It was found that CD4⁺ T cells of local draining lymph nodes played an important role in the regulation of these events. Stimulation of allergen-specific T cells requires interaction between major histocompatibility complex (MHC) class II molecules (expressed on antigen-presenting cells), peptide (presented on MHC) and the T cell receptor. Allergen sensitization stimulated T cells that expressed a restricted T cell receptor V β (TCR-V β) elements. Each allergen stimulated different V β elements, and sensitization to the same allergen resulted in a different pattern of TCR-V β stimulation in different lymphoid tissues. Some of these T cells had pro-allergenic effects, whereas others were able to inhibit the development of the allergic response, including the development of increased AR. These data indicate that the local T cell response regulates the type of immune response that evolves following local allergen sensitization.

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Regulation of immunoglobulin E (IgE) production and development of immediate hypersensitivity responses are controlled by CD4⁺ T cells (3). Recently, several steps of control mechanisms have been identified that play a critical role in the stimulation of IgE production. T cells are activated by antigen-presenting cells that present peptides on major histocompatibility complex (MHC) class II molecules to CD4⁺ T cells (33). The complex of MHC plus peptide is recognized by the T cell receptor (TCR) that is expressed on the surface of T cells. The TCR consists of two chains (α and β or γ and δ) comprising several different components and elements including the variable elements (V β) that are located closely to the contact side with the MHC/peptide complex. The composition of these different TCR elements determines the antigen specificity of this particular TCR and the individual T cell, respectively. Recognition of MHC plus peptide complex by the TCR results in stimulation and activation of T cells (13, 16). Additional co-stimulatory factors, such as interleukin 1 (IL-1) or ligation of CD28, are required to achieve full T-cell activation.

Some activated T cells produce (IL-4), which re-

presents an essential factor in the regulation of IgE (4, 5, 9). IL-4 binds to the IL-4 receptor (IL-4R), which is expressed on T and B cells and many other hematopoietic cell systems. IL-4 alone is not sufficient to induce productive messenger RNA ϵ -(mRNA) transcripts. Inducing ϵ -mRNA, which can be translated into IgE molecules, requires additional signals. Interaction between the CD40 receptor expressed on B cells and the corresponding ligand (CD40L, gp39) was recently identified to represent an important step in the stimulation of IgE (1, 7, 15, 25, 28). The CD40L is expressed on activated but not on resting CD4⁺ T cells.

These results indicate that a multidirectional interaction between several cellular components of the immune system is required to stimulate allergen-specific IgE production. Initial activation of the allergen-specific T cell represents a prerequisite and is controlled by the interaction between MHC, peptide and TCR, which assemble to a tri-molecular complex. In addition to these stimulatory factors, several factors have negative regulatory functions in this model of IgE regulation. Interferon γ (IFN- γ) is an important factor that inhibits IL-4 induced IgE

production and is secreted by CD8⁺ T cells and some CD4⁺ T cells as well (2, 11, 18).

Therefore, allergen-specific IgE production requires cognate T-B cell interaction. In addition, stimulation of B cells via noncognate pathways enhances polyclonal antigen nonspecific IgE production. Factors acting in a noncognate fashion include infection of B cells with Epstein-Barr virus (EBV), culture of cells with hydrocortisone and others. This article summarizes work aimed to identify factors and pathways resulting in the production of allergen-specific IgE production. To delineate cell-cell interaction under *in vivo* conditions, an animal model was established to study the development of immediate hypersensitivity responses in mice.

Results and discussion

Animal model of the localized allergic response

To study the development of allergen-specific and allergen-induced immediate hypersensitivity responses under *in vivo* conditions, a murine model of allergen sensitization was developed. Mice were sensitized via the most common route of allergen exposure in humans: allergen inhalation. Different protocols of allergen aerosolization were employed to induce an allergen specific IgE (and IgG1) response in IgE high-responder BALB/c mice. The response to two different allergens was analyzed. Mice sensitized to ovalbumin (OVA) or to ragweed allergen (RW, crude pollen extract) developed an allergen-specific IgE and IgG1 (particularly following long-term sensitization) response (20, 23). In order to study the functional role of these IgE antibodies, a skin test technique was developed, and the intradermal injection of small quantities of allergens resulted in immediate-type wheal formations (26). Maximal appearance of wheals was noted after about 20 min following application of allergen, and they disappeared spontaneously within the next 20 min.

These responses were paralleled by the development of increased airway responsiveness (AR), which was analyzed by two different methods. Increased AR could be demonstrated by measurement of airway resistance and conductance *in vivo* (20) as well as by analysis of AR *in vitro* (8). Using both methods, it was found that mice that developed an allergen-specific IgE/IgG1 response and immediate-type cutaneous hypersensitivity responses were hyperreactive. These data confirm previous reports from other groups indicating that local allergen exposure through the airways and the lung may result in the stimulation of IgE (6, 12). In addition, we found that stimulation of the IgE (6, 12) response was associated with the development of increased AR (Table 1). Histological analysis of larger airways

Table 1. Development of immediate hypersensitivity responses in allergen-sensitized mice

Strain	Allergen	IgE	anti-allergen IgG-1	anti-allergen IgG2a	AR	ST
BALB/c	OVA	↑↑	(↑)	—	↑↑	+++
SJL/J	OVA	—	—	↑↑	n	—
BALB/c	Ragweed	↑↑	↑↑	—	↑↑	+++

Different strains of mice were sensitized to ovalbumin (OVA) or ragweed by aerosolization of the allergen. After completion of a short- or long-term sensitization protocol, allergen-specific IgE, IgG1 and IgG2a antibody titers were measured in serum samples. In parallel, the presence of immediate cutaneous hypersensitivity responses was analyzed by skin testing (ST) with the respective allergens. Airway responsiveness (AR) was assessed by electrical stimulation of tracheal segments which were prepared from sensitized mice. Increased AR (↑) was measured only in OVA- or RW-sensitized BALB/c mice.

in sensitized mice also revealed a cellular infiltrate predominantly consisting of lymphocytes and some eosinophils (23). Taken together, the changes and effects seen in this animal model system closely resemble some of the findings in allergic asthma.

Development of IgE and increased airway responsiveness are T cell dependent

Histological examination of the airways and the lungs indicated that local sensitization resulted in a marked enlargement of local draining lymph nodes of large airways and the lungs: peribronchial draining lymph nodes (PBLN). Analysis by flow cytometry revealed that the enlargement was mainly due to an expansion of CD4⁺ T cells paralleled by an increase in B cell numbers. To further study the role of T cells in this system, lymphocytes from PBLN of sensitized mice were prepared and transferred from sensitized into nonsensitized recipients. The results from these transfer experiments clearly demonstrated that not only the IgE response and the subsequent development of cutaneous immediate hypersensitivity was lymphocyte-dependent, but also the development of allergen-induced airways hyper-reactivity could be transferred by these cells (8, 24).

These results led to the question of whether certain lymphocyte subpopulations and subsets could be identified that were responsible for the effects observed. Since the induction of an allergen-specific immune response requires interaction of T cell receptor with MHC and peptide, it was examined whether sensitization to an allergen may stimulate a restricted pattern of TCR-expressing T cells. The TCR repertoire was qualitatively and quantitatively assayed by measurement of the distribution of TCR-Vβ elements using flow-cytometric cell analysis.

Following the sensitization to OVA and RW, the stimulation of an restricted panel of TCR-Vβ-expressing T cell populations was detected in both

local draining lymph nodes and in the spleen of sensitized mice. Several characteristic results were obtained: First, each allergen stimulated a different pattern of V β -expressing T cell subpopulations (21, 23). For example, V β 8.1/8.2 positive T-cells were found in higher frequency following OVA sensitization; RW sensitization preferentially stimulated V β 8.1, V β 8.2 and V β 13 positive T cells (23). Second, in different compartments of the immune system (such as PBLN and spleen) a variable pattern of TCR-V β -positive T cells was observed. For example, RW sensitization stimulated V β 8.1, V β 8.2 and V β 13 T cells in PBLN, but an expansion of V β 8.1, V β 8.2, V β 8.3, V β 9 and V β 14 T cells was detected in the spleen (23).

Further examination of the functional role of these different V β -expressing T-cell populations revealed that they provide differential signals for Ig production by B cells. Co-culture experiments of highly purified V β - T-cells and sensitized B cells were carried out in order to study the production of immunoglobulin isotypes and subclasses via T- and B-cell interaction in an allergen-dependent culture system. In each allergen model, T cells bearing different TCRs provided preferential help for the production of different immunoglobulin isotypes and subclasses (21, 23).

These results were confirmed by transfer experiments with highly purified T-cell populations. Transfer of V β 8.1/8.2 T cells that were purified from OVA-sensitized mice and intravenously infused in nonsensitized recipients resulted in the stimulation of an allergen-specific IgE response and the development of increased AR, provided that the animals were challenged with the respective allergen via the

airways and the lung. In addition, transfer of OVA-responsive V β 2 T cells was not sufficient to induce any of these immediate-type allergic responses. Furthermore, when V β 2 and V β 8.1/8.2 T cells were simultaneously transferred, the development of IgE and airway hyperresponsiveness were blocked in the presence of V β 2 T cells (24).

Only V β 8 T cells from sensitized animals were able to provide the necessary signals for the stimulation of IgE and increased AR. When these T cells were prepared from control animals exposed to phosphate-buffered saline, they failed to mediate the development of an allergic response.

Model of T cell-dependent development of allergic responses

Local allergen exposure via the airways stimulated an allergen-specific immune response that could be detected in local draining lymph nodes of the respiratory tract. (Specialized) antigen-presenting cells may take up the allergen directly in the airways and present allergenic epitopes to T cells. Recently, Holt et al. (14, 32) identified and characterized an antigen-presenting cell population of the respiratory tract that seems to be involved in the regulation of the local immune response. They showed that intra-epithelial dendritic cells can take up allergen in the airways and present them to T cells in local lymph nodes.

Since the major T cell response to the allergen could be detected in the lymph node, it is possible that dendritic and other antigen-presenting cell populations induce the primary immune response in these lymphoid compartments. This model would be very similar to the way antigen is presented in the skin. Langerhans cells take up antigen (or allergens) in the skin, migrate to the local lymph nodes where they present epitopes to T cells. They then can travel back to their place of origination.

Sensitization to OVA resulted in the stimulation of a predominant T-cell population, which was characterized by the expression of a certain TCR phenotype (V β 8.1/8.2). Stimulation of a restricted pattern of TCRs following allergen exposure may be due to presentation of defined peptides by MHC class II molecules on antigen presentors. In fact, we found that V β 8.1/8.2 T cells recognized the OVA peptide 323-339, which is known as a major OVA epitope. Sensitization to this epitope alone also resulted in the development of immediate-type allergic responses similar to those measured following sensitization to whole OVA (22) (Fig. 1).

The result that T cells with different TCRs on their surface were found stimulated in different lymphoid tissues may be explained by the preferential presentation of different T-cell epitopes by antigen-

Table 2. Local allergen sensitization stimulates different TCR-V β -bearing T-cells in different compartments of the immune system

BALB/c			
Ovalbumin		Ragweed	
PBLN	Spleen	PBLN	Spleen
	V β 2		
V β 8.2*	V β 8.1/8.2	V β 8.1	V β 8.1
		V β 8.2	V β 8.2
			V β 8.3
			V β 9
		V β 13	
	V β 14		

BALB/c mice were sensitized to ovalbumin or ragweed via the airways and the lung. Sensitization resulted in the development of immediate hypersensitivity responses assessed by allergen-specific IgE (IgG1) production and increased airway responsiveness. In local draining lymph nodes of the airways and the lung (PBLN) and the spleen, the frequency of TCR-V β -bearing T cells was analyzed by flow cytometry, and the results were compared to the distribution of these subjects in PBLN and spleen of nonsensitized animals. *V β 8.2 T-cells were selectively stimulated following sensitization to OVA (323-339).

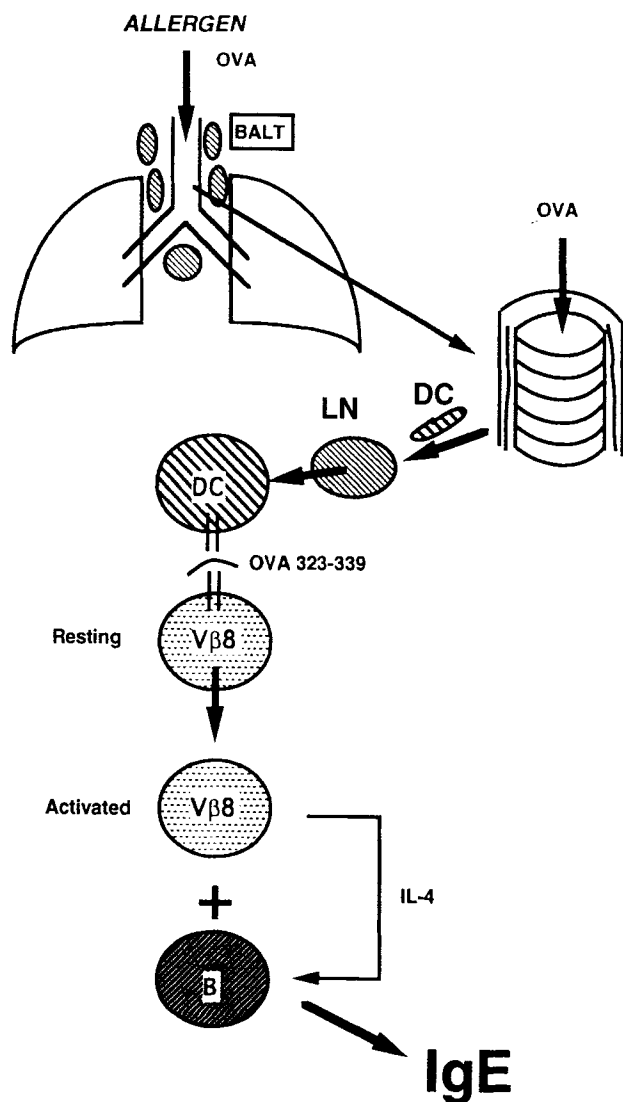


Fig. 1. Allergen-specific TCR-V β expressing T cells regulate the development of allergic sensitization, IgE production and the development of increased airway responsiveness (AR). For details, see text.

presenting cells homing in these compartments of the immune system. It may be that some specialized antigen-presenting cells can present certain epitopes and peptides to T cells better than others (29, 34). This would explain why sensitization to some antigens results in the stimulation of different TCR-expressing T-cell populations in different lymphoid tissues.

It is very unlikely that the TCR phenotype determines the type of immune response triggered by these T cells. In fact, our results demonstrated that the stimulation of the allergic response to OVA is not a function of all V β 8 T cells but was found only in the cells that had previously encountered the antigen. That means that allergen sensitization renders the cells competent to provide the necessary signals for

the stimulation of the allergic response. Stimulation of the cells, such as with mitogens, was not sufficient to provide these signals. In addition, for each allergen, different T cell populations expressing various V β elements were identified that triggered the development of the allergic response. This result also suggests that the TCR is not the critical element that determines the type of immune response (such as Th1 versus Th2 response). In addition to MHC class II, peptide and TCR interaction, other signals are required to push the immune response in the direction of an pro-allergic response. It is likely that these signals are provided by antigen-presenting cells. The nature of these signals, however, is still unclear and they need to be identified. Particularly, it remained unclear whether these are soluble factors or other (cognate) receptor-ligand interactions between T cells and antigen presentors.

It was recently reported that local allergen challenge on the skin resulted in the stimulation of a restricted TCR pattern in sensitized patients (10). It was found that during the delayed-type response following patch tests with grass pollen extract, T cells expressing a restricted TCR repertoire migrated into the site of the response. However, T cells from different individuals exhibited a differential pattern of TCR expression.

These data indicate that local sensitization stimulates a local immune response that can trigger the development of an allergic response. The type of immune response is controlled by T cells, which receive a number of different signals through antigen-presenting cells and other cell populations. The development of the allergic response is not only characterized by the induction of an allergen-specific IgE response. In addition, lymphocytes play also an important role in the development of increased airways responsiveness. In all cases, the presence of an IgE response was always paralleled by the development of airway hyperresponsiveness, indicating an interdependent relationship between the type of T cell response, the stimulation of an (allergen-specific) IgE response and the development of increased AR (17, 19, 27, 30, 31, 35).

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