

Immunoglobulin E regulation in humans, 1989–1994

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In 1989, when I had the privilege of receiving one of the Pharmacia Allergy Research Foundation awards, our group was just working out a new model for the induction of immunoglobulin E (IgE) synthesis in humans (22). According to this model, induction of IgE synthesis would always require 2 signals, one delivered by the cytokine interleukin 4 (IL-4) and the other by contact-dependent T- and B-cell interactions. Five years later, the two-signal model for IgE induction is indeed alive and well, and the underlying events are being characterized at the molecular level in more and more detail. I briefly review the major advances made in the past 5 years in the understanding of the signals required for the triggering of an IgE response.

CD40/CD40 ligand interactions play a crucial role in delivering the second (B cell-activating) signal

The role of CD40 in the induction of IgE synthesis was initially established in T cell-independent systems by demonstrating that anti-CD40 monoclonal antibody (mAb) and IL-4 synergize in the induction of IgE synthesis by highly purified human sIgE⁺ B cells (8). CD40 belongs to a newly defined family of cysteine-rich transmembrane receptors, which includes the receptor for nerve growth factor (NGF) and tumor necrosis factor α (TNF- α), CD27, CD30 and the *fas* antigen. This suggests that CD40 may be involved in the regulation of B-cell activation, growth and survival.

Progress in the understanding of the role of CD40-mediated interactions in IgE regulation was made with the cloning of the murine and human ligand for CD40 (1, 20). CD40 ligand is a type 2 membrane glycoprotein homologous to NGF, TNF- α and TNF- β , which is transiently expressed by T cells following activation. Cells transfected with this molecule are able to induce IgE synthesis, in the presence of IL-4, in both murine and human B cells. In other experiments, a soluble CD40-IgG fusion protein inhibited IL-4-dependent IgE synthesis in human mononuclear cells (5). Thus, CD40/ligand interactions are critical in delivering the second signal required for IgE production.

Molecular events in the two-signal model of IgE induction: IL-4 induces ϵ germline transcripts, whereas the B cell-activating signal induces DNA switch recombination

Molecular analysis has shown that induction of switching to a particular CH gene almost invariably correlates with the transcriptional activation of the same gene, in its germline configuration. The germline transcripts that result from this process initiate a few kb upstream of the switch (S) region, and proceed through one or more short exons (I exons) that are spliced to the first exon of the CH gene. Germline transcripts are unable to code for any mature protein of significant length, because the I exon contains multiple stop codons in all 3 reading frames. Therefore, germline transcripts are also referred to

as "sterile" transcripts. Alternatively, germline transcripts are referred to as "truncated" transcripts, because the I exon is usually 200–300 base pairs (bp) shorter than the VDJ exon present in mature transcripts. Although there is no significant conservation of the sequences of different germline CH transcripts, their overall structure is conserved.

The relationship between germline transcription units and regulation of isotype switching has been confirmed by gene knock-out experiments. Deletion of the I γ 1 (10) or I γ 2 (24) exons and their promoter resulted in inhibition of class switching to the corresponding genes. However, these experiments, while proving that the I γ regions are important for switching, did not establish the role of germline transcription and/or germline transcripts in regulating class switching.

To this purpose, the I ϵ region and its promoter were replaced with a B cell-specific promoter cassette containing the E μ intronic enhancer and a VH promoter, and this construct was introduced in embryonic stem (ES) cells. Heterozygous and homozygous mutant ES cells were then assayed in the RAG-2-deficient blastocyst complementation system (3). In this approach, mutated ES cells are injected into blastocysts from RAG-2 deficient mice, which are not capable of developing into mice that produce mature T and B cells. Therefore, in the resulting chimeras, the entire peripheral blood B and T cell repertoire is derived from the injected ES cells (3). Analysis of isotype switching in the mutant B lymphocytes that develop in this system showed substantial levels of transcription through the S ϵ region, even in the absence of I region sequences. These findings supported a model of class switch recombination in which transcription generates baseline levels of isotype switching in the absence of I region sequences. However, the optimal efficiency of the process requires the presence of the intact I region and/or I region promoter *in cis* (2). Therefore, although transcription through the S region may be a requirement for class switching, additional factors regulate the efficiency of the process.

A novel regulatory region has been recently identified that controls Ig heavy chain class switching. A segment of DNA approximately 15 kb downstream of the most 3' CH gene (C α in the mouse) was found to have weak transcriptional enhancing activity (15). Based on its location, a possible role of this element (termed the 3' Enhancer: 3'EH) in the control of isotype switching was considered. This hypothesis was tested by replacing a 5-kb segment of DNA spanning this enhancer with a neomycin resistance gene-targeted mutation in ES cells (4). Both 3'EH +/– and 3'EH –/– ES cells generated normal populations of splenic B cells following RAG-2 deficient blastocyst complementation. Analyses of

mutant pre-B cell lines and splenic B cells indicated that homozygous deletion of the 3'EH had no effect on VDJ recombination or generation of IgM + B cells. The 3'EH +/– cells were normal with respect to proliferation, switching and secretion of various immunoglobulin heavy chain isotypes following stimulation with lipopolysaccharide (LPS) and cytokines. Activated splenic B cells from homozygous mutant ES cells also proliferated normally and secreted normal levels of IgM. However, the homozygous mutant cells were severely deficient in IgG2a, IgG2b, IgG3 and IgE secretion, depressed in IgA secretion but normal for IgG1 secretion. *In vivo*, chimeras generated from homozygous mutant ES cells were severely deficient in serum IgG2a and IgG3 levels but had relatively normal levels of IgM, IgG1, IgG2b and IgA, suggesting the existence of alternative pathways for activation of class switching to IgG2b, and perhaps IgA. In all cases, failure to secrete particular heavy chain isotypes could be ascribed to the failure to induce germline transcription of those genes and, as a result, the failure to undergo class switch recombination to those genes (4).

These findings indicate that homozygous deletion of the 3'EH locus results in a general defect in the ability to induce germline transcripts and switch to all CH genes, except γ 1. Thus, sequences within the IgH 3' enhancer deletion are essential for regulating germline transcription and class switch recombination of 5 different CH genes spread over a 120-kb locus. Based on these data, it is thought that sequences within the 3'EH deletion may be an essential part of a locus control region that regulates LPS-stimulated germline transcription and/or accessibility of downstream CH genes. Finally, the parallels between the phenotype of the 3'EH mutants and selective isotype deficient humans is notable, suggesting that further studies of this region may also yield insight into such diseases.

Although IL-4 is by itself sufficient for the initiation of transcription through the ϵ locus, switch recombination and expression of mature C ϵ transcripts (containing VDJ spliced to C ϵ 1-4) require a second signal, such as T/B cell interactions via T cell receptor/MHC class II or CD40/CD40 ligand, transformation with Epstein-Barr virus (EBV) and glucocorticoids (21). DNA switch recombination events underlying IgE isotype switching have been demonstrated using nested primer polymerase chain reaction (PCR) amplification of ϵ switch fragments, followed by cloning and DNA sequencing (9, 18, 19). S μ /S ϵ switch fragments were amplified from B cells stimulated with EBV, anti-CD40 mAb, or hydrocortisone, in the presence of IL-4, but not from B cells stimulated with either signal alone. DNA sequencing revealed that deletional switch recombination had occurred with direct joining of S μ to S ϵ

in all fragments derived from B cells stimulated with IL-4 + EBV, and IL-4 + anti-CD40 mAb, and in 8 of 10 fragments from B cells stimulated with IL-4 + hydrocortisone. The recombination sites within S μ were clustered within 900 bp at the 5'-end of the S region, suggesting that there are hot spots for recombination within S μ . In contrast, the S ϵ recombination sites were scattered throughout the S ϵ region.

Switching from IgM to IgE can occur sequentially

Two switch fragments amplified from B cells stimulated with IL-4 and hydrocortisone contained insertions at the S μ /S ϵ junction that were derived from S γ 4 (9). The presence of an S γ 4-derived insertion at the S μ /S ϵ junction in one, and possibly two, of the sequenced fragments suggests that some B cells might have undergone sequential isotype switching from IgM to IgG4 to IgE. This is consistent with the capacity of IL-4 to induce isotype switching to IgG4, as well as to IgE, and may underlie the finding that single B cells can give rise to clones that secrete IgM and IgG4, as well as IgE (6). Evidence for sequential switching to IgE has been obtained in B cells from mice infected with *Nippostrongylus brasiliensis*, as demonstrated by the isolation of ϵ circles, which were recombinant between C γ 1 and C ϵ (23). More recently, Mills et al. (14) have demonstrated that PCR-amplified fragments of S μ /S ϵ junctions from B cells treated with EBV and IL-4 and from patients with hyper-IgE states hybridize with S γ probes.

The presence of direct S μ /S ϵ joining does not rule out the possibility that sequential switching might have previously occurred. A B cell that first switches to IgG4 and then to IgE would lose S γ 4-derived sequences if the second switch recombination event again involves the S μ rather than the S γ 4 region. Alternatively, nonsequential IgE switching in B cells that receive EBV or anti-CD40 mAb as second signals might reflect a different mode of activation of the recombination machinery compared with hydrocortisone.

Consecutive and IL-4-induced nuclear factors bind to the I ϵ region and are responsible for the induction of ϵ germline transcripts

Expression of ϵ germline transcripts is thought to be regulated by nuclear factors that bind to the I ϵ promoter and adjacent regions. Among the DNA binding proteins involved in ϵ germline transcription identified so far, some are constitutively expressed,

whereas others are induced by specific cytokines. The specificity in the induction of nuclear factors provides a molecular basis for the specificity of cytokine-directed isotype switching. It should be stressed that multiple nuclear factors, often combined to form supramolecular complexes, may potentially bind to the same region, such as upstream to the I ϵ promoter. Thus, the transcriptional state at each point in time depends on the protein binding pattern, i.e., on which nuclear factor(s) are bound to which DNA sequence(s).

In the mouse, an LPS- and IL-4-responsive element is located in a 179-bp segment of DNA that spans the I ϵ transcription initiation site. This element confers LPS- and IL-4-inducible expression to a heterologous reporter gene (16). Mutational analysis demonstrated that this expression depended on DNA sequences contained within a highly conserved region directly upstream from the I ϵ transcription initiation site. BSAP, a nuclear protein that is constitutively expressed in normal B cells, binds to the downstream end of the conserved sequence and is essential for ϵ germline transcription (13). Additional proteins (NF-BRE, HMG-I(Y) and STF-IL-4) that are specifically induced and/or activated by IL-4 bind close to the I ϵ transcription initiation site (11, 16, 17).

In humans, two contiguous IL-4 responsive elements have been identified in lymphoblastoid B cells, both of which are located upstream of the I ϵ exon, in the region of high human and mouse homology, but at different sites (7, 12). I have shown that a nuclear factor present in nuclear extracts of IL-4-stimulated normal B cells binds to the *Sma*I-*Sac*I region upstream of the initiation sites for ϵ germline transcripts (Vercelli D, unpublished observation). It remains to be established whether this nuclear factor is the human equivalent of murine BSAP. Interestingly, a nuclear DNA-binding protein, NFIL-4, has been recently identified in B cells and monocytes, which specifically interacts with an IL-4 responsive element 5' to the human I ϵ exon, as well as with a 9-bp binding motif in the promoter of CD23, another IL-4-regulated gene (12). The presence of homologous responsive elements 5' to different genes provides the mechanism for the concerted regulation of multiple genes by a single cytokine.

Conclusion

At present, in no other system are the signals required for isotype switching better understood than in the IgE system. IgE switching is therefore becoming a general model for "directed" isotype switching in mice and humans (to the unrestrained satisfaction of all IgE hackers like me).

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