SECONDARY RECEPTOR EDITING IN THE GENERATION OF AUTOIMMUNITY

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Abstract

Receptor editing is the process that replaces the heavy chain or light chain variable region genes in a B-cell immunoglobulin receptor that is already productively rearranged. It is a major mechanism in the bone marrow for maintaining B-cell tolerance to autoantigens. We propose that a pathological autoimmune process can use receptor editing to induce the *de novo* creation and activation of B cells with autoreactive receptors in the peripheral immune system.

Keywords

receptor editing; autoimmunity; B cell; autoreactivity

Receptor editing is the replacement of a productively rearranged immunoglobulin light chain or heavy chain variable region gene with another one, either on the same chromosome, on the homologous chromosome, or through an isotype switch (*i.e.,* κ light chain to λ light chain) [1]. Evidence from both transgenic and wild type mice have demonstrated that this process is an important mechanism in the maintenance of tolerance at the earlier stages of B-cell ontogeny in the bone marrow. If a developing B cell expresses a heavy chain/light chain combination that recognizes an autoantigen with sufficient affinity, it can be signaled to continue to express the Ig gene recombination machinery, including the *rag1* and *rag2* genes. It thereby undergoes further gene rearrangements that replace either the light chain or the heavy chain variable regions, so that a new B-cell receptor is produced that is not autoreactive.

Thus, the role of receptor editing in the bone marrow is well established in the suppression of autoimmunity. More controversial is the possibility of receptor editing in the peripheral lymphoid system, also termed receptor revision [2]. Although several laboratories have demonstrated the expression of the *rag* genes in the spleen and lymph nodes, particularly after an antigenic challenge, much of this phenomenon has been explained by the peripheralization of immature B cells [3]. *In vitro* experiments in mice have demonstrated that B cells can apparently be induced to upregulate *rag* genes by stimulation with LPS and IL-4 [4,5], although it cannot be ruled out that these observations are explained by selective survival and proliferation of immature B cells.

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In the setting of autoimmune disease, particularly lupus, the characterization of Ig gene usage by autoantibody producing B cells has shown increased receptor editing [6,7]. In general, however, it cannot be ascertained where and when in B-cell ontogeny this process might have occurred. It has been assumed that the increased editing is a result of a frustrated effort by the immune system to suppress autoimmunity, presumably in the bone marrow. However, some data are consistent with the maintenance of tolerance by peripheral receptor editing [8], and in one case the timing of somatic hypermutation in the Ig gene sequence suggested that peripheral receptor editing actually resulted in the creation of an autoantibody [9].

In this regard, work from Youinou and colleagues has provided striking evidence for the increased expression of the *rag* genes *in vivo* in the setting of human autoimmunity or through *in vitro* stimulation by anti-IgM and other signals, including IL-6 [10–13]. Whether this is truly a reinduction of the recombination machinery or a selective survival of *rag* expressing cells cannot be determined with certainty. However, the parallel between this work and the results in the mouse system (admittedly with a different cytokine) is provocative.

We have more recently approached this issue in a highly defined system in which we can differentiate more clearly processes that occur at various stages of B-cell ontogeny. The transfer of CD4 T cells from normal mice (bm12) into normal mice of another strain (C57BL/6) that differs only in the MHC class II locus induces a chronic graft-versus-host (cGVH) syndrome that produces autoantibodies and immunopathology that parallel spontaneous lupus [14]. The model depends on cognate interaction of the donor CD4 T cells with the recipient B cells [15]. We have modified this system such that we can separately transfer the stimulating CD4 T cells and the responding B cells to an immunodeficient (*rag1* knockout) mouse, and produce the same response of anti-DNA and anti-chromatin autoantibodies. Thus, we can preselect the transferred B cells in various ways, and determine which B cells are capable of losing tolerance in this system. We thereby have shown, perhaps surprisingly, that the B cells that respond best after transfer are mature peripheral B cells, *i.e.*, those that have lost the CD93 marker [16].

Some of our earlier work with the cGVH model had already revealed anomalies related to receptor editing. In one set of experiments, we utilized the hen egg lysozyme (HEL)-anti-HEL double transgenic system developed by Goodnow [17,18]. These mice (on a C57BL/6 background) have an IgM/IgD, κ, conventional transgene that results in a high affinity anti-HEL antibody. In the presence of a transgene that produces a soluble form of HEL, the double transgenic mice achieve tolerance largely by downregulating their surface Ig receptor. They also show evidence of increased light chain receptor editing in the bone marrow [19]. The transfer of MHC Class II incompatible CD4 T cells form bm12 mice into the HEL/anti-HEL double transgenic recipients breaks this 'self' tolerance, and results in the production of high levels of anti-HEL serum antibodies [17]. This is accompanied by a decreased level of receptor editing in the bone marrow, as shown by lower numbers of non-HEL binding B cells, decreased levels of endogenous κ chain rearrangements, and decreased *rag2* expression [20].

In another system, we have utilized recipient mice on a normal C57BL/6 background that also expressed a site-directed immunoglobulin heavy chain transgene that came from an anti-DNA monoclonal antibody. This transgene, named *56R*, had been modified by the insertion of an additional arginine residue, which resulted in even stronger binding with double-stranded DNA. In fact, very few light chains are able to pair with 56R to produce an immunoglobulin that *isn't* an anti-DNA autoantibody [21]. BALB/c mice with the 56R transgene undergo extensive light chain editing in order to express the 56R transgene with a

light chain that does not result in autoreactivity. In the C57BL/6 background, however, some 56R-expressing anti-DNA B cells do escape tolerance, in a T-independent process that is not yet understood [22].

Not unexpectedly, the transfer of MHC class II incompatible CD4 T cells from bm12 mice into C57BL/6.*56R* recipients resulted in increased levels of anti-DNA antibodies as part of the cGVH reaction [23]. Surprisingly, however, the serum antibody detected was produced not only by the chromosome containing the heavy-chain transgene (as marked by allotype), but also from the endogenous heavy chain chromosome. PCR typing of the heavy chain variable regions from spontaneous hybridomas from these cGVH mice showed a pattern of gene usage that was even more remarkable. Every one of the 56 IgG producing hybridomas failed to show evidence of the *56R* transgene, although 18/22 IgM hybridomas were transgene positive. Thirty-two of the IgG hybridomas were IgG2a anti-DNA (and thus could be tested serologically for allotype), and of these 30 used the endogenous Igh genes (IgG2a^b, or more properly designated as IgG2c). Two used the transgenic chromosome (IgG2a^a), and yet failed to reveal the transgene by PCR. Sequencing showed that they had rearranged a different VH gene, presumably by the process of receptor invasion at the site of a cryptic heptamer [2]. Somehow the loss of tolerance induced by the abnormal T-cell help of the allogeneic T cells in the cGVH bypassed the use of a pre-existing anti-DNA heavy chain gene and selected other heavy chain variable region genes from the repertoire.

How can the remarkable switch away from an anti-DNA site directed transgenic heavy chain gene be understood in the context of loss of B-cell tolerance to DNA forced by abnormal allogeneic T-cell help? As the transgene bearing cells are prevalent in C57BL/6.*56R* mice, the edited B cells clearly are undergoing selective activation in this model. We have shown in several mouse lupus models that the b allotype in heavy chains is indeed favored for anti-nuclear autoantibodies; however, this effect is modest, and it would not explain why even the a allotype anti-DNA heavy chain hybridomas no longer expressed the transgene [24,25]. It seems likely that the B cells that expressed heavy-chain edited anti-DNA antibodies must have been subjected to selection by the allogeneic T cells at a stage in B-cell ontogeny not readily available to the *56R$^+$* B cells. We propose that this process occurs in the peripheral lymphoid system. In the initial polyclonal activation of the peripheral B cells by the alloreactive T cells, receptor editing would occur and produce some combinations of new heavy and new (or old) light chains which are DNA reactive. In the ubiquitous presence of antigen and the ongoing help from alloreactive T cells, these anti-DNA B cells would become fully activated and secrete autoantibodies. In contrast, in the absence of peripheral editing, a B cell would either not express an anti-DNA Ig or would be in a state of anergy that would not be susceptible to activation by allogeneic help.

This switching of Ig chain usage in the periphery could occur by secondary receptor revision involving further gene rearrangements. This would imply either the persistent expression of the genetic recombination machinery, including the *rag1* and *rag2* genes, or its reinduction under the stimulus of the cGVH. This possibility is supported by Youinou's results with human cells [10–12]. It is also possible that some B cells may migrate to the periphery already harboring two productively rearranged Ig chains, one of which would result in an autospecificity and would not normally be expressed on the cell surface [26,27]. This would allow the dual expressing cell to escape tolerization, and the autoreactive Ig would be activated during the cGVH.

We are now testing the potential for receptor editing to occur in mature B cells under the stimulus of alloreactive T cell help. If this occurs, it would imply the *de novo* creation of anti-DNA B cells in the periphery, when most of the tolerance checkpoints have already been bypassed. An important final checkpoint would be the requirement for ongoing T cell

help. In the cGVH, the promiscuity of the alloreactive T cells would provide the mechanism to bridge this final checkpoint. We would postulate that such a process might also occur in spontaneous lupus, and that either abnormal T cell help or abnormal B-cell independence of such help would be key factors in this loss of tolerance that leads to anti-DNA autoantibody production (Figure).

Acknowledgments

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Take-home messages

- Autoreactive B cells in the bone marrow are frequently rescued by a secondary rearrangement of their heavy or light chain genes, which displaces the autoantigen specificity of their immunoglobulin receptor. This process is called *receptor editing*.

- Abnormalities in receptor editing have been described in human lupus and in mouse lupus models, and in other autoimmune diseases.

- In systemic autoimmunity, receptor editing in the periphery may actually create autoimmune B-cell receptors, and thereby bypass the checkpoints for B-cell tolerance.
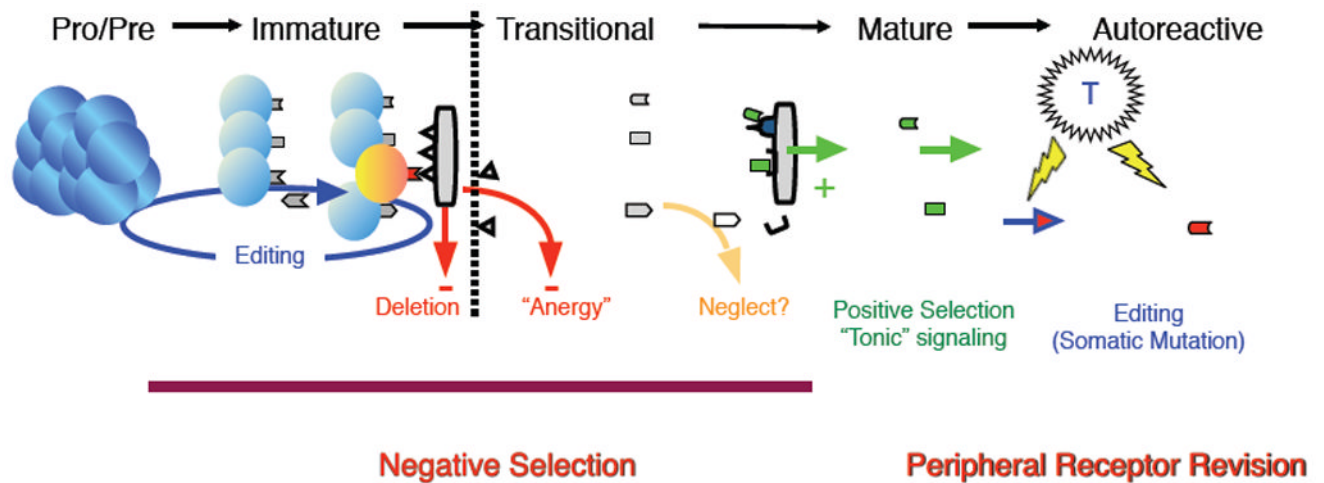
Figure 1. BCR specificity dictates selective outcome at multiple checkpoints
B cells that interact avidly with soluble autoantigens are purged from the active repertoire at several stages of ontogeny by negative selection. We propose an additional process whereby mature B cells can acquire a new autoreactivity by receptor revision or somatic mutation [28]. The creation and survival of such autoreactive cells would depend on abnormal immunoregulation, such as ongoing T-cell help. (Figure modified from M.P. Cancro [29]).