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Pillars of Immunology

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From RAG2 to T Cell Riches and Future Fortunes

Rebecca A. Glynn and Craig H. Bassing

Variable diversity joining recombination–mediated AgR gene assembly is the basis for adaptive immunity in jawed vertebrates. In this *Pillars of Immunology* article, Shinkai et al. (1) showed that RAG2 stimulates T cell development by promoting TCR gene assembly.

In 1993, evidence suggested a common recombinase assembled V region exons of all AgR genes from V, D, and J gene segments. This idea was supported by the observation that conserved recombination signal sequences (RSSs) flank and direct site-specific recombination of all *Ig* and *Tcr* gene segments (2). Furthermore, RSS-directed recombination was recognized to assemble the following: 1) complete TCR genes (*TCRβ* and *TCRα* or *TCRγ* and *TCRδ*) only in immature T cells, 2) complete BCR genes (*IgH* and *Igκ* or *Igλ*) only in immature B cells, and 3) *TCRβ* or *IgH* in prolymphocytes preceding *TCRα* or *Igκ* or *Igλ* in prelymphocytes (3). A screen for genes capable of recombining RSSs on plasmids in nonlymphoid cell lines identified *Rag1* and *Rag2*, which are expressed in immature T and B cells (4, 5). RAG1 weakly induced recombination, whereas RAG2 synergized with RAG1 to promote a 1000-fold increase in recombination activity (4, 5). The RAG proteins were named to encompass the uncertain potential function(s) of their products in activating versus being components of the recombinase. The inactivation of *Rag1* or *Rag2* was shown to fully block assembly of all AgR genes and development of T and B cells beyond prolymphocyte stages (6, 7). Yet, the potential cause/effect relationship between loss of V(D)J recombination and failure of lymphocyte differentiation remained unproven.

T and B cell development was known to proceed through discrete stages and transitions linked with assembly, expression, and signaling of AgR genes. In the case of αβ T cell development, $CD4^{-}CD8^{-}$ (double-negative [DN]) thymocytes assemble TCRβ genes first (8). Concomitant with TCRβ expression, DN cells proliferate, expand, and differentiate into $CD4^{+}CD8^{+}$ (double-positive [DP]) thymocytes that then assemble TCRα genes (8). The expression of αβ TCRs on DP thymocytes allows for positive selection on self-peptide/MHC ligands that activates TCR-mediated signaling to drive development of $CD4^{+}$ or $CD8^{+}$ (single-positive [SP]) thymocytes, which then exit the thymus as mature αβ T cells (8). Inactivation of *TCRβ* or *TCRα* causes a block in αβ T cell development at the DN or DP stage, respectively, suggesting that TCRβ and TCRα proteins each signal differentiation (9, 10). Consistent with this view, a preassembled TCRβ transgene partially rescued DN-to-DP thymocyte development in *Scid* mice, which have a mutation that impairs DNA double-strand break repair, completion of V(D)J recombination, and development of T and B cells (11). Additionally, it was recognized that disulfide-linked αβ TCR dimers noncovalently associate with invariant CD3 proteins (CD3-ε, -δ, -γ, -ζ, and -η) to form complete αβ TCR complexes (12). Studies in T cell lines indicated surface expression of αβ TCR dimers or CD3 complexes were dependent on the association of TCRβ, TCRα, and CD3 in the Golgi (13, 14). In line with this notion, TCRβ was not detected on DP cells lacking TCRα (9). Collectively, these observations raised important questions about how TCR β-chains are expressed on DN cells and signal DN-to-DP cell development.

This *Pillars of Immunology* article yielded important insights into relationships between the following: 1) TCR gene assembly and T cell development, and 2) TCRβ surface expression and signaling in DN cells (1). Shinkai et al. used flow cytometry to ascertain the effects of *TCRα*, *TCRβ* transgenes, or both on αβ T cell development in $Rag2^{-/-}$ mice. Whereas TCRα alone had no effect, TCRβ fully rescued the expansion and differentiation of DN into DP thymocytes but failed to develop SP cells. Coexpression of TCRα and TCRβ fully rescued the following: 1) expansion and differentiation of DN cells into DP cells, 2) differentiation of DP cells into $CD4^{+}$ SP cells, and 3) cellularity of $CD4^{+}$ αβ T cells because the transgenic αβ TCR used was MHC class II–restricted and signaled development of only $CD4^{+}$ cells (15). These data revealed the necessary and sufficient roles for expression of TCRβ in early αβ T cell development and of TCRα and TCRβ in late αβ T cell development. This work produced unequivocal evidence that the inability of Rag2 deficient mice ($Rag2^{-/-}$) to assemble TCRβ and TCRα genes causes the block of αβ T cell development. Consequently, Shinkai et al. cemented a fundamental advance that the main, and possibly only, function of RAG2 is to promote initiation of V(D)J recombination.

Moving beyond the role of RAG2 in V(D)J recombination, Shinkai et al. assessed effects of the TCRβ transgene on surface expression and formation of TCR/CD3 complexes. Unsurprisingly, flow cytometry failed to detect CD3ε on $Rag2^{-/-}$ DN cells (7). Conversely, transgenic TCRβ induced CD3ε and TCRβ expression on $Rag2^{-/-}$ DP cells. Next, Shinkai

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Abbreviations used in this article: DN, double-negative; DP, double-positive; RSS, recombination signal sequence; SP, single-positive.

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et al. used two-dimensional gel electrophoresis of thymocyte proteins immunoprecipitated with anti-TCRβ, anti-CD3ε, or anti-CD3ζ/anti-CD3η Abs to identify complexes. As expected, they detected TCRαβ–CD3γδεζ complexes in *TCRβ*$^{+}$:*Rag2*$^{+/+}$ DP cells. Interestingly, they found CD3γδε and CD3ζη complexes in *Rag2*$^{-/-}$ DN cells and TCRβ–CD3γδε complexes in *TCRβ*$^{+}$:*Rag2*$^{-/-}$ DP cells. These data revealed the following: 1) expression and interaction of CD3 subunits does not require RAG2 or TCRβ proteins, 2) TCRβ proteins can bind CD3γδε complexes independent of TCRα proteins, and 3) TCRα drives integration of CD3ζ in TCRαβ–CD3 complexes. Building on a model for B cells (16), Shinkai et al. proposed that TCRβ–CD3 complexes may interact with a surrogate TCR α-chain to signal expansion and differentiation of DN thymocytes.

The knowledge and reagents of this *Pillars of Immunology* article provided an invaluable foundation for elucidating molecular mechanisms that promote lymphocyte development and control V(D)J recombination (1). Subsequent work of Shinkai et al. (17) and another group showed that CD3 proteins are expressed on DN cells before TCRβ gene assembly and can be activated to signal DN-to-DP development (18). Thus, TCRβ signals through CD3 proteins, a finding that led to identification of pre-Tα as the surrogate TCR α-chain. This surrogate forms pre-TCRs with TCRβ–CD3γδε complexes to signal ligand-independent differentiation of TCRβ^{+} DN cells (19, 20). The approach of Shinkai et al. (1) was used to show that RAG2 drives B cell development by promoting assembly of *IgH* genes in pro–B cells and *Igκ* or *Igλ* genes in pre–B cells (21). These analogous studies gave unequivocal evidence for RAG2-dependent activity of a common recombinase for all AgR loci. This was proven by the demonstration that RAG1 and RAG2 are the only proteins required for cleavage of a V(D)J recombination substrate (22).

Since 1993, mice deficient for *Rag1* or *Rag2* alone and with AgR transgenes have been instrumental for identifying roles and elucidating functions of AgR locus *cis*-elements in regulating lymphocyte lineage- and developmental stage-specific V(D)J recombination (23). These mice also allowed discovery that RAG proteins bind to D and J segments, generating recombination centers that capture distal V segments to assemble complete AgR genes (24). Recently, such mice have revealed unexpected roles for RAG1 and RAG2 beyond simply initiating V(D)J recombination. RAG cleavage in developing T and B cells signals transcriptional activation of a genetic program that modulates cellular localization and includes genes important for proper lymphocyte selection (25). Moreover, RAG double-strand breaks induced in common lymphoid precursor cells cause heritable gene expression changes that facilitate the protective activities of adaptive and, surprisingly, innate lymphocytes (26). Overall, the observations planted by Shinkai et al. (1) continue to propel discoveries about the mechanisms that guide lymphocyte development and endow them with their unique functionalities.

Disclosures

The authors have no financial conflicts of interest.

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