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Receptor Selection in B and T Lymphocytes

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Abstract

The process of clonal selection is a central feature of the immune system, but immune specificity is also regulated by receptor selection, in which the fate of a lymphocyte's antigen receptor is uncoupled from that of the cell itself. Whereas clonal selection controls cell death or survival in response to antigen receptor signaling, receptor selection regulates the process of V(D)J recombination, which can alter or fix antigen receptor specificity. Receptor selection is carried out in both T and B cells and can occur at different stages of lymphocyte differentiation, in which it plays a key role in allelic exclusion, positive selection, receptor editing, and the diversification of the antigen receptor repertoire. Thus, the immune system takes advantage of its control of V(D)J recombination to modify antigen receptors in such a way that self/non-self discrimination is enhanced. New information about receptor editing in T cells and B-1 B cells is also discussed.

Keywords

immunoglobulin genes; T cell receptor; immune tolerance; receptor editing; gene rearrangement

INTRODUCTION

Lymphocytes often express a single antigen receptor despite their genetic potential to simultaneously express many. This fact has focused interest on how expression of multiple receptors is limited. It has recently come to light that cells bearing antigen receptors may actively attempt to express new ones, which can lead to expression of multiple receptors, inactivation of previously expressed receptors, or replacement of old receptors with new ones—a process called receptor editing. Receptor editing occurs through ongoing or renewed antigen receptor gene rearrangement. In this overview, we discuss features of both T cell receptor (TCR) and immunoglobulin (Ig) loci that promote secondary receptor gene rearrangements, and we review the evidence for receptor editing in B and T cells. Receptor editing has recently been the subject of several brief reviews (1–4).

SECONDARY REARRANGEMENTS

V(D)J recombinase rearranges and ligates together dispersed variable (V), diversity (D), and joining (J) minigene elements and brings the newly assembled V(D)J exon in proximity to the constant (C) exons of these genes (see accompanying article by Schatz, "Evolution and Mechanisms of VDJ Recombination," pp. 495–97). An important feature of some antigen receptor genes is the ability to undergo so-called secondary, or nested, rearrangements,

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Since the submission of the manuscript, the cloning by Zachau's group of the murine κ -locus has made extraordinary progress. For information on this fast-moving area and associated recent publications, the reader is referred to their web site: <http://www.med.uni-muenchen.de/biochemie/zachau/kappa.htm>.

which can salvage the functionality of loci with primary out-of-frame rearrangements and favor receptor editing, i.e., the modification of already functional genes. This process is affected by several factors including the number of gene segments in the locus, their organization along the DNA, and the organization and types of recombination signal sequence (RSSs) adjacent to the gene segments in question. Some typical organizations of receptor genes and their influence on secondary rearrangements are shown schematically in Figures 1 and 2. As we discuss below with respect to the well-studied mouse and human systems, some antigen receptor gene loci appear to be particularly specialized to carry out secondary rearrangements, whereas other loci may disfavor them. The organizations of mouse and human antigen receptor gene loci are shown schematically in Figure 3.

Loci That Favor Secondary Rearrangements

Ig- κ —Rearrangements in the mouse involve initial joining of one of ~140 V elements to one of four functional J elements (5) (Figure 3A) (6–12); a similar organization is seen in humans, who have about 66 V elements and 5 J s (13) (Figure 3B) (14). The locus lacks D gene segments; consequently, upon primary V J joining on one allele, secondary rearrangements between remaining upstream V s and downstream J s can occur in a single step (shown schematically in Figure 1B). In the mouse, J 1 and J 2 rearrangements are preferred (15), which retains downstream Js available for secondary rearrangement (16). Furthermore, because many V genes are placed in a transcriptional orientation opposite to the J elements, loci often rearrange by inversion, retaining thereby the entire repertoire of V s for subsequent rearrangements (shown schematically in Figure 2B, bottom). When the V gene segments are in the same transcriptional orientation as the segments to which they rearrange, deletional rearrangements excise intervening DNA, which is permanently lost from the chromosome (Figure 2B, top). Analysis of the Ig- loci in mouse or human B cell lines has shown that a single allele can undergo two or more successive V-J recombinations (16–23). Isolation of the excised circular DNA from sequences intervening in the V/J recombination revealed that they often contained independent V/J joins, indicating that secondary rearrangements are common in the mouse splenic B cells analyzed (24, 25). B cells of mice lacking the locus on one chromosome have increased usage of downstream J s on the remaining allele, consistent with displacement of out-of-frame VJ joins by nested rearrangement (23). Thus, locus structure permits replacement reactions, allowing receptor editing.

TCR α —At the TCR locus, any one of about 100–200 V segments can be joined to one of 50 J elements (Figure 3A, B) (26–29). A primary VJ rearrangement may yield an out-of-frame join that can be replaced by subsequent rearrangement of an upstream V segment with one of the remaining downstream J loci (30). T cells may undergo many nested V - J recombinations (31, 32). In addition, thymocytes rearranging TCR loci retain excised DNA circles containing the TCR locus, usually in a rearranged form (30, 33). Since these episomal DNAs are lost with cell proliferation (30, 34, 35), TCR rearrangements functionally exclude TCR expression. Furthermore, TCR rearrangements favor initial use of J 's at the 5' end of the locus (36), possibly suggesting progressive use over time of 5 - to-3 J 's. The TCR locus organization constrains rearrangement to occur by deletion rather than inversion (27) (shown schematically in Figure 1B) (28, 29).

Loci That Disfavor Secondary Rearrangements

Ig-H—In contrast to the Ig- and TCR loci, some loci are so constructed that they tend to suppress secondary rearrangements, at least through conventional recombination signal sequences. VH genes are arranged in the same transcriptional orientation as the D and J elements (37, 38), forcing VDJ assembly at the Ig-H locus to occur by deletion of the intervening DNA (38) (Figure 3). As a consequence, correction of nonfunctional VDJ

rearrangements by secondary rearrangement is prevented because no more D regions are available for recombination to upstream V_H s. While unrecombined upstream V_H s and downstream J_H s are typically retained, they both have recombination signal sequences carrying 23bp spacers, and therefore they cannot be joined together by recombinase (see Figure 1A).

Despite this, secondary rearrangements can sometimes occur on the Ig-H locus. Early in mouse B cell development, secondary D-to-J joining can replace primary D_H -to- J_H rearrangements by deletion or inversion (39), but this apparently does not occur in humans (40). Furthermore, V_H elements can sometimes undergo secondary recombination to preformed VDJ elements through V_H replacement by targeting noncanonical, but conserved, RSS-like sequences that are present in the 3' end of the rearranged V_H coding sequence (41). V_H replacement is discussed in a later section.

Ig-L λ Loci—The lambda locus in the laboratory mouse is composed of two linked miniclusters spanning about 200 kb (42–47). $V_2, V_{x/J} C_2 C_2$ lies upstream of $V_1/J_3 C_3, J_1-C_1$, but most rearrangements occur between V and J regions of the same cluster (Figure 3A). All rearrangements involve DNA deletions. The recombined $V_1-J_1-C_1$ gene is the most common type of rearrangement (48). Thus, combinatorial diversity is extremely limited in the mouse locus, and receptor editing is precluded (shown schematically in Figure 2A). The human λ -locus has an array of ~70 V genes upstream of seven J-C clusters; each J-C cluster has a single J, and only three of the clusters are functional (Figure 3B) (49). Unlike in the mouse λ -locus, this organization allows nested recombination and receptor editing (50).

Suppressive Rearrangements

RS/kde—An additional mechanism facilitated by receptor gene organization is the ability to preclude function of certain loci by nested rearrangements that can themselves have no functional product. One important example of a rearranging, but noncoding, gene element that illustrates this mechanism is the RS/kde element (“recombining sequence” in mouse, “kappa deleting element” in human) (51). This element inactivates the Ig- κ locus by deletional recombination (52). RS is found ~25 kb downstream of the mouse C κ exon (53) (Figure 3A). (RS should not be confused with the RSS, recombination signal sequence, carried by all rearranging receptor genes). The kde is in a similar position in the human κ -locus (54) (Figure 3B). Both RS and kde contain an RSS sequence with a 23-bp spacer that can recombine through a V(D)J recombinase-dependent process either to unrearranged V s or to noncanonical RSS sites in the J-C intron (25, 51, 55). RS/kde rearrangements occur in ~75% of mouse B cells that express κ -L-chain (56) and in an even higher fraction of human κ -producing cells (13, 57). RS rearrangements also occur on the second allele of approximately 12% of mouse κ -expressing cells (58). Because the RS/kde encodes no protein, these joins are nonfunctional (55, 59). It appears that the RS/kde elements have no other purpose than to inactivate κ -genes, many of which were previously functional (57). Since RS rearrangement appears concurrently with Ig- κ locus recombination, they may be predicted to clear the way for κ expression (59, 60). Thus, κ locus structure favors replacement reactions that allow receptor editing.

$\psi J\alpha/\delta rec$ —A similar process of deletional recombination can occur in the TCR locus, where the J element joins to rec, eliminating the entire TCR locus that is embedded in the TCR locus (Figure 3A,B) (61, 62). This type of non-protein coding recombination is reported to occur in ~70% of human T cells that go on to express TCR (63). Like the J element, RS and kde have homology to J regions (64), but unlike most antigen receptor

pseudogenes, they retain consensus RSSs (51, 52, 55), implying evolutionary conservation of rearrangement function.

QUASI-ORDERED REARRANGEMENTS IN DEVELOPMENT

B Cells

Rearrangements generating antigen receptor genes generally occur in a particular temporal sequence regulated by control of gene accessibility. In mouse B cell development, H-chain genes are assembled first. PreB cells bearing in-frame, functional IgM H-chains (μ -chains) are selected for clonal expansion and differentiation, whereas cells lacking μ -chains are eventually targeted for elimination (65). Cells lacking μ -chains often attempt rearrangements on the second allele, which, if functional, can rescue the cells. This selection process is a major developmental checkpoint that involves the testing of μ -chain based on its ability to associate with surrogate L-chain components λ 5 (66) and VpreB (67) and to mediate transmembrane signals through associated Ig- α / β signal transducers (68–71). Only after a proliferative burst of approximately six cell divisions (72) do cells exit cell cycle; then recombination activity is redirected to the Ig L-chain loci generating sIgM⁺ B cells (73).

Gene Assembly and Lineage Commitment in T Cells—In T cell development, TCR loci are similarly rearranged in a temporal sequence, beginning with DJ rearrangements at the TCR β loci, followed by V α -to-DJ rearrangements, a proliferative burst of those cells expressing functional α -chains, and subsequent redirection of recombinase to the TCR α loci (74–77). In a further parallel to B cell development, testing of the α -chain in T cell development involves protein association with a surrogate chain, pT β (78), and signaling through a preTCR complex (79). Thymocytes lacking α -chains, pT β , or molecules involved in preTCR signaling fail to develop further (80–82). Enforced expression of α -chain transgenes blocks endogenous α -chain gene assembly (83) and promotes development to TCR α gene rearrangement and expression (84).

Expression of a complete α / TCR is clearly insufficient for RAG downregulation (31, 32, 85, 86). α / T cells are subject to the process of positive selection (87), in which cells with a receptor biased to weak MHC self-reactivity are selected to advance in development. This maturation is coincident with the cessation of TCR β rearrangement (88, 89). RAG downregulation can be experimentally induced in thymocytes by antibody cross-linking of the TCR (90). The abundance of V genes and J genes in the TCR β locus allows cells to undergo multiple rearrangement attempts, which may be necessary for efficient positive selection (31, 32, 86).

T cell development in the thymus is further complicated by two factors. First, common precursors can give rise to either α or β T cells, a situation that is regulated in part by the functionality of rearrangements. And, although the antigen receptors of these cells use different protein chains, the TCR α locus is embedded in the TCR β locus (Figure 3) and is physically excised in the process of TCR β rearrangements (27, 29, 91). The α and β loci rearrange prior to α and β loci (92, 93), and an intact β receptor likely promotes development in much the same way as α /pT β heterodimers. Lineage determination is also under control of the Notch pathway (94). Likewise, it has been proposed that α - or β /pT β -mediated signals may affect the Notch pathway itself (95).

Regardless of the details of α vs. β lineage commitment, it is of interest to observe patterns common to the organization and rearrangement of different antigen receptor genes. First, in all lymphocytes, one chain partner gene is preferentially rearranged and expressed before the other. Two waves of RAG gene expression correspond to the points in development in which each locus is recombined (96, 97). After expression of the first chain,

cells are selected for proliferative expansion (78, 96). The initially expressed Ig-H, TCR- α , and TCR- β loci invariably include D elements, whereas loci rearranging later lack D regions (Figure 3). Absence of D regions facilitates receptor editing (Figure 1) but limits potential receptor gene diversity, suggesting that receptor editing may be an important selective force in evolution. The initially rearranging chains are each encoded in a single locus, as is the TCR- β locus, which should theoretically limit dual expression. However, in humans and mice the Ig-L chain is encoded by two or three loci, respectively, each with two alleles that rearrange independently of one another, compounding the problem of allelic exclusion with one of isotypic exclusion.

ALLELIC EXCLUSION

The vast majority of mammalian B cells bear a single antibody heavy and light chain (98–103), despite the ability to produce two, one encoded by each allele. In the mouse, most cells express a single light chain (104, 105), despite the ability to express six (2 alleles \times 3 isotype loci: κ , $2/\lambda$, $1/3$). As discussed below, allelic and isotypic exclusion are ensured by a variety of active processes. The evolutionary selective forces that make allelic exclusion desirable are not particularly clear. It has been argued that B cell monospecificity is important for both immune self-tolerance and efficient antibody effector function. In T lymphocytes, several mechanisms ensure that α receptors are excluded from β T cells and vice versa, despite the ability of both types of receptor to rearrange in the same cells (95). Furthermore, in β T cells the lack of allelic exclusion (allelic inclusion) of TCR α chains is rare. On the other hand, in these same cells coexpression of two TCR α chains is common (86, 106, 107). Allelic inclusion is also observed in TCR β (108) and TCR γ (109). Why exclusion should be strict in some situations but not in others is unclear. The presumed risk of autoimmunity that may be caused by relaxed allelic exclusion remains to be proved.

Mechanisms of Allelic Exclusion

Stochastic Factors—Several mechanisms in B cells have been proposed to explain the phenomena of allelic and isotypic exclusion (e.g., expression of only Ig- μ or Ig- δ , but not both). One major mechanism is inherent to the recombination machinery itself. Because recombination introduces small deletions and insertions at the coding join, about two thirds of rearrangements are out of frame. In some cases, the V, D, or J elements themselves may harbor stop codons, or such codons may be created in the process of recombination. In theory, these stochastic mechanisms alone reduce allelic inclusion of a single locus to less than 20%. This frequency drops further if a time limit is imposed on the rearrangement process, and further still if rearrangement is intrinsically preferred in one allele over another. Some evidence suggests both of these factors play a role in L-chain allelic and isotypic exclusion. In the mouse κ -light chain locus, DNA demethylation and concomitant accessibility to recombination is, at least initially, monoallelic (110), though this is clearly not a strict rule since about half of all B cells have rearrangements on both κ alleles (104). Furthermore, most μ -expressing cells lack δ rearrangements, whereas virtually all δ -producing cells bear μ rearrangements, usually on both alleles (13, 56, 111–113). During B cell development in the bone marrow, δ -bearing B cells emerge 24 h later than μ ⁺ B cells (114), and analysis of δ -producing hybridomas revealed that most μ ⁺ cells had rearranged a single δ -gene, suggesting that δ gene recombination is both inefficient and time limited (57). While these stochastic factors reduce allelic and isotypic inclusion, they are complemented by more active regulatory processes.

Selective Factors—A second mechanism that could enforce allelic exclusion in mature cells is counter selection against double producing cells. As originally conceived, excess H-chain expression was thought to be toxic to cells (115). Evidence suggesting this possibility

came from analysis of myeloma cells, in which L-chain loss is toxic if H-chains are produced. While this toxicity explanation is not supported by experiments in which double H-chain expressing B cells were generated by transgenesis *in vivo* (116), it is nevertheless possible that double producers are counterselected for reasons other than toxicity. If random H/L pairs are frequently self-reactive, as has been predicted on a priori grounds (117), cells bearing two different receptors would be much more likely to be autoreactive than cells with a single H/L pair. Consequently, counterselection of double producers may occur as a consequence of tolerance induction. This might explain the curious finding that in δ -deficient mice, which manifest poor allelic exclusion of H-chains in the preB compartment (118), allelic exclusion is restored in peripheral B cells (66).

Feedback Suppression of Recombination

Ig-H—While selective forces obviously play a major role in lymphocyte biology in general, a considerable body of evidence argues that instructive mechanisms have a dominant role in establishing allelic exclusion. In preB cells, functional μ -chains actively block further H-chain rearrangements (66, 119–121). This inhibition occurs through assembly of the surrogate L-chain components δ (120, 122) and VpreB (67) with the membrane-bound form of μ -chain (121, 123–125), followed by signaling via the associated Ig- μ complex (125–127). Forced expression of a heavy chain by transgenesis substantially blocks VDJ assembly on the H-chain locus (119, 123, 128, 129). Disruption of the preB cell receptor (preBCR) signaling complex inhibits both B cell development and allelic exclusion at this stage (103, 118). H-chains that fail to associate with surrogate L-chain, such as certain chains that include VH81X sequences, are essentially absent from the peripheral B cell pool (72, 130, 131). In normal B cells, many cells have incomplete, DJ rearrangements on the nonfunctional H-chain gene allele, suggesting that the rate of V-to-DJ recombination is slow compared to the ability of the cell to perceive functional H-chain assembly in a preBCR complex (132, 133). In addition, in the mouse, otherwise nonfunctional D μ chains generated from partial D-J rearrangements that involve D reading frame 2 can terminate recombination and block further development (65, 134, 135). Overall, there is compelling evidence for active feedback regulation of H-chain gene recombination that contributes to allelic exclusion.

TCR β —An analogous feedback process in thymocytes regulates allelic exclusion of the TCR locus. Pairing of TCR α chain with the invariant preTCR β chain generates a signaling complex with CD3 that signals cessation of V(D)J recombination and developmental progression (78, 79, 136, 137). Enforced expression of a β -chain transgene (Tg) blocks endogenous rearrangements and promotes developmental progression (83, 138).

Ig-L-Chain Allelic Exclusion

That Ig-L chain loci must similarly be subject to feedback suppression was predicted on a priori grounds, but the evidence for feedback suppression in light chain gene rearrangements is much less compelling or clear than the evidence for H-chain or TCR- α allelic exclusion. B cells from normal animals manifest substantial allelic exclusion (102), but sensitive techniques are able to identify double producing cells above background (139–141). In contrast to the ability of H-chain transgenic constructs to suppress H-chain rearrangements, enforced expression of L-chain transgenes led to mixed results. Initial experiments by Storb and colleagues using the MOPC 21 κ -chain Tg were consistent with a strict feedback regulation model, as hybridomas generated from these mice appeared to lack endogenous expression when H-chain was also expressed (142). Curiously, in this study many hybridomas lacked H-chain expression but expressed endogenous κ -chains (142). It was later found that κ -chain-producing hybridomas from these mice not only coexpressed the Tg but also often rearranged and expressed endogenous κ -loci (139). Some other transgenic

mouse lines apparently failed to exclude endogenous rearrangements because of insufficient protein expression (119), but this quantitative effect could not explain how in the MOPC 21 mice endogenous rearrangements were excluded in some B cells but not in others. Similarly, in MOPC-167 κ -gene transgenic mice, endogenous κ expression was suppressed in only a subset of B cells (123). It is unlikely, but not formally excluded, that the incomplete allelic exclusion observed in these κ -transgenic mouse experiments was solely the result of defective Tg expression. Certain conventional κ transgenic mice manifest excellent allelic exclusion in a substantial proportion of cells (143, 144).

Several lines of evidence suggest that in some cells even normal L-chain gene expression is not sufficient to suppress further L-chain gene rearrangement. Several sIg + B cell tumor lines and long-term IL-7-dependent bone marrow B cell lines express new receptor chains through secondary rearrangements (22, 145–149). In an analysis of episomal DNA present in mouse spleen cells, Harada & Yamagishi found that of 16 clones containing V J joins excised by nested κ rearrangements, 5 were in-frame and theoretically functional (24), suggesting that in these B cells the natural genes were frequently incapable of suppressing further rearrangements. Perhaps more convincingly, in mice in which functional V J κ -genes were targeted to the natural locus, endogenous κ rearrangements were inhibited in some, but not all, B cells (144, 150). Again, only a subset of cells, which varied in frequency depending upon the variable region gene used, appeared subject to feedback suppression. A criticism of these experiments is that even the targeted κ -genes may be aberrantly expressed owing to the premature juxtaposition of a V promoter near the C locus. This could conceivably target recombination to the κ locus abnormally early in development, perhaps in cells that lack H-chains and that therefore are unable to perceive the presence of light chain by BCR signaling. Conversely, if DNA accessibility in the κ -locus is stochastically controlled (110), the targeted gene may at first be transcriptionally silent in some cells that make the other allele accessible. The targeted allele might then be coexpressed at a later time, resulting in allelic inclusion of a significant fraction of cells, an unexpected but well-documented, property of targeted L-chain mice (144, 150, 151).

In contrast to these results, it appears that when expressed as transgenes, certain H/L pairs are extremely efficient at suppressing endogenous L- and H-chain protein expression and RAG expression in the bone marrow (152), suggesting that BCR specificity plays a critical role in development. One interpretation of these results is that, like T cells, B cells are positively selected by self-antigens that downregulate RAG expression. Some studies with cell lines are consistent with this idea (145, 146). However, if antigen-specific positive selection was associated with cessation of V(D)J recombination in B cells, then allelic inclusion would be predicted to occur frequently among normal B cells in vivo (as is the case for TCR α exclusion in T cells). On the contrary, as we discuss below, there is substantial evidence from the study of autoantibody transgenic mice that negative selection, i.e. tolerance mediated by encounter with self-antigens, blocks developmental progression and prevents recombinase downregulation. These results can be reconciled with the above-mentioned results by taking into account the influence of immune tolerance on feedback suppression of recombination.

RECEPTOR EDITING

Receptor Editing Monitored In Vivo in Transgenic Models of Immune Tolerance

The fact that H+L antibody transgenes could be used to generate mice in which most B cells had a defined specificity (119) stimulated studies analyzing immune tolerance in B and T cells (153). Transgenic mouse models using autoantibodies to HEL, MHC class I alloantigens, DNA, erythrocytes, and other antigens have been useful in defining a number of ways that self-reactivity is controlled (154). In transgenic autoantibody models in which

developing B cells were confronted with antigen in a multivalent form, autoreactive cells were absent from the peripheral lymphoid system, but a population of cells carrying a low level of receptor was present in the bone marrow (155–157). Two lines of evidence suggested that receptor editing, i.e. autoantigen-induced secondary Ig gene rearrangements, might be a mechanism of immune tolerance in these models.

In mice transgenic for the 3–83 antibody (anti-H-2K^{k,b}), B cells were numerous and they almost exclusively expressed the Tg-encoded antibody as a consequence of feedback suppression of recombination (158). But when cognate antigen was introduced by breeding the 3–83 transgenes to the appropriate MHC background, spleen and lymph node B cells were reduced in number, and those cells that remained lacked self-reactivity. B cells in the peripheral lymphoid organs retained surface expression of the 3–83 H-chain, but not the 3–83 L-chain, and an extremely high percentage of cells expressed endogenous κ -chain. In the bone marrows of antigen-expressing mice, transgenic sIg + B cells expressed high levels of recombinase mRNA and manifested rearrangements at the endogenous L-chain loci. Based on these findings, it was suggested that autoantigen binding by immature bone marrow B cells could reinduce or prolong L-chain gene rearrangements, allowing a cell to alter specificity and escape death (158). It was further postulated that the ability to undergo receptor editing was limited to early stages of B cell development because 3–83 mice that expressed cognate antigen only in the periphery, under control of a liver-specific K^b Tg, underwent profound B cell deletion without appreciable receptor editing.

Independent evidence for receptor editing was obtained in transgenic mice carrying H + L genes of dsDNA antibody 3H9 (159). Spleen cells of H + L Tg mice and hybridomas generated from them were analyzed for antibody specificity and endogenous rearrangements. While dsDNA-specific B cells were lacking from the hybridoma sample, indicating that specific tolerance was induced, splenic B cell numbers were surprisingly normal, particularly in older mice. Splenic B cells retained transgenic H-chain expression on the cell surface but lacked 3H9 L-chain expression as detected with specific anti-idiotypic antibody. The hybridomas expressed endogenous L-chains that apparently altered B cell specificity and extinguished dsDNA binding. Suppression of dsDNA specificity was reversible in one hybridoma, when the endogenous κ was lost, suggesting that binding of the transgenic H-chain with the endogenous L-chain outcompeted transgenic L-chain. In addition to this evidence for “phenotypic” allelic exclusion, among these hybrids an excessive use of endogenous V_H 12/13 genes associated with downstream J_H s was observed. In control mice transgenic for just the 3H9 L-chain, only 6/25 hybrids excluded endogenous L-chain expression, but the endogenous L-chains that were expressed had the typical distribution of V_H and J_H usage.

Radic et al (160) and Prak et al (23) extended this work to test for tolerance-induced receptor editing in 3H9 H-chain-only Tg mice. This test was possible because 3H9 H-chain binds DNA in association with diverse L-chain partners (161), but spleen hybridomas from 3H9 H-chain Tg mice lack reactivity to dsDNA (160, 162). Hybridoma analysis again revealed a strikingly limited usage of V_H genes in splenic B cells with excess use of V_H 12/13 and skewing to downstream J_H 's, particularly J_H 5. These studies also documented that in many cells multiple rearrangement attempts were made on a single chromosome. Assuming that additional attempts were required to replace κ -chains that conferred auto-reactivity, these results provide an explanation for skewing of J_H usage. This would predict that many VJ joins displaced by secondary rearrangements were in-frame and functional, an assumption that was not directly tested. An independent study by Eilat and colleagues of a different set of VH11 anti-DNA H-chain mice yielded similar results (163). Since in these studies no L-chain Tg was present, the later stages of B cell development should have been relatively normal. Furthermore, because simple apoptosis of autoreactive cells from an

initially random population would be unlikely to account for the observed skewing in J usage, these results suggested that autoreactive cells were rescued by ongoing Ig-recombination.

Analysis of Receptor Editing in Gene Targeted Antibody Gene Mice

VH Replacement—Gene targeted autoantibody Tg mice were generated to more closely mimic the natural genomic context and to test for V_H replacement *in vivo*. In theory, gene targeting to the natural locus should facilitate editing because secondary rearrangements on the targeted allele, should they occur, can silence or modify antibody expression. In conventional Tg mice, the suppression of Tg expression is highly inefficient because transgenes are typically inserted on chromosomes without access to potential rearrangement partners. A complicating feature of mice with targeted H-chain loci is that they retain upstream D regions, which are normally absent after VDJ assembly. These dangling Ds can rearrange to remaining J elements of the constructs or to the heptamers embedded in the targeted VH region. Early studies (164) failed to identify use of the conserved heptamer embedded in the expressed V_H, but destructive rearrangements caused by D joining to other heptamer-like sites in the gene were observed. Another study (165) found frequent editing by D-regions, but also found V_H-to-VDJ replacement events that rescued H-chain function, a result predicted from prior work with cell lines (41, 166). This and subsequent results from studies of other H-chain targeted mice (116, 163, 167, 168) have verified that such replacement events at the Ig-H locus occur at detectable frequency in these animals, indicating that nested rearrangements alter specificity *in vivo*. But there is debate about the frequency, stage specificity, and tolerance inducibility of the V_H replacement reaction (169). *In vitro* analyses indicate that the embedded heptamer is extremely inefficient at targeting V(D)J recombination relative to conventional RSSs (170). When V_H-to-VDJ replacements have been observed in mouse B cells, they almost always include additional “N” nucleotides derived from TdT activity, indicating that most V_H replacements occur at the proB stage, prior to L-chain gene rearrangements (116, 165, 167). This in turn suggests that tolerance signaling through intact surface immunoglobulin (Ig) does not drive the V_H-to-VDJ replacement response.

Editing at the κ-Locus—The possibility that editing on the H- or L-chain loci could be tolerance induced was more directly tested in models of H+L autoantibody gene targeted mice. Chen and colleagues analyzed mice coexpressing heavy and light chain targeted constructs encoding the 3H9H/V_κ4 anti-dsDNA antibody or the 3H9 H-chain with a different targeted L-chain (V_κ8) that conferred reactivity to ssDNA (152). The 3H9/V_κ4 dsDNA reactive combination was subject to extensive L-chain editing in the absence of editing on the H-chain locus. Ninety-eight percent of cells retained the expression of the 3H9 H-chain, and the vast majority of these failed to express the other H-chain allele, but at the L-chain locus extensive rearrangements occurred on both the targeted and untargeted V_κ-alleles. Only 4% of hybridomas lacked detectable secondary V_κ-rearrangements. L-chain editing occurred by inversional rearrangements in 57% of the hybridomas, deletional type rearrangement events in 28% of the cells, and inclusional rearrangements (i.e. on the opposite allele with an unaffected targeted allele) in only 11% of cells. In the 3H9H/V_κ8 mice, B cells bearing this receptor are often seen in the periphery, in an anergic state (162). But suggestive evidence of receptor editing was also observed in the 3H9H/V_κ8 B cells because in both conventional and 3H9H/V_κ8 gene-targeted mice a subset of B cell hybridomas showed exaggerated usage of V_κ12/13 along with skewed J_κ usage on the untargeted allele (152). (In the targeted 3H9H/V_κ8 mice the V_κ8 gene was joined to J_κ5, providing no additional functional rearrangement possibilities on that allele.) These results suggested that tolerance-induced receptor editing was focused on the L-chain loci and could be induced by both strong and weak toleragens, dsDNA and ssDNA, respectively.

In a second study, targeted α - and H-chain gene mice were generated encoding the 3–83 antibody, specific for self-MHC molecules H-2K^k and H-2K^b, and light chain editing in B cells was monitored (151). Quantitative southern blotting assessed the extent of recombination in the α -loci and indicated that in the auto-reactive combination at least 30% of targeted alleles and 59% of wild-type alleles were rearranged, while in B cells of α -only mice, these percentages were 10% and 27%, respectively. As a control, the 3–83 mice were bred with H-chain gene mice of an irrelevant, nonautoreactive specificity. (Unfortunately, in this study, it was not possible to compare 3–83 H+L targeted mice with and without antigen because the targeted H-chain gene was linked to a cross-reactive antigen locus contributed by the embryonic stem cell strain–129.) Most control cells appeared to retain expression of the targeted L-chain, whereas at least 85% of the autoreactive B cells lost expression of the autoreactive specificity on the cell surface. RS-type recombinations inactivating the α -loci were 60-fold more prevalent in the autoreactive context than in the presence of an innocuous receptor. In the bone marrows of mice with an innocuous H+L receptor, small preB cells were absent owing to accelerated developmental progression. However, in mice with autoreactive receptors that manifested receptor editing at the DNA level, this compartment was very large, suggesting that receptor editing specifically caused a retrograde step in development from sIg⁺ to sIg[–] stages. This is as one might predict from the high frequency of nonfunctional secondary rearrangements associated with inactivation of the targeted α -gene. In both this study and that of Chen et al (152), it appeared that receptor editing could allow a rather efficient rescue of previously autoreactive cells because peripheral B cell numbers were relatively normal.

Central B Cell Tolerance Is Associated with Developmental Block

It has been known for a long time that B cell production can be suppressed by anti-IgM treatment (171). Early transgenic mouse studies on B cell tolerance to membrane MHC molecules indicated that self-reactive B cells were absent from the peripheral lymphoid organs, but large numbers of newly formed autoreactive cells bearing a low density of sIg were retained in the bone marrow (155, 156). Because these data were superficially consistent with the clonal selection hypothesis and classical studies on B cell tolerance (172), they were interpreted to mean that autoreactive cells were eliminated at the preB-to-B cell transition. It now appears most likely that autoreactivity initially prevents B cell developmental progression and promotes receptor editing that allows some cells to change their specificities. While cells failing to alter their receptors eventually die, this tempo of cell death is essentially identical to the rate of turnover of preB cells that fail to generate any receptor at all. Thus, a cell death program succeeds a receptor editing phase.

An early indication that this might occur was provided in studies performed in the 1970s, in which bone marrow B cells were challenged with anti-IgM antibodies (173, 174). In these cultures, immature B cells rapidly lost sIg but were not reduced in number, as assessed by quantitation with anti-MHC class II antibodies; after removal of the anti-Ig stimulus, only a small subset reexpressed sIg. In contrast, mature splenic B cells rapidly reexpressed sIg after similar treatment. This “irreversible receptor modulation,” as it was called at the time, was probably the first evidence for editing, which is predicted to frequently lead to nonfunctional secondary rearrangements. More recently, in experiments in which highly purified bone marrow B cells, rather than unpurified bone marrow preparations, were challenged with anti-IgM antibodies, this stimulus was found to result in apoptosis (172, 175, 176). A possible resolution of the discrepancy between the two types of study is that the presence of bone marrow accessory cells may be required for the editing response (177). Experiments studying antigen-induced apoptosis in immature B cells of autoantibody transgenic mice are consistent with a model in which cell death is slow and delayed. Culture of anti-HEL Tg bone marrow B cells with membrane-bound HEL blocked developmental progression at the

fraction E (immature B cell) stage but failed to cause rapid cell death (178). The block was reversible upon antigen removal during the first 1–2 days, but at later times B cell death occurred. Enforced expression of Bcl2 in immature auto-reactive B cells was shown to further prolong survival at this developmental stage (178) but was unable to relieve the developmental block.

The receptor editing model provided a rationale for this delayed and reversible death program in immature B cells. In short-term cultures of 3–83 (anti-MHC) B cells, anti-BCR antibodies failed to accelerate B cell death during the initial 48 h of culture but stimulated secondary L-chain gene rearrangements in a considerable proportion of cells, estimated to be from 25% to 50% of all cells (179). This same study reported that anti-BCR treatment could also induce receptor editing in normal, nontransgenic bone marrow B cells. In another model system, immature bone marrow B cells of 3–83 Tg mice were expanded in IL-7 and challenged with antigen. Under these conditions, autoantigen blocked developmental progression and promoted receptor editing but did not appreciably accelerate cell death (180, 181). Consistent with this idea, Bcl-2 overexpression did not allow autoreactive receptor-bearing B cells to escape from the bone marrow but appeared to improve the efficiency of their escape by receptor editing, probably by prolonging the time window of secondary rearrangement (182). In an independent study, Bcl-2 overexpressing immature B cells spontaneously switched from κ to λ -chain expression in vitro (146). Somewhat analogous results were obtained in Bcl-x1-overexpressing anti-HEL/mHEL double Tg mice, in which central tolerance was apparently disrupted, yielding enhanced peripheralization of autoreactive B cells with an anergic phenotype along with enhanced receptor editing (183). Curiously, however, the same Bcl-x1 Tg had minimal effects in the 3–83 model of central tolerance (J Lang, D Nemazee, unpublished data). Overall, these data suggest that receptor editing is associated with a developmental checkpoint.

The notion that through secondary recombination autoreactive cells can overcome a developmental block implies that inhibition of secondary recombination in autoantibody Tg mice should prevent B cells from maturing to populate the periphery. This idea was directly tested in both the anti-H-2K^{k,b} and anti-DNA models by breeding Ig-H+L transgenes to a RAG-1- or RAG-2-deficient background (184, 185). B cell development did not advance past the late bone marrow stages, and B cells did not populate the peripheral lymphoid organs. The developmentally arrested RAG-deficient autoreactive cells underwent apoptosis in situ (185). In contrast, on an antigen-free (H-2^d), RAG-deficient background anti-H-2K^{k,b} B cells did develop and populated the spleen. These studies are consistent with a model in which developmental arrest is coupled to receptor editing, and cells that fail to appropriately modify their receptors die.

Locus Specificity of B Cell Receptor Editing

Several lines of evidence suggest that tolerance-induced receptor editing in immature B cells is focused primarily on the Ig-L chain loci, although some editing at the H-chain locus may also occur. One indication of this is that most autoantibody Tg mice exhibit excellent H-chain exclusion, but poor L-chain allelic exclusion (152, 158, 159). Other evidence has been gleaned from cell culture models of receptor editing. Bone marrow B cells of 3–83 Tg mice cultured in IL-7 maintain excellent H and L-chain exclusion in the absence of cognate antigen, but λ -rearrangements could be induced in over two thirds of cells upon anti-BCR treatment (181). In these same cells, VDJ assembly on the H-chain locus was not detectable by a sensitive PCR assay. However, as discussed above, conventional H-chain transgenic mice do not allow an assessment of V_H replacement events, and when targeted H-chain autoantibody mice have been analyzed, V_H-to-VDJ replacement occurred infrequently and usually included N-region additions indicative of recombination at the proB stage prior to sIgM expression (152).

Developmental Stage Specificity of Receptor Editing

Immature bone marrow B cells sensitive to tolerance-induced receptor editing rapidly lose this sensitivity as they mature, and that stage is succeeded by an apoptosis-sensitive one. These cells can be distinguished by a series of surface markers (186) and by the ability of the latter cells to migrate to the spleen (187). More detailed analysis has been facilitated by the ability to selectively expand receptor editing-sensitive B cells from bone marrow using IL-7 and to follow their differentiation after IL-7 withdrawal (146, 188). In these cultures, IL-7 withdrawal is required for recombinase expression, possibly because cell cycle events in proliferating cells suppress recombinase expression (189). RAG-2 protein is selectively degraded in cycling cells (189). Upon IL-7 withdrawal, cells express increasing amounts of sIg, and the ability to respond to antigen challenge by editing decreases and apoptosis sensitivity increases concomitantly. Since more mature cells presumably also must retain the option to fix their receptors and to participate in the immune response, the inability of these cells to be induced to receptor editing by antigen alone makes sense.

Is there a role for receptor editing in receptor diversification? Besides providing an elegant self-tolerance mechanism, receptor editing may have an additional biological benefit. V(D)J recombination is often far from random because differences exist within sets of variable and joining segments in their recombination signal sequences, promoter regions, and proximity to other *cis*-acting elements that affect the efficiency of recombination. As a result, overrepresentation of certain genes is common and perhaps inevitable. Secondary and higher order rearrangements may play an important role in promoting a higher degree of randomness in V and J gene usage than would occur if each locus underwent only a single recombination.

PERIPHERAL EDITING

Receptor Revision

Even mature B cells, it was shown recently, can undergo receptor editing during the immune response in germinal centers (190–193). The first indications of this were that RAG mRNA and protein were inducibly expressed in mature B cells under certain culture conditions or upon a germinal center immune reaction (190, 194). Histological analysis of spleens and lymph nodes of immunized mice indicated that the cell type expressing RAG *in vivo* was the centrocyte (190, 195). The centrocyte is a nondividing cell that has recently proliferated in the germinal center reaction and is intimately contacted and selected by the follicular dendritic cell network (196). Concerted treatment of splenic B cells with CD40 agonist and IL-4, agents that probably mimic T cell help and induce heavy chain class switching, could rapidly induce upregulation of RAG genes (193, 195, 197). Combined treatment with bacterial lipopolysaccharides and IL-4 had a similar effect (190, 195). Later studies suggested that IL-7 could substitute for IL-4 and was probably the critical cytokine *in vivo* because antibodies to IL-7R, though they permitted germinal center formation, prevented RAG expression in centrocytes (197).

RAG expression in mature B cells was strongly associated with B cell death both *in vivo* and *in vitro* and was often present in cells that were engulfed by macrophages (190, 195). In histological sections of germinal centers, RAG protein expression appeared to be localized to the cytoplasm rather than the nucleus (190, 194). RAG gene products induced *in vivo* and *in vitro* were nevertheless shown to cause double-stranded DNA breaks adjacent to recombination signal sequences (191) and new L-chain protein expression (192, 193, 198). Curiously, the cells undergoing these recombination reactions and receptor alterations did not manifest a loss of surface Ig expression (192), which would be predicted to be a common occurrence, suggesting rapid death of sIg-cells in these cultures.

Perhaps most intriguingly, the cell fractions that expressed recombinase also expressed other markers characteristic of B cell precursors, including surrogate L-chain components (199), IL-7R (197), the surface marker GL-7 (194), and, in human germinal center cells, TdT (199). The reexpression of surrogate L-chain components is particularly interesting because of the likelihood that many editing events would initially silence L-chain production, perhaps requiring surrogate L-chain to temporarily pair with H-chain. Expression of surrogate L-chain components is also observed in cycling, centroblast cells (199). These unexpected similarities between bone marrow and germinal center cells, first pointed out by Han, Kelsoe, and colleagues on the basis of RAG expression (194), suggest that many elements of B cell development are recapitulated during the immune response.

However, in the regulation of recombinase expression, there are critical differences as well. In the bone marrow microenvironment, immature B cells require only BCR ligation to undergo receptor editing, whereas different stimuli drive receptor editing in germinal center cells, and BCR stimulation under such conditions actively blocks the recombinase response (198, 199). In one study, *in vivo* RAG expression appeared to be present in a subset of germinal center centrocytes distinguishable by reduced CD45 levels and nonoptimal receptor gene usage for cognate immunogen (191). It is therefore unlikely that immune tolerance induces V(D)J recombination in germinal center cells. Instead, the data are most consistent with a role for receptor editing in the diversification of the receptors of antigen-reactive cells. However, as we discuss below, this interpretation also presents problems.

Very recently, data has been presented suggesting that receptor revision may occur concurrently with somatic hypermutation. Weigert and colleagues have identified a clone of anti-dsDNA reactive cells captured as hybridomas from MRL/lpr mice harboring targeted L-chain and H-chain genes encoding anti-DNA (F Brard, M Shannon, EL Park, S Litwin, M Weigert, *in press*). In this clone the L-chain Tg was heavily mutated, and all clone members shared a lethal stop mutation. The clone expressed a second L-chain on the other kappa allele that had acquired fewer mutations. Assuming that the new κ -gene rearrangement appeared some time after the initiation of somatic mutation, these results imply that revision can occur in cells that undergo point hypermutation and that editing may be specifically stimulated by the loss of BCR expression. In a second study, de Wildt et al (200) identified among 365 human primary IgG⁺ clones a pair of cells expressing an identical hypermutated H chain gene and two different expressed L-chain genes, which were presumably rearranged after these mutations. Since the L-chain genes themselves showed evidence of somatic mutation, hypermutation and receptor editing can apparently occur concurrently in these cells.

A second recent study argues that receptor revision can occur in mouse B-1 cells (201), which are not thought to take part in germinal center reactions. IgM⁺B220^{low} peritoneal cells, particularly those from the autoimmune-prone NZB strain, express RAG mRNA and possess double-strand breaks at J RSSs. In analysis of mice carrying functional replacements of IgH and IgL variable genes, B-1 cells lost idiotypic determinants, indicative of receptor editing at the protein level. The function of such modification is unclear and, as in the case of germinal center B cells, has important implications for the generation of autoantibodies.

Locus Specificity of Receptor Revision

Several studies documented renewed recombination of L-chain genes in mature B cells (191–193, 197, 199, 201), and a single study provided possible evidence of rearrangements at the IgH locus (192). This latter result was obtained in a VDJ replacement mouse in which both nonphysiological D-to-VDJ joins and potentially physiological V-to-VDJ replacement reactions are possible. Thus, either this experimental system may represent the ideal model

to reveal physiologically relevant receptor revision on the IgH-locus, or it may allow recombination events on the IgH-locus that rarely occur in normal cells. It will be important to determine if receptor revision can occur at the H-loci through V-to-VDJ replacements.

RECEPTOR EDITING IN T CELLS

As discussed earlier, TCR allelic exclusion essentially fails to occur because of ongoing rearrangements in cells prior to positive selection. This raises the question of whether an editing mechanism may play a role in thymocyte negative selection. Wang et al (202) established a targeted replacement mouse using a rearranged TCR VJ gene from a pigeon cytochrome c (PCC)-specific T cell hybridoma inserted in the 5' region of the germ-line J locus. This gene was expressed in most double positive cells but was lost, presumably through nested rearrangements, in all but ~3% of peripheral T cells. Retention of gene expression was greatly improved when a TCR Tg encoding the original hybridoma was coexpressed on a positively selecting background but was not improved on a nonselecting background. Most importantly, in the presence of PCC, negative selection was not associated with loss of thymic cellularity, in contrast to what was seen with conventional anti-PCC TCR transgenic mice, and nonautoreactive cells populated the periphery. While these results failed to prove that autoreactivity promoted further rearrangements, the possibility is not excluded, and this model should allow this question to be further tested. It is equally possible that the autoreactive cells in this system were positively selected and subsequently lost by apoptosis, but the compartment was filled with competing cells that had lost expression of the targeted TCR gene prior to antigen encounter.

Perhaps more remarkable is the recent suggestion that receptor editing can occur during peripheral T cell tolerance (203). In this experiment, a TCR α -chain (V 5) Tg mouse was constructed and tolerance to the endogenous mouse mammary tumor virus superantigen MTV-8 was assessed. In antigen-expressing mice most V 5⁺ cells were deleted, and the remainder were functionally inactivated. However, over time, a V 5⁻ population emerged. It was concluded that in a subset of the tolerant cells, extra-thymic V(D)J recombination occurred, altering and replacing their receptors because RAG expression and doublestranded breaks adjacent to TCR D 2 and J 50 were detected in a subset of peripheral T cells. Furthermore, the appearance of V 5⁻ cells, but not tolerance, was dependent on the presence of B cells, suggesting that a B:T interaction, such as a germinal center reaction, might be required for this process. A potential inconsistency between the data and this model was that the RAG-expression was detected in V 5⁻ rather than V 5⁺ cells. These points will no doubt be addressed in the near future as experimental systems are designed with these questions in mind (202).

Finally, it should be noted that the structure of the TCR loci is consistent with receptor editing, as active genes using the 5'-most D/J/C cluster can in theory be subsequently eliminated and replaced by V-to-DJ rearrangements to the 3' D/J/C cluster on the same chromosome (Figure 3). In addition, the spacers of their RSS elements are theoretically compatible with direct V-to-J rearrangement, but this has never been observed.

CONCLUSION

The ability of immune cells to turn V(D)J recombinase on and off, or to redirect its activity to new gene loci, appears to regulate lymphocyte specificity in a novel way distinct from, and complementary to, the control of cellular growth and survival. This regulation of recombinase is largely controlled by signaling through the antigen receptor itself and constitutes a feedback mechanism that allows cells to "repair" receptor genes whose products are dysfunctional, or of inappropriate specificity, including those with too high or

too low affinity for self-antigens. Receptor editing facilitates immune self-tolerance during B cell development and also permits developing thymocytes to screen multiple TCRs for potential positive selection. Mature B lymphocytes (and possibly also T cells) may re-express recombinase upon foreign antigenic stimulation, possibly allowing more rapid clonal evolution to high-affinity reactivity. The organization of antigen receptor genes appears to facilitate these processes, often at the cost of limiting potential receptor gene diversity and lymphocyte monospecificity. The full extent to which these receptor selection processes normally occur, or benefit the organism, remains to be elucidated.

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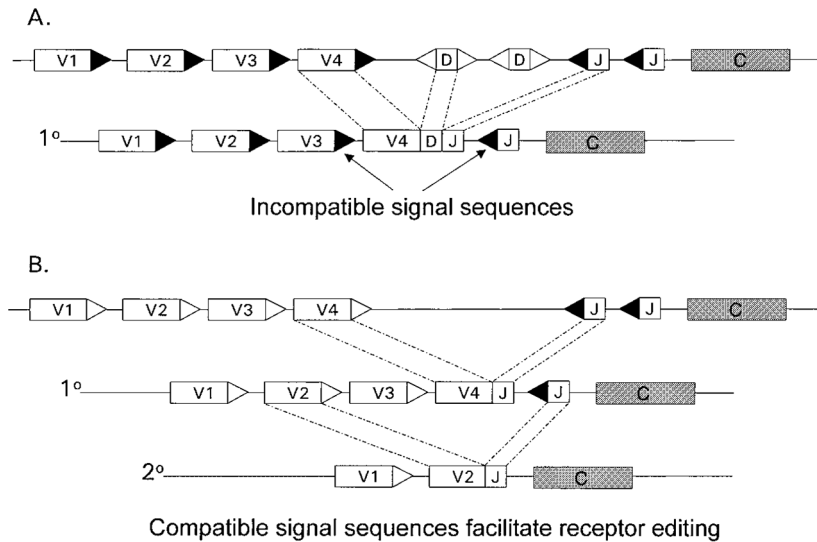


Figure 1.

Role of gene organization in facilitating or inhibiting receptor editing. V, D, and J coding elements are flanked by recombination signal sequences carrying 12-bp spacers (*open triangles*) or 23-bp spacers (*black triangles*) that constrain the range of possible rearrangements in *cis*. Recombinase joins elements with 12-bp spacers to those with 23 bp. Because of their overall organization, loci vary in their abilities to support receptor editing type rearrangements. (A) Cartoon of one type of gene organization similar to mouse and human Ig-H loci (see Figure 3 for details). The presence of D elements along with V genes in the same transcriptional orientation as the J/C cluster forces deletional rearrangements. Primary VDJ assembly cannot be replaced by recombination using conventional signal sequences. (B) In contrast, in loci without D elements, sequential rearrangements are often possible. In this example, a primary (1^0) V4-to-J join is replaced by a subsequent secondary (2^0) rearrangement between V2 and the downstream J. Such secondary rearrangement permits the replacement of potentially functional V4-to-J joins, i.e., receptor editing. This type of organization is also seen in mammalian TCR loci.

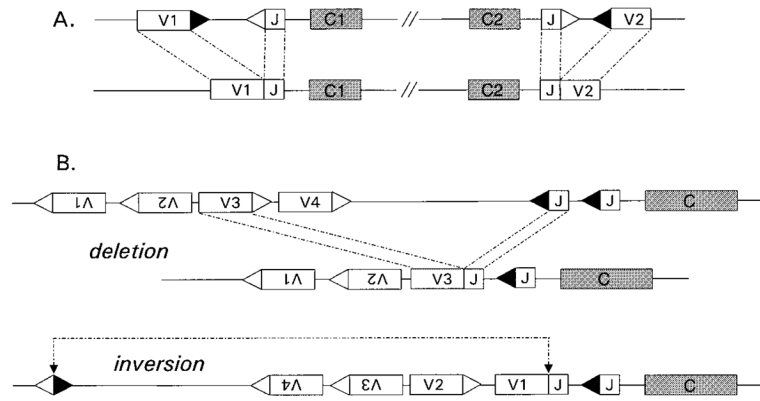


Figure 2.

Gene organizations that inhibit or facilitate receptor editing. (A) Cluster type receptor gene organization is used in many lower vertebrates and is retained in certain mammalian receptor gene loci, such as mouse *Ig-*. Rearrangements occur within clusters but not between adjoining clusters, preventing editing and potentially posing problems for isotype exclusion. (B) Inversional rearrangements are dictated by gene orientation. Variable gene segments in inverted transcriptional orientations relative to J/C clusters are indicated by upside-down Vs. Such elements join through inversion rather than excision of intervening DNA. Hypothetical V3 and V4 elements must undergo deletion during primary rearrangement to Js, whereas V1 and V2 elements rearrange by inversion. Note that subsequent secondary rearrangement can again occur through either deletion or inversion, but inversional rearrangements retain more V genes and change the orientations of V elements intervening the break points.

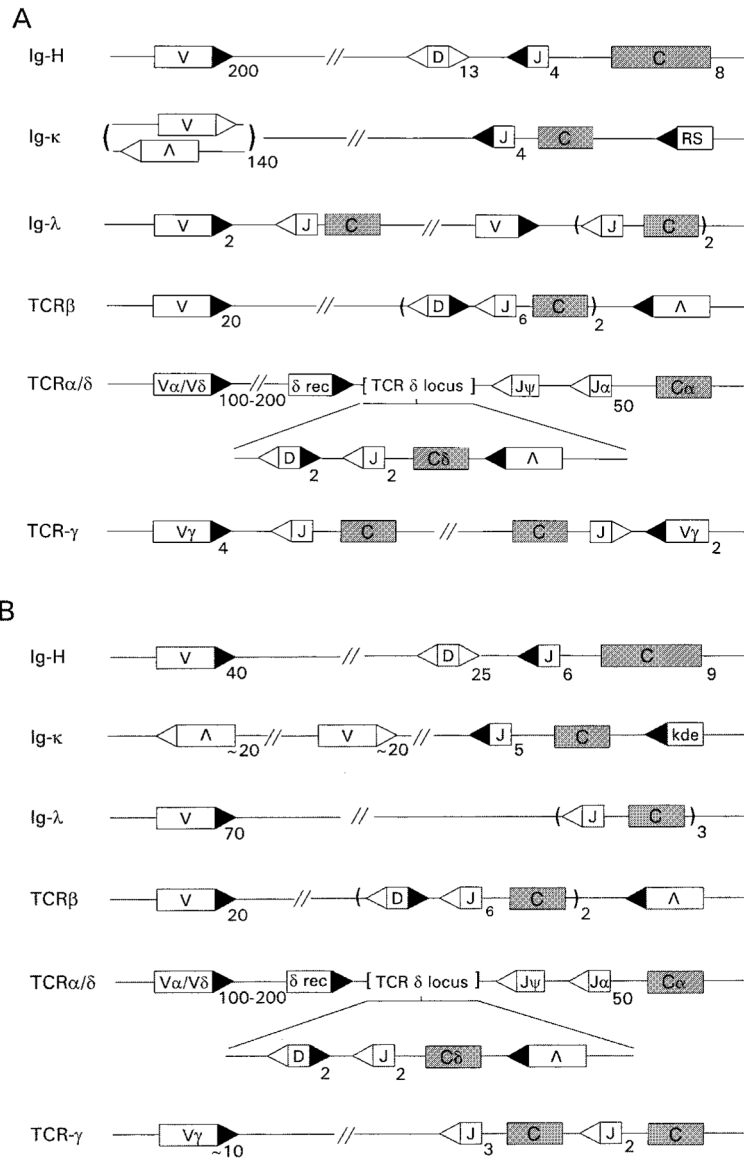


Figure 3. Antigen receptor loci of (A) mouse and (B) human. Note that Ig- γ , TCR- β , TCR- δ , and TCR- γ have structures that are compatible with secondary, replacement rearrangements in both mouse and human. Conventional V(D)J recombination disfavors receptor editing at the Ig-H locus of mouse or human because of the 12/23 rule and the arrangement of V_H elements in the same transcriptional orientation as the J_H/C_H cluster. In the mouse Ig- γ and TCR- δ loci, functionally rearranged genes cannot efficiently be altered by secondary rearrangements because of their cluster type organization, whereas editing is possible in the human versions of these loci provided that the 3' most J's are not initially used. In the TCR- δ locus of both mouse and human, TCR- δ rearrangements exclude TCR- δ expression.