

The role of mIgE during thymus-dependent and thymus-independent immune responses

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Introduction

Immunoglobulin E (IgE) was the last of the immunoglobulins to be detected (1, 2). It is present in very low amounts (nano- to microgram/ml range) in the serum of normal, healthy individuals and of normal laboratory mouse strains and has a very short half-life (3). This contrasts with the other immunoglobulin classes, which are present in much higher concentrations (micro- to milligram/ml range) and form a substantial component of serum proteins. Immunoglobulins play a role in homeostatic mechanisms, and they are a first line of defense against pathogenic organisms. Because IgE titers are elevated in helminth infestations, IgE was thought to play a role in the defense against parasitic worms. However, the value of this notion is debated because it was shown that high titers of IgE may even be detrimental during infestations with such worms (4, 5). Immunoglobulins can be found in two forms. In the secreted form (sIg), they represent the effector arm of the humoral immune system. They can also be expressed on the surface of a B cell in a membrane-bound form (mIg), and, in this physical state, they probably convey signals to steer the B cell along its differentiation pathway. The mIg transmembrane segments are about 25 amino acids long and have the potential for interaction with other polypeptides (6). The cytoplasmic domains of mIgs are different in size, ranging from only three amino-acid residues (KVK) in the case of mIgM and mIgD to 14–28 residues for the other mIg subclasses. The nature and effects of the signals generated by the antigen receptor are mostly unknown, but it has been speculated that they may control important mechanisms such as affinity maturation (7–10), class switch (11, 12), memory induction (13), and differentiation into plasma cells.

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Recently, we (14) and Kaisho et al. (15) have reported important functions for the transmembrane and cytoplasmic regions of immunoglobulins. In mutant mice that lack the transmembrane domains of IgE and IgG₁, serum IgE or IgG₁ are virtually absent, whereas in mice lacking the cytoplasmic domains of IgE or IgG₁, a severe impairment of T-cell-dependent immune responses is seen.

In this review, we discuss the role of mIgE in thymus-dependent and -independent immune responses. We will first briefly review the events that lead to membrane expression of IgE.

Regulation of the membrane expression of IgE

In the course of an immune response, B cells can express different immunoglobulin heavy-chain isotypes. Early in the response, B cells express IgM and IgD as antigen receptor, and they secrete IgM. Later, they can lose expression of IgM and IgD, and express other isotypes. This process is called isotype switch, is controlled by T cells, and takes place in a defined anatomical site, the germinal center. For a review, see Refs. 16, 17. Isotype switch allows an immunoglobulin to retain its antigenic specificity, residing in the variable regions of the heavy- and light-chain genes, but to vary the effector function of the antibody by exchanging the constant part of the heavy chain. The molecular events leading to isotype switch are quite complex and only partially understood. For an extensive review on isotype switching, see Refs. 18, 19. For the purpose of this review, it suffices to mention that the gene segments coding for the constant part of the different isotypes (except for IgD) are preceded by a switch (S) region. After the induction of switch recombination, a gene segment between two switch regions is removed, causing a double-strand break in the S regions, followed by repair. This results in a new, hybrid switch region, which is made up of the 5' and the 3' ends of the S regions involved, and which can be used again in a further switch recombination process (Fig. 1A).

Switch recombination is dependent on transcription starting from a promoter site upstream of the S region. The transcript includes a nonfunctional exon (I exon), located 5' of the S region. Successful switch recombination apparently depends on processing (i.e., splicing) of this transcript. Switch recombination is normally dependent on the close interaction with helper T cells (T_h cells) (12, 20). Upon priming, T_h cells secrete cytokines such as interleukin (IL)-4, IL-13, interferon (IFN)- γ , or transforming growth

factor (TGF)- β (21, 22). The cytokines initiate or suppress transcription from the promoters upstream of the I exons, thereby determining the isotype that will be involved in switch recombination. The priming of T cells is not dependent on antigen presentation by B cells; switch recombination, however, is dependent on class II MHC expression by B cells (23). Primed T cells express the CD40 ligand, which binds and cross-links the CD40 antigen on B cells (24). This interaction and others such as the CD28-CD80/CD86 interaction (25) take place in the germinal center (9, 16, 26) and are essential for the initiation of the switch recombination. Switch to IgE is induced by IL-4 (27) and IL-13 (28), and inhibited by IFN- γ (29). Interaction of IL-4 with its receptor causes the activation of STAT6 and its translocation to the nucleus, where it binds to an IL-4-responsive element in the promoter region 5' of the ϵ gene (30). Switch to IgE is also induced *in vitro* by lipopolysaccharides (LPS) and IL-4 (31). Recently, the receptor for LPS on B cells has been identified. It is the Toll-like receptor-4 (Tlr4), which belongs to the IL-1-receptor/Toll-like receptor family (32). CD40 and the Toll receptor family share a signal transduction pathway: they both induce the translocation to the nucleus of NF κ B (33). Among other activities, NF κ B can cooperate with STAT6 in the induction of germ-line ϵ transcripts (34).

In conclusion, switch recombination depends on direct T-B-cell interaction, the type of cytokine produced, transcriptional activation, RNA processing, DNA excision, and efficient DNA repair. The effects of some of these factors on the switch to IgE are well documented, like the effect of cytokines, others are hardly or not at all studied (RNA processing, DNA excision, and efficient repair). Yet, they are of essential importance in the number of cells that will be recruited in an IgE-mediated immune response.

As mentioned before, immunoglobulins are found as a secreted product and as a membrane-bound receptor. The constant parts of the heavy chains are coded by five or six exons. The last two exons code for the transmembrane and cytoplasmic domains. A 3' "external" polyadenylation-addition site is found downstream of these exons. The third or fourth exon, which is located 5' of the membrane exons, is a composite exon: it contains an internal splice donor site, which is used when mRNA for membrane-bound Ig is made, but it is also followed by an "internal" polyadenylation-addition site, which is used when mRNA for secreted Ig is made. Therefore, the production of the two types of RNA is determined by alternative splicing or, rather, alternative polyadenylation (Fig. 1B). The ratio of the amount of secreted to the amount of membrane-bound Ig that is

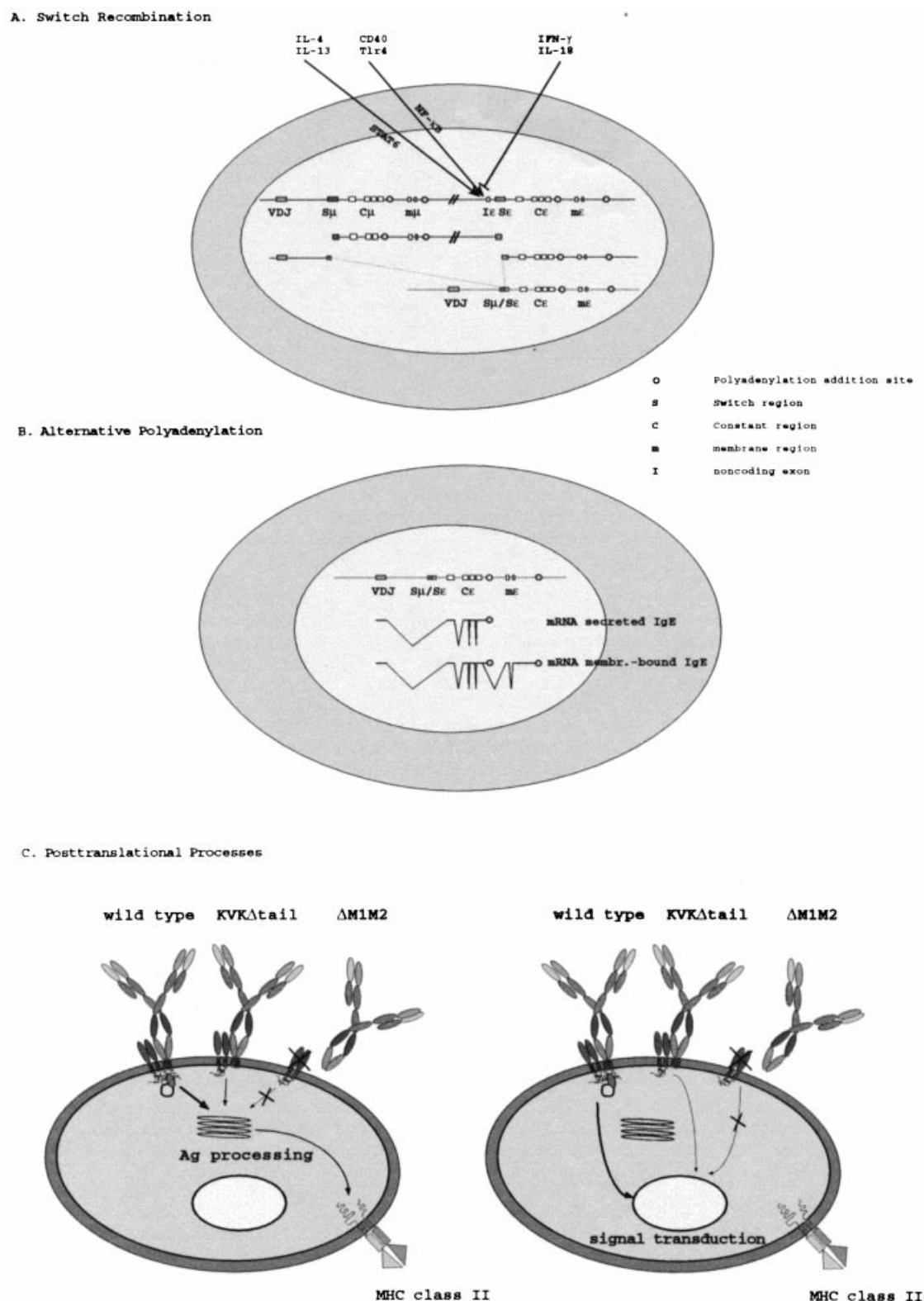


Figure 1. Critical stages in the development of a B cell towards IgE-secreting cell. See text and Ref. 14 for more details. A) Switch recombination. Critical events are transcriptional activation, DNA excision and DNA repair leading to switch recombination. B) Alternative polyadenylation. Critical events are regulation of polyadenylation and mRNA stability. C) Post-translational processes. In this diagram, possible explanations are given for the phenotype of mutant mice that lack the transmembrane and/or the cytoplasmic domain of IgE. The presence of proteins that could bind the cytoplasmic domain of IgE, and therefore modify signaling – or antigen presentation events via mIgE, is assumed.

produced by a single cell is therefore determined by the efficiency with which the internal or external polyadenylation sites are used and by the stability of the ensuing mRNAs. In resting B cells, the ratio is about 1, but more mRNA for secretory Ig can be found in activated lymphoblasts. This effect seems to be correlated with, but is not absolutely dependent on, a rise in concentration of a 64-kDa subunit of the cleavage stimulatory factor (CstF) and an increase of the stability of the mRNA (for recent reviews on this matter, see Refs. 35, 36). Whether the regulation of splicing for the IgE heavy-chain mRNA is similar to that described for IgG and IgA remains to be established. Preliminary data from our laboratories suggest a different regulation: expression of mRNA for the secreted form of IgE is favored over that for the membrane form in resting B cells. The factors that influence the alternative polyadenylation are largely unknown. However, because expression of mIgE is essential for recruitment of IgE-producing cells in the immune response, clarification of this issue is of great importance. It is noteworthy in this regard that the frequency of cells that have switched to IgE is 15-fold lower than the frequency of cells that have switched to IgG1 (37). In the secondary immune response, the number of IgE-producing cells is 500-fold lower than the number of IgG1-producing cells (G. Achatz, unpublished observations).

Biologic significance of mIgE

A step forward in the understanding of the role of mIgs other than mIgM or mIgD was achieved with two mouse lines with mutations in the ϵ heavy-chain gene. In the first, the intracellular domain of IgE was removed except for three amino acids (Lys, Val, Lys) (KVK Δ tail line). The cytoplasmic domain of IgE in these mice is the same as that of mIgM and mIgD. In the second line, both the intracellular and transmembrane domains of IgE (Δ M1M2 line) are lacking (Fig. 1C) (14, 38). In Δ M1M2 mice, serum IgE is reduced to less than 10% of normal mice, while KVK Δ tail mice show a reduction of 50%, reflecting a serious impairment of the IgE-mediated immune response. Class switch to IgE was not impaired by the targeting event. Upon stimulation of isolated spleen cells of wild-type, Δ M1M2 and KVK Δ tail mice with LPS and IL-4 *in vitro* (31, 39, 40), concentrations of IgE and IgG1 in the culture supernatants were comparable in wild-type and mutant mice. These results imply that the reduced IgE titers found in both mutant lines are solely a reflection of the loss of biologic activities associated with the transmembrane and cytoplasmic domains of IgE.

Therefore, we assessed the immune response of the mutant mice. First, mice were immunized with the T-cell-dependent antigen 2,4-dinitrophenyl-ovalbumin (DNP-OVA), and specific antibody titers were measured. Serum levels of specific IgG1 were comparable in wild-type and mutant mice. In contrast, in Δ M1M2 mice, DNP-specific IgE antibodies could barely be measured. In KVK Δ tail mice, titers were 50–80% lower than in control mice. In KVK Δ tail mice, a clear secondary response, as characterized by a strong and fast rise in specific antibody titer, was absent. A further indication of a qualitatively changed immune response was obtained when we compared the IgE responses before and after depletion of the sera with protein G. As shown before (39, 41), a high quantity of specific IgG antibodies can compete with specific IgE antibodies for recognition sites in the assay system and thus reduce the sensitivity of IgE detection. In control mice, the increase in titer was only slight after depletion, while in mice of the Δ M1M2 line, specific IgE antibody titers remained at detection levels. However, a doubling of the DNP-specific IgE titer was seen in the KVK Δ tail mice (Achatz & Lamers, unpublished observations). Our interpretation of this result is that the antigen-specific IgE antibodies of the KVK Δ tail mice compete less efficiently with IgG1 antibodies than those of wild-type mice, reflecting a lower general affinity of anti-DNP antibodies in KVK Δ tail mice. A more quantitative measurement of affinities was not possible due to the low absolute antibody titer of IgE.

With the help of an IgE-specific SPOT-ELISA, we found that the reduced IgE levels in the mutant mice were reflected in a lower number of IgE-secreting cells, indicating that a lower number of IgE-producing cells were effectively recruited in the immune response (14).

Our data clearly show that the transmembrane domain of IgE is indispensable for a T-cell-dependent IgE-mediated immune response, and that the cytoplasmic tail determines not only the absolute amount of IgE produced, but also the quality of the antibodies. How does this work?

Two hypotheses can be brought forward. The first implies that signals generated via membrane-bound Ig are needed at all times, not only for the maturation process, but also for the expansion of antigen-specific cells. The second hypothesis postulates that antigen presentation to T-helper cells is necessary at all times during an antibody response and that the antigen receptor is the only device for an effective antigen presentation. The hypotheses are not necessarily mutually exclusive. All Ig classes can associate with the Ig- α /Ig- β heterodimer (42), the signal-transducing unit of the B-cell receptor. Recent experiments have shown that an

intact antigen receptor on B cells is essential for the survival of B cells (43). From these experiments, it is not clear which function the antigen receptor performs: interaction with external ligands, maintenance of a tonic signal generated by the mere presence of the receptor in the membrane, or capture of antigen. Both the Ig- α /Ig- β sheath and the cytoplasmic tail of mIg (44) have been thought to be involved in guiding antigen bound via the receptor to the antigen-processing compartments. Key residues for internalization are present in the tails in the form of an YxxI/M motif. Because IgG can be expressed at the surface of a B cell without the Ig- α /Ig- β sheath (42) and in this condition is devoid of signaling capacity, such a receptor would serve as an efficient antigen-capturing unit, without eliciting a direct cellular response. This condition could be a prerequisite for processes that are highly dependent on T-B-cell interactions such as somatic mutation and affinity maturation and the generation of memory cells, but also processes such as the rescue from apoptosis and the induction of plasma cells. These considerations predict that the results we obtained in the KVK Δ tail and Δ M1M2 lines can be extended to the IgG isotypes, and perhaps to IgA. Indeed, Kaisho et al. (15) reached similar conclusions in studying mice carrying similar mutations in the γ 1 gene. However, the phenotype of the mIgG mutants is more prominent. A possible explanation is that IgE expression requires the Ig- α /Ig- β sheath, which may partly compensate for the loss of the IgE tail.

If the membrane domains are a prerequisite to recruit cells for the production of secreted IgE, how can we explain the fact that residual serum IgE is present in Δ M1M2 mice? As we have seen before, the isotype switch to IgE induced by IL-4 and the polyclonal B-cell activator LPS is not impaired in Δ M1M2 mice. The LPS-receptor, Tlr4, belongs to the Toll-like receptor family, a conserved family of proteins that is involved in innate immunity. It uses the same signal transduction pathway as CD40 to achieve a change in nuclear metabolism: NF- κ B is activated and translocated to the nucleus. It is conceivable that any activation which involves NF- κ B and STAT6 can induce switch recombination to IgE, even independently of the anatomical site. The residual IgE in Δ M1M2 mice could therefore stem from a polyclonal activation involving NF- κ B in the presence of sufficient IL-4. A living example of such a scenario could be the reaction to the helminth *Nippostrongylus brasiliensis*. *N. brasiliensis* induces a robust IgG1 and IgE production, both through a dominant activation of Th2 cells and through a strong, T-cell-independent activation of B cells (45, 46). We measured the serum levels of IgE after infestation with *N. brasiliensis*. Wild-type mice showed a 50-fold increase in serum levels, as

did the mutant mice. However, these mice started with a much lower serum IgE titer. After secondary infestation, 11 weeks after the first challenge with *N. brasiliensis*, a strong and fast IgE response was seen in the wild-type mice, indicative of a memory response. The IgE response in the KVK Δ tail was also substantial, but at 55% of the wild-type response. In the Δ M1M2 mice, IgE was now clearly measurable. Interestingly, when the IgE titers of control and mutant mouse lines are compared to the titers in infested animals, a rather constant ratio is observed (20:10:1, comparing wild type to KVK Δ tail and Δ M1M2, respectively). The results indicate that the IgE response to *N. brasiliensis* also needs a specific interaction with the IgE antigen receptor complex on the B cell, accompanied by strong Th2 helper activity. The measurable level of IgE secretion in the Δ M1M2 mice after the secondary challenge with *N. brasiliensis* can then be explained by an even stronger Th2 memory response together with a direct stimulation of B cells, leading to the activation of NF- κ B, switch recombination and IgE secretion, similar to the response to LPS plus IL-4 *in vitro*. An alternative explanation would be the relatively rare occurrence of an isotype switch from IgM to IgE indirectly via IgG1. The last pathway would rely on the control function of mIgG1, and therefore be independent of mIgE.

Membrane-bound IgE could be a target for therapeutic interference. Recently, the use of nonanaphylactic, humanized anti-IgE antibodies has emerged as a therapeutic strategy in allergic disease. For a review on this issue see Ref 47. These antibodies bind to the same site on the IgE molecule that interacts with the high-affinity IgE receptor, thereby interfering with the binding of IgE to this receptor without cross-linking the IgE on the receptor. Treatment with anti-IgE antibodies leads primarily to a decrease in serum IgE levels. As a consequence, the number of high-affinity IgE receptors on mast cells and basophils decreases, leading to a lower excitability of the effector cells. The antibodies can of course also interact with mIgE on B cells. Stimulation of B cells via the antigen receptor without appropriate T-cell help normally leads to apoptosis (48). Because the anti-IgE antibody is humanized, the immune system treats it as an autoantigen, and T cells are not recruited. Finally, membrane-bound IgE has also been identified as the target for the CD23-mediated suppression of the IgE response. Yu et al. (41) showed that CD23, the low-affinity IgE receptor, functions as a negative feedback regulator of IgE responses. If this process is B-cell autonomous, this inhibition can be achieved only by antigen cross-linking CD23 with mIgE.

Concluding remarks

Two major conclusions can be drawn:

- 1) The transmembrane domain of mIgE is indispensable for T-cell-dependent IgE secretion.
- 2) The cytoplasmic domain not only determines the absolute amount of IgE produced, but also influences the quality of the immunoglobulins.

Our knowledge of the regulation of the expression of membrane-bound IgE is, at best, limited. Unfortunately, a similar statement can be made regarding the function of the cytoplasmic domain of IgE. However, we do know that the

production of IgE is tightly regulated. This regulation is evident on the level of DNA recombination (switch), transcription, and RNA processing. It is not inconceivable that post-translational processes may also influence the expression of membrane-bound IgE. The consequences of a possible dysregulation, i.e., uncontrolled IgE secretion, warrant an in-depth study of these processes.

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