

Regulation of the IgE isotype switch: new insights on cytokine signals and the functions of ϵ germline transcripts

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In allergic responses, B cells are driven to undergo an immunoglobulin isotype switch, shifting from IgM to IgE synthesis. This process involves the rearrangement of germline DNA in the immunoglobulin heavy-chain locus and is stimulated by cytokines (IL-4 and IL-13) and CD40 activation. It is now evident that cytokine-induced 'germline' ϵ -RNA transcripts associate with DNA in the genomic switch region (S ϵ) to form DNA–RNA hybrid structures, which target nucleases in for deletional switch recombination. Alterations in cytokine production and signaling affect the efficiency of this process and are associated with inherited predisposition to allergy.

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Current Opinion in Immunology 2000, 12:618–623

0952-7915/00/\$ – see front matter
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Abbreviations

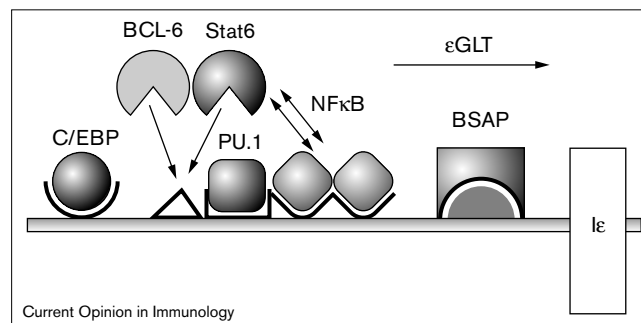
AID activation-induced cytidine deaminase
IL-4R α IL-4-receptor α chain

Introduction

The immunoglobulin isotype IgE is best known for its ability to activate mast cells via the high-affinity IgE receptor, Fc ϵ RI, thereby triggering immediate-hypersensitivity reactions. Additional functions for IgE have been identified, including the elimination of helminthic parasites and the regulation of its own receptors, Fc ϵ RI and CD23 [1]. Recently it has been established that allergen-specific IgE can facilitate antigen capture by B cells — via the low-affinity IgE receptor, CD23 — and that these B cells can serve as antigen-presenting cells, leading to augmented immune responses [2,3 $\bullet$]. In light of the pleiotropic functions of IgE in allergy, much effort has been focused on defining the mechanisms governing its production.

Initially, all B cells produce IgM antibodies. The V, D and J elements encoding the variable-region domains of the μ heavy chain are located adjacent to the C μ exons that encode the IgM C-regions at the 5' end of the immunoglobulin heavy-chain (IgH) locus. Following appropriate stimulation, B cells can alter the isotype of the antibodies they produce while retaining their antigenic specificity. In this process, genomic DNA is spliced and rejoined to juxtapose the VDJ elements to the C-region exons that encode the γ , ϵ and α chains of IgG, IgE and IgA isotypes, respectively; these C-region exons are located further downstream in the IgH region [4]. Intervening DNA is excised as the VDJ and a downstream

Figure 1



Structure of the I ϵ promoter. IgE class-switch recombination is invariably preceded by the transcription of ϵ germline transcripts (ϵ GLT) originating at the IL-4-responsive I ϵ promoter. The promoter contains sites for C/EBP, Stat6, PU.1, NF κ B (two sites) and BSAP (Pax5). In addition to binding adjacent promoter elements, Stat6 and NF κ B (PU.1 can also synergize with Stat6 but this is not shown) interact physically and affect transcription synergistically. The repressor, BCL-6, competes with Stat6 for promoter binding and can attenuate I ϵ transcription.

C sequence are joined, so the mechanism is referred to as deletional switch recombination.

In the case of IgE, two types of signals induce this process: the first is provided by cytokines (IL-4 and IL-13); the second is provided by activation of CD40 on B cells [5]. Recent studies, to be reviewed here, have shed light on the molecular mechanisms whereby cytokine-induced gene transcription precisely directs the cutting and pasting of germline DNA. In addition, ongoing genetic analyses have established that polymorphisms affecting the efficiency of this cytokine signaling can contribute to the predisposition to atopy.

The regulation of ϵ -chain germline transcription

The binding of IL-4 or IL-13 to their receptors on B cells leads to the activation of the transcription factor Stat6 and the induction of 'germline' transcription at the C ϵ locus. Germline transcripts originate from a promoter 5' of the I ϵ exon, which is located just upstream of the four C ϵ exons [6]. Mature germline mRNAs include the 140-basepair I ϵ exon and exons C ϵ 1–C ϵ 4 and have been referred to as 'sterile' because of the presence of stop codons in I ϵ [7]. The I ϵ promoter contains binding sites for several transcription factors, including Stat6, NF κ B (two sites), BSAP (Pax5) and C/EBP (Figure 1). Although activated Stat6 is clearly the critical regulator in C ϵ germline transcription, mutational analyses of the promoter have demonstrated that the NF κ B and BSAP elements must also be present for normal function (see Figure 1) [8–10]. Stat6 and NF κ B

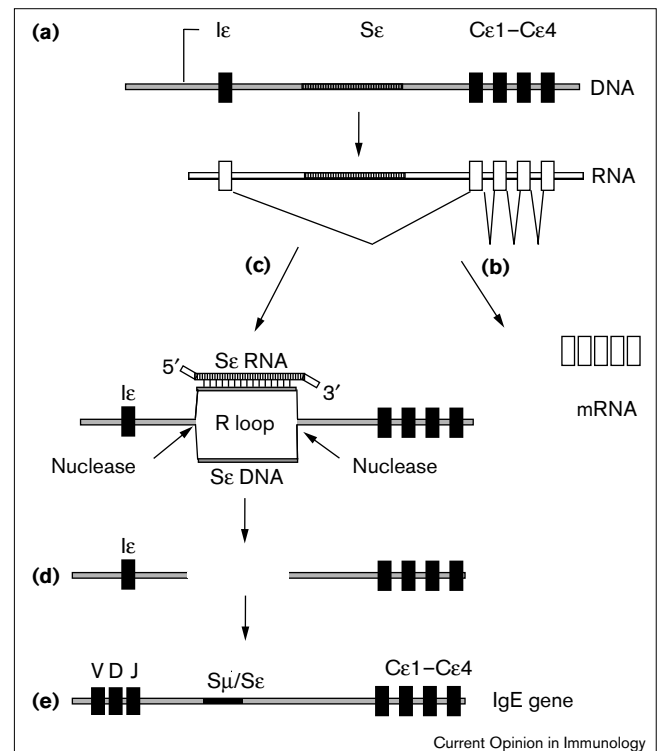
actually synergize in activating the promoter and this may be related to a physical interaction [11]. Isotype switching is impaired in NF κ B-p50^{-/-} mice [12]. Qiu and Stavnezer [13] have shown that BSAP overexpression will drive I ϵ transcription and promote IgE isotype switching. In the past year, a PU.1-binding element overlapping the distal NF κ B site has been characterized as well [14*]. Like NF κ B, PU.1 can also synergize with Stat6 in activating the promoter; Stütz and Woisetschlager [14*] report that either PU.1 or NF κ B function suffices to support Stat6-driven ϵ -germline transcription.

Some interesting recent observations have identified an important negative regulator of the I ϵ promoter and germline transcription. BCL-6, a POZ/zinc-finger transcription factor expressed in B cells, has a DNA target site similar to that of Stat6. Rothman and co-workers [15**] recently demonstrated that BCL-6 can bind to Stat6-binding sites and repress the induction of ϵ -chain germline transcripts by IL-4 [15**]. BCL-6^{-/-} mice have enhanced IgE isotype switching whereas BCL-6^{-/-}Stat6^{-/-} animals do not produce IgE, consistent with a model in which BCL-6 can block Stat6-mediated IL-4 activation of the I ϵ promoter. Previously, a restriction fragment length polymorphism (RFLP) had been identified that was significantly associated with atopy (i.e. with high IgE levels and multiple, strongly positive RASTs [these are markers of atopy]) but not with IgE levels alone [16]. This *HindIII* RFLP itself resides in the first intron of the BCL-6 gene but could be linked to mutations that modulate the Stat6-repressor function of the gene. Of course, IL-4-induced Stat6-mediated signaling is involved not only in IgE germline transcription but also in many other proallergic pathways. These include the induction of Th2-cell differentiation, expression of CD23 and upregulation of the adhesion molecule VCAM; thus the function of BCL-6 could exert broad effects on the development of allergic disease.

The function of germline transcription in deletional isotype-switch recombination

Although IL-4-induced ϵ -chain germline RNA invariably appears prior to IgE isotype switching in B cells, the molecular *raison d'être* of germline transcripts has, until recently, remained a mystery. It has been clear for some time that germline transcription is an indispensable part of the isotype switch process. Several groups have shown that targeted disruption of immunoglobulin I exons or their promoters completely blocks isotype switching even if the switch region remains intact [17–20]. Conversely, the insertion of inducible or constitutively active promoters in front of I exons can drive both germline transcription and isotype switching. We have observed (A Kisselgof, HC Oettgen, unpublished data) that transcription that is driven by the PGK-neo promoter at a targeted ϵ locus, in which the C ϵ exons are deleted but S ϵ sequences remain intact, is permissive for IL-4/LPS-induced S μ –S ϵ switch joining. Taken together with the data from the I-region mutants, this suggests that any

Figure 2



Model for germline transcript-mediated class-switch recombination. (a) In response to IL-4 (or IL-13), RNA is transcribed at the germline ϵ heavy-chain locus, originating at the I ϵ promoter. This RNA is spliced to generate germline ϵ -mRNA (b), leaving behind S ϵ RNA, which remains hybridized to one of the strands of S ϵ DNA (c). The resulting 'R loop' structure is a substrate for endogenous nucleases, which introduce double-strand DNA breaks (d). Joining can then occur between breaks upstream of the C ϵ cluster of exons and downstream of the VDJ exons, giving rise to an intact IgE heavy-chain gene (e). The piece of DNA containing I ϵ , as well as all the intervening DNA between S μ and S ϵ is excised, but part of S μ remains.

S ϵ -region-containing transcript might be sufficient to drive deletional switch recombination.

Findings published this year have shed some light on the molecular function of immunoglobulin germline transcripts. Previously, it had been observed — in *in vitro* transcription experiments — that S-region-containing RNAs remain associated with template DNA, forming DNA–RNA hybrids [21–23]. Using an *in vitro* transcription system, Tian and Alt [24*] showed that the S-region RNA and genomic S sequences formed stable 'R loops' — structures in which the S transcript hybridizes to the template DNA — leaving the opposite strand as single-stranded DNA. They further demonstrated that these R loops were susceptible to cleavage by the excision-repair nucleases, XPF-ERCC1 and XPG — endonucleases previously known to target duplex/single-strand junctions [24*]. These findings support a model in which intronic S-region-containing RNA remains associated with the C-rich DNA template following its excision during RNA processing (I ϵ –C ϵ splicing). The

RNA–DNA hybrid then serves as a nuclease substrate, giving rise to double strand DNA breaks which can then be annealed by DNA end joining (see Figure 2).

These studies do not establish that XPF-ERCC1 and XPG are the nucleases relevant to the deletional isotype-switch mechanism; the identity of the DNA-repair enzymes normally utilized by B cells for this process remains to be determined. Stavnezer and co-workers [25•] recently analyzed isotype switching *in vitro* in B cells from mice deficient in several mismatch-repair enzymes and showed 35–75% reduction in switching, depending on the isotype and the defective enzyme. Concurrent studies have shown that the susceptibility of transfected substrates to recombination is restricted to B cell lines in which the heavy-chain locus is susceptible to switch recombination and to LPS-activated primary B cells [26•].

Although many of the enzymes required for switching are likely to be constitutively expressed, there is recent evidence to suggest that some components of the deletional recombination apparatus can be induced by switch stimuli. In fact, it is well known from cellular immunological studies that IL-4-mediated signaling and germline transcription alone are not sufficient to drive deletional switch recombination. A second signal must be provided by CD40 and is likely to induce other critical components of the apparatus that mediates DNA ligation and repair. This concept is supported by the observation that the isotype switching in B cells that is triggered by IL-4 and CD40 ligand is cycloheximide-sensitive, indicating that these signals induce activation-specific proteins important in the process.

Honjo and co-workers [27••] have searched for such inducible components by subtraction of cDNAs derived from resting murine B cells from those exposed to switch stimuli and have identified a cytidine deaminase (activation-induced cytidine deaminase [AID]) that is expressed specifically in activated splenic B cells and in germinal centers. The enzyme has homology to the ApoB mRNA-editing enzyme, APOBEC (see also Update). Thus the data accumulated to date suggest that IL-4- or IL-13-induced germline transcription can generate the DNA–RNA hybrid structures, which direct nucleases to the appropriate heavy-chain S-region sequences. Subsequently, CD40 ligand is obligatory for the execution of the isotype switching and probably operates by inducing additional critical components of the switch recombination machinery.

IL-4, IL-13, their receptors and signal transduction

The central role of stat6-mediated signaling in driving germline transcription — the first signal for isotype switch recombination — would suggest that alterations in the structure or regulation of IL-4 or IL-13, or modifications in receptor structure that affect signaling efficiency, might influence atopy. Important observations on several

polymorphisms in these cytokine loci, as well as in the IL-4-receptor α chain (IL-4R α), and their correlation with atopy have been published in the past year.

Previously it had been established that there are strong linkages of total IgE levels (and other atopic phenotypes) to markers flanking the cytokine cluster on chromosome 5 (5q31). This locus contains the genes encoding IL-4 and IL-13 [28]. Links have been reported between total IgE levels and a polymorphism in intron 3 of the IL-4 gene (in the Japanese population this is linked to asthma as well) [29,30]. In addition, several polymorphisms have been identified in the 5' region of the IL-4 gene. One of these, –590T/C, is associated with high IgE levels as well as enhanced promoter function *in vitro* [31]. This year, polymorphisms that are linked to serum IgE levels have been identified in the IL-13 gene as well [32•]. Four of these polymorphisms are in tight linkage disequilibrium and include substitutions in the promoter region as well as an Arg→Gln substitution near the end of the coding region.

Alterations in IL-4R function, which could also affect the efficiency of delivery of the cytokine signal, have been under scrutiny for a while and have undergone extensive additional analysis this year. This work originated with a report by Hershey *et al.* [33], who identified a polymorphism, Arg551Gln, in IL-4R α . The substitution is associated with decreased binding of the cytosolic phosphatase SHP-1 [33]. This phosphatase dephosphorylates phosphotyrosines and has been implicated in the termination of signaling by cytokine receptors, so that decreased binding to the mutant allele could be associated with enhanced signal transduction. Several subsequent analyses did not confirm this association, however, and recent functional analyses have failed to confirm any effect of the isolated mutation on IL-4-mediated signaling as measured by tyrosine phosphorylation, cell proliferation, CD23 induction and protection from apoptosis [34,35,36•].

As work on the significance of Arg551Gln has progressed, additional IL-4R α polymorphisms have been identified and an extensive analysis of eight IL-4R α polymorphisms and their combined haplotypes was presented this year by Ober *et al.* [37••]. Their study populations included an inbred group, the Hutterites, as well as outbred White, Black and Hispanic individuals. Significant associations were established between IL-4R α alleles (or haplotypes), asthma and atopy both in the Hutterites and in the outbred populations — supporting the idea that variations in this gene influence allergic phenotypes. However, the correlations varied among the populations studied; no single substitution or haplotype was associated with atopy across the populations. This observation suggests that IL-4R α gene variation outside the coding region may also influence disease susceptibility. As with other complex diseases, susceptibility to atopy is probably conferred by the contributions of many individual, low-penetrant, common alleles.

IL-13R α genes are both encoded on the X chromosome. A report this year identified, firstly, a coding-region polymorphism — Gln110Arg, in IL-13R α 1 — that was associated with asthma and, secondly, a 5'-untranslated-region variant associated with IgE levels [38].

Conclusions

The critical role of cytokines in promoting IgE production has long been established. In the past year, the mechanisms whereby IL-4 and IL-13 direct switching to the IgE locus have come into clearer focus. ϵ germline transcripts, whose function was previously unclear, have now been shown to form hybrids with S ϵ genomic DNA at the IgE locus. These structures serve as substrates for nucleases, which then initiate double-stranded DNA breaks in the first step of deletional switch recombination. Alterations in signaling that affect the efficiency of this cytokine-driven process have now been shown to correlate with predisposition to atopic disease.

Cytokines alone, however, are not the only forces driving the IgE switch. Additional signal(s), provided by interactions between CD40 and CD40 ligand, are also absolutely necessary. Future work is likely to provide insights into the mechanisms of collaboration between IL-4 and CD40 signaling in bringing the deletional switch recombination process to completion.

Update

Recently, Honjo and co-workers [39] have generated mice with a targeted deletion of AID. This enzyme had previously been shown to be expressed specifically in B cells exposed to switch stimuli, suggesting a possible role in deletional isotype switching. The new AID $^{-/-}$ mice exhibit a complete block in isotype class switching, with elevated IgM levels and low or absent IgG and IgA. The animals also have decreased somatic V-region hypermutation during active antibody responses. In parallel studies, AID mutations have now been identified in humans with the autosomal recessive form of the hyper-IgM syndrome (HIGM2) [40]. These findings provide strong evidence for a role of AID both in deletional isotype switching and in affinity maturation of antibody responses by somatic hypermutation.

AID has homology to the RNA-editing enzyme, APOBEC. Therefore it is possible that AID is important in the processing of pre-RNA(s) encoding proteins (such as nucleases) that are involved in the mechanisms of switch recombination and hypermutation. Alternatively, AID might mediate the construction of ribozymes with nuclease activity or it could act directly on DNA substrates in the heavy-chain locus.

Acknowledgements

The author would like to thank Raif Geha, Talal Chatila and Donata Vercelli for their helpful comments. HCO is supported by the Pew Scholars Program in the Biomedical Sciences.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
- Oettgen HC, Geha RS: **IgE in asthma and atopy: cellular and molecular connections.** *J Clin Invest* 1999, **104**:829-835.
 - Heyman BT, Gustavsson LS: ***In vivo* enhancement of the specific antibody response via the low-affinity receptor for IgE.** *Eur J Immunol* 1993, **23**:1739-1743.
 - Gustavsson S, Wernersson S, Heyman B: **Restoration of the antibody response to IgE/antigen complexes in CD23 $^{-}$ deficient mice by CD23 $^{+}$ spleen or bone marrow cells.** *J Immunol* 2000, **164**:3990-3995.
- In an elegant series of papers, Heyman and Gustavsson have previously established that passively administered, antigen-specific IgE can augment humoral immune responses to that antigen when administered intravenously. This effect is CD23-dependent and the authors hypothesized that IgE-sensitized CD23 $^{+}$ B cells could take up antigen for processing and presentation. In the current study they demonstrate that transfer of CD23 $^{+}$ B cells to CD23 $^{-/-}$ mice reconstitutes IgE-augmented immune responses. This work further establishes this important immunomodulatory function of IgE.
- Shapira SK, Jabara HH, Thienes CP, Ahern DJ, Vercelli D, Gould HJ, Geha RS: **Deletional switch recombination occurs in interleukin-4-induced isotype switching to IgE expression by human B cells.** *Proc Natl Acad Sci USA* 1991, **88**:7528-7532.
 - Vercelli DH, Jabara K-I, Arai K, Geha RS: **Induction of human IgE synthesis requires interleukin 4 and T/B interactions involving the T cell receptor/CD3 complex and MHC class II antigens.** *J Exp Med* 1989, **169**:1295-1307.
 - Del Prete GE, Maggi P, Parronchi I, Chretien A, Tiri D, Macchia M, Ricci J, Banachereau J, de Vries S, Romagnani: **IL-4 is an essential factor for the IgE synthesis induced *in vitro* by human T cells clones and their supernatants.** *J Immunol* 1988, **140**:4193-4198.
 - Gauchat J-F, Leberman D, Coffman R, Gascan H, de Vries J: **Structure and expression of germline ϵ transcripts in human B cells induced by interleukin 4 to switch to IgE production.** *J Exp Med* 1990, **172**:463-473.
 - Rothman PSC, Li B, Gorham L, Glimcher F, Alt M, Boothby M: **Identification of a conserved lipopolysaccharide-plus-interleukin-4-responsive element located at the promoter of germline epsilon transcripts.** *Mol Cell Biol* 1991, **11**:5551-5561.
 - Thienes CP, De Monte L, Monticelli S, Busslinger M, Gould HJ, Vercelli D: **The transcription factor B cell-specific activator protein (BSAP) enhances both IL-4- and CD40-mediated activation of the human epsilon germline promoter.** *J Immunol* 1997, **158**:5874-5882.
 - Delphin SJ, Stavnezer J: **Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline epsilon promoter: regulation by NF-IL-4, a C/EBP family member and NF-kappa B/p50.** *J Exp Med* 1995, **181**:181-192.
 - Shen CH, Stavnezer J: **Interaction of stat6 and NF-kappa B: direct association and synergistic activation of interleukin-4-induced transcription.** *Mol Cell Biol* 1998, **18**:3395-3404.
 - Sha WC, Liou HC, Tuomanen EI, Baltimore D: **Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses.** *Cell* 1995, **80**:321-330.
 - Qiu G, Stavnezer J: **Overexpression of BSAP/Pax-5 inhibits switching to IgA and enhances switching to IgE in the I.29 mu B cell line.** *J Immunol* 1998, **161**:2906-2918.
 - Stütz AM, Woisetschlager M: **Functional synergism of STAT6 with either NF-kappa B or PU.1 to mediate IL-4-induced activation of IgE germline gene transcription.** *J Immunol* 1999, **163**:4383-4391.
- Previous analyses of the ϵ promoter had established the presence of Stat6, NF κ B, BSAP and C/EBP elements. The authors now demonstrate an additional functional ϵ promoter component, PU.1. The PU.1 site overlaps the distal NF κ B site. The authors report that mutation of the binding sites for either PU.1 or NF κ B alone has no effect on promoter function but that disruption of both abrogates cytokine-mediated inducibility. They conclude that Stat6-driven transcription requires the cooperation of either PU.1 or NF κ B.
- Harris MB, Chang CC, Berton MT, Danial NN, Zhang J, Kuehner D, Ye BH, Kvatyuk M, Pandolfi PP, Cattoretti G *et al.*: **Transcriptional repression of stat6-dependent interleukin-4-induced genes by**

BCL-6: specific regulation of I epsilon transcription and immunoglobulin E switching. *Mol Cell Biol* 1999, 19:7264-7275.

Most analyses of Iε promoter function to date have focused on the activating transcription factors, especially Stat6. BCL-6, a POZ/zinc-finger factor, has a target sequence with homology to the Stat6 target and, given evidence that it might function as a transcriptional repressor, Rothman's group assessed its effect on IL-4-inducible genes. They demonstrate that BCL-6 can repress IL-4-driven Iε transcription (but, interestingly, not CD23 transcription). This finding established a potentially important negative regulator of allergic responses and it will be interesting to see how the pathways regulating BCL-6-mediated repression of allergic responses unravel.

16. Adra CN, Gao PS, Mao XQ, Baron BW, Pauker S, Miki T, Shirakawa T, Hopkin JM: **Variants of B cell lymphoma 6 (BCL6) and marked atopy.** *Clin Genet* 1998, 54:362-364.
 17. Jung S, Rajewsky K, Radbruch A: **Shutdown of class switch recombination by deletion of a switch region control element.** *Science* 1993, 259:984-987.
 18. Zhang J, Bottaro A, Li S, Stewart V, Alt FW: **A selective defect in IgG2b switching as a result of targeted mutation of the I gamma 2b promoter and exon.** *EMBO J* 1993, 12:3529-3537.
 19. Bottaro A, Lansford R, Xu L, Zhang J, Rothman P, Alt FW: **S region transcription per se promotes basal IgE class switch recombination but additional factors regulate the efficiency of the process.** *EMBO J* 1994, 13:665-674.
 20. Lorenz M, Jung S, Radbruch A: **Switch transcripts in immunoglobulin class switching.** *Science* 1995, 267:1825-1828.
 21. Reaban ME, Griffin JA: **Induction of RNA-stabilized DNA conformers by transcription of an immunoglobulin switch region.** *Nature* 1990, 348:342-344.
 22. Stavnezer J: **Triple helix stabilization?** *Nature* 1991, 351:447-448.
 23. Daniels GA, Lieber MR: **RNA:DNA complex formation upon transcription of immunoglobulin switch regions: implications for the mechanism and regulation of class switch recombination.** *Nucleic Acids Res* 1995, 23:5006-5011.
 24. Tian M, Alt FW: **Transcription induced cleavage of immunoglobulin switch regions by nucleotide excision repair nucleases in vitro.** *J Biol Chem* 2000, 275:24163-24172.
- As work has progressed on models of DNA-RNA hybrids as substrates for nucleases in switch recombination, the identity of the endogenous nucleases that might cleave R-loop substrates has remained open. Tian and Alt again show that germline transcription leads to the formation of S-region R-loop DNA-RNA hybrids. They then demonstrate that the nucleotide excision-repair nucleases XPF-ERCC1 and XPG can cleave these R-loops, introducing double-strand DNA breaks.
25. Schrader CE, Edelmann W, Kucherlapati R, Stavnezer J: **Reduced isotype switching in splenic B cells from mice deficient in mismatch repair enzymes.** *J Exp Med* 1999, 190:323-330.
- Like the work of Tian and Alt [26], this study from Stavnezer's group delves into the question of which endogenous enzymes might participate in the DNA cutting and ligation reactions of the class-switch recombination mechanism. The ability of B cells from mice deficient in various mismatch-repair enzymes (Msh2, Mlh1, Pms2 or Mlh1/Pms2) to undergo cytokine-driven isotype switching was examined by fluorescence-activated cell sorting for B cell surface isotype. A 35-75% reduction in class-switching was observed, dependent upon the particular enzyme missing and the isotype. This analysis implicates the class of mismatch-repair enzymes in the isotype-switch recombination mechanism.
26. Shanmugam A, Shi MJ, Yauch L, Stavnezer J, Kenter AL: **Evidence for class-specific factors in immunoglobulin isotype switching.** *J Exp Med* 2000, 191:1365-1380.
- The authors have developed plasmid switch substrates that can be transiently transfected into B cells and used to measure recombination activity. Unlike some previously described constructs, recombination of these substrates occurs only in B cell lines (or LPS-activated splenocytes) that can undergo chromosomal isotype switching; thus they are valid probes for switch recombinase activity. Unlike chromosomal isotype-switching, the cell lines do not require cytokine activation to support switching-on of the substrates. This study also identifies evidence that recombination from μ to α or from μ to $\gamma 3$ sequences correlates with switch activity at the endogenous loci, providing evidence for the existence of isotype-specific switch factors.
27. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO, Honjo T: **Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing**

deaminase family in germinal center B cells. *J Biol Chem* 1999, 274:18470-18476.

The two-signal requirement (cytokine plus CD40 ligand) for the induction of class-switch recombination predicts that, in addition to cytokine-triggered germline transcription at the IgH locus, other activating events must occur, presumably driven via the CD40/TRAFF/NFκB pathway, which lead to the assembly of a functional switch recombination apparatus. In this report, subtractive cDNA cloning has been used to analyze the transcripts present in resting and switch-induced CH12F3-2 murine B lymphoma cells (more than 80% of which can be stimulated to switch to IgA). AID was isolated in this screen and found to have expression patterns and functional homologies that make it an attractive candidate as a participant in the switch recombination process. AID is expressed in developing germinal centers and in primary splenocytes stimulated to undergo switching. Importantly, IL-4 alone does not induce AID: a combination of IL-4 and CD40 ligand or IL-4 and LPS is required. AID has significant homology to an RNA-editing enzyme, APOBEC-1, which processes ApoB mRNAs. These observations suggest that AID may play a role in the genetic processes of switch recombination.

28. Shirakawa I, Deichmann KA, Izuahara I, Mao I, Adra CN, Hopkin JM: **Atopy and asthma: genetic variants of IL-4 and IL-13 signalling.** *Immunol Today* 2000, 21:60-64.
 29. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich KE, Schou C, Krishnaswamy G, Beaty TH: **Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations.** *Science* 1994, 264:1152-1156.
 30. Noguchi E, Shibasaki M, Arinami T, Takeda K, Maki T, Miyamoto T, Kawashima T, Kobayashi K, Hamaguchi H: **Evidence for linkage between asthma/atopy in childhood and chromosome 5q31-q33 in a Japanese population.** *Am J Respir Crit Care Med* 1997, 156:1390-1393.
 31. Rosenwasser LJ, Borish L: **Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10).** *Am J Respir Crit Care Med* 1997, 156:S152-S155.
 32. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritzsche C, Weiland SK, Erickson RP, von Mutius E, Martinez FD: **A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children.** *J Allergy Clin Immunol* 2000, 105:506-513.
- A number of studies have previously identified linkages between the cytokine locus on human chromosome 5q31, where IL-4 and IL-13 are encoded, and atopy. An allergy-associated polymorphism has been identified in the IL-4 promoter but no mutations affecting the structure of the cytokine have been identified. As IL-13 is also a potent inducer of IgE production and is implicated in allergic pathogenesis in animal models, associations between IL-13 mutations and allergic predisposition could also be anticipated. This collaborative study identified a cluster of seven polymorphisms in the IL-13 gene, four of them tightly linked to an Arg139Gln mutation near the carboxyl terminus. This substitution was shown to be strongly associated ($p = 0.000002$) with elevated serum IgE in three separate populations. The mutation might modify the interaction of the D helix of IL-13 with IL-13-receptor (R)α1.
33. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA: **The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor.** *N Engl J Med* 1997, 337:1720-1725.
 34. Grimbacher BS, Holland M, Puck JM: **The interleukin-4 receptor variant Q576R in hyper-IgE syndrome.** *N Engl J Med* 1998, 338:1073-1074.
 35. Kruse S, Japha T, Tedner M, Sparholt SH, Forster J, Kuehr J, Deichmann KA: **The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction.** *Immunology* 1999, 96:365-371.
 36. Wang HY, Shelburne CP, Zamorano J, Kelly AE, Ryan JJ, Keegan AD: **Cutting edge: effects of an allergy-associated mutation in the human IL-4R alpha (Q576R) on human IL-4-induced signal transduction.** *J Immunol* 1999, 162:4385-4389.

As several case/control studies have given rise to conflicting results regarding the association of the IL-4Rα Arg551Gln mutation with allergy, it has become very important to undertake functional analyses of the effects of this mutation. Wang *et al.* analyzed IL-4-mediated signaling in two different murine cell lines transfected with wild-type and mutant IL-4-receptor. No differences were observed in tyrosine phosphorylation, Stat6 activation, CD23 induction and protection from apoptosis. The authors propose that the associations with atopy observed for this mutation in some studies may be due to different, possibly linked, alterations in the IL-4 signaling pathway. It would also seem possible that expression of the constructs in murine cells and their requisite interaction with murine cytosolic signaling proteins

might not necessarily reproduce any alterations in signaling that might arise when all interacting components are of human origin.

37. Ober C, Leavitt SA, Tsalenko A, Howard TD, Hoki DM, Daniel R, Newman DL, Wu X, Parry R, Lester LA *et al.*: **Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations.** *Am J Hum Genet* 2000, **66**:517-526.

The inconsistent findings regarding the associations of IL-4R α polymorphisms and atopy reported by various groups, along with disparate findings regarding function, have given rise to some uncertainty about whether the Arg551Gln mutation has any association with atopy or effect on receptor function. Ober *et al.* provide an extensive analysis of all eight known coding polymorphisms and their various combinations (haplotypes) in several populations: the inbred Hutterites and outbred groups of Whites, Blacks and Hispanics. Their work confirms significant associations in each population between IL-4R polymorphisms, and asthma and atopy. The significantly linked alleles/haplotypes were

not consistent among populations. It is possible that associations with the markers could arise from variations in noncoding sequences.

38. Heinzmann A, Mao XQ, Akaiwa M, Kreomer RT, Gao PS, Ohshima K, Umeshita R, Abe Y, Braun S, Yamashita T *et al.*: **Genetic variants of IL-13 signalling and human asthma and atopy.** *Hum Mol Genet* 2000, **9**:549-559.
39. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T: **Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme.** *Cell* 2000, **102**:553-563.
40. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Catalan N, Forveille M, Dufourcq-Langelouse R, Gennery A *et al.*: **Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2).** *Cell* 2000, **102**:565-575.