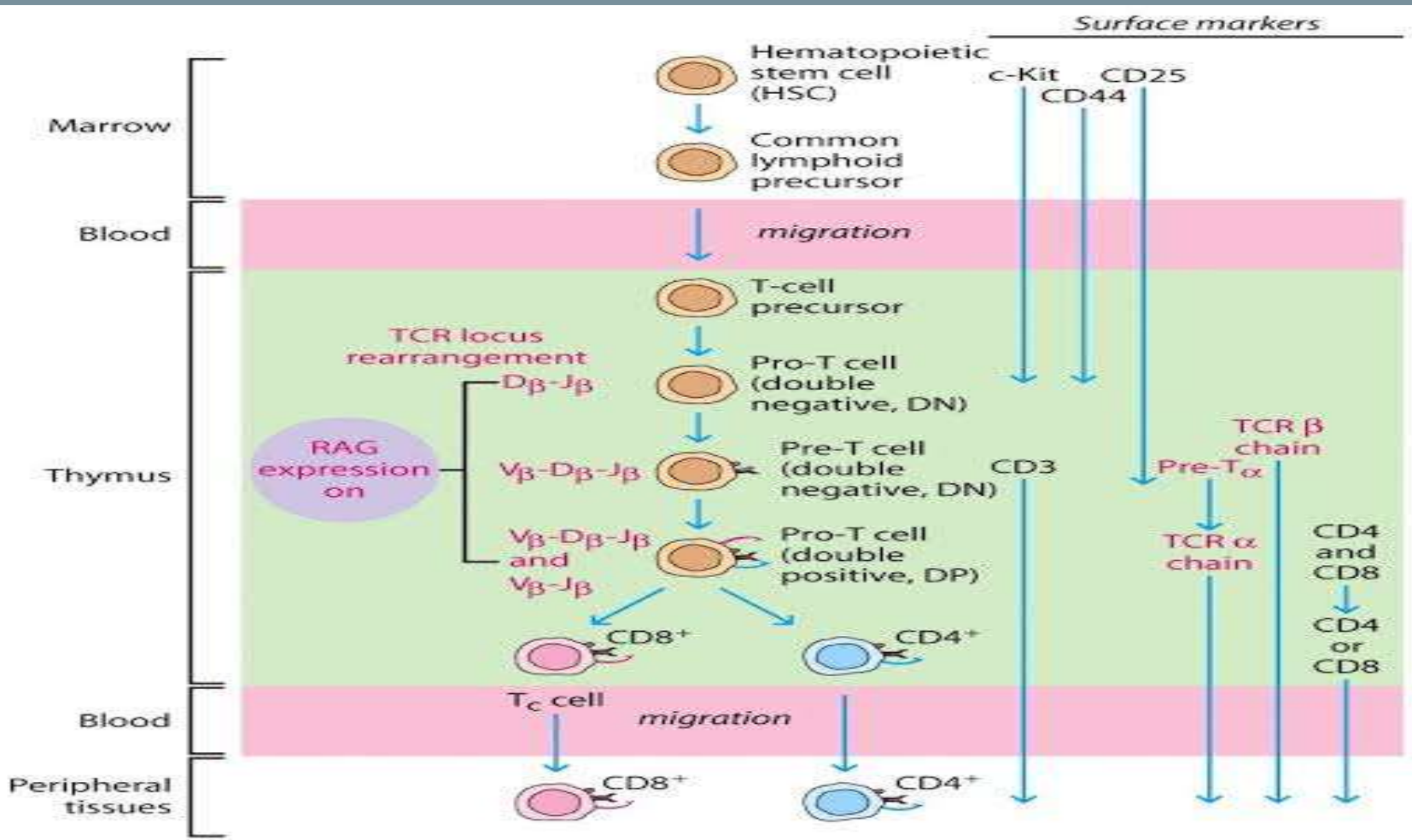
T cell maturation, Activation and Differentiation

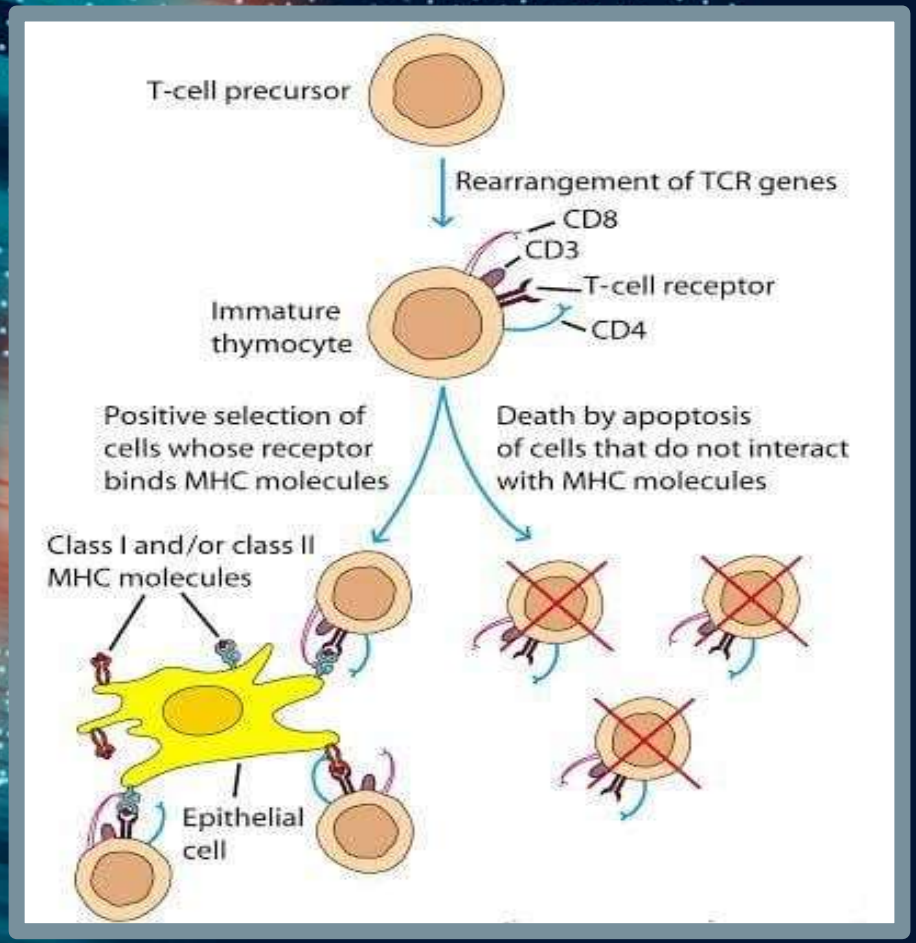
T-Cell Maturation

- Progenitor T cells from the early sites of hematopoiesis migrate to the thymus (Thymocytes)·
- T-cell maturation involves rearrangements of the germ-line TCR genes and the expression of various membrane markers.
- Progenitor T cells lack detectable CD4 and CD8 (double negative (DN) cells) but express c-Kit, CD44, and CD25Most double-negative thymocytes progress down the aß developmental pathway

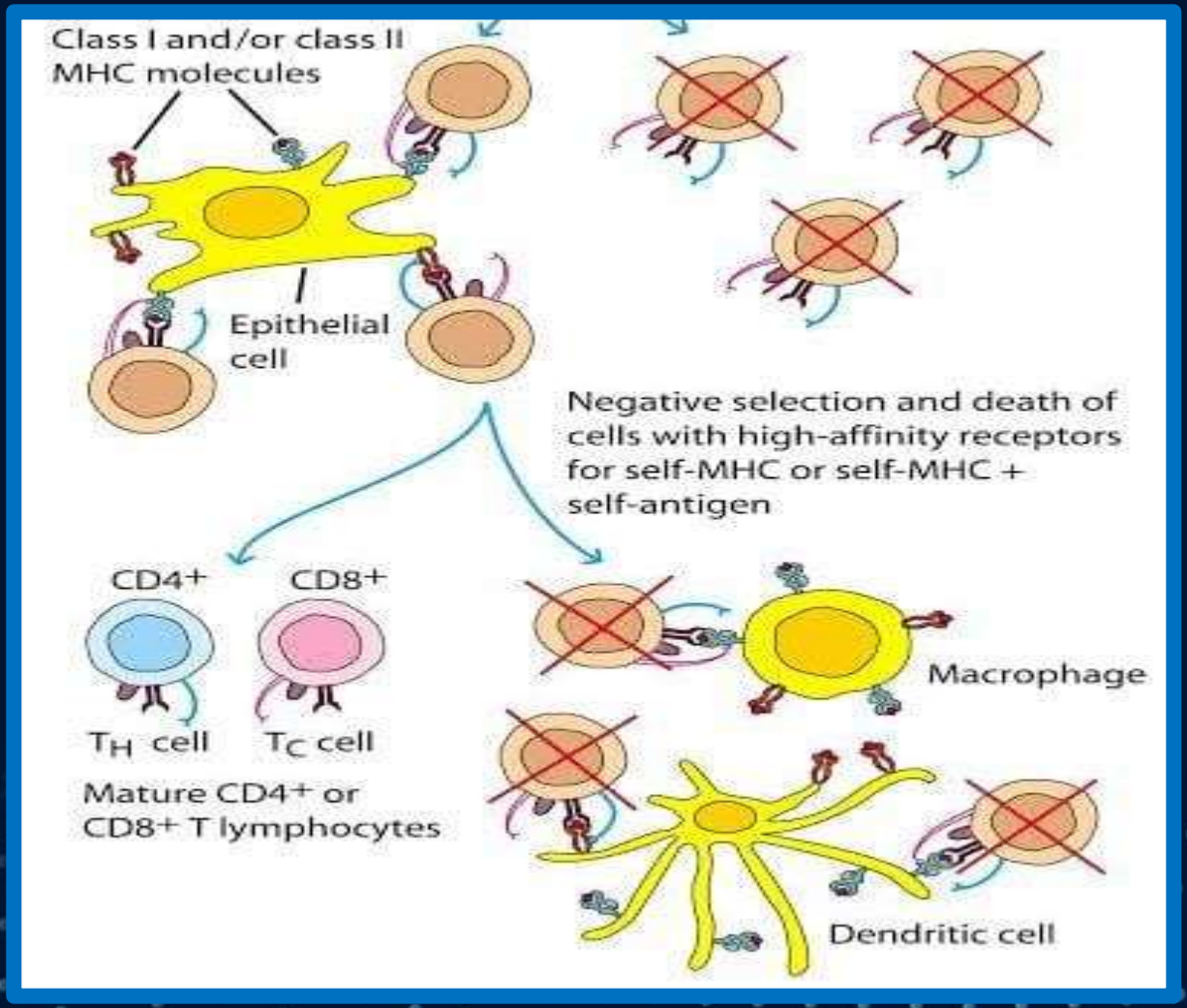
- Stop proliferation and start rearrangement of TCR ß chain genes and express the ß chain.
- Combine with a 33-kDa glycoprotein known as the pre-T a chain and associate with the CD3 group to form a novel complex called the pre-T-cell receptor or pre-TCR· Suppresses further rearrangement of TCR ß chain genes·
- Renders the cell permissive for rearrangement of the TCR a chain·
- Induces developmental progression to the CD4+ 8+ double positive state.
- Survive thymic selection develop into mature single-positive CD4+ thymocytes or single-positive CD8+ thymocytes


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

Thymic Selection



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


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

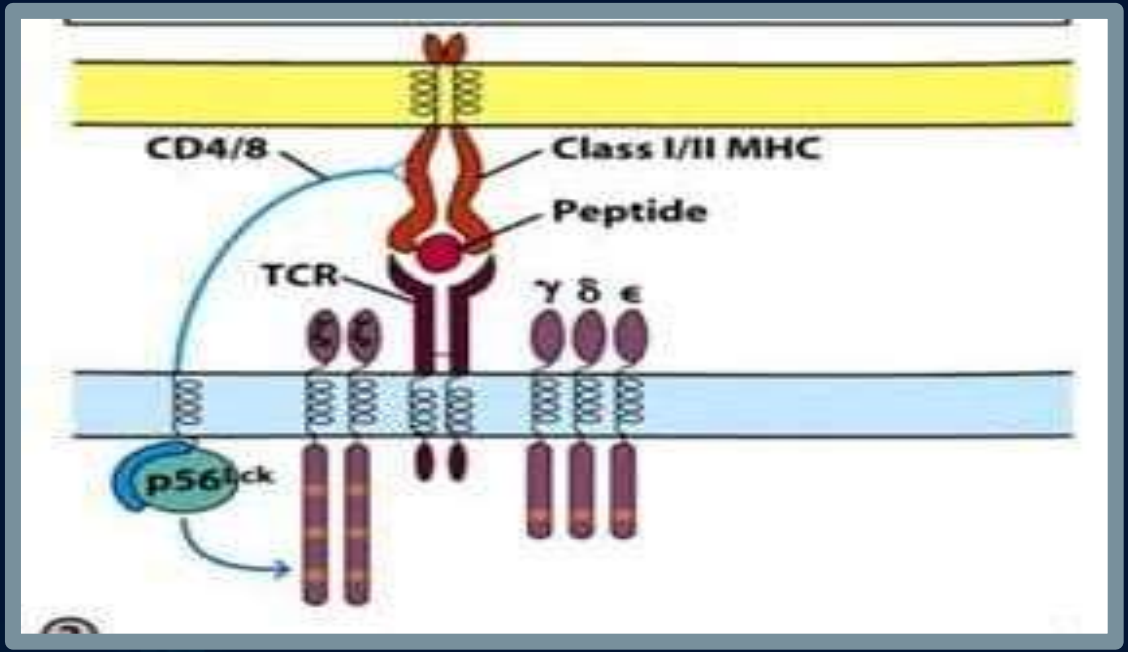
TH cell activation

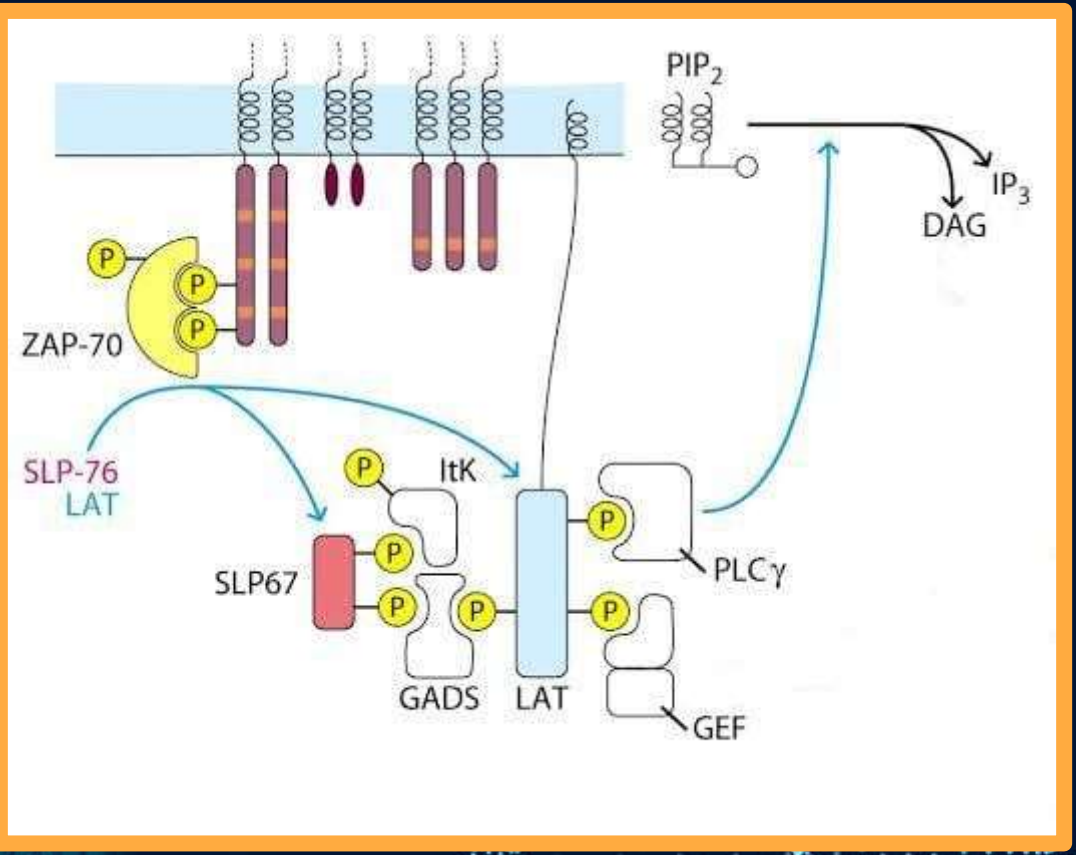
- Expression of genes:

  - Immediate genes (within 1 hr)
  - Early genes (within1-2 hrs)
  - Late genes (after 2 days)

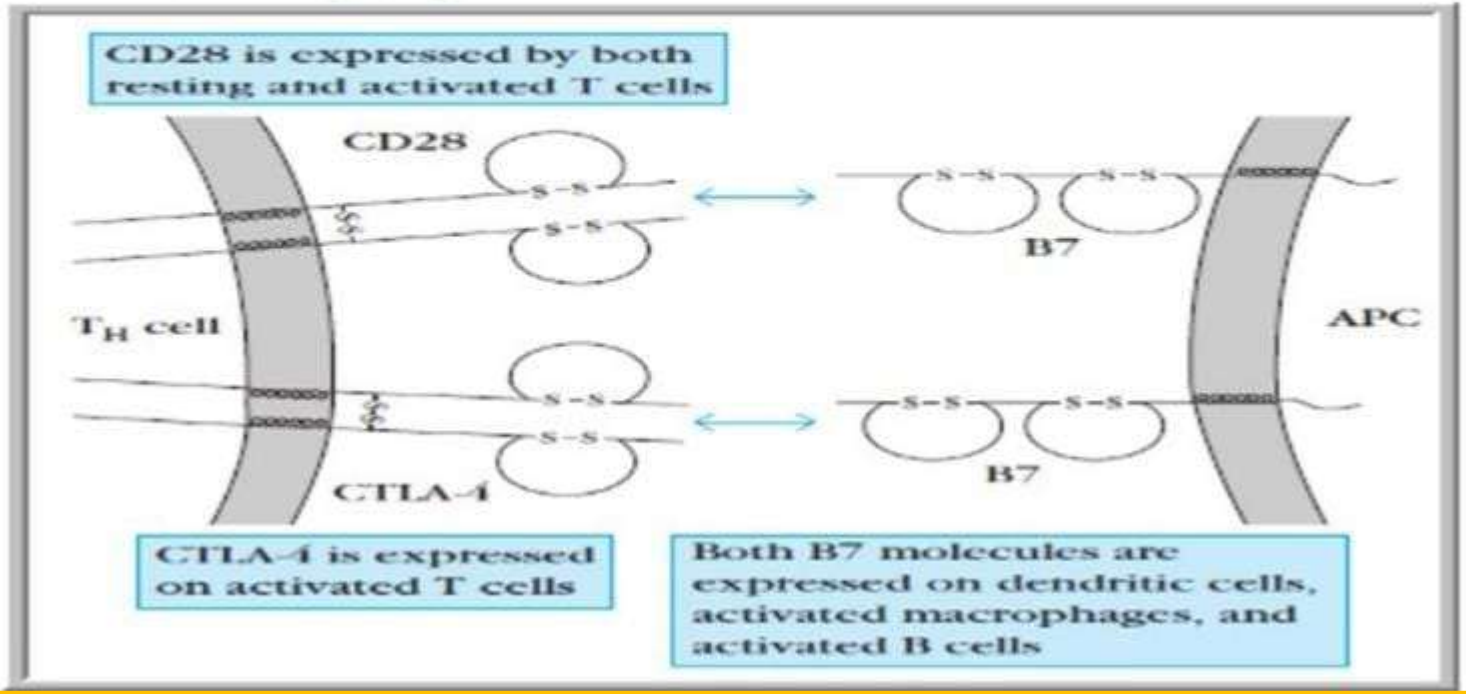
- Signalling pathway: Fyn and Lck protein kinases phosphorylate Tyr residues in ITAM motif→ these are docking sites for ZAP70 (at chain)

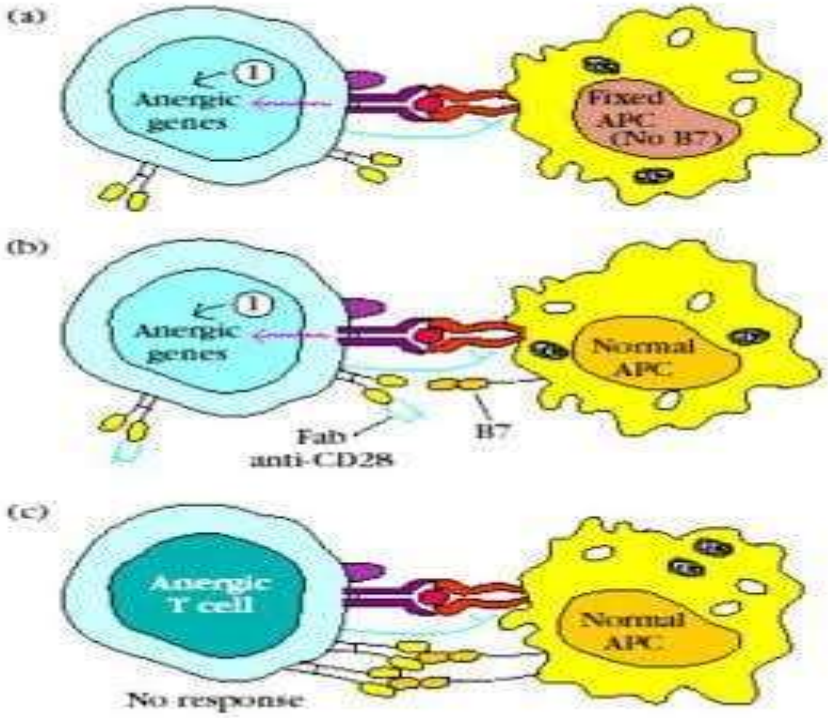
Signaling Molecule

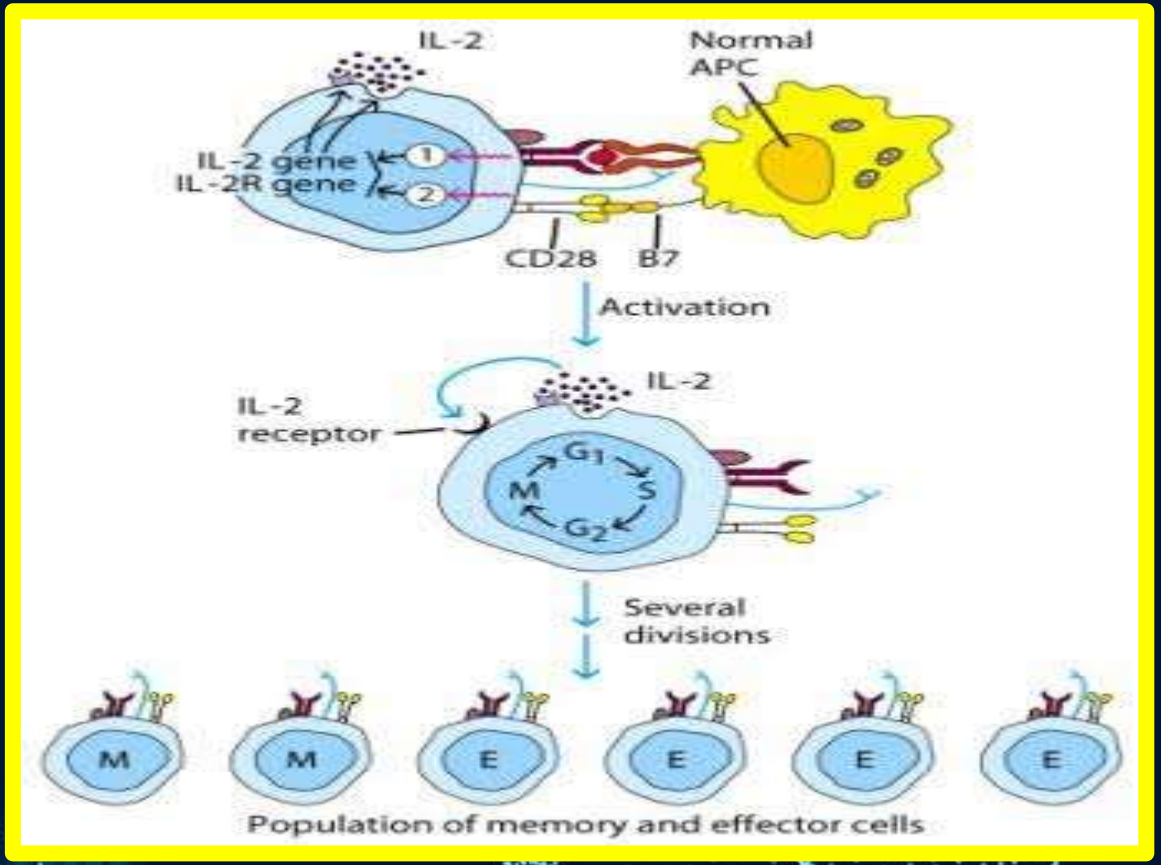


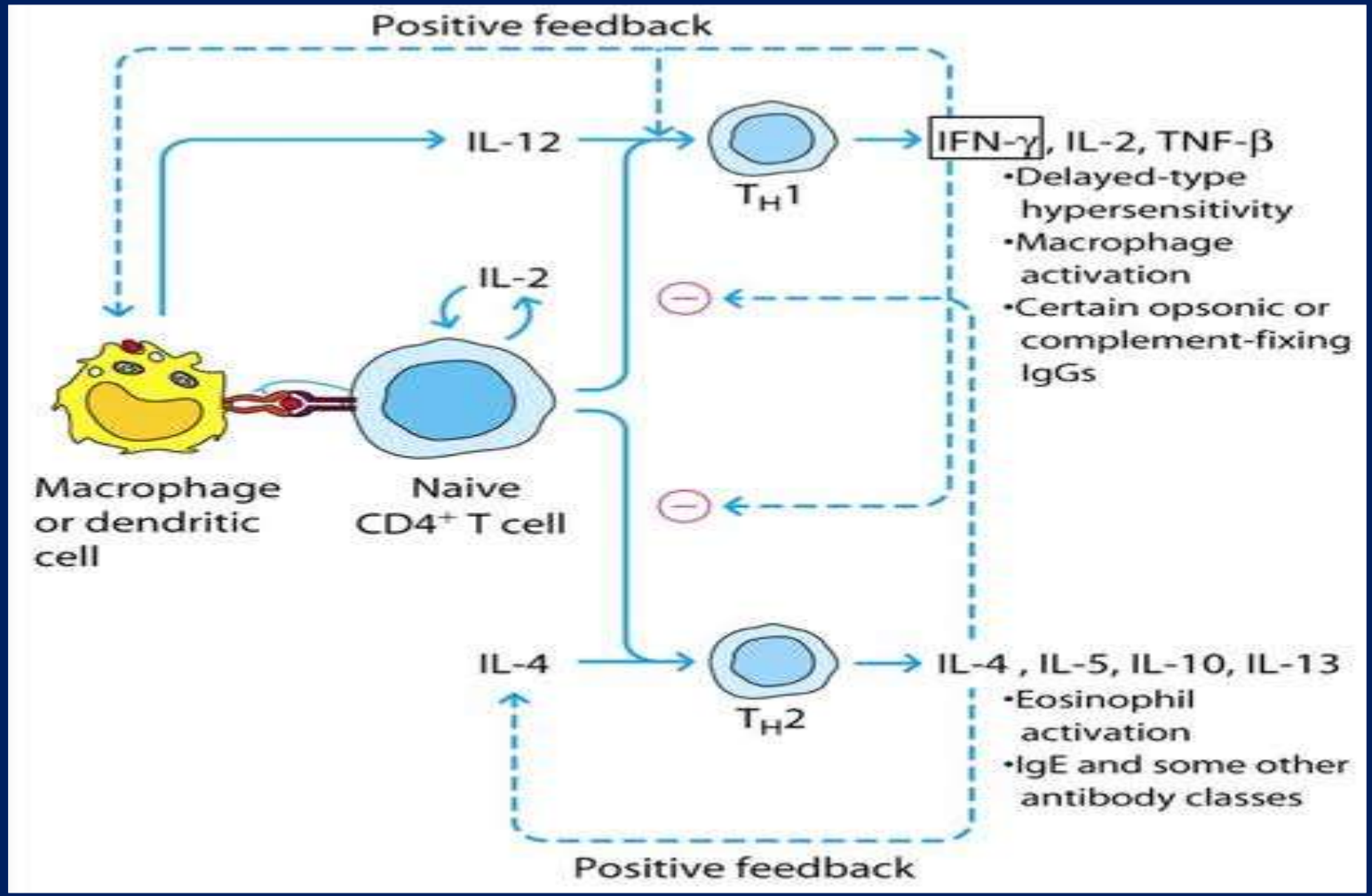

PIP2
IP3
DAG
P
ZAP-70
SLP-76
LAT
ItK
SLP67
PLCγ
GADS
LAT
GEF

- Key players:
  - CTLA-4/CD28
  - B7 family of proteins




(a)
Anergic genes
Fixed APC (No B7)
(b)
Anergic genes
Normal APC
Fab anti-CD28
B7
(c)
Anergic T cell
Normal APC
No response


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


Positive feedback
IL-12
T_H1
IFN-γ, IL-2, TNF-β
•Delayed-type hypersensitivity
•Macrophage activation
•Certain opsonic or complement-fixing IgGs
IL-2
Macrophage or dendritic cell
Naive CD4+ T cell
IL-4
T_H2
IL-4 , IL-5, IL-10, IL-13
•Eosinophil activation
•IgE and some other antibody classes
Positive feedback

Cell Mediated Immune Responses

Primary Function Of Cell Mediated Response

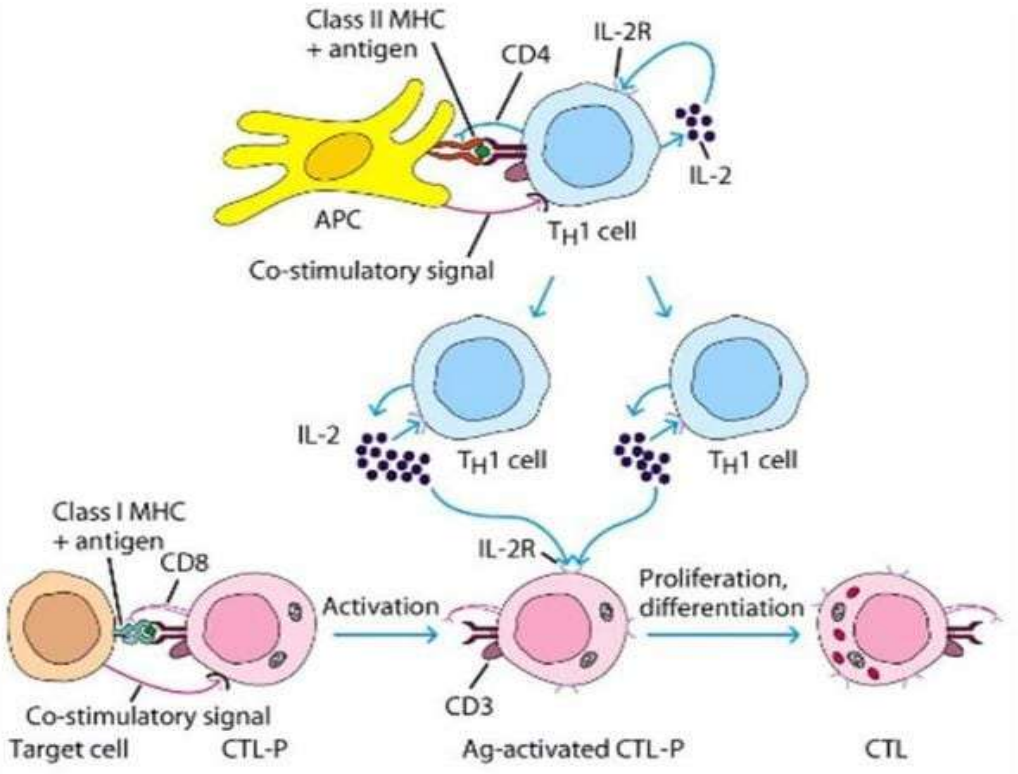
- Eliminate Intracellular Pathogens
- - Eliminate Tumor Cells·
- Both Ag Specific And Non-specific cells Are Involved - Ag Specific: CD8+ Cells (T) And TH (DTH) - Non-specific: Macrophages. Neutrophils. NK·
- Both Specific And Non-specific Require Cytokines· Humoral And Cell Mediated Do Collaborate
- - Ex. Macrophages use Abs as receptors to recognize target cells

Cell Mediated Immunity can be divided into 2 major categories

- - Effectors lyse target· 2 groups of cells: CTLs (specific) and NK.
- Macrophages (non specific)
- - Effectors which are CD4+ and mediate DTH
- Types of Effector cells: CD4+ (THI and TH_2) and CD8+ (CTLs)

Cytotoxic T Cells

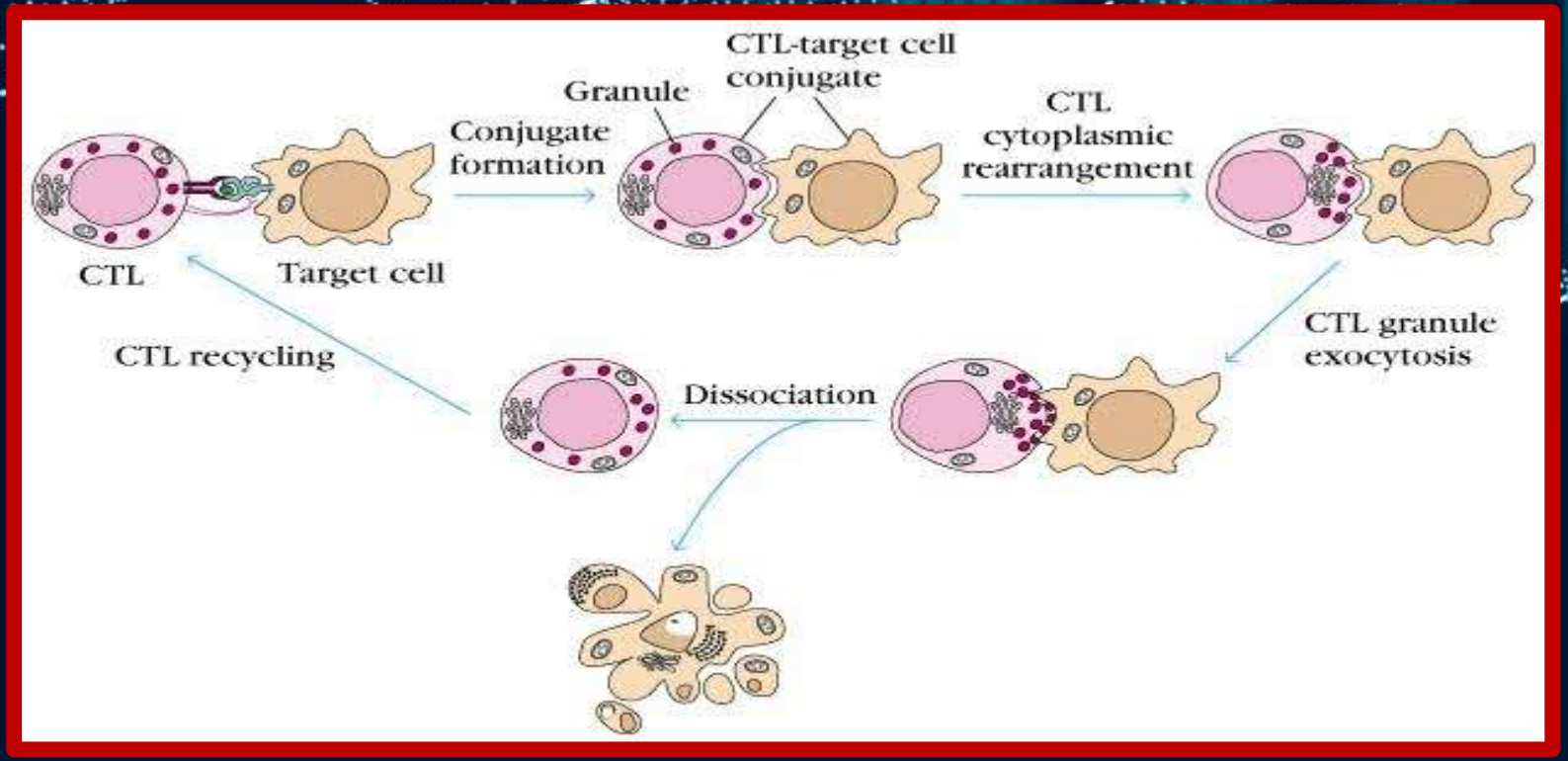
- CTLS recognize cells that have been infected
- Virus- Transformed to tumor. CTL activation is divided into 2 phases
- - Activation and differentiation of naive CTL
- - Effector recognizes Class I MHC/peptide and destroys target
- Naive CTLs cannot kill- Referred to as CTL-Ps (precursors)- 3 signals needed for activation•
- Ag specific signal through TCR/MHC I + Ag Co-stimulatory signal CD28(CTL) B7 (APC)•
- IL-2 signaling inducing proliferation (CTL-P do not express IL-2 R)IL-2 is provided by TH or CTL
- -P itself
- - IL-2R is expressed only after activation


Class II MHC
+ antigen
CD4
IL-2R
IL-2
APC
TH1 cell
Co-stimulatory signal
IL-2
TH1 cell
TH1 cell
Class I MHC
+ antigen
CD8
IL-2R
Activation
Proliferation,
differentiation
CD3
Co-stimulatory signal
Target cell
CTL-P
Ag-activated CTL-P
CTL

How CTLS Kill?

4 Phases in CTL killing

- Conjugate formation
- LFA-1 (CTL) binds ICAMs (Target)- LFA-1 changes to high avidity if Ag Is Recognized
- Activated LFA-1 persists for 5-10 mins
- Membrane attack Requires Ca and energy Granules release Perforins (65 kDa) and Granzymes (serine proteases) at the junctional space
- Perforins polymerize forming cylindrical pores (5-20 nm).
- Ca is needed Granzymes enter target cell
- Granzyme B can enter through mannose-6-phosphate receptor in a vesicle
- -DNA fragmentation
- CTL dissociation
- Target cell destruction
- Apoptotic death within a few hours:


CTL-target cell conjugate
Granule
Conjugate formation
CTL cytoplasmic rearrangement
CTL
Target cell
CTL granule exocytosis
CTL recycling
Dissociation

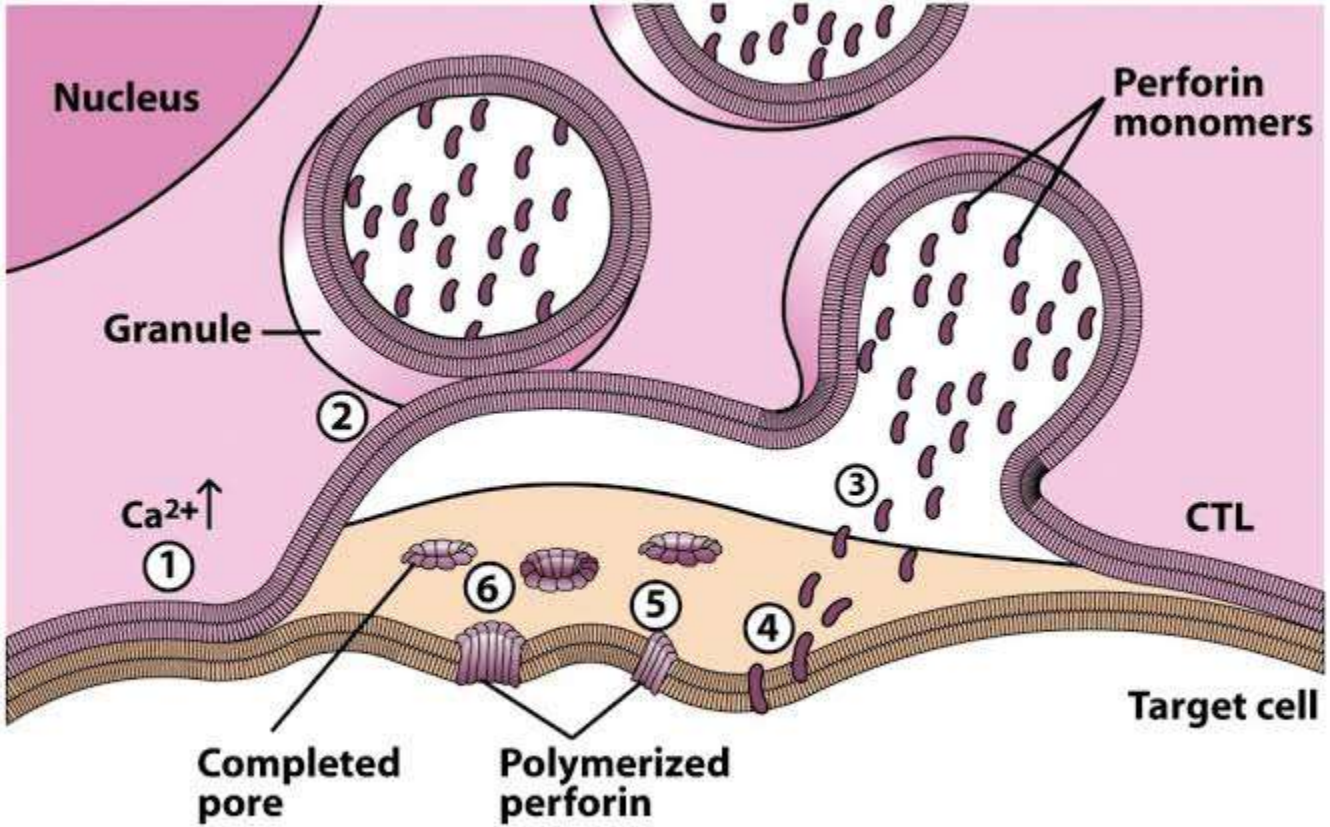


Figure 14-9a
Kuby IMMUNOLOGY, Sixth Edition

Fas L Mediated Cytotoxicity

- Some CTLs lack granzyme and perforin
- They kill using FasL-Fas interaction

1. - Fasl is found on CTLS
2. - Fas is found on target cell
3. - FasL-Fas interaction induces apoptosis·

- 2 Mechanisms are responsible for CTL induced apoptosis

1. - FasL-Fas (FADD Activation leading to pro-caspase 8 activation)
2. - Perforin and granzyme
3. - During apoptosis caspases (cysteine proteases that cleave aspartic acid) are activated
4. - Family of more than 12 caspases exist
5. - Activation of caspases results in orderly destruction of target cell

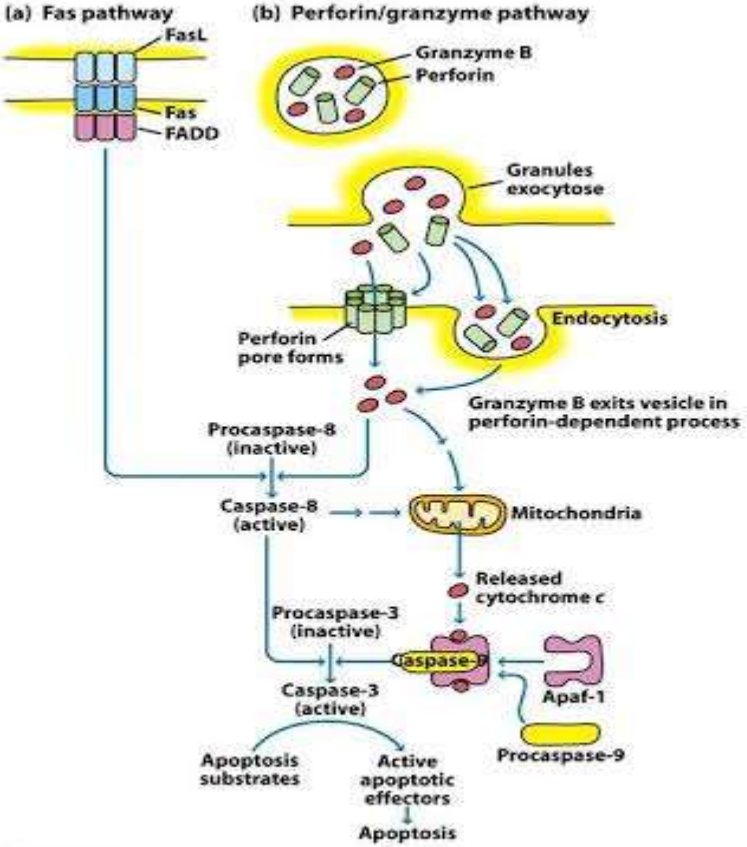


Figure 14-11
Kuby IMMUNOLOGY, Sixth Edition

Natural Killer Cells

- NK make up 5-10% of circulating lymphocytes
- - Major producers of IFNY
- - Through IFNy they influence innate immunity (Macrophage)
- - They also influence adaptive, favor THI and Eliminate viruses and tumor cells
- Early Responders to Viral Infections.
- - IFNG and IFNB produced by virus infected cells Stimulates Nk cell activity
- - IFNY production induces macrophages to make IL-12- IL-12 results in more IFNy pushing towards THI
- - TH1 through IL-2 induces CTL activation

- NK eliminate target cells same way CTLS
- Through perforin/granzyme and FasL/Fas
- However they are different from CTLS
- - No Ag Specific TCR
- - No CD3- No MHC restriction
- - No memory, same intensity regardless of repeated exposure

Target Recognition

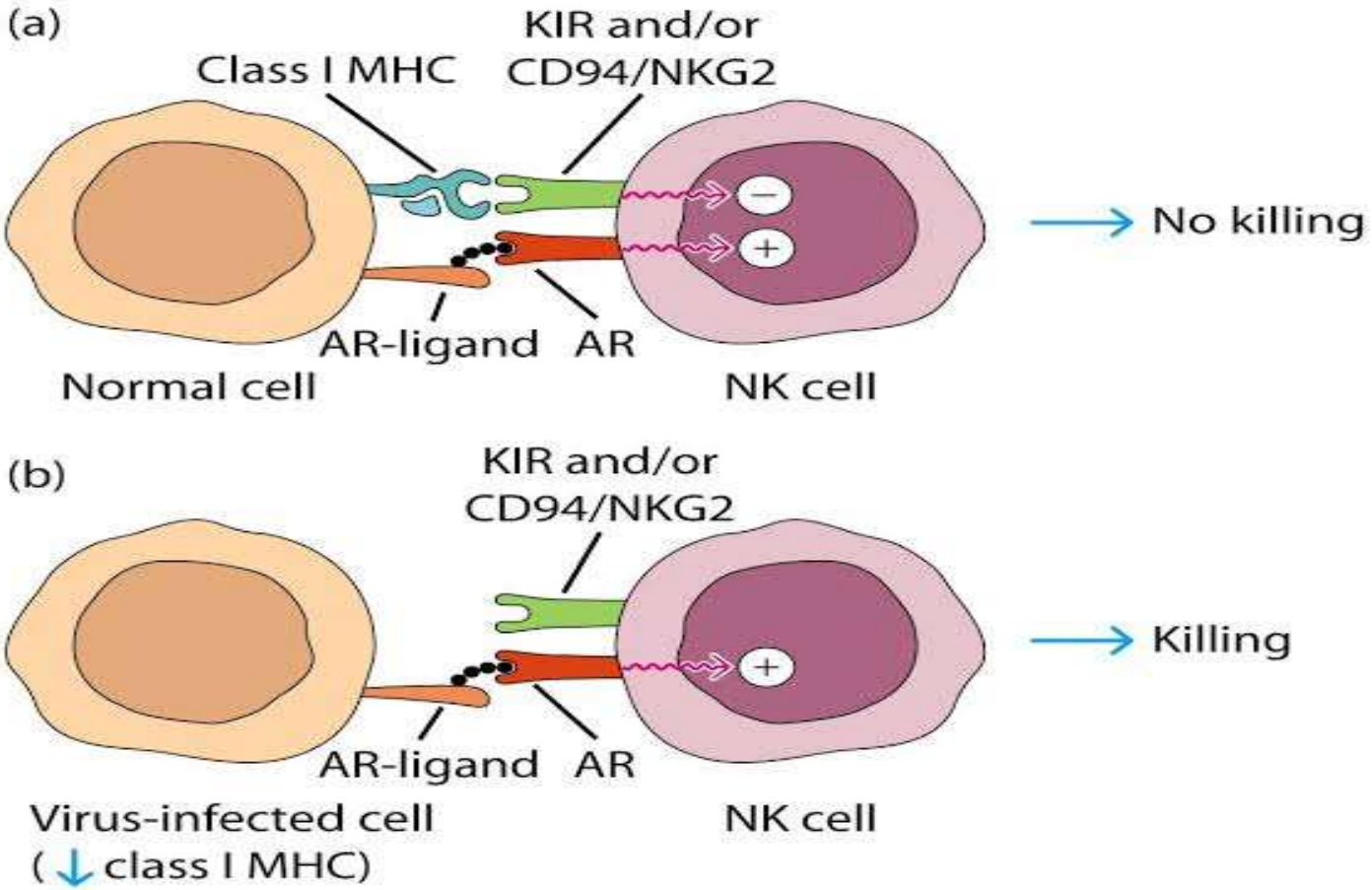
- Balance between activating and inhibiting molecules allows NK cells to differentiate normal from altered.

- Still not clear what the activating receptors are

- C-type lectins are candidates·

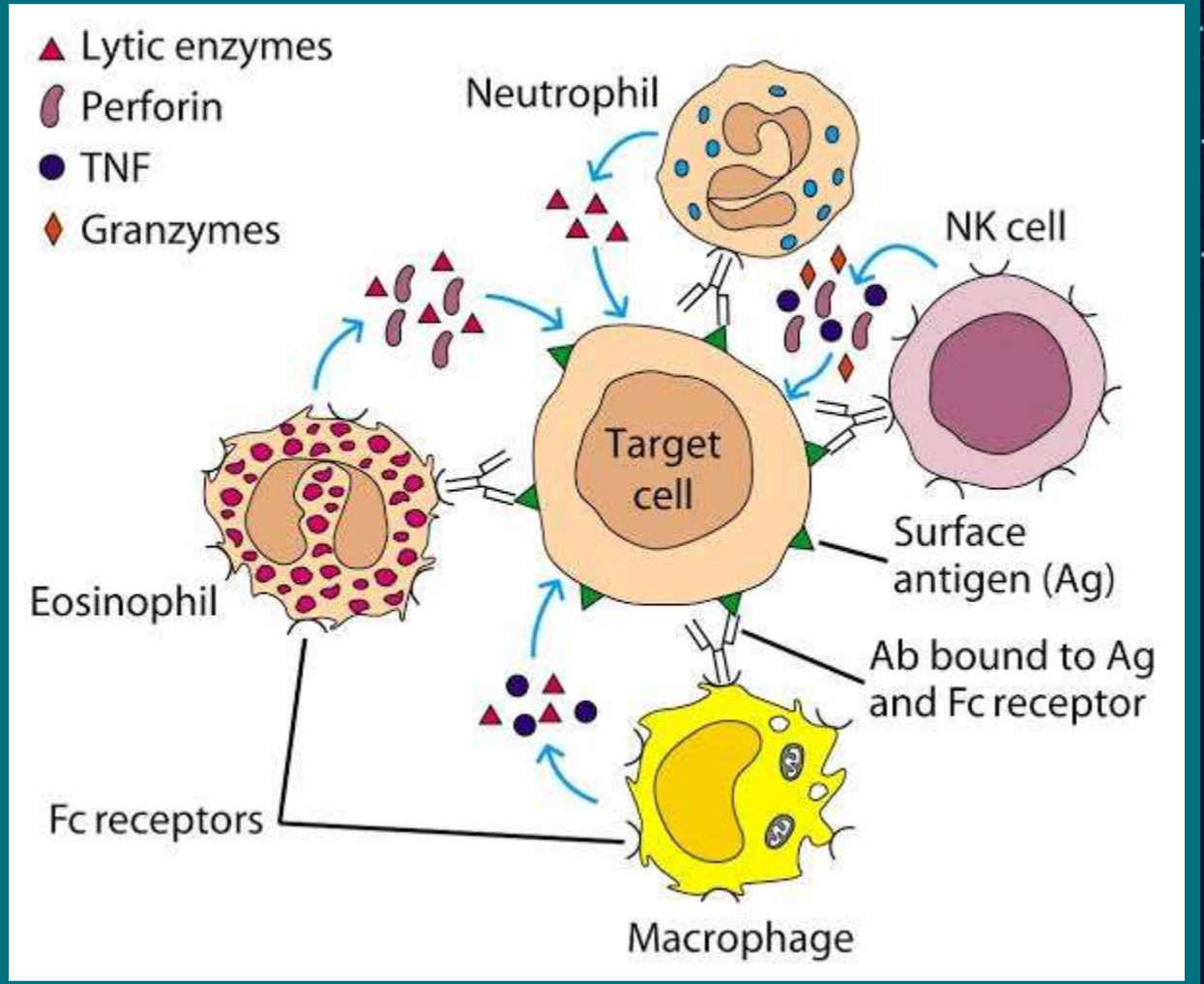
  - NKR-PIC
  - CD2 ((receptor for the adhesion molecule LFA-3)
  - CD16 (FeyRIII, Involved In Antibody Mediated Recognition)
  - NKp30.
  - NKp44 and NKP46

- Inhibitory Receptors
- MHC Molecules
- CLIR -CD94/NKG2 and KIRS recognize HLA-E


(a)
KIR and/or
CD94/NKG2
Class I MHC
No killing
AR-ligand
AR
Normal cell
NK cell
(b)
KIR and/or
CD94/NKG2
Killing
AR-ligand
AR
Virus-infected cell
(↓ class I MHC)
NK cell

Antibody Dependent Cell Mediated Cytotoxicity (ADCC)

- Cells capable of cytotoxicity express Fc receptors.
- Antibody binds target cell, cytotoxic cells bind Fc portion of Ab
- Antibody provides the specificity
- Examples of cells capable of ADCC
- Macrophage, NK, Neutrophils, eosinophils
- Killing of target is accomplished
- Through perforin, granzyme (NK, Eosinophils) TNF (Macrophage, NK)- Lytic enzymes (Macrophage, Neutrophils, Eosinophils, NK)


Lytic enzymes
Perforin
TNF
Granzymes
Neutrophil
NK cell
Target cell
Surface antigen (Ag)
Eosinophil
Ab bound to Ag and Fc receptor
Fc receptors
Macrophage

THANKS!