T Cell Differentiation in the Thymus

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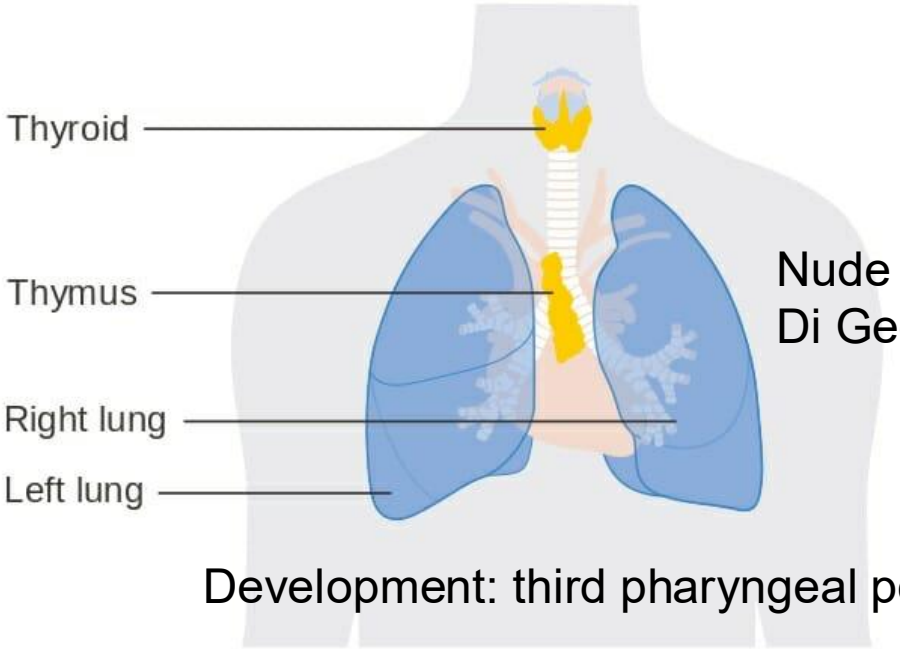
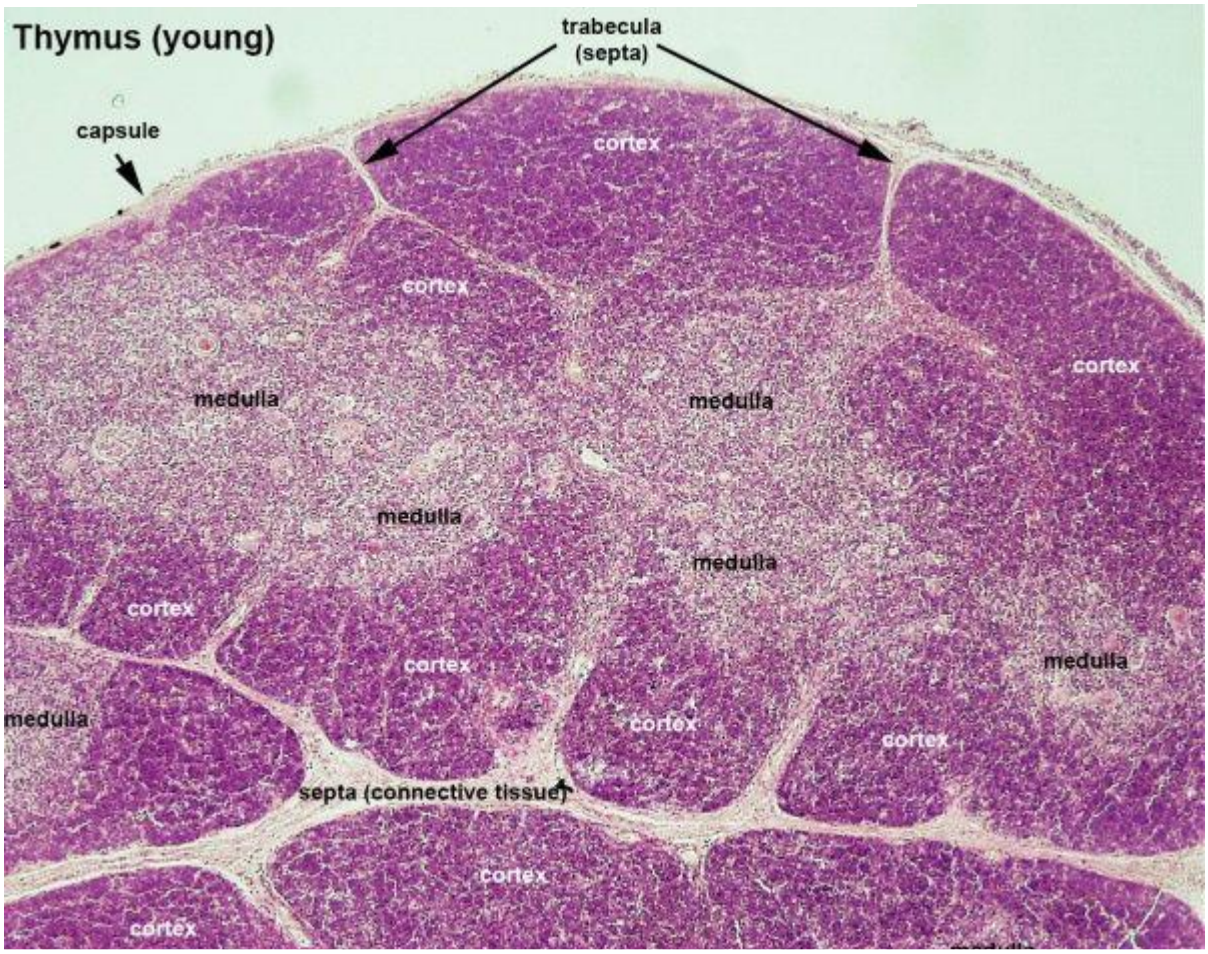
GSK Course, February 19, 2025

1. Stages of T cell development
2. Positive selection
3. Lineage commitment
4. Negative selection in the thymus
5. Peripheral tolerance
6. Regulatory T cells



Nude (mice and rats)
Di George syndrome

Development: third pharyngeal pouch



T Cell Differentiation in the Thymus

T cell Progenitors → Thymus → Mature T cells committed to specialized functions

3 weeks

Early thymocyte progenitors (ETP) colonize subcapsulary area of the thymus and progressively mature

Major steps in the thymic T cell differentiation:

1. Two lineages of T cells are generated expressing different receptors: $\alpha\beta$T cells (major population) and $\gamma\delta$T cells (minor population).

2. $\alpha\beta$TCR are selected for two properties:

- **Positive selection**: based on the ability to bind self-peptide-MHC complexes → select those that bind the 'self' MHC alleles from the 10^{10}-10^{15} random TCRs generated by rearrangement events (MHC restriction is established in the thymus).

- **Negative selection**: elimination of cells expressing TCRs that respond strongly to self-MHC molecules presenting self-antigen.

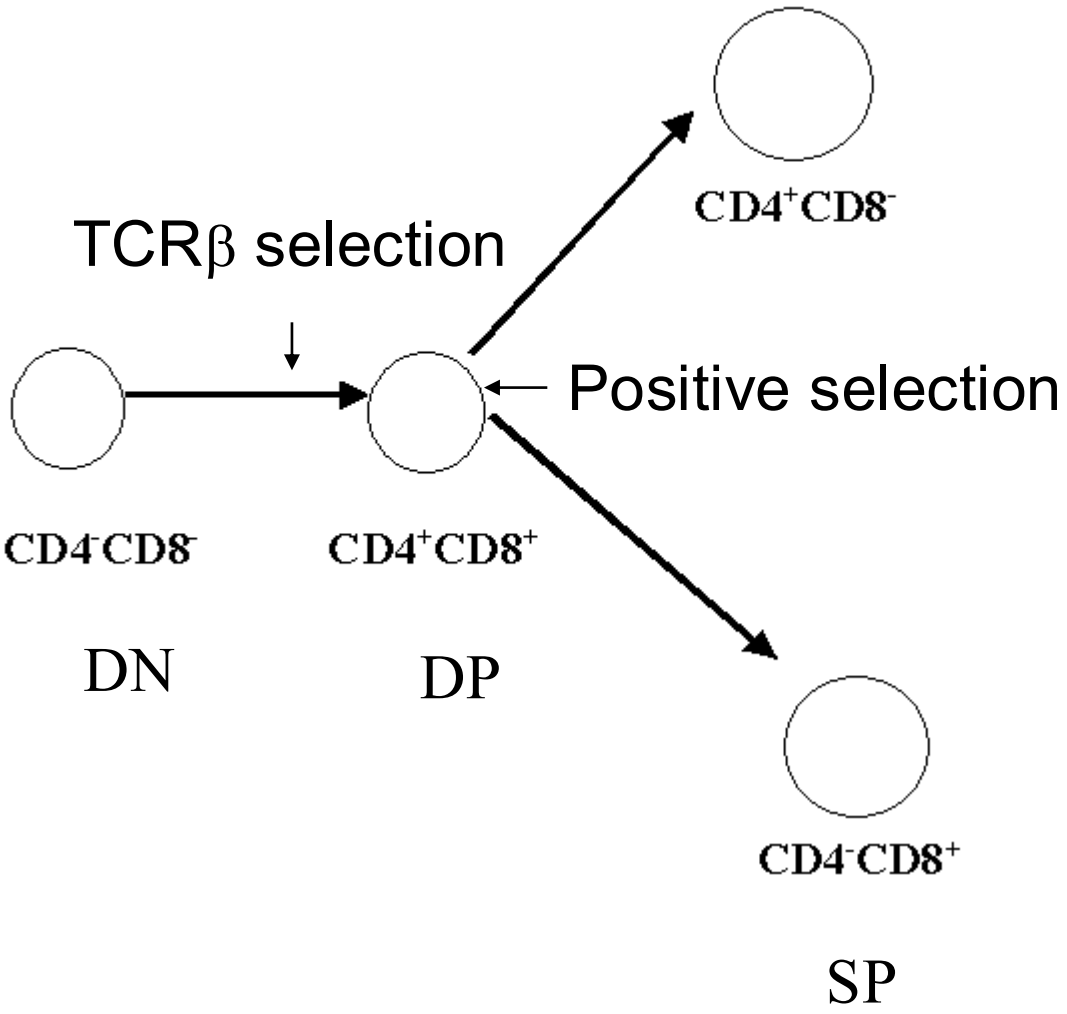
3. $\alpha\beta$ thymocytes differentiate into either $CD4^+$ T helper cell (recognize peptide/MHC class II) or $CD8^+$ cytotoxic T cells (recognize peptide/MHC class I).

General Overview of T Cell Differentiation

- Thymus is divided into distinct compartments with different stages of differentiation occurring in different locations. Thymocytes migrate upon successfully completing a required maturation step (positional/migratory cues CXCR4, CCR7, CCR9, p-selectin expression).
- Thymocyte development is divided into distinct stages defined by critical checkpoints.
- Differentiation begins with rearrangement of β, γ, δ TCR genes. The newly formed β-chain pairs with a pre-formed pre-TCRα (pTα) chain. The TCRα genes rearrange at a later stage.
- The expression of the co-receptors CD4 and CD8 is coordinated with the TCR specificity.
- Quality control of newly formed receptors

Two major checkpoints in thymocyte development: a) the proper assembly of TCR signaling complex in the thymocyte; b) TCR ability to interact with MHC ligands on thymic stromal cells.

Stages of Thymocyte Development



Early thymocyte differentiation and commitment to $\alpha\beta$ and $\gamma\delta$ T cell lineages

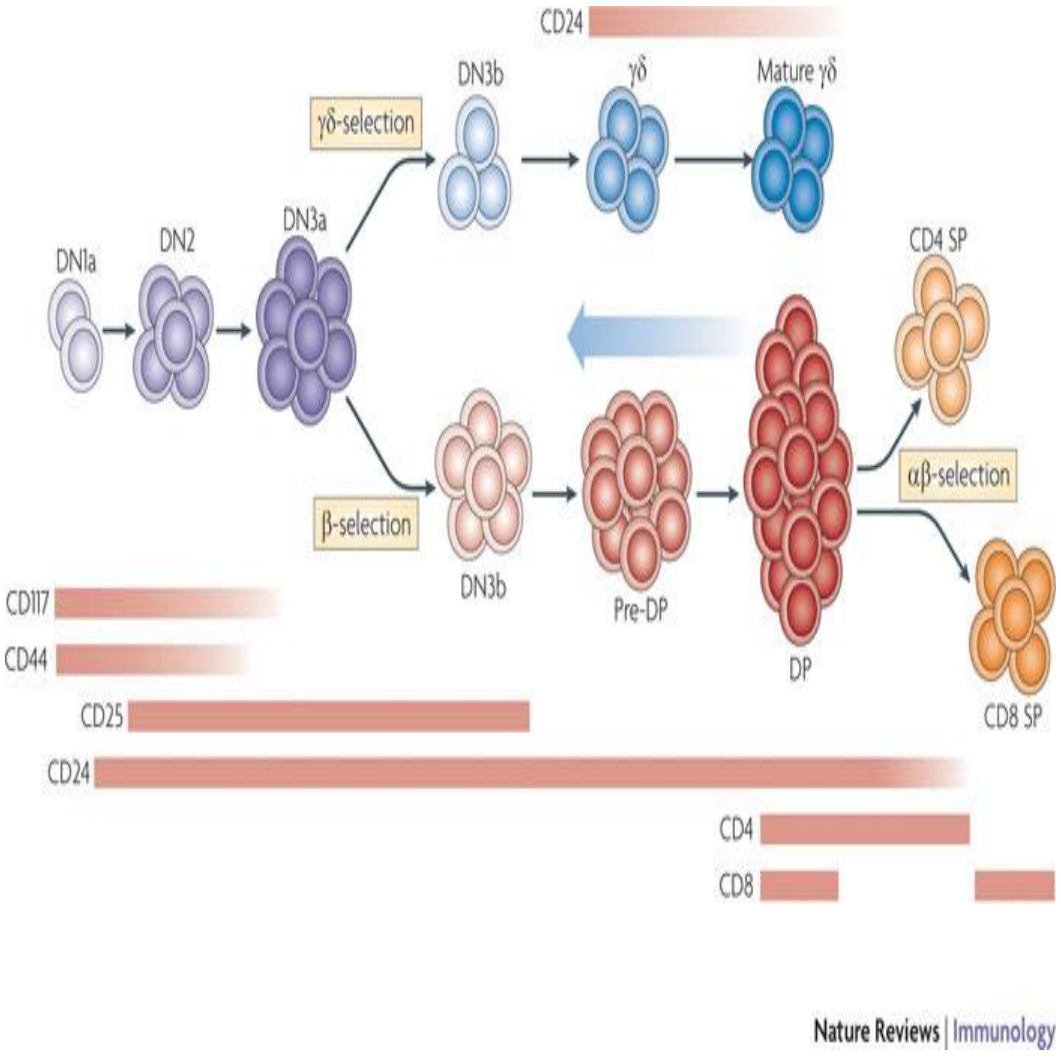


Figure 1 | **Stages of γδ and αβ T cell development. Schematic representation of cell surface markers used to** distinguish specific developmental subsets. The expression pattern of these commonly used markers is shown below or above each stage. The main T cell receptor (TCR)-mediated checkpoints are indicated, with the main αβ:γδ lineage divergence step taking place at the double-negative 3a (DN3a) to DN3b transition.

Step 1: Commitment to αβ or γδ lineage

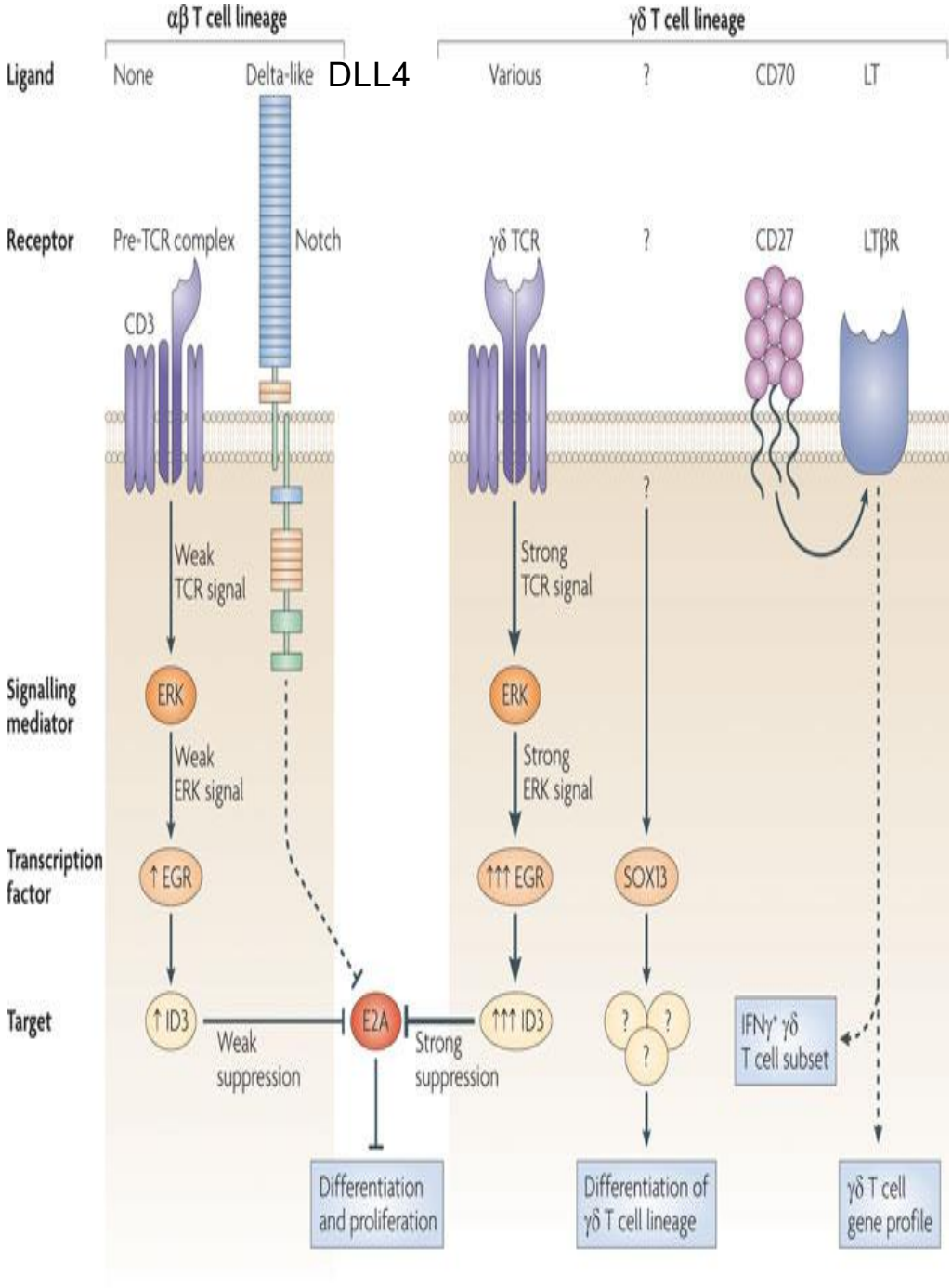
CD4$^-$CD8$^-$ (Double Negative) thymocytes

(Notch – DLL4)

- Early up-regulation of of RAG-1/RAG-2 enzyme followed by γ, δ and β genes rearrangement early and appear to rearrange simultaneously.
- Pre-Tα chain is also expressed during this stage. Pre-Tα chain pairs only with TCRβ chain and is invariant, i.e. does not undergo rearrangement.
- Successful TCR rearrangement is monitored by transmitting a signal:

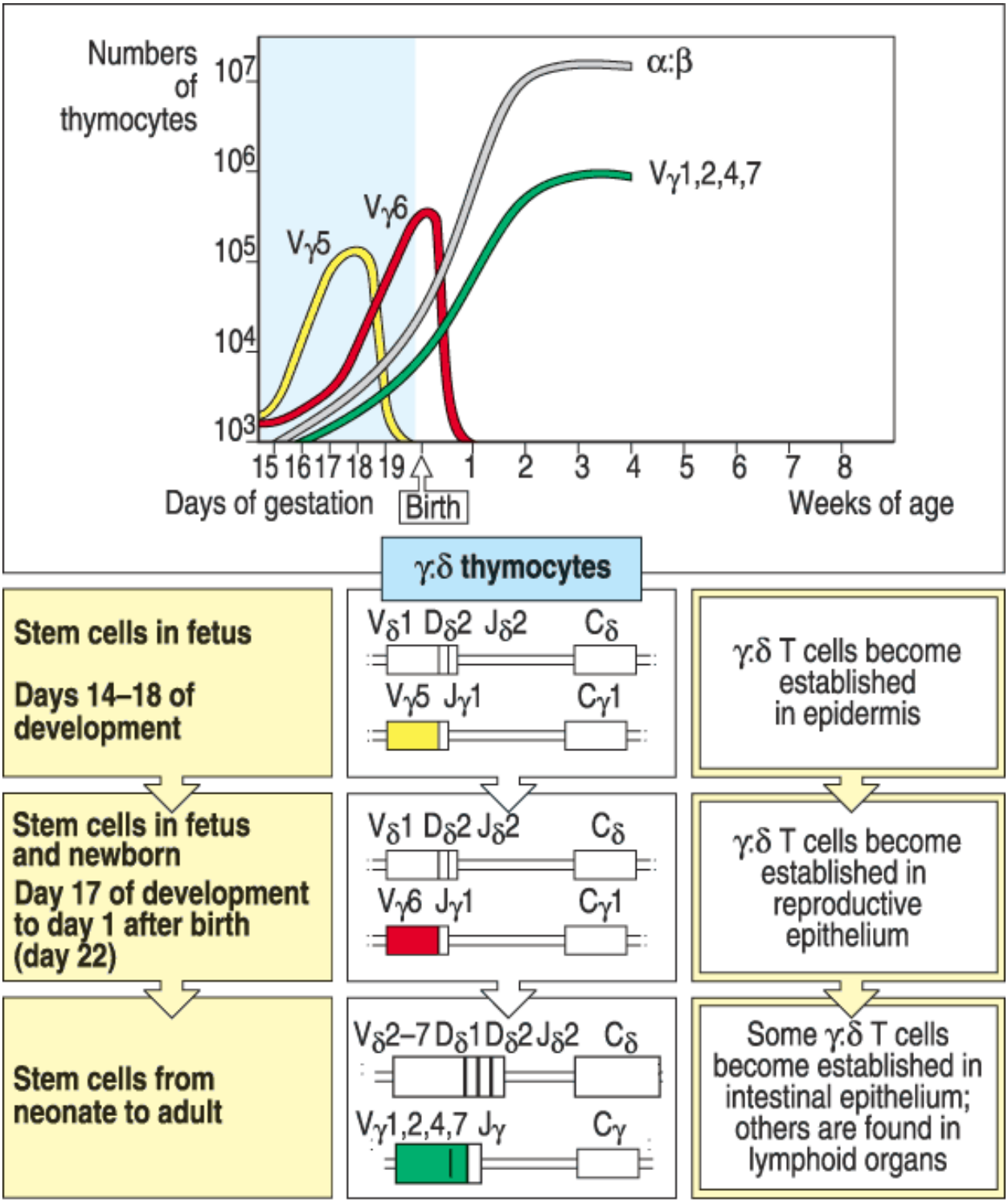
  •The pre-TCRα/β complex signals via lck and Zap70 to commit the cell to the αβ pathway;

  •A signal transmitted by a γδTCR may commit the thymocyte to the γδ lineage.



Nature Reviews | Immunology

The requirement for and influence of microenvironmental signals as part of ligand–receptor interactions involved in αβ and γδ T lineage cell commitment are outlined. Both lineage commitment (that is, differentiation) and proliferation depend on passing the E protein E2A-mediated double-negative 3 (DN3) checkpoint, which is different for each lineage. Higher levels of extracellular signal-regulated kinase (ERK), early growth response (EGR) and inhibitor of DNA binding 3 (ID3), which result in a strong suppression of E2A, are induced downstream of the γδ T cell receptor (γδ TCR) compared to αβ T lineage cells. By contrast, αβ T lineage cells require Notch signalling in addition to pre-TCR-mediated signals to inhibit E2A. In addition, γδ T cells are uniquely dependent on SRY-related high mobility group box transcription factor 13 (SOX13) and also make use of CD27 and lymphotoxin-β receptor (LTβR) signals (trans-conditioning-derived) to facilitate γδ T cell maturation and differentiation
interferon-γ.



Poorly defined ligands; MHC unrestricted; butyrophilin-like (Skint1, BTN3A1, BTN3A2)

Step 2: Cessation of TCR rearrangements

Once Double-Negative (DN) cells successfully commit to either the $\alpha\beta$ or $\gamma\delta$ lineage by productively rearranging either a β chain or $\gamma\delta$ chains TCR rearrangements stop – **allelic exclusion**.

Signaling through TCR results in degradation of RAG-2 and initiates proliferation in a Src family kinase lck-dependent manner.

During proliferative stage, TCR$\alpha\beta$ T cell precursors begin to express the CD4 and CD8 becoming Double-Positive (DP) thymocytes. There are no TCR-MHC interactions at this stage as cells have not rearranged TCRα chain genes.

Step 3: TCRα gene rearrangements.

RAG genes are re-expressed in the $CD4^+CD8^+$ thymocytes and Vα-Jα rearrangement begins.

Step 4: Positive Selection

Generation of self-MHC restricted TCR repertoire.

•The newly expressed TCR interact with self MHC (class I or class II) peptide complexes expressed on the surface of cortical epithelial cells in the thymus.

•If there is enough complementarity between the TCR and the MHC ligand to allow a weak interaction $\rightarrow$ signal to continue differentiation.

•If no signal through a TCR is received, cells die by apoptosis.

•A TCR that transmits a weak signal with self-antigen + self MHC will generate a strong signal with an unknown foreign antigen + self-MHC.

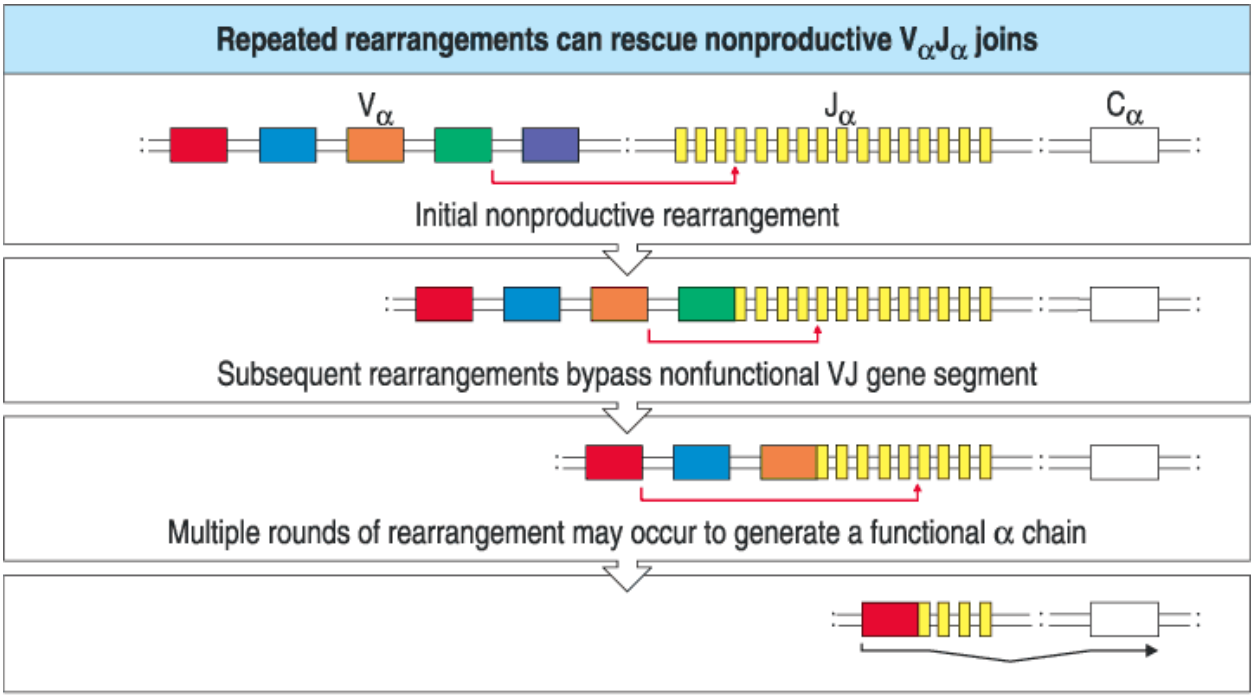
Positive Selection

100 precursors/day $\rightarrow$ 5 x 10^7 DP/day $\rightarrow$ 1 x 10^6 T cells exit

98% of thymocytes that begin differentiation are eliminated at the $CD4^+CD8^+$ stage due to a failure to be positively selected.

Positive selection of self-MHC restricted TCR repertoires

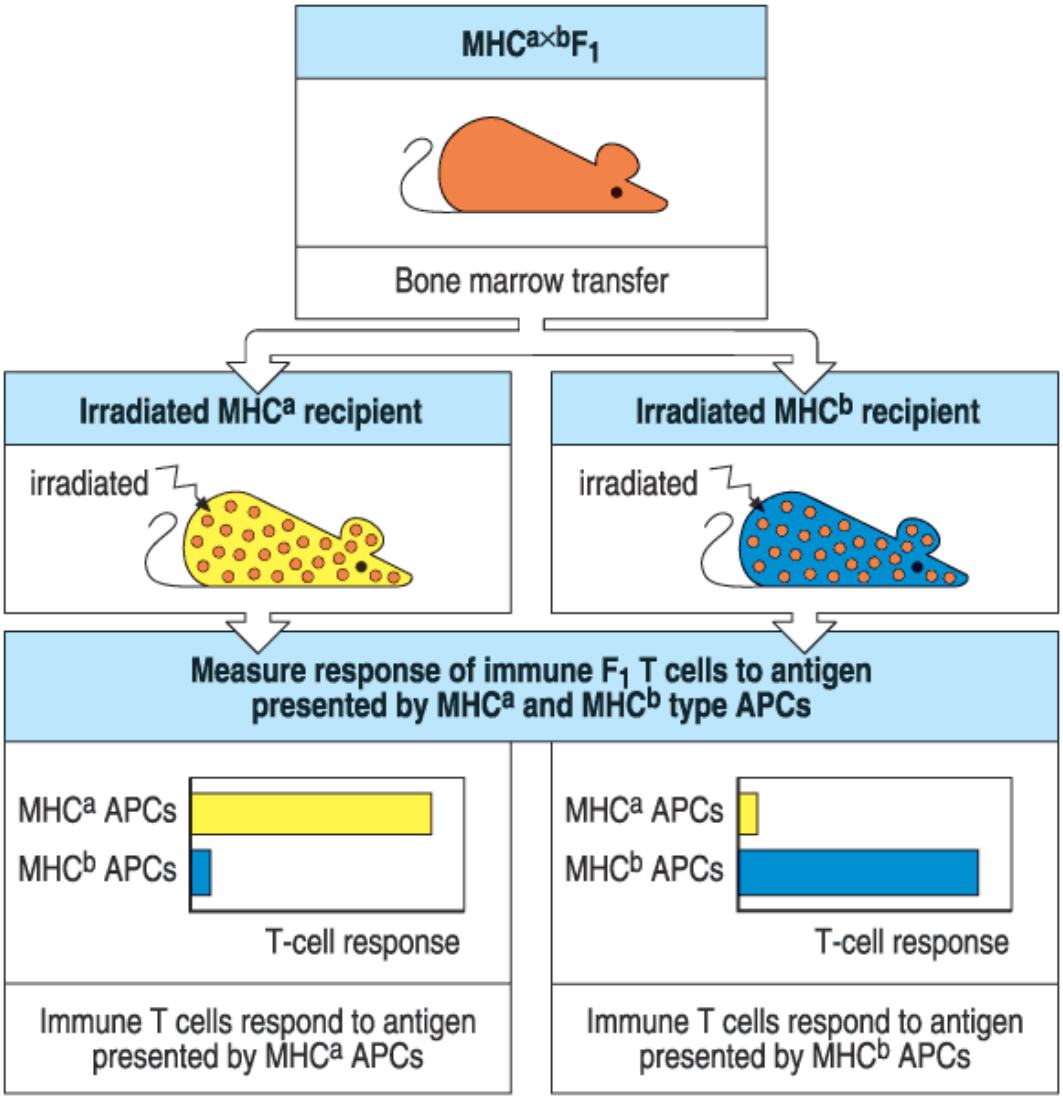
- Hundreds of allelic variants of MHC molecules exist.
- Diverse repertoire of TCRs is generated by random rearrangements in each thymus.
- TCR V regions have intrinsic affinity for MHC molecules.
- Continuing rearrangements of Vα genes maximizes the chances that a Vα chain paired with the single Vβ chain would recognize self-peptide-MHC complex.



Unlike TCR β-chains, expression of TCRα-chains is not allelically excluded.

"Self" Is Defined By MHC Molecules Present In The Thymus

Thymus is where T cells mature and are selected: MHC molecules expressed on thymic epithelium are what they consider "self".



Positive selection is mediated by MHC molecules expressed by thymic cortical epithelium

Differentiation into CD4 or CD8 T Cell Lineage

DP thymocytes expressing both CD4 and CD8 first test their TCRs for the ability to recognize MHC ligands in the thymus displayed by thymic cortical epithelial cells.

During positive selection, individual TCRs will find a match with either a peptide/MHC class I or peptide/MHC class II complex but not both. If it fits best to an MHC class I complex, CD8 contributes to the interaction. Vice versa for MHC class II complexes.

Thymocytes that were positively selected by an MHC class II ligand retain expression of CD4. MHC class I-selected thymocytes retain expression of CD8.

Peptide-MHC class I or II recognition and lineage commitment

Strength of signal model:

- Both CD4 and CD8 are associated with lck, but CD4 binds lck more efficiently.
- CD4 transmits a stronger lck signal than CD8
- Stronger signals result in CD4 SP thymocytes while weaker signals induce CD8 SP

Duration of signal (Singer et al) – transient CD8 down-modulation during DP-SP transition

CD4 commitment – induction of ThPOK
CD8 commitment – induction of Runx3

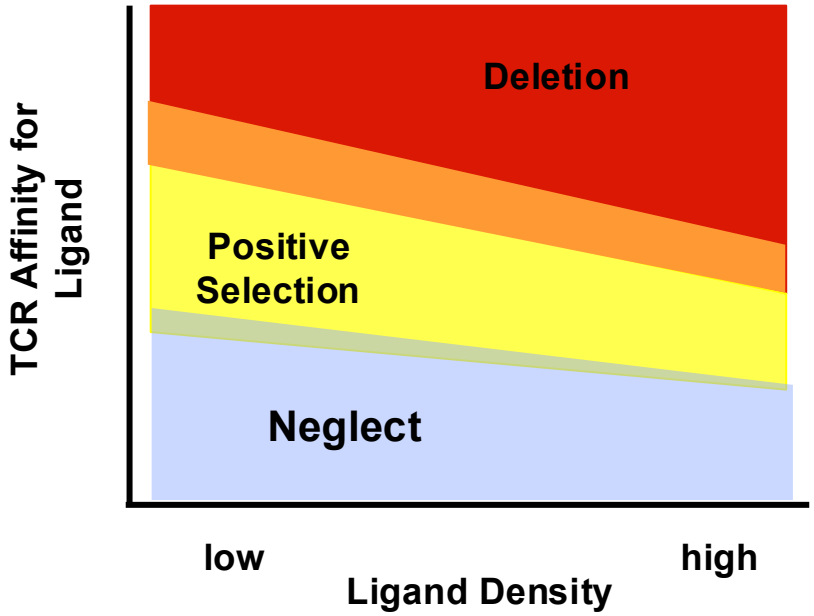
Negative Selection

Eliminates or inactivates T cell precursors that can recognize self-peptide-MHC complexes with a high avidity.

- Negative selection - strong TCR signaling induced by peptide-MHC complexes displayed by thymic APCs.
- Thymocytes receiving a strong signal are triggered to die by apoptosis, increase activation threshold or become unresponsive to TCR triggering (anergic).

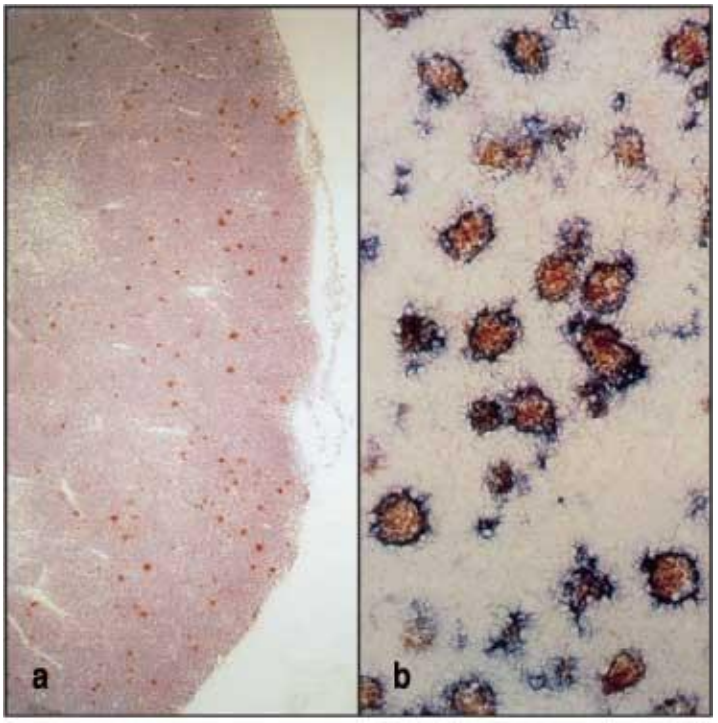
How Do Thymocytes Distinguish Between a Weak And Strong Signal?

•Different intracellular signaling pathways are recruited in response to positively and negatively selecting peptide-MHC complexes.



•Transient (negative selection) or persistent (positive selection) activation of Erk kinase in response to strong and weak TCR signals.

Specialized “agonist’ selected T cell lineages:
NKT cells
Treg cells
“Innate” CD4 T lymphocvtes
CD8αα T cells



Compartmentalization of selection:

Positive Selection: cortex, CD4CD8 DP stage.

Negative Selection: cortical medullary junction (late CD4CD8 DP stage) and medulla (SP stage).

Different proteases promote generation of distinct peptide-MHC complexes involved in positive and negative slection:

cTEC: cathepsin L, TSSP, β5t thymo-proteosome

mTEC, BM-derived thymic APC: cathepsin S, immunoproteosome.

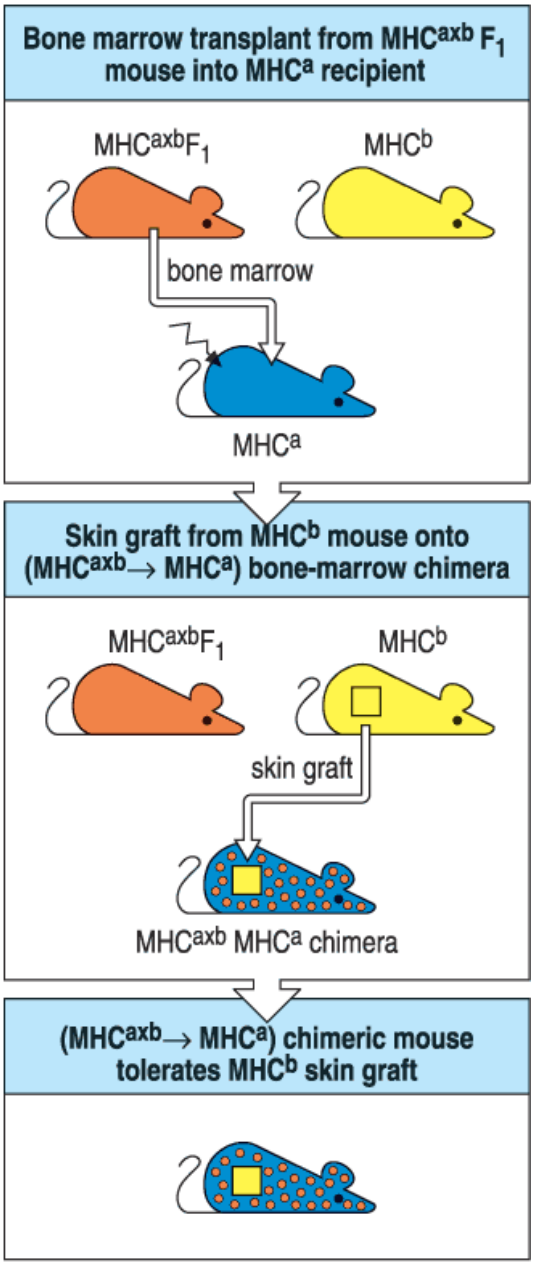
Cells That Mediate Negative Selection

Bone marrow-derived cells (dendritic cells, macrophages, B cells) predominately mediate negative selection.

Dendritic cells represent the most efficient type of APC mediating negative selection in the thymus. They present antigens synthesized in the thymus (cross-presentation) and may bring antigen to the thymus that they phagocytosed in the periphery.

Dendritic cells express high levels of MHC class I and class II molecules, co-stimulatory molecules (CD80 and CD86), and cell adhesion molecules.

Bone-Marrow-Derived Cells Mediate Negative Selection



Negative selection of T cells specific for tissue-specific self antigens

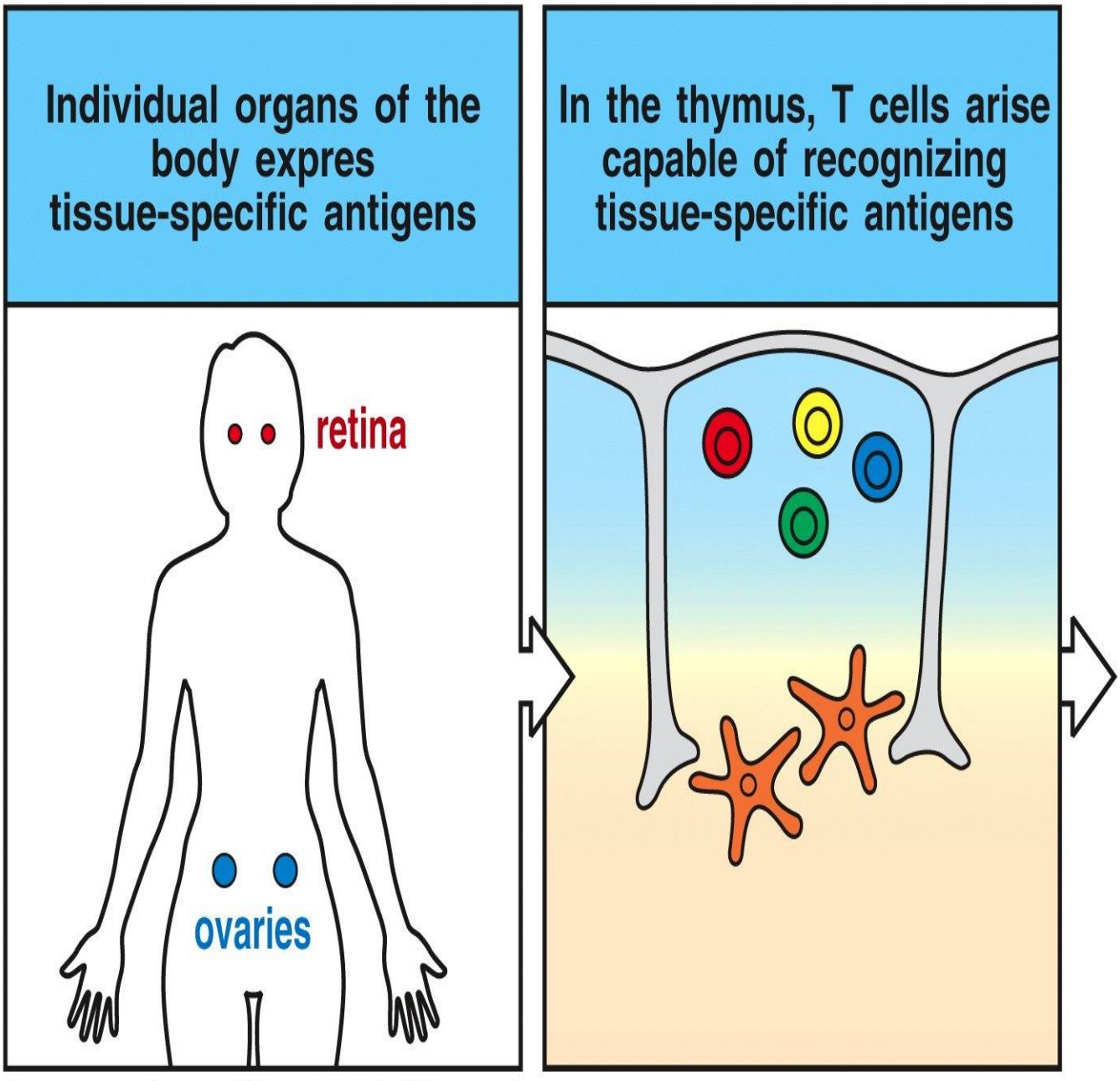
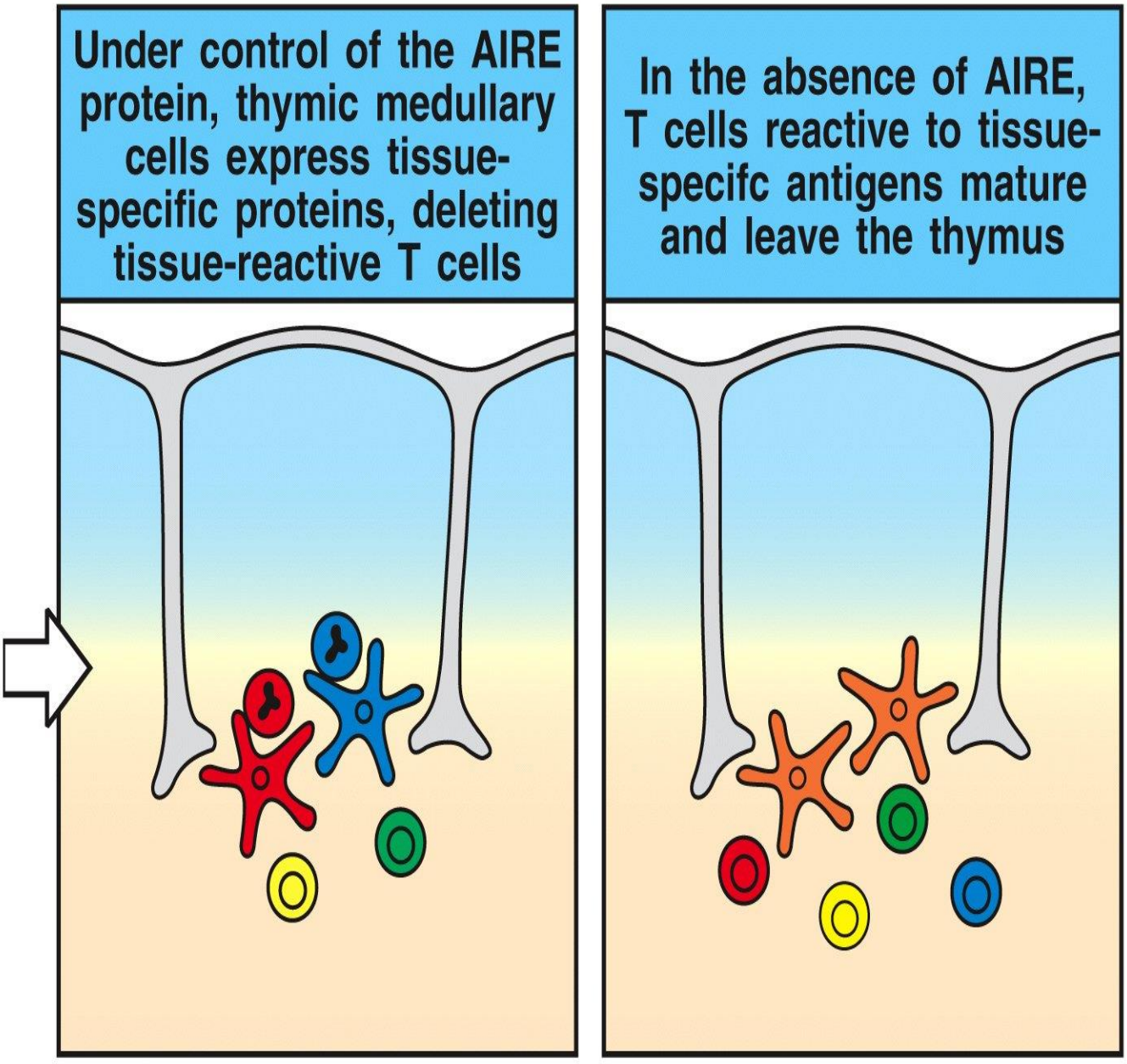
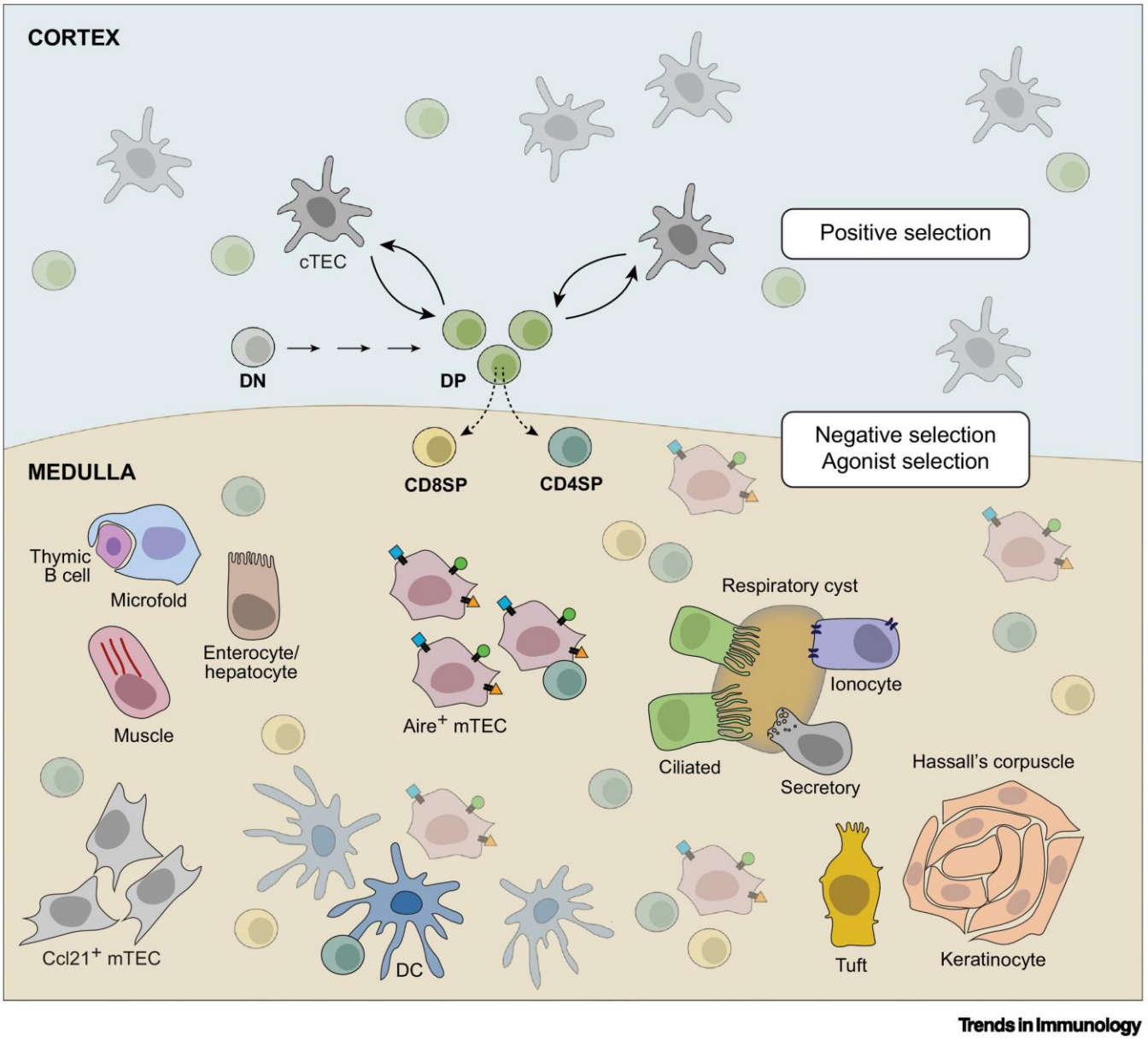


Figure 13-9 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)



Specialized medullary epithelial cells also mediate negative selection in the thymus. These cells appear to "randomly" activate transcription of proteins that are usually found only in the peripheral tissues, e.g. pancreas. Transcription factor AIRE has been implicated in this process. AIRE deficient mice and human patients (APECED) develop autoimmunity.

Aire^{+} mTECs and thymus mimetic cells



Michelson et al., Cell, 2022
Nusser et al., Nature, 2025

Peripheral Tolerance

- Central tolerance mechanisms are not 100% efficient at eliminating self-reactive T cells.

- Some self-antigens may be expressed only in the peripheral tissues and not in the thymus where central tolerance occurs.

- Some antigens may be developmentally expressed such that T cells in the periphery may encounter newly expressed antigens that were not present during T cell maturation (e.g., proteins related to puberty, pregnancy, lactation, etc.).

Regulatory T cells

Specialized subset of suppressive CD4 T cells, essential for preventing autoimmunity and inflammation.

- Mice thymectomized at three days of age develop T cell-mediated autoimmune responses directed toward multiple tissues as adults. Autoimmunity can be prevented by transferring $CD25^{+}CD4^{+}$ T cells from mice that were not thymectomized.
- Transferring naïve T cells into T cell-deficient mice causes autoimmunity that can be prevented by co-transfer of Treg cells into the recipients.
- X-linked transcription factor Foxp3 is specifically expressed by Treg cells and is required for their differentiation and function.
- Foxp3 deficient mice and humans, lacking regulatory T cells, develop fatal autoimmune syndrome.