

Molecular Mechanisms of IgE Class Switch Recombination

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Abstract Immunoglobulin (Ig) E is the most tightly regulated of all Ig heavy chain (IgH) isotypes and plays a key role in atopic disease. The gene encoding for IgH in mature B cells consists of a variable region exon—assembled from component gene segments via V(D)J recombination during early B cell development—upstream of a set of IgH constant region C_H exons. Upon activation by antigen in peripheral lymphoid organs, B cells can undergo IgH class switch recombination (CSR), a process in which the initially expressed IgH μ constant region exons (C μ) are deleted and replaced by one of several sets of downstream C_H exons (e.g., C γ , C ϵ , and C α). Activation of the IL-4 receptor on B cells, together with other signals, can lead to the replacement of C μ with C ϵ resulting in CSR to IgE through a series of molecular events involving irreversible remodeling of the IgH locus. Here, we discuss the molecular mechanisms of CSR and the unique features surrounding the generation of IgE-producing B cells.

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1 Introduction

B cell immunoglobulin (Ig) E production is a tightly regulated mediator of allergic disease as well as host defense. As B cells develop, Ig heavy (IgH) and light (IgL) chain loci undergo V(D)J recombination and somatic hypermutation (SHM), which defines antibody specificity to antigen. The IgH locus can uniquely undergo an additional process that defines antibody function, namely IgH class switch recombination (CSR). CSR to IgE positions the variable region exon next to constant region exons that encode for IgE (C ϵ). The regulation of CSR to IgE involves transcriptional control, enzymatic modification of DNA leading to DNA double-stranded breaks (DSB), DNA repair processes, and permanent deletion of the previously expressed C exons (Wu and Zarrin 2014; Geha et al. 2003). In this review, we provide an overview of the intracellular events related to CSR with a focus on unique aspects of CSR to IgE (Table 1).

Table 1 Different regulation of transcription factors in I ϵ promoter and I γ 1 promoter

Transcription factor	I ϵ promoter	I γ 1 promoter	Species	Reference
NF- κ B	+	+	Human and mouse	Delphin and Stavnezer (1995), Dryer and Covey (2005), Lin and Stavnezer (1996), Messner et al. (1997)
STAT6	+	+	Human and mouse	Stütz and Woisetschläger (1999), Linehan et al. (1998), Messner et al. (1997)
PU.1	+	?	Human	Stütz and Woisetschläger (1999)
Pax5	+	+	Human and mouse	Thienes et al. (1997), Max et al. (1995)
E2A	+	*	Mouse	Sugai et al. (2003)
NFIL3	+	*	Mouse	Kashiwada et al. (2010)
C/EBP β	+	–	Human and mouse	Mao and Stavnezer (2001), Shen and Stavnezer (2001), Lundgren et al. (1994)
Bcl6	–	–	Mouse	Harris et al. (1999, 2005)
Id2	–	*	Mouse	Sugai et al. (2003)
AP-1	+	*	Mouse	Shen and Stavnezer (2001)
SWAP-70	+	*	Mouse	Audzevich et al. (2013)

+ positive regulation; – negative regulation; * no effect; ? not clear

2.1 $V(D)J$ Recombination and B Cell Development

The C_H gene segments consist of several sets of exons each encoding an individual antibody constant region (C_μ for IgM, C_δ for IgD, C_γ for IgG, C_ε for IgE, and C_α for IgA). As depicted in Fig. 1, C_μ is most proximal to the V region gene segments, while the most distal C regions, namely C_ε and C_α, are over 100 kb downstream of C_μ in both mouse and human. Each C_H region, except C_δ, is a transcription unit equipped with a promoter and several downstream exons. The first intron of these C_H transcription units—located between the so-called I epsilon exon and downstream C_H exons—is called the switch (S) region and contains large stretches of repetitive DNA elements. As discussed in more detail below, activation



Fig. 1 Schematic presentation of IgH locus in human and mouse. The unassembled (germline) IgH variable region consists of V_H, D_H, and J_H clusters. The IgH constant region consists of several C_H gene segments. Each C_H gene segment, except for Cδ, is a noncoding transcription unit that includes an initial I exon (I), a large first intron termed the switch region (S), and a set of C_H exons (C) that contain open reading frames when in context of a transcript driven from a V region promoter, but not in the context of a transcript arising from the I region promoter. I promoters as well as 3' regulatory region (3'RR in mouse and 3'α1 RR and 3'α2 RR in human) and other cis regulatory elements provide binding sites for transcription factors and other regulatory elements that affect transcription and IgH CSR. ψε and ψγ depict pseudogenes

of these C region transcription units, referred to as I region germline transcription, is required for CSR (Chaudhuri and Alt 2004).

Upon activation, mature naïve B cells can participate in further Ig diversification reactions including somatic hypermutation (SHM) and IgH class switch recombination (CSR). Both SHM and CSR are dependent upon the enzyme activation-induced cytidine deaminase (AID). SHM is a process wherein high-frequency point mutations occur within both IgH and IgL V region exons in germinal center (GC) B cells. These mutations result in altered binding Ig specificities that provide substrates for GC selection processes leading to Ig molecules with higher affinity to antigen in a process termed affinity maturation (Odegard and Schatz 2006; Shlomchik and Weisel 2012). IgH CSR replaces initially expressed C μ exons with a set of downstream exons (C γ , C ϵ , and C α) leading to a switch from IgM to other IgH isotypes such as IgG, IgE, or IgA. As the IgH constant region isotype determines antibody function, IgH CSR positions the IgH V region of selected B cells in a different functional context.

2.2 S Regions and Unique Structure Features of S ϵ

CSR occurs between two S regions. In mouse, individual core sequences of S regions vary in size from 1 to 10 kb and are organized in the order 5'-VDJ-S μ (4.0 kb), S γ 3 (2.5 kb), S γ 1 (8 kb), S γ 2b (5.0 kb), S γ 2a (2.5 kb), S ϵ (1.0 kb), and S α (4.2 kb) (Zarrin et al. 2005). As discussed in more detail below, S regions are guanine rich on the non-template strand. Based on the type of repetitive sequences as well as homology in non-template strands, S regions can be classified into two groups. S μ , S ϵ , and S α contain 10–80 bp repeats and share motif GGGGT and GAGCT, while S γ 1, S γ 2a, S γ 2b, and S γ 3 contain 49–52 bp repeats and share motif AGCT which is also in S μ , S ϵ , and S α (Chaudhuri and Alt 2004). The AGCT motif is evolutionarily conserved in vertebrates such as amphibians, birds, and mammals and is a key target for the CSR machinery. In mammals, motifs containing G's in clusters of 2–5 together are required (Hackney et al. 2009). S region length and repetitiveness appear to influence IgH CSR efficiency in that more efficient S regions are longer in length and contain more repeats (Zarrin et al. 2005). In this regard, the S ϵ region is shortest in length and has the fewest amount of repetitive sequences compared to other S regions in mouse, making it the least efficient S region in general (Hackney et al. 2009).

3 Mechanism of CSR

AID initiates the CSR process through DNA deaminase activity. In this respect, AID deaminates deoxycytosine (dC) into deoxyuridine (dU) on a single-stranded DNA template. The resulting dC-to-dU mutation can then be processed via a number of

pathways. The mutation may be repaired by a mismatch repair protein 2 (MSH2)-dependent process involving exonuclease activity and resynthesis of flanking DNA by an error-prone polymerase resulting in spreading of mutations from the original site of AID action. The mutation can also be replicated into a dC→dT mutation resulting in a point mutation at the site of AID action. Or the mutation may be processed into a DNA break through the action of uracil-*N*-glycosylase (UNG) and apyrimidinic endonuclease (APE1), which can remove the dU:dG mismatch resulting in a single-stranded DNA break. Double-stranded DNA breaks (DSBs) may result from multiple single-stranded DNA breaks in close proximity on both template and non-template DNA strands (Chaudhuri and Alt 2004; Chaudhuri et al. 2007). DSBs in both S μ and a downstream S region—such as S ϵ in the case of CSR to IgE—can lead to deletion of the intervening DNA and joining of the S μ and S ϵ breaks together through classical nonhomologous end-joining (C-NHEJ), or alternative end-joining (A-EJ) repair pathways (Yan et al. 2007). In terms of IgE, the post-CSR hybrid S μ /S ϵ junction is located within an intron located between the V region exon and newly positioned C ϵ exons, which is spliced out of transcripts emanating from the V region promoter to produce productive IgE message. Alternative splicing of constant region exons can generate a membrane-bound or secreted Ig.

3.1 I Region Germline Transcription in CSR

Early studies showed that induction of CSR to a downstream C_H region correlated closely with antecedent transcription of the corresponding C_H region. Transcription of the C ϵ region proceeds through I ϵ exon/S ϵ region/C ϵ exons and terminates at a poly A site. I ϵ region germline transcript (ϵ GLT) is spliced, capped, and transported out of the nucleus, but does not encode for protein due to lack of an open reading frame (Rothman et al. 1990). The importance of I epsilon exon and transcription in CSR was implicated decades ago when it was demonstrated that removal of 5' flanking sequences of S γ 1 region abolished CSR to IgG1 (Jung et al. 1993). CSR to IgG2b was also abrogated in B cells lacking the I γ 2b promoter and I exon in spite of an intact S γ 2b region (Zhang et al. 1993). Replacement of I γ 2b with a strong transcriptional promoter (PKG-neo^r) was able to rescue CSR to IgG2b in I γ 2b-deficient cells (Seidl et al. 1998). Together, these results show that transcription is required for CSR.

3.2 Role of S Region Structure in AID Targeting

AID was discovered over a decade ago as the key mediator of both SHM and CSR (Muramatsu et al. 2000). AID is highly conserved in mouse and human with 92 % homology in sequence (Muto et al. 2000). Ectopic expression of AID in fibroblasts can induce CSR in a transcribed artificial switch construct reaching levels that

parallel CSR in stimulated B cells, and the CSR is completely ablated in AID-deficient cells, indicating that AID is key for CSR (Muramatsu et al. 2000; Okazaki et al. 2002). Patients with mutations in AID also show a lack of IgH isotypes other than IgM (Revy et al. 2000).

In vitro observations have shown that AID deaminates dC into dU in single-stranded DNA (ssDNA) but not double-stranded DNA (dsDNA) or RNA (Ramiro et al. 2003; Chaudhuri et al. 2003; Dickerson et al. 2003; Pham et al. 2003) and AID is involved in stabilizing target sequences through the recruitment of replication protein A (RPA) (Chaudhuri et al. 2004; Basu et al. 2005). When AID is phosphorylated on serine 38, this induces the recruitment of RPA to transcribed S regions and enhances the activity of AID by virtue of its ability to stabilize single-stranded target DNA (Vuong et al. 2013). Experimental findings and current models discussed below account for AID acting on single-stranded DNA in the context of transcription.

Clustering of G's on the non-template strand in S regions can lead to RNA-DNA hybrids that are formed during transcription through S regions (Roy et al. 2008). Newly transcribed S region RNA is G rich and pairs with the C-rich template DNA with greater stability compared to its DNA complement, thus leaving a portion of the non-template G-rich DNA single stranded. This single-stranded loop of G-rich DNA is referred to an R loop and provides a model for how AID can target the single-stranded non-template DNA strand (Tracy et al. 2000; Yu et al. 2003; Roy et al. 2008). Substitution of only 5 % G with C by changing GGGGG into GGG and GGGG into GGG—while maintaining GC density—reduced the frequency of R loops dramatically from 6.7 to 0.23 %. When continuous G's changed into GG or G, no R loops were detected (Roy et al. 2008).

R loops have been detected in both S_{μ} and downstream S regions in vivo, including kilobase-sized R loops in $S_{\gamma 3}$ and $S_{\gamma 2b}$ regions (Huang et al. 2007; Yu et al. 2003). Replacement of the endogenous $S_{\gamma 1}$ region with an otherwise identical, but inverted sequence resulted in a 4-fold reduction of CSR to IgG1. Endogenous $S_{\gamma 1}$ region is G rich on the non-template strand, which can form R loops in physiological orientation due to the favorable energetics of G-rich RNA interacting with C-rich DNA. Inverted S regions, which are C rich on the non-template strand, do not exhibit these characteristics and thus do not form detectable R loops (Shinkura et al. 2003). Replacement of $S_{\gamma 1}$ region with *Xenopus* S_{μ} region, which is AT rich and similarly does not form R loops, results in a 4-fold decrease of CSR (Zarrin et al. 2004). These results suggest that although not required, R loops magnify the efficiency of CSR.

Although S regions in general are required for CSR, there appears to be flexibility regarding the requirements for specific S region sequences. In this context, substitution of S_{α} sequences with S_{ϵ} and S_{γ} sequences did not change target specificity of CSR (Kinoshita et al. 1998). As mentioned above, while inverted $S_{\gamma 1}$ and amphibian S_{μ} were still permissible for CSR, substitution of S_{μ} with vertebrate telomere sequences abolishes CSR completely (Junko et al. 2001). Inverted $S_{\gamma 1}$ and vertebrate telomere sequences are both G rich, but the telomere sequences do not contain palindromic sequences, whereas both inverted $S_{\gamma 1}$ and amphibian S_{μ} do.

As palindromic sequences can form secondary structures, such as stem loops, these findings may suggest that some form of secondary structure within S regions may be required. Other DNA structures are also reported in S region DNA sequence such as G quartets, in which guanines from four DNA strands are stabilized by G–G Hoogsteen Bondings and form a G4 planar structure (Dempsey et al. 1999). More complex structures involving AID and other factors during transcription have also been described (Yu and Lieber 2003; Shinkura et al. 2003). Although the roles of primary, secondary, and more complex tertiary structures remain to be fully elucidated, complex S region DNA structures are thought to lead to stalling of RNA polymerase II (RNA pol II)—to which AID is linked (Nambu et al. 2003)—thus providing CSR factors increased transcription-coupled S region access (Pavri et al. 2010; Xu et al. 2012; Kenter 2012).

Using an $MSH2^{-/-}$ $UNG^{-/-}$ genetic model system—in which AID-catalyzed deamination events can be tracked directly by C→T transitions—mutations are detected at equal frequencies in both template and non-template S region DNA (Xue et al. 2006), indicating that AID directly catalyzes deamination events on both template and non-template DNA. As template strand DNA does not form R loops, how AID targets the template DNA strand is an active area of research. In this regard, three models have been proposed to explain how AID may gain access to the template strand. One suggests that RNA polymerase generated DNA topological changes in S regions during transcription—where negatively supercoiled DNA is generated upstream of the active transcriptosome complex—provides AID targets to both DNA strands (Shen and Storb 2004; Besmer et al. 2006). Another model hypothesizes that RNase H leads to exposure of template DNA by degrading RNA transcripts in R loops (Huang et al. 2007; Lieber 2010). A third model implicates a role for the RNA exosome, which can degrade nascent RNA transcripts in R loops thus facilitating AID access to the template DNA strand (Basu et al. 2011; Nambu et al. 2003; Willmann et al. 2012; Pavri et al. 2010). In all of these models, non-template and template DNA strands in S regions must be accessible in the form of single-stranded DNA long enough for AID-mediated deamination to take place.

4 Unique Features of CSR to IgE

4.1 Transcriptional Control of $I\epsilon$

As discussed above, accessibility of $S\epsilon$ to AID requires transcription of the germline $I\epsilon/S\epsilon/C\epsilon$ unit controlled by the $I\epsilon$ promoter upstream of $S\epsilon$, as well as other cis regulatory elements, including a set of enhancer elements located at the 3' end of the IgH locus termed the 3' regulatory region (3'RR). Mouse IgH has one 3'RR downstream of the IgH C_H regions containing four enhancer segments, while human IgH has two 3'RR downstream of $C\alpha 1$ and $C\alpha 2$, respectively (Fig. 1). Each human 3'RR contains enhancer segments homologous to mouse (Pinaud et al. 2011).

either through LPS stimulation or through T-cell interaction through CD40 signaling. The two transcription factors bind to adjacent areas on the I ϵ promoter and act synergistically to activation transcription (Shen and Stavnezer 1998).

The I ϵ promoter contains two binding sites for E2A proteins termed E boxes, which are both required to fully activate I ϵ transcription (Sugai et al. 2003). E2A proteins are negatively regulated by Id2, which is a helix-loop-helix transcription factor constitutively expressed in resting B cells (Ishiguro et al. 1995; Becker-Herman et al. 2002) and can be induced by transforming growth factor- β 1 (TGF- β 1) (Sugai et al. 2003). Id2^{-/-} mice show an increased level of ϵ GLT and elevated levels of IgE. Id2 negatively regulates I ϵ promoter by binding to activated transcription factor E2A, thus interfering with its binding to E boxes in I ϵ promoter. Although Id2 negatively regulates CSR to IgE, it does not appear to affect IgG1 (Sugai et al. 2003).

The zinc finger-containing transcriptional repressor B-cell lymphoma 6 (Bcl6) also plays an inhibitory role in IgE CSR (Harris et al. 2005). Bcl6-deficient mice undergo an increased CSR to IgE and IgG1 after IL-4 activation in vitro. There are Bcl6 binding sites within the I ϵ promoter and these were shown to overlap with the binding sites for STAT6 (Harris et al. 1999).

Several basic leucine zipper (bZIP) family members, such as ATF, AP-1, CCAAT/enhancer-binding protein (C/EBP β), and IL-3-regulated (NFIL3, also called E4BP4), also bind to and can regulate the I ϵ promoter. After activation of the CD40 signaling pathway, AP-1 is rapidly upregulated and synergizes with STAT6 to activate the I ϵ promoter in mice. AP-1 recruits histone acetyl transferase, p300/CBP, which produces active histone modifications that may prolong the effect of AP-1 as it is induced quickly and transiently (Mao and Stavnezer 2001; Shen and Stavnezer 2001). In humans, C/EBP β synergizes with STAT6, whereas mouse C/EBP β may inhibit the synergism of AP-1 and STAT6 (Shen and Stavnezer 2001) and has been suggested to negatively regulate NF- κ B on the I γ 1 promoter (Mao and Stavnezer 2001).

In response to stimulation with LPS plus IL-4, mouse B cells deficient in nuclear factor interleukin-3 regulated (NFIL3) greatly reduce the production of ϵ GLT and CSR to IgE, while CSR to IgG1, IgM, IgG2a, IgG2b, and IgG3 are essentially unchanged (Kashiwada et al. 2010). Furthermore, unlike other bZIP transcriptional regulators, NFIL3 was found to bind to the I ϵ promoter but not other I promoters, indicating that in terms of C region regulation, NFIL3 may uniquely affect CSR to IgE (Kashiwada et al. 2010). Notably, NFIL3 has been shown to be involved in signaling circuits related to the circadian clock. The levels of NFIL3 are opposite to that of proline and acidic amino acid-rich (PAR) proteins, which are a group of transcription factors important in core circadian clockwork and fluctuate in a circadian fashion (Mitsui et al. 2001). Recently, the histone methyltransferase MLL3, which catalyzes active histone modification (H3K4me) in IgH locus I region promoters, was also found to be regulated by the circadian circuitry (Li et al. 2013; Valekunja et al. 2013). The protein levels of NFIL3 and MLL3 are both at their

peak during the first half of day, suggesting that they may work together to add a circadian dimension to the transcriptional regulation of $I\epsilon$ and CSR to IgE. Such a dimension is an intriguing possibility that remains to be explored.

4.2 Dual Activation of IgG1 and IgE

IL-4 in combination with either CD40 activation or LPS stimulation induces murine B cell transcription of both the $I\epsilon$ and the $I\gamma 1$ promoters and thus may lead to switching to both IgG1 and IgE. Observations from over two decades ago that $S\gamma 1$ sequences can be found within $S\mu/S\epsilon$ junctions of both mouse and human IgE^+ cells indicated that CSR can occur in a stepwise fashion. The first step is CSR from IgM to IgG, and the second step is a CSR reaction from IgG to IgE (Yoshida et al. 1990; Mills et al. 1992). It was later shown that B cells stimulated in vitro with IL-4 plus LPS, secreted IgG1 first, and then IgE after a delay of several hours. A similar delay also occurred in the production of membrane-bound IgG1 (mIgG1) versus IgE (mIgE). A small number of intermediate cells containing both mIgG1 and mIgE can be detected, indicating B cell passage through a step where IgG1 protein is still present after having undergone CSR from $C\gamma 1$ to $C\epsilon$ at the DNA level with freshly produced IgE (Mandler et al. 1993). A similar pattern of IgG1 before IgE was observed in mature B cells upon stimulation with IL-4 plus a stimulating antibody against CD40 (anti-CD40) (Wesemann et al. 2011). The physiologic reasons for dual $I\gamma 1$ and $I\epsilon$ activation and the molecular coordination of CSR to IgG1 and IgE are areas of active research.

Although the frequency of sequential CSR to IgE through IgG1 is dominant over direct CSR to IgE in mature B cells (Jung et al. 1994), immature and transitional B cells have a preference to undergo CSR to IgE through a mechanism involving more direct IgM to IgE CSR (Wesemann et al. 2011). While the $I\gamma 1$ and $I\epsilon$ promoters are both induced substantially by IL-4 plus anti-CD40 in mature B cells, the preference for direct CSR from IgM to IgE in immature cells is explained molecularly by the relative preservation of $I\epsilon$ inducibility compared to the abrogated inducibility of the $I\gamma 1$ promoter in immature B cells (Wesemann et al. 2011). In addition, as young mice have a natural abundance of immature and transitional B cells in the periphery, splenic B cells from young mice demonstrate increased propensity to undergo CSR to IgE. The extent to which this mechanism contributes to the elevated IgE seen in some immune deficiencies as well as in youth compared to adults (Monroe et al. 1999; Melamed et al. 1998; Grundbacher 1976) remains to be uncovered.

$S\mu$ contains the highest density of AID hotspots, and DSBs are more frequent in $S\mu$ compared to any other downstream S region (Schrader et al. 2003, 2005). Although the evolutionary forces leading to $S\mu$ as the most powerful S region are not fully known, we speculate that it may speak to the importance of simultaneous activation of two or more acceptor S regions. In the context of IL-4 plus anti-CD40 or LPS signaling, high DSB activity in the donor $S\mu$ region appears to drive CSR to

simultaneously activated $S\gamma 1$ and/or $S\epsilon$. In this respect, the limiting DSB activity in the acceptor S regions may control CSR outcome by competing for “next best” status—in terms of transcriptional activation, AID targeting, and DSB activity—compared to $S\mu$. In the case of the simultaneously active triplex of $S\mu$, $S\gamma 1$, and $S\epsilon$, excess DSB in $S\mu$ may ensure that selection of the downstream acceptor S region resides within the respective relative strengths of $I\gamma 1/S\gamma 1$ and $I\epsilon/S\epsilon$ to become transcriptionally active, recruit AID and form DSBs. If $S\mu$ DSBs were not able to drive CSR in the setting of simultaneous activation of multiple S acceptor regions, the acceptor regions may undergo recombination themselves and thus reduce the flexibility and diversity of CSR outcomes. Such a scenario was observed in mice in which $S\mu$ was weakened substantially through deletion of most of the core AID targets ($S\mu^{-/-}$ mice). $S\mu^{-/-}$ mice exhibited impaired CSR to IgG1, IgG2a, IgG2b, and IgG3 (Luby et al. 2001; Khamlichi et al. 2004). However, IL-4 plus anti-CD40-induced CSR to IgE is nearly the same levels in $S\mu^{-/-}$ mice as wild-type mice (Zhang et al. 2010) due to downstream $S\gamma 1$ -to- $S\epsilon$ CSR occurring before involvement of the weakened $S\mu$. The $S\gamma 1$ -to- $S\epsilon$ CSR results in deletion of the $C\gamma 1$ exons leaving a hybrid $S\gamma 1/S\epsilon$ S region as a second-step recombination partner with the inefficient, truncated $S\mu$ sequence. Thus, as $C\gamma 1$ is located in between $C\mu$ and $C\epsilon$, the B cell loses the ability to undergo CSR to $C\gamma 1$ in $S\mu^{-/-}$ mice because $S\gamma 1$ and $S\epsilon$ recombination—and $C\gamma 1$ exon excision—will occur before either has a chance to recombine with the weakened $S\mu$, thus severely limiting the probability of $C\gamma 1$ as a CSR outcome due to its position along the IgH locus.

In addition to $S\mu$ as the most powerful S region, at least three other regulatory mechanisms likely play a role in controlling CSR outcomes in the setting of simultaneous activation of $S\mu$, $S\gamma 1$, and $S\epsilon$. Firstly, as discussed above, $S\epsilon$ is the weakest S region in terms of length, number of repeat elements, and AID target motifs, and $S\gamma 1$ is one of the strongest S regions given its large size. Hence, these primary structural DNA features would favor CSR from $S\mu$ to $S\gamma 1$ first (unless $I\gamma 1/S\gamma 1$ cannot be transcriptionally activated as in the case of immature B cells). Evidence of this notion was derived from experiments wherein the $S\epsilon$ region was enhanced by replacing it with $S\mu$ sequence. This resulted in more direct CSR to IgE in vitro and elevated IgE in vivo (Misaghi et al. 2013). Secondly, the $S\gamma 1$ region may play a role in actively repressing $S\epsilon$ accessibility to participate in CSR as deletion of $S\gamma 1$ results in increased levels of IgE in vitro and in vivo (Misaghi et al. 2010). However, deletion of the $I\gamma 1$ promoter did not affect the level of CSR to IgE (Jung et al. 1993, 1994). Thus, perhaps the $S\gamma 1$ region, but not the $I\gamma 1$ promoter, may be involved in regulating CSR to $C\epsilon$. Thirdly, $S\epsilon$ appears to become accessible with delayed rate, even after $S\mu/S\gamma 1$ recombination has taken place. When activated, IgG1⁺ B cells proceed from IgG1 to IgE with similar kinetics compared to IgM to IgE (Wesemann et al. 2012), indicating that regulatory mechanisms delay the timing of CSR to IgE.

Production of IgE through direct CSR or indirect CSR may differ in the extent to which affinity maturation may occur. In this regard, direct CSR to IgE tends to generate low-affinity IgE, while sequential CSR to IgE inherits V region somatic mutations and affinity selection that occurs during the IgG1 stage (Xiong et al. 2012).

After activation by IL-4, purified IgG1 cells derived from germinal centers and memory B cells underwent CSR to IgE, indicating that sequential CSR can be interrupted by an IgG1 stage that can be rounds of cellular proliferation and differentiation before switching to IgE (Erazo et al. 2007). So far, high-affinity IgE derived from memory IgM B cells has not been observed, and IgG1 deficient mice tend to produce low-affinity IgE indicating that B cells produce high-affinity IgE by way of IgG1 (Erazo et al. 2007; Xiong et al. 2012). Years to come will assuredly uncover a deeper molecular and cellular understanding of the developmental pathways that generate IgE.

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