

Molecular regulation of IgE switching: let's walk hand in hand

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In contributing to a *Festschrift* to honor the 30th anniversary of the discovery of IgE, one would like to start by asking some of the great questions that the discovery of our favorite molecule has raised. Perhaps the biggest question of them all is, why does our body take the trouble to produce IgE in the first place? Actually, most researchers interested in IgE and IgE regulation think they know the answer; i.e., IgE is there to protect creatures originally surfaced in parasite-infested Africa. However, I, for one, am not so sure that this answer is adequate, for reasons that have much to do with my own skepticism about biologic explanations based on finalism and optimality, and even more with some of the most recent work of our laboratory on the coregulation of switching to IgE and IgG4 (1).

Thus, I would rather walk on firmer ground and address an issue which we can now tackle with much understanding, even though the very richness and complexity of the issue itself tells us that there are more layers, more facets to it than we imagined at first glance. What I would like to discuss here is, not why, but how is it that a B cell decides to produce IgE rather than any other isotype in the immunoglobulin locus. Interestingly enough, the work done to define the mechanisms responsible for the choice of the IgE isotype has been instrumental in shedding light not only on the commitment to IgE but, much more generally, on the very process of switching.

One way to look at it is that the reason why a B cell with an allergen-specific immunoglobulin receptor switches to IgE is that it finds itself

interacting with an allergen-specific T cell that is capable of producing interleukin (IL)-4 or IL-13 upon full activation. It became clear very early that without IL-4 (or possibly IL-13) no substantial IgE synthesis is possible (2, 3), even though some murine knockout models are somewhat leaky in this respect (4). Thus, there is a T-cell-centric view of IgE isotype switching that focuses on the Th1/Th2 paradigm, the properties that make an antigen an allergen, and the stimuli required to start IL-4 production. And there is a B-cell-centric view of IgE regulation that seeks to understand how a B cell needs to be stimulated to decide in favor of IgE. This is the view I subscribe to, and this is the tack I am going to take.

It is well known that during an immune response, a B cell can express different immunoglobulin heavy-chain isotypes sharing the same variable region. This process (isotype switching) allows a single B-cell clone to produce antibodies with the same fine specificity, but different effector functions. More work than one could possibly quote (reviewed by Vercelli [5]) has shown that, in order to switch to a particular isotype, a B cell needs two signals. Signal 1 is cytokine-dependent, results in the activation of transcription at a specific region in the immunoglobulin locus, and thus dictates isotype specificity. Signal 2 is delivered by CD40 engagement, activates the recombination machinery, and results in DNA switch recombination.

The signals required for isotype switching are provided to the B cell by a complex series of interactions with an antigen (allergen)-specific T cell. A

B cell that expresses IgM specific for the allergen binds the allergen via surface immunoglobulins, processes it, and presents it to an allergen-specific, Th2-like T cell; i.e., a T cell programmed to secrete IL-4, but not interferon-gamma.

Engagement of the T-cell receptor/CD3 complex by MHC class II molecules results in the rapid expression of the CD40 ligand (CD40L), which engages CD40, the counterreceptor constitutively expressed on B cells. T/B cell interactions mediated via CD40/CD40L are amplified by interactions between accessory molecules, particularly the CD28/B7 ligand/receptor pair. Engagement of CD40 results in the expression of B7 molecules on B cells. B7 engages CD28, inducing high-rate secretion of IL-4. IL-4 binds the heterodimeric IL-4 receptor (IL-4R), which consists of an α chain (shared with the IL-13R) and a γ chain (shared with the receptors for IL-2, IL-7, IL-9, and IL-15) (6). At this stage, the B cell is receiving both signals required for IgE switching: IL-4 triggers ϵ germline transcription, thereby targeting the ϵ switch region for recombination. Cross-linking of CD40 by CD40L activates DNA recombination to the targeted ϵ switch region, leading to IgE isotype switching and IgE secretion.

In molecular terms, isotype switching results from a DNA recombination event that juxtaposes different downstream constant heavy chain (C_H) genes to the expressed VDJ gene. Isotype switching is not a random event, but is directed by cytokines in conjunction with the regulation of B-cell proliferation and differentiation. Molecular analysis has shown that cytokine-dependent induction of isotype switching to a particular C_H gene almost invariably correlates with the transcriptional activation of the same gene in germline configuration, leading to the expression of germline transcripts (reviewed by Stavnezer [7]). These transcripts begin from TATAA-less promoters a few kilobases upstream of the switch (S) region, and proceed through short exons (I_H exons), the S regions, and C_H exons. The I_H exon is then spliced to the first exon of the C_H gene. Induction of correctly spliced transcripts is thought to be necessary to target the appropriate switch region for recombination and switching (8). During recombination, the region containing the germline promoter and the I_H exon is deleted as part of a switch circle (9).

Although cytokines (IL-4, IL-13) are sufficient for the initiation of transcription through the ϵ locus, a second signal (engagement of CD40 by CD40L) (10) is required for the activation of switch recombination (11). The recombination machinery – a complex apparatus that includes RAG-like proteins (12), DNA-dependent protein kinase (13), and Ku80 (14) – is thought to be the same for all

isotypes, and is targeted to the appropriate S region because the latter is made accessible by germline transcription. DNA rearrangement at the targeted S region generates chimeric $S\mu/S\epsilon$ regions (composed of the 5' $S\mu$ joined to the 3' portion of the targeted $S\epsilon$ region) (15–17) and switch circles, their reciprocal products, that are rapidly degraded.

Because transcription through the I_H exon and the S region seems to be required to target the appropriate S region for recombination and switching, the induction of germline transcripts is a key step in determining the isotype specificity of the switching event. Different cytokines specifically induce different nuclear factors that activate transcription at the appropriate germline promoter. A number of transcription factors have been identified that control the activation of the human ϵ germline promoter in response to signals (IL-4, CD40 cross-linking) delivered at the B-cell surface.

The picture that is emerging from our studies on the transcriptional regulation of the human ϵ germline promoter is one of great complexity – in fact, even greater than we initially imagined, and such that our long-held concept of how the signals needed for switching cooperate in order for the event to occur has begun to look simplistic. Indeed, we have identified at least four binding sites in the promoter that are essential for its activity: one for the IL-4-inducible protein Stat6, two for NF- κ B (18), and one for a B-cell-specific, constitutively expressed factor, B-cell-specific activator protein (BSAP) (19, 20). While the work on the binding of Stat6 and its effects is still ongoing, the situation is already quite clear as far as NF- κ B and BSAP are concerned. What is surprising is that both factors turn out to be necessary not only for the response to CD40 engagement (NF- κ B is known to be activated via the CD40 pathway, and BSAP binds to the promoter very close to the downstream NF- κ B site and may well interact directly with the NF- κ B protein), but also for the response to IL-4 alone. Mutation of the upstream NF- κ B site reduces IL-4-dependent activation of an ϵ germline reporter vector by $\approx 60\%$, whereas mutation of the site for BSAP inhibits transcription by 50% (18). The simultaneous mutation of both the NF- κ B and the BSAP binding sites kills the promoter, which becomes completely unresponsive.

The reason why these results are surprising is that neither NF- κ B nor BSAP has ever been reported to be involved in IL-4 responses, which are classically thought to be mediated mostly through Stat6 (21–23). Here, an IL-4-responsive promoter is wiped out without interfering (at least, *prima facie*) with Stat6 binding. One reasonable way to explain these findings is to assume that these factors interact directly with Stat6. Of course, the

ϵ promoter, like all immunoglobulin germline promoters in both man and mice, is TATAA-less and is therefore typically one of those promoters whose activation depends on strict cooperation between different transcription factors recruited through different pathways. All slots need to be filled for the gene to fire, as the saying goes. Nonetheless, these findings are intriguing in that they seem to suggest that our concept of the mechanisms underlying the synergism between the IL-4- and the CD40-delivered signals is plainly inadequate. In most current models (alas, including our own), *first* a B cell sees IL-4 and starts expressing ϵ germline transcripts, and *then* CD40 gets engaged, upregulates transcription, makes targeting more efficient, and finally activates the recombination machinery triggering switch recombination. If we look at transcription factors, the same set of proteins seems to be necessary to mediate the effects of both signals. Thus, these two pathways are more intimately intertwined and their synergism goes much deeper than we initially postulated. It will be interesting to see what our current analysis of the role of Stat6 will add to this already very complex picture. In any case, it is clear that, at the molecular level, the signals delivered by IL-4 and CD40 cross-linking walk hand in hand all the way through.

Acknowledgments

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