T Cell Development

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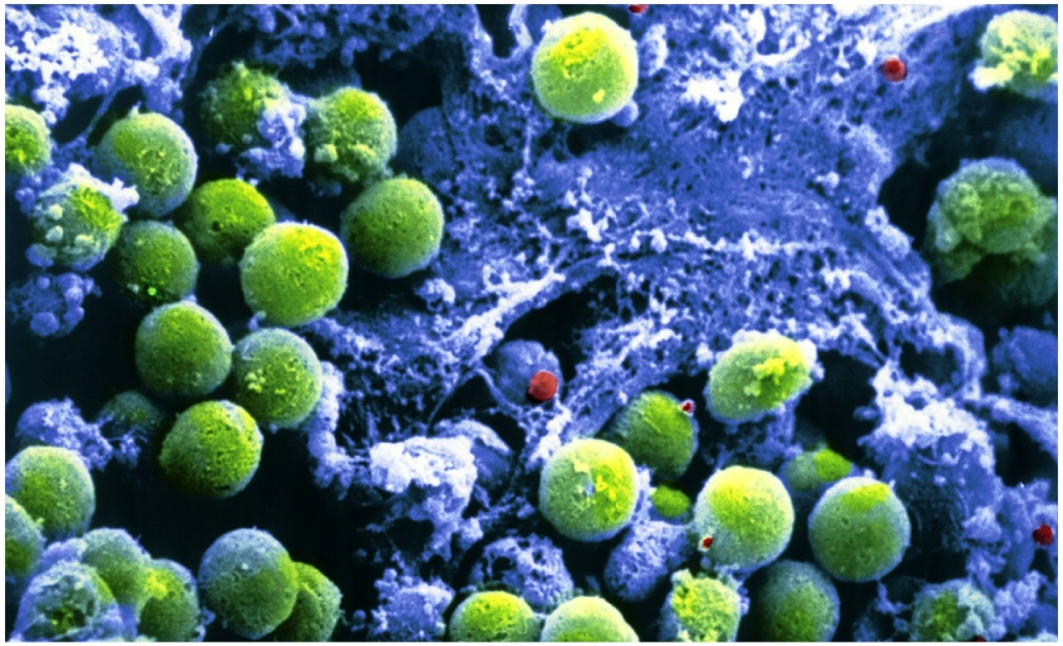
Thymocytes in the cortex of the thymus



Chapter 9 Opener
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- Early thymocytes development
- Positive and negative selection
- Lineage commitment
- Exit from the thymus and final maturation
- Other mechanisms maintaining self-tolerance
- Apoptosis
- Alloreactivity

Development of T cells from bone marrow stem cells

Nude mice fail to develop thymus and lack T cells

In earlier years, fetal thymic organ culture (FTOC) was developed as an *in vitro* microenvironment to study T cell development.

Later breakthrough in 2002, a bone marrow stromal cell line, ectopically expressing the Notch ligand, was found to be able to induce the differentiation of BM progenitor cells into CD4+CD8+, CD4+, and CD8+ T cells.

In the absence of Notch1 ligand, lymphoid precursors would develop into B cells.

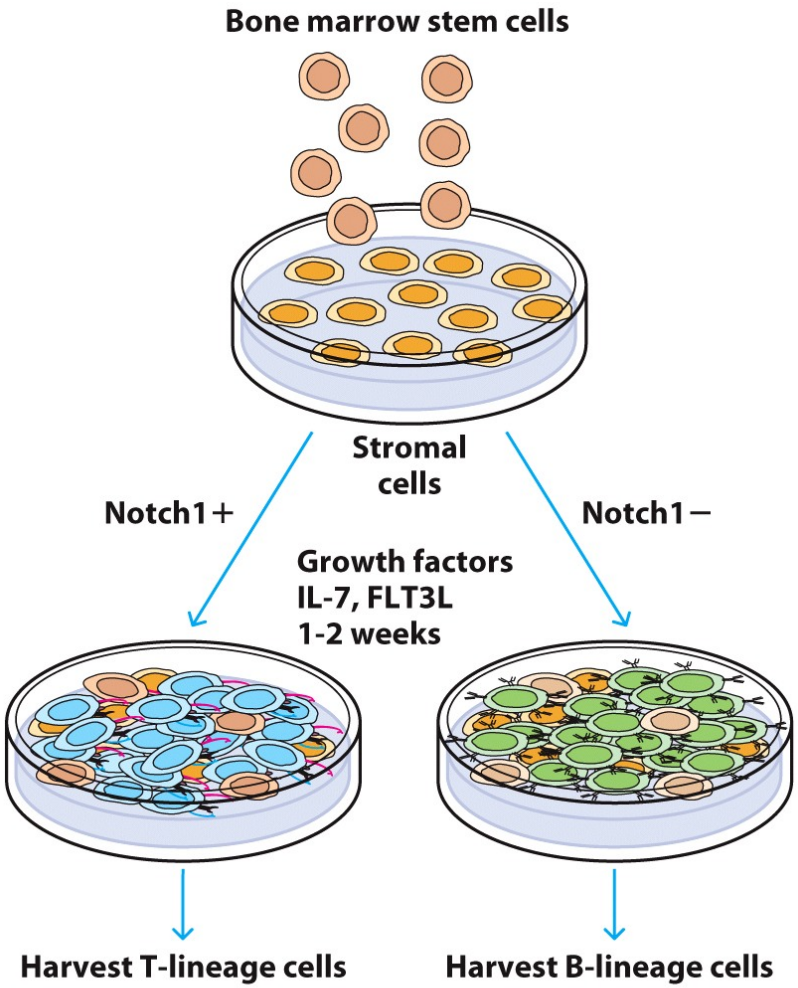

Figure 9-2
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T cell development in the mouse

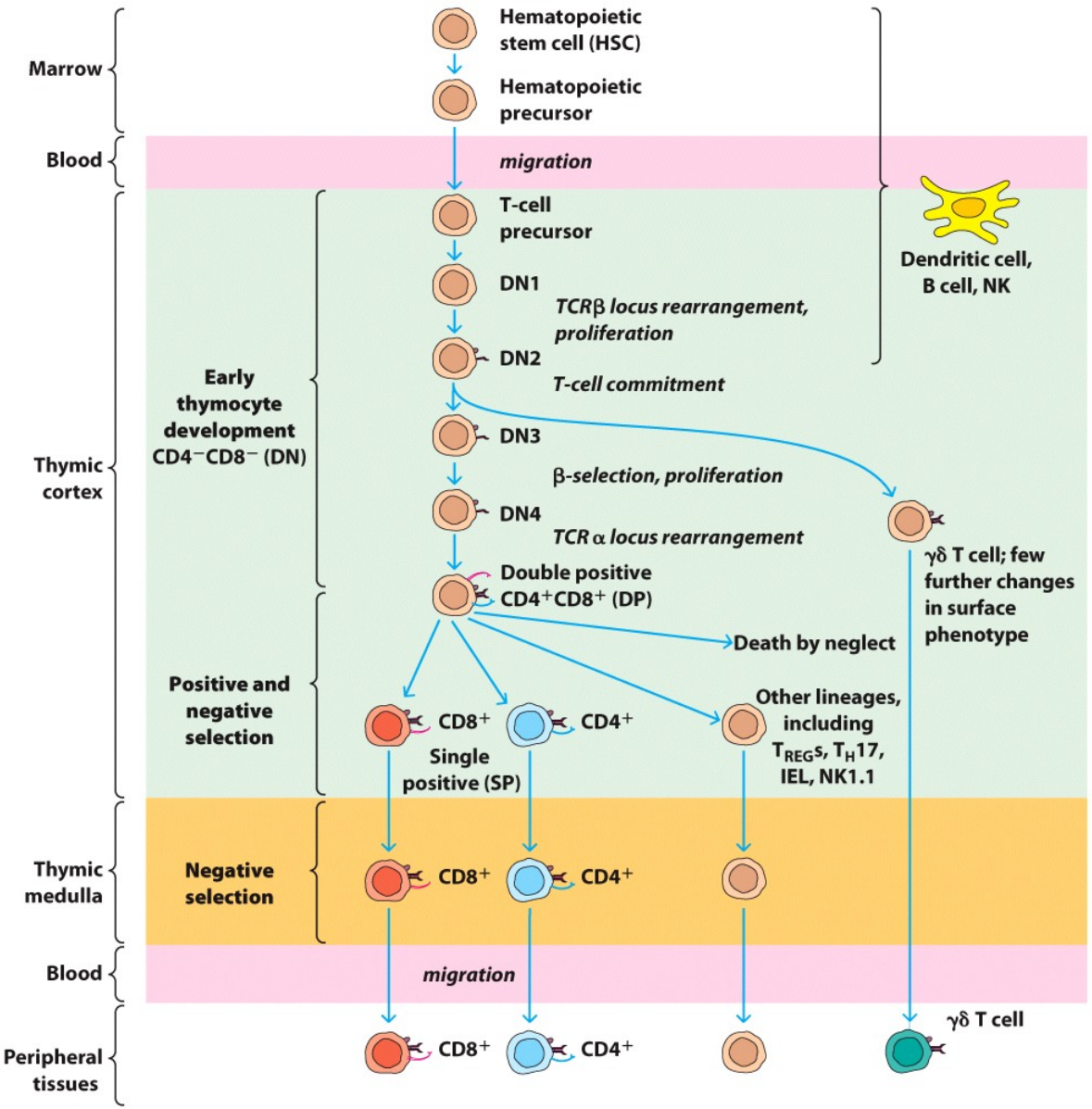

Figure 9-1
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T-cell precursors do not express TCR, CD3, CD4 or CD8, and have not expressed RAG-1/-2 that are required for TCR gene rearrangement

Double-negative (DN) cells are CD4–CD8–, and are characterized into 4 developmental stages.

TABLE 9-1 Double-negative thymocyte development

	Genotype	Location	Description
DN1	c-kit $(CD117)^{++}$, $CD44^{+}$, $CD25^{-}$	Bone marrow to thymus	Migration to thymus
DN2	c-kit $(CD117)^{++}$, $CD44^{+}$, $CD25^{+}$	Subcapsular cortex	TCRγ, δ, and β chain rearrangement; T-cell lineage commitment
DN3	c-kit $(CD117)^{+}$, $CD44^{-}$, $CD25^{+}$	Subcapsular cortex	Expression of pre-TCR; β selection
DN4	c-kit $(CD117)^{low/-}$, $CD44^{-}$, $CD25^{-}$	Subcapsular cortex to cortex	Proliferation, allelic exclusion of β-chain locus; α-chain locus rearrangement begins; becomes DP thymocyte

Table 9-1
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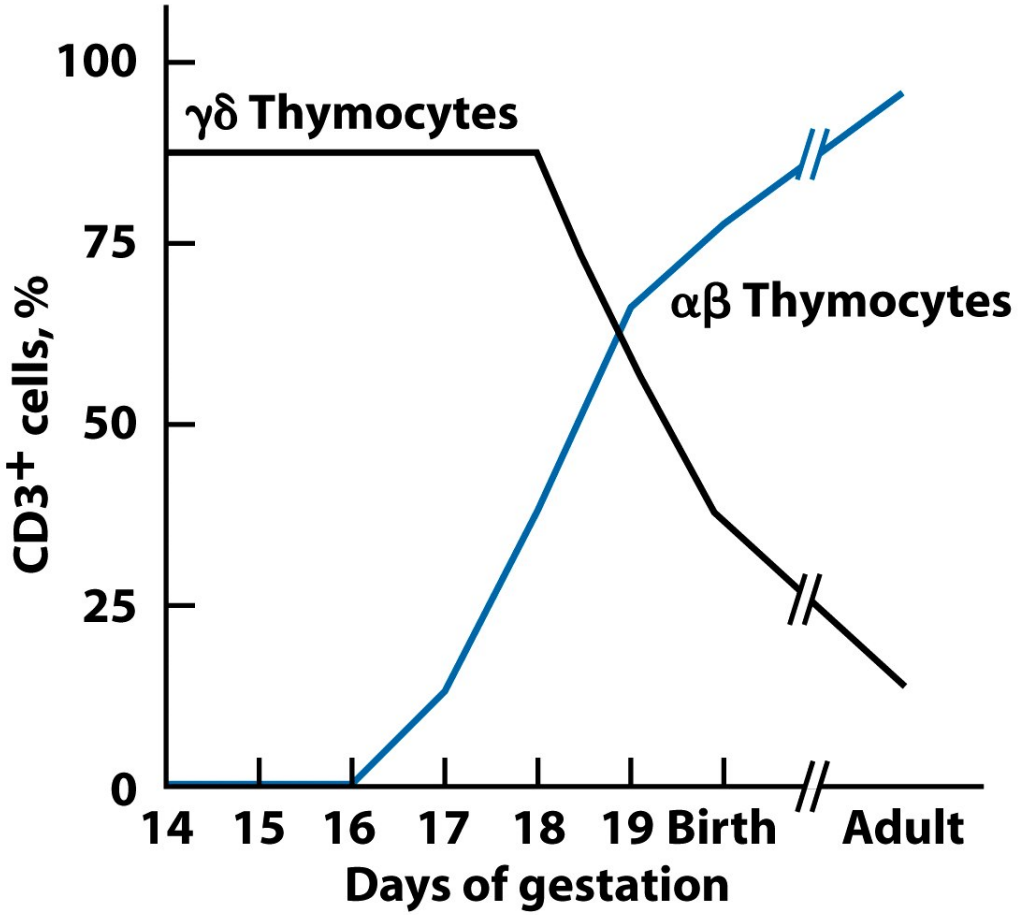

FIGURE 10-3 Time course of appearance of γδ thymocytes and αβ thymocytes during mouse fetal development. The graph shows the percentage of $CD3^+$ cells in the thymus that are double negative ($CD4^-8^-$) and bear the γδ T-cell receptor (black) or are double positive ($CD4^+8^+$) and bear the αβ T-cell receptor (blue).

Pre-TCR is a complex that appears in DN3 (c-Kit–, CD44–, CD25+), consists of a newly synthesized ß chain, a pre-Tα chain and CD3. Formation of pre-TCR activates a variety of processes

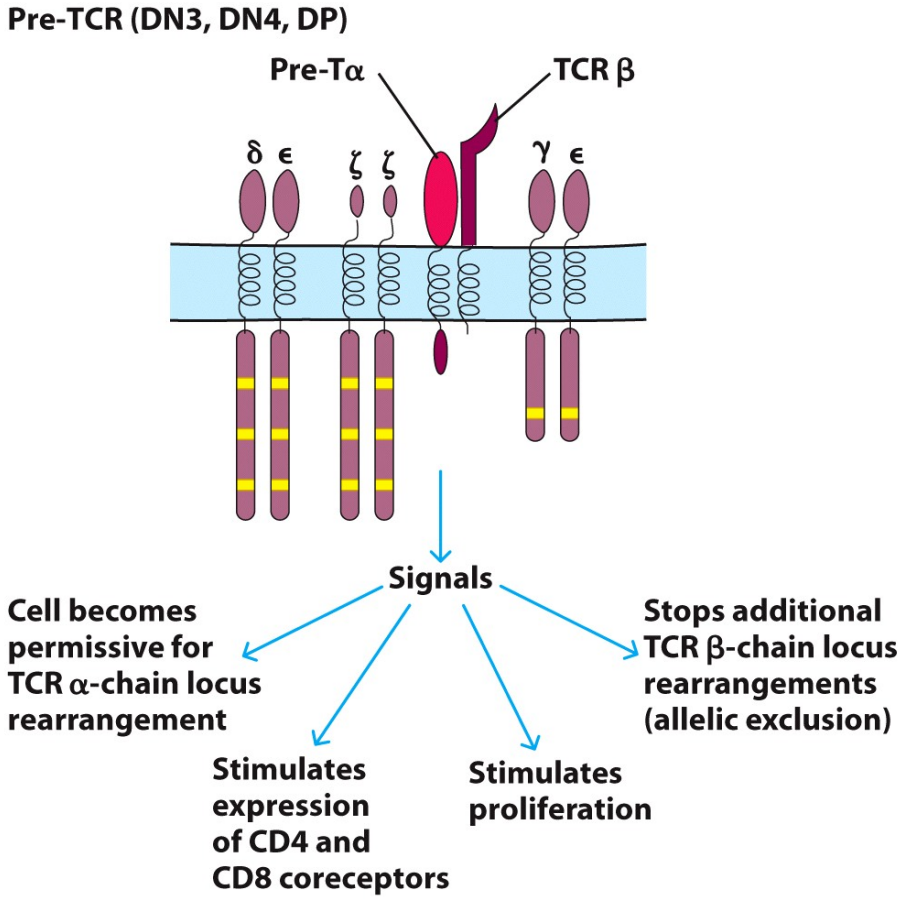

Figure 9-4a
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Notch proteins plays a critical role at this point in T-cell development: cells that do not express Notch do not mature past this stage.

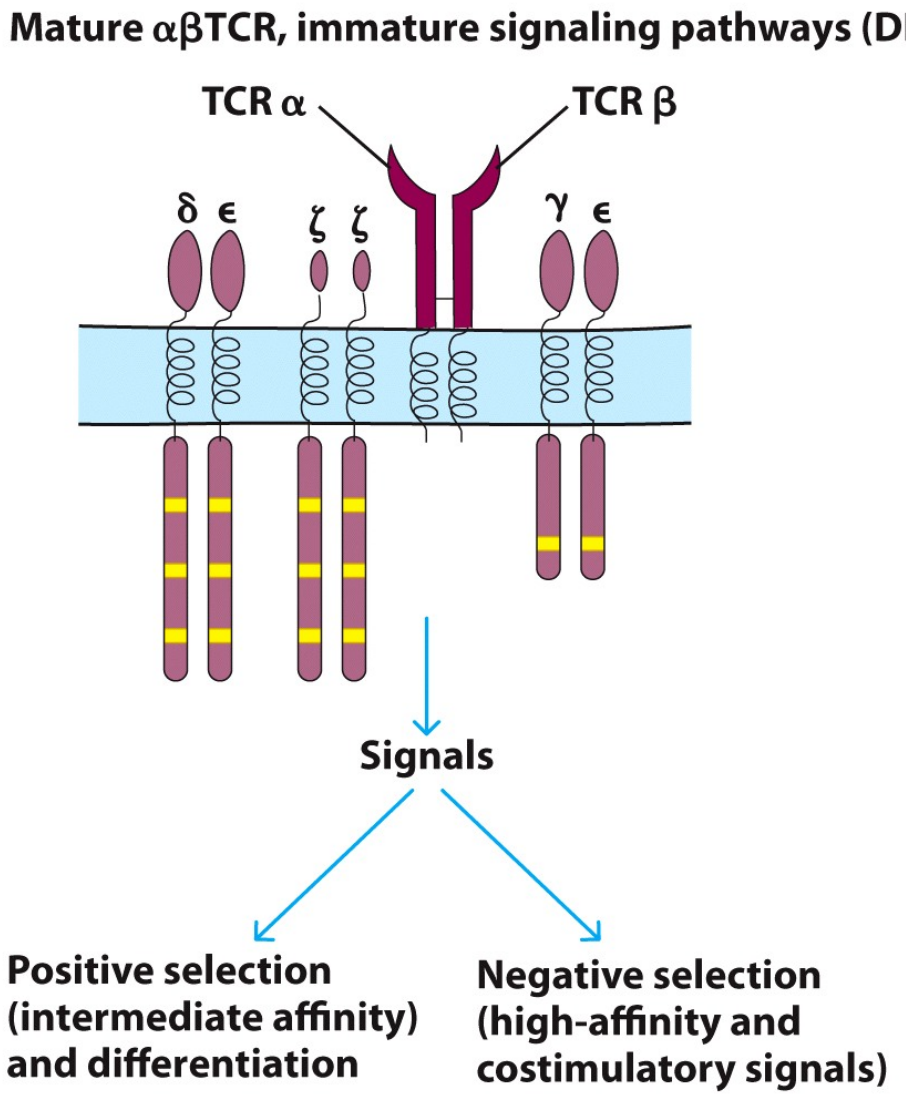

Figure 9-4b
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- Mature αβTCR is expressed at the DP stage.
- Once a DP thymocyte has successfully rearranged a TCRα chain, it will dimerize with TCRβ, replacing the pre-Tα chain.
- This mature αβTCR is now capable of interacting with self MHC, leading to either positive or negative selection.
- TCRα chain has a shorter intracellular region than pre-Tα chain and cannot generate intracellular signals independently.

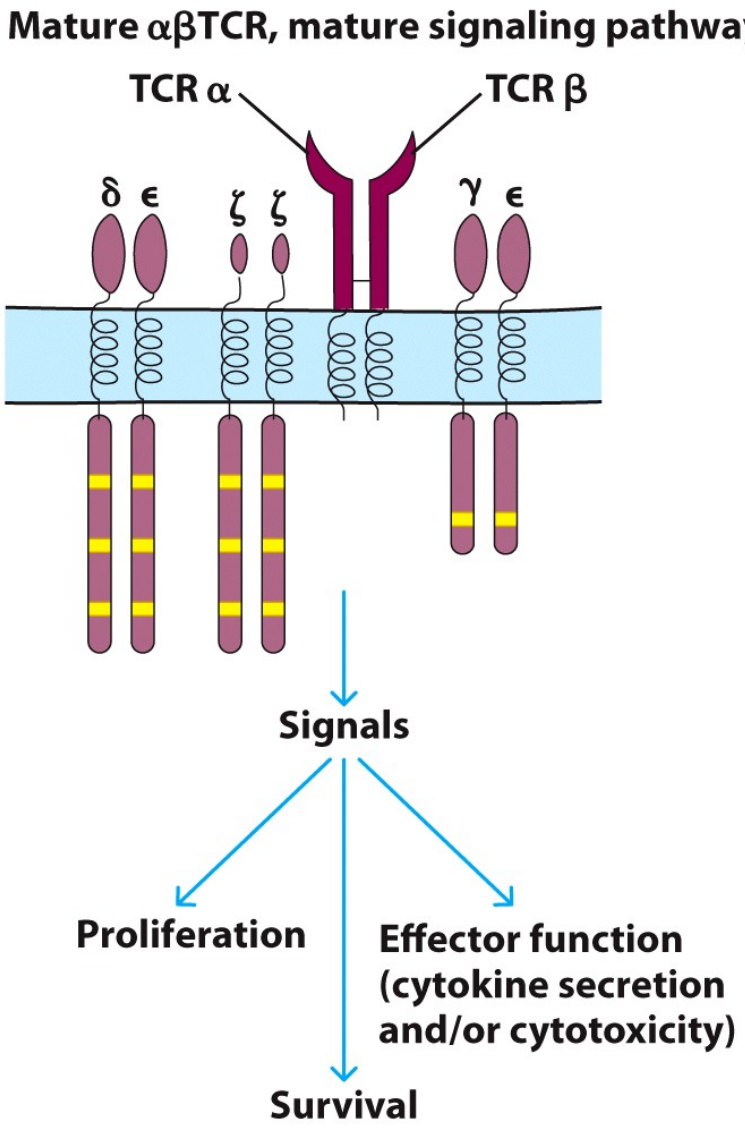

Figure 9-4c
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- After selection, αβTCR/CD3 complex is structurally the same as that expressed by the thymocytes before selection.
- It now responds to high-affinity engagement by initiating proliferation, activation and effector function.

T Cell Selection

In contract to B cell maturation in the bone marrow, T cells mature and differentiate in the thymus, where T cells diversify and develop into an effective primary T cell repertoire by 2 selection processes

Positive selection permits the survival of only those T cells with TCRs capable of recognizing self MHC molecules

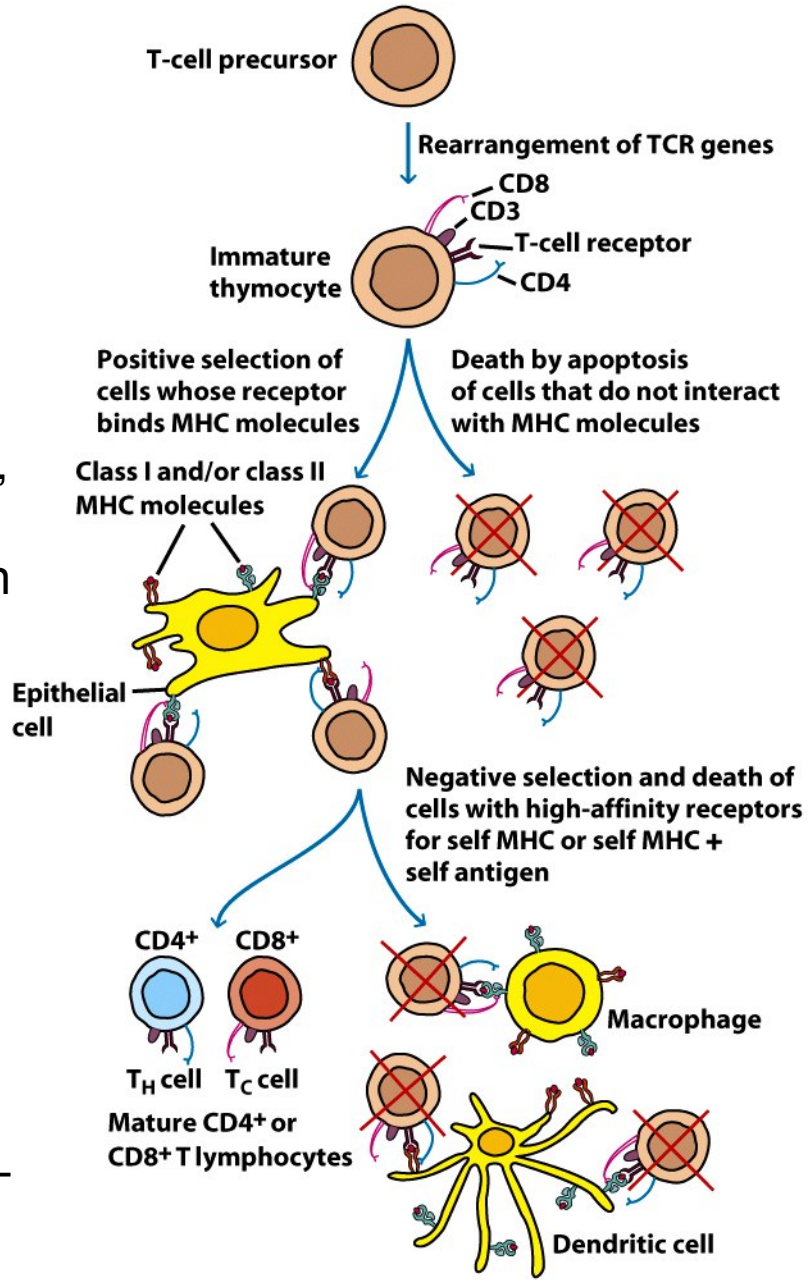
Negative selection eliminates T cells that react too strongly with self MHC or with self MHC plus self peptides

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.

Positive selection for Thymocytes bearing TCRs capable of binding self-MHC molecules, which results in **self-MHC restriction**. Cells that fail positive selection are eliminated within the thymus by apoptosis - death by neglect.

Negative selection that eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC, which results in **self-tolerance**.

Thymic selection involves stromal cells (epithelial cells, dendritic cells, macrophages) and results in mature T cells that are both self-MHC restricted and self-tolerant.



Overview of positive and negative selection of thymocytes in the cortex and medullar of thymus

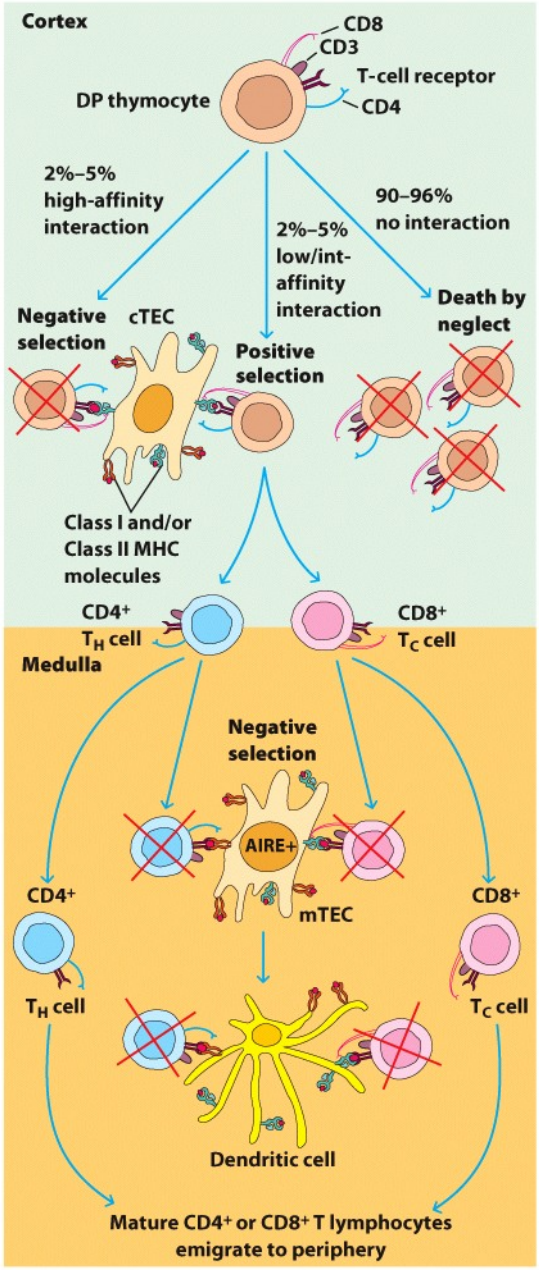


Figure 9-5
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Positive and negative selection of thymocytes in the thymic cortex

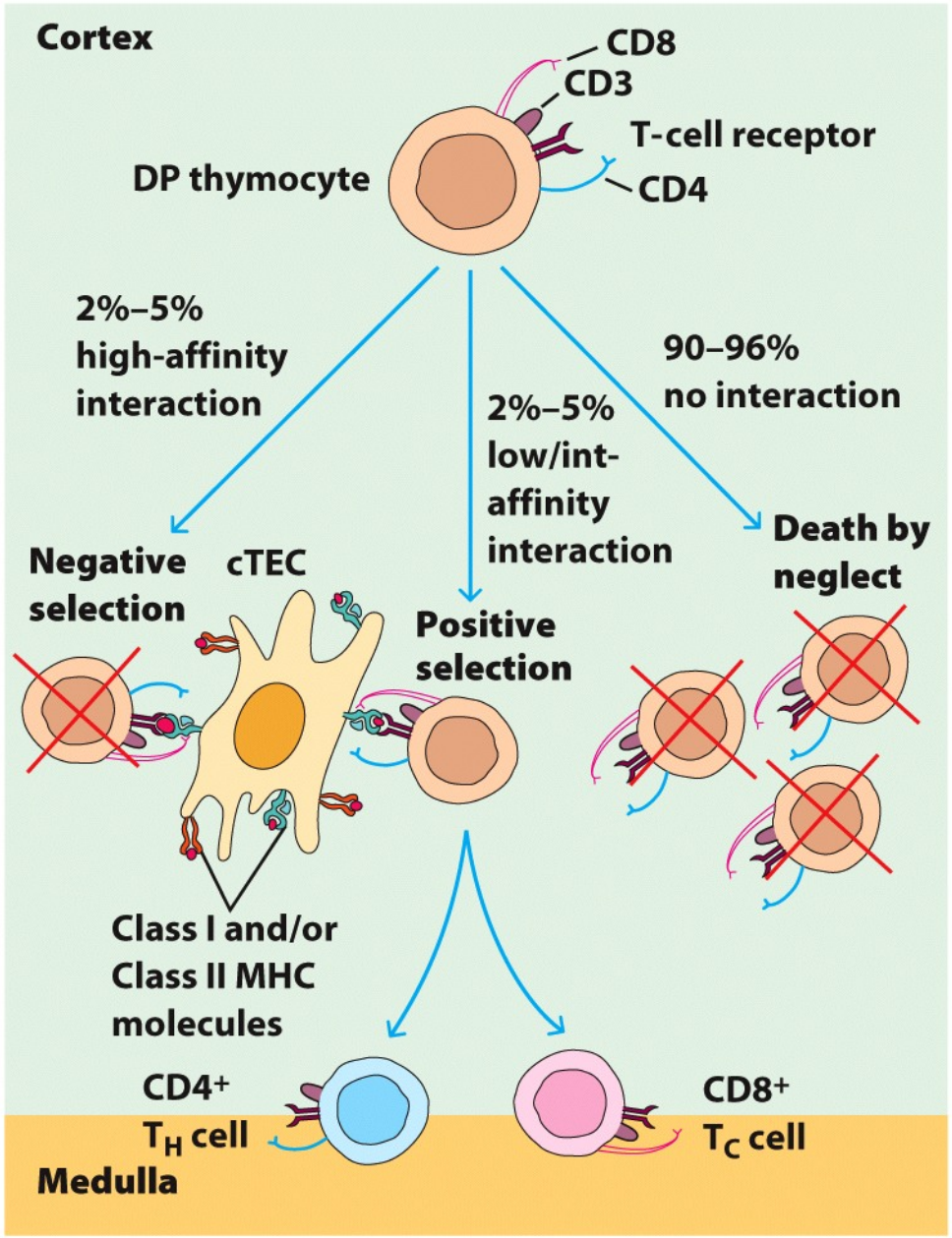

Figure 9-5 part 1
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Negative selection of SP thymocytes in the thymic medulla

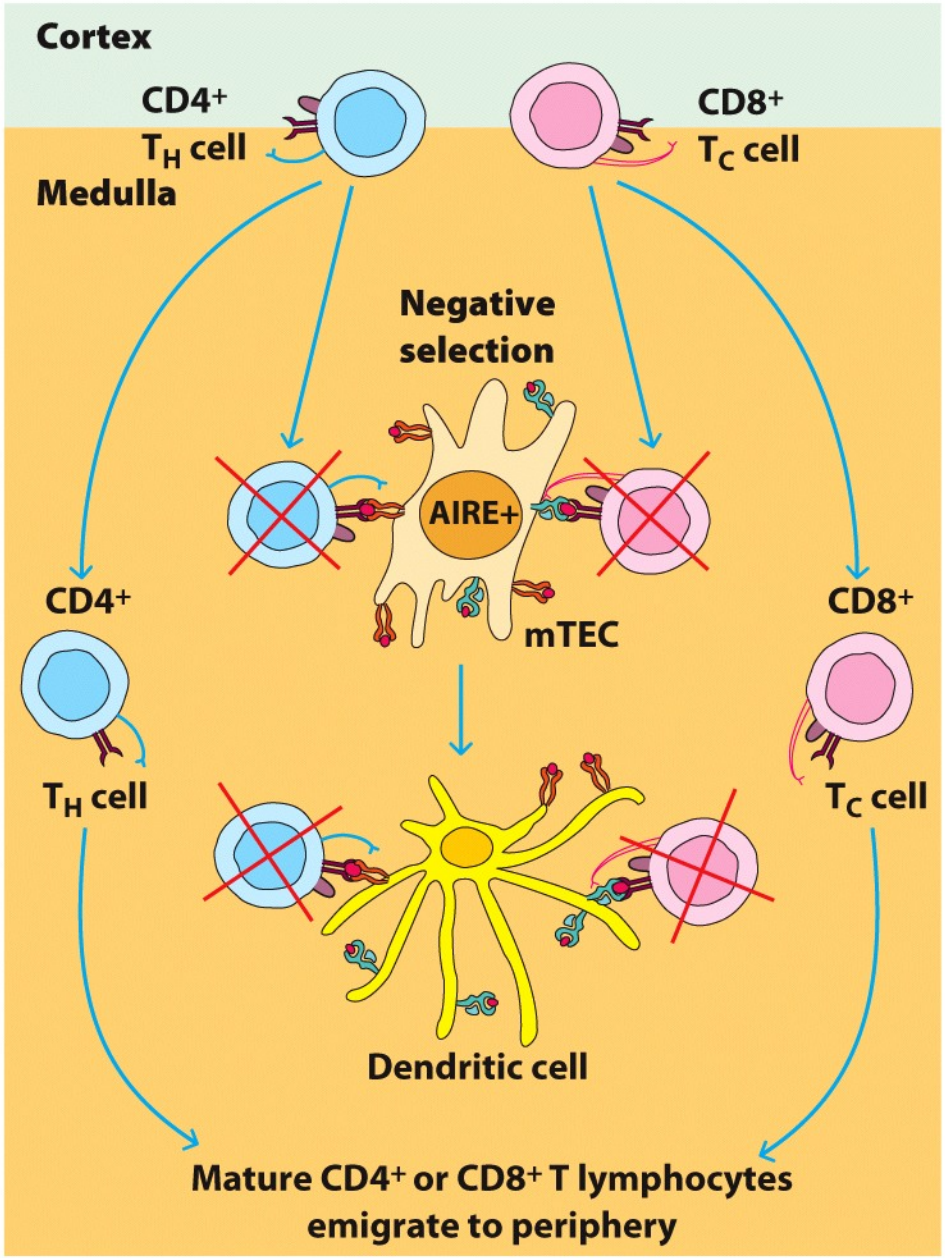

Figure 9-5 part 2
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Positive selection requires MHC I or II molecules

TABLE 10-1 Effect of class I or II MHC deficiency on thymocyte populations*

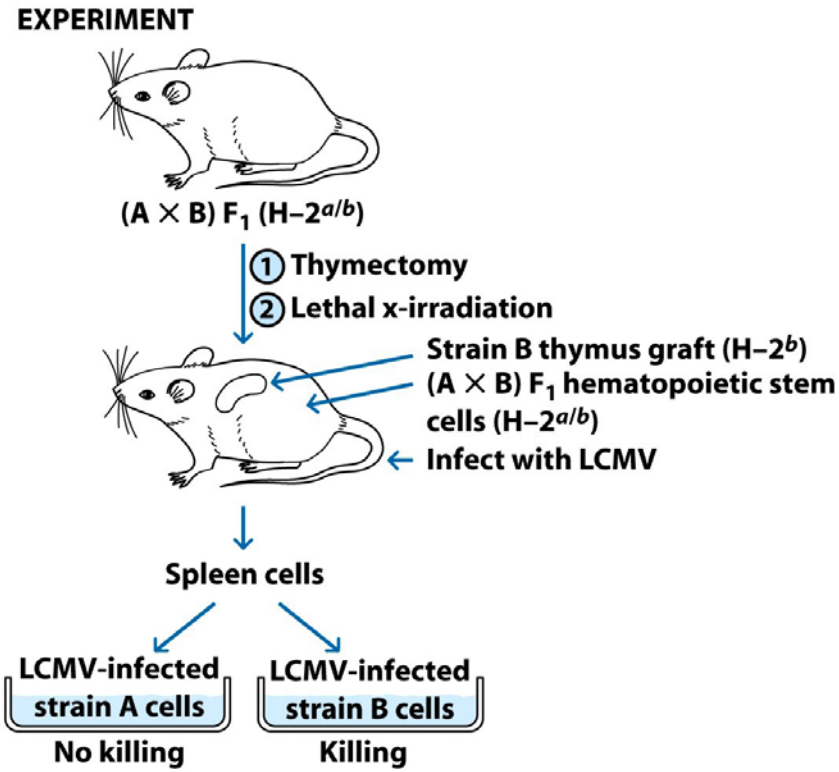
Cell type	Control mice	KNOCKOUT MICE: Class I deficient	KNOCKOUT MICE: Class II deficient
$CD4^-CD8^-$	+	+	+
$CD4^+CD8^+$	+	+	+
$CD4^+$	+	+	–
$CD8^+$	+	–	+

*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.

MHC I-deficient mice have a normal distribution of double-negative, double-positive, and CD4+ thymocytes but failed to produce CD8+ thymocytes; MHC Class II-deficient mice had double-negative, double-positive, and CD8+ thymocytes but lacked CD4+ thymocytes.

The absence of class I or II MHC molecules prevents positive selection of CD8+ or CD4+ T cells, respectively.

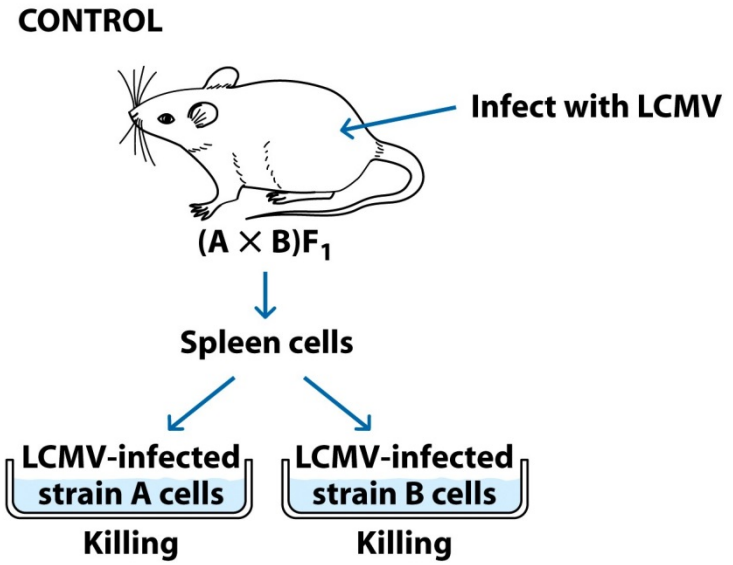
Chimera experiment to show that thymus selects only those T cells with TCRs recognizing antigens presented on the same haplotype of the thymus



Only strain B target cells were killed, suggesting that the H-2b grafted thymus had selected for maturation only those T cells that could recognize antigen combined with H-2b MHC molecules

To be certain that the thymus graft did not contain any mature T cells, it was irradiated before being transplanted

T-cell progenitors from the (A X B) F1 bone marrow transplant mature within a thymus that expresses only B-haplotype MHC molecules on its stromal cells



Transgenic system to show that that TCR interaction with MHC molecules is required for **positive selection**.

Rearranged αß-TCR transgenes derived from a CD8+ T-cell clone specific for influenza antigen plus H-2k MHC I molecules were injected into fertilized eggs from two different mouse strains, H-2k and H-2d.

Thymocytes expressing the TCR transgenes mature into CD8+ T cells only in H-2k mice, but not in H-2d mice, because the transgenic TCRs react with only the H-2k MHC I, but not the H-2d MHC I molecules.

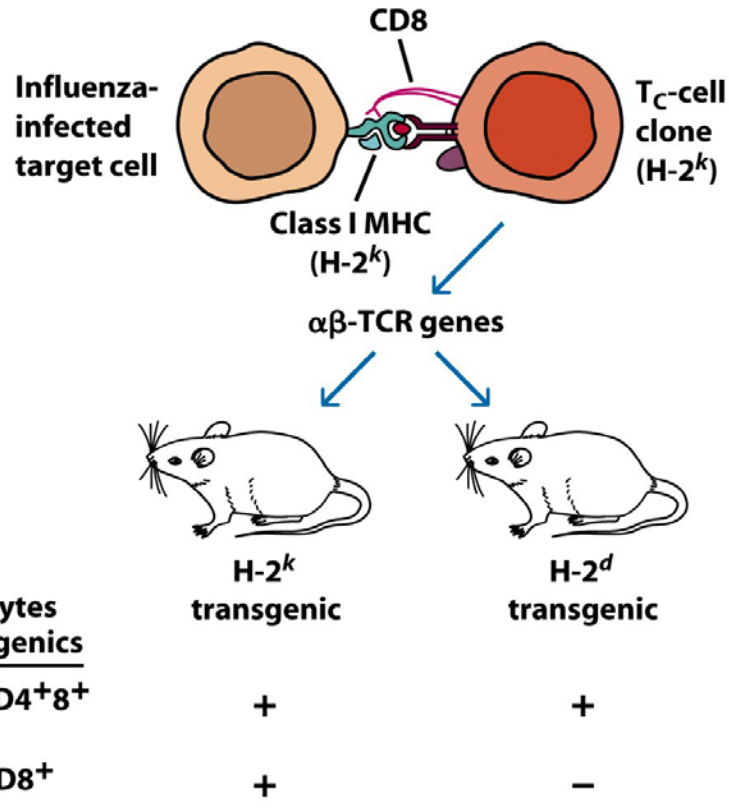
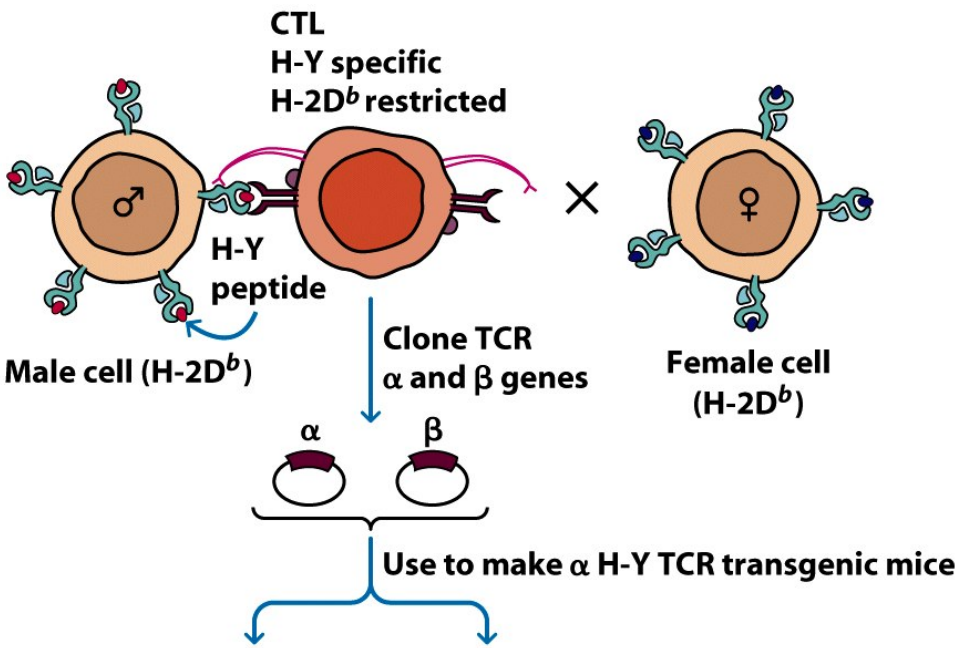


FIGURE 10-7 Effect of host haplotype on T-cell maturation in mice carrying transgenes encoding an $H\text{-}2^b$ class I–restricted T-cell receptor specific for influenza virus. The presence of the rearranged TCR transgenes suppressed other gene rearrangements in the transgenics; therefore, most of the thymocytes in the transgenics expressed the αβ T-cell receptor encoded by the transgene. Immature double-positive thymocytes matured into $CD8^+$ T cells only in transgenics with the haplotype ($H\text{-}2^k$) corresponding to the MHC restriction of the TCR transgene.

Negative selection requires both self antigen and self MHC



	 Male H-2D^b	 Female H-2D^b
H-Y expression	+	–
Thymocytes		
CD4$^-$8$^-$	++	+
CD4$^+$8$^+$	+	++
CD4$^+$	+	+
CD8$^+$	–	++

Thymocyte maturation was analyzed in transgenic mice bearing an αß-TCR transgene specific for the MHC I H-2Db plus H-Y antigen (a small protein encoded on the Y chromosome and is therefore a self molecule only in male mice).

The MHC haplotype of the transgenic mice was H-2Db, the same as the MHC restriction of the transgene-encoded receptor.

Mature CD8+ T cells expressing the transgene were absent in the male mice but present in the female mice, suggesting that thymocytes reactive with the self H-Y antigen are deleted during thymic selection.

Relationship between TCR affinity and selection

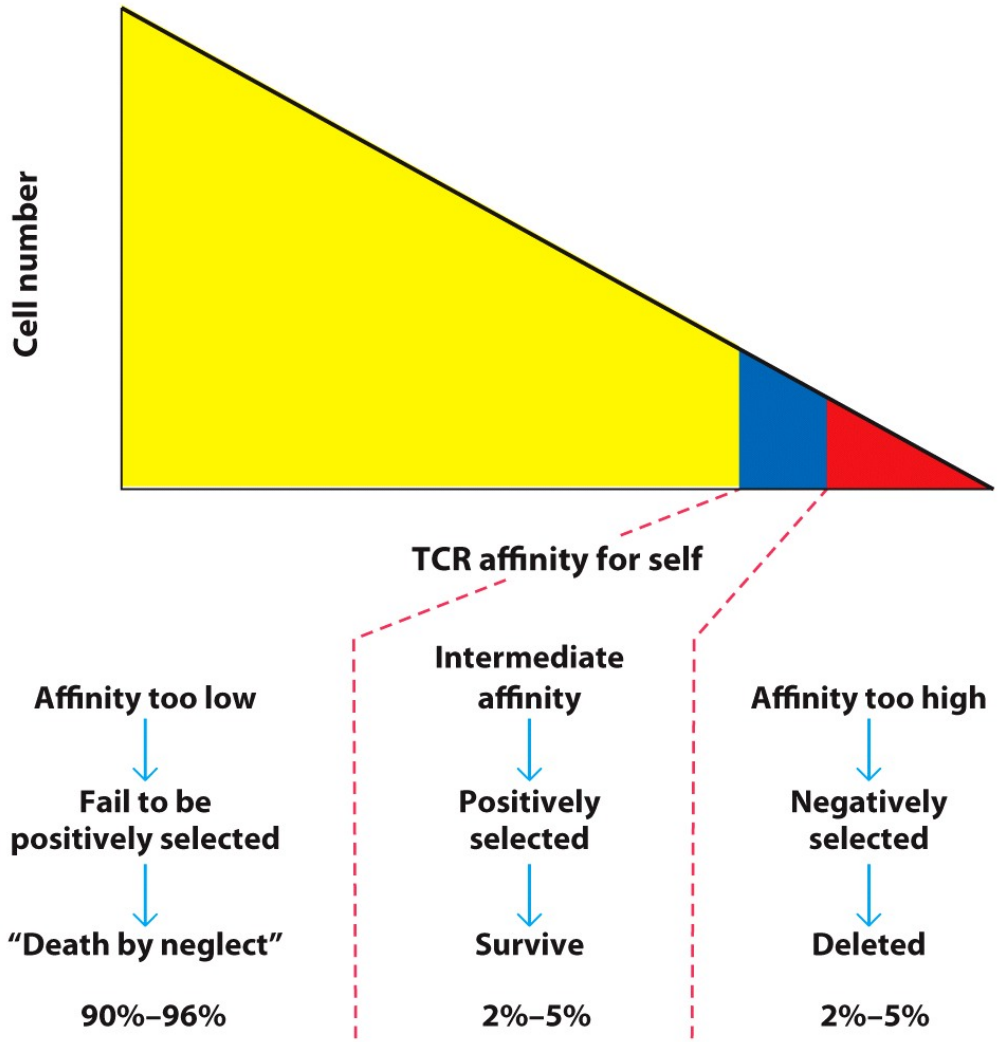


Figure 9-7
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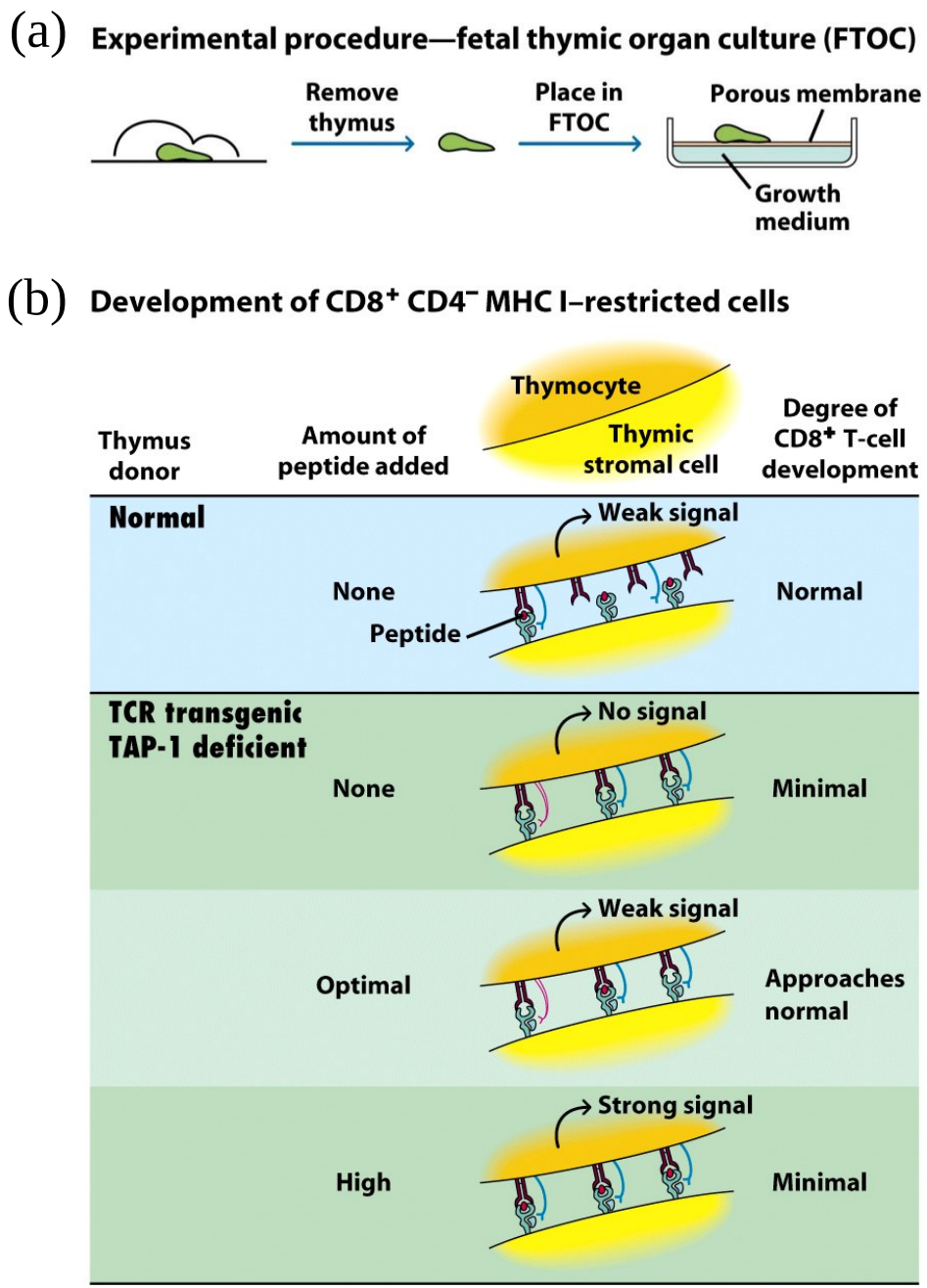

FIGURE 10-9 Role of peptides in selection. Thymuses harvested before their thymocyte populations have undergone positive and negative selection allow study of the development and selection of single positive ($CD4^+CD8^-$ and $CD4^-CD8^+$) T cells. (a) Outline of the experimental procedure for in vitro fetal thymic organ culture (FTOC). (b) The development and selection of $CD8^+CD4^-$ class I–restricted T cells depends on TCR peptide–MHC I interactions. TAP-1 knockout mice are unable to form peptide-MHC complexes unless peptide is added. The mice used in this study were transgenic for the α and β chains of a TCR that recognizes the added peptide bound to MHC I molecules of the TAP-1 knockout/TCR transgenic mice. Varying the amount of added peptide revealed that low concentrations of peptide, producing low avidity of binding, resulted in positive selection and nearly normal levels of $CD4^-CD8^+$ cells. High concentrations of peptide, producing high avidity of binding to the TCR, caused negative selection, and few $CD4^-CD8^+$ T cells appeared. *[Adapted from P. G. Ashton Rickardt et al., 1994,* Cell ***25:651.****]*

TAP1 (Transporter associated with antigen processing) transports antigen peptides into the lumen of RER, where they bind to MHC I molecules

Proposed models for CD4+ and CD8+ T cell lineage commitment

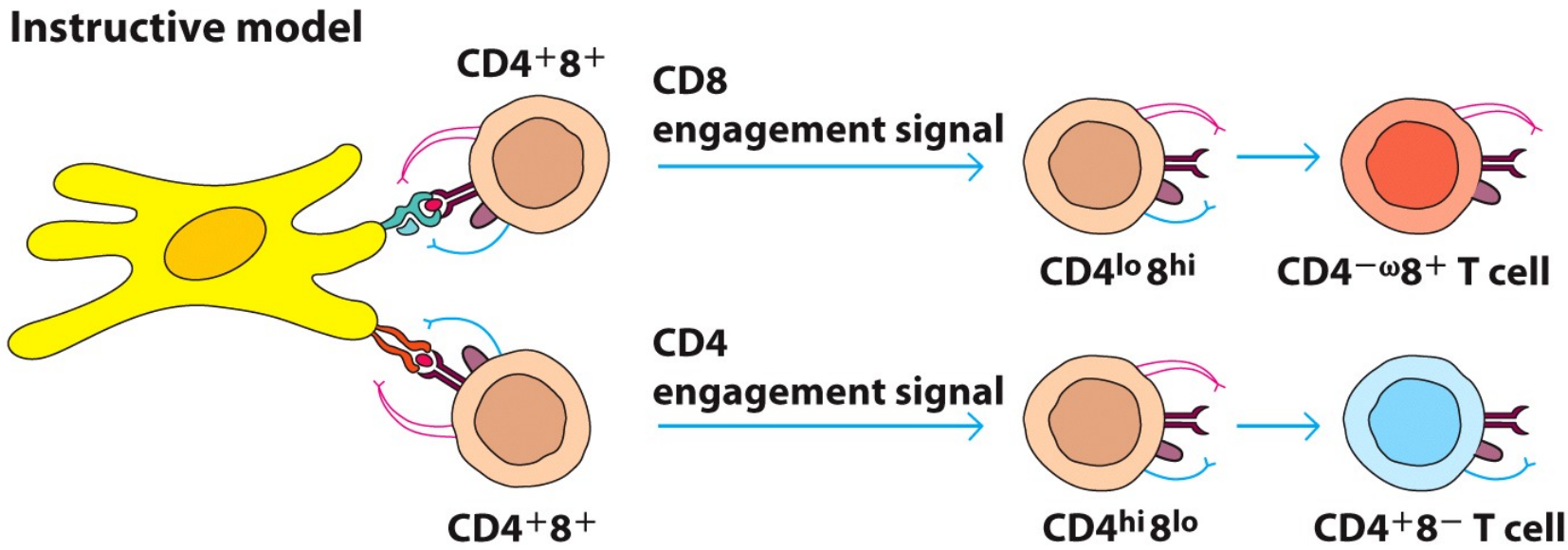

Figure 9-9a
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Instructive model – Interaction of one co-receptor with MHC on stromal cells leads to down-regulation of the other co-receptor

Proposed models for CD4+ and CD8+ T cell lineage commitment

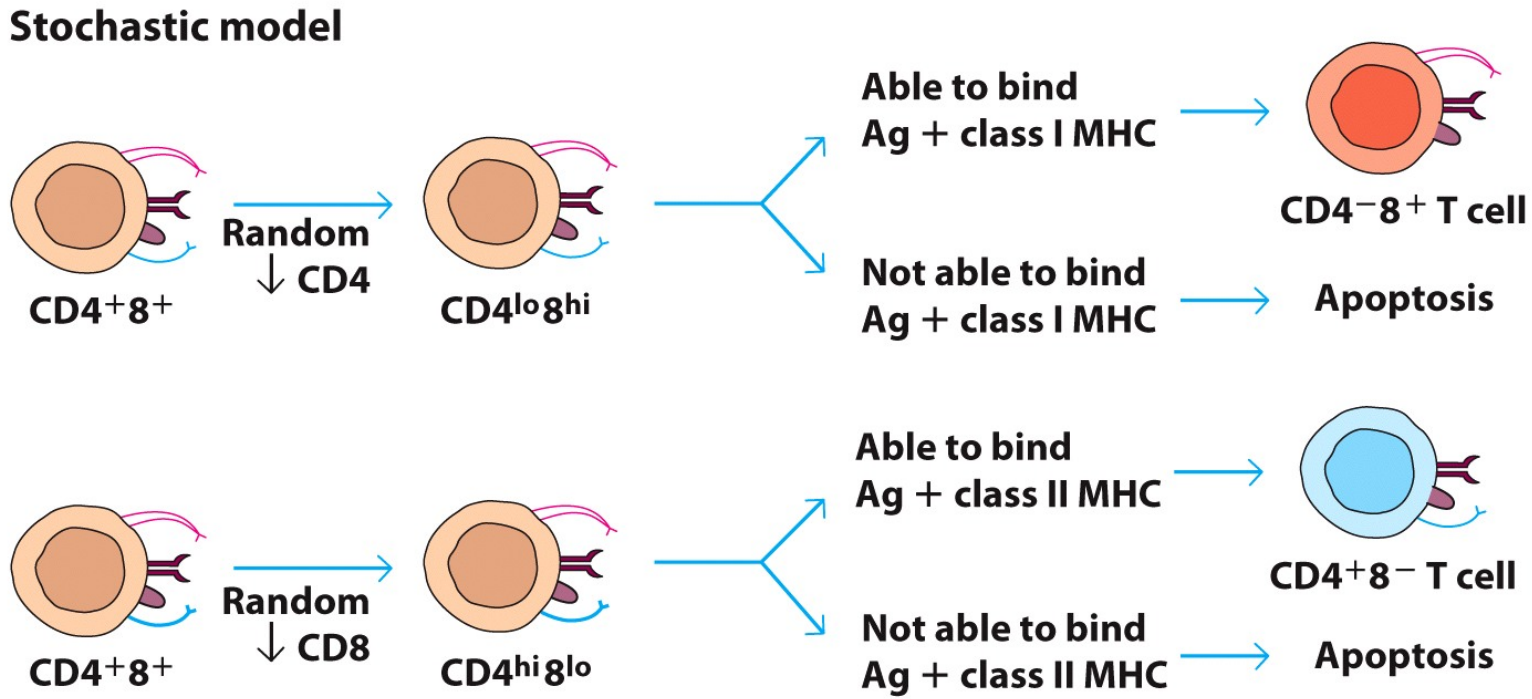

Figure 9-9b
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Stochastic model: Down-regulation of CD4 or CD8 is a random process

Proposed models for CD4+ and CD8+ T cell lineage commitment

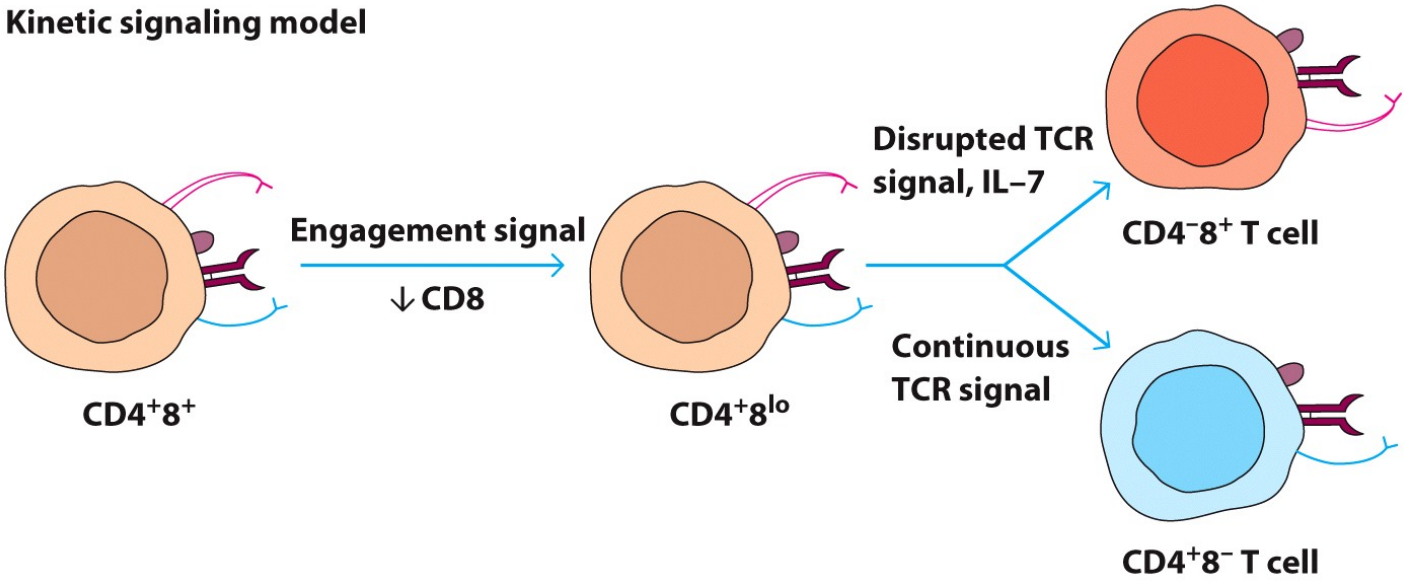

Figure 9-9c
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- The decision to commit is based on the continuity of TCR signal that a thymocyte receives.
- Positive selection results in down-regulation of CD8 on all thymocytes. This will not alter the intensity of a TCR/CD4/MHC II signal, which allows for continuing development to the CD4 SP lineage.
- Down-regulation of CD8 diminishes a TCR/CD8/MHC I signal, which sends a cell toward the CD8 SP lineage. IL7 is required to seal the CD8 commitment.

Regulatory T cells maintain peripheral tolerance to self antigens

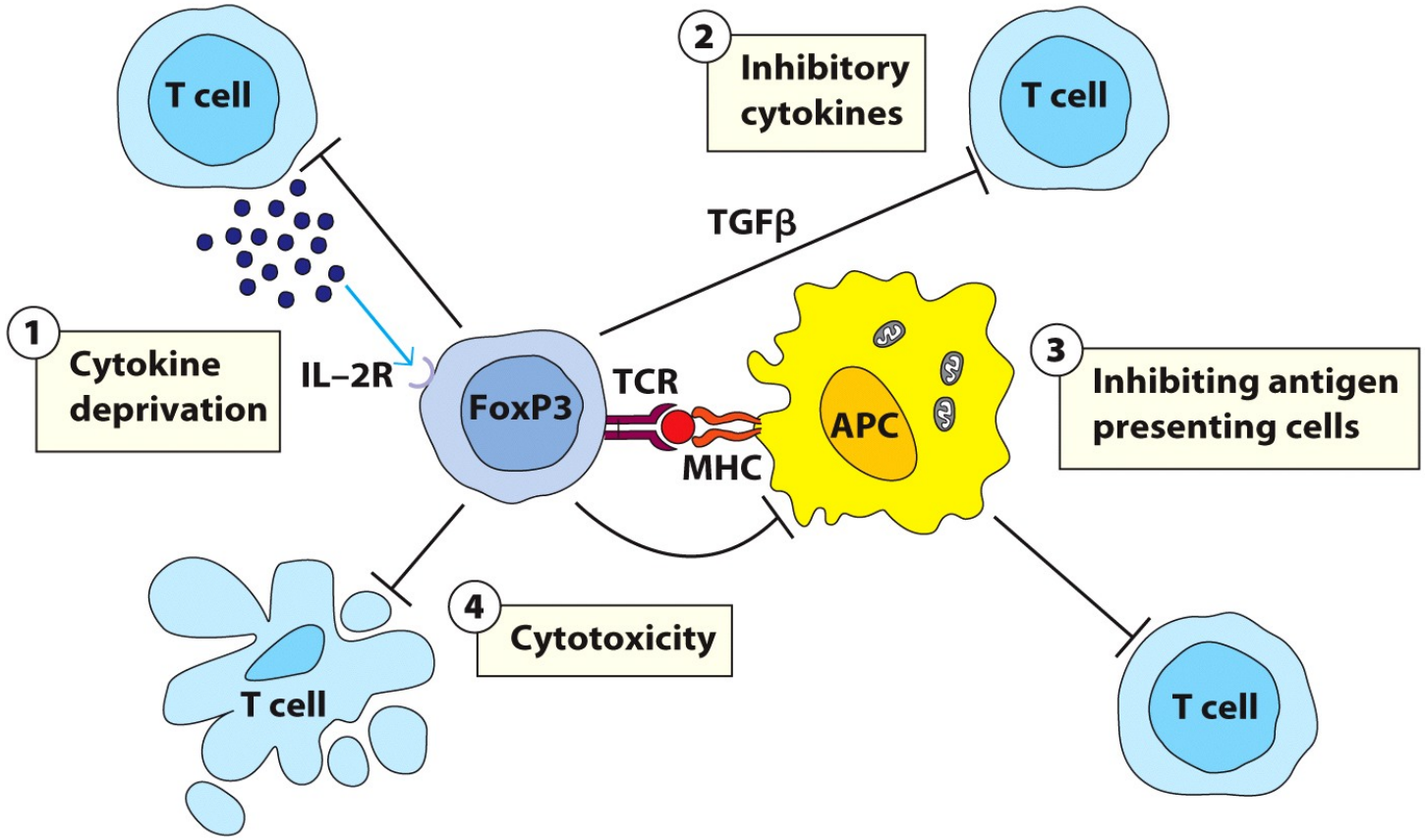

Figure 9-10
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Apoptosis allows cells to die without triggering an inflammatory response

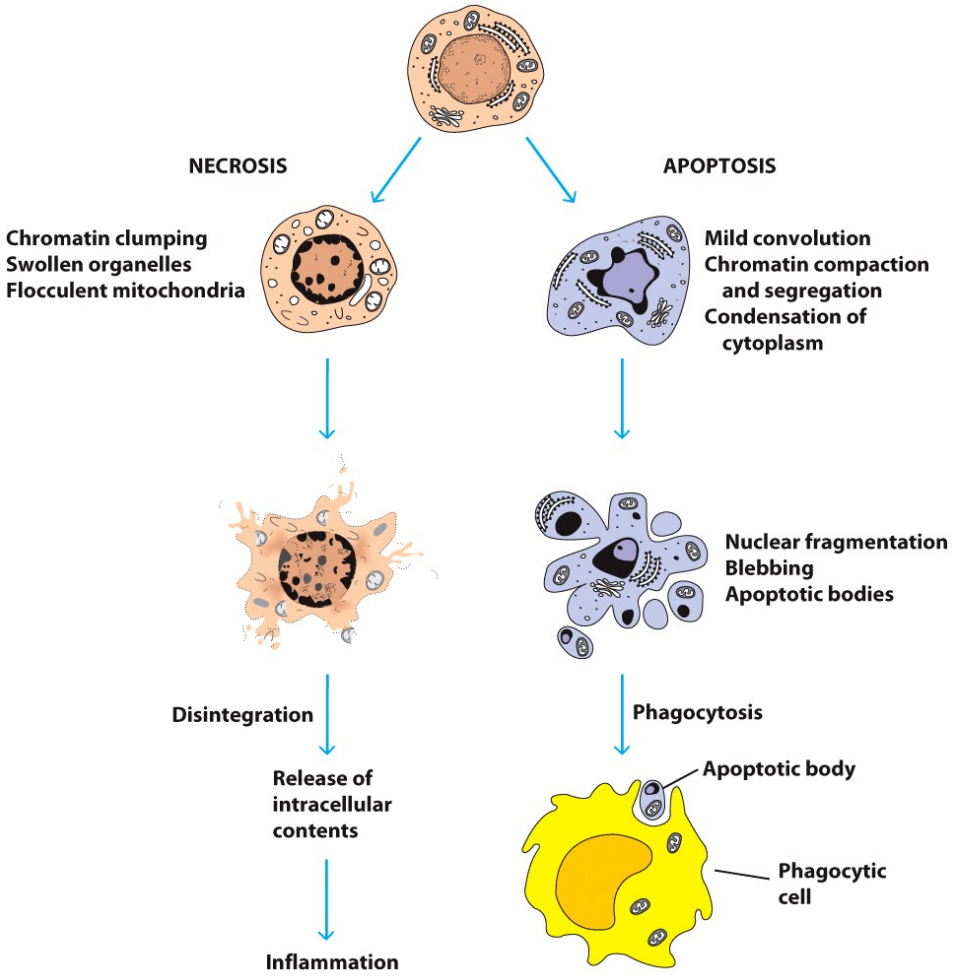

Figure 9-11a
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- Positive and negative selection of lymphocytes
- Immune cell homeostasis
- Immune cell-mediated elimination of pathogen-infected cells or transformed cancer cells

Morphological changes during apoptosis

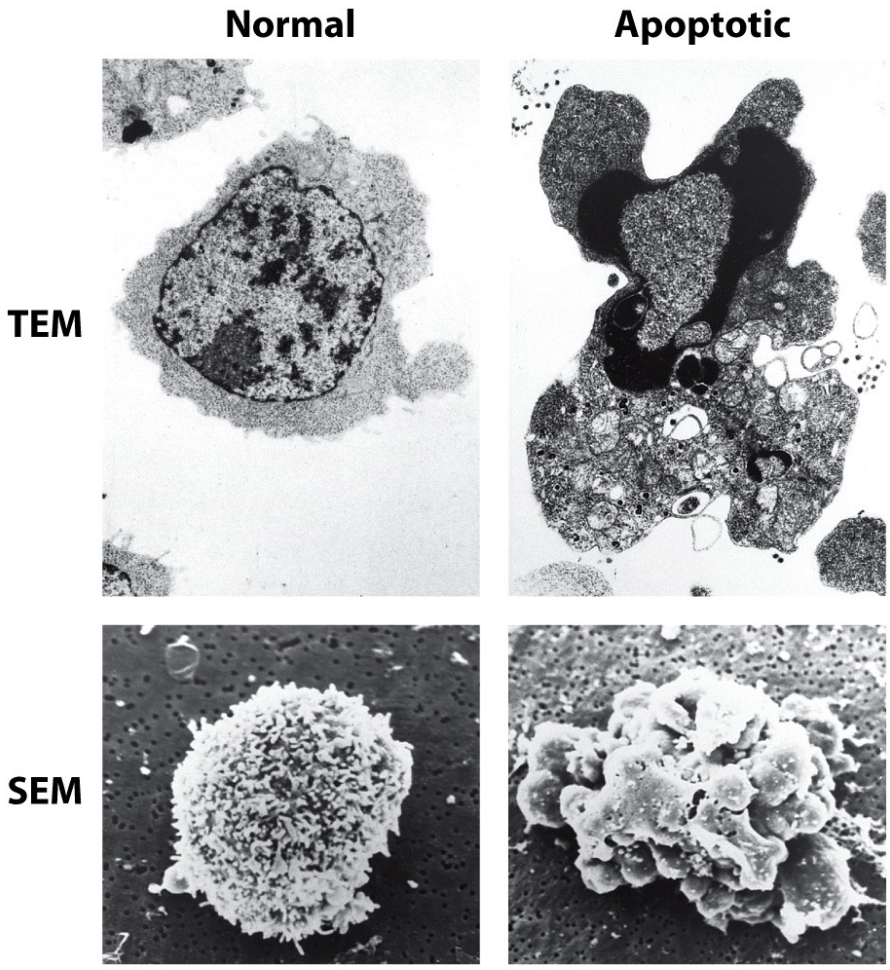

Figure 9-11b
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TABLE 9-2 Proteins involved in apoptosis

Protein	Location in cell	Function	Role in apoptosis
Death receptors (e.g., Fas, TNFR)	Membrane	Activates caspase cascade after binding ligand (e.g., FasL, TNF)	Promotes
Initiator caspase (e.g., caspase-8, caspase-9)	Cytosol	Protease; cleaves and activates effector caspases	Promotes
Effector caspase (e.g., caspase-3)	Cytosol, nucleus	Protease; cleaves and activates enzymes, cleaves and disassembles structural proteins	Promotes
Cytochrome *c*	Intermembrane space, mitochondria	Participates in formation of apoptosome	Promotes
Apaf-1	Cytosol	Participates in formation of apoptosome	Promotes
Anti-apoptotic Bcl-2 family members (e.g., Bcl-2, Bcl-x_L)	Mitochondria, ER	Regulates cytochrome *c* release	Inhibits
Pro-apoptotic Bcl-2 family members (e.g., Bax, Bak)	Mitochondria	Regulates cytochrome *c* release; opposes Bcl-2, Bcl-x_L	Promotes
BH3 proteins (e.g., Bim, Bid, PUMA)	Cytosol and mitochondria	Opposes activity of anti-apoptotic Bcl-2 family members at mitochondria	Promotes

Table 9-2
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Two pathways to apoptosis

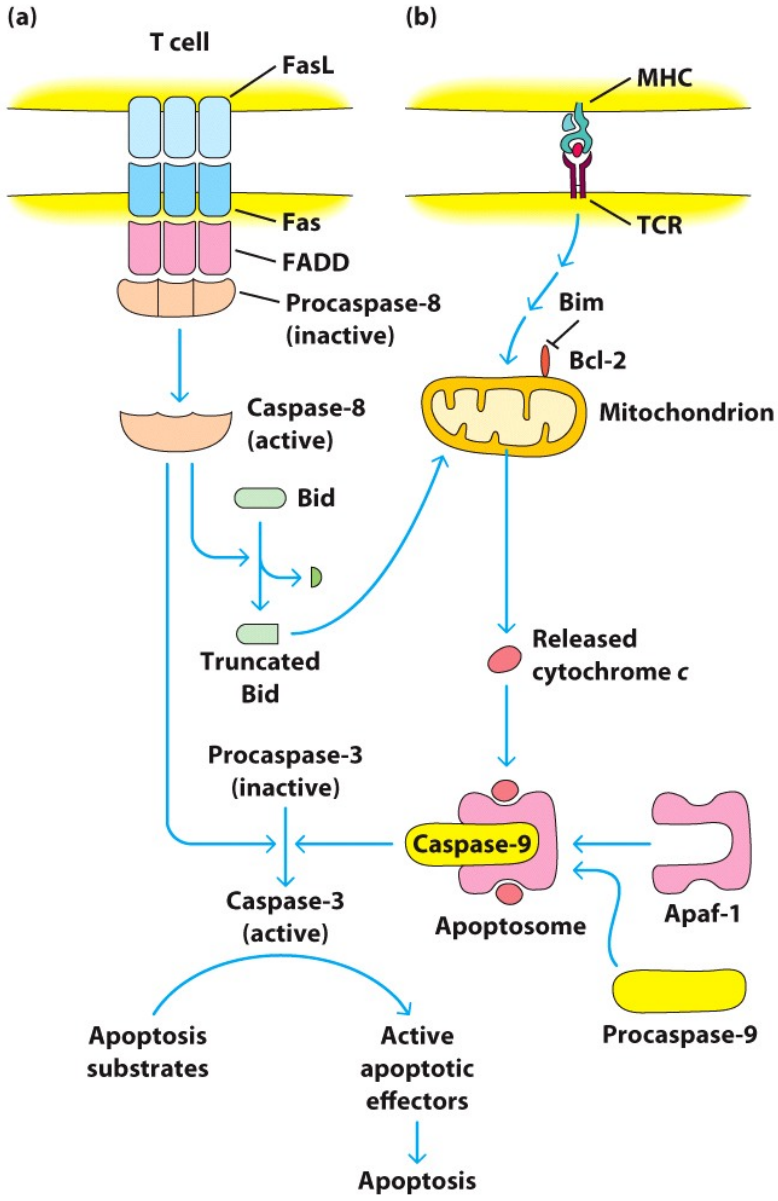

Figure 9-12
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Alloreactivity of T cells

Allogeneic is a term used to describe genetically different individuals of the same species.

MHC molecules were initially identified because of their role in rejection of foreign tissues. MHC are highly polymorphic, most individuals in the same species have unique sets of MHC molecules (histocompatibility antigens).

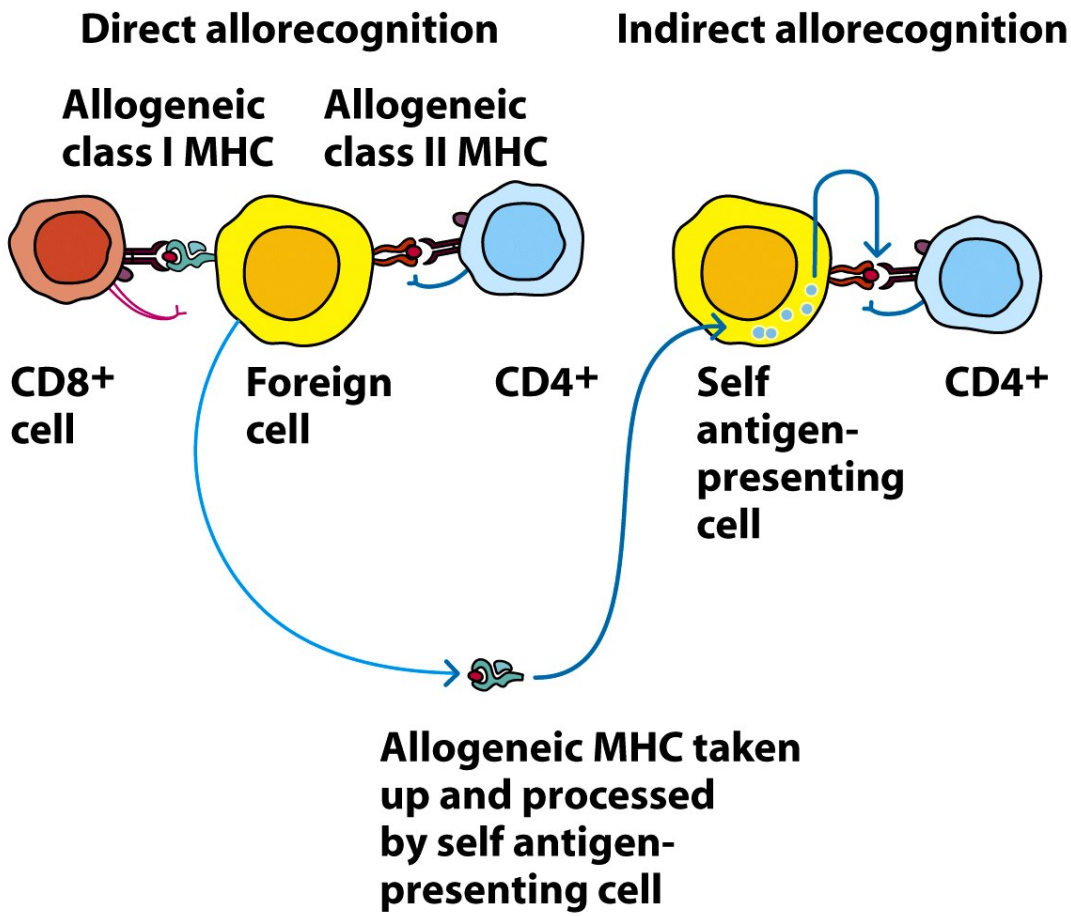
Graft rejection and graft versus host diseases are the clinical manifestations of humoral and cellular immune responses to allogeneic tissues.

T cells are essential for allogeneic immune responses; suppression of T cell function is the main approach used to control alloreactivity in transplant patients.

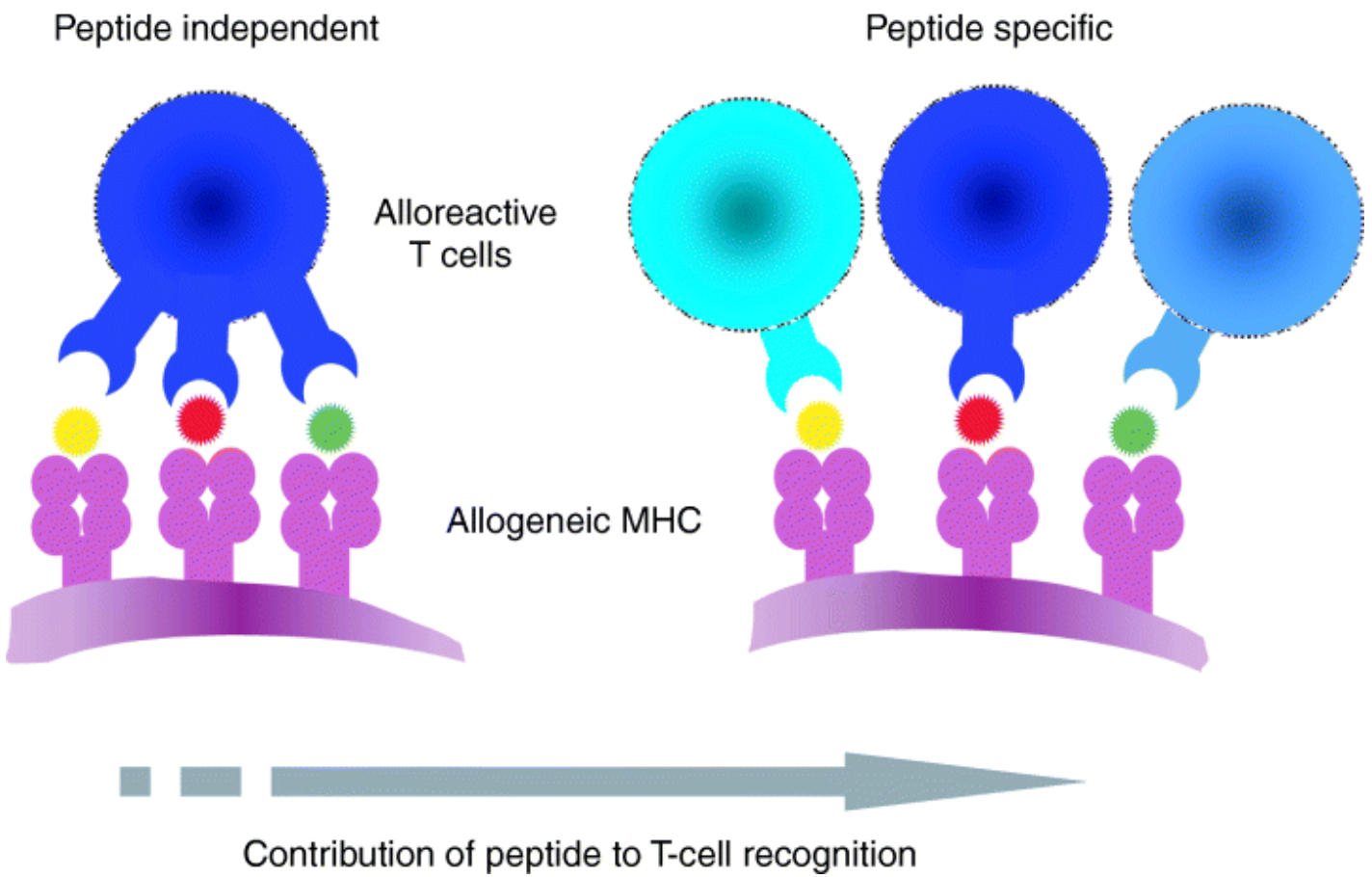
Mechanisms of T cell alloreactivity

Direct: T cells recognize allogeneic MHC I and II molecules on foreign cells.

Indirect: T cells recognize peptides derived from allogeneic MHC molecules processed and presented by self-MHC molecules.

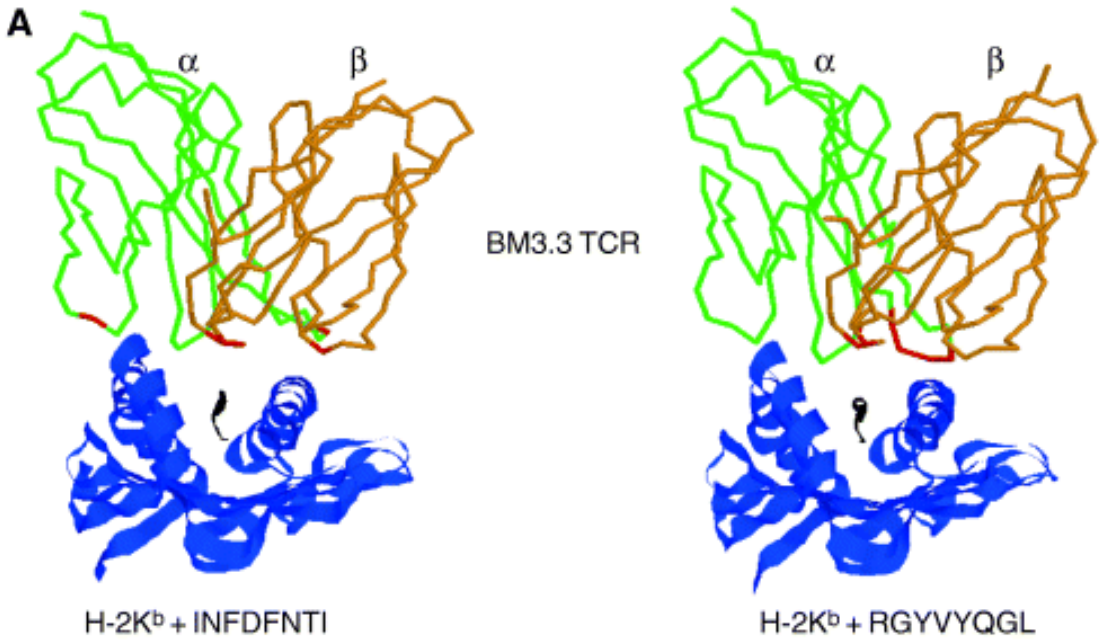


Direct alloreactivity could be antigen dependent or independent



Antigen-processing-deficient cells whose MHC molecules express a very limited range of peptides are not recognized by the majority of alloreactive T cells. Adding peptides can restore allorecognition, and many alloreactive T-cell clones exhibit selective peptide recognition, indicating that they are peptide specific.

Crossreactivity as the basis for allorecognition: T cells are selected to react to antigens presented by self or auto MHC; can also react to allogeneic MHC (plus antigens) with a similar structure

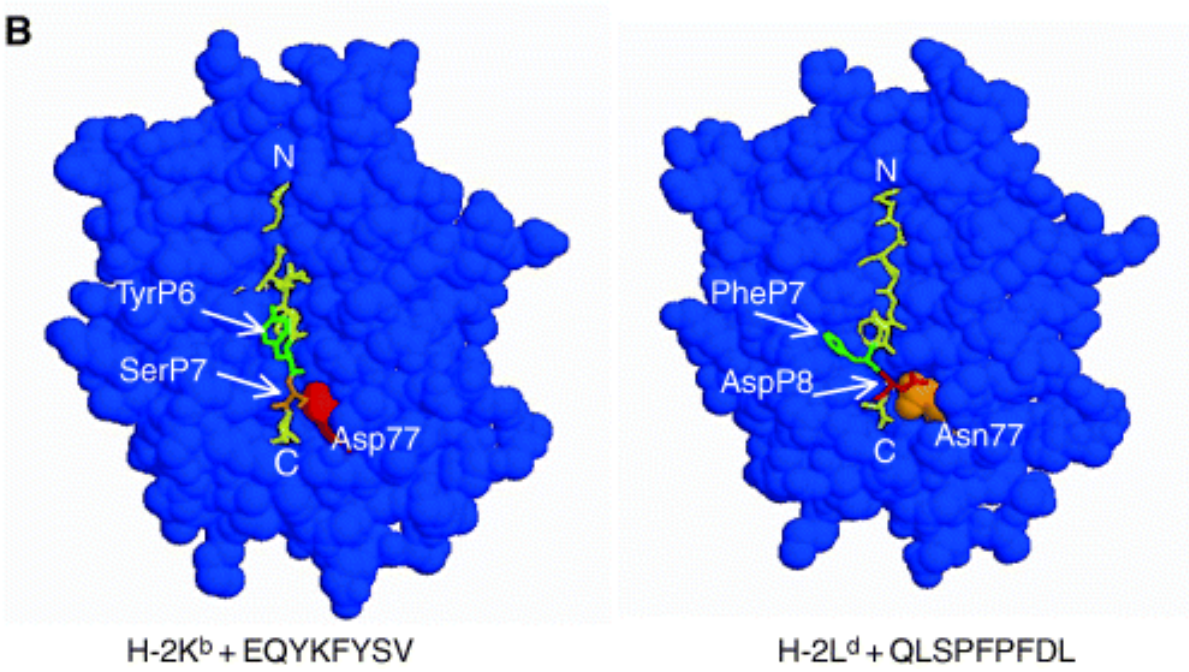


Example of the structural basis for TCR **crossreactivity**.

Flexibility of the TCR facilitates recognition of two different peptides presented by allogeneic H-2Kb. TCR residues positioned within 5Å of the allogeneic MHC molecule are shown in red to illustrate that there are differences in the residues that come in close proximity to each complex of H-2Kb and bound peptide.

Molecular mimicry between two different complexes of MHC-peptide can also contributes to the crossreactivity of the same TCR.

A shared region of negative charge shown in red is contributed by Asp 77 of H-2Kb and by position 8 of the QLSPFPFDL peptide bound by H-2Ld. Both bound peptides possess a large aromatic hydrophobic side chain shown in green at equivalent positions.



H-2K^{b} + EQYKFYSV

H-2L^{d} + QLSPFPFDL

Three-dimensional structure of TCR-peptide-MHC complex

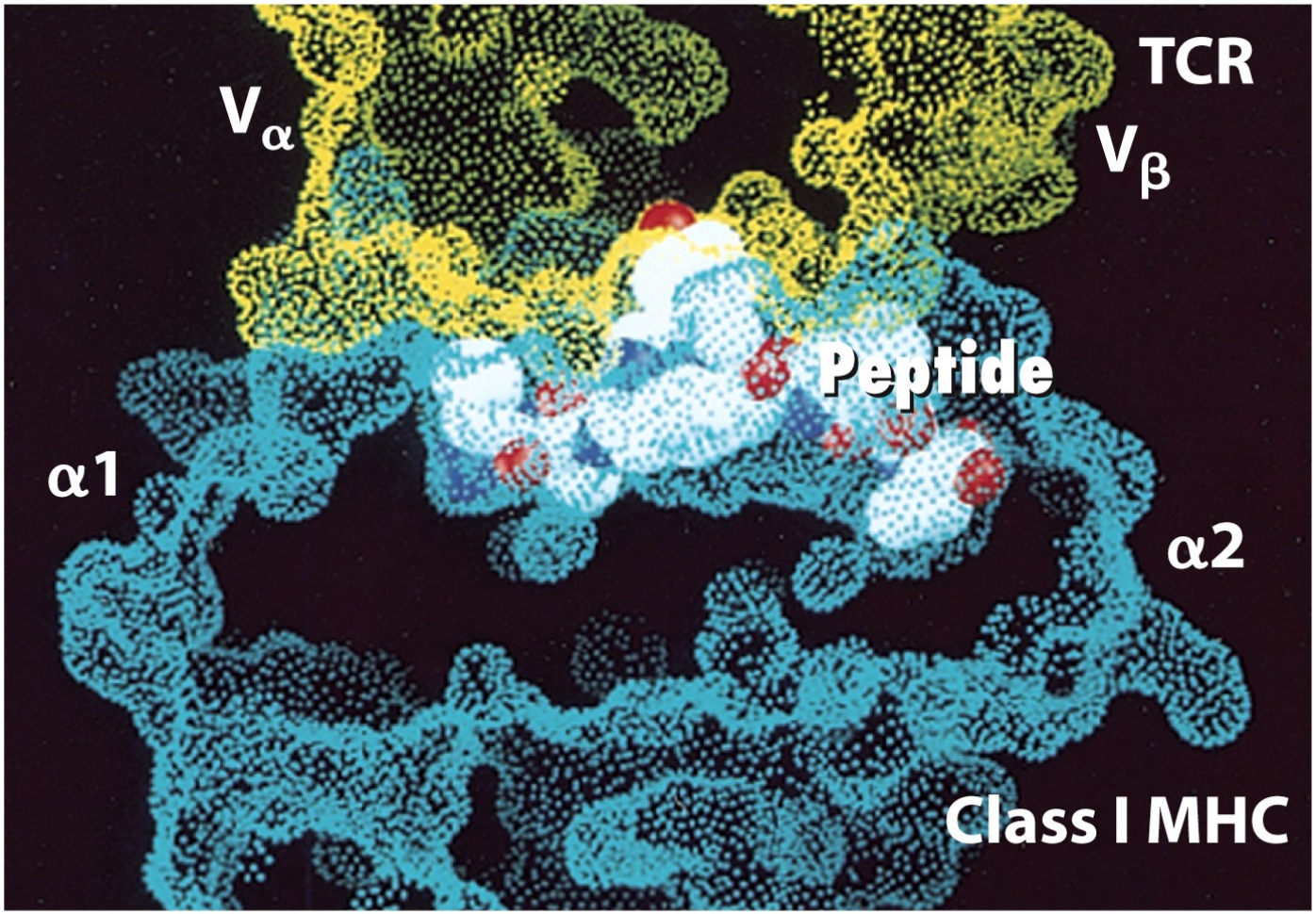


Figure 9-14a
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