T cell development & selection:

T-cell development can be divided into two clusters of events:

Early thymocyte development, during which a dizzyingly diverse TCR^+ population of immature T cells is generated, and *selection events* that depend on TCR interactions to shape this population so that only those cells that are self-restricted and self tolerant will leave to populate the periphery. Early thymocyte development is T-cell receptor independent and brings cells through uncommitted $CD4^-$ $CD8^-$ (double negative, DN) stages to the T-cell receptor-expressing, $CD4^+$ $CD8^+$ (double positive, DP) stage.

The specific events in this early stage include:

1. Commitment of hematopoietic precursors to the T cell lineage,
2. The initiation of antigen receptor gene rearrangements, and
3. The selection and expansion of cells that have successfully rearranged one of their T-cell receptor genes (β-selection).

The second phase of T-cell development is largely dependent on T-cell receptor interactions and brings cells to maturity from the $CD4^+CD8^+$ stage to the $CD4^+$ or $CD8^+$ single positive (SP) stage. The events in this last phase of development include:

1. **Positive selection**, selection *for* those cells whose T-cell receptors respond to self-MHC,
2. **Negative selection**, selection *against* those cells whose T-cell receptors react strongly to self-peptide/MHC combinations, and
3. **Lineage commitment**, commitment of thymocytes to effector cell lineages, including $CD4^+$ helper or $CD8^+$ cytotoxic populations.

Early thymocyte development:

T-cell development occurs in the thymus and begins with the arrival of small numbers of lymphoid precursors migrating from the bone marrow and blood into the thymus, where they proliferate, differentiate, and undergo selection processes that result in the development of mature T cells.

Commitment of hematopoietic precursors to the T cell lineage

Studies revealed that commitment to the T-cell lineage was dependent on a receptor, **Notch.** Notch, regulates the decision of a lymphoid precursor to become a T versus a B lymphocyte. When a constitutively active version of Notch1, one of four versions of Notch, is overexpressed in hematopoietic cells, T cells rather than B cells develop in the bone marrow. Investigators reveal that the transcription factor GATA-3 is a critical participant in Notch-mediated T-cell commitment.
After arriving in the thymus from the bone marrow via blood vessels at the cortico-medullary boundary, T-cell precursors encounter Notch ligands, which are abundantly expressed by the thymic epithelium.

The earliest T cells lack detectable CD4 and CD8 and are therefore referred to as **double-negative** (**DN**) cells. DN T cells can be subdivided into four subsets (DN1-4) based on the presence or absence of other cell surface molecules, including **c-kit** (CD117), the receptor for stem cell growth factor; **CD44**, an adhesion molecule; and **CD25**, the α chain of the IL-2 receptor.

The initiation of antigen receptor gene rearrangements

DN1 thymocytes are the first to enter the thymus and are still capable of giving rise to multiple cell types. They express only c-kit and CD44 (c-kit^{++} CD44^{+} CD25^{-}), but once they encounter the thymic environment and become resident in the cortex, they proliferate and express CD25, becoming **DN2** thymocytes (c-kit^{++} CD44^{+} CD25^{+}).

During this critical stage of development, the genes for the TCR γ, δ, and β chains begin to rearrange; however, the TCR α locus does not rearrange, presumably because the region of DNA encoding TCR α genes is not yet accessible to the recombinase machinery.

At the late DN2 stage, T-cell precursors fully commit to the T-cell lineage and reduce expression of both c-kit and CD44. Cells in transition from the **DN2** to **DN3** (c-kit^{+} CD44^{-} CD25^{+}) stages continue rearrangement of the TCR γ, TCR_δ, and TCR_β chains and make the first major decision in T-cell development: whether to join the TCR γδ or TCR αβ T-cell lineage.

Those **DN3** T cells that successfully rearrange their β chain and therefore commit to the TCR αβ T-cell lineage lose expression of CD25, halt proliferation, and enter the final phase of their DN stage of development, **DN4** (c-kit $^{low/-}$ CD44^{-} CD25^{-}), which mature directly into CD4^{+} CD8^{+} DP thymocytes.

To a large extent, the choice to become a αβ or γδ T cell is dictated by when and how fast the genes that code for each of the four receptor chains successfully rearrange.

The selection and expansion of cells that have successfully rearranged one of their T-cell receptor genes (β-selection)

Double-negative (DN) thymocytes that have successfully rearranged their TCR β chains are valuable, and are identified and expanded via a process known as **β-selection.** A 33-kDa invariant glycoprotein known as the **pre–T α chain** is uniquely expressed at this stage of development. Pre-T α acts as a surrogate for the real TCR α chain, which has yet to rearrange, and assembles with a successfully rearranged and translated β chain, as well as CD3 complex proteins. This precursor TCR/CD3 complex is known as the **pre-TCR** and acts as a sensor by initiating a signal transduction pathway.

Pre-TCR signaling results in the following cascade of events:

1. Maturation to the DN4 stage (c-kit $^{low/-}$ CD44^{-} CD25^{-})
2. Rapid proliferation in the subcapsular cortex

3. Suppression of further rearrangement of TCR β-chain genes, resulting in allelic exclusion of the β-chain locus
4. Development to the $CD4^+$ $CD8^+$ **double-positive (DP)** stage
5. Cessation of proliferation
6. Initiation of TCRα chain rearrangement

Once a young double-positive (DP) thymocyte successfully rearranges and expresses a TCR α chain, this chain will associate with the already produced TCR β chain, taking the place of the surrogate pre-TCR α chain, which is no longer actively expressed. This TCR αβ population now expresses both CD4 and CD8, and is ready for the second stage of T-cell development: selection.

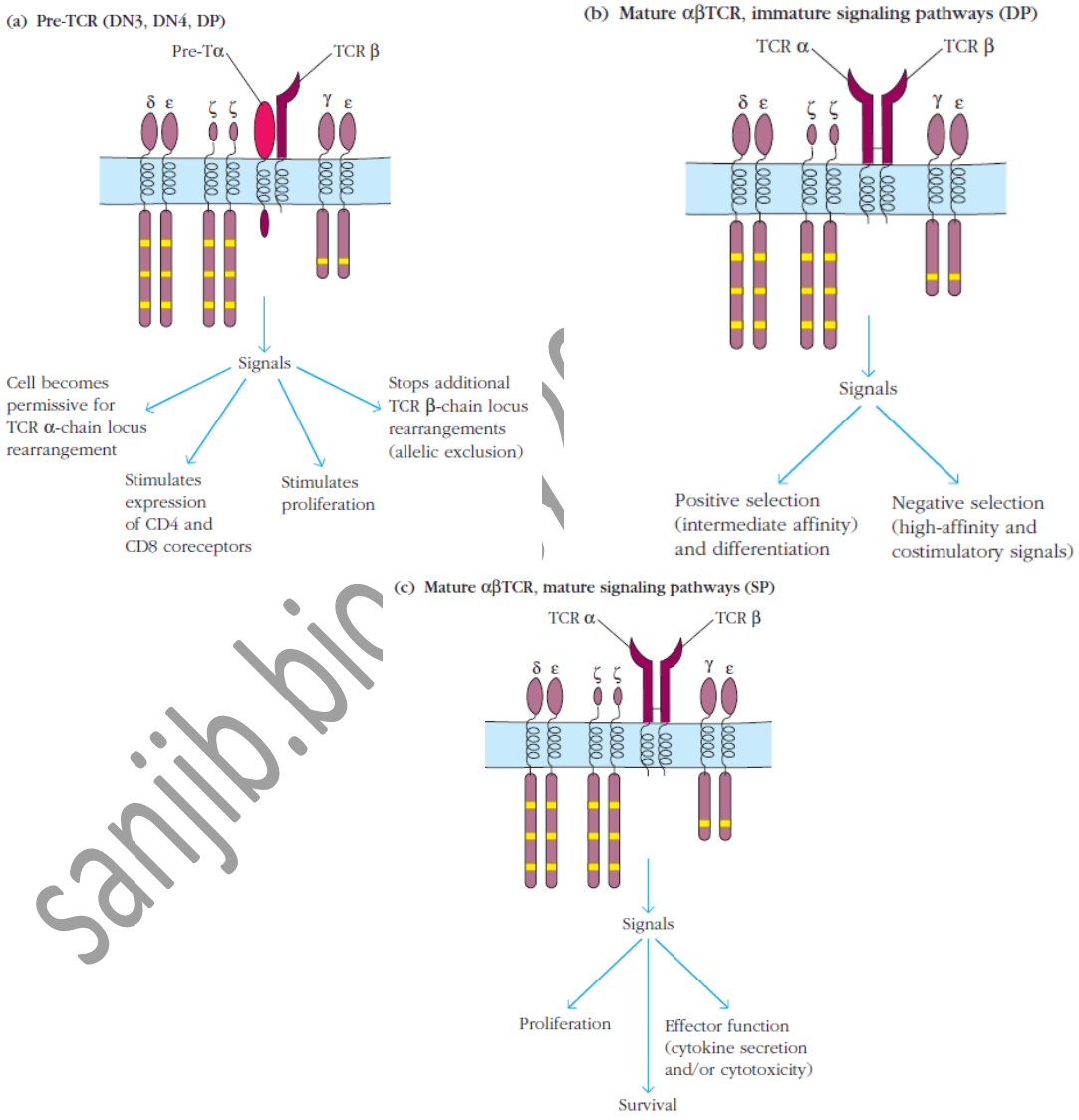


Fig: Changes in the structure and activity of the T-cell receptor through T-cell development

Positive and Negative Selection:

$CD4^+CD8^+$ (DP) thymocytes, small, nonproliferating cells that reside in the thymic cortex, are the most abundant subpopulation in the thymus, comprising more than 80% of cells. Most important, they are the first subpopulation of thymocytes that express a fully mature surface TCR αβ/CD3 complex and are therefore the primary targets of thymic selection. Thymic selection shapes the TCR repertoire of DP thymocytes based on the affinity of their T-cell receptors for the MHC/peptides they encounter as they browse the thymic cortex.

Two distinct selection processes are required:
• Positive selection, which selects for those thymocytes bearing receptors capable of binding self-MHC molecules, resulting in **MHC restriction**
• Negative selection, which selects against thymocytes bearing high-affinity receptors for self-MHC/peptide complexes, resulting in **self-tolerance**.

The vast majority of DP thymocytes (~98%) never meets the selection criteria and dies by apoptosis within the thymus. The bulk of DP thymocyte death (~95%) occurs among thymocytes that fail positive selection because their receptors do not specifically recognize self-MHC molecules. These cells do not receive survival signals through their TCRs, and die by a process known as **death by neglect**. A small percentage of cells (2%–5%) are eliminated by negative selection. Only 2% to 5% of DP thymocytes actually exit the thymus as mature T cells.

In the thymus, thymocytes come into contact with thymic epithelial cells that express high levels of class I and class II MHC molecules on their surface. These self-MHC molecules present self-peptides, which are typically derived from intracellular or extracellular proteins that are degraded in the normal course of cellular metabolism. DP thymocytes undergo positive and negative selection, depending on the signals they receive when they encounter self-MHC/self peptide combinations with their TCRs.

Positive Selection Ensures MHC Restriction

If a $CD4^+CD8^+$ thymocyte recognizes a self-MHC/peptide complex on the cortical epithelial cells that they browse, it will undergo positive selection, a process that induces both survival and differentiation of DP thymocytes. Remarkably, the majority of newly generated thymocytes do not successfully engage the MHC/peptides they encounter with their TCRs and have "failed" the positive selection test and die by apoptosis within 3 to 4 days.

Negative Selection (Central Tolerance) Ensures Self-Tolerance

Autoreactive $CD4^+CD8^+$ thymocytes with high-affinity receptors for self-MHC/self-peptide combinations are potentially dangerous to an organism, and many are killed by negative selection in the thymus. Most negative selection occurs via a process known as **clonal deletion**, where high-affinity TCR interactions directly induce apoptotic signals. Clonal deletion of DP thymocytes appears to be optimally mediated by the same cells (APCs) and same interaction (high-affinity TCR engagement coupled with costimulatory signals) that activate mature T cells. Thymic dendritic cells and macrophages, which are found in multiple areas of the thymus, clearly have the ideal features to mediate negative selection; both the cortex and the medulla have the potential to induce negative selection.

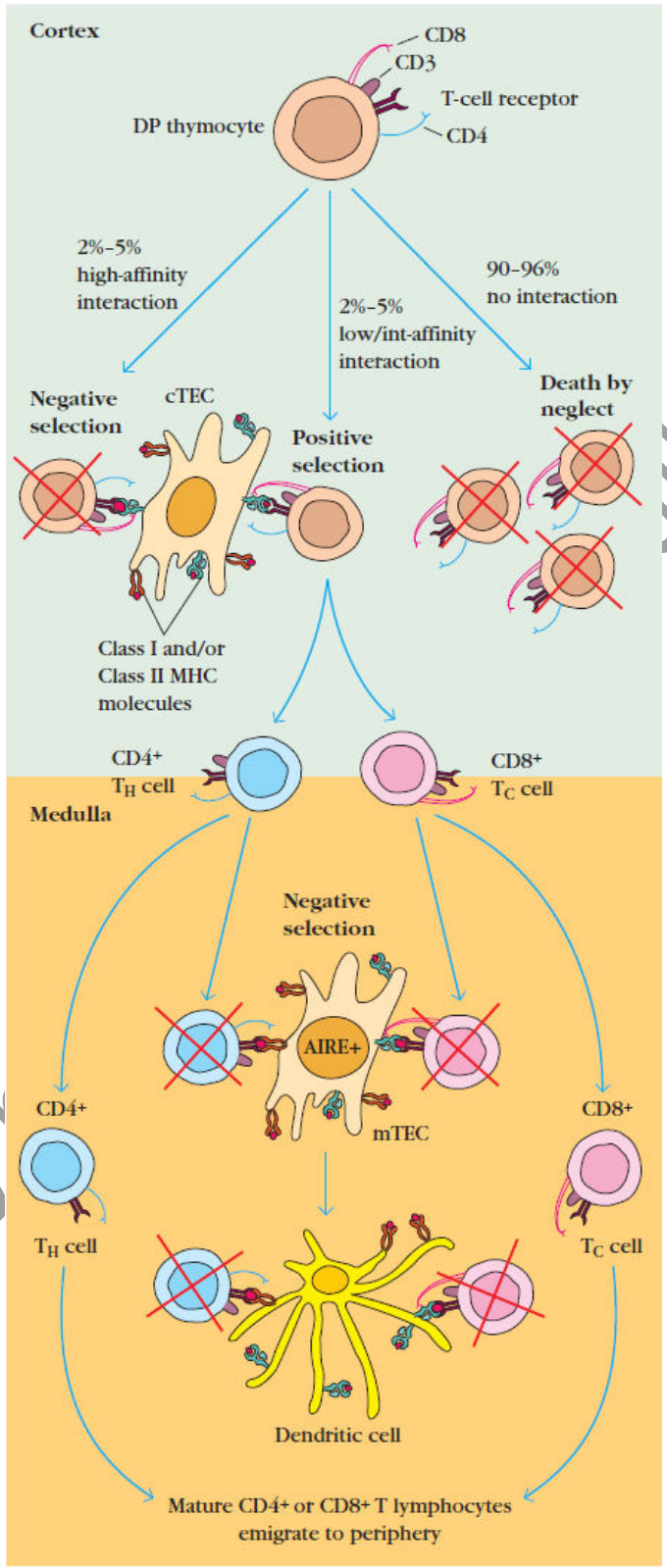


Fig: Positive and Negative Selection of Thymocytes in the Thymus.

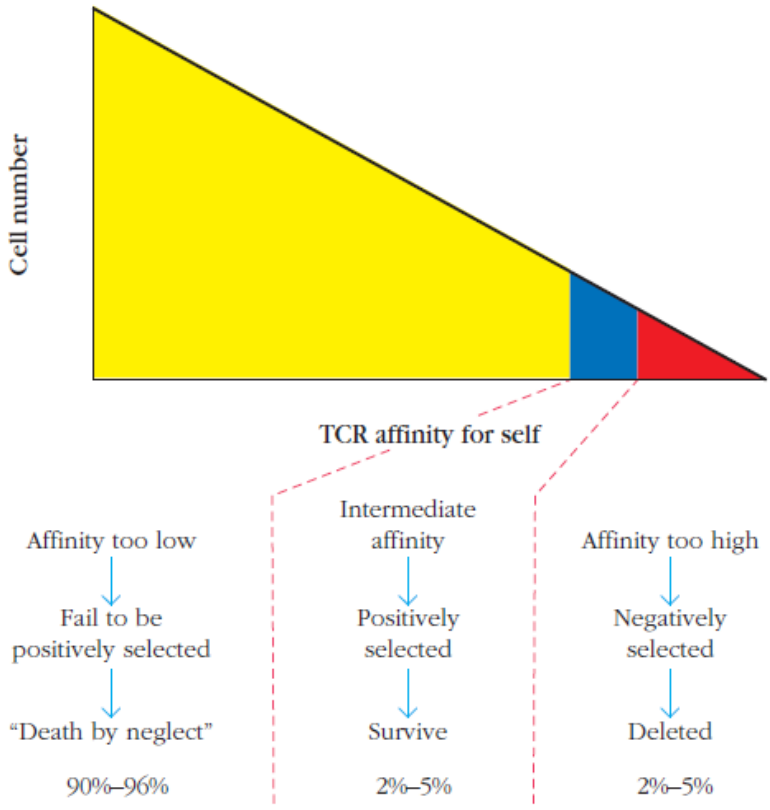


Fig: Relationship between TCR affinity and selection.

Lineage Commitment

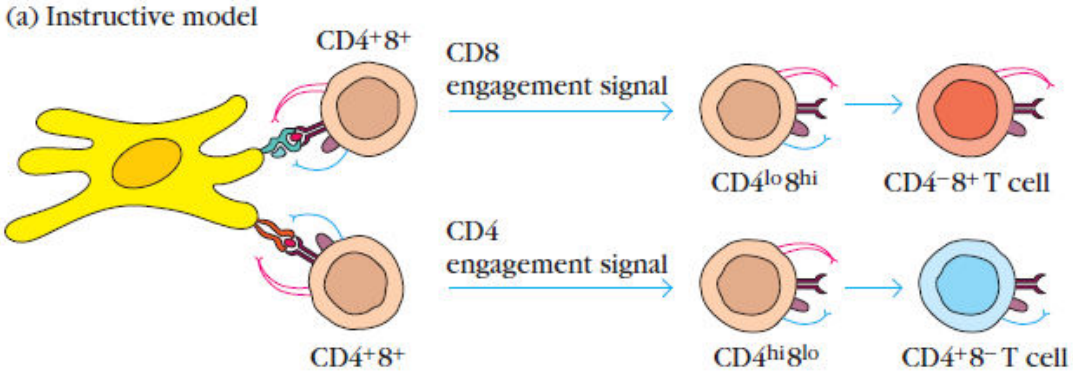
As thymocytes are being screened on the basis of their TCR affinity for self-antigens, they are also being guided in their lineage decisions. Specifically, a positively selected double positive thymocyte must decide whether to join the $CD8^+$ cytotoxic T-cell lineage or the $CD4^+$ helper T-cell lineage. Lineage commitment requires changes in genomic organization and gene expression that result in

a. Silencing of one coreceptor gene (CD4 or CD8) as well as

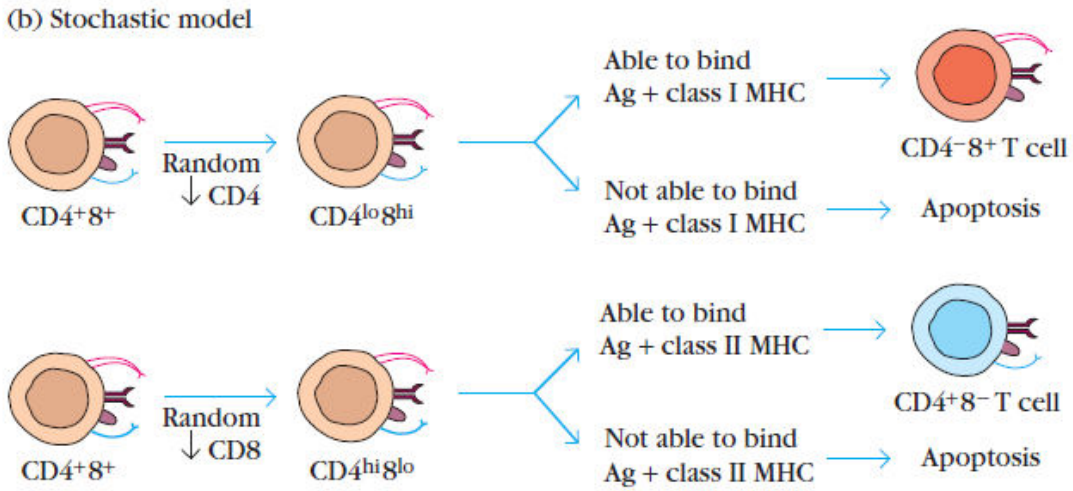
b. Expression of genes associated with a specific lineage function.

Investigators advanced two simple testable models to explain how developing T cells "matched" their TCR preference with their coreceptor expression.

In the **instructive model**, TCR/CD4 and TCR/CD8 coengagement generates unique signals that directly initiate distinct developmental programs. For example, if a thymocyte randomly generated a TCR with an affinity for MHC Class I, the TCR and CD8 would bind MHC Class I together, and generate a signal that specifically initiated a program that silenced CD4 expression and induced expression of genes specific for cytotoxic T-cell lineage function. Likewise, TCR/CD4 coengagement would generate a unique signal that initiated CD8 silencing and the helper T-cell developmental program.

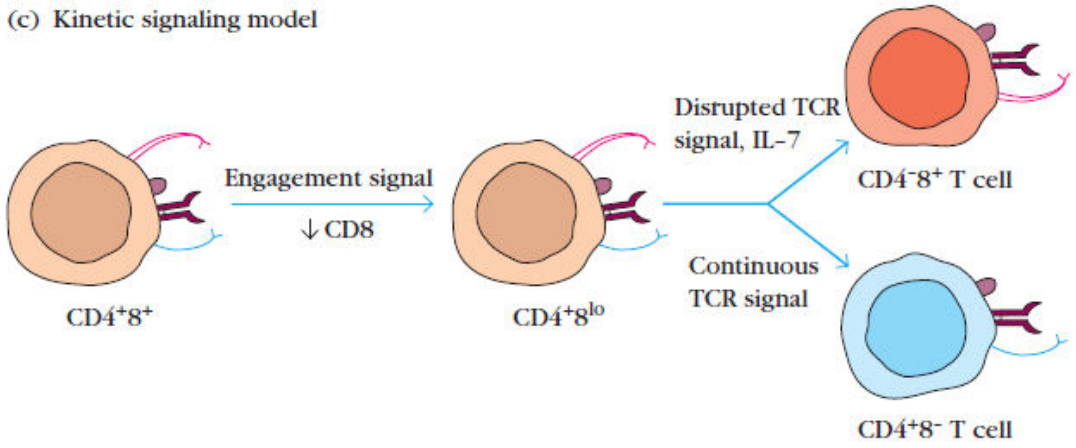


In the **stochastic model**, a positively selected thymocyte randomly down-regulates CD4 or CD8. Only those cells that express the "correct" coreceptor—the ones that can coengage MHC with the TCR—generate a TCR signal strong enough to survive to mature. In this model, TCR/CD4 and TCR/CD8 coengagement does not necessarily generate distinct signals. Unfortunately, studies that followed the consequences of such mismatches confounded researchers by providing evidence in support of both models! Clearly they were too simplistic.



The *strength of signal* model suggested that stronger positive selecting TCR signals resulted in CD4 lineage commitment, and weaker positive selecting TCR signals resulted in CD8 lineage commitment. Researchers were consistent with the observation that the intracellular tail of CD4 interacts more effectively with the tyrosine kinase lck than the intracellular tail of CD8. Therefore, a TCR/CD4 coengagement is likely to generate stronger signals than TCR/CD8 and result in CD4+ T-cell commitment.

Alfred Singer and colleagues have proposed the **kinetic signaling model**, they propose that thymocytes commit to the CD4+ T-cell lineage if they receive a continuous signal in response to TCR/coreceptor engagement, but commit to the CD8 lineage if the TCR signal is interrupted. All CD4+CD8+ thymocytes down-regulate surface levels of CD8 in response to positive selection. Given this response, only MHC Class II restricted T cells will maintain continuous TCR/CD4/MHC Class II interaction, and therefore develop to the CD4 lineage. However, with the loss of CD8 expression, MHC Class I restricted T cells will lose the ability to maintain TCR/CD8/MHC Class I interactions.



Exit from the Thymus and Final Maturation

Once a thymocyte successfully passes through selection and makes a lineage decision, it enters a quiescent state and leaves the thymus. Mature T cells that exit the thymus are referred to as **recent thymic emigrants (RTEs)**. Current observations suggest that a cascade of events controls these final stages of maturation: positive selecting TCR signals up-regulate Foxo1, a transcription factor that controls expression of several genes related to T-cell function. Foxo1 regulates expression of Klf2, which, in turn, up-regulates SIPR. Foxo1 also up-regulates both IL-7R, which helps to maintain mature T-cell survival, and CCR7, the chemokine receptor that directs mature T-cell traffic to the lymph nodes.

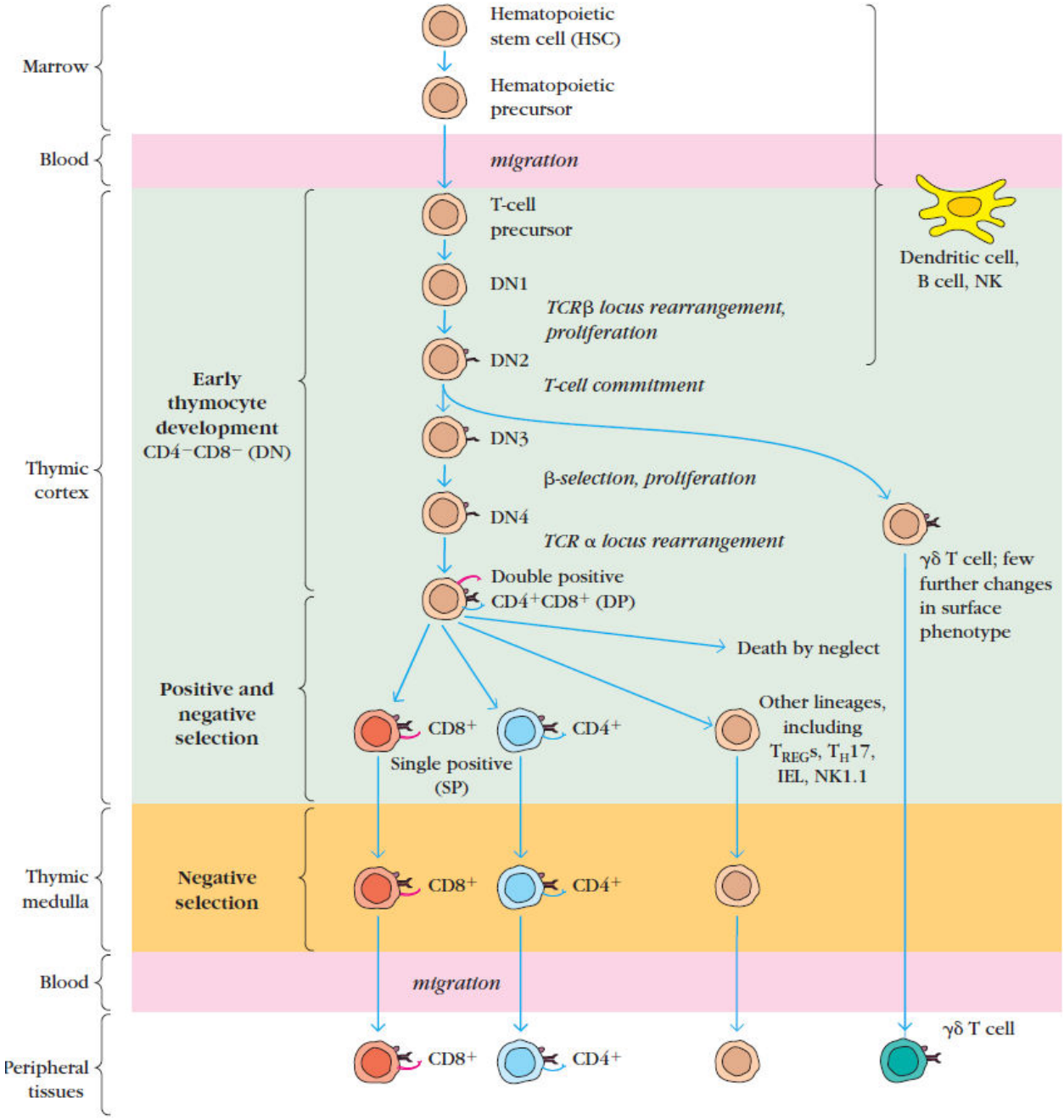


Fig: Development of T cells in the mouse

References:

- **Kuby Immunology Seventh edition.**