Introduction to Immunology

T Cell Development

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Thymic stroma cells provide the microenvironment for T-cell development.

- **T cells** develop from progenitors that are **derived from pluripotent stem cells located in the bone marrow**.
- T-cell development takes place in the **thymus**.
- The thymus is located in the **anterior mediastinum**.
- Hystologically the thymus has two regions:
  - **Peripheral cortex**: densely populated with lymphoid cells
  - **Central medulla**: less lymphocyte rich, contains dendritic cells and macrophages.

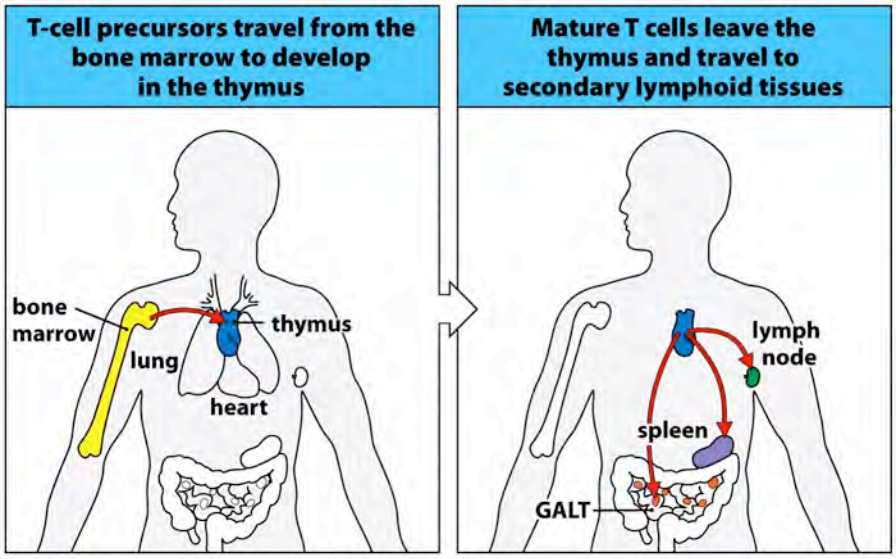
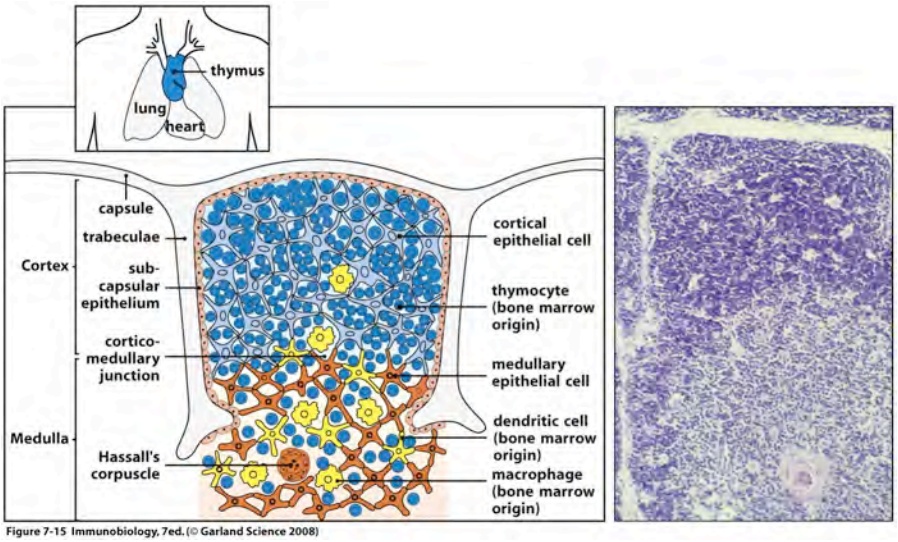


Figure 7.1 The Immune System, 3ed. (© Garland Science 2009)



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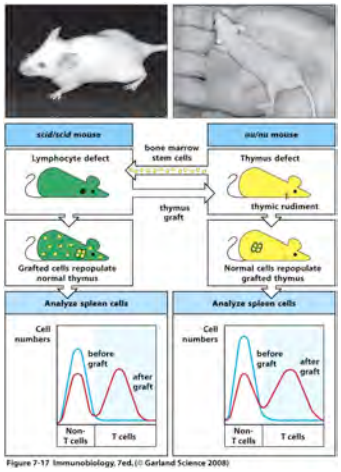
Thymic stroma cells provide the microenvironment for T-cell development.

- T-cell progenitors enter the thymus in the cortico-medular region
- Signals from the stroma promote T-cell comittment , differentiation and proliferation.
- A key signal is activation of the NOTCH1 receptor.

Both lymphoid progenitors and the thymic stroma need to be fully functional for effective T-cell development

SCID: defect of lymphoid progenitors.

Nude: defects of thymic epithelial cells.



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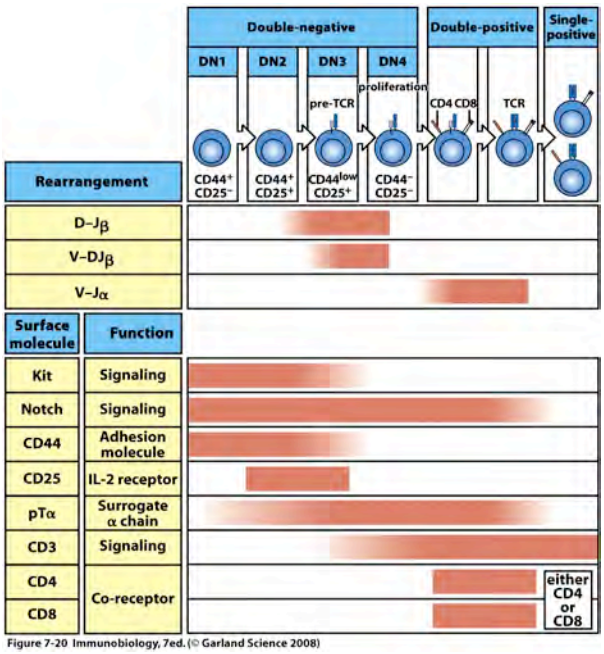
SCID bone marrow does not rescue the Nude phenotype

Nude bone marrow rescues the SCID phenotype

Early stages of T-cell development

- Initially early thymocytes (DN) are not committed to the T-cell lineage.
- DN1→**Kit+ CD44 +**CD25-
- DN2→**Kit+ CD44 +CD25+**
- DN3→**Kit+** CD44 -**CD25+**
- DN4→Kit-CD44 -CD25-
- DN T-cell development is driven by signals from stroma such as NOTCH1, IL7 and cKit.



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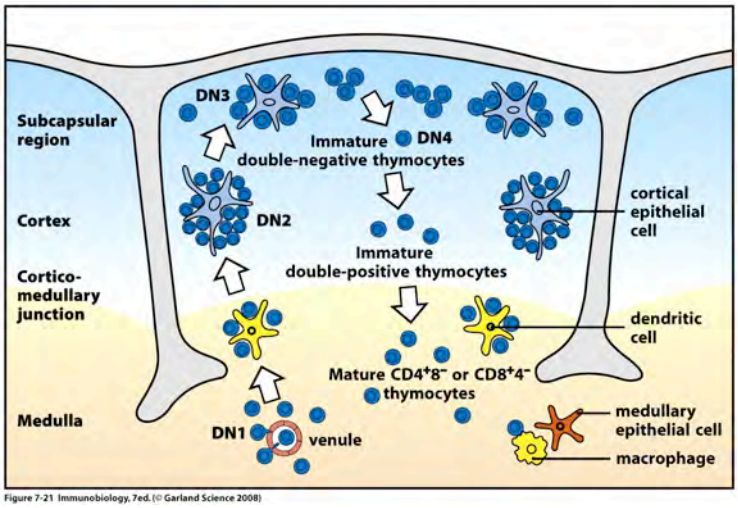
T-cell development is coupled with the rearrangement of the T-cell receptor genes

- DN2→start rearranging TCRβ (D-J)
- DN3→continues TCTβ rearrangement (V-DJ)
- DN4→TCRβ-preTCR signaling blocks further TCRβ rearrangement
- DP→TCRα rearrangement and positive selection
- SP→ Negative selection and exit from the thymus
- T-cell development from DN to SP takes 3 weeks.

Thymocytes migrate during T-cell development



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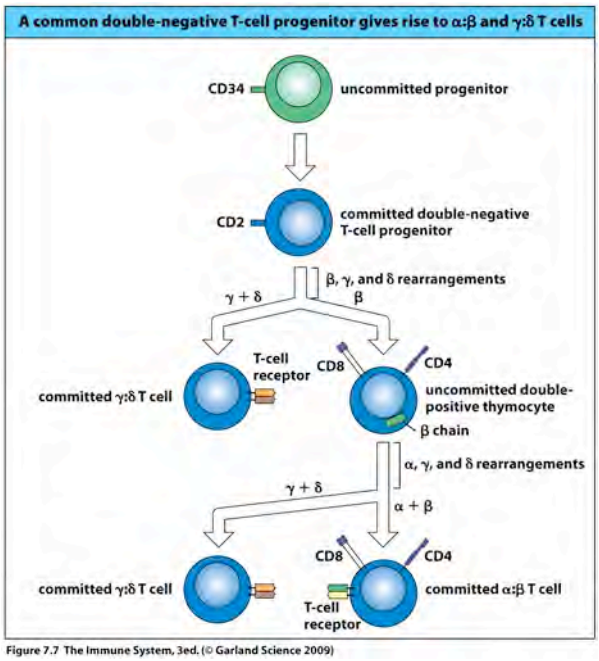
Lineage choice between gamma-delta and alpha-beta T-cells

- Gamma, delta and beta are all rearranged at the same time.
- A successful gamma-delta rearrangement drives a strong signal and promotes gamma-delta T-cell development.
- A successful TCRβ rearrangement drives a weaker preTCR signaling that drives alpha-beta lineage.
- TCRα rearrangement deletes TCR delta, blocking development into gamma-delta lineage.
- The strength of the NOTCH signal also influences alpha-beta vs. gamma-delta choice.



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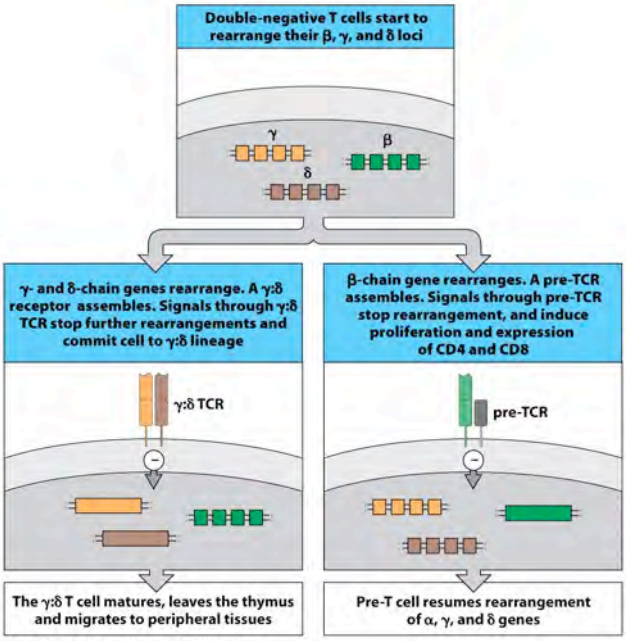

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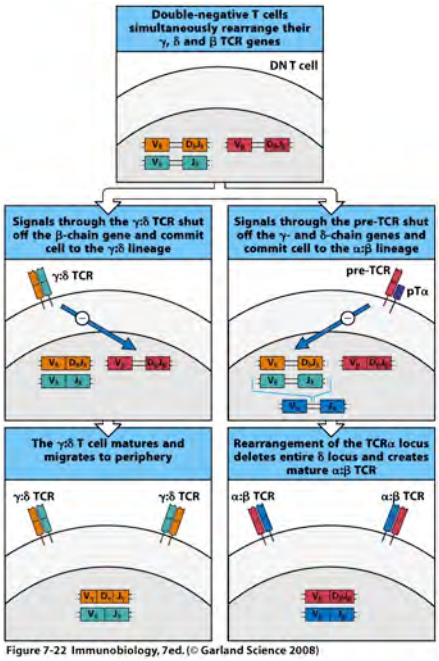

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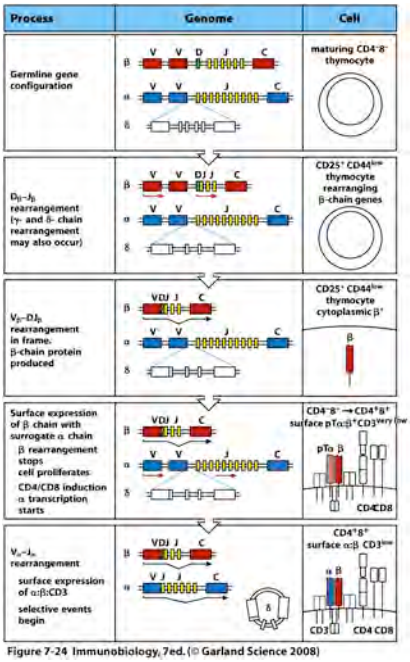
TCRβ rearrangement

- DJ are joined in DN2, then V joins to the already rearranged DJ in DN3.
- If successful a TCRβ is expressed with the preTCRα in DN4
- The structure of TCRβ with two D J clusters and C chains allows two full rounds of recombination.
- If unsuccessful and no effective gamma-delta recombination the cell dies.



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PreTCR signaling

- Beta chain plus invariable preTCRα chain in association with signaling complex proteins
- Signals through the LCK tyrosine kinase.
- Triggers degradation of rag2 blocking further rearrangement of the beta chain
- Induces proliferation during DN4
- Drives expression of CD4 and CD8.
- PreTCR generates a pool of cells with a single beta chain that will rearrange alpha.
- Mature T-cells can have the same beta chain and different alpha chains.
- After preTCRα signaling cells have to stop proliferating to rearrange alpha.

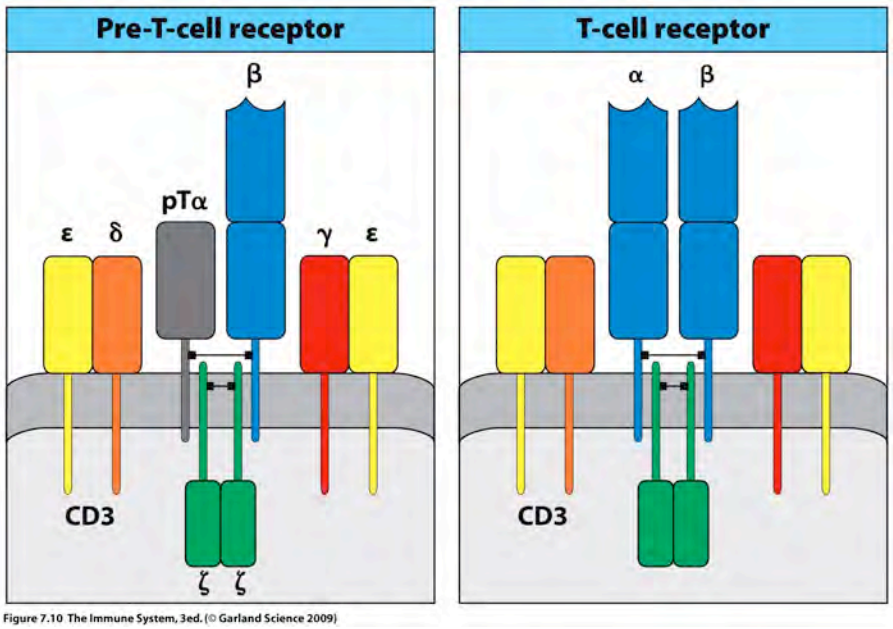


Figure 7.10 The Immune System, 3ed. (© Garland Science 2009)

TCRα rearrangement

- Does NOT have D segments, V-J only
- Recombination occurs only if beta is successfully expressed.
- 60 J segments and lots of V segments allow multiple rounds of recombination.
- A successful TCRα rearrangement does not block recombination.
- TCRα recombination is blocked by recognition of self-MHC complexes during positive selection.
- DP cells live for only 3-4 days unless rescued by TCR stimulation and positive selection.

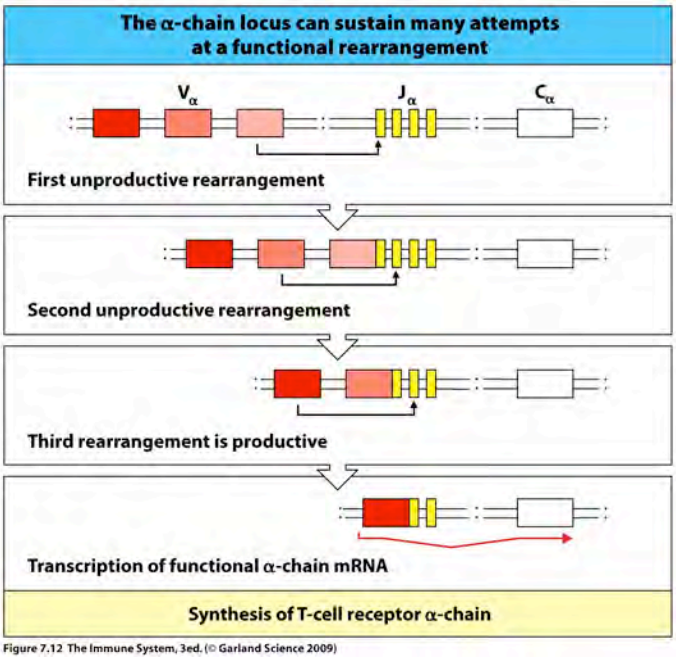


Figure 7.12 The Immune System, 3ed. (© Garland Science 2009)

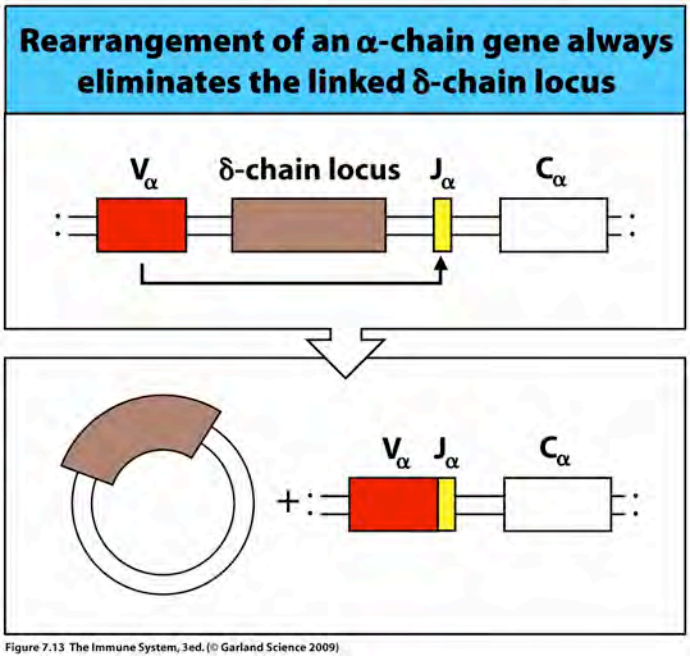
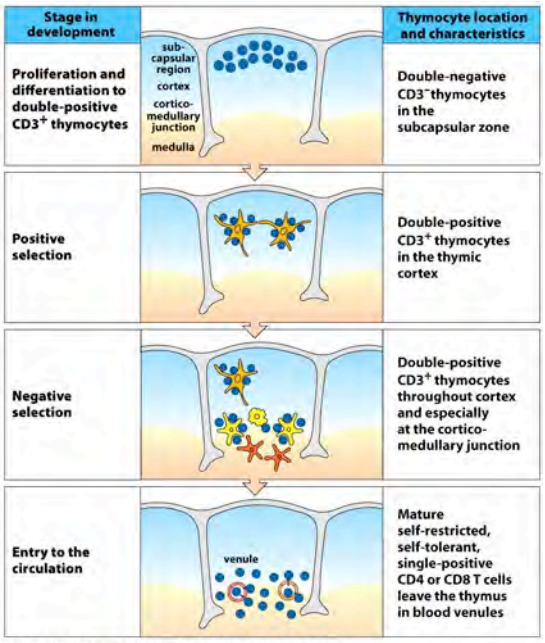


Figure 7.13 The Immune System, 3ed. (© Garland Science 2009)

T-cell selection

- The process of rescue from cell death and maturation to SP CD4 or SP CD8 cells upon recognition of MHC-self peptides.
- Negative selection TCR receptors that respond too strongly to self peptide are eliminated as potentially self reactive.



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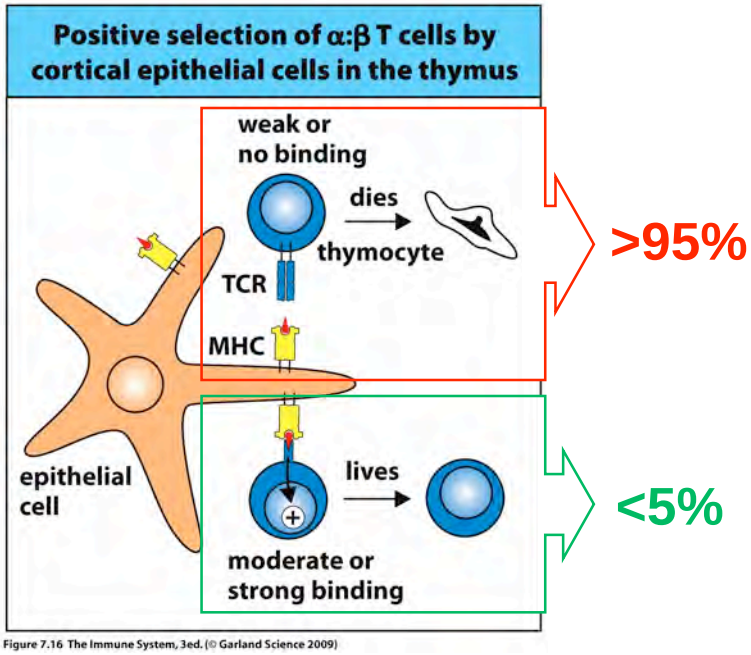

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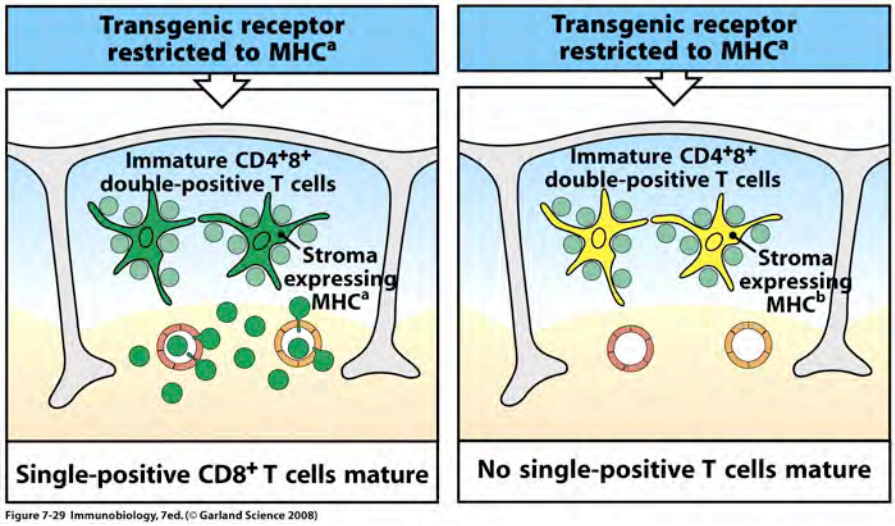
Positive selection

- Positive selection occurs selectively in the CORTEX
- A CD4CD8 T cell expresses TCR and encounters an EPITHELIAL cell expressing MHC class I:self peptide
  - i) Successful interaction drives positive selection and generates a CD8 SP (cytotoxic)
  - ii) Unsuccessful interaction drives continuous rearrangement of alpha to generate a different TCR and try again
- A CD4CD8 T cell expresses TCR and encounters an EPITHELIAL cell expressing MHC class II:self peptide→
  - i) Successful interaction drives positive selection and generates a CD4 SP (cytokine secreting)
  - ii) Unsuccessful interaction drives continuous rearrangement of alpha to generate a different TCR and try again
- If after multiple encounters there is no self recognition and the alpha chain runs out of recombination options the cell dies by apoptosis: >95% of the cells fail to be positively selected and undergo programmed cell death.



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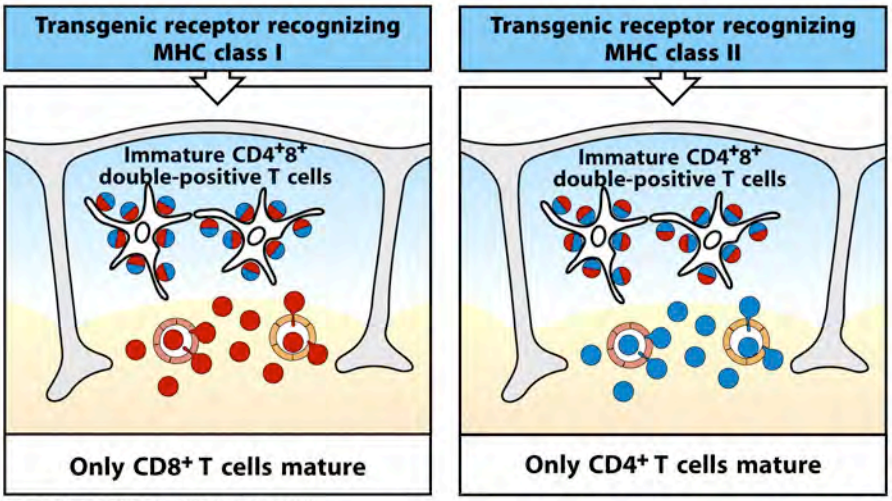

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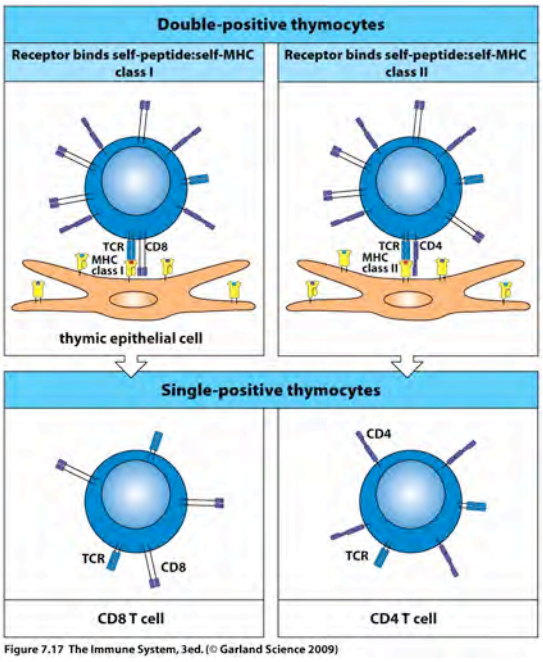
CD4 CD8 lineage fate

- Positive selection is coupled with CD4 or CD8 cell fate specification.
- If the CD4CD8 cell TCR recognizes MHC I the T-cell must have CD8 to be functional during MHC I immune reaction.
- If the CD4CD8 cell TCR recognizes MHCII the T-cell must have CD4 to be functional during MHCII immune reaction.
- CD4 cells will have an expression program that allows cytokine secretion while CD8 cells will have an expression program that makes them capable of cell killing.



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Negative selection

- Interaction of TCR with an MHC-peptide in the thymus triggers cell death.
- The thymic stroma is fully competent to eliminate autoreactive clones.
- AIRE (autoimmune regulator) drives expression of extrathymic antigens such as insulin in the thymic stroma.
- Negative selection is mainly driven by bone marrow derived dendritic cells and macrophages in the medulla.

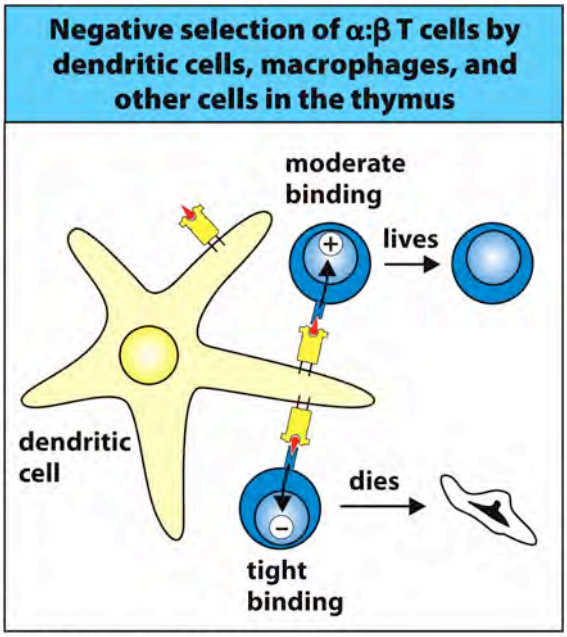

Figure 7.18 The Immune System, 3ed. (© Garland Science 2009)

Summary

- T cells arise in the thymus from progenitors that are derived from pluripotent bone marrow cells.
- The peripheral cortex of the thymus which is densely populated with lymphoid cells, hosts most of T-cell development and is responsible for positive selection.
- The central medulla of the thymus is less lymphocyte rich, contains dendritic cells and macrophages which are the main mediators of negative selection.
- Early thymocytes lack expression of CD4 and CD8 and are not committed to the T-cell lineage (they can generate B-cells and NK cells).
- Thymocytes can differentiate along two main lineages to become either gamma-delta T-cells or alpha-beta T-cells depending on the type of TCR they express.

Summary

- T-cell development goes in parallel with the rearrangement of the T-cell receptor genes
- DN2→start rearranging TCRβ (D-J)
- DN3→continues TCRβ rearrangement (V-DJ)
- DN4→TCRβ-preTCR signals drive proliferation and blocks further TCRβ rearrangement
- Small DP cells upregulate the expression of CD3, rearrange TCRα and undergo positive and negative selection.
- Only <5% of total DP thymocytes mature to single positive CD4 or CD8 cells and leave the thymus the rest (>95%) die by apoptosis.

Summary

- Gamma, delta, and beta TCR gene loci are all rearranged at the same time.
- If the cell gets a successful gamma-delta rearrangement, this drives a strong signal that promotes gamma-delta T-cell development.
- If the cell gets a successful TCRβ chain this drives preTCR complex formation and a weaker signal that drives alpha-beta lineage.

Summary

- If successful, a TCRβ is expressed with the preTCRα invariable chain
- PreTCR signaling blocks further rearrangement of the beta chain and drives proliferation and differentiation to DP cells.
- When DP cells stop dividing they rearrange their alpha chain.
- TCRα allows multiple rounds of rearrangements.
- A successful TCRα rearrangement does not stop the recombination process.
- The maturation of TCRα deletes TCRδ, so once alpha starts rearranging there is no way for the cell to go into the gamma-delta lineage.

Summary

- Positive selection**: survival signal** allows DP cells to mature to SP CD4 or SP CD8 cells if they can recognize self-MHC in cortical epithelial cells of the thymus. Signal is weak and sustained.

Summary

Positive selection:

- A $CD4^+CD8^+$ T cell expresses TCR and encounters a cell expressing MHC class I:self peptide→if positive selection the cell becomes a cytotoxic CD8 T-cell
- A $CD4^+CD8^+$ T cell expresses TCR and encounters a cell expressing MHC class II:self peptide→ if positive selection the cell becomes a cytokine-secreting CD4 T-cell.
- If after multiple encounters there is no self recognition and the alpha chain runs out of recombination options the cell dies by apoptosis.
- Overall >95% of cells fail to be positively selected and die.
- Positive selection occurs selectively in the CORTEX

Summary

- Negative selection: **death signal** triggered by recognition of self peptide:MHC complexes. Signal is strong.

Summary

Negative selection

- The thymic stroma cells express antigens from all tissues thanks to the activity of a gene called AIRE (autoimmune regulator).
- AIRE drives expression of genes such as insulin in the thymus so that no T-cells with specificity against insulin leave the thymus.
- Cells with TCRs that recognize MHC-self antigen with high affinity undergo apoptosis.
- This is mainly driven by bone marrow derived dendritic cells and macrophages in the medulla.