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B cell receptor editing in tolerance and autoimmunity

Hilla Azulay-Debby[1], Doron Melamed[1,2]

[1] Department of Immunology, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Israel [2] The Rappaport Family Institute for Research in the Medical Sciences, Israel

TABLE OF CONTENTS

1. ABSTRACT

The random assembly of immunoglobulin (Ig) genes often creates a B cell receptor that is self-reactive, and such cells are subjected to negative selection. A primary mechanism to extinguish this self-reactivity is receptor editing, which allows continued recombination of Ig genes and replacement of the self-reactive receptor with a new innocuous receptor. Recent data now suggest that receptor editing may also promote autoimmunity in an autoimmune context. This mechanism has also been implicated in the process of B cell positive selection and maturation. Here we discuss the contribution of receptor editing in B-lymphopoiesis and its importance for B cell tolerance and autoimmunity.

2. INTRODUCTION

All individuals are tolerant of their own potentially antigenic substances, and failure of this self tolerance is the fundamental cause of autoimmunity. Autoimmune diseases can be systemic (such as Systemic lupus erythematosus – SLE or rheumatoid arthritis - RA) or tissue specific (such as diabetes and multiple sclerosis – MS), and are thought to result from three interacting components: genetic variants, environmental triggers and immune dysregulation (1). B lymphocytes are important mediators of autoimmunity as autoantibodies are the primary cause of many systemic (such as SLE and RA) and organ-specific (such as Graves' disease) diseases. In addition, B lymphocytes promote the evolution and differentiation of effector T cells, regulating the T-B cell interaction and the function of antigen-presenting dendritic cells (2-4). Recently, it has been proposed that B cells have more essential functions in regulating immune responses than had previously been appreciated (3), and abnormalities of these functions can contribute to the development of autoimmune diseases. These abnormalities can result from intrinsic factors, such as altered B cell receptor (BCR) signaling intermediaries and co-receptor expression (reviewed in (5,6)), or extrinsic factors, such as availability and responsiveness to BAFF (7), leading to increased survival and/or hyper-responsiveness of autoreactive B cells. However, the mechanism by which B cell autoimmunity initiates is not known and several mechanisms have been proposed, including antigenic

mimicry and bystander activation of autoreactive clones that is mediated by products of microorganisms such as LPS and bacterial DNA, acting as adjuvants (4,8,9). Such clones normally comprise part of the peripheral repertoire (10,11), but are kept in low frequency and mostly in an unresponsive state due to central and peripheral mechanisms of self tolerance (12-18). Here, we review the importance of one of these mechanisms, i.e. receptor editing, in establishing self-tolerance and its potential contribution to autoimmunity.

3. B CELL DEVELOPMENT AND TOLERANCE ESTABLISHMENT

The B cell repertoire is encoded in the genome and is constructed through two processes of genomic modifications. The first process occurs during lymphocyte development, where V(D)J recombination randomly assembles variable (V), diversity (D) and joining (J) BCR genes. The second process is somatic hypermutation (SHM), which substitutes single nucleotides of BCR genes during an immune response (12). These random processes of genomic modifications often result in the construction of self-reactive receptors, which are eliminated by tolerance mechanisms (19). B cell development in the bone marrow (BM) is guided by the successive attempts to assemble and express surface immunoglobulin (Ig). During their development, B-lineage precursors proliferate and progress through highly regulated selection check-points leading to the generation of immature, IgM-expressing B cells (20,21). At this stage, B cells can interact with self-antigen and are more sensitive to tolerance induction and negative selection than are mature stage B cells (22,23). Both mathematical models and experimental data show that 55-75% of the BCRs generated in the BM are self-reactive (19,24). Most of this self-reactivity is extinguished in the BM and referred as central tolerance. It has been proposed by Burnet's clonal selection theory (25), and proved experimentally later, that autoreactive B cells are eliminated by apoptosis in a mechanism called clonal deletion. It has later been shown that clonal deletion is a major mechanism for membrane-immobilized autoantigens (14,26,27), whereas soluble low-avidity self-antigens impose a state of functional inactivation, a mechanism called clonal anergy (28,29). More recent studies proposed a third mechanism, receptor editing, to operate during negative selection. This mechanism allows autoreactive B cells to escape clonal deletion by revising their antigen receptors through a process of secondary Ig gene rearrangements at the light chain gene locus (16,30). Secondary rearrangements have been demonstrated by re-induction or persistence of RAG-1 and RAG-2 as well as alternation in Jκ gene usage and increased λ-expressing cells in normal and transgenic (Tg) immature B cells undergoing negative selection (31-35). B cells surviving central tolerance migrate from the BM to the spleen (called transitional B cells) to complete their development, a process referred as positive selection, and this transition is regulated by "tonic" ligand-independent BCR signaling competence (36-38). Recent studies have shown that developing B cells failing to achieve appropriate tonic signals are developmentally arrested and undergo extensive receptor editing (37-40). Thus, receptor editing appears to be an important mechanism contributing to both negative and positive selection of BM B cells. Despite of all these tolerance mechanisms, some mature B cells bearing autoreactive receptors are found in the periphery. Many of these cells, however, express low-affinity antibodies or are functionally inactivated (41). In lupus patients, however, these autoreactive cells can reach 25-50% of the peripheral B cells suggesting a defective central tolerance mechanism (42). Autoimmunity of these cells, as well as of B cells acquiring self reactivity by SHM during germinal center reaction is, in most cases, prevented by lack of T-cell help and through Fas-mediated apoptosis, which are referred to as peripheral tolerance mechanisms (12-15).

4. SECONDARY DNA REARRANGEMENTS AND RECEPTOR EDITING AT IMMUNOGLOBULIN GENE LOCI

B cell development is guided by the successive attempts to assemble and express Ig genes, as mice defective in recombinase machinery (43-46), or incapable to express μH (47,48), have a complete block in B lymphopoiesis at the pro-B stage. Several selection check-points 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 non-self receptors that are functionally responsive. It has been first thought that B cells who fail to fulfill both requirements abort developmental progression and undergo apoptosis in the BM. However, it has later been shown that such B cells can undergo secondary Ig gene rearrangements as an alternative salvage pathway. These studies suggested that B cells expressing defective and/or self-reactive receptors undergo secondary Ig gene rearrangement to replace this receptor, rather then 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 (16). Secondary V(D)J recombination has been demonstrated both at the heavy chain (HC) and the light chain (LC) loci, though through different mechanisms. At the kappa locus, secondary recombination replaces the entire pre-existing VκJκ by recombination of an upstream Vκ to a downstream Jκ (Figure 1). 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 utilizes cryptic recombination signal sequences, embedded in many V_H genes, to allow upstream V_H genes to recombine with the existing VDJ and to produce hybrid V_H genes (Figure 1) (16). Recently, direct VH-JH recombination has also been demonstrated in DH-deficient mice, thereby violating the 12/23 role, but this process appears to be inefficient and out-competed by a wild-type allele (49).

4.1. The heavy chain locus

Assembly of the V, D and J genes allows synthesis μH protein that pairs with the surrogate light (SL) chain components, VpreB and λ5, and to be expressed as pre-BCR (reviewed in (17,20)). The signals generated by the pre-BCR are necessary for all pre-B-cell-dependent

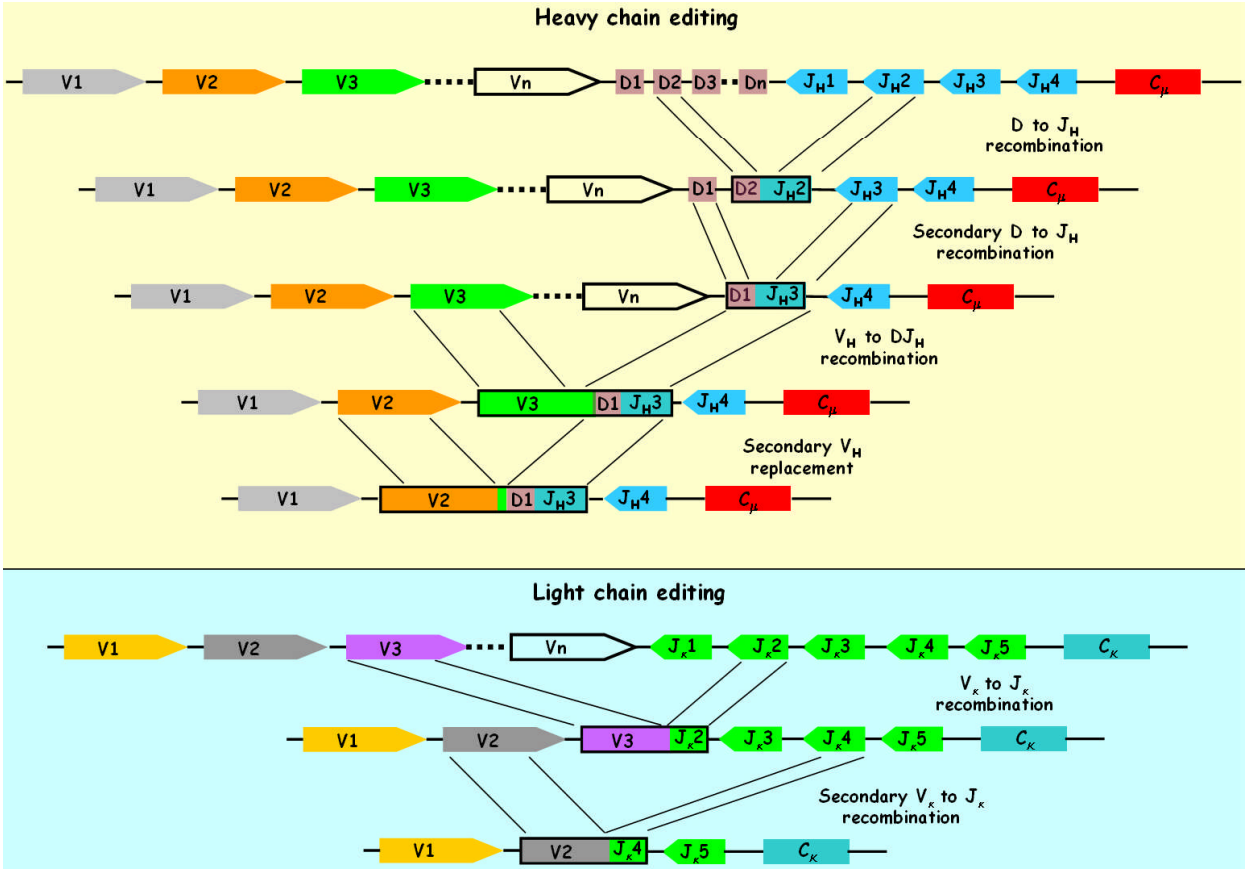

Figure 1. Implications of receptor editing at the heavy and light chain loci. At the HC locus, primary DJ can be replaced by secondary recombination of an upstream D to downstream J by deletion DNA. V_H replacement can occur by secondary recombination of an upstream V_H into an embedded heptamer found at the 3' end of many V_H genes and replacing most of the VDJ. At the kappa chain locus primary VκJκ can be replaced by secondary recombination of an upstream Vκ to downstream Jκ. This can occur by deletion (shown here) or inversion (not shown), depending on the transcriptional orientation of the recombining genes.

processes including IgH allelic exclusion, positive selection and proliferative expansion and proB-to-preB developmental progression (20,50,51). It is not clear whether these signals are generated from pre-BCR aggregation (17), or mediated by an interaction with putative ligand expressed on stromal cells (52). However, failure to express the pre-BCR abrogates allelic exclusion and allows ongoing V(D)J recombination (17,20), and lack or insufficient pre-BCR signaling imposes developmental arrest in several signaling mutated mice (reviewed in (53-55)). Hence, expression and signaling of the pre-BCR are critical for positive selection of pro-B cells, and inappropriate expression and/or signaling of the pre-BCR may lead to secondary Ig gene rearrangements in an attempt to replace it.

Secondary rearrangements at the HC locus have first been shown in a pre-B cell line carrying a primary D_HJ_H rearrangement. In these cells the primary D_HJ_H was replaced by secondary D_H-to-J_H recombination (56) (Figure 1). As D_HJ_H encodes a Dμ protein, which pairs with SL chain proteins and transmits signals that are essential for the Dμ counter-selection and abortion of B cell development (57,58), it is possible that secondary rearrangements replace Dμ elements that are read in the counter-selected reading frame 2 (57). Another form of secondary Ig gene rearrangement at the IgH locus is by V_H segment replacement, which was first demonstrated in pre-B lymphoma cells. Such V_H replacement has been shown on both productive and non-productive alleles (59,60). Secondary rearrangements by V_H replacements are mediated by conserved heptameric sequences embedded at the 3' end of many V_H genes (60), which are carried by most of human V_H genes (61) (Figure 1). However, the embedded heptamer is far less efficient in mediating V(D)J recombination relative to the conventional sequence (62).

The biological significance of V_H replacement was then revealed in studies of a knock-in mouse carrying an anti-DNA antibody IgH transgene in the J_H locus. Editing of the inserted transgene was carried out by recombination of upstream endogenous V_H, or D_H or both genes, replacing the transgenic V_H gene (63). In these knock-in mice, B cell development is almost normally mediated through pre-BCR, with locus specific competence to undergo receptor editing (64). In vivo V_H gene

replacement has also been described in other gene-targeted mouse models (65-68) and in human rheumatoid arthritis (69). It was first thought that V_H replacement in these mice is triggered by tolerance signaling. However, several studies have demonstrated that V_H to VDJ recombination includes additional "N" nucleotides, implicating that the majority of V_H replacement events occur at the pro-B stage, in which L chain gene segments are still in germline configuration (63,66,68). Therefore, it is likely that tolerance signaling through the BCR does not mediate V_H replacement. Instead, it is possible that V_H replacement is activated in cells that fail to express competent pre-BCR. One cause for this is when the produced μH chain is unable to associate with the SL chain. There are several findings supporting this assumption. First, about 10% of pre-B cells express μH chains in the cytoplasm, which half of them fail to pair with SL chains (20,70). Second, various μH chains that cannot associate with SL chains have been identified (70). Third, approximately 20% of pre-B cells that express μH chains use the V_H81x, but this V_H is rarely found in peripheral B cells (20,70), probably because V_H81x μH chain cannot associate with SL chain (70). Furthermore, transgenic mice carrying a VH81x μH Tg, cannot form a pre-BCR, and mature B cells in these mice do not express the transgene (71). V_H replacement may also be activated in cells expressing signaling-incompetent BCR, which fail to undergo positive selection. Studies have shown that CD19 deficiency (72) or mutated pre-BCR (73) generate inappropriate tonic signaling, and B cell development in these mice is impaired. However, the extent of V_H replacement in these models has not been studied. Another explanation for the occurrence of V_H replacement during pre-BCR formation is repertoire diversification (74). This is supported by demonstrating V_H replacements in non-autoimmune context obtained in gene targeted mice (67,75) and by the fact that formation of such V_H hybrids extends the CDR3 diversity.

4.2. The light chain locus

Most developing B cells rearrange light chain genes at the pre-B stage, after a productive and functional μH chain is produced. Studies have shown that some light chain rearrangements occur before or at the same time with heavy chain rearrangements (76), and such light chains can replace the SL chain and rescue some B lymphopoiesis in λ5-deficient mice (77). A productive light chain pairs with the heavy chain and replaces the SL chain to construct the IgM antigen receptor and to promote further development into an immature stage. Spontaneous class switch recombination (CSR) may also occur in B lymphopoiesis, thus generating IgG-expressing B cells in the BM (78,79). The newly formed BCR is critical for two major processes: 1) negative selection of self-binding specificities, and, 2) the generation of appropriate tonic signals to promote B cell maturation and migration from the BM to the spleen, a process referred as positive selection. Studies show that development of B cells that fail negative or positive selection is aborted and such cells primarily activate the receptor editing mechanism in an attempt to modify the BCR (35,37).

Both mathematical models (24) and recent experimental data (19) indicate that the majority of the pre-selected antibody repertoire in the BM is autoreactive (55-75%). This autoreactivity is purged by immune tolerance during development and maturation. Hence, in negative selection, receptor editing is induced in immature B cells by BCR ligation with self-ligand. It is efficiently stimulated by membrane antigens with affinities as low as $5x10^4M^{-1}$ (80). The regulation of RAG genes expression during the induction of receptor editing has recently been shown to occur at the transcription level and to depend on NFκB/rel activation by BCR signaling (81) and the adaptor protein BASH (82). These secondary Ig gene rearrangements also require expression and function of E2A proteins (83). Modification of the BCR signals by lack of CD19, CD45, or Btk, have no effect on receptor editing stimulated by multivalent membrane-bound antigen (84-86). This activation appears to be independent of c-Myc expression, an important oncoprotein that is activated in BCR-induced apoptosis (87). Studies have shown that this tolerance-mediated receptor editing is developmentally regulated and lost in late immature cells in the BM (88), coinciding with the acquisition of BCR signaling properties typical of mature cells (22,89). However, in the case of soluble Ag, this editing "window" is extended and may also include late immature B cells in the spleen (90). Other studies have shown that activation of receptor editing depends on BM micro environment, which is provided by $Thy1^{dull}$ cells (91), and that the efficiency of receptor editing depends on the number of available Jκ on the expressed Vκ allele (92). Hence, receptor editing in negative selection of immature B cells is thought to occur within a "window-time" during BM lymphopoiesis (93). The size of this window may vary depending on the type and concentration of antigen, the avidity, the time and duration of antigen encounter (5). Factors that extend this "window" such as overexpression of Bcl2 (94) or the presence of protecting $Thy1^{dull}$ cells (91), can enhance the receptor editing efficiency.

Receptor editing in negative selection has first been shown in Ig Tg mice specific to MHC class I or DNA (31,33). It was not activated in these mouse models when bred on RAG-deficient background, confirming that alternation of BCR specificity is due to secondary recombination (95). Appreciation of receptor editing as a main mechanism in negative selection has been established utilizing different in vitro bone marrow culture systems (34,35), and utilizing autoantibody targeted mouse models (knock-in). These mouse models have shown that primary autoreactive-encoding VκJκ is inactivated by RS recombination or replaced by new recombination of an upstream Vκ to downstream Jκ, in 85-95% of the peripheral B cells (64,98) (Figure 1). Further, in central tolerance mouse models, where light chain is targeted into its physiological locus, it has recently been shown that most of B cell tolerance is imposed by receptor editing, in the absence of significant clonal deletion (96,97). Thus, receptor editing is very efficient in rescuing autoreactive B cells, although multiple rearrangements are often required until appropriate Vκ editor is selected (99). High frequency of receptor editing was also demonstrated in normal mouse and human B cells (100,101), in mice transgenic for

membrane-expressed anti-Cκ single-chain antibody fusion (102), and in peripheral human B cells expressing SL chain and autoreactive receptors (103). Using mice that carry human and mouse Cκ genes it was estimated that 25% of the antibody molecules are produced by light chain gene replacement, providing receptor editing with a major contribution in generating the antibody repertoire (93). Recently it has been shown that editing is the main mechanism in B cell negative selection to membrane autoantigen, but its efficiency is limited by the number of tries (96). As V(D)J recombination is affected by factors such as differences within recombination signal sequences, promoter regions and other cis-acting elements, certain V_L genes are overrepresented (46). Under these circumstances, the importance of receptor editing is also to promote representation of Ig genes that are disfavored by the primary V(D)J recombination (93).

B cell maturation is accompanied with extensive cell death. Osmond and colleagues have estimated that mice produce about 2 x 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 (104). Although many of these cells are the targets of negative selection, accumulated data now suggest that B cell maturation and migration from the BM is limited by positive selection (reviewed in (18,105)), and that these signals are transmitted by the BCR and its signaling components (106,107). Recent data suggest that these signals are ligand-independent (reviewed in (18,53)), and generated by the proper assembly and expression of an oligomeric BCR (17,53,108). This tonic activity has been implicated with the physiologic gene expression programs and the induction of RAG genes in developing B and T lymphocytes (109,110), and in positive selection of B-1 versus B-2 B cell development (111). In BCR transgenic mice lacking the CD19 co-receptor (37) or the src kinase Lyn (40), there is an increased number of edited B cells. Further studies revealed that these cells have inappropriate tonic signals, they fail positive selection and undergo developmental arrest, and consequentially activate receptor editing. These studies have later been extended to show the same phenomenon in non-Tg B cells (38), to suggest that receptor editing is an important mechanism in B cell positive selection. This suggestion is strengthen by showing that receptor editing is directly stimulated by modifying BCR tonic signaling in immature B cells (109). Later studies have confirmed these observations by showing activation of receptor editing in immature B cells upon ablation of the BCR by Cre-loxP recombination (39). Thus, failure of positive selection activates secondary light chain gene recombination in order to edit and express a new receptor that is signaling competent.

Another form of receptor editing has been described in peripheral B cells and referred as receptor revision. Several reports showed RAG mRNA and protein expression in germinal centers and in vitro IgD^+ B cells activated by CD40 (or LPS) and IL4 (112,113). These RAG B cells express surface markers characteristic of pro-B or pre-B cells such as λ5, VpreB, TdT, GL7, low levels of B220, and IL7R (112,114-118). However, the similarities between bone marrow and peripheral RAG^+ B cells raised the possibility that these cells might in fact represent B cell precursors that have migrated to the periphery. Using different RAG-GFP indicator mouse models, three independent studies supported this hypothesis (119-121). These mouse models were later used to show that RAG-expressing early B cell precursors are recruited from the BM into the germinal center during an immune response, but this recruitment is antigen-independent (122,123). Interestingly, receptor editing in peripheral B cells has recently been shown in non-autoimmune mice immunized with a peptide mimetope of DNA, suggesting that this revision mechanism may function to maintain peripheral tolerance (10).

5. CONTRIBUTION OF RECEPTOR EDITING TO AUTOIMMUNITY

While receptor editing has been first described as a physiological relevance mechanism for immune tolerance, recent studies suggest that editing might also promote autoimmunity. These studies have used conventional or site directed transgenes encoding autoantibody to suggest two possibilities for promoting autoimmunity by receptor editing. One possibility is defective editing in the BM, which does not substitute the autoreactive light chain, but rather, results in an expression of a second light chain that is not autoreactive and can promote further development and maturation. The second possibility is the acquisition of autoreactivity by receptor revision in the periphery (Figure 2).

Autoreactivity in the BM is tested in the immature B cell check-point and further developmental progression of these cells is aborted (35,124). One mechanism by which defective receptor editing can rescue these cells is by rearranging a second light chain that pairs with the H chain, but unlike the first light chain, encodes an innocuous receptor. This has been demonstrated in mice targeted with H chain that encodes self-reactivity when paired with most light chains (99,125) and in anti-MHC knock-in mice (126), resulting in allelic or isotypic inclusion of the autoreactive light chain. It is hypothesized that the multireactive B cell is immune from further editing as it has reached a state of tolerance, and can utilize the non-autoreactive "editor" light chain to "dilute" autoreactivity and to promote development and maturation (127) (Figure 2, upper panel). In the contest of autoimmune-prone mutations, these cells have been shown to proliferate and to secrete autoantibodies (128). A similar diluting effect has been proposed for continued expression of SL chain in human B cells expressing autoreactive receptors (103). The use of defective editing for escaping of tolerance has recently been proven in a B cell nuclear transfer mouse model (129). Hence, receptor editing may promote autoreactivity by creating dual receptor expressing cells and allelic inclusion of autoreactive light chains, which may lead to the production of autoantibodies.

There are several mouse models -carrying an Ig gene encoding autoantibodies that have been used in order to understand how B cells producing these autoantibodies

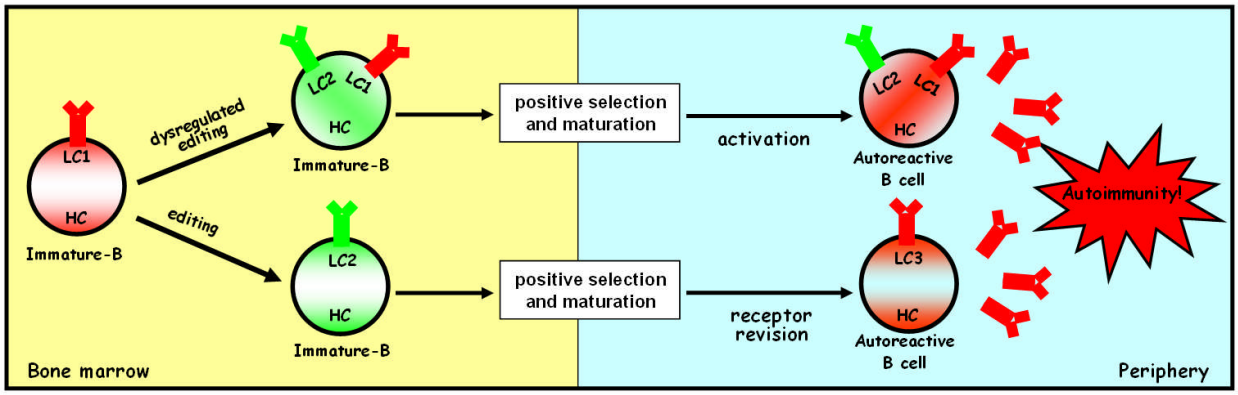

Figure 2. Possible models for promoting B cell autoimmunity by receptor editing. Immature B cells expressing an autoreactive BCR, composed by a heavy chain (HC) and light chain 1(LC1), undergo dysregulated receptor editing, resulting in the expression of a second, non-autoreactive, "editor" light chain (LC2) (upper pathway). This editor light chain "dilutes" the cell autoreactivity and allows positive selection and maturation. Upon activation in the periphery, this cell may secrete autoantibodies. An alternative possibility is that immature B cells undergo appropriate editing in the BM and replace the autoreactive LC1 with an innocuous LC2 (lower pathway). This non-autoreactive B cell may undergo dysregulated receptor revision in the periphery and replace the innocuous LC2 with a new, autoreactive LC3, which may lead to the secretion of autoantibodies.

are controlled in healthy individuals, and how they contribute to the generation of an autoimmune disease (130). When the autoantibody transgenes are derived from SLE disease-associated specificities, such as anti-double-stranded DNA, and the mouse genetic background is normal, tolerance can be induced in one of the three optional mechanisms (deletion, anergy or receptor editing), depending on the mouse model. However, induction of chronic graft-versus-host disease (GVHD), or breeding on an autoimmune-prone genetic backgrounds results in the production of autoantibodies (131-136). Interestingly, most of the autoantibodies produced in these models are edited and utilized endogenous H or L chains (131,132,137). On the other hand, the same process in the tolerant anti-hen egg lysozyme (HEL)-soluble HEL double Tg model resulted in breakdown of self-tolerance, but also in reduced levels of receptor editing in the BM (138). A possible interpretation of these results is that antinuclear autoantibodies are generated in these mice as a consequence of dysregulated receptor revision in peripheral non-autoreactive or low-affinity B cell population (139,140) (Figure 2, lower panel). This is supported by studies showing that in the non-autoimmune animal, high affinity anti-DNA-expressing B cells are centrally deleted in the BM very efficiently (141), and, in contrast, low affinity anti-DNA B cells can reach the periphery as anergic cells (92,142). The efficient central deletion of autoreactive B cells is also expected to be intact in animal models of SLE, as has been shown for membrane-bound antigen (143), in which the autoimmune driving force is confined to the periphery by processes such as cGVH (134,137). Therefore, in these SLE models the high affinity autoreactive B cells are likely to be generated in the periphery, as has been suggested (134,136).

The possibility that deficiencies in central or peripheral receptor editing could play a role in generating human autoimmunity has been suggested. Defective receptor editing was found in the vast majority of lupus patients (144,145). For example, editing at the Vκ locus is used more apparently in a patient with untreated SLE than in healthy individuals (146). Similarly, B-cells that display features consistent with receptor editing are enriched in the joints of patients with rheumatoid arthritis (116). Evidence for receptor editing events was also found in B cells from cerebrospinal fluid of multiple sclerosis patients (147). Dysfunctional receptor editing can also occur during autoantibody generation in autoimmune thyroid diseases like Graves' disease and Hashimoto's thyroiditis (147). In patients with Sjogren's syndrome defects in editing Ig receptors may contribute to the emergence of autoimmunity (148). There is no evidence for a major molecular abnormality in receptor editing in different autoimmune diseases, nor in patients with a specific disease. However, abnormalities in subsequent events, such as the degree of receptor editing/receptor revision, somatic hypermutation, and positive and negative selection, are responsible for modifying the composition of the peripheral B-cell repertoire. Identification of the mechanisms that control the Ig variable gene and B-cell repertoire may allow new therapeutic approaches for autoimmunity.

6. PERSPECTIVE

Receptor editing is activated during B lymphopoiesis in both positive and negative selection check-points. In most cases, this mechanism is appropriately regulated and is beneficial for normal homeostasis. However, if dysregulated it can be harmful and may promote autoimmunity. The driving biochemical signals that stimulate receptor editing in developing B cells are not fully understood. Also it is unclear whether or not receptor editing is indeed linked to the BM or may also be a physiologically relevant event in the periphery. This is highly important to better understand the biological perspectives of human autoimmune diseases and the selection processes of autoimmune B cell clones. Another important aspect is the role of editing in positive selection,

and how this editing may or may not be different than that stimulated during negative selection.

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Send correspondence to: Dr Doron Melamed, Department of Immunology, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel, Tel: 972-4-829-5237, Fax: 972-4-829-5245, E-mail: melamedd@techunix.technion.ac.il

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