

T-cell receptor V β elements regulate murine IgE production and airways responsiveness

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Introduction

Immediate type allergic reactions play a pivotal role in the pathogenesis of atopic diseases such as atopic dermatitis and bronchial asthma. Effector cells of allergic type I reactions have receptors on the cell surface which bind IgE with high (mast cells, basophils) or low affinity (eosinophils, monocytes, macrophages) (1–7), and the effector phase is initiated by crosslinking of receptor-bound IgE, resulting in cell degranulation and mediator release (8–10). It is now well established that T lymphocytes regulate the production of IgE by providing at least two distinct signals.

The cascade of regulatory events for IgE synthesis is initiated by the activation of CD4⁺ T cells (Fig. 1). Subsequently, these activated T cells (11) stimulate resting, antigen-specific B cells (12–14). This step requires physical (cognate) interaction between T and B cells through binding between the T-cell receptor (TCR) complex and MHC class II molecules on the B-cell surface (signal 1) (15). This initial activation enhances the responsiveness of B cells to the second signal.

The second signal is provided in a non-cognate fashion by a T-cell product or interleukin (IL). Of particular interest for the regulation of IgE production is the involvement of IL-4 (16–20) and IFN- γ (21–25), both products of activated T cells. It has been demonstrated initially in the mouse (26) and confirmed in human studies (27–30), that different

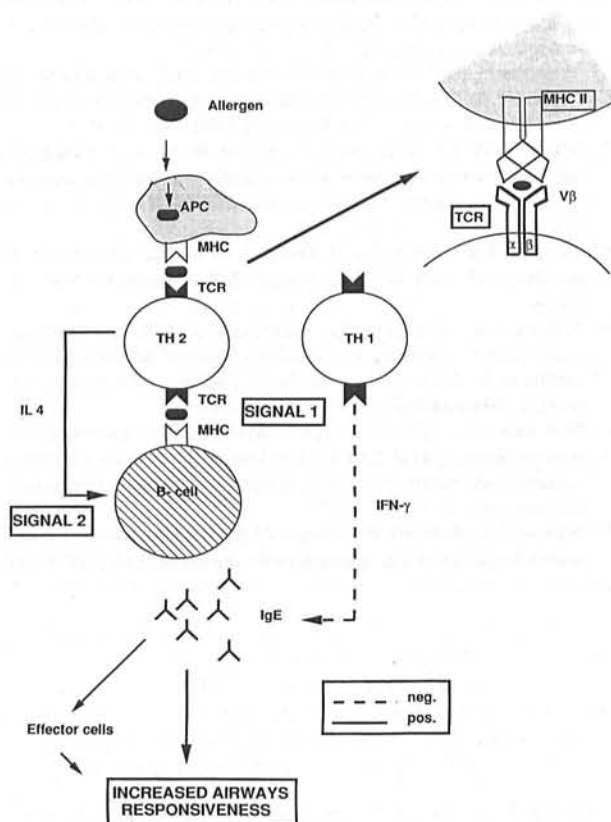


Fig. 1. Model of IgE regulation. Antigen-presenting cells (APC), T-helper cell (TH) population and B cells are involved in the regulation of IgE production. TH cells deliver at least two sets of signals (Signal 1 and Signal 2) to B cells for the stimulation of IgE.

T-cell clones exhibit a biased pattern of IL secretion. From on these observations, T-cell clones which produce IL-4 but not IFN- γ have been termed T-helper (TH) 2 cells and, analogously, T-cell clones secreting IFN- γ , but not IL-4, are defined as TH1 type cells. Although these data are supportive in terms of the identification and characterization of functionally distinct CD4⁺ T-cell subpopulations, the distinction between TH1 and TH2 type lymphocytes has not yet been demonstrated on freshly isolated antigen-specific lymphocytes.

IL-4 has two major activities on activated B cells: it stimulates B-cell proliferation (31, 32) and acts as a switch factor for IgE (33–35) (signal 2). In contrast, IFN- γ inhibits IL-4-induced IgE production (21–25). In addition to the observation that IFN- γ acts directly at the B-cell level (21), an effect on T cells has also been demonstrated (11).

Recent observations from several groups indicate that the induction of functional ϵ -mRNA is dependent on the presence of at least two signals, one signal provided by IL-4 and the second signal provided by CD4⁺ activated T cells (15, 35, 36). Stimulation of purified human B cells with IL-4 alone in the absence of autologous T cells induces only ϵ germline transcripts of 1.7 to 1.9 kb (34, 35, 37–39). Further experiments have shown that this germline ϵ transcript contains all four C ϵ exons and an I ϵ exon. Because the I ϵ exon contains several stop codons, this transcript cannot be translated into mature IgE protein. From these data it was concluded that IL-4 alone is not sufficient to induce productive ϵ -mRNA (35). The induction of mature protein requires the transcription of a second species of ϵ -mRNA with a size of about 2.2 kb. This second form of ϵ -mRNA is induced only when both signals, IL-4 and a second T-cell signal which has not yet been defined, were provided in the culture system. This 2.2 kb ϵ -mRNA is then translated into secreted IgE. Kinetic experiments indicate that the appearance of the 2.2 kb mRNA was always preceded by the 1.7 kb ϵ -mRNA transcript (35). Although the presence of a "sterile", non-productive mRNA is not unique for the ϵ -locus, as it has been described for other murine isotypes (such as γ 1 and γ 2b) (37, 38, 40–44), the functional role of this smaller transcript in isotype switching remains to be defined.

These data support the conclusion that IgE production is strictly controlled by T cells. Although the majority of these experiments focused on the regulatory pathways for polyclonal IgE production, there are few studies which address the functional characterization of T cells which regulate allergen-specific IgE production. Antigen- or allergen-specific activation of T cells requires the engagement of the TCR with antigenic fragments (peptides), presented by MHC class II molecules on antigen-presenting cells

(45, 46). The arrangement of the different TCR elements on the α and β chains determines which peptides may be recognized by a particular T cell. The antigen specificity of this recognition process is determined, in addition to other TCR elements, by the amino acid sequences of the variable elements of the TCR α and β chains (V α and V β).

Since several of the major allergens have been cloned and sequenced (47–54), it is now possible to determine and identify individual T-cell epitopes within these proteins. The results, although limited, indicate that allergenic proteins contain several epitopes with different sequences, recognized by individual T-cell subsets (53, 55–60). Different allergenic epitopes may be detected by specific V β sequences on the TCR. If, sensitization with a single allergen does result in the stimulation of some distinct T-cell population(s) expressing a unique V β TCR, we must then inquire as to the role of these distinct T-cell subpopulations in IgE production and their role in the development of increased airways responsiveness (AR) in bronchial asthma.

Results and discussion

These questions were addressed in the mouse where sensitization following antigen exposure through the airways, in the absence of adjuvant, resulted in IgE production (61). The mouse was chosen because of the availability of monoclonal antibodies against most V β elements. This permitted analysis of the usage of the TCR repertoire in a quantitative as well as qualitative way under *in vitro* and *in vivo* conditions. Furthermore, the genetic characterization of inbred mouse strains regarding the TCR and MHC repertoire provides an opportunity to analyze the role of these systems in IgE production in a more detailed way. Some of the strains have already been identified as IgE high- or low-responders (62–69).

An allergen-specific IgE response was induced in BALB/c mice, IgE high-responders (65), by ultrasonic nebulization of ovalbumin (OVA) diluted in PBS. Following an established protocol, the mice were exposed to OVA for 20 min each day for 10 consecutive days, or alternatively, once a week for 4 weeks. Both protocols resulted in proportionately high IgE anti-OVA response (relative to IgE antibody), measured by ELISA in serum samples obtained shortly after the sensitization protocol was completed (Table 1).

Since sensitization to allergen inhalation induced an IgE anti-OVA response, we determined whether these immunologic changes may also be associated with changes in airways responsiveness (AR). Within the last few years, several techniques have been developed that allow the study of lung function in mice (61, 70, 71). Two different methods were employed

Table 1. Ultrasonic nebulization of OVA but not PBS stimulated an anti-OVA IgE response and induced increased airways responsiveness

	PBS	OVA
Anti-OVA IgE	<20	1885±396
Anti-OVA IgG	<20	215±24
GL-ED ₅₀	81.5±38.8	27.3±11.8*
EFS-ES ₅₀	4.2±0.3	2.7±0.2**

Sensitization of BALB/c mice with OVA resulted in the stimulation of an anti-OVA IgE response which was accompanied by relatively low IgG anti-OVA antibody titers (expressed as arbitrary ELISA units). Measurement of airways conductance (GL) by body plethysmography revealed a decreased (* $p < 0.05$) ED₅₀ in sensitized versus non-sensitized mice. ED₅₀ is expressed as methacholine dose in µg/kg. In parallel, determination of airways responsiveness by electrical field stimulation (EFS) indicated a decreased ED₅₀ (expressed in Hz) in sensitized mice versus PBS-exposed controls (** $p < 0.001$).

to assess the status of AR in our mouse model. One approach was based on the measurement of changes in airway conductance *in vivo* by body plethysmography after intravenous challenge with increasing concentrations of methacholine (61, 70). In this way we showed heightened bronchoconstrictive responses in sensitized, as compared with non-sensitized mice, following intravenous challenge with methacholine.

A second method was employed to study AR *in vitro*. Tracheal smooth muscle was prepared from sensitized- and non-immunized controls and exposed to electrical field stimulation (71) at a constant voltage but with increased electrical frequency. The ES₅₀ (the electrical stimulation which resulted in half maximal constriction of airways smooth muscle) was calculated from a dose-response curve which was generated for each tracheal segment after stimulation with increased frequencies. Electrical field stimulation of tracheal smooth muscle preparations revealed that a significantly lower frequency was required to achieve half maximal airways constriction in sensitized mice than in non-sensitized controls. Thus, both approaches to the study of AR in this model revealed that sensitization to OVA by inhalation results in an increase in AR (Table 1).

Induction of the IgE response and the development of increased AR were paralleled by characteristic changes in the lymphocyte system. This means of sensitization resulted in a marked enlargement of local draining lymph nodes of the airways and the lung (peribronchial draining lymph nodes; PBLN) (61). The enlargement resulted from a 5-fold increase in total lymphocyte numbers, and further analysis of T-cell subsets showed a significant increase in CD4⁺ T cells. To analyze the functional capacity of these T cells in more detail, transfer experiments were performed where PBLN lymphocytes from sensitized mice were transferred intravenously into syngeneic, non-sensitized mice. Immediately following the

transfer the recipients were challenged with a single nebulization of OVA, which by itself had no effect on IgE production or AR. The control groups received spleen cells from PBS-exposed animals. Several days later the recipients were analyzed for IgE production and AR. The transfer of PBLN lymphocytes from sensitized into non-sensitized animals resulted in the stimulation of an anti-OVA IgE response. In addition the recipients developed increased AR. These data indicate that lymphocytes from PBLN of sensitized mice, but not from spleens of control animals, exhibit the capacity to stimulate allergen-specific IgE production and, subsequently, to increase AR in non-sensitized recipients (71).

The T-cell populations involved in these responses were assessed next. To analyze TCR-Vβ usage in this system, initial experiments were carried out to determine the TCR-Vβ repertoire of OVA-responsive T cells. For this purpose, several sets of OVA-responsive T-cell hybridomas were generated from OVA-sensitized mice, and the TCR expression of these hybridomas was studied by flow cytometry, using monoclonal antibodies against Vβ elements. The results indicated that OVA-responsive T-cell hybridomas primarily express four types of Vβ elements: Vβ8.1, Vβ8.2, Vβ2 or Vβ14. The majority of the hybridomas were positive for Vβ8.1 and 8.2 (72).

Based on these data, the frequency of individual Vβ-expressing T-cell populations was analyzed in freshly prepared lymphocytes from PBLN of sensitized mice and was compared with the distribution in PBLN of non-immunized mice. We found that sensitization to OVA resulted in an increase in numbers of Vβ8.1/8.2 T cells. In contrast, the percentages of Vβ2- and Vβ14-expressing cells were relatively unchanged. These findings led to the hypothesis that Vβ8 T cells may be involved in the regulation of IgE production and that they may contribute to the development of increased AR in this model. To study the effect on immunoglobulin production, Vβ8 and Vβ2 cells were purified from sensitized mice and co-cultured with B cells, primed *in vivo*, in the presence of OVA. At the end of a 9-day culture period, the supernatants were analyzed for total IgE production. We found that IgE production by the primed B cells was stimulated only in the presence of Vβ8.1/8.2 cells and that Vβ2 cells lacked the capacity to stimulate IgE production. Further, Vβ2 cells inhibited the Vβ8-stimulated IgE production. This phenomenon was found when a mixture of equal numbers of Vβ8 and Vβ2 cells were cultured with the B cells. Since we demonstrated similar antigen responsiveness (by proliferation to OVA) in both Vβ8.1/8.2 and Vβ2 cells, it appeared unlikely that the differential effects on IgE production were due to differences in reactivity to differing concentrations of OVA.

There are several possible explanations for the effects of different V β -bearing T cells on IgE production. Enumeration of IgE-secreting B cells by ELISA spot assay (73) indicated that V β 8 T cells increased the numbers of IgE-producing B cells rather than increasing individual B-cell production of IgE. Since the population of primed B cells contained some IgE-secreting B cells, it is possible that V β 8 T cells led to an expansion of IgE-committed B cells. Another explanation is that V β 8 cells induced switching to IgE in non-committed B cells. Further experiments at the molecular level will determine which of these mechanisms is triggered by V β 8 T cells.

T cells regulate B-cell function in a number of different ways. T cells are able to deliver signals to B cells through a physical interaction with these cells. This interaction may take place on the level of TCR-MHC class II interaction, but may not be restricted to this pathway. In addition, soluble T-cell factors, such as IL-2, IL-4, IL-5 and IL-6 (16–20, 74) influence B-cell function by acting at different levels of the B-cell maturation pathway. B-cell activation, proliferation or differentiation is influenced by many of the same ILs. Of particular interest is the role of IL-4, which induces Ig class switching to IgE. Although several T-helper cell populations have been identified which differ in their capacity to secrete IL-4 (TH 2 cells), a restricted pattern of cytokine production has not yet been attributed to T cells expressing different TCRs on their surface.

V β 8 T cells from BALB/c mice are known to respond well to OVA. One immunogenic epitope for these lymphocytes is the OVA peptide 323–339 (75). There are known associations between the expression of certain TCR and antigen presentation. It is possible that V β 8 and V β 2 T cells recognize different epitopes of the OVA molecule. Antigen-presenting cells may present different epitopes with varying efficiency and, subsequently, V β 8 and V β 2 cells may recognize antigen presented on different APC. Since it has been shown that the induction of an IgE response to OVA is dependent on several variables, including the route of sensitization (65), the amount of antigen (67), and the length of exposure to antigen (65, 68), it is also possible that antigen, presenting cells in different compartments of the immune system (e.g. local draining lymph nodes of the airways versus the spleen) may preferentially stimulate T cells bearing different TCRs simply by presenting different OVA peptides with varying efficiency.

At the present time, we have no evidence that V β 8 T cells always stimulate IgE production. This observation is presently linked only to the allergen OVA. Analysis of the sensitization profile to other allergens will determine which T-cell populations are stimu-

lated with different allergens. It currently appears unlikely that the expression of the V β element goes beyond the antigen recognition unit and determines the functional capacity of T cells to modulate Ig production. This would require that intrathymic differentiation direct thymocytes bearing different TCRs along different pathways. Although such differentiation pathways are established for CD4⁺ T cells with class II and for CD8⁺ T cells with class I restricted responses, no evidence is known of selected pathways within the population of CD4⁺ T cells.

The functional relevance of these *in vitro* findings for the regulation of IgE production by V β 8 and V β 2 cells was examined in two *in vivo* situations. In the first set of experiments, a mouse strain which is genetically deleted of V β 8 T cells from the TCR repertoire (SJL/J) was sensitized to OVA employing the same sensitization protocol established in BALB/c mice (71). Stimulation of IgG anti-OVA antibodies in these animals indicated that nebulization of OVA resulted in sensitization to the allergen. In contrast to V β 8-expressing BALB/c mice, the SJL/J animals did not mount an IgE anti-OVA response. Furthermore, analysis of AR indicated that sensitized SJL/J mice had an identical dose-response curve to electrical field stimulation as non-immunized SJL/J or BALB/c mice. In addition to the differences in V β repertoire between BALB/c and SJL/J mice, both strains also express different MHC class II haplotypes (Table 2). The variation in MHC class II molecules regulates, at least in part, the recognition of peptides by T cells. *In vitro* studies demonstrated that the expression of certain MHC class II gene products influences both peptide binding and, subsequently, interaction with the TCR (46). From these observations, it remains to be analyzed whether variation in IgE responses among different mouse strains may be related to differences in the TCR repertoire or whether the expression of different MHC class II molecules may also determine the type of immune response following allergen sensitization.

Table 2. Sensitization to OVA resulted in different response patterns in BALB/c and SJL/J mice

Strain	TCR	Anti-OVA		AR
		IgG	IgE	
BALB/c	V β 8 ⁺	(↑)	↑↑	increased
SJL/J	V β 8 [−]	↑↑↑	—	normal

BALB/c and SJL/J mice developed differential immunologic changes after sensitization to OVA. On day 12, serum samples were analyzed for anti-OVA IgE and IgG titers by ELISA, and airways responsiveness (AR) was measured by electrical stimulation by tracheal smooth muscle. In contrast to SJL/J mice, the BALB/c strain expresses V β 8⁺-bearing T cells in the T-cell repertoire.

In the second *in vivo* system, transfer experiments were performed where V β 8 T cells from OVA-sensitized BALB/c mice were transferred into non-sensitized, age- and sex-matched syngeneic recipients. The results from these experiments indicated that the sole transfer of V β 8.1/8.2 T cells from sensitized into non-immunized recipients stimulated an IgE anti-OVA response. Furthermore, analysis of AR demonstrated that these IgE-responding mice also developed increased AR. These changes were found only in mice which received V β 8 cells from OVA-sensitized mice but were not detected in animals which were injected with V β 8 cells prepared from control animals. These data demonstrate the functional capacity of different V β -expressing T-cell populations to regulate IgE production and AR *in vivo*, confirming our *in vitro* findings.

Conclusion

These data demonstrate the relationship between T-cell function, IgE production and development of increased AR (Fig. 2). Sensitization of mice with a well-characterized allergen resulted in the induction of an allergen-specific IgE response. This response was achieved simply by exposure to the allergen through the airways and the lung in the absence of adjuvant. In local draining lymph nodes, this means of antigen exposure resulted in the stimulation of allergen-specific T cells and these T cells, could be distinguished by the profile of V β elements expressed. We may speculate that individual allergens can indeed trigger a unique spectrum of TCR which are able to recognize epitopes presented on antigen-

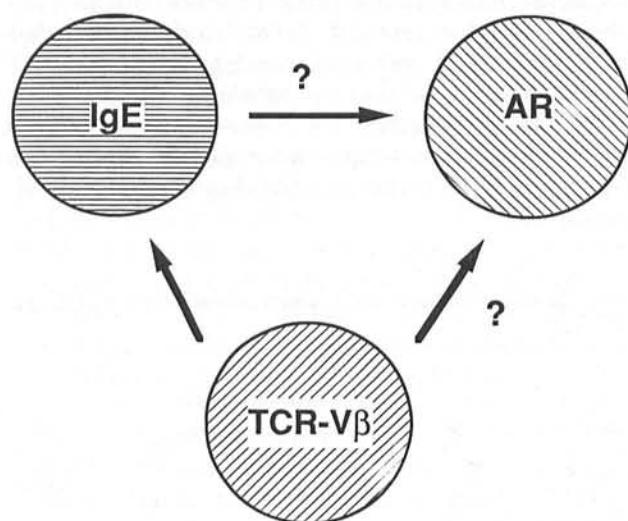


Fig. 2. In an allergen-driven system, T cells expressing certain V β elements stimulate IgE production and increased airways responsiveness (AR). The exact mechanisms by which T cells regulate AR need to be defined. It is possible that T cells directly influence AR or that IgE directly affects smooth muscle function.

presenting cells. At least in this unique model of OVA sensitization, we show that a particular V β -expressing T-cell subpopulation stimulated an IgE response under *in vitro* as well as *in vivo* conditions. The same T-cell subpopulation also was associated with the induction of changes in AR, confirming the link between IgE and AR in allergen-induced IgE responsiveness and bronchial hyperreactivity in man (76–78). This mouse model should allow us to delineate the underlying immunologic mechanisms which govern increased AR and IgE production. Although it is conceivable that IgE directly affects the smooth muscle or related systems which regulate AR, it appears that T cells may have a more direct effect on AR, for example, by secreting soluble factors which interfere with the physiologic pathways regulating AR.

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