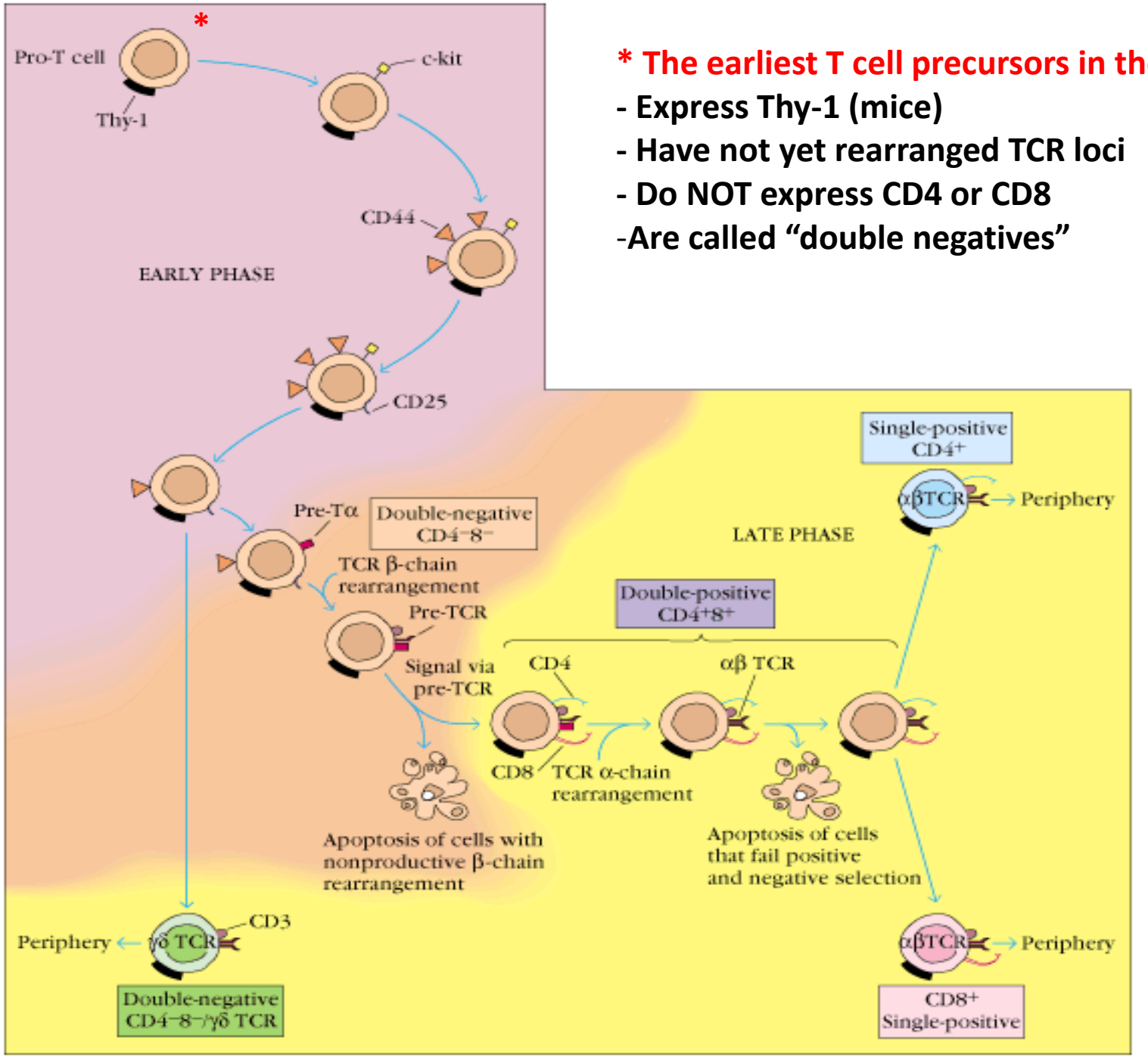
T cell activation



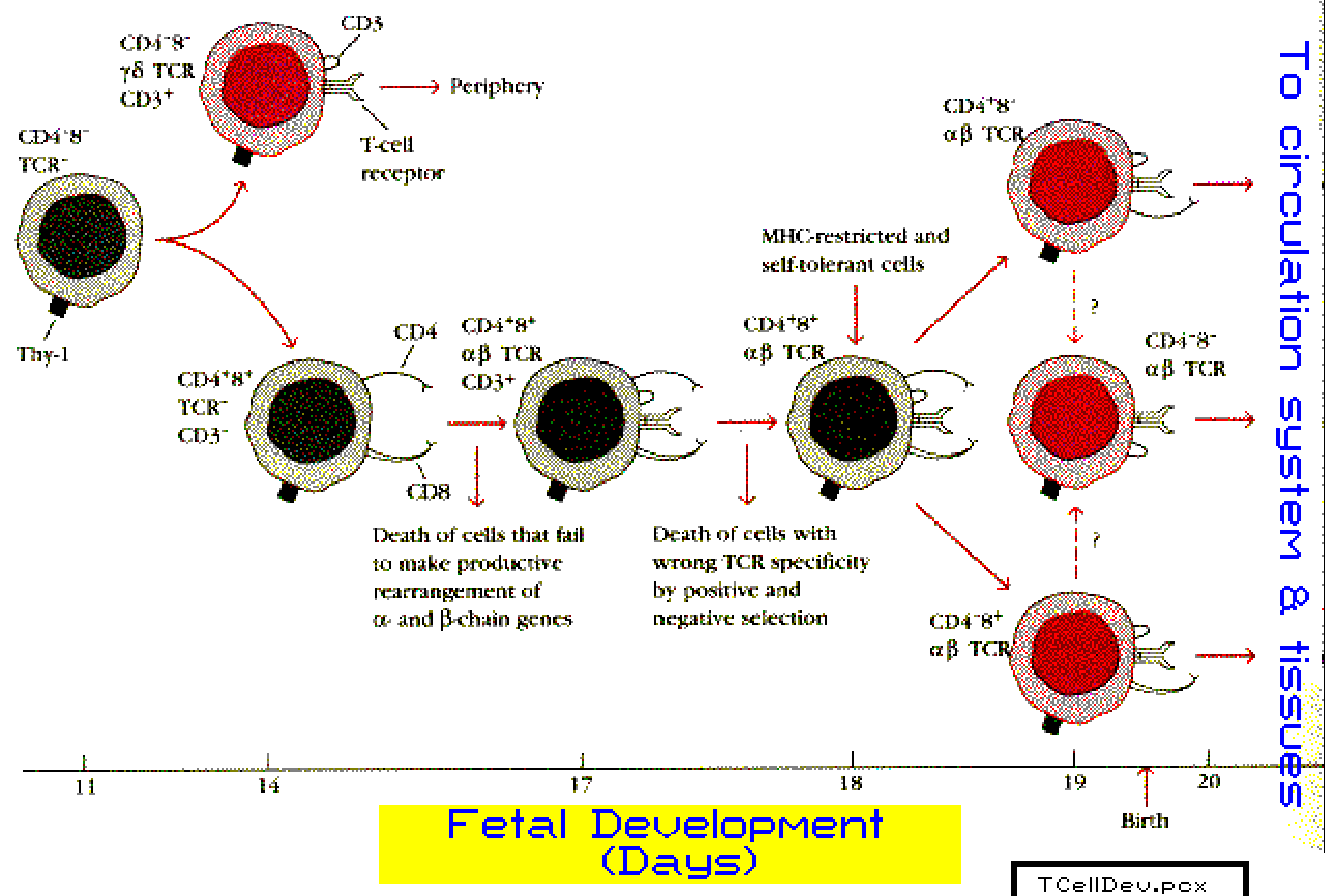
*** The earliest T cell precursors in the thymus:**

- Express Thy-1 (mice)
- Have not yet rearranged TCR loci
- Do NOT express CD4 or CD8
- Are called "double negatives"

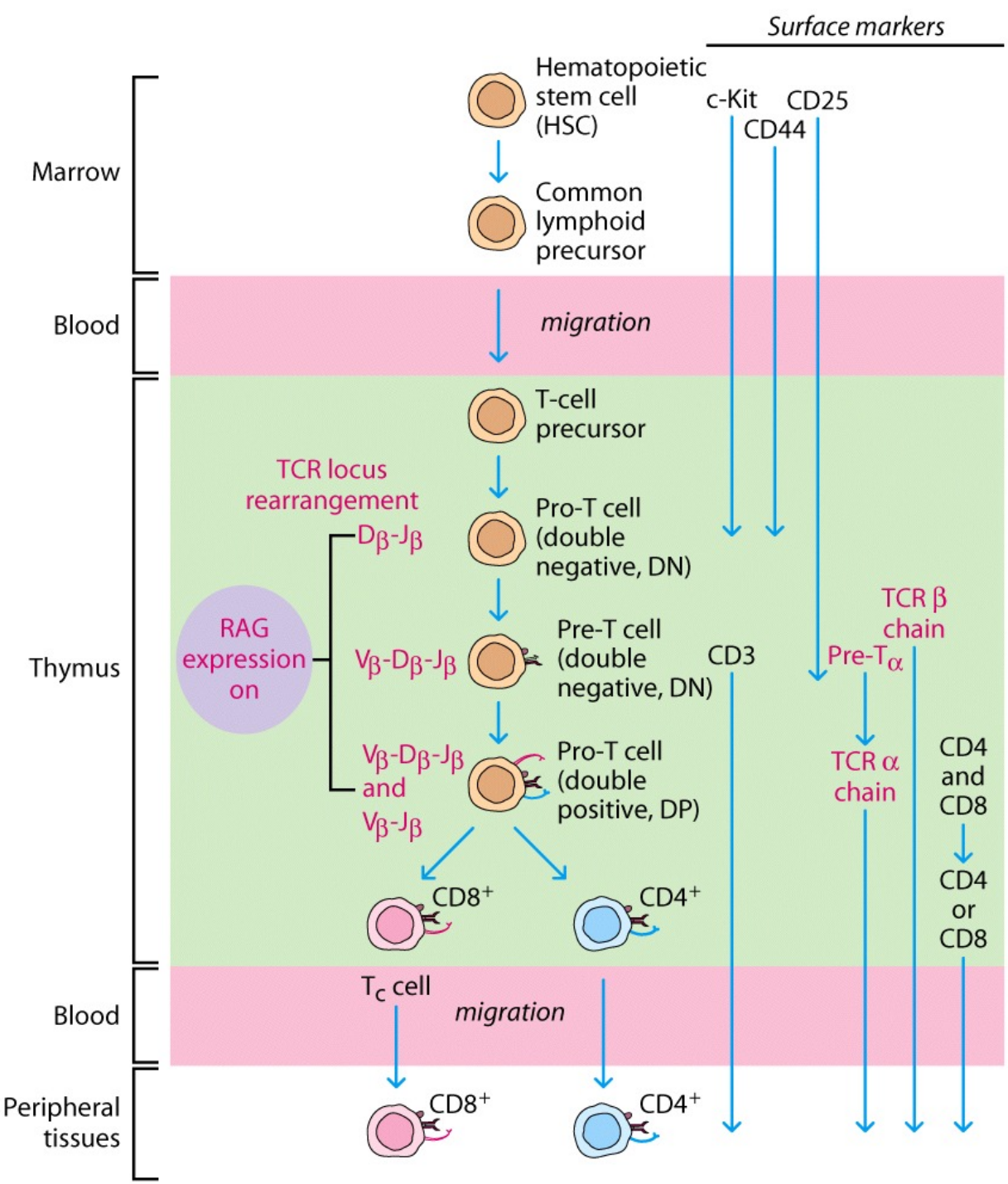
MARKERS:

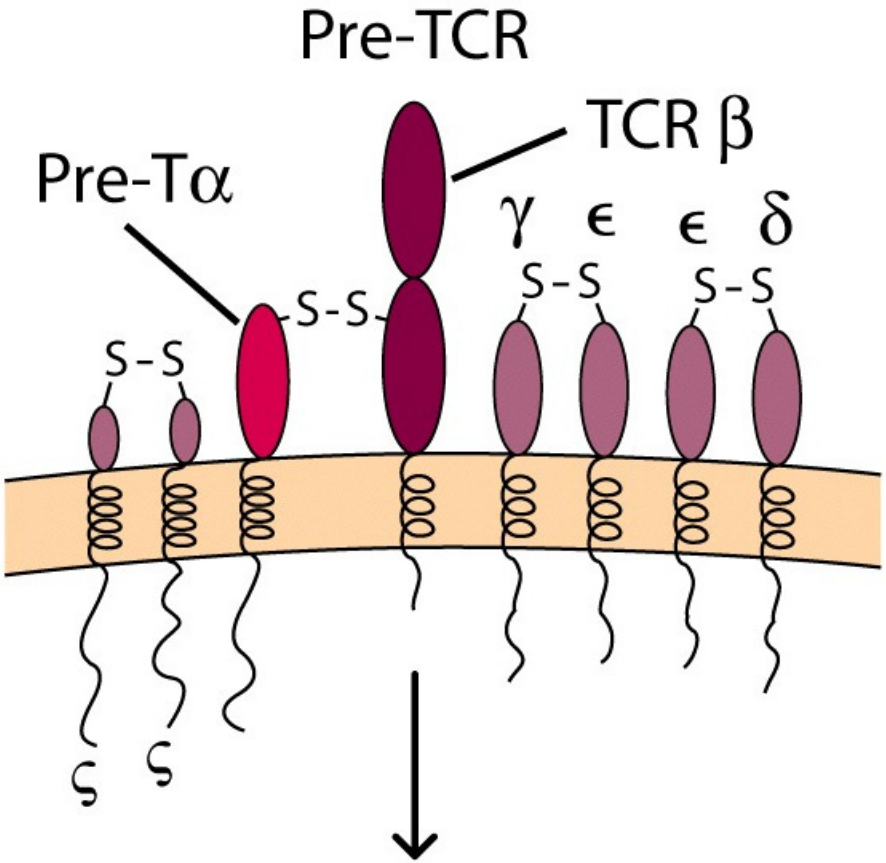
- **C-KIT**- Receptor for Stem Cell Growth Factor
- **CD44** - Adherance Molecule. Homing to thymus
- **CD25** - Alpha chain of IL-2 receptor

Double Negative



TCellDev.pcx
Figure 12-1
Kuby, 2nd Ed.


Surface markers
Marrow
Blood
Thymus
Blood
Peripheral tissues
Hematopoietic stem cell (HSC)
Common lymphoid precursor
c-Kit
CD25
CD44
migration
T-cell precursor
TCR locus rearrangement
Dβ-Jβ
Pro-T cell (double negative, DN)
RAG expression on
Vβ-Dβ-Jβ
Pre-T cell (double negative, DN)
CD3
TCR β chain
Pre-Tα
Vβ-Dβ-Jβ and Vβ-Jβ
Pro-T cell (double positive, DP)
TCR α chain
CD4 and CD8
CD4 or CD8
CD8+
CD4+
Tc cell
migration
CD8+
CD4+

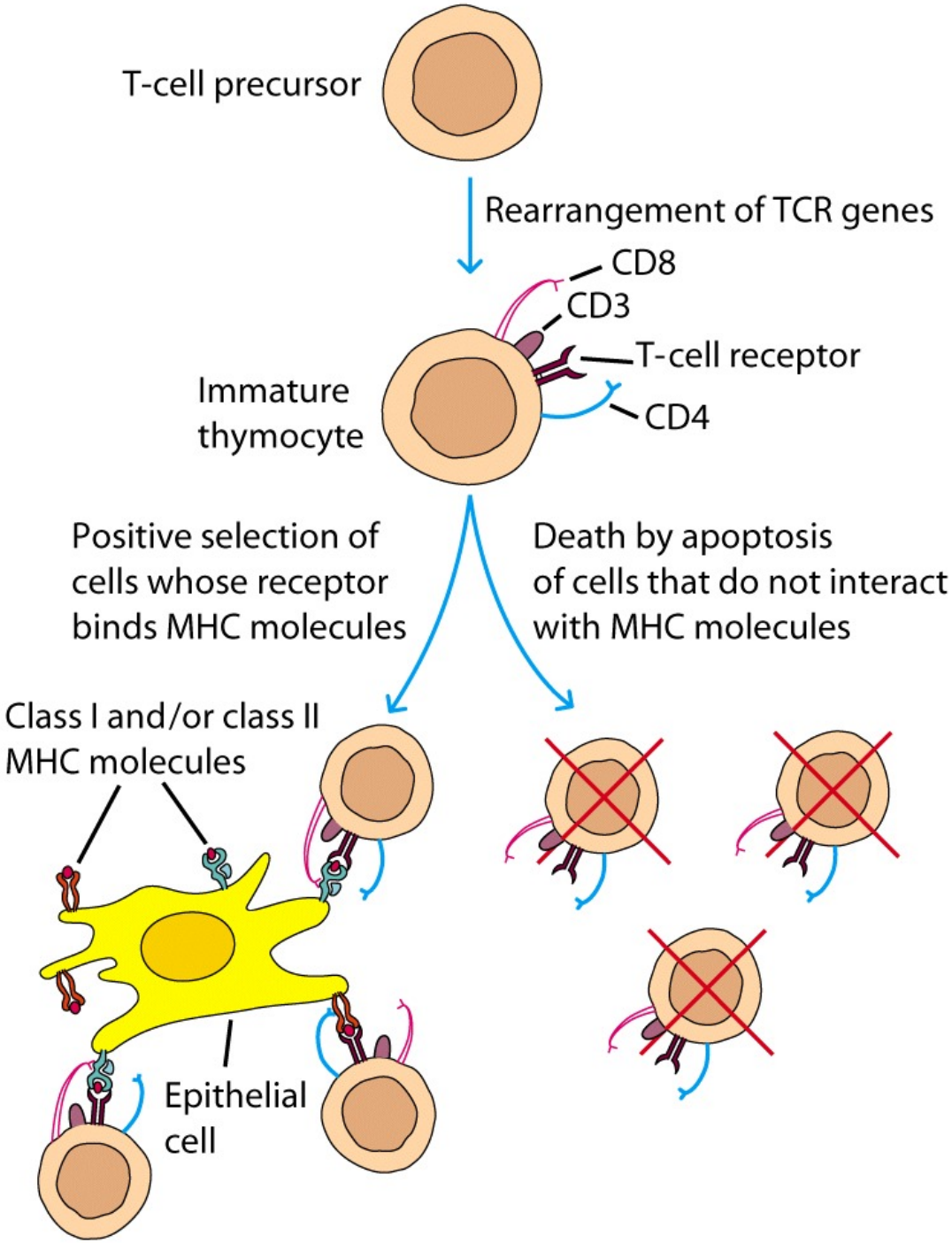


1) Made productive TCR chain re-arrangement
2) Signals for proliferation (similar chain) and maturation
3) Suppresses further chain re-arrangement (allelic exclusion)
4) Signals for TCR a chain to undergo re-arrangement
4) Induces development to CD4+8+ (double positive) stage

Positive and Negative Selection of T cells:

-**Positive selection**: occurs in the **cortex** and allows only those T cells that are able to bind to self-MHC molecules in the thymus to mature

-- **Negative selection**: occurs in the **medulla** and removes T cells whose TCR strongly recognize (high affinity) self-MHC (or self self-peptides plus self MHC). Die of apoptosis within the thymus.


T-cell precursor
Rearrangement of TCR genes
CD8
CD3
T-cell receptor
CD4
Immature
thymocyte
Positive selection of
cells whose receptor
binds MHC molecules
Death by apoptosis
of cells that do not interact
with MHC molecules
Class I and/or class II
MHC molecules
Epithelial
cell

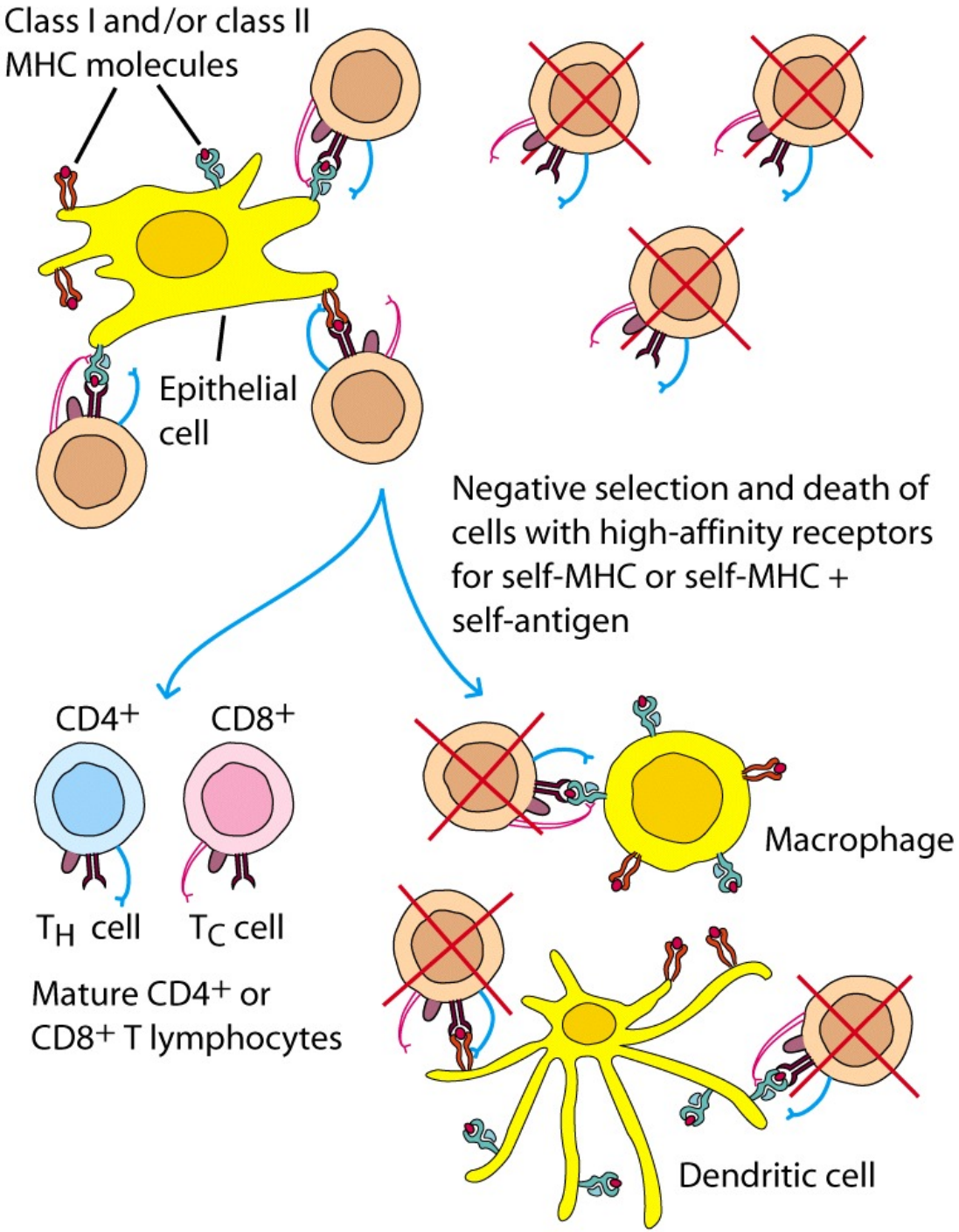
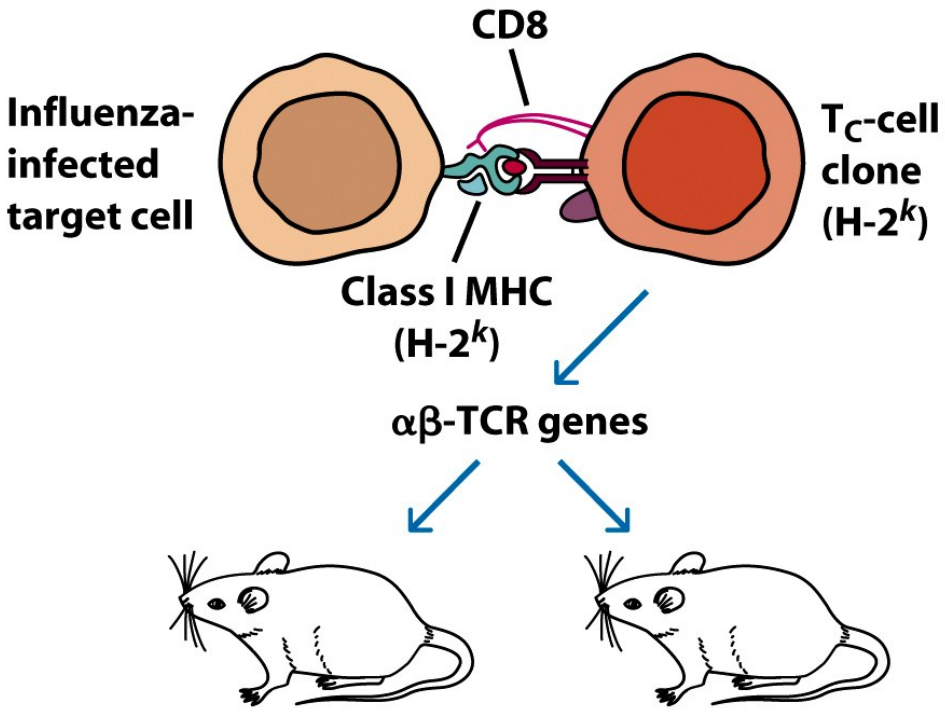

Class I and/or class II
MHC molecules
Epithelial
cell
Negative selection and death of
cells with high-affinity receptors
for self-MHC or self-MHC +
self-antigen
CD4+
CD8+
TH cell
TC cell
Mature CD4+ or
CD8+ T lymphocytes
Macrophage
Dendritic cell

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.

Negative selection by thymocytes:



Thymocytes in transgenics	H-2^k transgenic	H-2^d transgenic
TCR$^+$/CD4$^+$8$^+$	+	+
TCR$^+$/CD8$^+$	+	–

Figure 10-7
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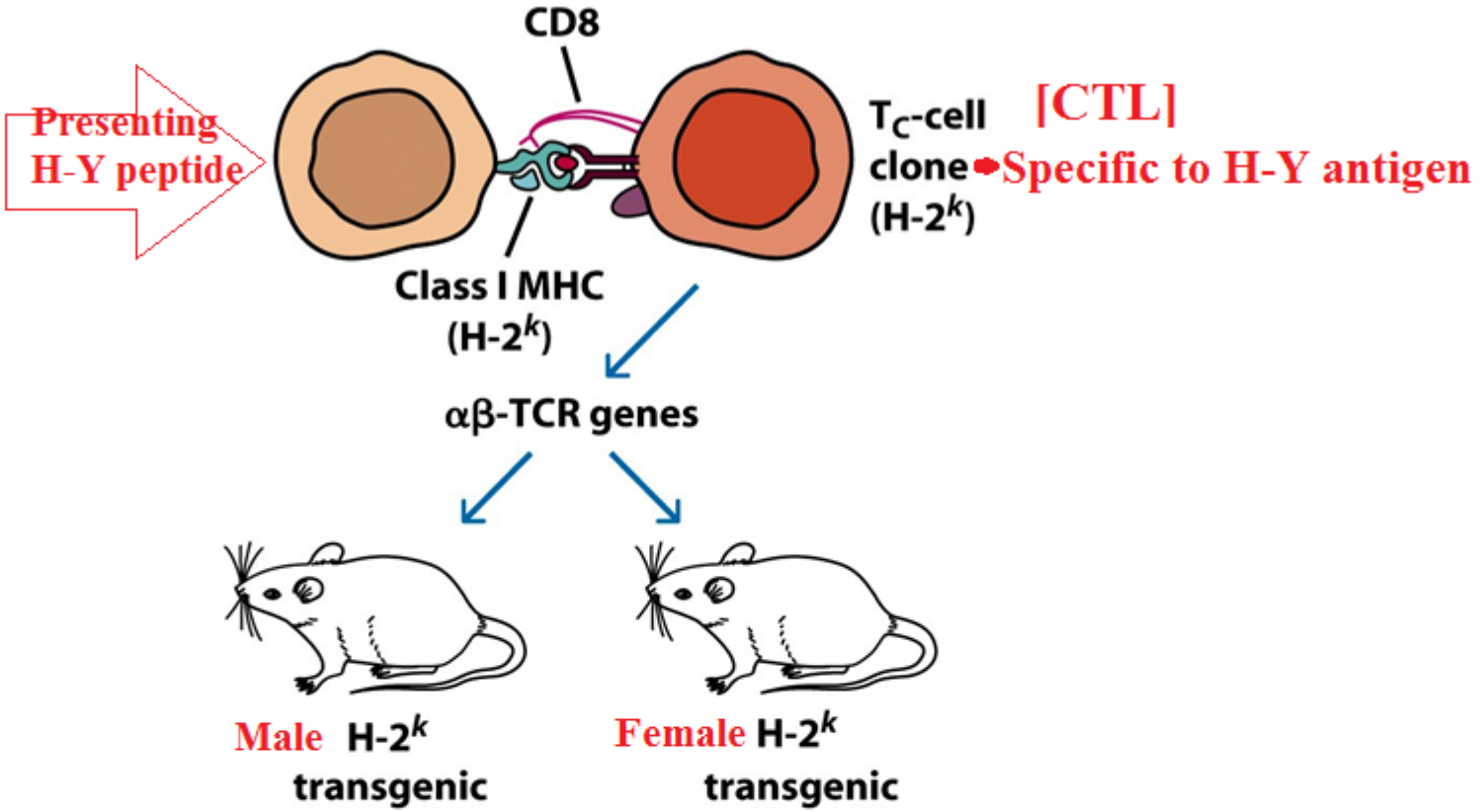
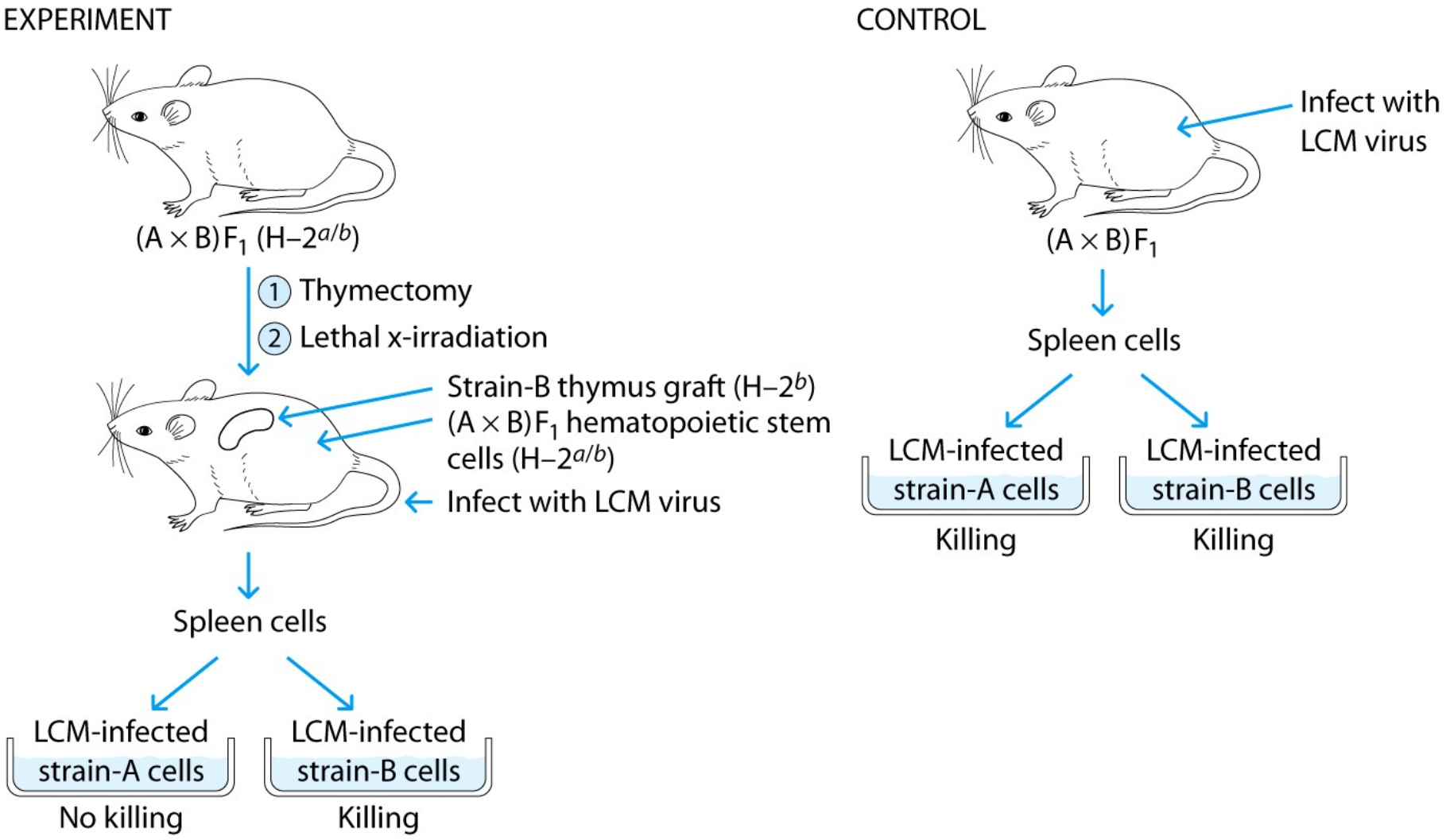
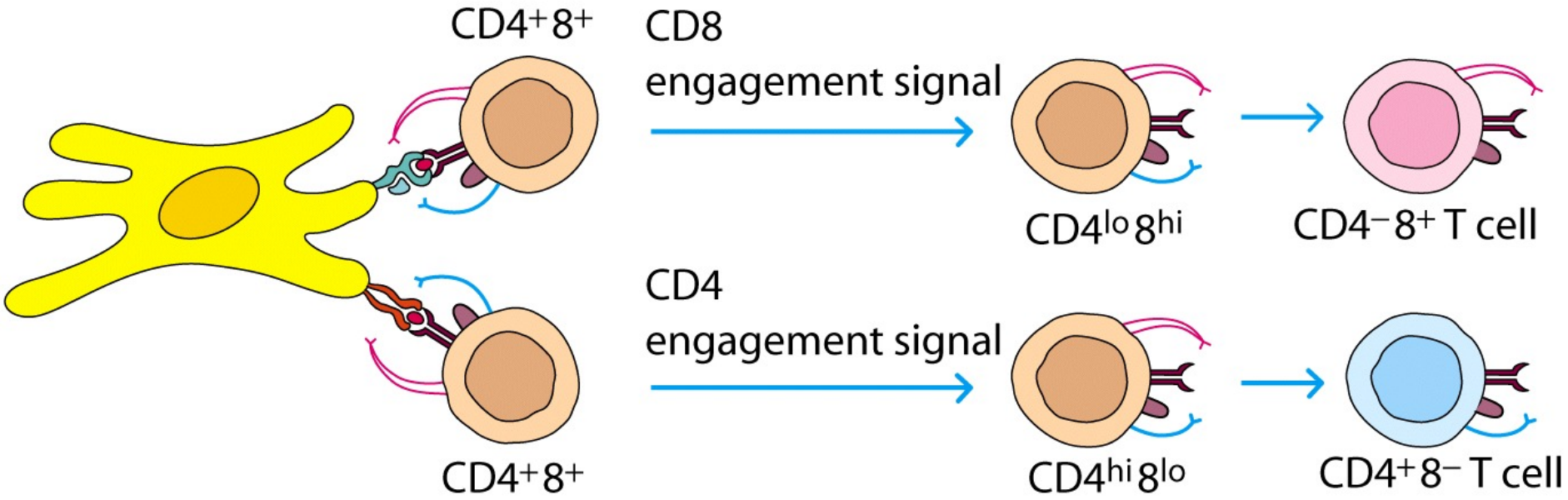
Negative selection by thymocytes:

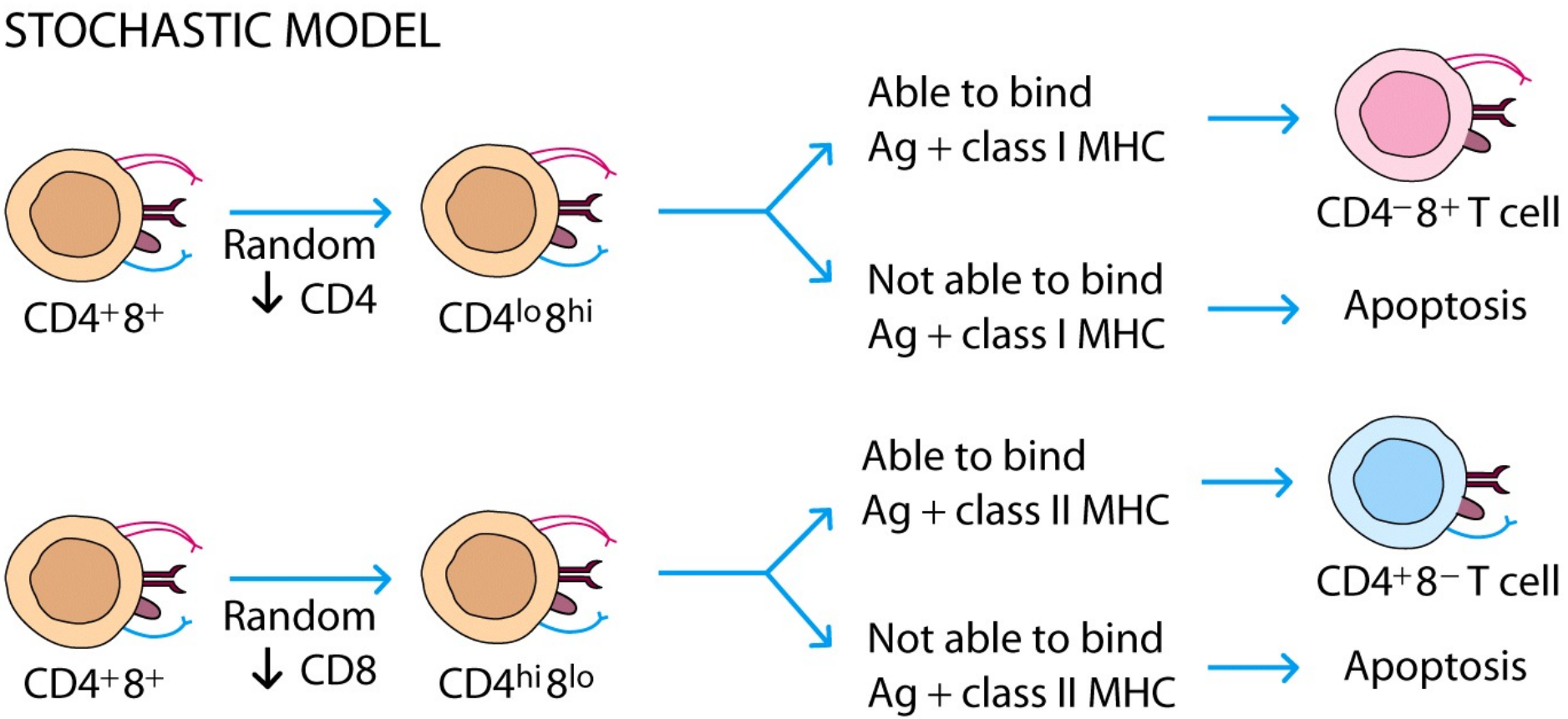


H-Y antigen	+	-
Thymocytes in transgenics		
$CD4^+8^+$	+	+
$CD4^+$	+	+
$CD8^+$	–	++

Positive selection

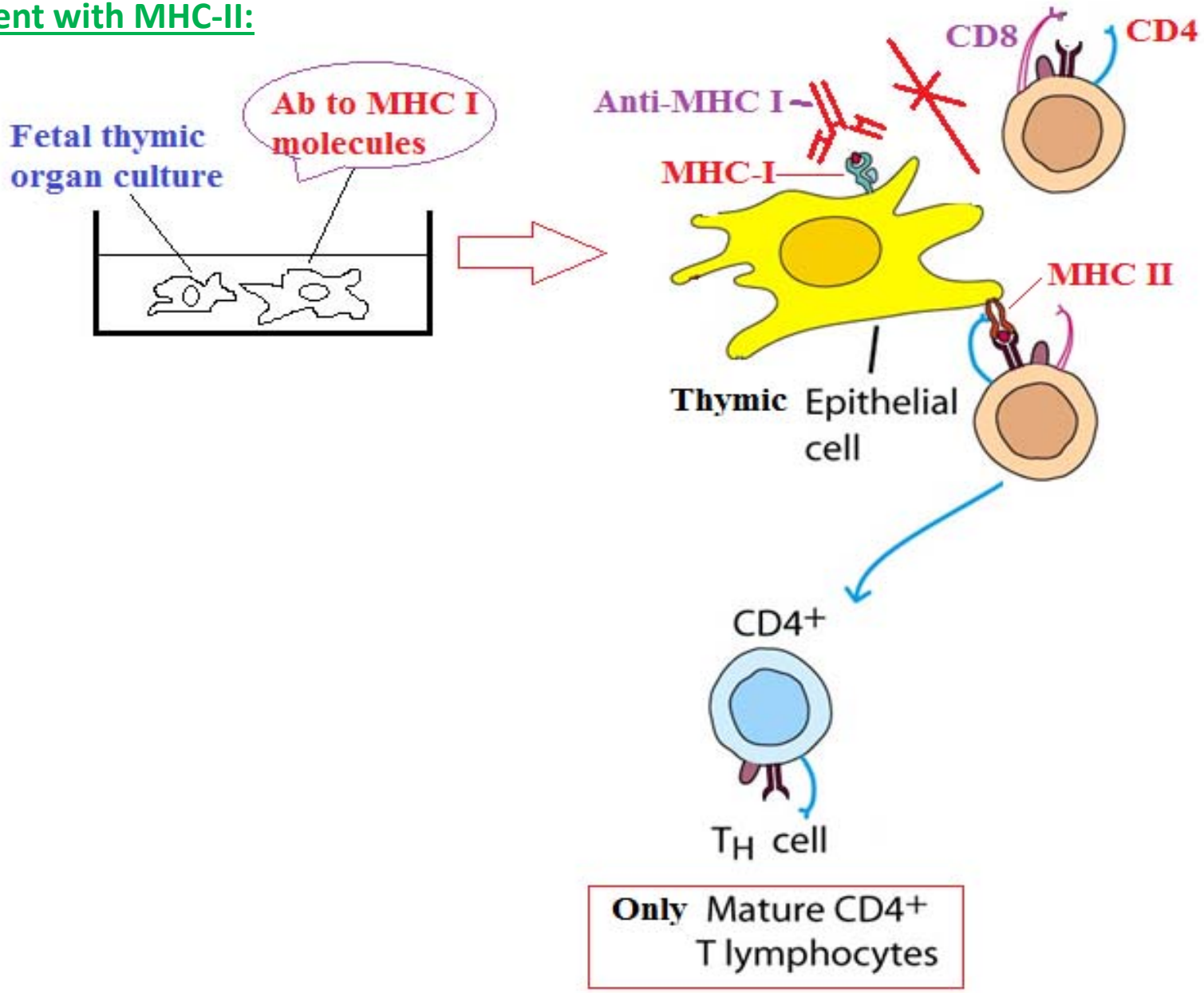



INSTRUCTIVE MODEL
CD4+8+
CD8
engagement signal
CD4lo8hi
CD4−8+ T cell
CD4
engagement signal
CD4+8+
CD4hi8lo
CD4+8− T cell

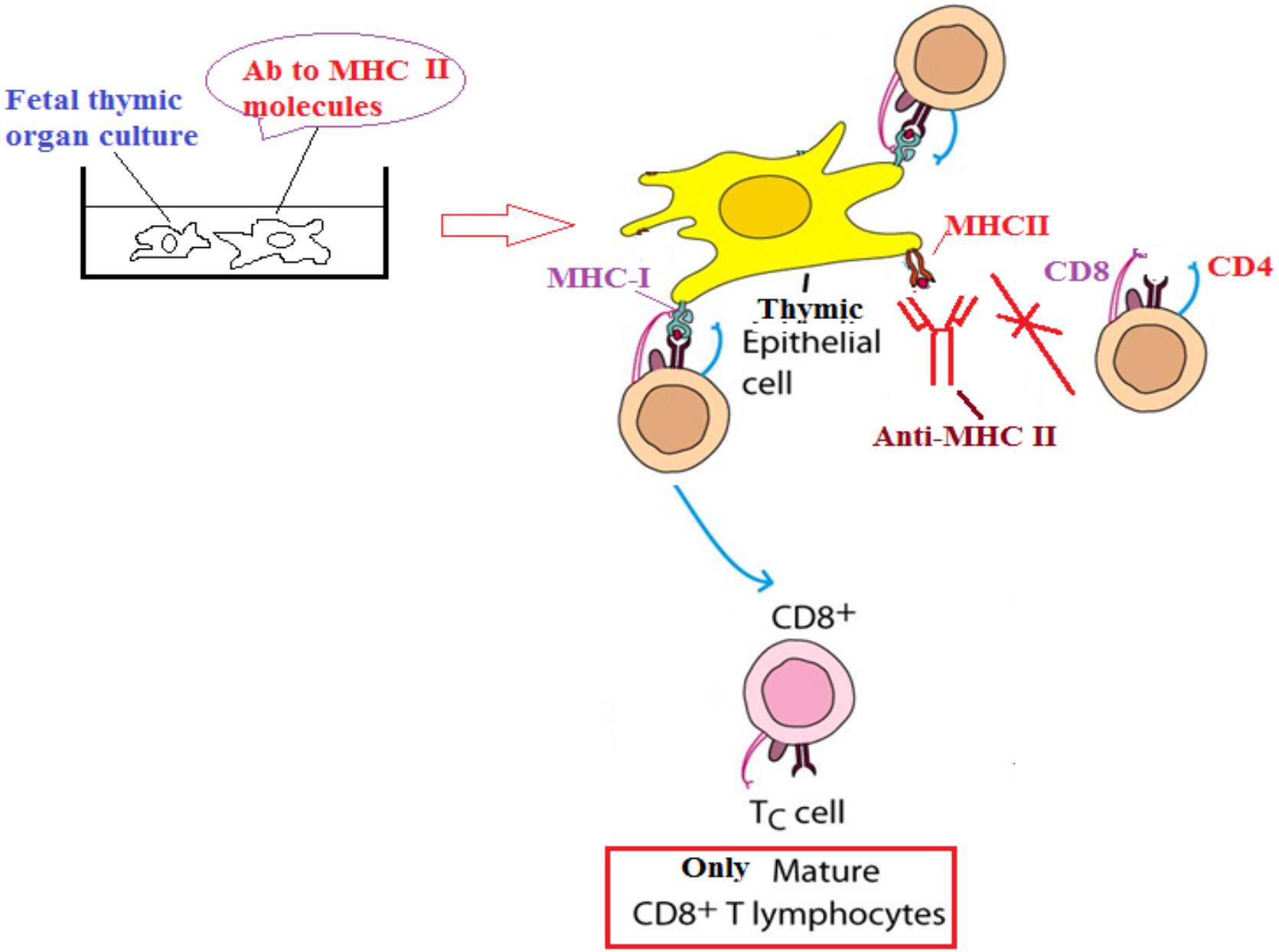

STOCHASTIC MODEL
CD4+8+
Random
↓ CD4
CD4lo8hi
Able to bind
Ag + class I MHC
CD4−8+ T cell
Not able to bind
Ag + class I MHC
Apoptosis
CD4+8+
Random
↓ CD8
CD4hi8lo
Able to bind
Ag + class II MHC
CD4+8− T cell
Not able to bind
Ag + class II MHC
Apoptosis

Maturation to T cells depends on Interaction of immature thymocytes with MHC I/II molecules:

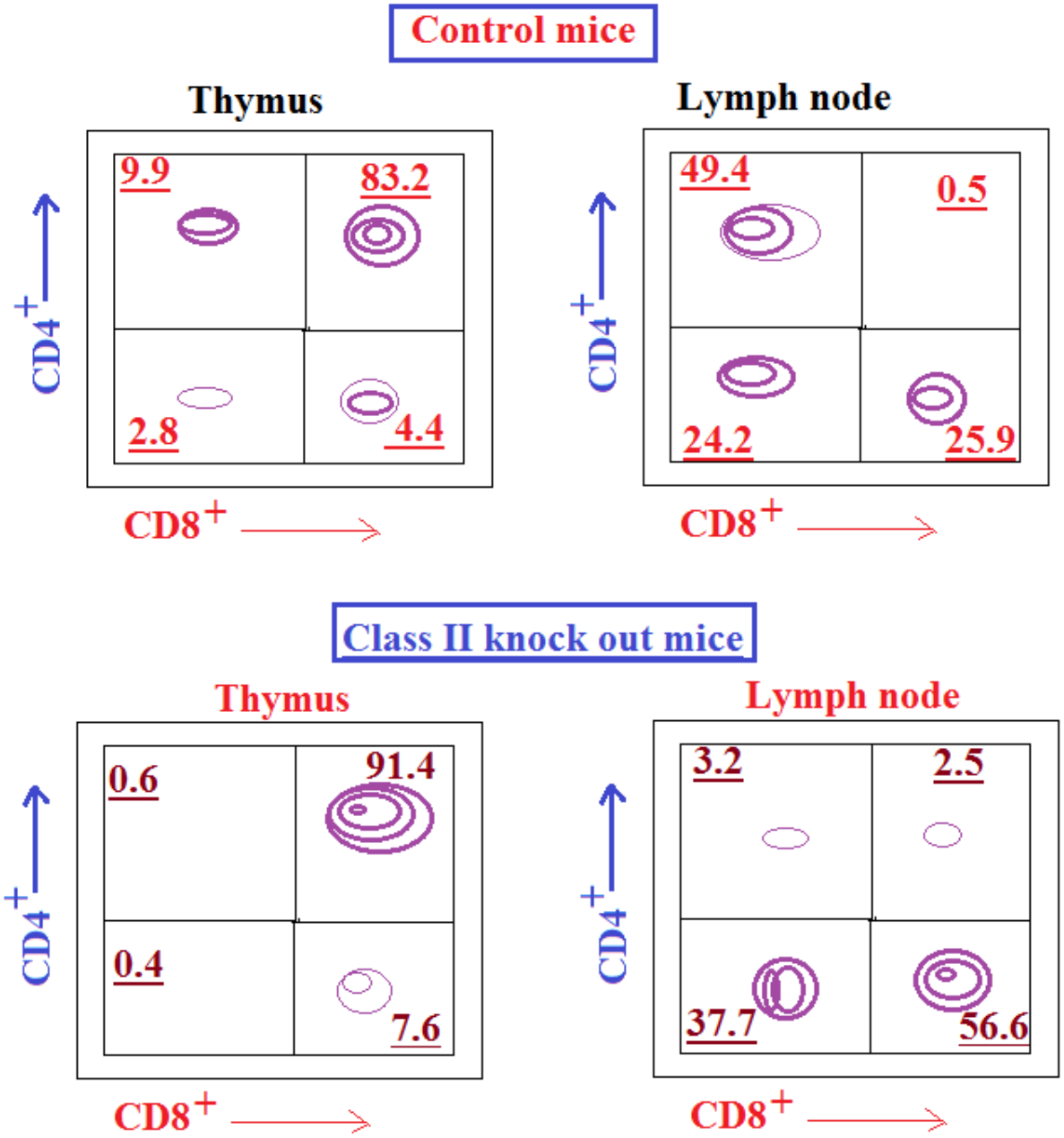
Engagement with MHC-II:

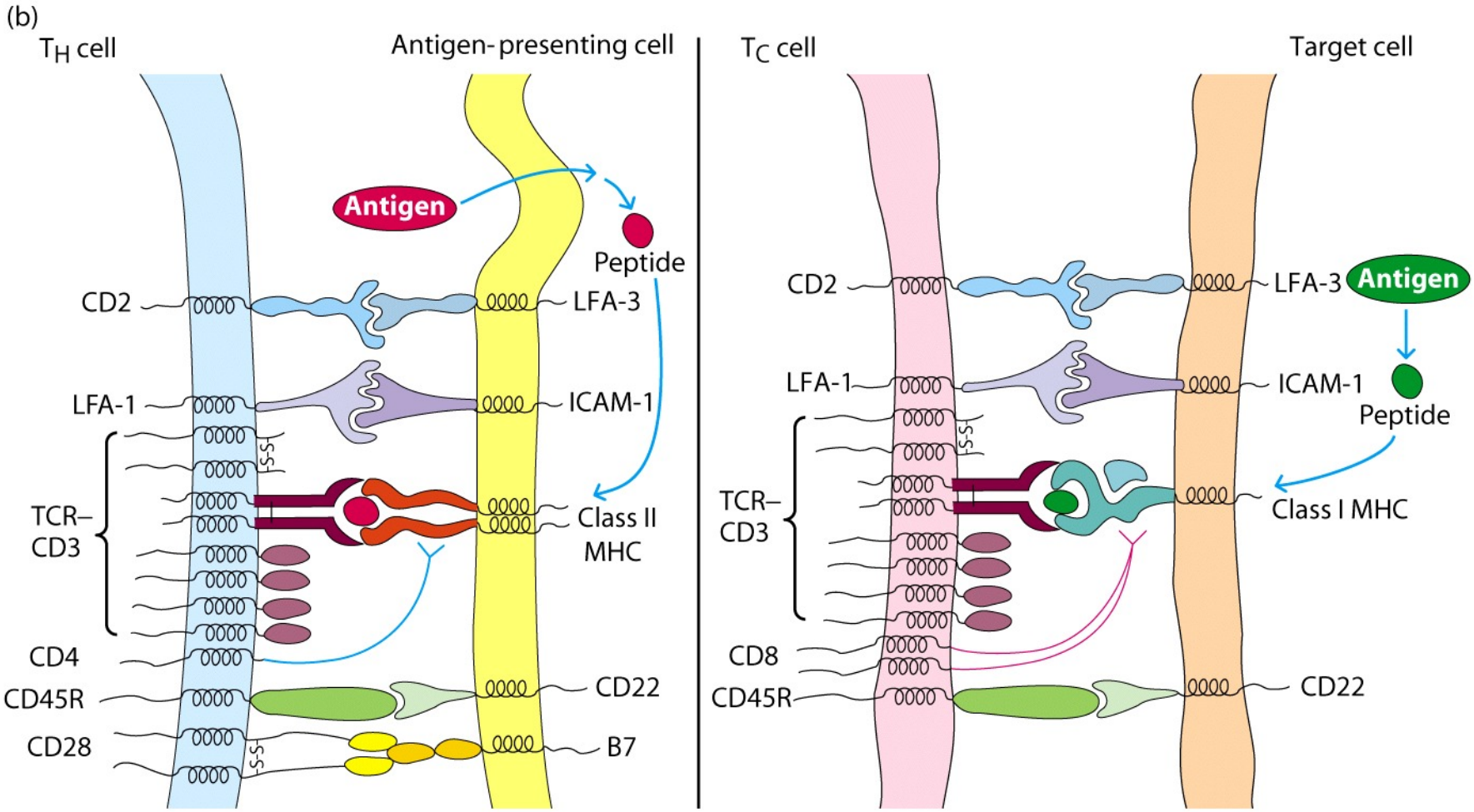


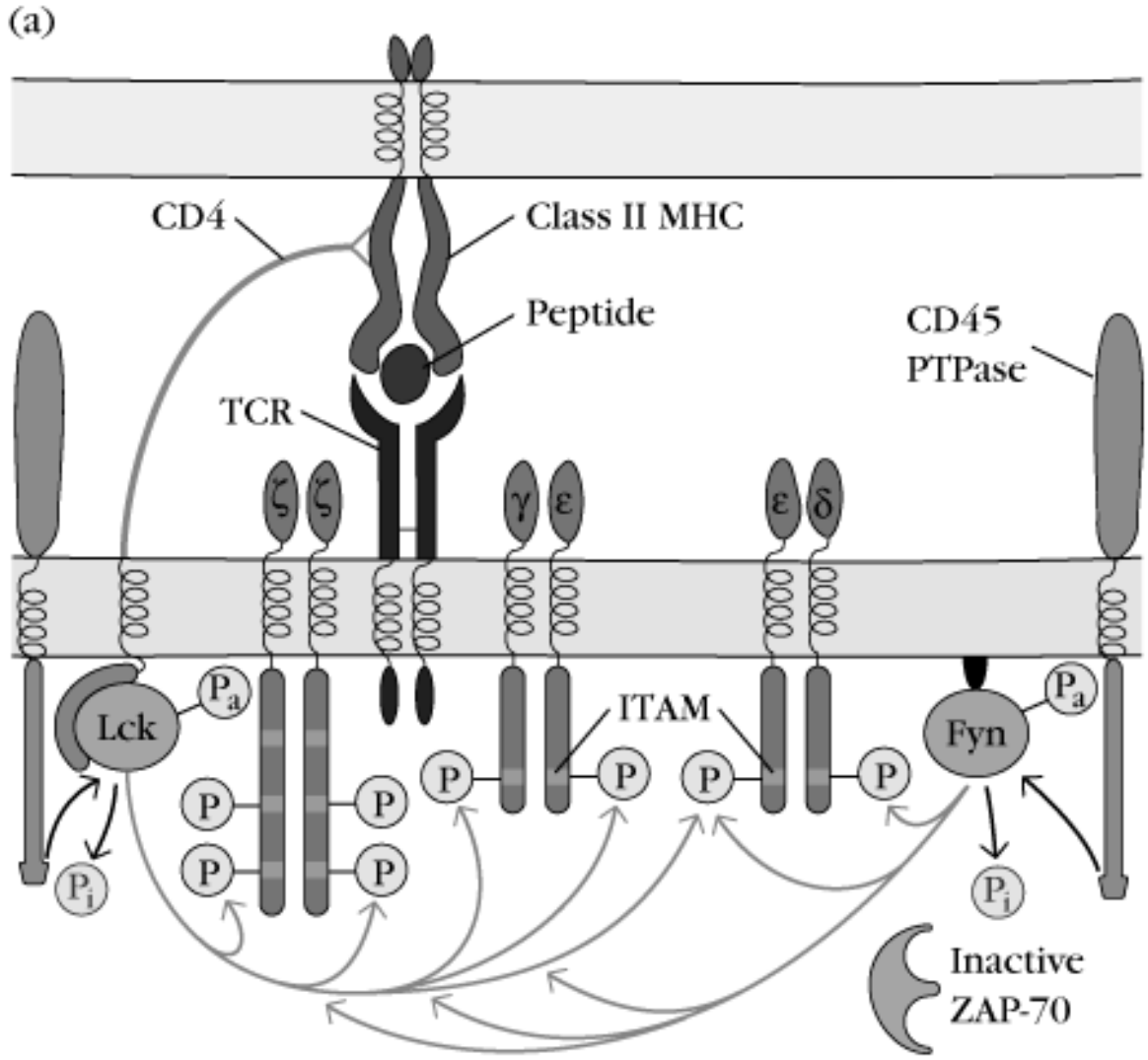
Engagement with MHC-I molecules:

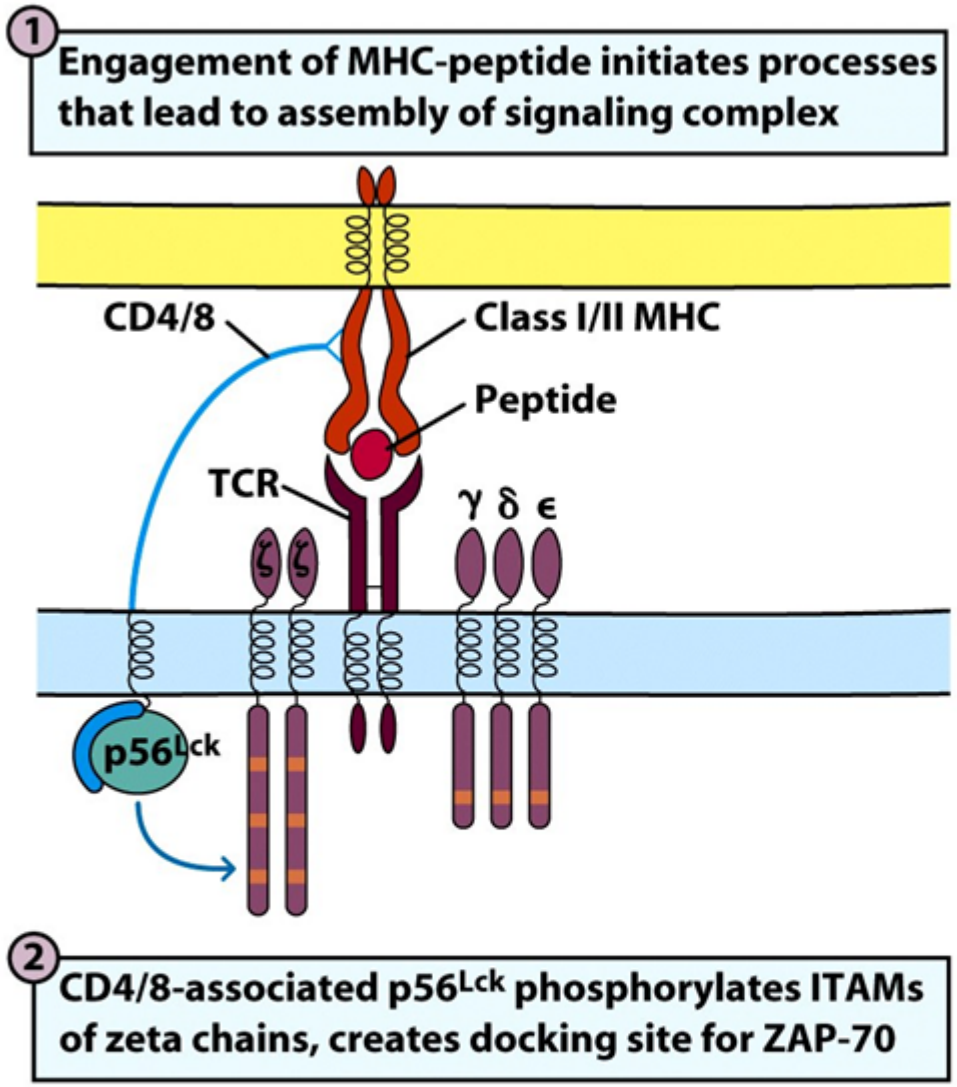


FACS analysis of thymocytes and peripheral lymph node T cells from normal mice and MHC-II Knock-out mice.




(b)
TH cell
Antigen-presenting cell
Antigen
Peptide
CD2
LFA-3
LFA-1
ICAM-1
TCR–
CD3
Class II
MHC
CD4
CD45R
CD22
CD28
B7
TC cell
Target cell
CD2
LFA-3
Antigen
LFA-1
ICAM-1
Peptide
TCR–
CD3
Class I MHC
CD8
CD45R
CD22


(a)
CD4
Class II MHC
Peptide
CD45
PTPase
TCR
ζ
ζ
γ
ε
ε
δ
Lck
Pa
P
P
P
P
ITAM
P
P
P
P
Fyn
Pa
Pi
Pi
Inactive
ZAP-70



Phosphorylation = addition of P

Figure 10-11 part 1
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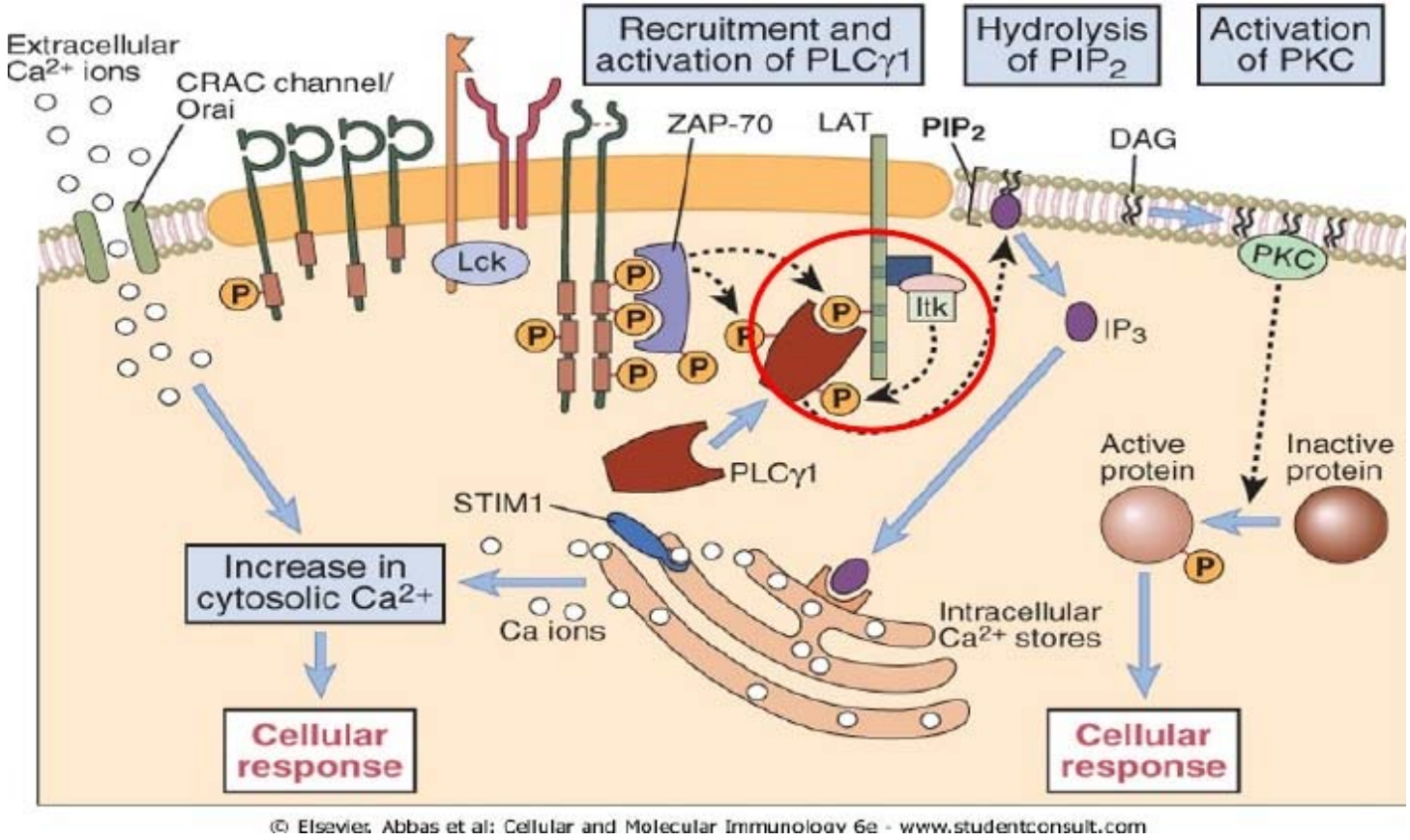
T cell signaling downstream of PLCγ1



Signals through the TCR, CD3 and CD4/8 activate a PTK cascade

- - CD4/8 are associated with a cytoplasmic tyrosine kinase enzyme – Lck (lymphocyte kinase)
- 1.- TCR-MHC-Peptide activates the phosphatase CD45
- 2.- CD45 activates Fyn and Lck
- 3.- Activated Fyn and Lck phosphorylate ITAMs in CD3 chains
- 4.- Phosphorylated ITAM motifs on the CD3zz chains become a docking site for the PTK ZAP-70.
- 5.- Binding of CD4 to MHC allows Lck to phosphorylate and activate ZAP-70 to become an active PTK.

- **Activation of ZAP-70 initiates a cascade of events that results in LAT activation and phosphorylation (activation) of phospholypase C (PLCγ1).**
- **PLCγ1 converts phosphatidyl inositol 4,5-biphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3):**
- **IP3 triggers the release of calcium from intracellular storage vesicles into the cytosol, thus raising cytoplasmic calcium levels.**

The calcium:

1) synergizes with DAG to activate protein kinase C (PKC)

2) PKC activates the transcription factor NF-kB

3. and PKC activate IkB kinase (IKK), which phosphorylates IkB, releasing the transcription factor NF-kB – which translocates to the nucleus.
4. acts together with calmodulin to activate calcineurin (phosphatase)
5. Calcineurin activates the cytoplasmic component of the transcription factor NFAT (NFATc), causing it to translocate to the nucleus, where it combines with NFATn

- **TRANSCRIPTION FACTORS- lead to gene transcription, cell proliferation and differentiation.**

Calcineurin phosphatase plays a crucial role in T cell activation. Dephosphorylation of the nuclear factors of activated T cells (NFATs) by calcineurin is essential for activating cytokine gene expression and, consequently, the immune response.

Current immunosuppressive drugs are based mainly on calcineurin inhibitors, cyclosporine A and FK506, which inhibits dephosphorylation of NFATc.

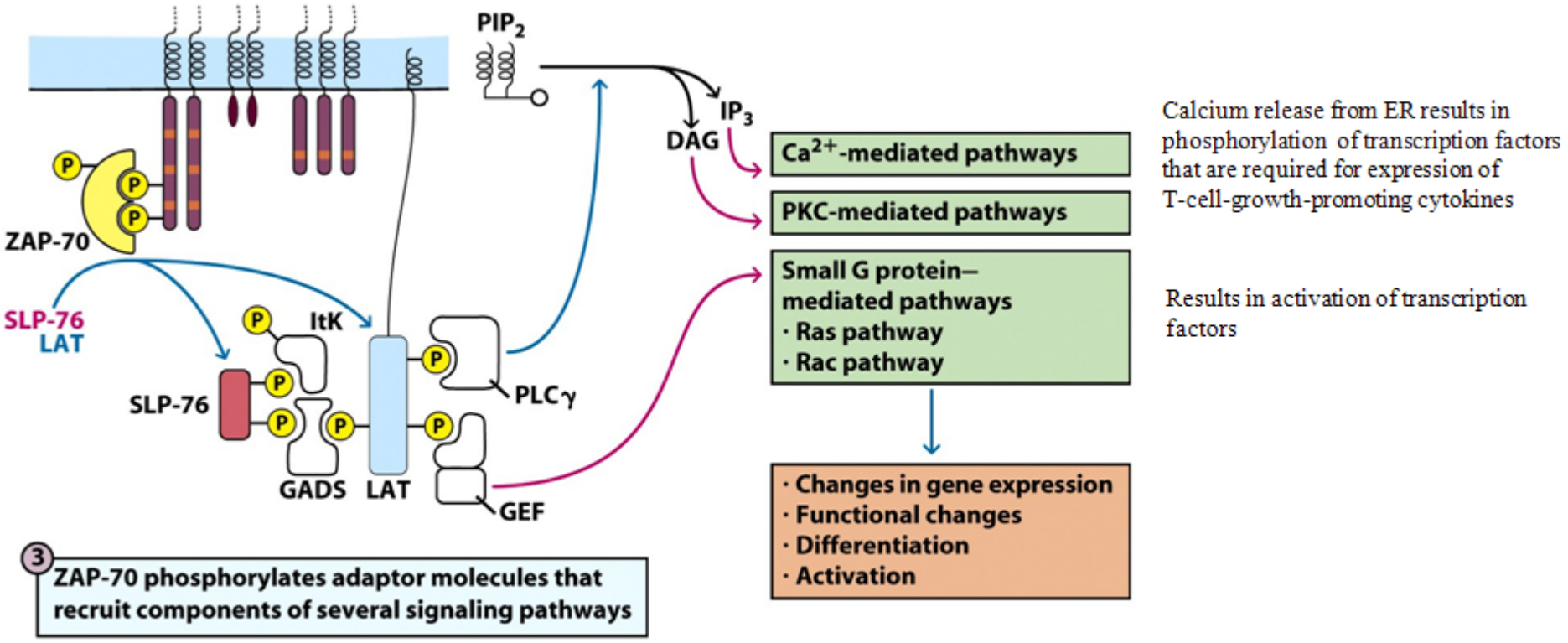


Figure 10-11 part 2
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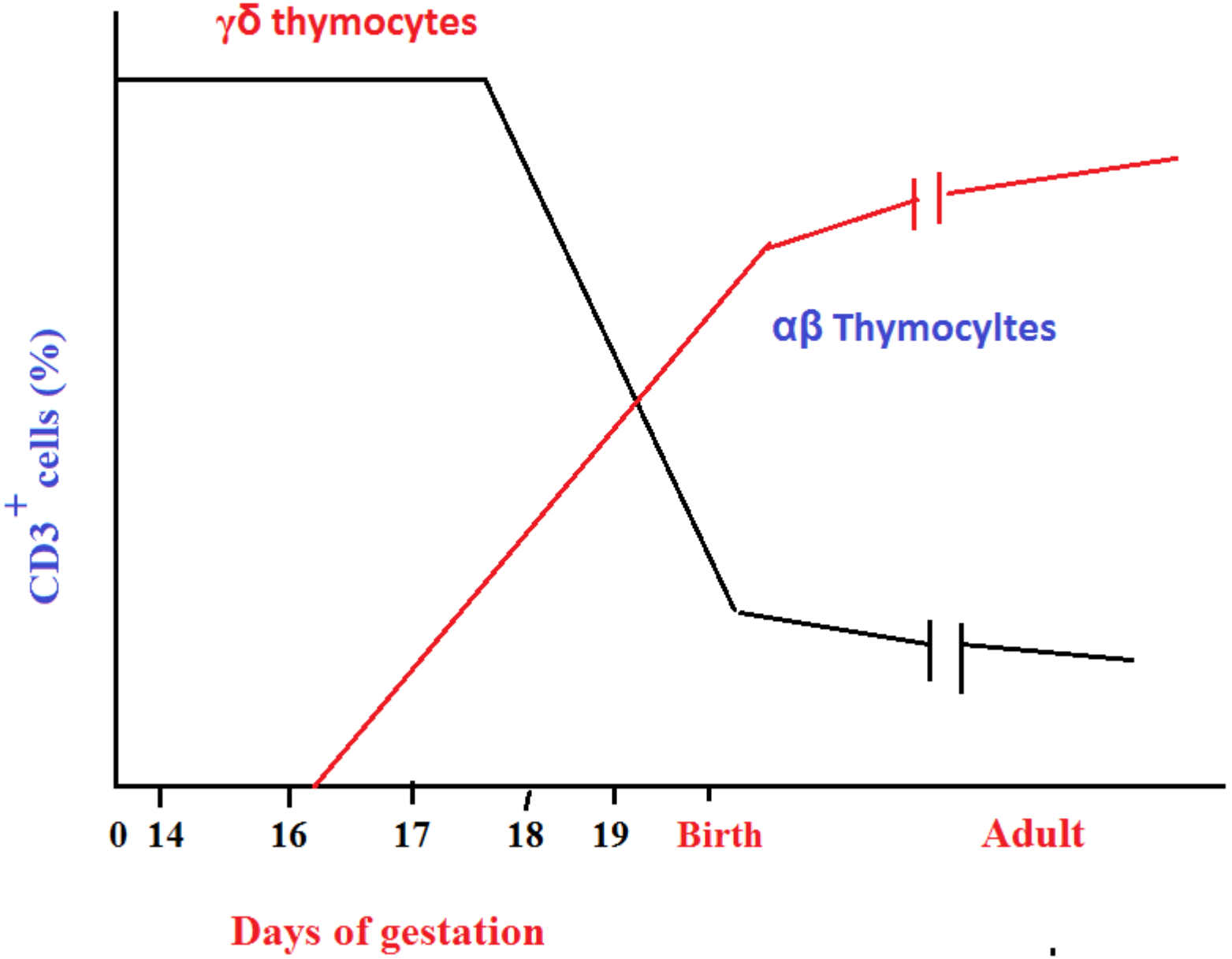
ADAPTOR PROTEINS - SLP76 (SH2-domain containing leukocyte protein of 76 KDa and LAT (Linker of activated T cells).

ADAPTOR PROTEINS: 1) Serve as links for proteins, 2) Promote assembly of membrane proteins


γδ thymocytes
αβ Thymocyltes
CD3+ cells (%)
0
14
16
17
18
19
Birth
Adult
Days of gestation

γδ T cells (peripheral)

Intra-epidermal lymphocytes: **$CD4^-CD8^-$ $CD3^+$ γδ-$TCR^+$$Thy1^+$** (In the skin of mice and rat)

Intra-epithelial lymphocytes (IELS): **$CD4^-CD8^+$ $CD3^+$ γδ-$TCR^+$$Thy1^+$** (In the intestinal epithelial cells of mice)

γδ T cells can bind directly to the antigens without antigen processing and presentation together with MHC; similar to antibody molecule.

Function of γδ T cells:

1. **Can mediate tumor cell lysis like NK cells**
2. **Can respond to mycobacterial antigen such as purified protein derivative (PPD).**
3. **PPD belongs to a group of highly conserved proteins found in all organisms, called HSP.**
4. **γδ T cells might have some roles to eliminate damaged cells/microbial invaders.**

NK1-T cells (NKT):

Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and natural killer cells.
The best-known NKT cells differ from conventional αβ T cells in that their T-cell receptors are far more limited in diversity ('invariant' or 'type 1' NKT). They and other CD1d-restricted T cells ('type 2' NKT) recognize lipids and glycolipids presented by CD1d molecules, a member of the CD1 family of antigen-presenting molecules, rather than peptide-major histocompatibility complexes (MHCs).

1. αβ TCR
2. **This TCR interact with MHC-like molecules called CD1 rather than with MHC-I/MHC-II.**
3. **Can kill cells (NKT cells are important in recognizing glycolipids from organisms such as *mycobacterium*, which causes tuberculosis.)**
4. **Natural killer T cells can share other features with NK cells, as well, such as CD16 and CD56 expression and granzyme production**

Superantigens

- **Proteins produced by pathogens**
- **Not processed by antigen presenting cells**
- **Intact protein binds to the variable region on TCR of T cells and to MHC class II on antigen presenting cells (APC)**
- **Large numbers of activated T cells and APC release cytokines having pathological effects**

Superantigens

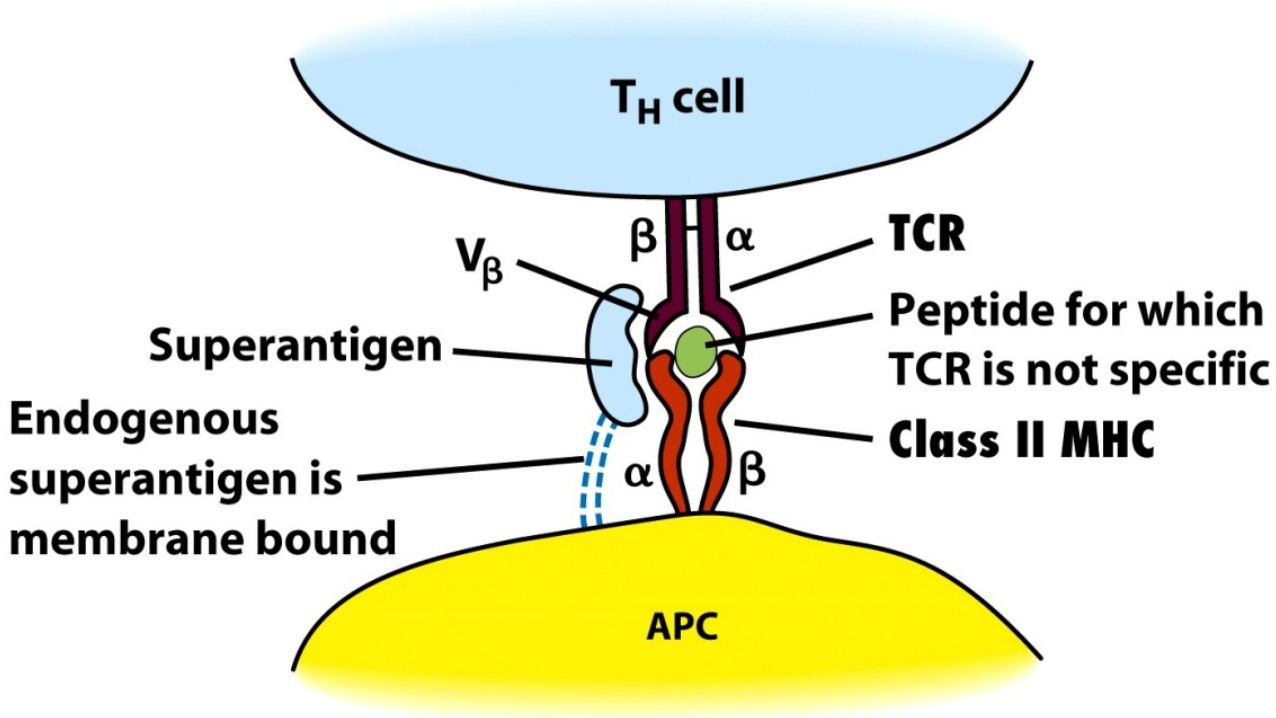


Figure 10-16
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TABLE 10-3 Exogenous superantigens and their V_β specificity

Superantigen	Disease*	V_β SPECIFICITY	
		Mouse	Human
Staphylococcal enterotoxins			
SEA	Food poisoning	1, 3, 10, 11, 12, 17	nd
SEB	Food poisoning	3, 8.1, 8.2, 8.3	3, 12, 14, 15, 17, 20
SEC1	Food poisoning	7, 8.2, 8.3, 11	12
SEC2	Food poisoning	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	Food poisoning	7, 8.2	5, 12
SED	Food poisoning	3, 7, 8.3, 11, 17	5, 12
SEE	Food poisoning	11, 15, 17	5.1, 6.1–6.3, 8, 18
Toxic shock syndrome toxin (TSST1)	Toxic shock syndrome	15, 16	2
Exfoliative dermatitis toxin (ExFT)	Scalded skin syndrome	10, 11, 15	2
Mycoplasma arthritidis supernatant (MAS)	Arthritis, shock	6, 8.1–8.3	nd
Streptococcal pyrogenic exotoxins (SPE-A, B, C, D)	Rheumatic fever, shock	nd	nd

*Disease results from infection by bacteria that produce the indicated superantigens.

Table 10-3
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Superantigens

Consequences:

- **Because they cross-link the V domain of the TCR with the V domain of the MHC-II, this results in non-specific proliferation and activation.**
- **Over production of Th cytokine leading to systemic toxicity (IFN-g, TNF-) and inflammatory mediators.**
- **Deletion (negative selection) of thymocytes bearing V domains recognized by the superantigen---- beneficial?**

Endogenous superantigens:

Minor lymphocyte stimulating (Mls) determinant ► retroviral proteins such as mouse mammary tumor virus (MTV).

Mls allele	Retroviral carrier	Chromosome location	Vβ specificity
Mls1	**MTV-7**	**1**	**6, 7, 8.1, 9**
Mls2	MTV-3	4	3
Mls3	MTV-6	16	3, 5
Mls 4	MTV-1	7	3

Negative selection of thymocytes mediated by Mls1:

	Strain	Mls1	Vβ 3	Vβ5	Vβ 6	Vβ 7	Vβ8.1	Vβ9
Immature αβ thymocytes	**AKR (MTV-7 On mouse Chr-2)**	+	+	+	+	+	+	+
	B10.BR	-	+	+	+	+	+	+
Mature peripheral αβ T cells	**AKR(MTV-7 On mouse Chr-2)**	+	+	+	-	-	-	-
	B10.BR	-	+	+	+	+	+	+

The interaction of CD28 with B7 sends additional activation signals.

- **CTLA-4 is not initially expressed, but is expressed after T cell activation.**

- **CTLA-4 has a higher affinity for B7 than CD28.**

- **Interaction of CTLA-4 with B7 is thought to down regulate T cell activation.**

- **There are actually two related molecules: B7.1 and B7.2**

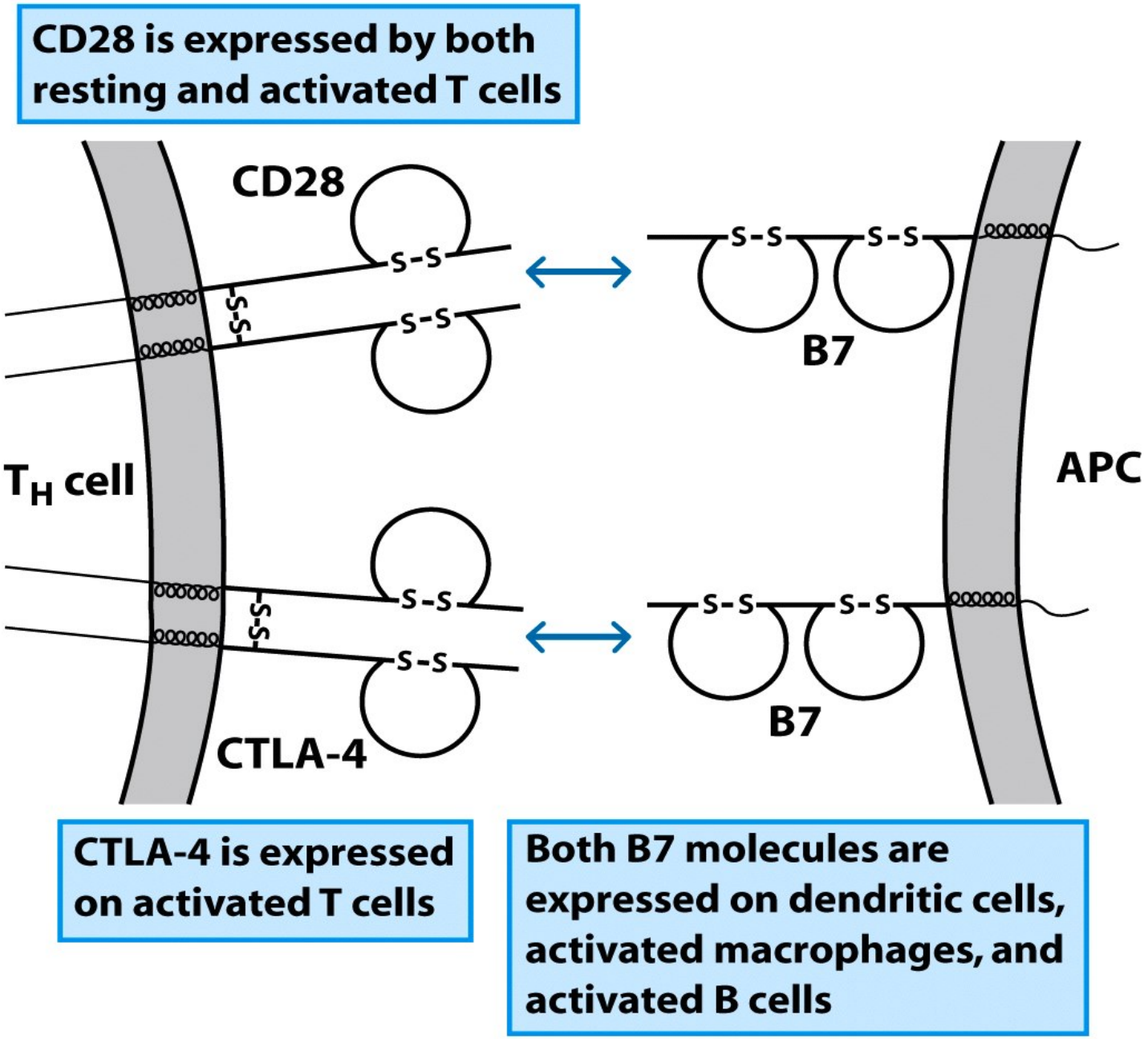
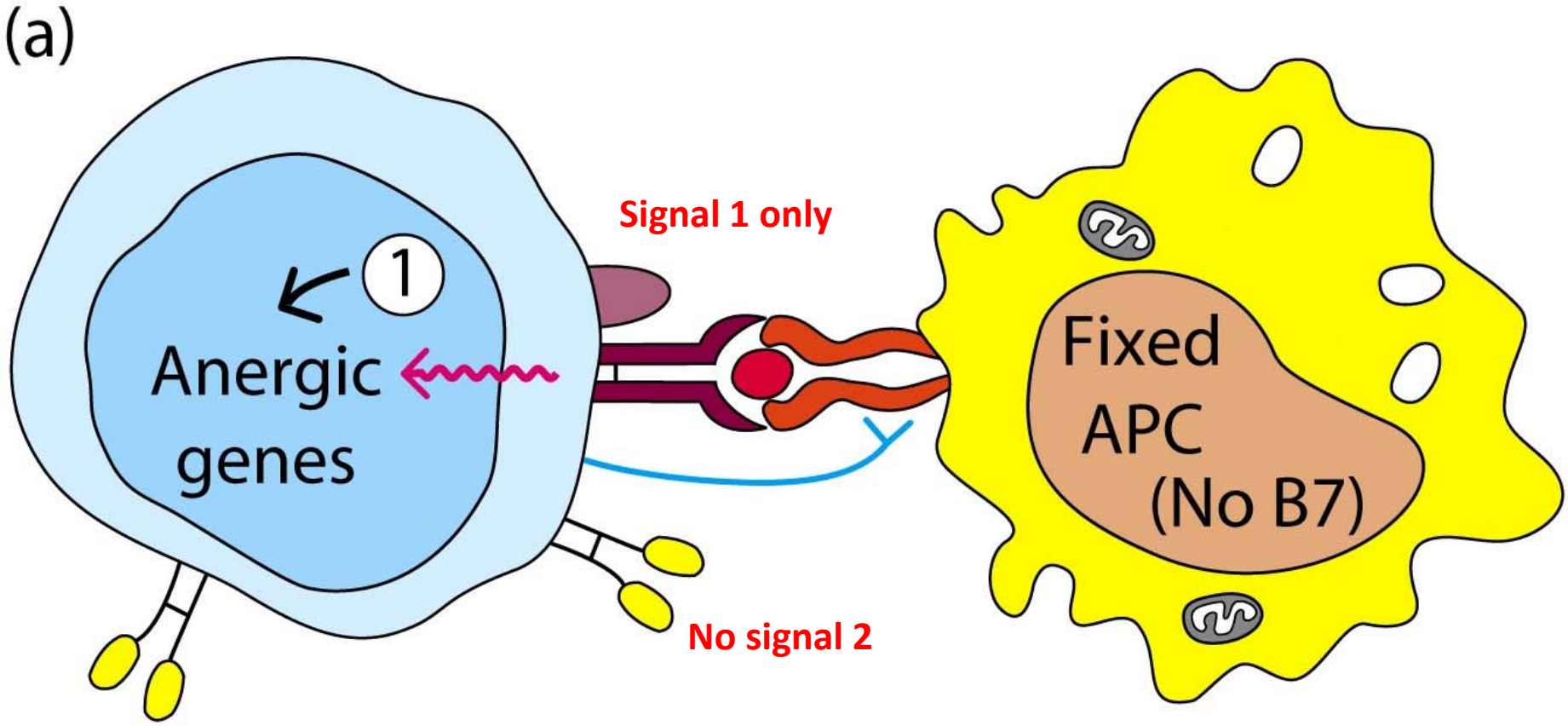
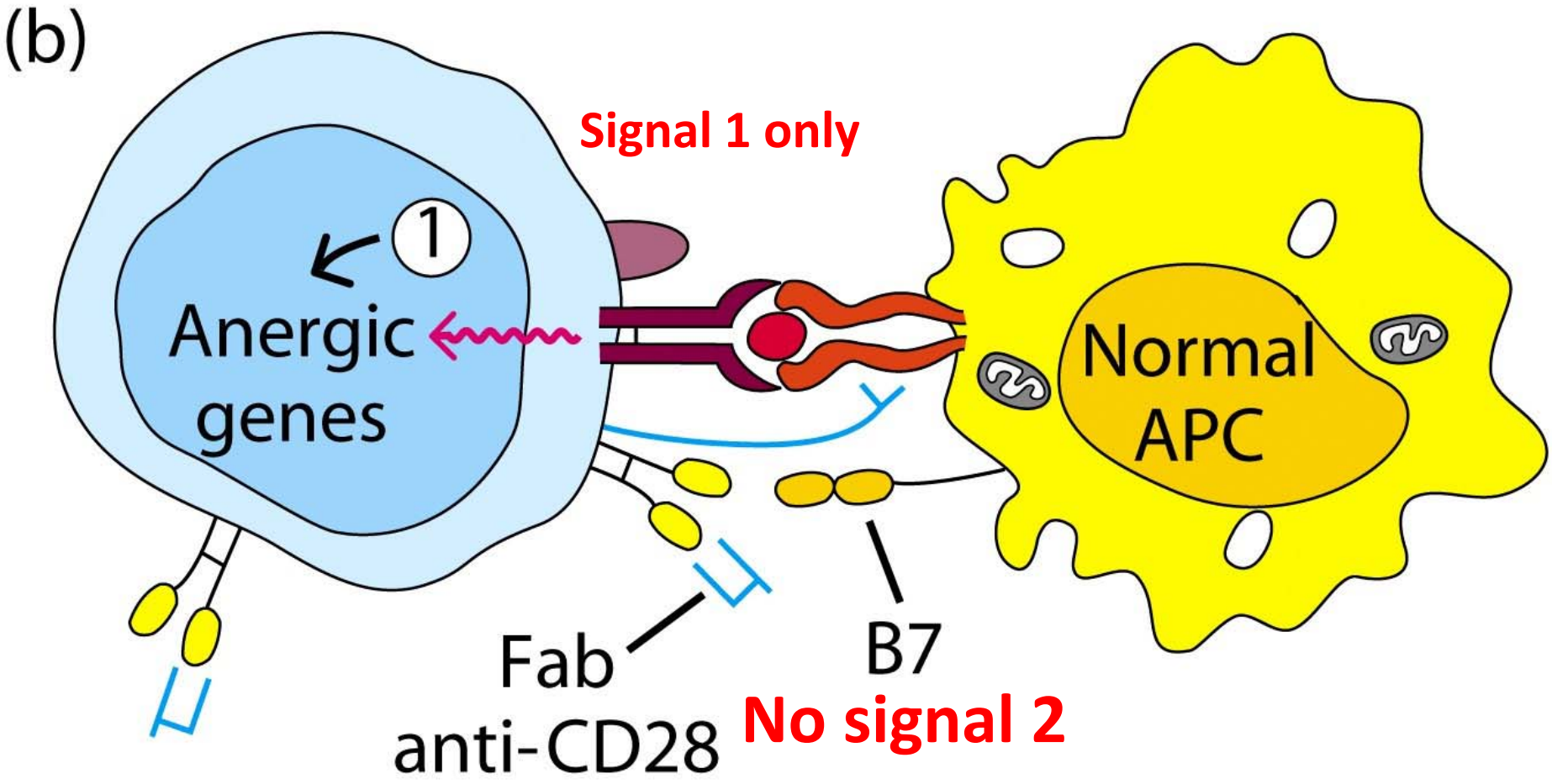


Figure 10-14
Kuby IMMUNOLOGY, Sixth Edition

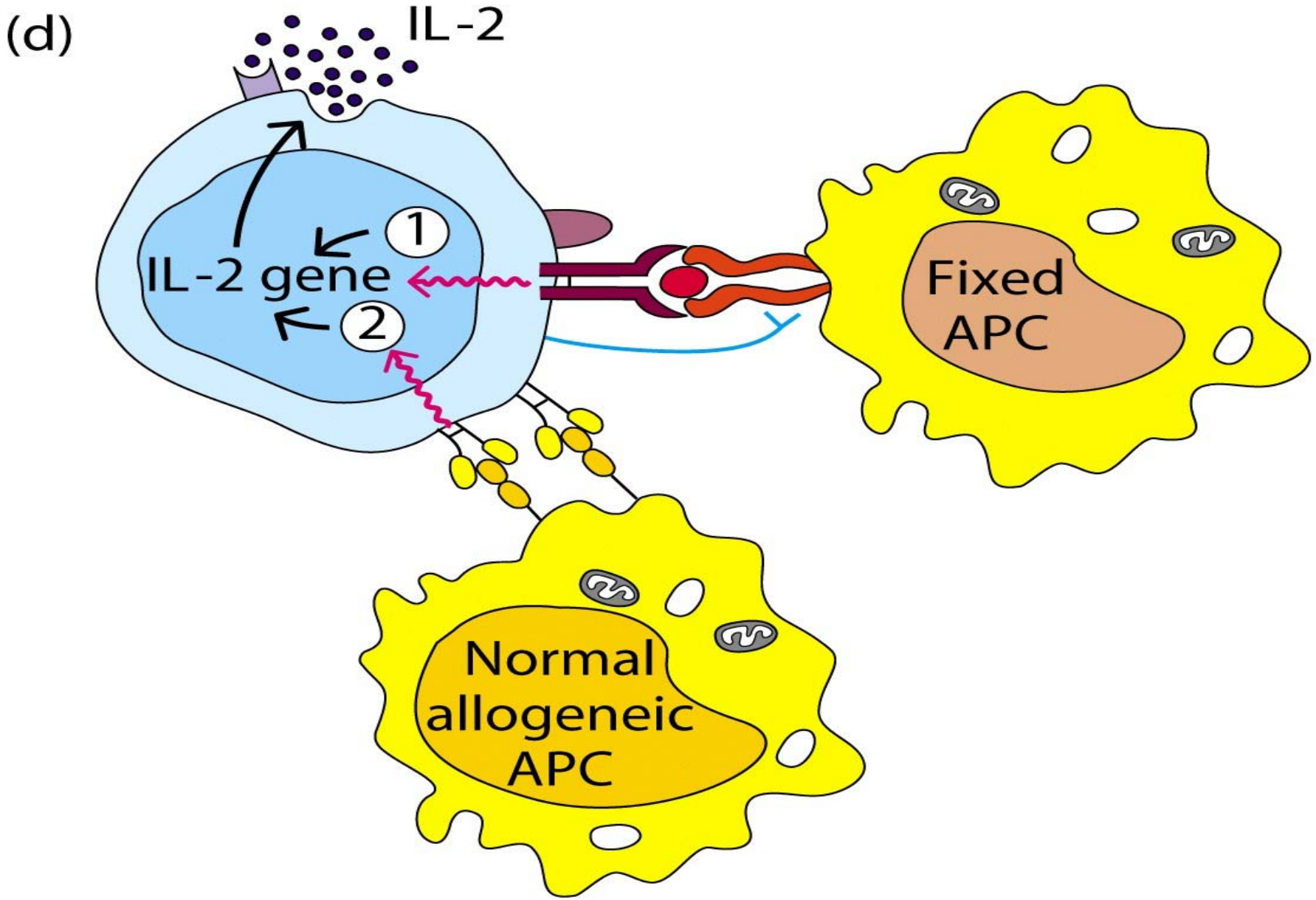
CLONAL EXPANSION vs CLONAL ANERGY= inability of T cell to respond after TCR-MHC/peptide stimulation



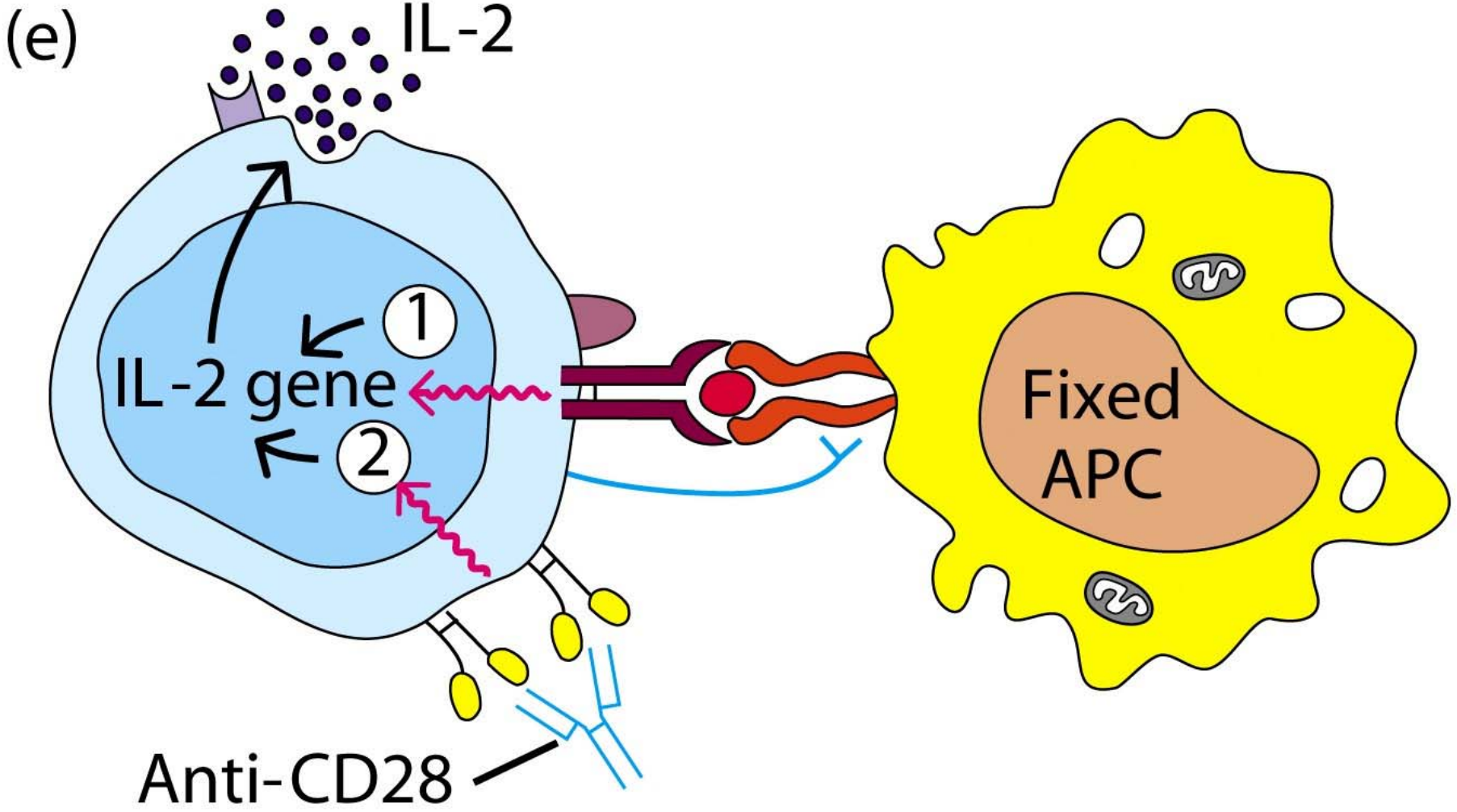
Anergy will develop



Anergy will develop

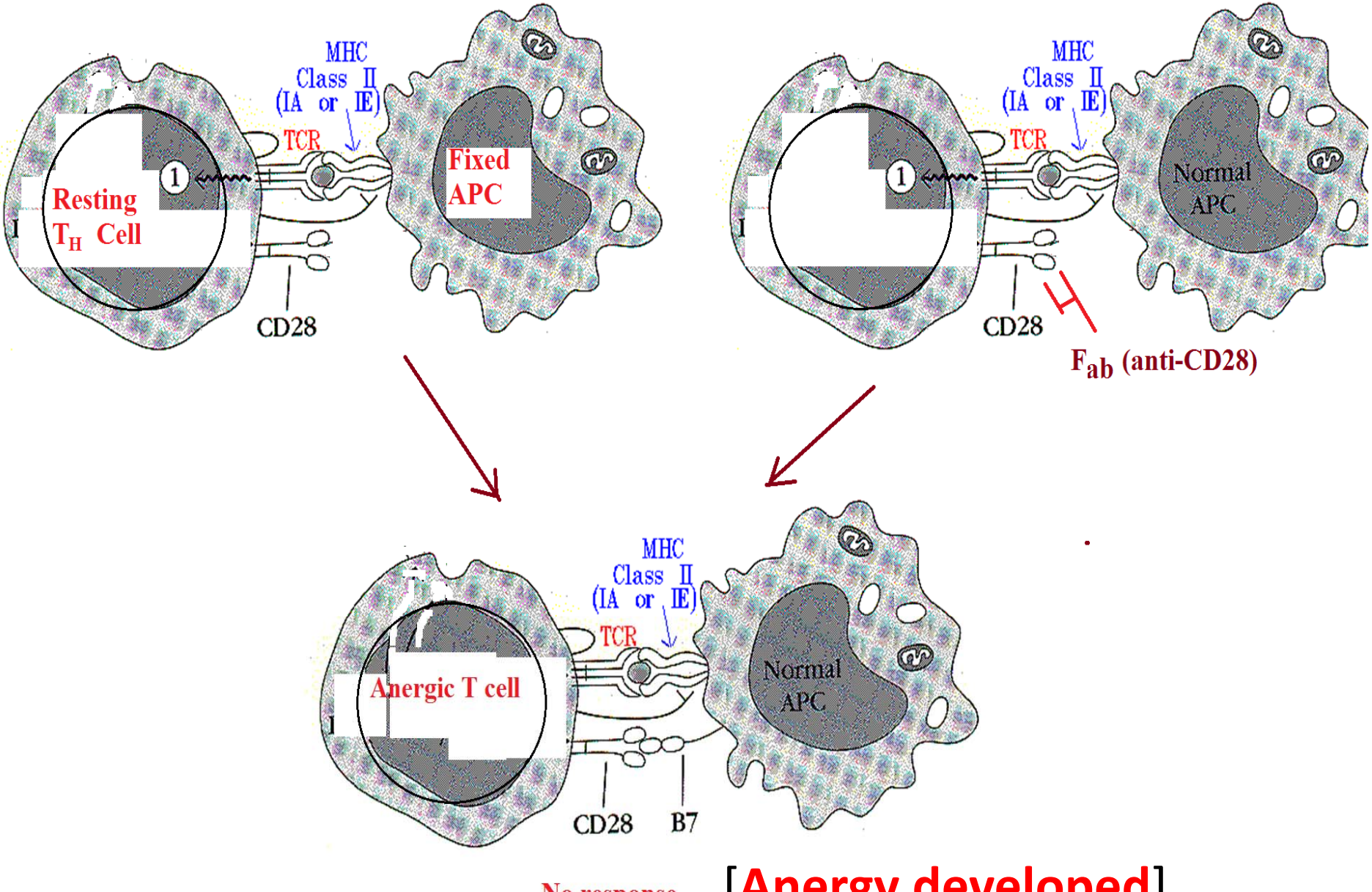


CLONAL EXPANSION!!!!

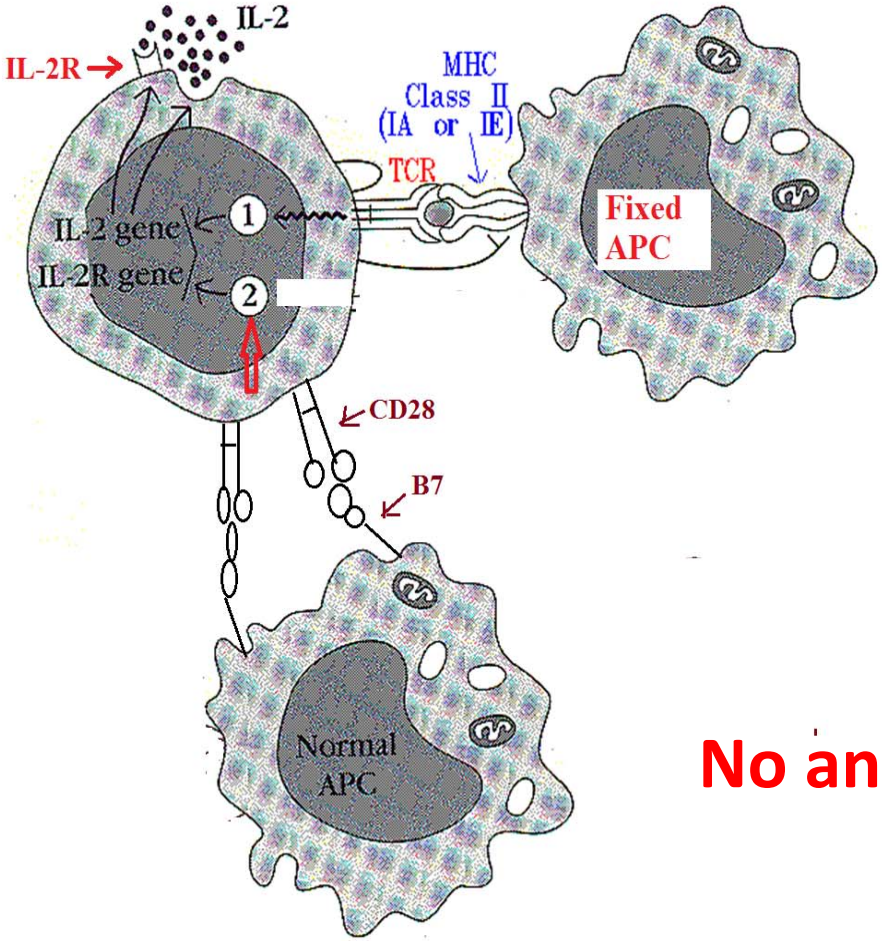


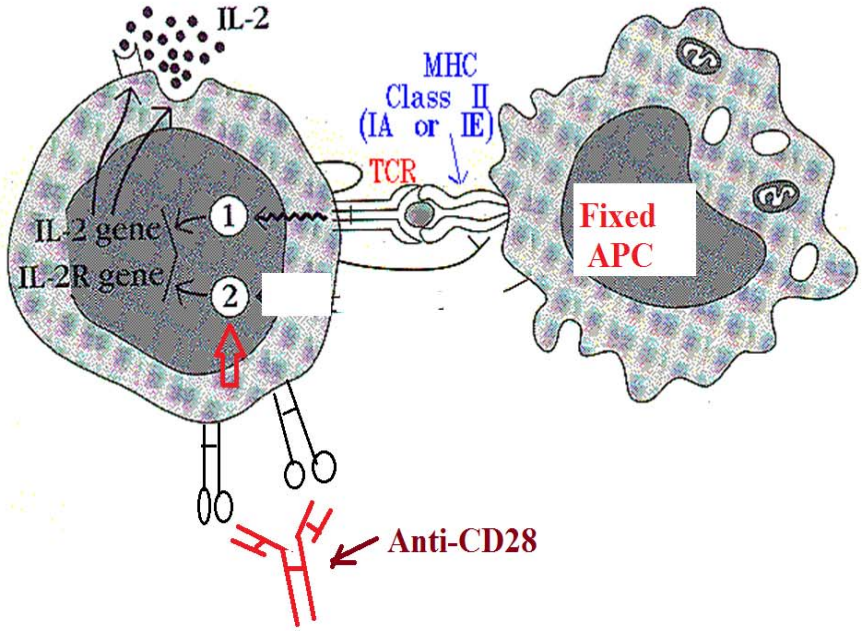
CLONAL EXPANSION!!!!

Clonal anergy:

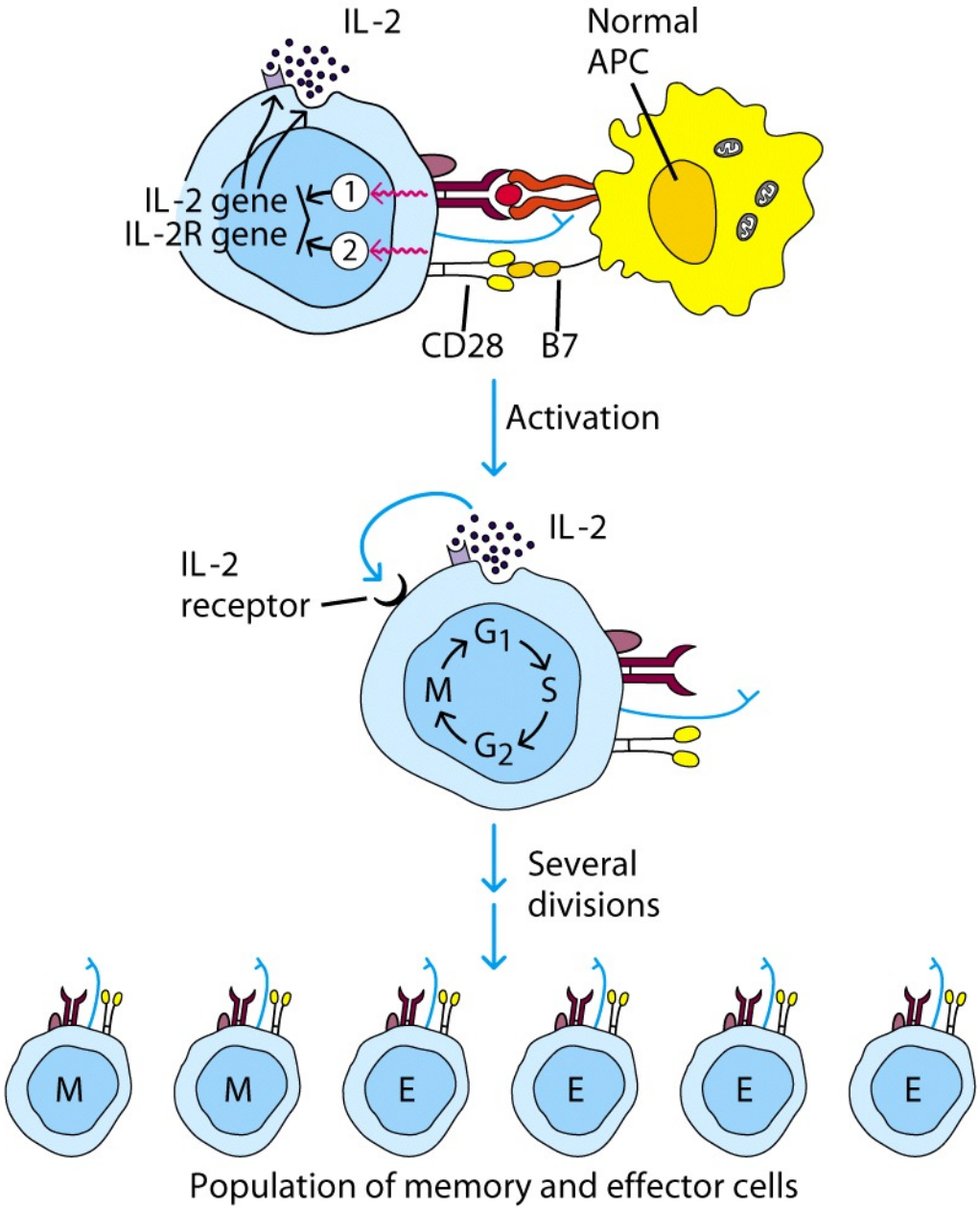


[**Anergy developed**]

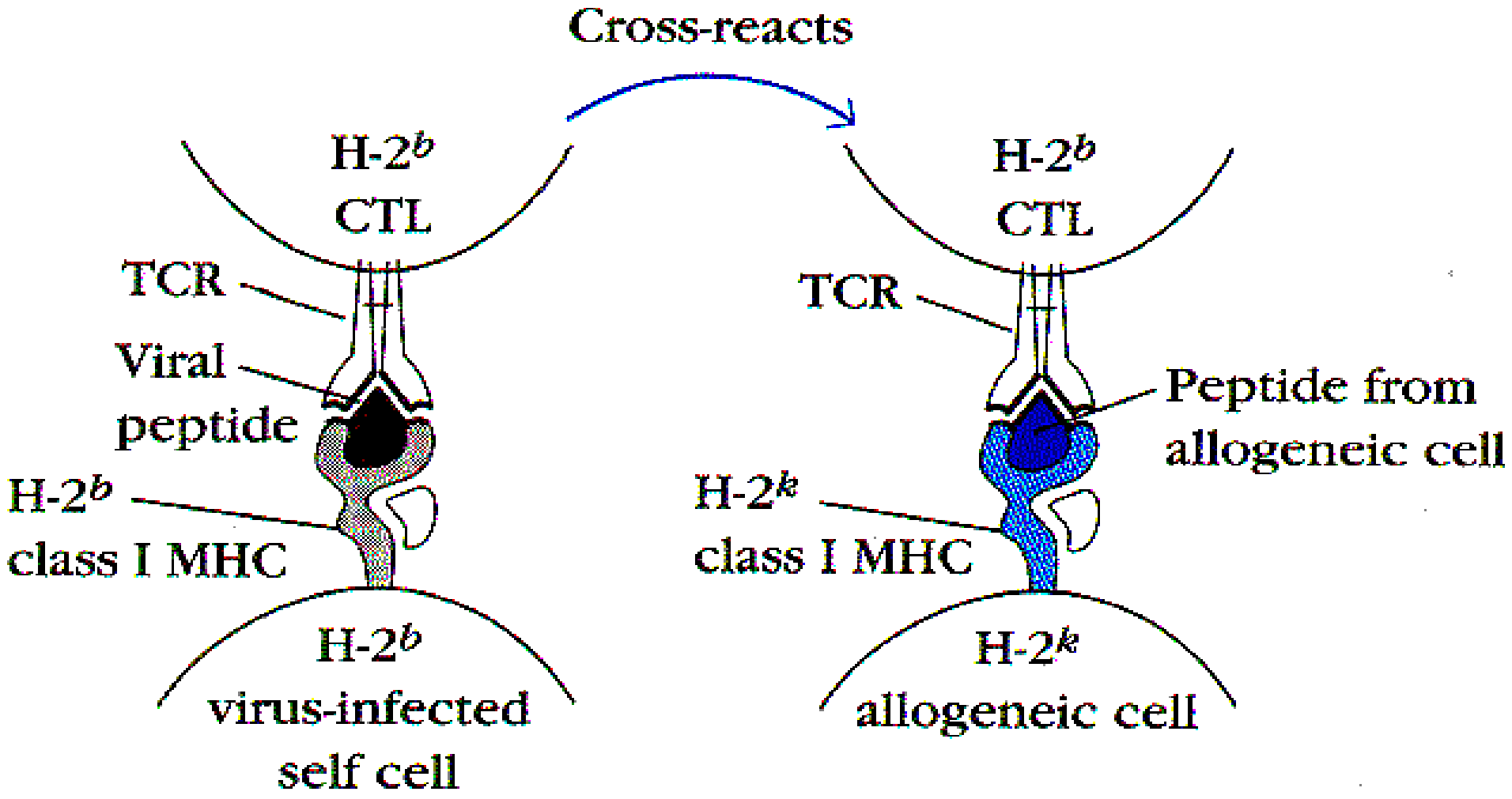




No anergy developed


IL-2
Normal
APC
IL-2 gene
IL-2R gene
1
2
CD28
B7
Activation
IL-2
IL-2
receptor
G1
S
G2
M
Several
divisions
M
M
E
E
E
E
Population of memory and effector cells

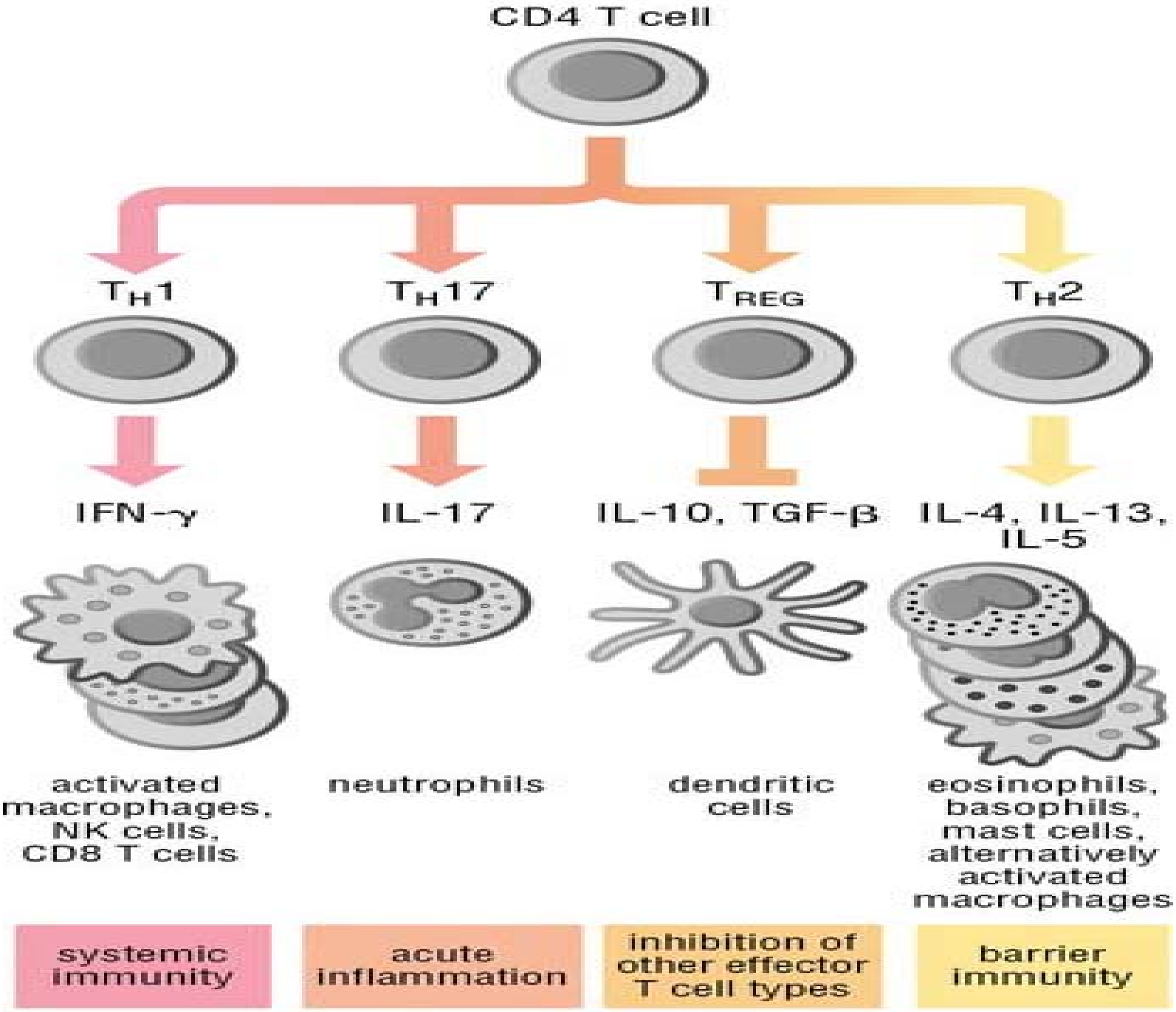
Allo-Reactivity of T-Cells: or Why Do T-cells Frustrate Transplant Surgeons?



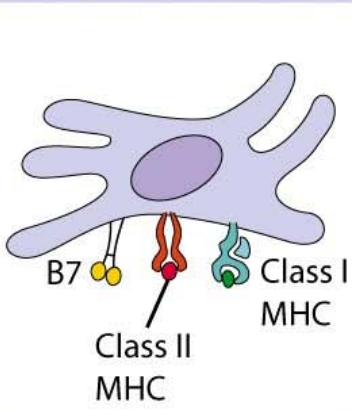
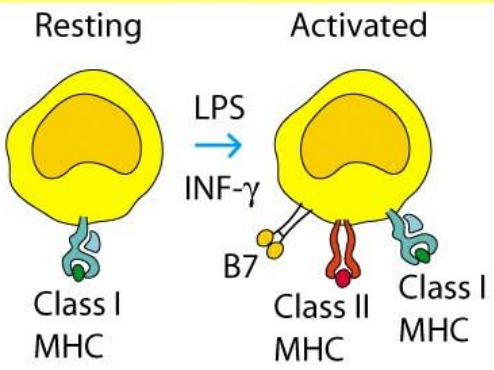
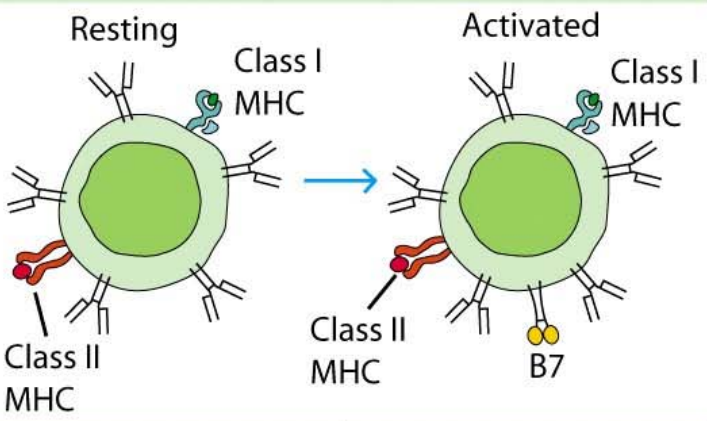
T cell differentiation:

- Naïve T cells continually re-circulate between the blood and lymph system search for appropriate antigen
- Once activated (signal 1 and 2) Primary response where T cells proliferate and differentiate into effector and memory T cells.
- **CD4 effector T cells can form two subpopulations** based on cytokine production: **TH1 subset** (IL-2, IFN-γ) and **TH2 subset** (IL-4, IL-5, IL-10)
- **TH1: associated with cell-mediated functions** inflammation (delayed-type hypersensitivity, activation of CD8 T cells);

TH2: associated with Bcell activation.



Why are DC better APCs?

	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (–)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (–)	Inducible B7 (++)	Inducible B7 (–)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(–)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells