

The Road to the Production of IgE Is Long and Winding

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THE REGULATORY FUNCTION OF MEMBRANE-BOUND IMMUNOGLOBULIN

Immunoglobulins are found in two forms. As secreted proteins (sIg) they represent the effector arm of the humoral immune system. They are also found as integral membrane proteins on B lymphocytes (mIg). In this physical state they are connected to signal transduction cascades in the cell. They convey signals to guide the B cell along its differentiation pathway and allow it to respond to antigen. The mIg transmembrane segments are about 25 amino acids long and have the potential for interaction with other polypeptides (1). The cytoplasmic domains of mIgs are different in size and range from only three amino acid residues (KVK) in the case of mIgM and mIgD to 14 residues for IgA and 28 residues for the other mIg subclasses.

The nature and effects of the signals generated by the antigen receptor, in particular mIgM and mIgD, are under intense study. For this article the role that was established for mIgM in the early development of the B cell, in the pre-B cell stage, is of significance: The expression of mIgM is an absolute prerequisite for further development (2). Also, later in development mIgM is essential for survival (3).

About the function of the intracellular domains, little is known as to date. It is speculated that they can control mechanisms such as affinity maturation (4, 5), class switch (6, 7), memory induction (8), and differentiation into plasma cells.

A step forward in the understanding of the role of mIgs other than mIgM or mIgD was achieved with mouse lines that carried mutations in the ϵ (9) or $\gamma 1$ (10) heavy chain gene. In mutant mice that lack the transmembrane domains of IgE or 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. Class switch to IgE or IgG was not impaired by the targeting event. These results imply that the reduced IgE or IgG₁ titers found in the mutant lines are solely a reflection of the loss of biological activities associated with the transmembrane and cytoplasmic domains of IgE and IgG₁.

These data, and the data of Loder and colleagues from our own laboratory (11), unambiguously stress the importance of the signalling function of membrane-bound Ig for the survival and differentiation of the B cell.

In this review we discuss events that lead to membrane expression of IgE.

REGULATION OF THE MEMBRANE EXPRESSION OF IgE

B cells can express different immunoglobulin heavy chain isotypes, depending on differentiation stage or activation state. As mentioned earlier, in all conditions expression of mIg is fundamental for further differentiation or survival. Early in

their differentiation pathway, as immature B cells in the bone marrow, B cells express only mIgM. After a specificity check of the receptor and the removal of autospecificities, B cells exit from the marrow into the periphery, and undergo further maturation steps toward fully immunocompetent, mature B cells. The expression of a second membrane-bound immunoglobulin, mIgD, seems to signal the successful completion of this process. Early in an immune response, B cells continue to express mIgM and to a lesser extent mIgD, and they secrete IgM. Later, they can lose expression of IgM and IgD, and express other isotypes. This process, called *isotype switch*, is controlled by T lymphocytes and takes place in a defined anatomical site, the germinal center (for more information see References 12 and 13). Isotype switch allows a B cell 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 secreted antibody by exchanging the constant part of the heavy chain.

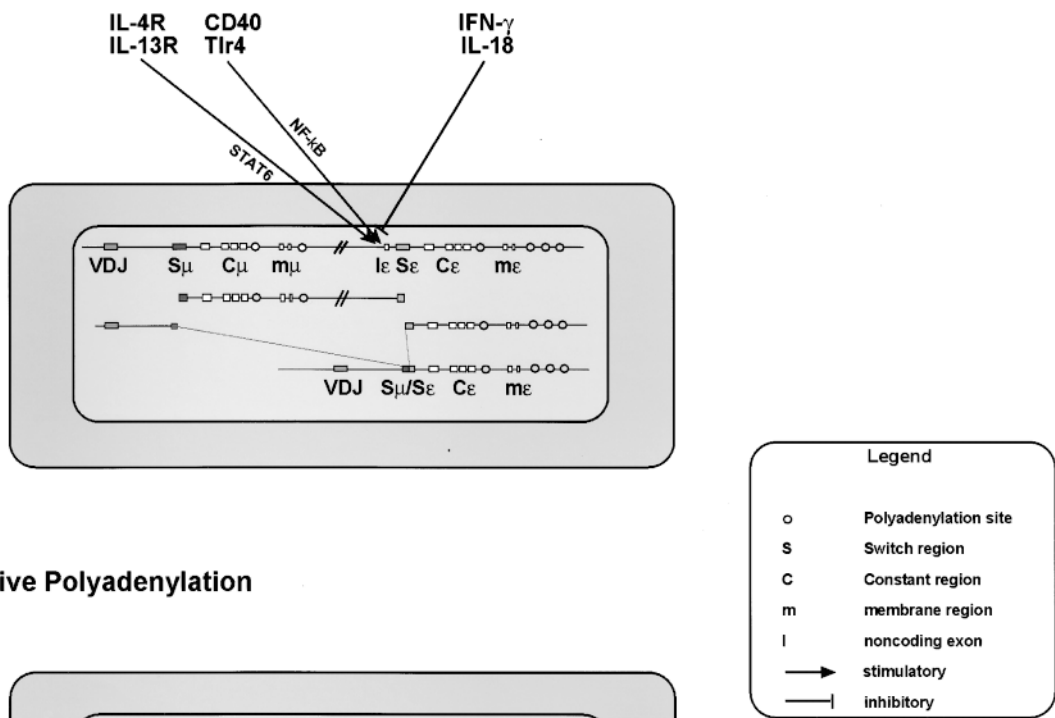
The molecular events leading to isotype switch are complex and only partially understood. For extensive reviews on isotype switching, the reader is referred to References 14 and 15. For the purpose of this article it suffices to note that the gene segments encoding the constant part of the different isotypes (except for IgD) are preceded by repetitive DNA sequences (switch [S] regions). Induction of switch recombination leads to apposition of two S regions, followed by removal of the gene segment between them. This causes double-strand breaks in the S regions, which must be repaired to warrant cell survival. The result is a new, hybrid S region that is made up of the 5' end of the upstream and the 3' end of the downstream S region involved, respectively. The newly formed hybrid S region can be used again in a further switch recombination process (Figure 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, that is noncoding. Successful switch recombination apparently depends on processing of this transcript, that is, splicing of the pre-mRNA (16) or polyadenylation (17). *In vitro* class switching to IgG₃, IgG_{2b}, IgG_{2a}, and IgE, and to a lesser extent to IgG₁ and IgA, depends on the presence of a long-range regulatory region 3' of the Ig locus, the 3' Ig enhancer (18, 19). This enhancer resembles a locus control region (19). It regulates transcription arising from the respective heavy chain isotype loci. *In vivo* the dependence on the 3' enhancer seems to be less absolute as evidenced by lower, but not absent, serum Ig levels in mice with mutations in the 3' enhancer (19).

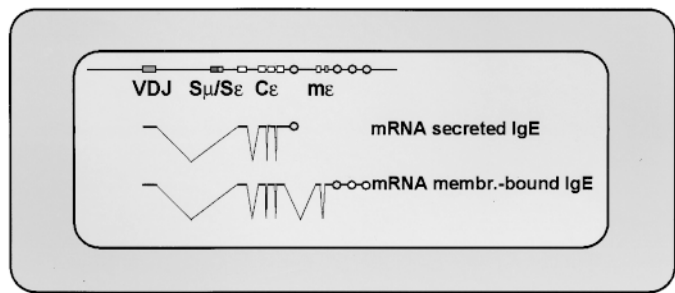
Switch recombination is further dependent on the close interaction with helper T cells (Th cells) (7, 20). After contact with antigen and presentation of the processed antigen to Th cells, these cells secrete cytokines such as interleukin 4 (IL-4), IL-13, interferon γ (IFN- γ), or transforming growth factor β (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 recom-

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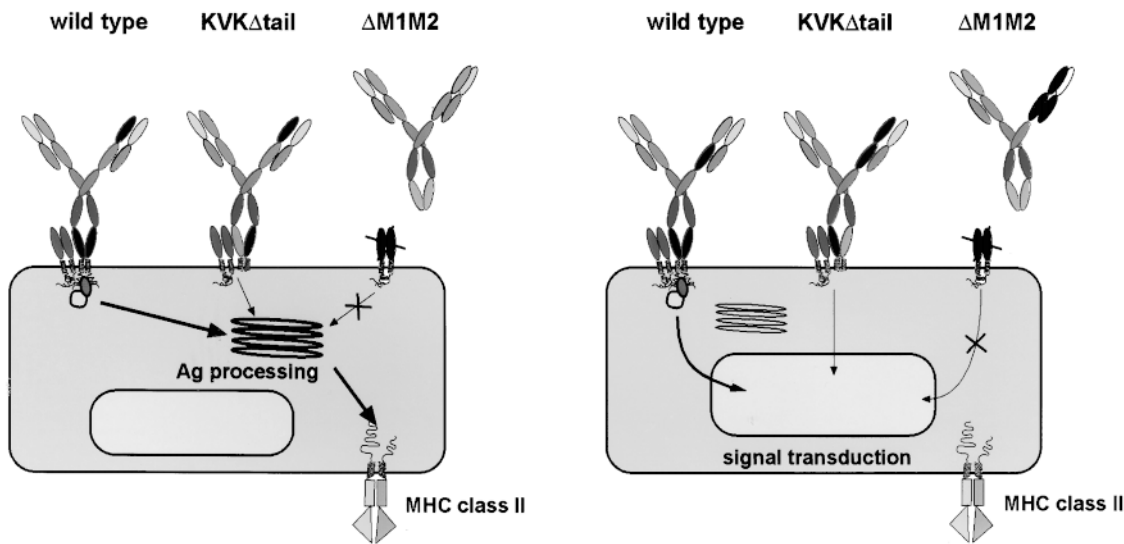
A. Switch Recombination



B. Alternative Polyadenylation



C. Posttranslational Processes



bination. Priming of T cells is not dependent on antigen presentation by B cells, and therefore not dependent on the antigen-focusing capacity of the B cell receptor (BCR). 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 (13) and are essential for the initiation of the switch recombination. Switch to IgE is induced by IL-4 (26) and IL-13 (27) and inhibited by IFN- γ (28). Binding of IL-4 to its receptor causes the activation of the signal transducer and activator of transcription 6 (STAT-6) and its translocation to the nucleus (29), where it binds to an IL-4-responsive element in the promoter region 5' of the $I\epsilon$ exon (30). Switch to IgE is also induced *in vitro* by lipopolysaccharides (LPS) and IL-4 (31). 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 an intracellular effector pathway: they both release the transcription factor NF- κ B from its inhibitor (I- κ B) in the cytoplasm and induce the translocation to the nucleus of functionally competent NF- κ B (33). Among other activities, NF- κ B binds to the Sy1 region and can cooperate with STAT-6 in the induction of germ line epsilon transcripts (29, 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, such as 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 for the efficiency of the recombination process and for the survival of the cell during switch recombination and therefore for the number of cells that will be recruited in an IgE-mediated immune response.

As mentioned previously, immunoglobulins are found as secreted products and as membrane-bound receptors. How is the expression of the two different forms regulated? The constant parts of Ig heavy chains are encoded by five (IgG, IgD and IgA) or six (IgM and IgE) exons, of which always the last two exons encode the transmembrane and cytoplasmic domains. A 3' "external" polyadenylation site is found downstream of these exons. The exon that 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 site, which is used when mRNA for secreted Ig is made (Figure 1B). Therefore, the production of the two types of RNA is determined by alternative splicing or, rather, by alternative polyadenylation (for reviews on this matter we refer the reader to References 35 and 36). The ratio between the amount of secreted versus the amount of membrane-bound Ig that is 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 in activated lymphoblasts and plasma cells more mRNA for secretory Ig can be found. This effect seems to be correlated with, but not absolutely dependent on, a rise in concentration of a 64-kD subunit of the cleavage stimulatory factor (CstF) (37) and an increase in the stability of the mRNA. These results were obtained in experiments with IgM-, IgG-, or IgA-producing B cells and B cell lines. Whether the regulation of splicing for the IgE heavy chain mRNA is similar to that described for IgM, IgG, or 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 group of Burrone (38, 39) has drawn attention to unconventional polyadenylation events taking place during the formation of the membrane form of ϵ mRNA. However, the polyadenylation signals they have identified (AGTAAA and AAGAAA) are very much in disagreement with consensus polyadenylation signals (40). By extending the sequence analysis into the ϵ - α intragenic region we have identified a consensus ATTAAA polyadenylation site about 1.6 kb 3' of the stop codon in exon 6 of the ϵ gene (our unpublished observations, 1999; see also Reference 41). Factors that influence alternative polyadenylation and the stability of the different mRNA species, in particular those for IgE, are largely unknown. 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 switch recombination to IgG₁ and IgE in mice (or IgG₄ and IgE in humans) seems to depend on similar stimuli, yet the frequency of cells that can switch to IgE *in vitro* is 15-fold lower than the frequency of cells that have switched to IgG₁ (42). When we look *in vivo* at a secondary immune response, a further loss is seen: The number of IgE-producing cells is 500-fold lower than the number of IgG₁-producing cells (Reference 9; and G. Achatz, unpublished observations, 1999). Of course, processes other than those involving transcription, splicing, and translation could be involved. It is fully conceivable that cooperation with other cellular proteins, or signaling or transport processes, play a role in the success with which an individual IgE-producing cell can be recruited in the pool of antibody-secreting cells (see Figure 1C).

CONCLUSION

The following conclusions can be drawn.

1. The molecular events leading to switch recombination are complex, and form a potential hazard for the switching cell.
2. Transmembrane and cytoplasmic domains of mIgE are indispensable for an effective recruitment of IgE-producing cells in the immune response and for the quality of the immune response.
3. The regulation of splicing events is an important factor in determining the expression of mIgE.
4. The recruitment of IgE-producing cells in the immune response is far lower than that of IgG₁-producing cells. It is

Figure 1. Schematic representation of events leading to the expression of membrane IgE (mIgE). (A) Switch recombination. Receptors for the main stimulatory cytokines are depicted together with the transcription factors that are translocated from the cytoplasm to the nucleus after cytokine binding. Inhibitory cytokines are also depicted. (B) mRNA transcripts that can be generated from the epsilon locus after switch recombination. (C) Possible functions of mIgE. To explain the phenotype of the mutant mice described by Achatz and coworkers (9), we hypothesize that additional molecules interact with the intracellular domain of mIgE.

not clear whether this reflects a tighter regulation or a less efficient switch recombination or polyadenylation process.

5. It is not inconceivable that also posttranslational processes may influence the expression of membrane-bound IgE.

The consequences of a dysregulated IgE secretion, that is, allergic reactions, warrant an in-depth study of all processes that lead to IgE secretion.

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DISCUSSION

Renz: What factors determine whether a B cell makes IgG₄ in the human (or IgG₁ in the mouse), or, alternatively, IgE? As you said, IL-4 can induce the switching to both.

Lamers: The regulation is apparently on the level of transcription of the IgE locus. We only have the final readout, the cells that make the switch. About 1% of all B cells make the switch

and produce the secreted form of IgE. The switch recombination is very ineffective in the IgE locus and more effective in the IgG₁ locus. This may be because the recombination event itself is more effective or the repair event is more effective. The dose of IL-4 may have an effect, but when we do the experiment, we flood the cells with IL-4. One thing is very important and we never looked at that *in vitro*, something that you can summarize as “parallel input.” When we do the experiments *in vitro*, we give IL-4 and LPS. We know this activates Stat-6 and NF- κ B. When you do these experiments *in vivo*, we say: CD40 and IL-4, but that is really a simplification, because it normally happens in a germinal center, where there is input from everywhere. For instance, NF- κ B induction is not activated only by CD40, it is activated by oxygen radicals, by the TNF receptor, the IL-1 receptor, etc.; we do not have a clue what is really happening there or what the other signal transduction pathways are doing. We know that MAP kinases are involved, but how this all is integrated, I don’t think anybody knows.

Vercelli: We will have to reconsider the way we look at IgE regulation. We have been focusing very much on IL-4 and how it induces germ line transcripts and how that works. That is essential, there is no question. But it is also very clear that this does not exhaust the possibilities. There must be other levels of control post germ line transcription. Alternative splicing is probably one. About how IgG₁ and IgG₄ relate, respectively, to IgE in mice and man, I am not sure how well we can compare that, simply because IgG₁ and IgG₄ don’t have relative to IgE the same position in the locus. It is also possible that there are position effects that govern long-range DNA-protein interactions. I wonder, for instance, whether long-range interactions with the three enhancers in the human locus work with these different loci. Qualitative, yes, but quantitative, I am not sure.

Lamers: There is now a knock-in of ϵ into the γ 1 locus in the mouse. It was not well conceived, because they have taken only the coding part without all the regulatory parts, so it will probably behave like γ 1. If you want to do it in a constructive way, you would have to take it part by part, first only the 5’ end, then the 5’ end plus the coding regions, then the same plus the 3’ end and do some more; that is not something a small laboratory can do, but that would solve this question once and for all.

Aalberse: Data now available, including your data, indicate that IgE-producing B cells do not seem to proliferate much, or perhaps don’t proliferate at all. So, after the switch IgE-producing B cells are different from other B cells. A B cell has to survive, and you indicated that that was one problem: Some B cells don’t survive and of course then they disappear; they may differentiate or proliferate; the differentiation may go into different directions. They may develop into short-lived plasma cells or into long-lived plasma cells. Your *in vivo* model would enable you to distinguish these possibilities. Our chairman has detailed information about short-lived and long-lived plasma cells. Do you have any information? Do these constructs make a difference in this respect?

Lamers: IgE responses in mice are usually extremely short-lived, so the induction of long-lived plasma cells is probably not very effective. That is a very good point. Your point about

the clone size of the IgE-producing B cells is difficult to study, because of the extremely low number of IgE-producing cells. We may now have a strategy to study this. We glued the green fluorescent protein to the cytoplasmic tail of IgE, which would allow us to get one single cell out of a mouse, then adoptively transfer it to another mouse. So, we may have a model where we can follow individual IgE-positive B cells and assess their proliferative potential.

Savelkoul: You said that when you prevent the membrane expression of IgE, you interfere not only with the IgE production, but also with the affinity maturation of IgE. We know that the IgE isotype is not particularly good in affinity maturation, possibly related to the fact that the B cells, once they start making IgE, probably do not proliferate very much. A long time ago we estimated the clonal size to be in the order of 32 cells. I would like to tickle your brain about sequential switching. You find normal IgG₁ production, despite the blockade in subsequent switching to IgE. The situation in mice seems to be different from that in the human, where there seems to be more direct switching from IgM to IgE. Another point I would like to raise is the distribution of the IgE-producing cells. You have focused on the spleen, but we know that the peripheral lymph nodes end up with 10,000 specific IgE-producing cells, which are short-lived. In addition, you will find a few, not many, long-lived IgE plasma cells in the bone marrow.

Lamers: The point about the spleen and the lymph nodes is well taken. The only reason why we have taken the spleen is to have an organ where we can count reproducibly. If you take the lymph nodes, it is always dependent on how reproducibly the mouse was immunized in the local area; this may bias your experiment and you would need many mice to get reproducible results. I don’t give much for the sequential switch. When γ 1 switching is prevented, the switch to IgE is more prominent. At best you can say that γ 1 takes B cells away from the switch to IgE. As far as affinity maturation is concerned, IgE seems to be very different from γ 1. But these are complicated studies since the number of IgE-producing cells is so low that you have to sort many cells to get sufficient RNA to do these experiments.

Holt: May I make one comment on these long-lived plasma cells? There are in fact a number of immunization protocols in the mouse where you can elicit high numbers of strongly radiation-resistant long-lived E-producing plasma cells in bone marrow, and these have never been really studied. With the technology that is available now it may very well be worthwhile to go back and reexamine these as a separate entity, because I believe they really do exist.

Daha: I wonder whether you had a chance to see whether these mice, especially the tail KOs, give any type of pathology following OVA sensitization.

Lamers: We have not looked at pathology. There are the total IgE KOs discussed in the first presentation, which indicated that if you don’t have IgE, you still may have pathology. What we are interested in in the partial tail KOs is affinity maturation, because that is likely to influence the outcome of certain effects. If you want to find out what IgE really is for, you have to find a disease where skin infiltration is important. The model we are currently looking at is *Borrelia* infections.