Mechanisms for generation of T cell receptor diversity

- TCR Diversity Is Generated Like Antibody Diversity but Without Somatic Mutation

Although TCR germ-line DNA contains far fewer V gene segments than Ig germ-line DNA, several mechanisms that operate during TCR gene rearrangements contribute to a high degree of diversity among T-cell receptors. Figure1 compare the generation of diversity among antibody molecules and TCR molecules. ***Combinatorial joining*** of variable-region gene segments generates a large number of random gene combinations for all the TCR chains, as it does for the Ig heavy- and light chain genes (Figure1 a). For example, 100 Vα and 50 Jα gene segments can generate 5×10^3 possible VJ combinations for the TCR α chain. Similarly, 25 Vβ, 2 Dβ, and 12 Jβ gene segments can give 6×10^2 possible combinations. Although there are fewer TCR Vα and Vβ gene segments than immunoglobulin VH and Vα segments, this difference is offset by the greater number of J segments in TCR germ-line DNA. Assuming that the antigen-binding specificity of a given T-cell receptor depends upon the variable region in both chains, random association of 5×10^3 Vα combinations with 6×10^2 Vβ combinations can generate 3×10^6 possible combinations for the αβ T-cell receptor. Additional means to generate diversity in the TCR V genes are described below, so 3×10^6 combinations represents a minimum estimate.

As illustrated in Figure 9-8b, the location of one-turn (12-bp) and two-turn (23-bp) recombination signal sequences (RSSs) in TCR β- and δ-chain DNA differs from that in Ig heavy-chain DNA. Because of the arrangement of the RSSs in TCR germ-line DNA, alternative joining of D gene segments can occur while the one-turn/two-turn joining rule is observed. Thus, it is possible for a V_β gene segment to join directly with a J_β or a D_β gene segment, generating a $(VJ)_\beta$ or $(VDJ)_\beta$ unit. *Alternative joining of δ-chain gene segments* generates similar units; in addition, one D_δ can join with another, yielding $(VDDJ)_\delta$ and, in humans, $(VDDDJ)_\delta$. This mechanism, which cannot operate in Ig heavy-chain DNA, generates considerable additional diversity in TCR genes.

The joining of gene segments during TCR-gene rearrangement exhibits **junctional flexibility.** As with the Ig genes, this flexibility can generate many nonproductive rearrangements, but it also increases diversity by encoding several alternative amino acids at each junction (Figure1c). In both Ig and TCR genes, nucleotides may be added at the junctions between some gene segments during rearrangement. Variation in endonuclease cleavage leads to the addition of further

nucleotides that are palindromic. Such **P-region nucleotide addition** can occur in the genes encoding all the TCR and Ig chains. Addition of **N-region nucleotides,** catalyzed by a terminal deoxynucleotidyl transferase, generates additional junctional diversity. Whereas the addition of N-region nucleotides in immunoglobulins occurs only in the Ig heavy-chain genes, it occurs in the genes encoding all the TCR chains. As many as six nucleotides can be added by this mechanism at each junction, generating up to 5461 possible combinations, assuming random selection of nucleotides (see Figure 1d). Some of these combinations, however, lead to nonproductive rearrangements by inserting in-frame stop codons that prematurely terminate the TCR chain, or by substituting amino acids that render the product nonfunctional. Although each junctional region in a TCR gene encodes only 10–20 amino acids, enormous diversity can be generated in these regions. Estimates suggest that the combined effects of P- and N-region nucleotide addition and joining flexibility can generate as many as 1013 possible amino acid sequences in the TCR junctional regions alone.

The mechanism by which diversity is generated for the TCR must allow the receptor to recognize a very large number of different processed antigens while restricting its MHC recognition repertoire to a much smaller number of self-MHC molecules. TCR DNA has far fewer V gene segments than Ig DNA. Unlike the Ig genes, the TCR genes do not appear to undergo extensive somatic mutation. That is, the functional TCR genes generated by gene rearrangements during T-cell maturation in the thymus have the same sequences as those found in the mature peripheral T-cell population. The absence of somatic mutation in T cells ensures that T-cell specificity does not change after thymic selection and therefore reduces the possibility that random mutation might generate a self-reactive T cell.

T-Cell Maturation and the Thymus

Progenitor T cells from the early sites of hematopoiesis begin to migrate to the thymus at about day 11 of gestation in mice and in the eighth or ninth week of gestation in humans. In a manner similar to B-cell maturation in the bone marrow, Tcell maturation involves rearrangements of the germ-line TCR genes and the expression of various membrane markers. In the thymus, developing T cells, known as **thymocytes,** proliferate and differentiate along developmental pathways that generate functionally distinct subpopulations of mature T cells. The thymus occupies a central role in T-cell biology and aside from being the main source of all T

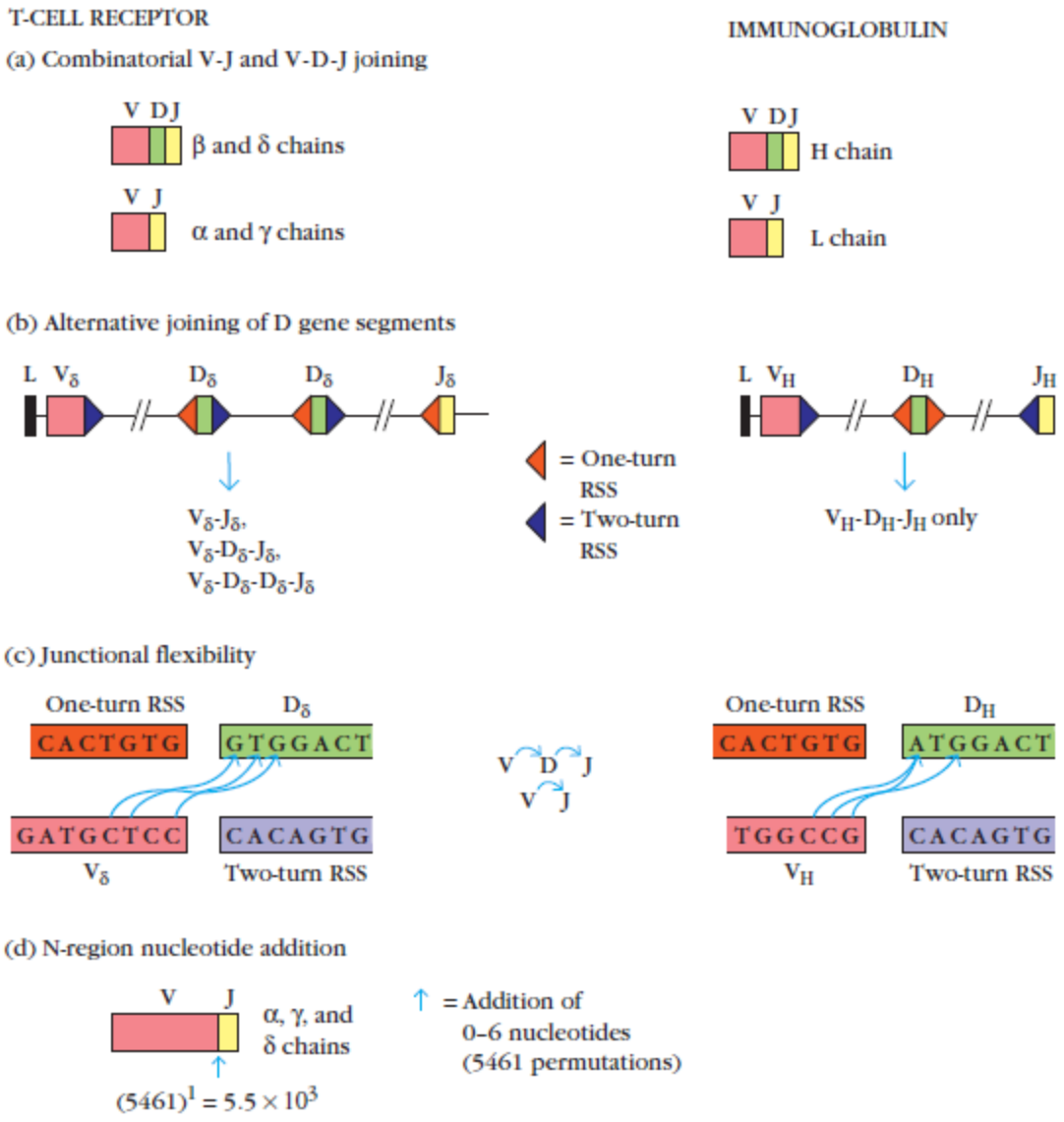

Figure1. Comparison of mechanisms for generating diversity in TCR genes and immunoglobulin genes. In addition to the mechanisms shown, P-region nucleotide addition occurs in both TCR and Ig genes, and somatic mutation occurs in Ig genes. Combinatorial association of the expressed chains generates additional diversity among both TCR and Ig molecules.

cells, it is where T cells diversify and then are shaped into an effective primary T-cell repertoire by an extraordinary pair of selection processes. One of these, **positive selection,** permits the survival of only those T cells whose TCRs are capable of recognizing self-MHC molecules. It is thus responsible for the creation of a self-MHC-restricted repertoire of T cells. The other, **negative selection,** eliminates T cells that react too strongly with self-MHC or with self-MHC

plus self peptides. It is an extremely important factor in generating a primary T-cell repertoire that is self-tolerant (Means doen't react with self antigens or tolerate them).

As shown in Figure 2, when T-cell precursors arrive a tthe thymus, they do not express such signature surface markers of T cells as the T-cell receptor, the CD3 complex, or the coreceptors CD4 and CD8. In fact, these progenitor cells hav enot yet rearranged their TCR genes and do not express proteins, such as RAG-1 and RAG-2, that are required for rearrangement. After arriving at the thymus, these T-cell precursors enter the outer cortex and slowly proliferate. During approximately three weeks of development in the thymus, the differentiating T cells progress through a series of stages that are marked by characteristic changes in their cell surface phenotype.

For example, as mentioned previously, thymocytes early in development lack detectable CD4 and CD8. Because these cells are $CD4^-$ $CD8^-$, they are referred to as **double-negative (DN)** cells. Even though these coreceptors are not expressed during the DN early stages, the differentiation program is progressing and is marked by changes in the expression of such cell surface molecules as c-Kit, CD44, and CD25. The initial thymocyte population displays c-Kit, the receptor for stem-cell growth factor, and CD44, an adhesion molecule involved in homing; CD25, the β-chain of the IL-2 receptor, also appears on early-stage DN cells. During this period, the cells are proliferating but the TCR genes remain unrearranged. Then the cells stop expressing c-Kit, markedly reduce CD44 expression, turn on expression of the recombinase genes *RAG-1* and *RAG-2* and begin to rearrange their TCR genes. Most double-negative thymocytes progress down the αβ developmental pathway. They stop proliferating and begin to rearrange the TCR β-chain genes, then express the β chain. Those cells of the αβ lineage that fail to productively rearrange and express β chains die.Newly synthesized β chains combine with a 33-kDa glycoprotein known as the pre-Tα chain and associate with the CD3 group to form a novel complex called the **pre-T-cell receptor** or **pre-TCR (**Figure 3).

Some researchers have suggested that the pre-TCR recognizes some intra-thymic ligand and transmits a signal through the CD3 complex that activates signal-transduction pathways that have several effects:

- indicates that a cell has made a productive TCR β-chain rearrangement and signals its further proliferation and of distinctive cell-surface markers.

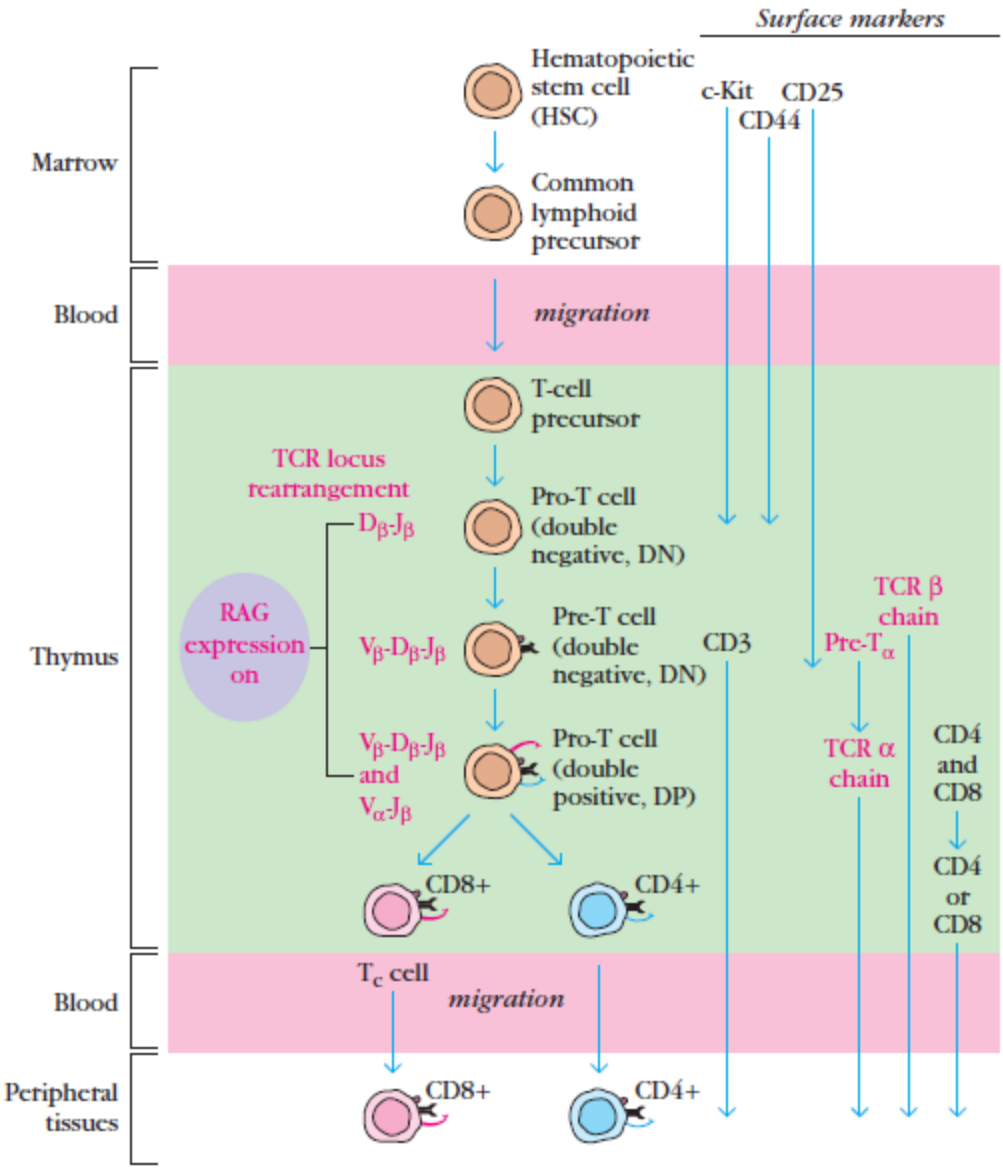

Figure 2: Development of αβ T cells in the mouse. T-cell precursors arrive at the thymus from bone marrow via the bloodstream, undergo development to mature T cells, and are exported to the periphery where they can undergo antigen-induced activation and differentiation into effector cells and memory cells. Each stage of development is characterized by stage-specific intracellular events and the display maturation.

- Suppresses further rearrangement of TCR β-chain genes, resulting in allelic exclusion.
- Renders the cell permissive for rearrangement of the TCR α chain.
- Induces developmental progression to the $CD4^+8^+$ *double-positive* state.

After advancing to the **double-positive (DP)** stage, where both CD4 and CD8 coreceptors are expressed, the thymocytes begin to proliferate. However, during this proliferative phase, TCR α-

chain gene rearrangement does not occur; both the *RAG-1* and *RAG-2* genes are transcriptionally active, but the RAG-2 protein is rapidly degraded in proliferating cells, so rearrangement of the α-chain genes cannot take place. The rearrangement of α-chain genes does not begin until the double-positive thymocytes stop proliferating and RAG-2 protein levels increase. The proliferative phase prior to the rearrangement of the α-chain increases the diversity of the T-cell

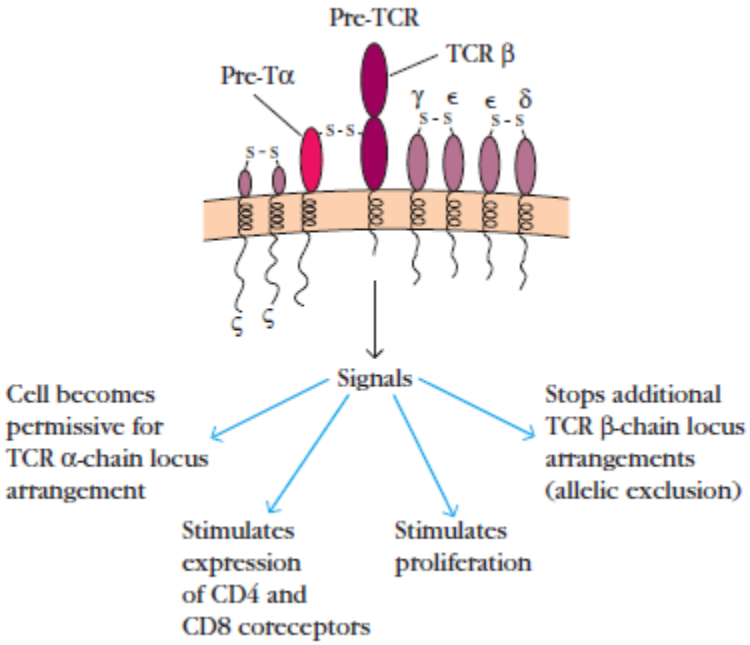

Figure3: Structure and activity of the pre–T-cell receptor (pre- TCR). Binding of ligands yet to be identified to the pre-TCR generates intracellular signals that induce a variety of processes.

repertoire by generating a clone of cells with a single TCR β-chain rearrangement. Each of the cells within this clone can then rearrange a different α-chain gene, thereby generating a much more diverse population than if the original cell had first undergone rearrangement at both the β- and α-chain loci before it proliferated.

The possession of a complete TCR enables DP thymocytes to undergo the rigors of positive and negative selection. T-cell development is an expensive process for the host. An estimated 98% of all thymocytes do not mature—they die by apoptosis within the thymus either because they fail to make a productive TCR-gene rearrangement or because they fail to survive thymic selection. Double-positive thymocytes that express the αβ TCR-CD3 complex and survive thymic selection

develop into immature **single-positive CD4$^-$** thymocytes or **single-positive CD8$^-$** thymocytes. These single-positive cells undergo additional negative selection and migrate from the cortex to the medula, where they pass from the thymus into the circulatory system.