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Antigen Receptor Allelic Exclusion: An Update and Reappraisal
Brenna L. Brady,¹ Natalie C. Steinel,¹ and Craig H. Bassing

Most lymphocytes express cell surface Ag receptor chains from single alleles of distinct Ig or TCR loci. Since the identification of Ag receptor allelic exclusion, the importance of this process and the precise molecular mechanisms by which it is achieved have remained enigmatic. This brief review summarizes current knowledge of the extent to which Ig and TCR loci are subject to allelic exclusion. Recent progress in studying and defining mechanistic steps and molecules that may control the monoallelic initiation and subsequent inhibition of V-to-(D)-J recombination is outlined using the mouse TCRβ locus as a model with frequent comparisons to the mouse IgH and Igk loci. Potential consequences of defects in mechanisms that control Ag receptor allelic exclusion and a reappraisal of the physiologic relevance of this immunologic process also are discussed. The Journal of Immunology, 2010, 185: 3801–3808.

Antigen receptor allelic exclusion is defined as the surface expression of Ig or TCR chains from a single allelic copy of corresponding genetic loci. Pernis et al. (1) identified this phenomenon in the 1960s while studying Ig expression on rabbit lymphocytes, providing evidence for the “one lymphocyte–one antigen receptor” concept of Burnet’s clonal selection theory. Analyses of Ig rearrangements in the early 1980s suggested that the assembly and expression of an Ag receptor chain from one allele inhibit further V-to-(D)-J recombination on the other allele (2). Evidence for such feedback regulation was provided over the next decade by demonstrations that preassembled Ig or TCR transgenes enforce allelic exclusion through inhibiting V-to-(D)-J rearrangements (2). These observations helped establish the current dogma that allelic exclusion is maintained by feedback regulation to ensure virtually every lymphocyte exhibits monospecific Ag recognition.

Although Ag receptor allelic exclusion has been investigated for almost 50 y, the importance of this process and the precise mechanisms by which it is achieved remain largely unknown. Monoallelic gene expression is a general phenomenon critical for normal biology. This regulation is pervasive during genetic imprinting and X chromosome inactivation, enforcing gene silencing in all cell types (3). Defects in X chromosome inactivation and imprinting were found to cause human disorders, which defined the relevance of these processes and facilitated their investigation by studying clear phenotypes. In contrast, defects in olfactory or Ag receptor allelic exclusion, which silence tissue-specific genes in distinct lineages, have not been linked unequivocally to any symptoms in humans (3). However, dysfunction of these tissue-specific processes may result in subtle phenotypes or may be compensated by additional mechanisms, either of which would mask their significance and provide obstacles for investigation.

Allelic exclusion, self tolerance, and autoimmunity
A long-standing tenet of adaptive immunity is that virtually all lymphocytes express surface TCR or Ig chains from one allele to ensure monospecific Ag recognition and suppress autoimmunity by facilitating central tolerance to self-reactive lymphocytes. However, this notion is not supported by current knowledge. Flow cytometry reveals IgH allelic inclusion in only 0.01% of mouse B cells (4), but allelic inclusion of Igk and TCR loci in at least 1–10% of mouse lymphocytes (Table I) (5–9). Thus, a significant fraction of normal mouse (and where assayed human) lymphocytes express surface Igk or TCR chains from both alleles, refuting the “one lymphocyte–one antigen receptor” concept. In addition, recognition of multiple distinct ligands is known now to be a general and inherent property of T and B cell Ag receptors (10), meaning that far more than the 1–10% of allelically included lymphocytes exhibits poly-specific Ag recognition. Because primary TCR and BCR repertoires include receptors capable of binding self-Ags, organisms with adaptive immune systems must possess central ability to tolerate the generation of autoreactive lymphocytes and thereby prevent autoimmunity. Central tolerance mechanisms include deletion, stalled maturation, anergy, or receptor editing (9). In the 1990s, analyses of TCR and Ig transgenic mice demonstrated that dual expression of self-reactive and non–self-reactive receptors enables developing...
T and B cells to escape deletion and differentiate into mature lymphocytes that possess autoreactive potential in vitro, yet generally fail to cause autoimmunity in vivo (11–13). These additional tolerance mechanisms restrain the systemic activation of lymphocytes expressing both self-reactive and non–self-reactive receptors. A separate line of investigation demonstrated that the destructive potential of autoreactive lymphocytes that escape central tolerance is restrained through dominant peripheral tolerance mechanisms, such as those controlled by regulatory T and B cells (14, 15). In this context, allelic exclusion might function as an early cell-autonomous tolerance mechanism to reduce the frequency of developing lymphocytes with expression of two or more poly-specific Ag receptors and thereby facilitate central tolerance. If defects in allelic exclusion overwhelmed central tolerance mechanisms, peripheral tolerance checkpoints would be expected to function as an additional barrier to restrain the destructive potential of allelically included lymphocytes expressing autoreactive Ag receptors. Consistent with this notion, environmental factors such as viral infections can break down peripheral tolerance mechanisms and trigger autoimmunity driven by peripheral T cells expressing dual αβ TCRs (16). However, allelic inclusion also can be beneficial because T lineage cells expressing dual αβ TCRs protect against infection by increasing the diversity of receptors that recognize foreign Ags (17).

Multiple mechanisms affect Ag receptor allelic exclusion

Although feedback inhibition is a major component by which allelic exclusion is achieved, the sequence analyses of assembled TCR and Ig genes have revealed that additional mechanisms contribute to affect Ag receptor allelic exclusion. Because 1/3 of V-(D)-J rearrangements occur in-frame, only 1/9 of developing lymphocytes can assemble and express a particular Ag receptor gene from both alleles in the absence of feedback regulation. Of the remaining developing cells, 4/9 would assemble one in-frame and one out-of-frame gene and 4/9 would assemble out-of-frame genes on both alleles. Because the expression of an Ag receptor chain is required for continued lymphocyte differentiation, the 4/9 cells that fail to assemble a productive gene on either allele are eliminated by apoptosis. Consequently, 1/5 is the theoretical maximum of mature lymphocytes that can express a particular Ag receptor gene from both alleles and exhibit allelic inclusion in the absence of feedback regulation and selection for or against such cells. Single-cell sequence analysis revealed that, of the 2–4% of splenic B cells containing two in-frame IgH genes, only one was functional (Table I) (18). In contrast, sequencing demonstrated that ~30% of αβ T cells contain two in-frame TCRα genes (Table I) (9); yet, TCRα/TCRβ pairing constraints and regulated TCRα-chain turnover reduce the frequency of TCRα allelic inclusion to ~10% (Table I) (9, 19). Similar sequence analyses of Igk, TCRβ, TCRγ, and TCRδ rearrangements revealed two in-frame genes in 2–35% of cells depending upon the locus (Table I) (5, 7, 20–22). These percentages are higher than the corresponding allelic inclusion frequencies, suggesting that pairing restrictions or other mechanisms contribute to Igk, TCRβ, TCRγ, and TCRδ allelic exclusion. Consistent with this notion, silencing of in-frame V-D-J-Cβ genes at the transcriptional and posttranscriptional levels contributes to TCRβ allelic exclusion mouse αβ T cells (20, 23). These data indicate that multiple mechanisms function in a successive manner to limit the frequency of cells with surface expression of Ig or TCR chains from both allelic copies of corresponding loci. In this context, defects in mechanisms that control feedback inhibition could be countered by pairing restrictions, transcriptional silencing, and posttranscriptional silencing to limit allelic inclusion and facilitate central tolerance. Yet, dysfunction of these downstream allelic exclusion mechanisms, for example as an organism ages, could increase the frequencies of allelically included mature lymphocytes expressing autoreactive Ag receptors that need to be restrained by peripheral tolerance checkpoints.

Developmental stage-specific control of allelic exclusion through feedback regulation

Most reviews of mechanisms that control Ag receptor allelic exclusion involve IgH and Igk loci, probably because studying B lineage cells led to the discoveries of allelic exclusion, V(D)J recombination, and feedback regulation. In this work, the mouse TCRβ locus (Fig. 1) will serve as a paradigm for discussion of the developmental stage-specific control of allelic exclusion through feedback regulation because many recent advances in this area have come from studying V-to-D-Jβ recombination. Frequent comparisons to the IgH and Igk loci (Fig. 1) also are provided.

Recombinational accessibility control of V-to-(D)-Jβ recombination

Correlations between transcription, rearrangement, and nuclelease sensitivity of Ig gene segments led to the hypothesis that modulation of chromatin accessibility regulates V(D)J recombination within the contexts of allelic exclusion (24). Consequently, the majority of studies investigating mechanisms that control Ag receptor allelic exclusion have focused on the potential differential regulation of RAG accessibility between loci on homologous chromosomes. For many years, recombinational (RAG) accessibility has been measured indirectly by germline transcription, general nuclelease accessibility, and histone modifications associated with transcription (24). RAG accessibility also has been quantified by the expression of reporter genes inserted into endogenous Ag receptor loci (25–28); however, this indirect approach requires
knowledge of local transcriptional regulation for unequivocal conclusions (27, 28). In addition to RAG accessibility, the V-to-(D)-J recombination step requires physical juxtaposition of RAG-accessible gene segments, RAG binding to a combination signal sequence (RSS) flanking at least one of these segments, and capture of the other RSS to form productive synaptic cleavage complexes. Recent advances in techniques and reagents have enabled methods to investigate higher-order structural conformations of Ag receptor loci (29), RAG binding to chromatin over genomic locations (30), RAG activity at a particular gene segment (31), and RAG cleavage within individual Ag receptor loci (32). Unfortunately, these assays have inherent limitations that temper conclusions and can measure only a single mechanistic step required for V-to-(D)-J rearrangement. Such difficulties are substantial obstacles for elucidating the precise mechanisms that control V-to-(D)-J recombination within the contexts of feedback regulation and allelic exclusion.

Ag receptor locus transcriptional enhancers and promoters are required for V-to-(D)-J recombination; however, the precise mechanisms by which these cis elements direct V rearrangements and their potential role in allelic exclusion remain unknown. The IgH and Igk loci each contain two enhancers, whereas the TCRβ locus contains only one known enhancer (Fig. 1). Promoters are known to reside upstream of each V segment within these loci, as well as upstream of the DQ52, Jk1, DB1, and DB2 segments (Fig. 1). The molecular mechanisms by which enhancers and promoters direct V-to-(D)-J rearrangements are understood most for the TCRβ locus. The TCRβ enhancer (Eβ) and the DB1 promoter (pDB1) form a holoenzyme complex that directs D-to-Jβ1 and V-to-Dβ1 rearrangements, as well as germline transcription and chromatin accessibility of DB1 and Jβ1 segments (33). Neither Eβ nor pDB1 controls VB germline transcription or chromatin accessibility (34), which appear regulated at least in part by VB promoters (35). Collectively, these data suggest that Eβ and pDB1 may direct VB rearrangements only through promoting accessibility of D–Jβ complexes so the RAG proteins can bind 5′DB RSSs and capture VB RSSs. The IgH and Igk enhancers and promoters most likely direct V-to-(D)-J recombination through similar regulation of D–JH1 and Jk segments. Consistent with this notion, experiments using a cleavage-incompetent Rag1 protein have demonstrated that RAG binding is detectable over germline D and J, but not V, segments within TCRβ, IgH, and Igk loci (30). Yet, definitive conclusions require similar analyses with TCRβ and IgH alleles containing preassembled D–J complexes and incorporation of assays that measure RAG/RSS interactions and juxtaposition and synopsis of V and D–J segments. Because enhancers and promoters function together to direct V-to-(D)-J recombination, it is logical to assume that the enforcement of allelic exclusion could involve mechanisms that modulate the activities of these cis elements. Support for this notion is provided by the observation that IgH allelic exclusion depends upon the IgH intronic enhancer maintaining high expression of V-D-J-Cα genes during the pre-B to immature B cell transition (36).

Initiation of allelic exclusion

The V-to-(D)-J recombination step of Ag receptor loci subject to allelic exclusion is thought to occur on one allele at a time, with the assembly and expression of a functional TCR or Ig gene from the first allele inhibiting V-to-(D)-J rearrangement within the corresponding locus on the second allele (37). To enforce allelic exclusion by feedback inhibition, only one allele can initiate V-to-(D)-J recombination during the time window required for feedback signals to exert their cellular effects. Monoallelic initiation of V rearrangement could occur at any of the mechanistic steps required for V-to-(D)-J recombination.

The correlation between transcription and rearrangement led to studies suggesting that the differential positioning of TCRβ, IgH, and Igk loci at nuclear regions known to repress transcription might affect monoallelic initiation of V-to-(D)-J recombination (Fig. 2) (29). For example, TCRβ loci associate with inner nuclear membrane lamina or pericentromeric heterochromatin at a higher frequency in CD4−/CD8− (double-negative [DN]) thymocytes than in embryonic stem cells, B lineage cells, or CD4+/CD8+ (double-positive [DP]) thymocytes (38, 39). TCRβ alleles with an ectopic enhancer that promotes TCRβ allelic inclusion are localized less frequently at nuclear membrane lamina and pericentromeric heterochromatin (39), providing indirect evidence that association of Ag receptor loci with these transcriptional repressive nuclear regions may suppress V-to-(D)-J rearrangement. Despite frequent association of TCRβ loci with nuclear membrane lamina and pericentromeric heterochromatin, D-to-Jβ rearrangements, germline VB transcription, and VB RAG accessibility each occur on both TCRβ alleles in developing thymocytes (31, 37). In addition, the association of IgH loci

**FIGURE 1.** Genomic configuration of mouse TCRβ, IgH, and Igk loci. Schematic diagrams of germline TCRβ, IgH, and Igk loci depicting the relative locations of V segments (blue rectangles), D segments (yellow rectangles), J segments (green rectangles), C regions (gray rectangles), enhancers (red ovals), and promoters (blue circles). The relative location of the Igk Sis element (blue square) also is shown. The loci are not drawn to scale, particularly with regard to the genomic distances between V and D–J segments.
with pericentromeric heterochromatin does not inhibit transcription of germline or rearranged alleles (32, 40). Collectively, these data suggest that the positioning of TCR and Ig alleles at inner nuclear membrane lamina or pericentromeric heterochromatin may suppress V-to-(D)-J rearrangements by inhibiting the juxtaposition of V and D–J segments, rather than through suppressing transcription or RAG accessibility (Fig. 2). Consistent with this notion, germline and D–J-rearranged loci positioned at pericentromeric heterochromatin do not exhibit contraction by looping between Vb and DJb segments as do unrearranged TCRβ loci residing away from these nuclear regions (38). Identification of the cis elements and trans factors that control the association of TCR and Ig loci with inner nuclear membrane lamina and pericentromeric heterochromatin is required to elucidate the potential function of nuclear positioning in regulating V-to-D-J rearrangements and allelic exclusion. Logical candidates have been provided by the discoveries of cis elements between V and J segments within the TCRβ, IgH, and Igκ loci (41–43), and the demonstration that this Igκ Sis element binds the Ikaros transcriptional repressor, targets Igκ transgenes to centromeric heterochromatin, and inhibits V-to-Jκ rearrangement (42).

Monoallelic initiation of V-to-(D)-J recombination also could be affected by developmentally regulated conformation changes of Ag receptor loci that control the juxtaposition of RAG-accessible V and D–J segments (Fig. 2). Because germ-line TCRβ, IgH, and Igκ loci span large chromosomal distances, the positioning of V and D–J segments in proximity by locus contraction most likely facilitates or is required for primary V-to-(D)-J rearrangements within these loci. Germ-line and/or D–J-rearranged TCRβ, IgH, and Igκ loci exhibit monoallelic contraction by chromosome looping between V and DJ segments at a higher frequency in lymphocytes of the lineage and stage where these loci rearrange as compared with in other cells (29, 38, 39). Data revealing that rearrangements of VB and VH segments inserted just upstream of DB or DJ segments cause allelic inclusion and/or are not subject to normal feedback inhibition provide indirect evidence that locus contraction/decontraction may regulate the V-to-D-J recombination step within TCRβ and IgH loci (23, 44).

These Ag receptor locus conformational changes may be regulated by mechanisms that direct association of TCR and Ig loci with repressive nuclear regions and/or by distinct cis elements and trans factors such as enhancer/promoter interactions (45) and CCCTC-binding factor/cohesin proteins (46). However, factors in addition to locus decontraction must control the juxtaposition of accessible V and D–J segments within TCRβ and Igκ loci to regulate secondary V-to-(D)-J rearrangements across short distances and recombination of the VB14 segment, which resides in close proximity to D–Jκ segments.

In addition to RAG accessibility and juxtaposition, intrinsic properties of RSSs may contribute to affect allelic exclusion. The inherent inefficiency of VB and VH RSSs could restrain the overall rate at which accessible and juxtaposed V and D–J segments bind RAG and/or form productive synaptic complexes such that the frequency of synchronous V-to-D-J rearrangements between TCRβ or IgH alleles is rare (47). VBs
RAG access to D–J complexes, with little or no Vβ-to-Jβ joining due to beyond 12/23 compatibility RSS joining restrictions (37). These RSS restrictions limit the number of potential Vβ rearrangements on each allele to 70 (35 Vβs × 2 DBs), as compared with the 840 (35 Vβs × 2 DBs × 12 Jβs) potential Vβ rearrangements allowed by the 12/23 rule. Together, joining restrictions and inefficiencies of TCRβ RSSs may lower the probability that both alleles can initiate Vβ rearrangement in the time window required for feedback inhibition. Consistent with this notion, replacement of the Vβ14 RSS with the 10-fold more efficient 3′ DB1 RSS, which rearranges to 5′ DB or Jβ RSSs, short-circuits TCRβ feedback signals, and increases the frequency of TCRβ allelic inclusion (C.H. Bassing, unpublished observations). However, these data also support the notion that TCRβ feedback signals activate 5′DB RSS-binding trans factors to prevent RAG accessibility and V-to-D–Jβ rearrangements (48, 49), analogous to inhibition of V-to-DB rearrangements by c-fos binding 3′DB RSSs (37). Considering that TCRβ and IgH, but not TCRα genes are assembled through D–J intermediates, such inhibition of RAG access to 5′DB RSSs could account for why feedback regulation inhibits the assembly of V-D-Jβ and V-D-JβH exons more stringently than V-D-Jβ exons (Table I) (7, 18, 20–22).

Feedback inhibition

The assembly and expression of a functional TCRβ or IgH gene in DN thymocytes or pro-B cells, respectively, activate intracellular pathways that signal feedback inhibition, cessation of RAG expression, cellular proliferation/expansion, and differentiation into DP thymocytes or pre-B cells (Fig. 2). Although the pathways and mechanisms through which TCRβ and IgH chains signal these processes remain largely unknown, experiments have revealed that DN thymocytes employ distinct pathway(s) to signal feedback inhibition through mechanisms involving the E47 and Ets-1 transcription factors (49–51). TCRβ-signaled downregulation of E47 appears to inhibit V-to-D–Jβ rearrangements in DN cells through rendering Vβ segment RAG inaccessible (50). Ets-1 binds to and represses Eβ (52), which is not required for the expression of assembled V-D-J-Cβ genes in DN cells or TCRβ-mediated DN-to-DP expansion and differentiation (53). Thus, TCRβ feedback signals could modulate Ets-1 activity to inhibit Eβ in DN cells and thereby downregulate RAG accessibility of 5′DB RSSs on the D–Jβ-rearranged allele while enabling continued TCRβ expression from the V-D–Jβ-rearranged allele. This hypothetical mechanism also would explain how TCRβ feedback signals suppress Vβ14-to-D–Jβ rearrangements without downregulating RAG accessibility of Vβ14 segments (31). The use of alleles with preassembled D–Jβ complexes or V-D–Jβ-C genes along with assays of RAG binding, juxtaposition, and synopsis should facilitate elucidation of the mechanisms through which TCRβ and IgH feedback signals inhibit V-to-D–Jβ rearrangements in DN thymocytes and pro-B cells.

In addition to the activation of mechanisms that directly inhibit V-to-D–Jβ rearrangements in DN or pro-B cells, TCRβ and IgH signals may indirectly promote feedback inhibition through cessation of RAG expression, cellular proliferation/expansion, and differentiation into DP thymocytes or pro-B cells. For example, TCRβ and IgH signals that drive DN thymocytes or pro-B cells from G0/G1 and into S phase may contribute to affect allelic exclusion by shortening the time window for additional V-to-D–Jβ rearrangements (Fig. 2). In addition, considering that TCRβ-driven cellular proliferation is required for progressive silencing of TCRγ transcription during the DN-to-DP transition (54), similar TCRβ- and IgH-dependent epigenetic mechanisms may silence germline V segment transcription to help suppress V-to-D–Jβ rearrangements in DP thymocytes and pre-B cells. Finally, TCRβ and IgH signals that inhibit RAG expression also could contribute to allelic exclusion by preventing DN or pro-B cells from continuing V-to-D–Jβ recombination (49).

Maintenance of allelic exclusion

To maintain TCRβ and IgH allelic exclusion, V-to-D–Jβ rearrangements must remain suppressed on D–Jβ-rearranged alleles following RAG re-expression in DP thymocytes and pro-B cells. Evidence supports that developmental stage-specific inhibition of RAG access to germline V segments and juxtaposition of V and D–J segments most likely cooperate to maintain feedback inhibition of V-to-D–Jβ recombination on D-Jβ-rearranged alleles (Fig. 2) (37–39, 55, 56). Studies investigating mechanisms that maintain TCRβ feedback inhibition have been conducted using DP cells of RAG-deficient mice either treated with anti-CD3 Abs or expressing a TCRβ transgene to drive DN-to-DP differentiation. One finding often ignored in these experiments is that germline Vβ transcripts and Vβ chromatin marks associated with active transcription are present at higher levels in DP cells of RAG-deficient mice treated with anti-CD3 Abs as compared with DP cells from RAG-deficient mice expressing a TCRβ transgene (41, 57). Germline Vβ14 transcripts exhibit the largest difference. These data indicate that anti-CD3 treatment and TCRβ transgenes do not equally activate pre-TCR signaling pathways or thresholds required for Vβ silencing; determining which represents the more physiologic condition would be important. Germline transcription of D–Jβ segments in DP thymocytes has been interpreted that D–Jβ complexes remain RAG accessible (41, 57); however, analysis of RAG binding to 5′DB RSSs on alleles with preassembled D–Jβ complexes is required for definitive conclusions.

Maintenance of TCRβ and IgH allelic exclusion in DP thymocytes and pro-B cells also must involve the suppression of secondary V rearrangements on alleles that have assembled out-of-frame V-D–Jβ-C genes in DN or pro-B cells (Fig. 2). The 12/23 rule and beyond 12/23 restrictions inhibit, but do not block V-to-JβH and V-to-Jβ rearrangements in pro-B cells and DN thymocytes (48, 58). Such RSS joining restrictions might contribute to suppress V rearrangements over out-of-frame V-D–Jβ-C genes in pre-B or DP cells (Fig. 2), particularly if the downstream J segments remain RAG accessible. Because Vβ rearrangements to Dβ2–Jβ2 complexes are not restricted by RSS joining constraints, additional factors must suppress such recombination events on alleles that have assembled out-of-frame V-D–Jβ-C genes in DN thymocytes (Fig. 2). Germline Vβ segments located immediately upstream of V-D–Jβ-C genes remain transcribed in DP thymocytes (37, 59), yet Vβ rearrangements to Dβ2–Jβ2 complexes are largely suppressed (37). This data indicate that V-to-D–Jβ rearrangements must be controlled by developmental stage-specific factor(s) that controls RAG binding to RSSs, juxtaposition, and/
or synopsis in either DN or DP thymocytes (37). One such candidate is the E47 transcription factor because forced expression of this factor promotes Vβ rearrangements to DB2–JB2 complexes in DP cells (50). Notably, V-to-DB2 recombination intermediates are detectable in DP thymocytes of wild-type mice, and intermediates involving VB14, which resides close to DB–JB segments, are observed in DP cells of TCRβ transgenic mice (55, 60). Although such V(D)J recombination intermediates could arise from contaminating DN cells, these observations suggest that Vβ rearrangements to DB2–JB2 complexes on alleles with V-D-J-Cβ1 genes are not completely blocked in DP thymocytes. Such secondary Vβ rearrangements on alleles with out-of-frame V-D-J-Cβ1 genes could lead to TCRβ allelic inclusion either before or after positive selection of αβ TCRs containing TCRβ-chains from the other allele. In addition, secondary VB rearrangements on alleles with in-frame V-D-J-Cβ1 genes during positive selection could lead to the loss of an αβ TCR or the replacement of a selected αβ TCR with an autoreactive receptor. The ability of DNA sequences to form boundaries between active and inactive VB chromatin domains upstream of assembled V-D-J-Cβ1 genes may have evolved to suppress the frequency of such deleterious VB rearrangements in DP thymocytes (59).

Interaction of self-Ags with autoreactive immature B cells or naive αβ T lymphocytes sustains or induces RAG expression, respectively, to promote V rearrangements that replace in-frame V-Jk or V-D-Jb exons. To maintain allelic exclusion of TCRβ, Igκ, and possibly IgH loci during αβ TCR revision and BCR editing, V rearrangements need to be restricted on alleles with in-frame V-(D)-J-C genes. Studies of mechanisms that potentially suppress V-to-(D)-J rearrangements on alleles lacking or containing out-of-frame V-(D)-J-C genes to maintain allelic exclusion during BCR editing and αβ TCR revision are lacking. Yet, one study indicates that V-to-Jk rearrangements occur at equal frequency on both alleles during BCR editing, leading to allelic inclusion in ~10% of cells (5). Considering that the frequency of αβ T cells exhibiting TCRβ allelic inclusion progressively increases as mice age (8), V-to-D-Jβ rearrangements also might occur with equal probability on both alleles during TCRβ revision. Because BCR editing and αβ TCR revision will regenerate self-reactive receptors that could be edited/revised or restrained by peripheral tolerance checkpoints, the immunologic consequences of allelic inclusion during these processes may not exert physiologic pressure to restrict V rearrangements on alleles with in-frame V-(D)-J-C genes.

Potential physiologic consequences of defects in allelic exclusion

Defects in mechanisms that control Ag receptor allelic exclusion could have deleterious consequences for host organisms in addition to causing autoimmunity. TCRβ and IgH gene rearrangements proceed through the programmed induction of RAG DNA double-strand breaks (DSBs) in G1 phase DN thymocytes or pro-B cells with expression from in-frame V-D-J-C genes driving cells into S phase and through multiple cell cycles. The ataxia telangiectasia mutated (ATM) and p53 tumor suppressor proteins inhibit the persistence of RAG DSBs throughout the cell cycle (61); however, some fraction of RAG DSBs generated during V-to-D-Jβ rearrangements normally evades the G1/S checkpoint and induces apoptosis of DN thymocytes at the G2/M checkpoint (62). These RAG DSBs could arise because the mechanisms that control monoallelic initiation and feedback inhibition of Vβ rearrangements are only effective in 90–99% of DN thymocytes, as evidenced by TCRβ allelic inclusion and biallelic in-frame V-D-J-Cβ rearrangements in 1–10% of αβ T cells (20). Failure to enforce monoallelic initiation or feedback inhibition of V-to-D-J rearrangements at normal levels could result in the generation of RAG DSBs, whereas TCRβ or IgH signals are driving developing lymphocytes from G0/G1 through G1 and into S phase. This would lead to increased elimination of DN or pro-B cells that express functional TCRβ or IgH chains and compromise host immunity by shrinking the repertoire of Ag receptors expressed on mature lymphocytes. Because RAG DSBs that persist into S phase can result in genomic instability, deficiencies in these mechanisms controlling monoallelic initiation and feedback inhibition of V-to-D-J rearrangements also might cause an increased predisposition to lymphomas driven by TCRβ or IgH translocations. Accordingly, monoallelic initiation and feedback inhibition of V-to-D-J rearrangement may have evolved through pressure to generate broad Ag receptor repertoires and restrain oncogenic translocations by enforcing regulation upon a random process. In this context, the more stringent allelic exclusion observed at the IgH locus as compared with the TCRβ, TCRγ, and TCRβ loci (Table I) may have evolved in response to the greater oncogenic potential of RAG DSBs introduced at the IgH locus than at these other loci (63). In the B cell lineage, similar pressure combined with greater cellular proliferation upon the rearrangement and expression of IgH genes than Igλ genes may have led to more strict enforcement of allelic exclusion at the IgH locus as compared with the Igκ and Igλ loci.

Evidence for lateral inhibition of V(D)J recombination

Since the discovery that allelic exclusion is regulated by feedback inhibition, many experiments have been conducted to evaluate whether the monoallelic initiation of V rearrangements occurs through deterministic versus stochastic mechanisms. TCRβ, IgH, and Igκ loci each replicate asynchronously in lymphocytes, with the order between alleles randomly determined and clonally maintained (64). The early

**FIGURE 3.** Potential lateral inhibition of V(D)J recombination may contribute to enforce allelic exclusion. RAG cleavage of Ag receptor loci in developing lymphocytes activates ATM-dependent signals that may transiently inhibit additional V(D)J recombination events. This regulation may enable time for DNA repair, transcription, translation, surface expression, and signaling required for feedback inhibition. These putative ATM signals could directly prevent V-to-(D)-J rearrangements or indirectly, such as through downregulation of RAG activity.
replicating Igk allele is preferentially demethylated, rendered RAG accessible, and selected for V-to-Jk rearrangement in pre-B cells (64), yet no connection has been reported between asynchronous TCRβ or IgH locus replication and determination of monoallelic V-to-D-J rearrangement. In contrast, available data suggest that stochastic association of TCRβ alleles with inner nuclear membrane lamina and pericentromeric heterochromatin reduces the probability that biallelic V-to-D-Jβ rearrangements occur before feedback inhibition (37). These Igk deterministic and TCRβ stochastic models each assume that a static time window exists between monoallelic initiation and feedback inhibition of the V-to-(D)-J recombination step.

One neglected proposal of the original feedback inhibition model is that V(D)J recombination events on one allele could activate signals that transiently inhibit rearrangements on the other allele (65). In response to RAG DSBs, the ATM kinase phosphorylates numerous proteins and activates a genetic program that includes genes encoding proteins that regulate transcription and chromatin accessibility (66). ATM-deficient lymphocytes exhibit increased RAG cleavage of IgH and Igk loci in G1 phase cells and biallelic Igk chromosome breaks and translocations upon re-entry into the cell cycle (32). These data are consistent with the notion that RAG cleavage on one allele activates ATM-mediated lateral inhibition signals to suppress recombination events on the other allele (33) until DNA repair and termination of the DNA damage response, although other interpretations are possible. Although no obvious effect of ATM deficiency was observed upon IgH allelic exclusion in bone marrow cells of young mice (32), the frequency of TCRβ allelic inclusion in ATM-deficient thymocytes and peripheral αβ T cells is greater than in wild-type cells (N.C. Steinel and C.H. Bassing, unpublished observations). This difference could reflect the relative dependence of TCRβ and IgH loci upon ATM for ensuring monoallelic RAG cleavage and/or suppressing aberrant rearrangement events that prevent the assembly of functional in-frame coding joins. However, confirmation and identification of these potential ATM-activated signaling pathways and the elucidation of their potential function in controlling V-to-(D)-J rearrangements and contributing to the initiation of allelic exclusion are required for unequivocal conclusions.

Conclusions

In the past few years, studies incorporating advances in techniques and reagents have revealed that Ag receptor allelic exclusion most likely is controlled by multiple redundant and successive mechanisms (Fig. 2). The next step within the field is to identify the DNA elements, protein factors, and potential RNA molecules that may control each of these individual mechanisms. These efforts should be guided by the more advanced knowledge of the related X chromosome inactivation and imprinting processes in mammals, as well as the general cellular mechanisms known to silence developmentally regulated site-specific DNA recombination events in Schizosaccharomyces pombe and Tetrahymena thermophila. Subsequent steps will be to inactivate/mutate these candidate cis elements and trans factors to confirm their potential role in regulating allelic exclusion and determine the precise mechanisms through which they function in this capacity. To reach unequivocal conclusions, the design and interpretation of these experiments will need to consider potential regulation at all mechanistic steps, not just the one being assayed. In the long-term, experiments that manipulate all of the redundant and successive mechanisms that regulate Ag receptor allelic exclusion will be required to reveal the biological relevance of this phenomenon that has mystified immunologists for half a century.

Disclosures

The authors have no financial conflicts of interest.

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