Receptor Editing in Positive and Negative Selection of B Lymphopoiesis

Efrat Edry and Doron Melamed

J Immunol 2004; 173:4265-4271; doi: 10.4049/jimmunol.173.7.4265
http://www.jimmunol.org/content/173/7/4265

References

This article cites 92 articles, 47 of which you can access for free at:
http://www.jimmunol.org/content/173/7/4265.full#ref-list-1

Subscription

Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Receptor Editing in Positive and Negative Selection of B Lymphopoiesis

Efrat Edry* and Doron Melamed2†

In B lymphopoiesis, Ag receptor expression and signaling are critical to determine developmental progression, survival, and activation. Several positive and negative selection checkpoints to test this receptor have been described in B lymphopoiesis, aiming to ensure the generation of functionally competent, nonautoimmune repertoire. Secondary Ag receptor gene recombination allows B lymphocytes to replace an inappropriate receptor with a new receptor, a mechanism called receptor editing. This salvage mechanism uncouples the Ag receptor fate from that of the cell itself, suggesting that B cell repertoire is regulated by a process of receptor selection. Secondary rearrangements are stimulated in different stages of B cell development, where editing of the receptor is necessary to fulfill stage-specific requirements. In this study, we discuss the contribution of receptor editing in B lymphopoiesis and its regulation by positive and negative selection signals. The Journal of Immunology, 2004, 173: 4265–4271.

B lymphopoiesis is guided by Ig gene rearrangement and construction of the Ag receptor. Several selection checkpoints have been described along the developmental pathway aiming to test the competence of the new receptor and to ensure the generation of mature B cells expressing nonself receptors that are functionally responsive. Early immunological models predicted that a B cell that fails to fulfill both requirements aborts developmental progression and undergoes apoptosis in the bone marrow (BM). Studies showing an alternative salvage pathway for these cells have been published at the late 1980s and early 1990s. These studies suggested that B cells expressing a defective and/or self-reactive receptor undergo secondary Ig gene rearrangement to replace this receptor, rather than undergoing a default apoptosis. This salvage mechanism was first characterized in central tolerance of immature B cells undergoing negative selection and was termed receptor editing (1). Secondary V(D)J recombination has been demonstrated both at the H chain (HC) and the L chain (LC) loci, though through different mechanisms. At the κ locus, secondary recombination replaces the entire pre-existing VκJκ by recombination of an upstream Vκ to a downstream Jκ. Such mechanism of secondary recombination is not applicable at the HC locus, as no more D regions are left available after primary VDJ formation. Instead, receptor editing at the HC locus uses cryptic recombination signal sequences (RSS), embedded in many VH genes, to allow upstream VH genes to recombine with the existing VDJ and to produce hybrid VH genes (Fig. 1) (1). It was later shown that B cell development also depends on BCR expression and appropriate signaling, a process referred as positive selection (2–6). Developing B cells expressing defective and/or inefficient receptors are developmentally arrested, but can be rescued by secondary recombination that replaces the HC or the LC. Thus, receptor editing, mediated by secondary V(D)J recombination, is an important mechanism contributing to both positive and negative selection in B lymphopoiesis.

HC receptor editing in selection of early B cell precursors

B lymphocytes rearrange HC V, D, and J gene segments at the pro-B stage. A productive VDJ encodes μH chain that pairs with the surrogate L (SL) chain components, VpreB and A5, and is expressed as pre-BCR (reviewed in Refs. 3 and 5). Signaling from the pre-BCR is required to suppress V(D)J recombination and to establish allelic exclusion at the HC locus (7, 8). Appropriate pre-BCR also signals for positive selection and proliferative expansion (5, 7). It is not clear whether these signals are generated from pre-BCR aggregation (3), or mediated by an interaction with putative ligand expressed on stromal cells (9). Failure to express the pre-BCR abrogate allelic exclusion and allows ongoing V(D)J recombination (3, 5). Also, lack or insufficient pre-BCR signaling imposes developmental arrest in several signaling mutated mice (reviewed in Refs. 10–12). Hence, expression and signaling of the pre-BCR are critical for positive selection of pro-B cells.

The HC locus, due to its construction, disfavors secondary rearrangements at least through conventional RSS. Despite these, secondary rearrangements can take place at the IgH coding region. Early studies with a pre-B cell line carrying a primary D1JH rearrangement showed that secondary D1JH-to-JH recombination substitutes the primary D1JH (13) (Fig. 1). D1JH encodes a Dμ protein, which pairs with SL chain proteins and

*Department of Immunology, Faculty of Medicine, Technion, and 1Rappaport Family Institute for Research in the Medical Sciences, Haifa, Israel
2 Address correspondence and reprint requests to Dr. Doron Melamed, Technion, Faculty of Medicine, Department of Immunology, Haifa 31096, Israel. E-mail address: melamedd@vs.technion.ac.il
3 Abbreviations used in this paper: BM, bone marrow; HC, H chain; LC, L chain; RS, recombination sequence; RSS, recombination signal sequences; SL, surrogate L; Tg, transgenic.
transmits signals that are essential for the D\textsubscript{H} counterselection and abortion of B cell development (14, 15). It is possible that secondary rearrangements at this stage may allow replacement of rearranged D\textsubscript{H} elements that are read in the counterselected reading frame (14) (Fig. 2).

Another mechanism of receptor editing at the HC locus is by VH segment replacement. Studies of the IgH coding region in pre-B lymphoma cells show that secondary recombination allows replacement of the existing VH with an upstream germline VH segment, and that such replacement occurs on both productive and nonproductive alleles (16, 17). Secondary rearrangements by VH replacements are mediated by conserved heptameric sequences embedded at the 3’\textsubscript{H} end of many VH genes (17). In human, 40 of 44 functional VH genes carry such an embedded heptamer (18) (Fig. 1). Although detected at significant frequencies, in vitro analysis revealed that the embedded heptamer is far less efficient in mediating V(D)J recombination relative to the conventional sequence (19).

The in vivo observation of VH replacement has been demonstrated in gene-targeted autoantibody transgenic (Tg) mouse models. In these mice, the Tg VDJ is targeted into the endogenous HC locus, and therefore, is subjected to the appropriate regulatory elements. This allows almost normal B cell development, which is mediated by the pre-BCR expression and signaling, as well as locus specific competence, to undergo receptor editing (20). Using a site-directed Tg mouse model in which the J\textsubscript{H} locus has been replaced with a VDJ exon coding for the HC of an anti-DNA Ab, Chen and colleagues (21) showed the occurrence of receptor-editing events at the HC locus. Editing of the inserted transgene was conducted by recombination of upstream endogenous V\textsubscript{H}, or D\textsubscript{H} or both genes replacing the Tg VH\textsubscript{H} gene (21). In vivo VH\textsubscript{H} gene replacement has also been described in other gene-targeted mouse models (22–25) and in human rheumatoid arthritis (26). As most of the gene-targeted mice produce B cells expressing a self-reactive BCR and used for tolerance studies, it was first thought that VH replacement in these models is triggered by tolerance signaling through intact surface BCR complex. However, several studies have demonstrated that V\textsubscript{H} to VDJ recombination includes additional “N” nucleotides, implicating that the majority of VH replacement events occur at the pro-B stage, in which LC gene segments are still in germline configuration (21, 23, 25). Therefore, it is likely that tolerance signaling through the BCR does not mediate VH replacement.

Because pre-BCR expression and signaling are critical for positive selection of pro-B cells, the mechanism of VH\textsubscript{H} replacement...
can be linked to the appropriate formation of the pre-BCR. \( \text{V}_{\text{H}} \) replacement can be stimulated in pro-B cells where the produced \( \mu \text{H} \) chain is unable to associate with the SL chain (Fig. 2). There are several findings supporting this assumption. First, \( \sim 10\% \) of pre-B cells express \( \mu \text{H} \) chains in the cytoplasm, which halt of them fail to pair with SL chains (5, 27). Second, various \( \mu \text{H} \) chains that cannot associate with SL chains have been identified (27). Third, \( \sim 20\% \) of pre-B cells that express \( \mu \text{H} \) chains use the \( \text{V}_{\text{H}}18x \), but this \( \text{V}_{\text{H}}14 \) is rarely found in peripheral B cells (5, 27), probably because \( \text{V}_{\text{H}}18x \) \( \mu \text{H} \) chain cannot associate with SL chain (27). Furthermore, Tg mice carrying a \( \text{V}_{\text{H}}18x \) \( \mu \text{H} \) Tg cannot form a pre-BCR, and mature B cells in these mice do not express the transgene (28). An alternative possibility, but not mutually exclusive, for \( \text{V}_{\text{H}}14 \) replacement in pre-BCR formation is repertoire diversification (29). This is supported by demonstrating \( \text{V}_{\text{H}}14 \) replacements in nonautoimmune context obtained in gene-targeted mice (24, 30), and by the fact that formation of such \( \text{V}_{\text{H}}14 \) hybrids extends the CDR3 diversity. Interestingly, in many autoantibodies produced in MRL mouse strain and in human autoimmune diseases such unusual hybrids are found (reviewed in Ref. 31).

**Receptor editing in positive selection of immature B cells**

Productive LC recombination promotes B cell development to an immature stage and allows surface IgM expression. At this stage of development, immature B cells can interact with self Ags and face the strict process of negative selection (see below). In contrast, nonself BCR signals for allelic exclusion at the LC locus (7, 8), but it is unclear whether nonself-reactivity is sufficient for further developmental progression and maturation. Osmond and colleagues (32) have estimated that mice produce \( \sim 2 \times 10^7 \) immature B cells per day in the BM that migrate to the spleen. Most of these cells die shortly after and only a small number of cells are selected into the long-lived pool (32). Although many of these cells are the targets of negative selection, there are several evidences that this process also reflects positive selection. First, there are differences in the \( \text{V}_{\text{H}} \) gene repertoire of BM B cells relative to peripheral B cells (33). Second, B cell maturation is accompanied with stage-dependent differences in BCR signaling molecules and in the BCR ability to enter into lipid rafts (34, 35). Third, B cell maturation is severely impaired in many BCR-signaling mutated mice, such as Btk, SLP-65, Lyn, and Vav, and in mice lacking functional IgB cytoplasmic tail (reviewed in Refs. 10–12). These data suggest that B cell maturation is limited by a process of positive selection. Additional studies have shown that these positive selection signals are transmitted by the BCR and its signaling components (36, 37). The nature of these signals is not completely understood. It is thought that proper assembly and expression of an oligomeric BCR generates some basal phosphorylation that is required for positive selection (3, 4, 10). This basal-tone or steady-state level of signaling is the result of a dynamic equilibrium between positive and negative signaling regulatory molecules and is also called “tonic” signals (38, 39). Data showing that these signals are ligand independent support this hypothesis (40), although others propose an unknown ligand in generating these signals (41). Lately, we and others have shown that in B and T lymphocytes, tonic activity of signaling pathways independent of receptor ligation determines the physiologic gene expression programs and the induction of \( RAG \) genes. In both systems these ligand-independent signals are mediated by ERK kinase (39, 42). A recently published study has shown that basal signal strength also determines positive selection of B-1 vs B-2 B cell development (43). Collectively, these findings implicate tonic signals with the establishment of allelic exclusion and positive selection of B cells.

Failure of positive selection imposes developmental arrest (reviewed in Refs. 10–12), but the fate of these B cells has not been studied in most of these signaling mutated mice. In mice deficient of CD19, B cell development, activation, and positive selection into the marginal zone is impaired (6, 44). Because CD19 function as an important positive regulator of BCR signaling, lack of CD19 results in receptor signaling incompetence (45). Using the 3-83Tg mouse model, we showed that immature 3-83Tg CD19–/– B cells fail positive selection, as a result of BCR signaling incompetence, and undergo developmental arrest. These cells up-regulate recombination genes and undergo receptor editing, as demonstrated in vivo and in vitro (46) (Fig. 2). Similarly, 3-83Tg mice deficient of Lyn had increased number of edited B cells (47). Thus, failure of positive selection activates secondary LC gene recombination to edit and express a new receptor that is signaling competent. Recently, we have shown that this receptor editing is directly stimulated by inappropriate tonic signaling in immature B cells. We found that elevation of tonic signals in nonediting 3-83Tg immature B cells stimulates RAG expression, whereas lowering tonic signals in the editing 3-83Tg CD19–/– cells suppress RAG expression (42). In addition, we found that B cell maturation in this model is limited to cells that were able to compensate for the lack of CD19 by coexpression of an endogenous receptor or by elevation of BCR surface density, leading to the generation of appropriate tonic signals (42, 46). It would be interesting to assess the extent of receptor editing in the other signaling mutated mouse models where developing B cells fail positive selection. Receptor editing has also been shown to be active in cases when underexpressed BCR fail to promote positive selection due to insufficient signals (48, 49). Taken together, these results suggest that Ag-independent BCR signaling threshold mediates positive selection of immature B cells. Signals that are too high or too low block developmental progression and activate receptor editing, whereas appropriate signals promote it (Fig. 3).

It is important to note that interaction with low-affinity self Ag can modify these BCR signals. As a result of such interaction, cells expressing signaling incompetent receptors are positively selected rather than die, and may generate autoimmunity. This has been demonstrated in CD45-deficient, CD22-deficient, and lyn-deficient mouse models (reviewed in Ref. 50), as well as in B cells expressing low density of self-reactive BCR (51). The interaction with self Ag has also been shown to promote B1 cell development (52). Hence, interaction with self Ag may modify tonic signals and alter the positive selection threshold, which determines the B cell fate.

**Receptor editing in negative selection**

Immature B cells can interact with self Ag in the BM. Early mathematical models predicted that most of the generated B cell specificities are self-reactive (53), a prediction that was experimentally confirmed recently (54). Tg mouse models using autoantibodies to HEL, MHC class I, DNA, erythrocytes, and other Ags were used to show that self-reactivity in the B cell compartment is extinguished by three main mechanisms: receptor editing, apoptosis, and anergy. It was later shown that
this negative selection depends on avidity interactions and BCR signaling threshold (50).

Although IgM expression should signal for LC allelic exclusion (7, 8), early studies in B cell lines and normal B cell precursors revealed that surface IgM expression does not terminate V(D)J recombination (55, 56). These studies suggested that ongoing V(D)J recombination can replace an existing V_{\kappa}J_{\kappa} with a new recombination of an upstream V_{\kappa} to a downstream J_{\kappa}. Using Ig-Tg mouse models, it was later suggested that secondary recombination is in fact tolerance induced and resulting from interaction with self-tissue, aiming to alter the BCR specificity. Because of the locus organization and accessibility at this stage of development (8), tolerance-induced receptor editing is primarily obtained at the LC locus (57). Secondary \kappa rearrangements can occur by deletion or inversion of the intervening DNA, depending on the transcriptional orientation of the recombining elements (Fig. 1) (1). In addition, a conserved recombination sequence (RS) element is present at the \kappa locus and functions to promote receptor editing. Upon recombination, the RS element inactivates the active \kappa allele and directs V(D)J recombination to the second \kappa allele or to the \lambda loci (3, 18). Hence, replacing of an existing V_{\lambda}J_{\lambda} by receptor editing can occur by secondary V_{\lambda}J_{\lambda} recombination or by RS recombination.

The role of receptor editing in negative selection was first studied in H+L Ig-Tg mice expressing BCR specific to DNA or to MHC. Hybridoma cell analysis from anti-dsDNA Tg mice (3H9/H_{\kappa}V_{\kappa}4) revealed extensive \kappa-chain editing with no HC editing (58). In the anti-MHC mouse model (3–83), it was demonstrated that B lymphocytes encountering self Ag are stimulated to undergo V(D)J recombination and secondary LC rearrangement (59). Receptor editing was not activated in these mouse models when bred on RAG-deficient background, confirming that alternation of BCR specificity is due to secondary recombination (60). Appreciation of receptor editing as a main mechanism in negative selection has been established using different in vitro culture systems (Fig. 2). It was demonstrated that anti-HEL and anti-MHC Tg immature B cells encountering self Ag undergo developmental arrest (57, 61). As a consequence, the majority of these cells (>60%) are stimulated to undergo LC receptor editing (57, 62). A better in vivo estimation for the efficiency of receptor editing emerged from several studies using autoantibody-targeted mouse models (knockin). These mouse models have shown that primary autoreactive-encoding V_{\kappa}J_{\kappa} is inactivated by RS recombination or replaced by new recombination of an upstream V_{\kappa} to downstream J_{\kappa} (20, 63). Despite the autoreactive specificity encoded by the targeted Ab, these mice have essentially normal B cell numbers. Hybridoma analysis of anti-DNA 3H9/V_{\kappa}4 revealed that 98% of the cells had undergone LC editing (63), and at least 85% of the peripheral B cells lost autoreactive specificity in the anti-MHC model (20). Similar results were obtained in anti-DNA H chain-only targeted mice where LC editing has been assessed at the endogenous nonmutated \kappa locus (22, 64). Thus, receptor editing is very efficient in rescuing autoreactive B cells, although multiple rearrangements are often required until appropriate V_{\kappa} editor is selected (65). High frequency of receptor editing was also demonstrated in normal mouse B cells (66), and in peripheral human B cells expressing SL chain and autoreactive receptors (67). Using mice that carry human and mouse C_{\kappa} genes, Nussenzweig and colleagues (68) have shown that 25% of the Ab molecules are produced by LC gene replacement, providing receptor editing with a major contribution in generating the Ab repertoire. Because V(D)J recombination is affected by factors such as differences within RSS, promoter regions, and other cis-acting elements, certain V_{\kappa} genes are overrepresented (69). Under these circumstances, the importance of receptor editing is by promoting representation of Ig genes that are disfavored by the primary V(D)J recombination (68).

Several factors have been shown to regulate the stimulation of receptor editing in negative selection. A central regulatory function relates to the BCR signal transduction, which undergoes significant changes during developmental progression (34). BCR signaling following interaction with Ag is profoundly different relative to the tonic signals required to promote positive selection both in level of phosphorylation and pattern of phosphorylated proteins (Fig. 3) (42). Modification of these signals by lack of CD19 or CD45 coreceptors or Btk have no effect on receptor editing stimulated by multivalent membrane-bound Ag (45, 70, 71). Analysis of affinity threshold revealed that receptor editing was efficiently stimulated by MHC Ags with affinities of $>5 \times 10^4$ M$^{-1}$ (72). This activation appears to be independent of c-Myc expression, an important oncprotein that is activated in BCR-induced apoptosis (73). We have previously shown that apoptosis and receptor editing are developmentally regulated. Using an in vitro culture system and an Ig-Tg model, we found that immature B cells progress from receptor-editing competent apoptosis-resistant stage into receptor-editing incompetent apoptosis-sensitive stage (74). Because c-Myc responsiveness to BCR ligation increases with developmental progression (73), it may explain the obtained compartmentalization of the mechanisms contributing to B cell negative selection. Using a different anti-DNA-targeted mouse model, Eilat and colleagues have shown that receptor editing efficiency depends on the number of available J_{\kappa} on the expressed V_{\kappa} allele (75). Studying the stimulation of receptor editing in response to soluble Ag yielded some additional results.
Tze et al. (76) have used the HEL Tg mouse model and showed that receptor editing in immature B cells can also be stimulated in response to soluble Ag at a later developmental stage. Despite the distinct differences between the anti-HEL and anti-MHC Tg systems, these data propose that the size of the editing “window” in B cell development may also depend on the nature of the Ag. However, it is difficult to interpret the results by Tze et al. (76) because the majority of these IgMhigh late immature cells (70%) have undergone apoptosis even in the absence of Ag. In contrast, earlier studies have shown that transitional B cells in the spleen, which have a similar IgMhigh phenotype, rapidly die after Ag encounter (77), perhaps suggesting that receptor editing, although it can be stimulated, is not the major mechanism in this cell population. This heightened sensitivity to apoptosis could also result from differences in microenvironment between the BM and the spleen. It is also suggested that BM, but not spleen, microenvironment protects immature B cells from apoptosis and allows stimulation of receptor editing in response to BCR ligation (78). Thy1dull cells were later shown to provide apoptosis and allows stimulation of receptor editing in response to BCR ligation (78). Thy1dull cells were later shown to provide this microenvironment in the BM, proposing that receptor-editing stimulation depends also on the site of Ag encounter (79). It is now thought that stimulation of receptor editing in negative selection of immature B cells occurs within a “window-time” during BM lymphopoesis (68). The size of this window may vary depending on the type and avidity of Ag, and the avidity, the time, and duration of Ag encounter (50). Factors that extend this window, such as overexpression of Bcl2 (80) or the presence of protecting Thy1dull cells (79), can enhance the receptor-editing efficiency.

Receptor editing (revision) in mature B cells

Studies published in the mid-1990s suggested that V(D)J recombination can be reinduced in mature B cells to stimulate further Ig gene rearrangement, a process referred as receptor revision (Fig. 2). These studies have shown RAG induction and DNA double-strand breaks in germinal center B cells after antigenic stimulation, or in vitro upon stimulation with a combination of IL-4 and LPS or IL-4 and CD40 (reviewed in Ref. 1). That these secondary rearrangements are driven by Ag is supported by showing that GC B cells underwent receptor revision after somatic mutation was initiated (30, 81). It is suggested that receptor revision is not tolerance-driven but is rather induced in B cells exposed to Ags to which their receptors bind at low affinity (1), providing an important contribution to the generation of high affinity Abs during affinity maturation (31). However, while receptor revision can significantly expand the B cell repertoire, expression of a new HC or LC may also provide these B cells with self-reactivity as described in several autoimmune models (reviewed in Ref. 31).

Phenotypic analysis of B cells undergoing receptor revision revealed many similarities with immature BM B cells including expression of GL-7, IL-7R, SL chain components, and TdT (reviewed in Refs. 1 and 31). These observations suggest that secondary recombination occurs in immature B cells that have migrated to the periphery. Using different RAG-indicator mouse models, where expression of RAG can be monitored by coexpression of GFP, three independent studies show that RAG/GFP appears only in peripheral B cells with an immature phenotype (82–84). These mouse models were later used to show that RAG-expressing immature B cells are recruited into the germinal center during an immune response, but this recruitment is Ag-independent (85, 86). The conclusion suggested by these studies is that the Ig rearrangements in the GC arise from immature B cells migrating from the BM. To reconcile this conclusion with the detection of receptor revision in cells undergoing somatic hyper mutation (SHM), one would assume that immature B cells can participate in the GC response, or that receptor revision is a very rare event. Studies in Fas-deficient mice revealed that IgM− cells, generated by a stop mutation in its expressed Igk gene, are able to participate in the germinal center reaction (81), and that μ-deficient pro-B cells could undergo class switch recombination and mature in μMT/Δpr mice (87). However, sequencing analysis of human B cells revealed that LC receptor revision occurs at very low frequency (<3%), and that V_{H} replacement does not accompany somatic mutations or that such cells are counterselected (88).

An alternative explanation for the induction of V(D)J recombination in GC B cells is the lack of positive selection. As BCR expression and signaling is critical for B cell survival (89), it is possible that acquisition of SHM may affect the interaction between HC and LC. This may result in improper assembly of the BCR and failure to maintain the continuous positive selection signal required for survival (89). There are several examples that changes applied to the CDR3 region (3, 5, 90, 91), or mutation in the A1 constant region (92), affect HC and LC pairing, the process of B cell selection, and the Ab repertoire. In developing B cells, such defective signals stimulate receptor editing (46). It is yet to be directly shown whether such events can also stimulate V(D)J recombination in mature cells.

Conclusions

Positive and negative selection checkpoints are set in B lymphopoiesis to test the competence of the BCR. Cells that fail to fulfill appropriate receptor requirements are developmentally arrested. Such cells stimulate secondary V(D)J recombination and undergo receptor editing to express a new receptor. Receptor editing is thought to “repair” receptor genes encoding a defective and/or self-reactive BCR, and is stimulated by BCR signals that are too low or too high (Fig. 3). Hence, appropriate BCR tonic signals are required for B cell development and survival. Modification of these signals, such as interaction with self Ag or the expression of signaling incompetent receptor, blocks developmental progression and stimulates receptor editing.

References


