Annu. Rev. Immunol. 2004. 22:485–501
doi: 10.1146/annurev.immunol.22.012703.104707

First published online as a Review in Advance on November 10, 2003

RAGs AND REGULATION OF AUTOANTIBODIES

Mila Jankovic,[1] Rafael Casellas,[2] Nikos Yannoutsos,[1] Hedda Wardemann,[1] and Michel C. Nussenzweig[1,3]

[1]Laboratory of Molecular Immunology, [3]Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021; email: jankovm@mail.rockefeller.edu; wardemh@mail.rockefeller.edu; Yannoun@mail.rockefeller.edu; nussen@mail.rockefeller.edu

[2]Division of Biology, California Institute of Technology, Pasadena, California 91125; email: casellas@caltech.edu

Key Words B cell development, tolerance, receptor editing, RAG1 and RAG2 expression, antiself antibodies

■ **Abstract** Autoreactive antibodies are etiologic agents in a number of autoimmune diseases. Like all other antibodies these antibodies are produced in developing B cells by V(D)J recombination in the bone marrow. Three mechanisms regulate autoreactive B cells: deletion, receptor editing, and anergy. Here we review the prevalence of autoantibodies in the initial antibody repertoire, their regulation by receptor editing, and the role of the recombinase proteins (RAG1 and RAG2) in this process.

INTRODUCTION

Antigen receptor diversity is generated by random rearrangements between variable (V) diversity (D) and joining (J) gene segments in developing B cells and T cells (1). This receptor diversity ensures efficient immune responses to a universe of rapidly evolving potential pathogens. However, random gene reassortment can also produce self-reactive antibodies that could cause "horror autotoxicus," as pointed out by Ehrlich (2). Burnett, Talmage, and Lederberg provided a framework for understanding how self-reactive lymphocytes might be removed from the nascent repertoire as part of the clonal selection theory (3–5): They proposed that any self-reactive antibody producing lymphocytes would be deleted during development. In the past 20 years transgenic experiments in mice have shown that at least three mechanisms account for silencing of self-reactive antibodies during B cell development in the bone marrow: receptor editing, deletion, and anergy (6–9). One of these mechanisms, receptor editing, relies on V(D)J recombination to obviate autoantibody production in vivo. Here we review recent work on aspects of V(D)J recombination relevant to production and regulation of autoreactive antibodies in vivo.

MOST NASCENT B CELLS PRODUCE AUTOANTIBODIES

How B cells produce a diverse antibody repertoire while maintaining self-tolerance has been an important question since the time of Ehrlich (2). Landsteiner's finding that the antibody repertoire is almost infinitely diverse further stressed the potential importance of avoiding self-reactivity and raised the problem of prevalence of autoantibodies in the initial repertoire (10).

A number of investigators found that the majority of nascent immature B cells are deleted before they reach the mature B cell compartment, suggesting that autoreactive B cells might be a large part of the initial repertoire (11–15). Only 10%–20% of the bone marrow immature cells reach the periphery, and as few as 3% contribute to the mature B cell pool (14, 16–18). Furthermore, deletion was not random because there was a specific shift in the representation of antibodies in the repertoire between the immature and mature B cell stages (19–21).

The number of self-reactive antibodies was determined for human B cells by single cell analysis of the bone marrow and blood (22) (Figure 1). Two types of autoreactive antibodies were measured by ELISA, antinuclear antibodies (ANAs) and antibodies to defined antigens such as DNA, insulin and LPS. Antibodies that bound to more than one of these defined antigens were considered polyreactive. The ANAs were assayed by ELISA on HEp-2 cell lysates and verified by indirect immunofluorescence. Fifty-five percent to 75% of all antibodies in the early immature B cell compartment (B cells with the surface phenotype of pre-B cells that contained in-frame Igκ or Igλ chains) produced self-reactive antibodies (22). The self-reactive antibodies were lost from the B cell repertoire at two discrete checkpoints in development. The first checkpoint was between the early immature B cell stage and the immature B cell stage. Nearly all antibodies (poly)reactive with defined antigens were lost in this early transition in the bone marrow. ANA antibodies were lost from the repertoire in the immature B cell compartment and also in the periphery at the transition between the immature and mature B cell pool. Therefore, the majority of nascent human B cells is self-reactive. Future experimentation should focus on understanding changes in the efficiency of autoantibody removal that might lead to increased susceptibility to autoimmunity.

RAG EXPRESSION AND ANTIBODY ASSEMBLY IN DEVELOPING B CELLS

RAG1 and RAG2 proteins catalyze V(D)J recombination and antibody gene assembly (23, 24). RAGs bind to and cleave DNA at conserved recombination signal sequences (RS) that flank immunoglobulin (Ig) and T cell receptor (TCR) gene segments (25). These proteins are essential for the recombination reaction, and loss of either one in mice or humans results in complete block of lymphocyte development (26–28).

RAG1 and *RAG2* are only coexpressed in developing lymphocytes, and the level of expression is regulated in a developmental stage–specific manner (29, 30)

(see Figure 1). The first B cells to express *RAG1* and *RAG2* are early lymphoid committed progenitors found in fraction A1 (31, 32). These cells are $Lin^{-}CD27^{+}c\text{-}kit^{Hi}Sca^{Hi}$ and express low levels of RAGs and exhibit low levels of recombinase activity as measured by recombination of antibody D and J gene segments (33). *RAG*s are further induced in pro-B cells where Ig heavy chain gene assembly begins (31, 32, 34). Successful heavy chain gene recombination leads to expression of membrane Ig heavy chains (μ), which in turn induce clonal expansion, *RAG* downregulation, heavy chain allelic exclusion, and progression to the pre-B cell stage (35–40). It is essential for heavy chain allelic exclusion to be established at this stage of B cell development because persistent recombination might inactivate the productively rearranged heavy chain or produce a cell with two heavy chains and multiple antibody specificities. *RAG1* and *RAG2* downregulation is likely an important initial aspect of establishing allelic exclusion at the heavy chain locus. However, exclusion is maintained in later B cell stages by RAG-independent changes in heavy chain accessibility that appear to be induced through IL-7 (41, 42). *RAG1* and *RAG2* expression is downregulated in cycling pre-B cells but it is not known whether this downregulation is mediated at the transcriptional level or at the level of mRNA stability (43). Decreased transcription is suggested by decreased GFP expression in mice that carry YFP and GFP inserted into the *RAG1* or *RAG2* genes, respectively (32, 44). RAG protein levels are also regulated by phophorylation of the RAG protein, which decreases the half-life of the protein (45).

A second wave of *RAG* expression is induced when pre-B cells stop dividing, and it is associated with onset of high levels of Ig light chain gene recombination (35, 46). Upon successful expression of an Ig light chain gene and assembly of a functional BCR, pre-B cells become immature B cells. Immature B cells are the first B cells to express surface IgM, and it is in this compartment that antibodies are initially tested for self-reactivity and that central B cell tolerance is established by receptor editing or deletion (6, 7). Immature B cells display two features that facilitate induction of central tolerance: persistent *RAG* expression that allows for receptor editing, and apoptosis in response to high levels of BCR cross-linking that promotes deletion (32, 44, 47, 48).

PERSISTENT *RAG* EXPRESSION AND RECEPTOR EDITING

Receptor editing involves replacement of self-reactive antibody V region (7, 49) genes by V(D)J recombination (6). Gene replacement by persistent V(D)J recombination was first described in B cell lines in vitro (50–52). In mice, it was noted that Igκ transgenes did not induce high levels of allelic exclusion and that there was frequent persistent V(D)J recombination with some antibody transgenes (53, 54). However, the potential physiologic significance of secondary recombination of Ig genes in maintaining tolerance was only appreciated from studies of transgenic mice carrying anti–double stranded DNA or anti-MHC antibodies (6, 7). In these mice, immature B cells are exposed to multivalent self-antigens at early stages

of development (8, 55). Most of these B cells are deleted, but a small cohort of B cells escapes deletion and migrates to the spleen and lymph nodes where they accumulate with age (6). It was shown that while the transgenic heavy chain was present in the B cells that escape deletion, they expressed an endogenous, non-transgenic κ or λ gene, and the resulting chimera was no longer self-reactive (6, 7, 49). Thus, the nontransgenic κ or λ gene was said to "edit" the original transgenic self-reactivity.

Further insight into light chain editing came from the analysis of the transgenic mice that carry only the 3H9 heavy chain of an anti-DNA antibody (49, 56). 3H9 produces anti-DNA when combined with most light chains but a small group of light chains with low isoelectric points such as Vκ12A block anti-DNA reactivity. Despite the high level of reactivity of 3H9 with DNA, splenic B cells from these mice did not produce anti-DNA antibodies. 3H9 peripheral B cells showed highly skewed Vκ and Jκ usage with a large number of cells expressing Vκ12A, and the majority of the Vκ genes were joined to Jκ5, indicating that multiple recombination events had occurred on the same chromosome (49). Efficient light chain "editors" differed from other κ light chains in that their CDRs are very acidic and have the capacity to prevent DNA binding by neutralizing the arginines in the CDR3 of 3H9 (57). Enhanced editing was associated with high levels of *RAG* expression, suggesting that editing induced or prolonged *RAG* expression (7).

The efficiency of editing was assessed using mice produced by gene targeting to express anti-MHC (H-2K$^{k\text{-}b}$) or anti-DNA heavy and light chains (58, 59). In contrast to the severely reduced numbers of mature B cells reported in conventional anti-MHC transgenic mice, the bone marrow and peripheral B cell compartments of these knock-in mice were nearly normal, suggesting that editing was highly efficient (58, 59).

IgV_H genes can also be replaced by secondary V(D)J recombination through the use of cryptic recombination signal sequences embedded in the V_H gene (52, 60–62). Studies in mice and humans have shown evidence for V_H replacement in autoantibody-producing B cells (63–65). However, edited heavy chains frequently show N nucleotide addition, suggesting that this process can occur in progenitor B cells before light chain assembly (63, 66, 67). Such replacements would not be a response to self-antigen binding. Nevertheless, it has been estimated that 5%–12% of the human antibody repertoire is produced by V_H replacement (68).

SITE OF EDITING

RAG expression was believed to be upregulated for a third time in immature B cells that carry self-reactive receptors. In vitro experiments with both transgenic and wild-type B cells showed increased RAG mRNA levels in cultures of developing B cells following BCR cross-linking (69, 70). In addition bone marrow cells from mice that carry anti-MHC (H-2K$^{k\text{-}b}$) antibody transgenes show higher levels of *RAG* expression in the self-reactive background (71). Whether this increase in RAG

expression is due to increased transcription or selection for cells that express high levels of RAG remains to be determined. Immature B cells are not a homogeneous population with respect to the level of RAG or BCR expression (Figure 1). Analysis of the *RAG*2-GFP indicator mice showed that RAG levels were inversely correlated with the density of the surface B cell receptor and B cell maturation (44, 72). More mature B cells with higher levels of cell surface BCR expression showed lower levels of RAG expression, suggesting that RAG expression is gradually turned off as B cells mature (44, 72). Immature B cells that express high levels of surface IgM are also more susceptible to negative selection (47, 48). Thus, the apparent upregulation of RAG mRNA by BCR cross-linking in vitro could also be a consequence of the selective survival of the cells that expressed higher levels of RAGs and lower levels of BCR to begin with. Similarly, increased RAG expression in transgenic B cells altered B cell maturation and enrichment for less mature B cells that have lower levels of surface BCR and higher RAG expression.

To visualize the editing compartment in vivo, an Igκ allelic polymorphism was created by targeted replacement of the mouse constant region gene (mCκ) by its human homologue (hCκ) (73). In mice heterozygous for both a prerecombined light chain (at the mCκ locus) and the hCκ allele, edited B cells were distinguished as those cells that lost expression of the prerecombined mCκ allele (73). Kinetic studies showed that B lymphocytes undergoing editing were arrested for at least two hours at the pre-BII cell stage when compared to unedited counterparts. The delayed and edited B cell population showed higher levels of *RAG* expression than unedited cells (73). Similar kinetic data have been obtained using mice that carry a prerecombined Vλ gene instead of hCκ (74). These observations are consistent with a model where self-recognition activates secondary gene recombination by inducing a developmental arrest at a stage where *RAG1* and *RAG2* are expressed. In this model, cross-linking of the cell surface receptor by self-antigen does not reinduce but sustains *RAG1* and *RAG2* expression and allows for secondary V(D)J recombination. The model further clarifies how light chain allelic exclusion is maintained in the presence of high levels of editing: B cells expressing innocuous receptors transit rapidly from this stage to a compartment where *RAG* expression and V(D)J recombination are turned off. Thus, B cells with a nonself-reactive receptor are not exposed to prolonged V(D)J recombination, and the self-reactive cells that are trapped in the editing compartment remain so only until they produce a nonself-reactive receptor. In this regulated model for allelic exclusion, both Igκ alleles could be accessible to the V(D)J recombinase simultaneously without interfering with allelic exclusion.

EXTENT OF EDITING

Several lines of evidence suggest that secondary gene rearrangements are a common occurrence. For example, analysis of hybridomas generated from Vκ4Jκ4 and Vκ8Jκ5 knock-in mice shows that both prerearranged light chains are replaced by

editing in a large number of B lymphocytes (75). Likewise, the Vκ3-83Jκ2 light chain is edited by secondary recombination occurring at the targeted allele and this is accompanied by V(D)J recombination at the wild-type allele (58). Editing has also been examined in Igλ expressing nontransgenic B cells in mice and man (76, 77). Forty-seven percent of all mouse Igλ expressing B cells that have had their Igκ genes inactivated by RS rearrangements show in-frame and potentially functional Igκ genes (77). In man, almost all λ expressing B cells also carry a rearranged κ locus, whereas only 2% of κ expressing B cells carry rearrangement Ig λ genes (76). Furthermore, inactivated in-frame κ joints are found in 30% of human λ expressing cells (76). However, it was not determined why the in-frame Igκ alleles were replaced in mouse or man.

A direct measurement of the extent of receptor editing was made using the hCκ and Vλ knock-in alleles (73, 74). These studies revealed that on average 25% of the repertoire is the result of secondary gene replacements, indicating that editing makes an important contribution to the normal antibody repertoire.

TRANSCRIPTION AND SECONDARY V(D)J RECOMBINATION

Ig gene expression has been proposed to be essential for primary V(D)J recombination based on the direct correlation between the onset of germline transcription and antibody gene recombination (78–80). In support of this model, targeted deletion of transcription regulatory elements and some transcription factors impairs both expression and recombination of TCR and Ig genes in vivo (81–85). In addition, V(D)J recombination can only be induced in nonlymphoid cells when Ig genes are transcribed (86). However, experiments with cell lines, transgenic recombination substrates, and Pax5$^{-/-}$ B cells show that transcription does not always correlate with recombinational accessibility (87–91). Furthermore, the question of whether there is a role of transcription per se as opposed to indirect enhancement of locus accessibility has not been resolved.

The role of transcription in secondary recombination was investigated in mice mutant for the transcriptional coactivator OcaB. OcaB (also known as Bob-1 and OBF-1) (92–94) forms a ternary complex with the transcription factor Oct-1 (or Oct-2) (95) on octamer motifs present in some Ig gene promoters. This octamer/Oct-1(2)/OcaB ternary complex is thought to activate Ig gene transcription by specific interactions with the TATA-box-associated basal transcription machinery (96, 97). A direct correlation was found between light chain promoter transcription and receptor editing (98). In the absence of OcaB, the Igκ chromatin domain is accessible as measured by DNA demethylation, histone acetylation, promoter loading of Oct-1, and low level transcription (98). However, open chromatin and low level transcription were not sufficient for gene replacement by V(D)J recombination. Only strongly transcribed promoters were replaced (98). Thus, a specific threshold of Vκ gene transcription is required for secondary gene recombination.

TERMINATION OF *RAG* EXPRESSION

RAG expression was initially thought to be confined to pro-B, pre-B, and immature B cells in the bone marrow. However, several reports showed RAG mRNA and protein expression in germinal centers and in vitro activated B cells (99, 100). RAG1 and RAG2 mRNAs were detected in IgD^+ B cells cultured in the presence of CD40 (or lipopolysaccharide) and IL4, whereas splenic, Peyer's patches, or lymph node germinal center B cells showed abundant RAG1 protein expression as determined by immunostaining (99, 100). Unlike most peripheral B cells, RAG^+ lymphocytes (in both mice and humans) expressed several cell surface markers characteristic of pro-B or pre-B cells such as λ5, VpreB, TdT, GL7, low levels of B220, and IL7R (99, 101–105). Ongoing recombination in these peripheral cells was implied by the presence of DNA double-stranded break V(D)J intermediates (106–108) and de novo formation of Vλ1-Jλ1 signal joints. However, the similarities between bone marrow and peripheral RAG^+ B cells raised the possibility that these cells might in fact represent B cell precursors. Experiments with transgenic and GFP knock-in mice supported this hypothesis (44, 72). In both cases, GFP expression in developing B and T cells mimics endogenous *RAG*s with the caveat that the GFP expression level in the transgenic mice was one order of magnitude higher than in the knock-in mice. Despite this, the results obtained with both models were similar in that they showed that RAG-GFP expression in spleens was restricted to immature new immigrant B cells (44, 72). Furthermore, adoptive transfer experiments showed that mature B cells could not be induced to re-express RAGs during an immune response in vivo or by culturing them in the presence of LPS and IL4 (44, 72). Interestingly, the RAG^+ immature B cell compartment in the spleen was increased in animals immunized with antigen, infectious agents, or adjuvant only at late stages of the immune response (44, 72, 109, 110). Increase in production and export of immature B cells from the bone marrow (109, 110) accounted for accumulation of these RAG^+ immigrants with kinetics similar to germinal center formation (99, 100).

The levels of RAG expression in developing B cells decreased as they acquired higher levels of surface IgM expression (see above). New immigrant B cells in the periphery express substantially lower levels of RAG than immature B cells in the bone marrow (44, 72). Nevertheless, alterations in the kinetics of immature B cell migration to the periphery under conditions of immunization might explain reports on RAG expression in peripheral B cells even in the absence of reinduction.

FROM EDITING TO DELETION

Termination of *RAG* expression prevents further V(D)J recombination and receptor editing. To maintain tolerance, B cells that cannot edit self-reactive receptors must be deleted or rendered anergic.

The idea that self-reactive clones are deleted during development was put forward by Joshua Lederberg as a refinement of the clonal selection theory (5). This

concept was confirmed by experiments in which mice were treated with anti-Igs from birth and were found to be B cell depleted (111). The assumption linking this result to tolerance was that anti-IgM antibodies must mimic autoantigens by cross-linking the cell surface receptor. Likewise, bone marrow B cells challenged in vitro with anti-IgM antibodies downregulate surface Ig and undergo apoptosis (11, 13, 15, 19). In transgenic mice, expression of certain self-specificities also induced clonal B cell deletion (8, 55, 112–114). In anti-MHC expressing B cells exposure to antigen induced a developmental block that was concurrent with secondary VL gene recombination and replacement of cell surface receptors (48, 69, 70). Cells that failed to undergo successful editing were destined for death by apoptosis. This idea was confirmed in experiments with *RAG*-deficient mice that carried anti-DNA or self-MHC antibodies (115, 116). In the absence of secondary recombination all autoreactive B cells were deleted and there were no peripheral B cells (115, 116). Thus, antigen-induced apoptosis is a relatively late event in bone marrow B cell development that is preceded by an editing permissive stage that appears to be dependent on the bone marrow microenvironment (21, 48, 117).

CIS REGULATION OF *RAG* EXPRESSION

Highly regulated *RAG* expression is required for successful assembly of Ig and TCR genes and normal lymphocyte development. On the other hand, silencing RAGs in mature lymphoid cells is necessary in order to ensure genomic stability.

The *RAG* locus is peculiar in that *RAG1* and *RAG2* are adjacent but convergently transcribed genes that entered the vertebrate genome at the time of emergence of jawed fish (23–25, 118, 119). This unusual evolutionarily conserved genomic organization and the finding that RAG proteins can mediate transposition in vitro lead to the hypothesis that these genes originate from a mobile element that entered the vertebrate genome at the time of emergence of jawed fish (120, 121). Regulation of *RAG* expression has been studied by transfection in cell lines in vitro and in vivo using bacterial artificial chromosome (BAC) transgenic mice, using green or yellow fluorescent protein (GFP or YFP) as reporters in reconstitution experiments with transfected ES cells, and by gene targeting. The disadvantage of the transfection systems is that the transfected cells represent static stages in B or T cell development and therefore the dynamic aspects of *RAG* regulation in developing lymphocytes cannot be explored. Nevertheless, much has been learned about the chromatin structure and transcription factors that bind the murine and human *RAG1* and *RAG2* TATA-less promoters in vitro (122–126). The *RAG1* promoter is not active or tissue specific in transient transfections in the absence of a heterologous enhancer such as the Ig Eμ (125). In contrast, the murine *RAG2* promoter is active in transient transfection experiments in B and T cell lines, and the activity is dependent on PAX5 and GATA3 (127, 128). However, stable transfectants showed little activity for either *RAG1* or *RAG2* promoters in the absence of exogenous enhancers (129).

A second method used to evaluate RAG regulation was *RAG2*$^{-/-}$ blastocyst reconstitution by transfected *RAG2* transgenes (130). These experiments suggested

that all of the information required for *RAG2* regulation was found in an 18 kb genomic fragment extending from 9 kb upstream of the *RAG2* promoter to 2.4 kb 3′ of the untranslated region because it rescued thymocyte development (130). Similar rescue of RAG2$^{-/-}$ mice was also obtained with a yeast artificial chromosome (*RYAC*) that contained both functional *RAG* genes and 12 kb of DNA 5′ of *RAG2* (131). The double positive (DP) compartment was fully reconstituted, and mature CD4^{+}CD8^{-} and CD4^{-}CD8^{+} thymocytes were present at one third of normal levels in the *RYAC/RAG2*$^{-/-}$ mice (131). However, this reconstitution of the DP and single positive (SP) stage differed significantly from the normal wild-type T cell development in that the mature cells were reduced by up to 70% and that the TCRα rearrangements were restricted to only the initial recombination at the 5′ end of the Jα locus (131). Reconstitution was nonphysiological because *RYAC* failed to induce detectable *RAG1* or *RAG2* transcription in DP T cells. Thus, neither the 18 kb genomic fragment (130) nor the *RYAC* contained sequences required for RAG expression in DP T cells (131), and the *RAG2*$^{-/-}$ blastocyst reconstitution is not a sensitive assay to study *RAG* regulation in vivo.

Transgenic mouse and gene targeting experiments have confirmed the requirement for distal enhancers in regulating *RAG* expression in vivo. Two elements with *RAG* promoter-enhancing activity have been identified: One element is active in B cells and the second in DP T cells (32, 129, 131). Both of these elements are on the *RAG2* side of the locus, and both elements have effects on *RAG1* and on *RAG2* promoters, suggesting a mechanism for achieving coordinate regulation of the two genes by one set of genetic elements (32).

The B cell specific element is found in a 2.3 kb area located approximately 22 kb 5′ of *RAG2*. In cell lines this element enhances transcription over background levels in a highly variegated manner (36% of the transfected cells show no expression at all and only 10% of the clones show high levels of expression) (129). Nevertheless, enhancement is found only in pro-B cells and is absent from T, adult B, or nonlymphoid cell lines. Targeted deletion of this element from the mouse genome leads to a B cell specific tenfold and a twofold decrease in RAG*1* and RAG*2* mRNA levels, respectively, with no effect on *RAG* expression in DN or DP cells (129). Although the targeted deletion shows that this element is required for complete regulation, no ectopic or developmentally aberrant expression is reported in its absence. It remains to be determined whether this element is sufficient to drive tissue-specific and developmentally regulated expression of *RAG*, as is the case for other enhancers such as the Igμ enhancer.

The T cell specific element was identified in transgenic experiments with bacterial artificial chromosomes (BACs) that carry fluorescent protein indicator genes in place of the *RAG* genes (32). These experiments showed that the transcription of both *RAG1* and *RAG2* in DP thymocytes is coordinated by one or more cis element(s) located between 32 and 87 kb 5′ of *RAG2*. In addition to regulating expression in DP thymocytes, this region was also required to prevent position effect variegation in developing T cells and B cells. Therefore, an element (or elements) in this region is a required part of a traditional locus control region (132, 133).

Like developing B cells, thymocytes express RAGs in two waves. V(D)J recombination is initiated with the first wave of RAG expression at the TCRβ locus in $CD4^{-}CD8^{-}$ (DN) T cells. The TCRβ locus is analogous to the Ig heavy chain locus, and DN T cells are analogous to pro-B cells (134). Once a TCRβ chain is expressed, it combines with pre-Tα and CD3 components to produce the pre-TCR (135, 136). Similar to the pre-BCR, the pre-TCR downregulates *RAG* expression and induces T cells to mature to the $CD4^{+}CD8^{+}$ double positive (DP) stage. Upon entering the DP stage there is a second wave of *RAG* expression and progressive V(D)J recombination along the Jα locus (29, 131, 137–139). V(D)J recombination is only turned off in DP cells when they express a TCR that recognizes self-MHC with an affinity that allows for positive selection (140, 141). Thus, TCR cross-linking in DP T cells is the signal that extinguishes *RAG* expression. This is different from pre-B cells where receptor cross-linking delays extinction of *RAG* expression and prolongs recombination leading to receptor editing (73).

We speculate that the separate B cell and DP T cell specific elements for *RAG* regulation may provide a molecular explanation for why T cells turn off *RAG* expression upon TCR cross-linking, whereas immature B cells prolong *RAG* expression and induce receptor editing upon BCR cross-linking.

The *Annual Review of Immunology* is online at http://immunol.annualreviews.org

LITERATURE CITED

1. Tonegawa S. 1983. Somatic generation of antibody diversity. *Nature* 302:575–81
2. Silverstein AM. 2001. Autoimmunity versus horror autotoxicus: the struggle for recognition. *Nat. Immunol.* 2:279–81
3. Burnet MF. 1959. *The Clonal Selection Theory of Acquired Immunity*. Cambridge, UK: Cambridge Univ. Press
4. Talmage DW. 1959. Tumor development in immunologically deficient nude mice after exposure to chemical carcinogens. *Science* 129:1643–48
5. Lederberg J. 1959. Genes and antibodies. *Science* 129:1649–53
6. Gay D, Saunders T, Camper S, Weigert M. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med.* 177:999–1008
7. Tiegs SL, Russell DM, Nemazee D. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.* 177:1009–20
8. Nemazee DA, Burki K. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature* 337:562–66
9. Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* 334:676–82
10. Landsteiner K. 1936. *The Specificity of Serological Reactions.* New York: Dover
11. Sidman CL, Unanue ER. 1975. Receptor-mediated inactivation of early B lymphocytes. *Nature* 257:149–51
12. Teale JM, Klinman NR. 1980. Tolerance as an active process. *Nature* 288:385–87
13. Nossal GJ. 1983. Cellular mechanisms of immunologic tolerance. *Annu. Rev. Immunol.* 1:33–62
14. Allman DM, Ferguson SE, Lentz VM, Cancro MP. 1993. Peripheral B cell maturation. II. Heat-stable antigen (hi) splenic B cells are an immature developmental

intermediate in the production of long-lived marrow-derived B cells. *J. Immunol.* 151:4431–44
15. Norvell A, Mandik L, Monroe JG. 1995. Engagement of the antigen-receptor on immature murine B lymphocytes results in death by apoptosis. *J. Immunol.* 154:4404–13
16. Rolink AG, Andersson J, Melchers F. 1998. Characterization of immature B cells by a novel monoclonal antibody, by turnover and by mitogen reactivity. *Eur. J. Immunol.* 28:3738–48
17. Forster I, Rajewsky K. 1990. The bulk of the peripheral B-cell pool in mice is stable and not rapidly renewed from the bone marrow. *Proc. Natl. Acad. Sci. USA* 87:4781–84
18. Hao Z, Rajewsky K. 2001. Homeostasis of peripheral B cells in the absence of B cell influx from the bone marrow. *J. Exp. Med.* 194:1151–64
19. Raff MC, Owen JJ, Cooper MD, Lawton ARd, Megson M, et al. 1975. Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin-induced immunoglobulin suppression in vitro. Possible implications for B-cell tolerance to self. *J. Exp. Med.* 142:1052–64
20. Gu H, Tarlinton D, Muller W, Rajewsky K, Forster I. 1991. Most peripheral B cells in mice are ligand selected. *J. Exp. Med.* 173:1357–71
21. Sandel PC, Monroe JG. 1999. Negative selection of immature B cells by receptor editing or deletion is determined by site of antigen encounter. *Immunity* 10:289–99
22. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. 2003. Predominant autoantibody production by early human B cell precursors. *Science* 301(5638):1374-77
23. Schatz DG, Oettinger MA, Baltimore D. 1989. The V(D)J recombination activating gene, RAG-1. *Cell* 59:1035–48
24. Oettinger MA, Schatz DG, Gorka C, Baltimore D. 1990. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 248:1517–23
25. McBlane JF, van Gent DC, Ramsden DA, Romeo C, Cuomo CA, et al. 1995. Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* 83:387–95
26. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, et al. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 68:869–77
27. Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, et al. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* 68:855–67
28. Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, et al. 1996. RAG mutations in human B cell-negative SCID. *Science* 274:97–99
29. Wilson A, Held W, MacDonald HR. 1994. Two waves of recombinase gene expression in developing thymocytes. *J. Exp. Med.* 179:1355–60
30. Grawunder U, Leu TM, Schatz DG, Werner A, Rolink AG, et al. 1995. Down-regulation of RAG1 and RAG2 gene expression in preB cells after functional immunoglobulin heavy chain rearrangement. *Immunity* 3:601–8
31. Allman D, Li J, Hardy RR. 1999. Commitment to the B lymphoid lineage occurs before DH-JH recombination. *J. Exp. Med.* 189:735–40
32. Yu W, Misulovin Z, Suh H, Hardy RR, Jankovic M, et al. 1999. Coordinate regulation of RAG1 and RAG2 by cell type-specific DNA elements 5′ of RAG2. *Science* 285:1080–84
33. Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW. 2002. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* 17:117–30
34. Alt FW, Yancopoulos GD, Blackwell TK, Wood C, Thomas E, et al. 1984. Ordered rearrangement of immunoglobulin heavy

chain variable region segments. *EMBO J.* 3:1209–19
35. Li YS, Hayakawa K, Hardy RR. 1993. The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. *J. Exp. Med.* 178:951–60
36. Ehlich A, Martin V, Muller W, Rajewsky K. 1994. Analysis of the B-cell progenitor compartment at the level of single cells. *Curr. Biol.* 4:573–83
37. Nussenzweig MC, Shaw AC, Sinn E, Danner DB, Holmes KL, et al. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin mu. *Science* 236:816–19
38. Manz J, Denis K, Witte O, Brinster R, Storb U. 1988. Feedback inhibition of immunoglobulin gene rearrangement by membrane mu, but not by secreted mu heavy chains [published erratum appears in *J. Exp. Med.* 1989 June 1. 169(6):2269]. *J. Exp. Med.* 168:1363–81
39. Kitamura D, Rajewsky K. 1992. Targeted disruption of mu chain membrane exon causes loss of heavy-chain allelic exclusion. *Nature* 356:154–56
40. Melchers F, Rolink A, Grawunder U, Winkler TH, Karasuyama H, et al. 1995. Positive and negative selection events during B lymphopoiesis. *Curr. Opin. Immunol.* 7:214–27
41. Bassing CH, Swat W, Alt FW. 2002. The mechanism and regulation of chromosomal V(D)J recombination. *Cell* 109(Suppl.):S45–55
42. Chowdhury D, Sen R. 2001. Stepwise activation of the immunoglobulin mu heavy chain gene locus. *EMBO J.* 20:6394–403
43. Melchers F. 1995. The role of B cell and pre-B cell receptors in development and growth control of the B-lymphocyte lineage. In *Immunoglobulin Genes*, ed. T Honjo, FW Alt. pp. 33–56. London: London Academic Ltd. 3rd ed.
44. Monroe RJ, Seidl KJ, Gaertner F, Han S, Chen F, et al. 1999. RAG2:GFP knockin mice reveal novel aspects of RAG2 expression in primary and peripheral lymphoid tissues. *Immunity* 11:201–12
45. Li Z, Dordai DI, Lee J, Desiderio S. 1996. A conserved degradation signal regulates RAG-2 accumulation during cell division and links V(D)J recombination to the cell cycle. *Immunity* 5:575–89
46. Ehlich A, Schaal S, Gu H, Kitamura D, Muller W, et al. 1993. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. *Cell* 72:695–704
47. Carsetti R, Kohler G, Lamers MC. 1995. Transitional B cells are the target of negative selection in the B cell compartment. *J. Exp. Med.* 181:2129–40
48. Melamed D, Benschop RJ, Cambier JC, Nemazee D. 1998. Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection. *Cell* 92:173–82
49. Radic MZ, Erikson J, Litwin S, Weigert M. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. *J. Exp. Med.* 177:1165–73
50. Feddersen RM, Van Ness BG. 1985. Double recombination of a single immunoglobulin kappa-chain allele: implications for the mechanism of rearrangement. *Proc. Natl. Acad. Sci. USA* 82: 4793–97
51. Levy S, Campbell MJ, Levy R. 1989. Functional immunoglobulin light chain genes are replaced by ongoing rearrangements of germline V kappa genes to downstream J kappa segment in a murine B cell line. *J. Exp. Med.* 170:1–13
52. Kleinfield R, Hardy RR, Tarlinton D, Dangl J, Herzenberg LA, et al. 1986. Recombination between an expressed immunoglobulin heavy-chain gene and a germline variable gene segment in a Ly 1+ B-cell lymphoma. *Nature* 322:843–46
53. Ritchie KA, Brinster RL, Storb U. 1984. Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in kappa transgenic mice. *Nature* 312:517–20

54. Rusconi S, Kohler G. 1985. Transmission and expression of a specific pair of rearranged immunoglobulin mu and kappa genes in a transgenic mouse line. *Nature* 314:330–34
55. Erikson J, Radic MZ, Camper SA, Hardy RR, Carmack C, et al. 1991. Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature* 349:331–34
56. Prak EL, Trounstine M, Huszar D, Weigert M. 1994. Light chain editing in kappa-deficient animals: a potential mechanism of B cell tolerance. *J. Exp. Med.* 180:1805–15
57. Li H, Jiang Y, Prak EL, Radic M, Weigert M. 2001. Editors and editing of anti-DNA receptors. *Immunity* 15:947–57
58. Pelanda R, Schwers S, Sonoda E, Torres RM, Nemazee D, et al. 1997. Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification. *Immunity* 7:765–75
59. Chen C, Prak EL, Weigert M. 1997. Editing disease-associated autoantibodies. *Immunity* 6:97–105
60. Kleinfield RW, Weigert MG. 1989. Analysis of VH gene replacement events in a B cell lymphoma. *J. Immunol.* 142:4475–82
61. Reth M, Gehrmann P, Petrac E, Wiese P. 1986. A novel VH to VHDJH joining mechanism in heavy-chain-negative (null) pre-B cells results in heavy-chain production. *Nature* 322:840–42
62. Wilson PC, Wilson K, Liu YJ, Banchereau J, Pascual V, et al. 2000. Receptor revision of immunoglobulin heavy chain variable region genes in normal human B lymphocytes. *J. Exp. Med.* 191:1881–94
63. Chen C, Nagy Z, Prak EL, Weigert M. 1995. Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. *Immunity* 3:747–55
64. Pewzner-Jung Y, Friedmann D, Sonoda E, Jung S, Rajewsky K, et al. 1998. B cell deletion, anergy, and receptor editing in "knock in" mice targeted with a germline-encoded or somatically mutated anti-DNA heavy chain. *J. Immunol.* 161:4634–45
65. Itoh K, Meffre E, Albesiano E, Farber A, Dines D, et al. 2000. Immunoglobulin heavy chain variable region gene replacement as a mechanism for receptor revision in rheumatoid arthritis synovial tissue B lymphocytes. *J. Exp. Med.* 192:1151–64
66. Sonoda E, Pewzner-Jung Y, Schwers S, Taki S, Jung S, et al. 1997. B cell development under the condition of allelic inclusion. *Immunity* 6:225–33
67. Cascalho M, Ma A, Lee S, Masat L, Wabl M. 1996. A quasi-monoclonal mouse. *Science* 272:1649–52
68. Zhang Z ZM, Wang YH, Munfus D, Huye LE, Findley HW, et al. 2003. Contribution of Vh gene replacement to the primary B cell repertoire. *Immunity* 19:21–31
69. Hertz M, Nemazee D. 1997. BCR ligation induces receptor editing in IgM+IgD- bone marrow B cells in vitro. *Immunity* 6:429–36
70. Melamed D, Nemazee D. 1997. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. *Proc. Natl. Acad. Sci. USA* 94:9267–72
71. Nemazee D, Buerki K. 1989. Clonal deletion of autoreactive B lymphocytes in bone marrow chimeras. *Proc. Natl. Acad. Sci. USA* 86:8039–43
72. Yu W, Nagaoka H, Jankovic M, Misulovin Z, Suh H, et al. 1999. Continued RAG expression in late stages of B cell development and no apparent re-induction after immunization. *Nature* 400:682–87
73. Casellas R, Shih TA, Kleinewietfeld M, Rakonjac J, Nemazee D, et al. 2001. Contribution of receptor editing to the antibody repertoire. *Science* 291:1541–44
74. Oberdoerffer P, Novobrantseva TI, Rajewsky K. 2003. Expression of a targeted lambda 1 light chain gene is developmentally regulated and independent of

Ig kappa rearrangements. *J. Exp. Med.* 197:1165–72
75. Prak EL, Weigert M. 1995. Light chain replacement: a new model for antibody gene rearrangement. *J. Exp. Med.* 182:541–48
76. Brauninger A, Goossens T, Rajewsky K, Kuppers R. 2001. Regulation of immunoglobulin light chain gene rearrangements during early B cell development in the human. *Eur. J. Immunol.* 31:3631–37
77. Retter MW, Nemazee D. 1998. Receptor editing occurs frequently during normal B cell development. *J. Exp. Med.* 188:1231–38
78. Blackwell TK, Moore MW, Yancopoulos GD, Suh H, Lutzker S, et al. 1986. Recombination between immunoglobulin variable region gene segments is enhanced by transcription. *Nature* 324:585–89
79. Schlissel MS, Baltimore D. 1989. Activation of immunoglobulin kappa gene rearrangement correlates with induction of germline kappa gene transcription. *Cell* 58:1001–7
80. Yancopoulos GD, Alt FW. 1985. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. *Cell* 40:271–81
81. Chen J, Young F, Bottaro A, Stewart V, Smith RK, et al. 1993. Mutations of the intronic IgH enhancer and its flanking sequences differentially affect accessibility of the JH locus. *EMBO J.* 12:4635–45
82. Serwe M, Sablitzky F. 1993. V(D)J recombination in B cells is impaired but not blocked by targeted deletion of the immunoglobulin heavy chain intron enhancer. *EMBO J.* 12:2321–27
83. Xu Y, Davidson L, Alt FW, Baltimore D. 1996. Deletion of the Ig kappa light chain intronic enhancer/matrix attachment region impairs but does not abolish V kappa J kappa rearrangement. *Immunity* 4:377–85
84. Whitehurst CE, Schlissel MS, Chen J. 2000. Deletion of germline promoter PD beta 1 from the TCR beta locus causes hypermethylation that impairs D beta 1 recombination by multiple mechanisms. *Immunity* 13:703–14
85. Corcoran AE, Riddell A, Krooshoop D, Venkitaraman AR. 1998. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. *Nature* 391:904–7
86. Romanow WJ, Langerak AW, Goebel P, Wolvers-Tettero IL, van Dongen JJ, et al. 2000. E2A and EBF act in synergy with the V(D)J recombinase to generate a diverse immunoglobulin repertoire in nonlymphoid cells. *Mol. Cell* 5:343–53
87. Alvarez JD, Anderson SJ, Loh DY. 1995. V(D)J recombination and allelic exclusion of a TCR beta-chain minilocus occurs in the absence of a functional promoter. *J. Immunol.* 155:1191–202
88. Okada A, Mendelsohn M, Alt F. 1994. Differential activation of transcription versus recombination of transgenic T cell receptor beta variable region gene segments in B and T lineage cells. *J. Exp. Med.* 180:261–72
89. Cherry SR, Baltimore D. 1999. Chromatin remodeling directly activates V(D)J recombination. *Proc. Natl. Acad. Sci. USA* 96:10788–93
90. Angelin-Duclos C, Calame K. 1998. Evidence that immunoglobulin VH-DJ recombination does not require germ line transcription of the recombining variable gene segment. *Mol. Cell Biol.* 18:6253–64
91. Hesslein DG, Pflugh DL, Chowdhury D, Bothwell AL, Sen R, et al. 2003. Pax5 is required for recombination of transcribed, acetylated, 5′ IgH V gene segments. *Genes Dev.* 17:37–42
92. Gstaiger M, Knoepfel L, Georgiev O, Schaffner W, Hovens CM. 1995. A B-cell coactivator of octamer-binding transcription factors. *Nature* 373:360–62
93. Strubin M, Newell JW, Matthias P. 1995. OBF-1, a novel B cell-specific coactivator that stimulates immunoglobulin promoter

activity through association with octamer-binding proteins. *Cell* 80:497–506
94. Luo Y, Fujii H, Gerster T, Roeder RG. 1992. A novel B cell-derived coactivator potentiates the activation of immunoglobulin promoters by octamer-binding transcription factors. *Cell* 71:231–41
95. Staudt LM, Lenardo MJ. 1991. Immunoglobulin gene transcription. *Annu. Rev. Immunol.* 9:373–98
96. Luo Y, Roeder RG. 1995. Cloning, functional characterization, and mechanism of action of the B-cell-specific transcriptional coactivator OCA-B. *Mol. Cell Biol.* 15:4115–24
97. Schubart DB, Rolink A, Kosco-Vilbois MH, Botteri F, Matthias P. 1996. B-cell-specific coactivator OBF-1/OCA-B/Bob1 required for immune response and germinal centre formation. *Nature* 383:538–42
98. Casellas R, Jankovic M, Meyer G, Gazumyan A, Luo Y, et al. 2002. OcaB is required for normal transcription and V(D)J recombination of a subset of immunoglobulin κ genes. *Cell* 110:575–85
99. Han S, Zheng B, Schatz DG, Spanopoulou E, Kelsoe G. 1996. Neoteny in lymphocytes: Rag1 and Rag2 expression in germinal center B cells. *Science* 274:2094–97
100. Hikida M, Mori M, Takai T, Tomochika K, Hamatani K, et al. 1996. Reexpression of RAG-1 and RAG-2 genes in activated mature mouse B cells. *Science* 274:2092–94
101. Giachino C, Padovan E, Lanzavecchia A. 1998. Re-expression of RAG-1 RAG-2 genes and evidence for secondary rearrangements in human germinal center B lymphocytes. *Eur. J. Immunol.* 28:3506–13
102. Girschick HJ, Grammer AC, Nanki T, Mayo M, Lipsky PE. 2001. RAG1 and RAG2 expression by B cell subsets from human tonsil and peripheral blood. *J. Immunol.* 166:377–86
103. Hikida M, Ohmori H. 1998. Rearrangement of lambda light chain genes in mature B cells in vitro and in vivo. Function of reexpressed recombination-activating gene (RAG) products. *J. Exp. Med.* 187:795–99
104. Meffre E, Papavasiliou F, Cohen P, de Bouteiller O, Bell D, et al. 1998. Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B cells. *J. Exp. Med.* 188:765–72
105. Meffre E, Davis E, Schiff C, Cunningham-Rundles C, Ivashkiv LB, et al. 2000. Circulating human B cells that express surrogate light chains edited receptors. *Nat. Immunol.* 1:207–13
106. Hikida M, Nakayama Y, Yamashita Y, Kumazawa Y, Nishikawa SI, et al. 1998. Expression of recombination activating genes in germinal center B cells: involvement of interleukin 7 (IL-7) and the IL-7 receptor. *J. Exp. Med.* 188:365–72
107. Papavasiliou F, Casellas R, Suh H, Qin XF, Besmer E, et al. 1997. V(D)J recombination in mature B cells: a mechanism for altering antibody responses. *Science* 278:298–301
108. Han S, Dillon SR, Zheng B, Shimoda M, Schlissel MS, et al. 1997. V(D)J recombinase activity in a subset of germinal center B lymphocytes. *Science* 278:301–5
109. Gartner F, Alt FW, Monroe RJ, Seidl KJ. 2000. Antigen-independent appearance of recombination activating gene (RAG)-positive bone marrow B cells in the spleens of immunized mice. *J. Exp. Med.* 192:1745–54
110. Nagaoka H, Gonzalez-Aseguinolaza G, Tsuji M, Nussenzweig MC. 2000. Immunization and infection change the number of recombination activating gene (RAG)-expressing B cells in the periphery by altering immature lymphocyte production. *J. Exp. Med.* 191:2113–20
111. Lawton AR 3rd, Asofsky R, Hylton MB, Cooper MD. 1972. Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to μ-chain. *J. Exp. Med.* 135:277–97
112. Chen C, Nagy Z, Radic MZ, Hardy RR,

Huszar D, et al. 1995. The site and stage of anti-DNA B-cell deletion. *Nature* 373:252–55

113. Hartley SB, Cooke MP, Fulcher DA, Harris AW, Cory S, et al. 1993. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. *Cell* 72:325–35
114. Okamoto M, Murakami M, Shimizu A, Ozaki S, Tsubata T, et al. 1992. A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med.* 175:71–79
115. Spanopoulou E, Roman CA, Corcoran LM, Schlissel MS, Silver DP, et al. 1994. Functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1-deficient mice. *Genes Dev.* 8:1030–42
116. Xu H, Li H, Suri-Payer E, Hardy RR, Weigert M. 1998. Regulation of anti-DNA B cells in recombination-activating gene-deficient mice. *J. Exp. Med.* 188:1247–54
117. Sandel PC, Gendelman M, Kelsoe G, Monroe JG. 2001. Definition of a novel cellular constituent of the bone marrow that regulates the response of immature B cells to B cell antigen receptor engagement. *J. Immunol.* 166:5935–44
118. Fugmann SD, Lee AI, Shockett PE, Villey IJ, Schatz DG. 2000. The RAG proteins and V(D)J recombination: complexes, ends, and transposition. *Annu. Rev. Immunol.* 18:495–527
119. Flajnik MF. 1998. Churchill and the immune system of ectothermic vertebrates. *Immunol. Rev.* 166:5–14
120. Hiom K, Melek M, Gellert M. 1998. DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. *Cell* 94:463–70
121. Agrawal A, Eastman QM, Schatz DG. 1998. Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. *Nature* 394:744–51
122. Zarrin AA, Fong I, Malkin L, Marsden PA, Berinstein NL. 1997. Cloning and characterization of the human recombination activating gene 1 (RAG1) and RAG2 promoter regions. *J. Immunol.* 159:4382–94
123. Kurioka H, Kishi H, Isshiki H, Tagoh H, Mori K, et al. 1996. Isolation and characterization of a TATA-less promoter for the human RAG-1 gene. *Mol. Immunol.* 33:1059–66
124. Kitagawa T, Mori K, Kishi H, Tagoh H, Nagata T, et al. 1996. Chromatin structure and transcriptional regulation of human RAG-1 gene. *Blood* 88:3785–91
125. Fuller K, Storb U. 1997. Identification and characterization of the murine Rag1 promoter. *Mol. Immunol.* 34:939–54
126. Brown ST, Miranda GA, Galic Z, Hartman IZ, Lyon CJ, et al. 1997. Regulation of the RAG-1 promoter by the NF-Y transcription factor. *J. Immunol.* 158:5071–74
127. Lauring J, Schlissel MS. 1999. Distinct factors regulate the murine RAG-2 promoter in B- and T-cell lines. *Mol. Cell. Biol.* 19:2601–12
128. Kishi H, Wei XC, Jin ZX, Fujishiro Y, Nagata T, et al. 2000. Lineage-specific regulation of the murine RAG-2 promoter: GATA-3 in T cells and Pax-5 in B cells. *Blood* 95:3845–52
129. Hsu LY, Lauring J, Liang HE, Greenbaum S, Cado D, et al. 2003. A conserved transcriptional enhancer regulates RAG gene expression in developing B cells. *Immunity* 19:105–17
130. Monroe RJ, Chen F, Ferrini R, Davidson L, Alt FW. 1999. RAG2 is regulated differentially in B and T cells by elements 5′ of the promoter. *Proc. Natl. Acad. Sci. USA* 96:12713–18
131. Yannoutsos N, Wilson P, Yu W, Chen HT, Nussenzweig A, et al. 2001. The role of recombination activating gene (RAG) reinduction in thymocyte development in vivo. *J. Exp. Med.* 194:471–80
132. Grosveld F, van Assendelft GB, Greaves DR, Kollias G. 1987. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. *Cell* 51:975–85

133. Li Q, Peterson KR, Fang X, Stamatoyannopoulos G. 2002. Locus control regions. *Blood* 100:3077–86
134. Godfrey D, Kennedy J, Mombaerts P, Tonegawa S, Zlotnik A. 1994. Onset of TCR-beta gene rearrangement and role of TCR-beta expression during CD3-CD4-CD8-thymocyte differentiation. *J. Immunol.* 152:4783–92
135. von Boehmer H, Fehling HJ. 1997. Structure and function of the pre-T cell receptor. *Annu. Rev. Immunol.* 15:433–52
136. Kruisbeek AM, Haks MC, Carleton M, Wiest DL, Michie AM, et al. 2000. Branching out to gain control: how the pre-TCR is linked to multiple functions. *Immunol. Today* 21:637–44
137. Petrie H, Livak F, Schatz D, Strasser A, Crispe I, et al. 1993. Multiple rearrangements in T cell receptor alpha chain genes maximize the production of useful thymocytes. *J. Exp. Med.* 178:615–22
138. Shinkai Y, Koyasu S, Nakayama K, Murphy KM, Loh DY, et al. 1993. Restoration of T cell development in RAG-2-deficient mice by functional TCR transgenes. *Science* 259:822–25
139. Guo J, Hawwari A, Li H, Sun Z, Mahanta SK, et al. 2002. Regulation of the TCRalpha repertoire by the survival window of CD4(+)CD8(+) thymocytes. *Nat. Immunol.* 3:469–76
140. Turka LA, Schatz DG, Oettinger MA, Chun JJ, Gorka C, et al. 1991. Thymocyte expression of RAG-1 and RAG-2: termination by T cell receptor cross-linking. *Science* 16:778–81
141. Borgulya P, Kishi H, Uematsu Y, von Boehmer H. 1992. Exclusion and inclusion of α and β T cell receptor alleles. *Cell* 69:529–37

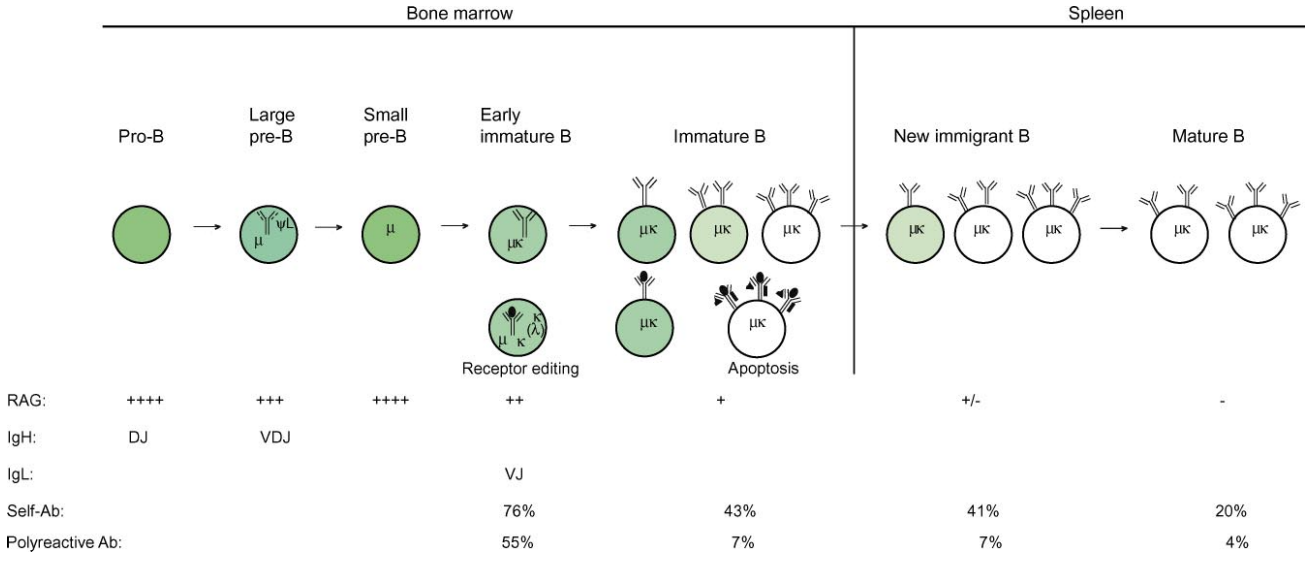

Figure 1 B cell development. Stages, status of the immunoglobulin heavy (IgH) and light chain (IgL) loci, expression of the RAG proteins (the shades of green represent the intensity of expression of the RAG2 protein in mouse RAG2-GFP indicator strains). Expression of the pre-B and B cell receptors, induction of receptor editing, and apoptosis by self-antigens.