T cells and T cell-mediated immune responses

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Protective Immunity to Microorganisms

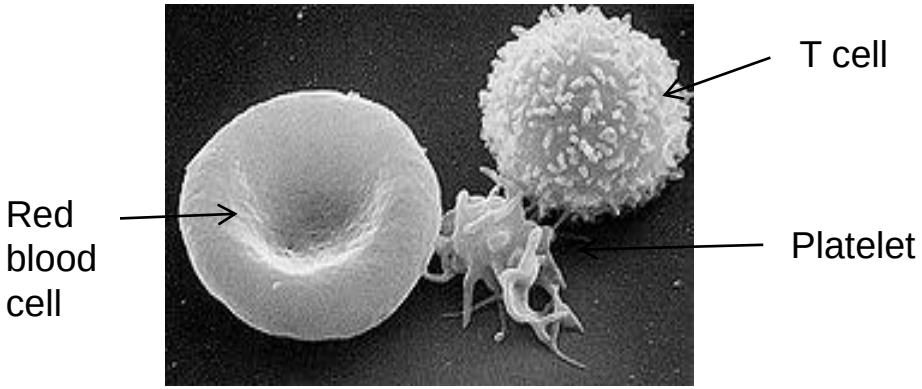
Defense against microbes is mediated by:

Innate immunity and acquired immunity

Through both

- **Humoral immunity (antibodies)**
  (B cells, antibodies, complements, some antimicrobial peptides..)
- **Cell mediated immunity (CMI)**
  (T cells, macrophages, NK cells…)

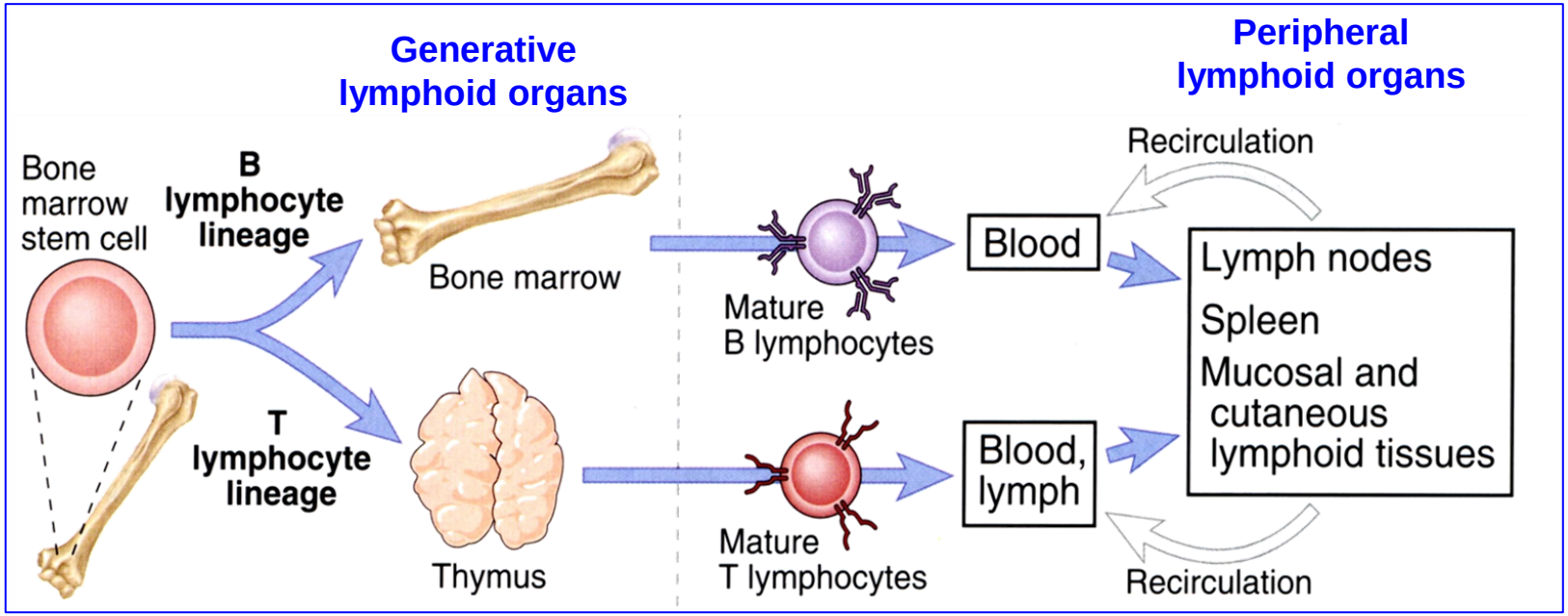
I: T Lymphocytes / T cells



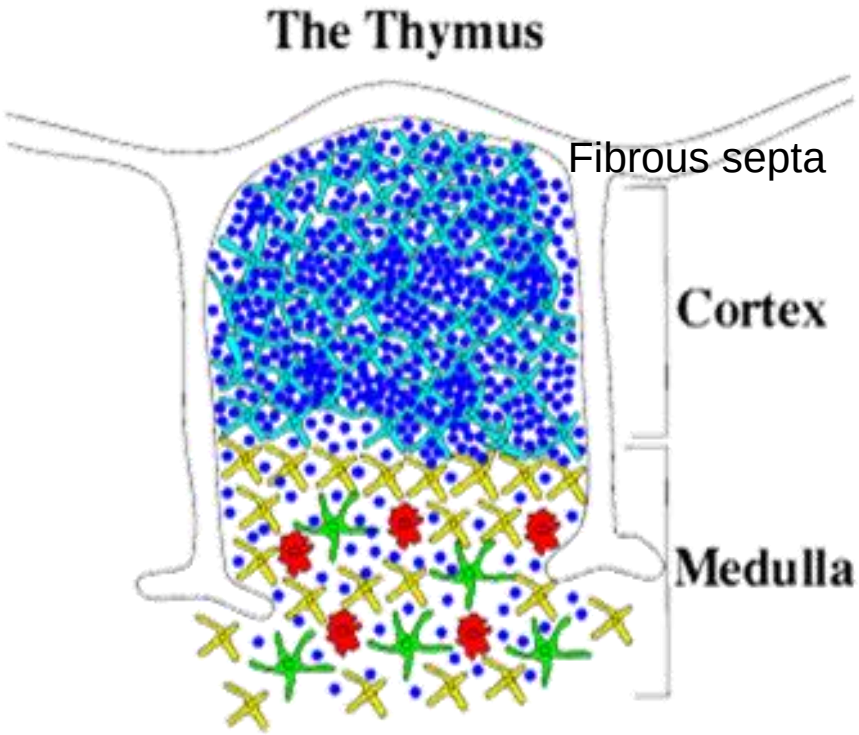
- The abbreviation *T*, in *T cell*, stands for thymus, since this is the principal organ responsible for the T cell's maturation
- T cells play a central role in cell-mediated immunity **
- They can be distinguished from other lymphocyte types, such as B cells or NK cells by the presence of a special receptor on their cell surface called T cell receptor (TCR)
- Several different subsets of T cells have been discovered, each with a distinct function

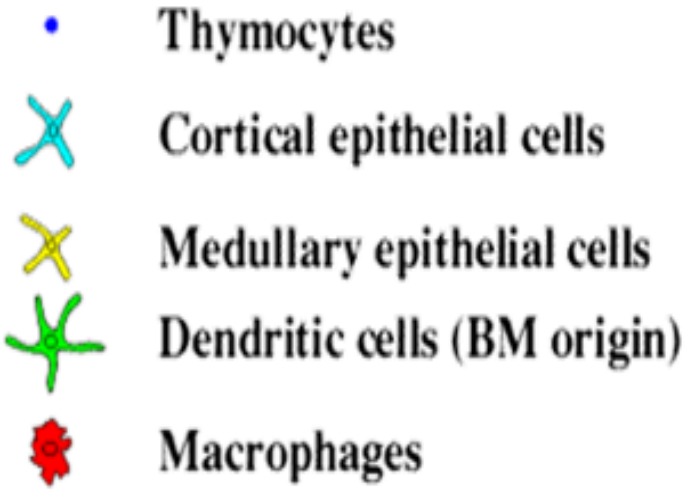
1: T Cell Ontogeny

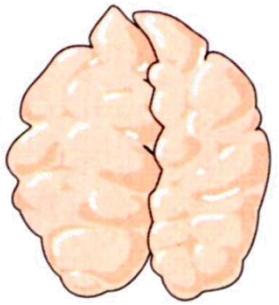
- All T cells originate from hematopoietic stem cells (HSC) in the bone marrow
- Hematopoietic progenitors derived from HSC populate the thymus and expand by cell division to generate a large population of immature thymocytes
- T cell's proliferation, differentiation, maturation and apoptosis all occur in process of immigration from cortex to medulla of thymus
- Environment of Thymus (nurse cells, matrix, hormone and cytokines) provide critical conditions for T cell development



2: The Structure of the Thymus






Thymus

Thymus

Bone marrow precursor

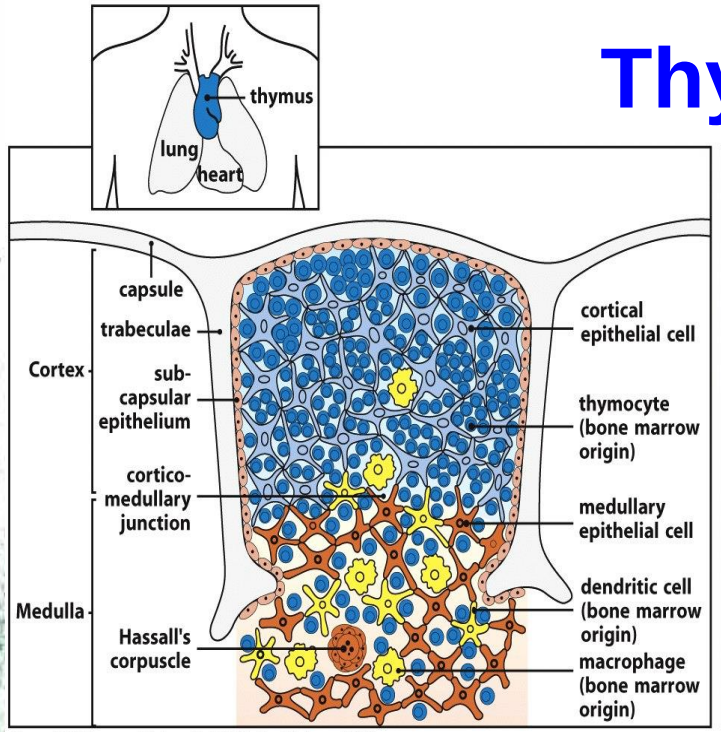
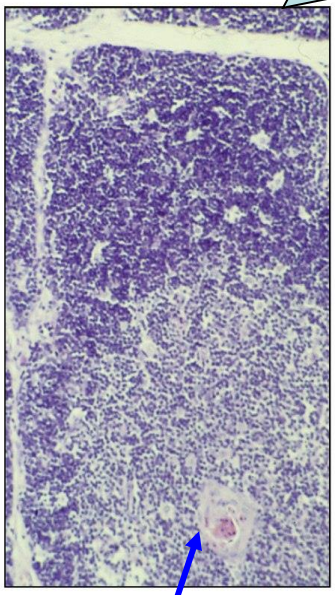


Figure 7-15 Immunobiology, 7ed. (© Garland Science 2008)



Hassall's corpuscule (Cell destruction?)

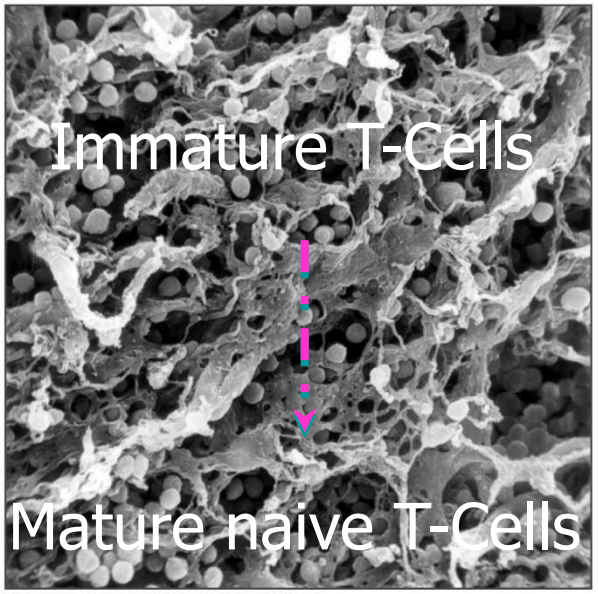


Figure 7-9 Immunobiology, 6/e. (© Garland Science 2005)

Blood stream

- T cell selection takes place in the thymus
- Requirement for antigen presentation to T cells
- Positive/negative selection
- Differentiate into self tolerant CD4 T and CD8 T cells

3: Stages of T Cell Maturation

① Pro-T cell stage — $TCR^-CD4^-CD8^-$

Double-Negative Thymocytes (DN)

② Pre-T cell stage — $pre\text{-}TCR^+CD4^-CD8^-$

DN Thymocytes

$TCR^+CD4^+CD8^+$

③ Double-Positive Thymocytes (DP)

Positive selection

T cells obtain ability to recognize MHC restricted antigens

$TCR^+CD4^+CD8^-$ $TCR^+CD4^-CD8^+$

④ Immature Single-Positive T Cells (SP)

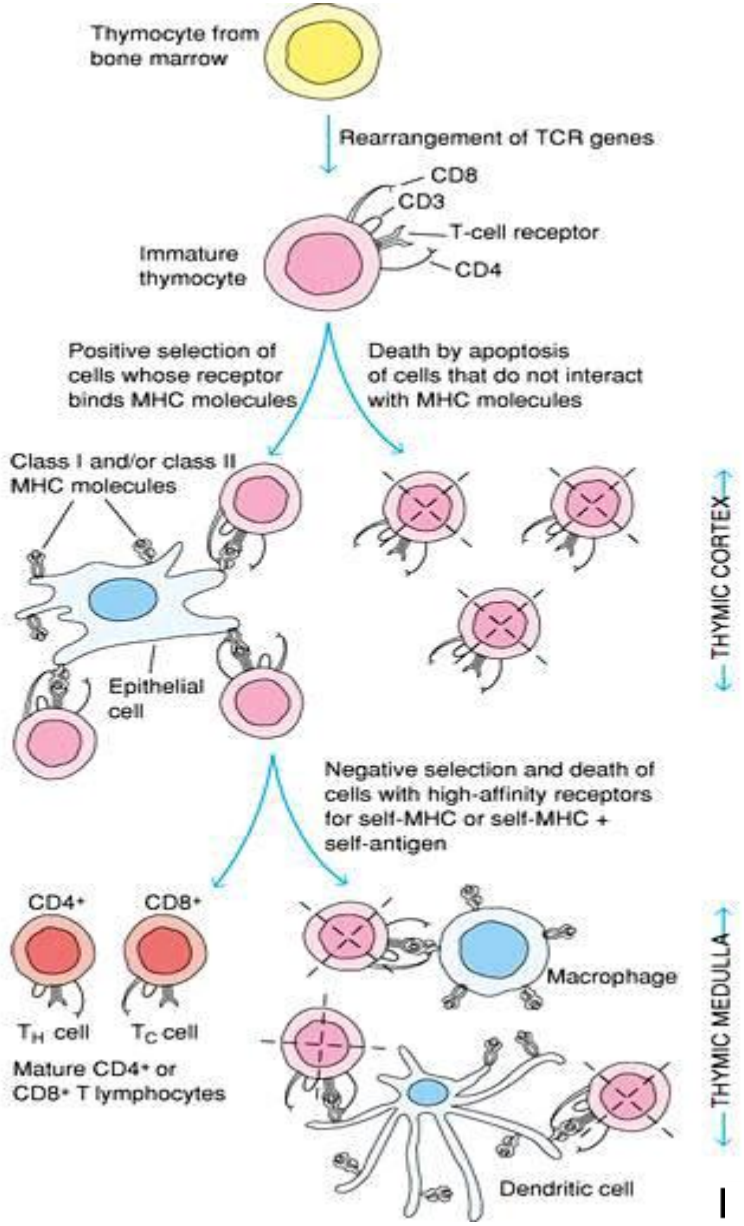
Negative selection

T cells obtain immunological tolerance to self antigen

$TCR^+CD4^+CD8^-$ $TCR^+CD4^-CD8^+$

⑤ Mature SP T Cells

II: Positive and Negative Selections of Thymocytes **



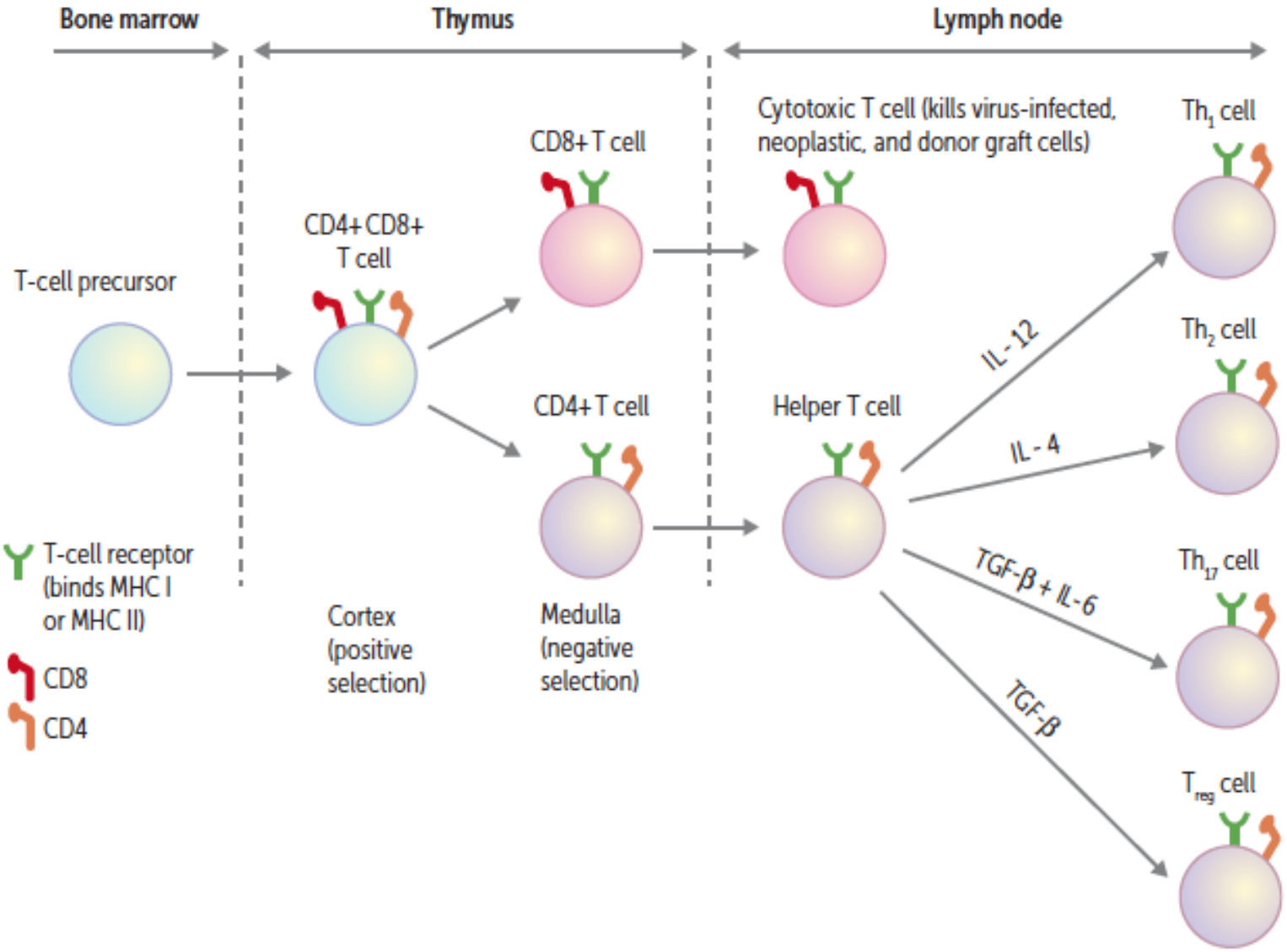
Positive selection: **

In deep cortex of thymus, CD4 or CD8 molecules on CD4+/CD8+ (DP) pre-T cells bind to MHC II or I molecules on thymic epithelial cells and further differentiate immature single positive (CD4+ or CD8+) T cells. Pre-T cells unbinding to epithelial cells cannot survive (undergoing apoptosis)

Negative selection: **

In the boundary of the thymic cortex and medulla immature T cells undergo to apoptosis or stop development if their Ag receptors bind to self-peptide-MHC complex on dendritic cells or macrophages. The remaining cells unrecognized such complex continue to survive, differentiate and exist thymus as mature naïve T cells

Differentiation of T cells



Positive selection	Thymic cortex. T cells expressing TCRs capable of binding surface self MHC molecules survive.
Negative selection	Medulla. T cells expressing TCRs with high affinity for self antigens undergo apoptosis.

Consequences of Selections

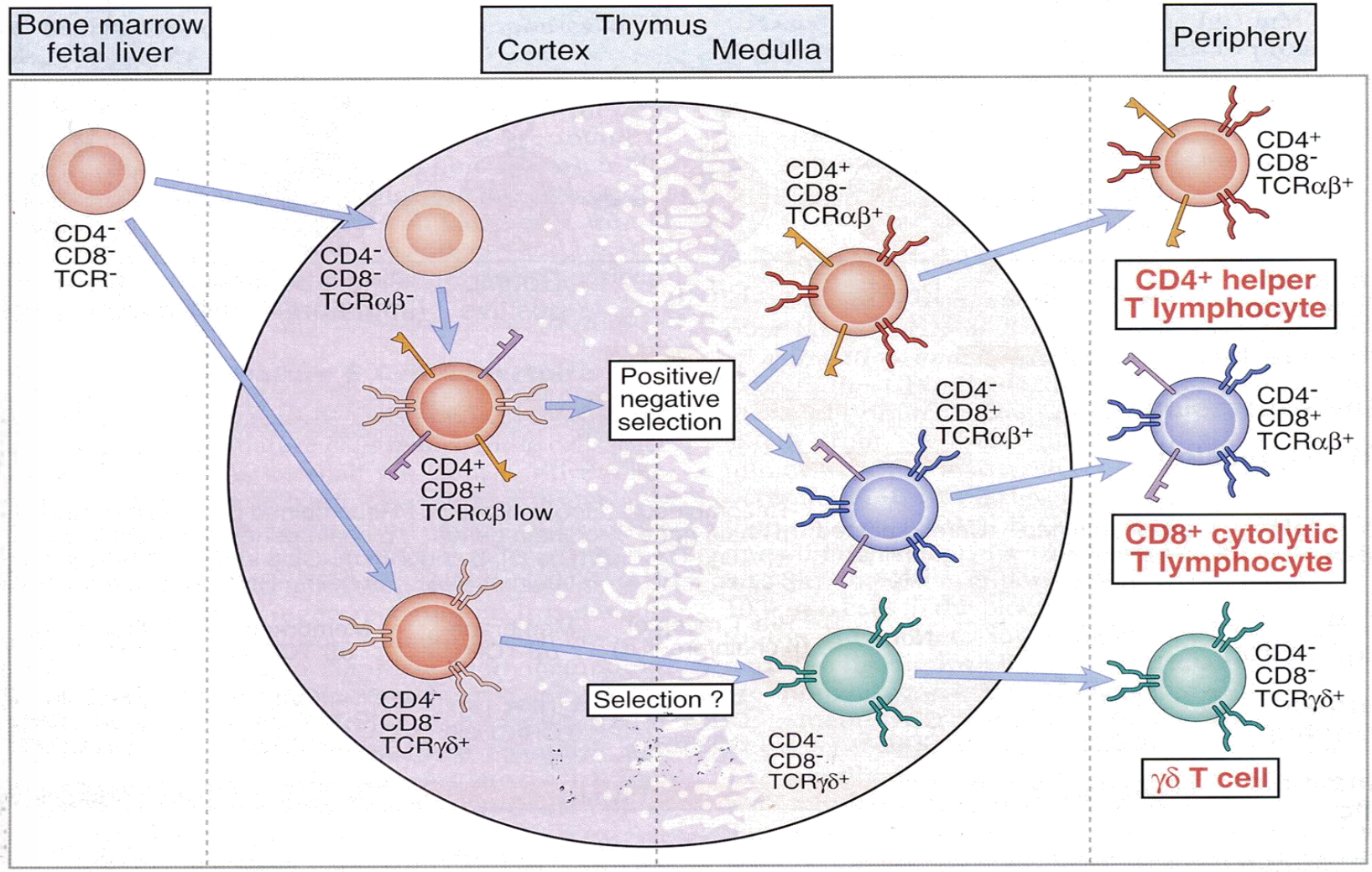
Positive Selection **

- TCR on immature single positive T cells become to maturation, obtain ability to recognize antigen peptides
- T cells obtain ability to recognize MHC restricted antigens

Negative Selection **

- This process is an important component of immunological tolerance
- Remove thymocytes that are capable of strongly binding with "self" peptides presented by self MHC molecules, which prevent the formation of self-reactive T cells that are capable of generating autoimmune diseases in the host
- Pre-T cells which are capable of recognizing non-self antigens develop into mature T cells.

After selections, mature T cells distribute into peripheral compartments



III: Surface Molecules of T Lymphocytes

- **Major surface molecules of T cells**:

1: TCR –CD3 complex

2: CD4 and CD8 coreceptors

3: Costimulatory molecules (CD28 and CTLA-4)

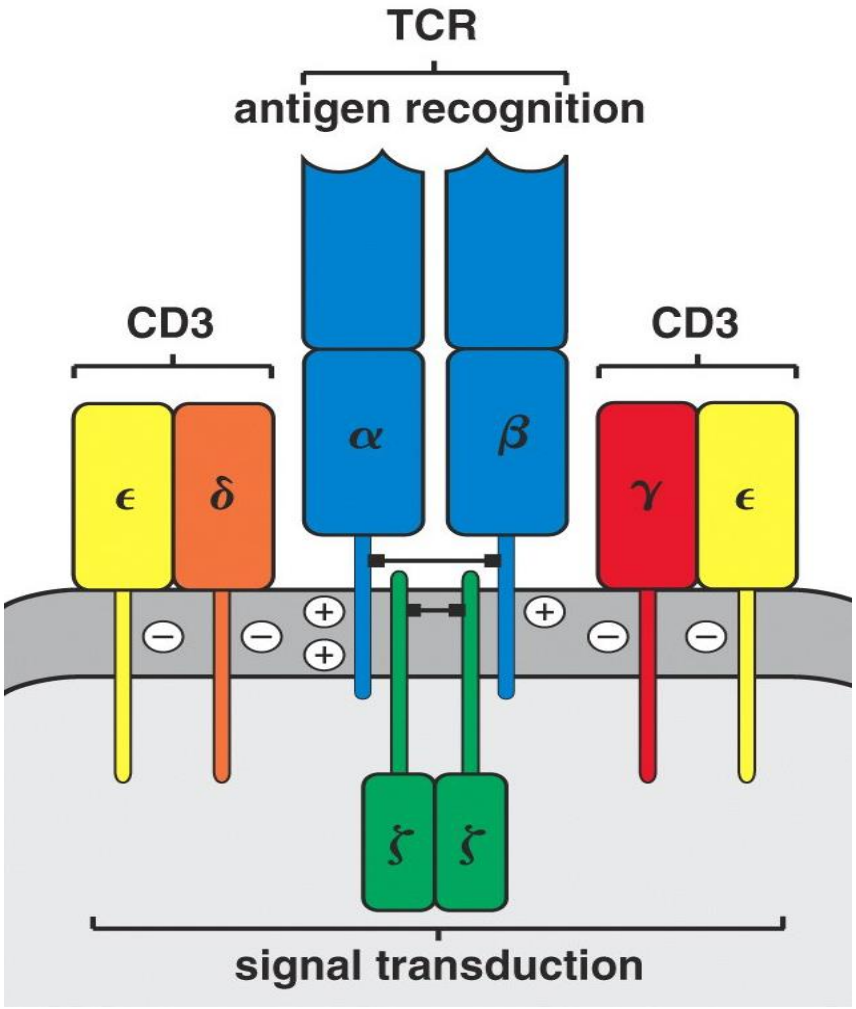
4: Mitogen receptors

5: Cytokine receptors

6: Molecules for apoptosis and homing

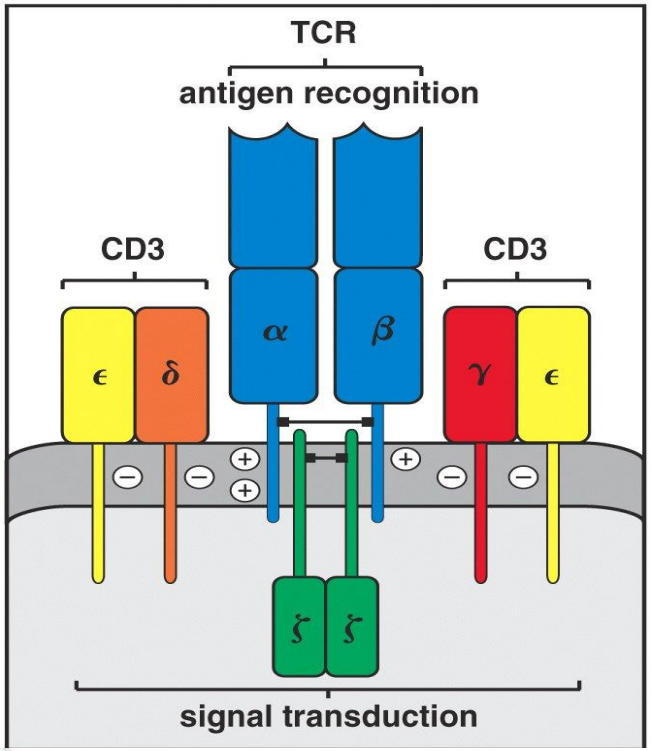
1: TCR-CD3 complex**

- TCR (T cell receptor) is a receptor recognizing antigens bounds to MHC molecules on the surface of APCs
- The biochemical signals that are triggered in T cells by antigen recognition are transduced not by the TCR itself but by invariant proteins called CD3
- CD3 are noncovalently linked to TCR to form the ***T cell receptor complex*** (TCR-CD3 complex)



The cytoplasmic tail of the TCR is extremely short, making it unlikely to participate in signaling.

Structure of TCR-CD3 complex



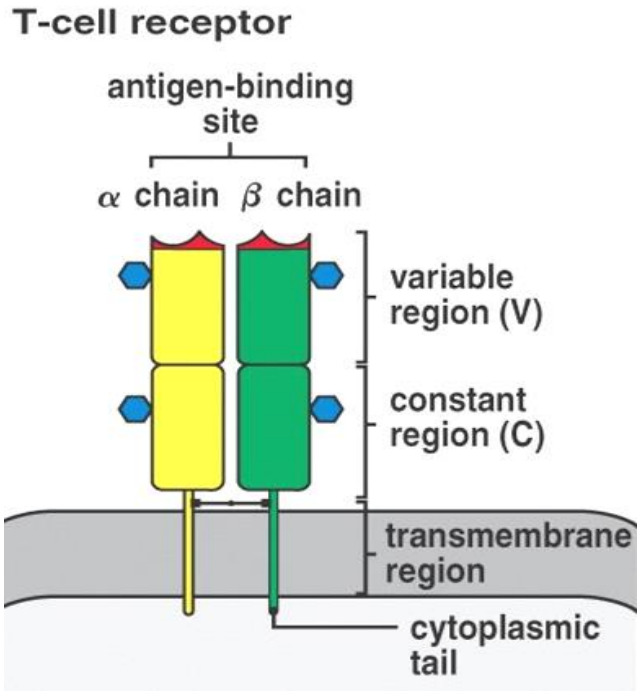
- TCR: { α β chains / γδchains

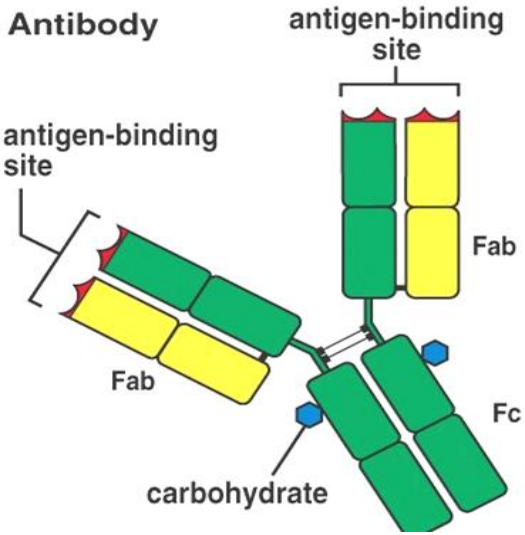
  It is a heterodimer consisting of an α and β chain in 95% of T cells, whereas 5% of T cells have TCRs consisting of γ and δ chains, covalently linked to each other by disulfide bonds.

- CD3: three dimers:

  - gamma epsilon (γε): heterodimers
  - delta epsilon (δε): heterodimers
  - two zetas (ζζ) : homodimer
  - or a zeta/eta (ζη): heterodimers

Comparison of αβTCR and antibody





- TCR is a member of the Ig superfamily, with Ig-like domains. Like Ig, each chain has a variable and a constant region; variable regions have CDR (complementarity-determining regions) which define the antigen-binding specificity and framework residues.
- TCR is encoded in gene segments that undergo somatic recombination during T cell development to generate antigen-binding diversity. Each T cell bears a single specificity.

Function of TCR-CD3 complex**

TCR:

• **TCR** is a molecule found on the surface of T cells that is, in general, responsible for recognizing antigens bound to MHC molecules

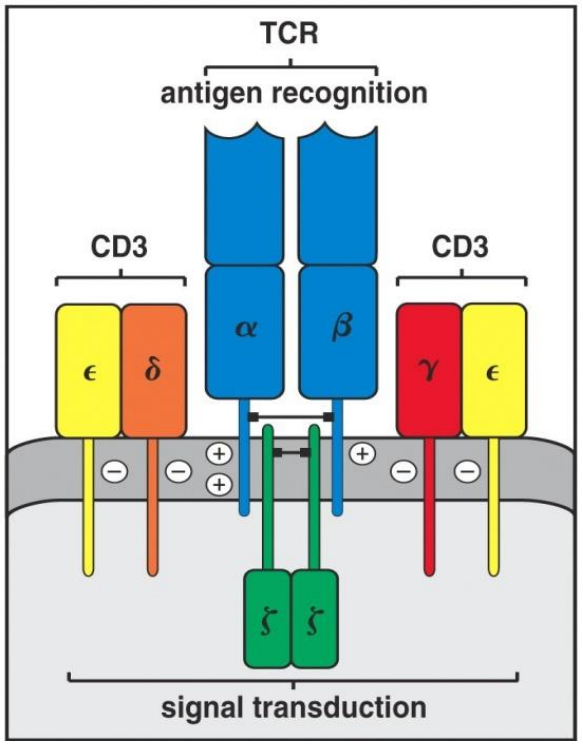
T cells have a dual specificity:

• They recognize polymorphic residues of self MHC molecules, which accounts for their MHC restriction

• They also recognize residues of peptide antigens displayed by self MHC molecules, which is responsible for their specificity

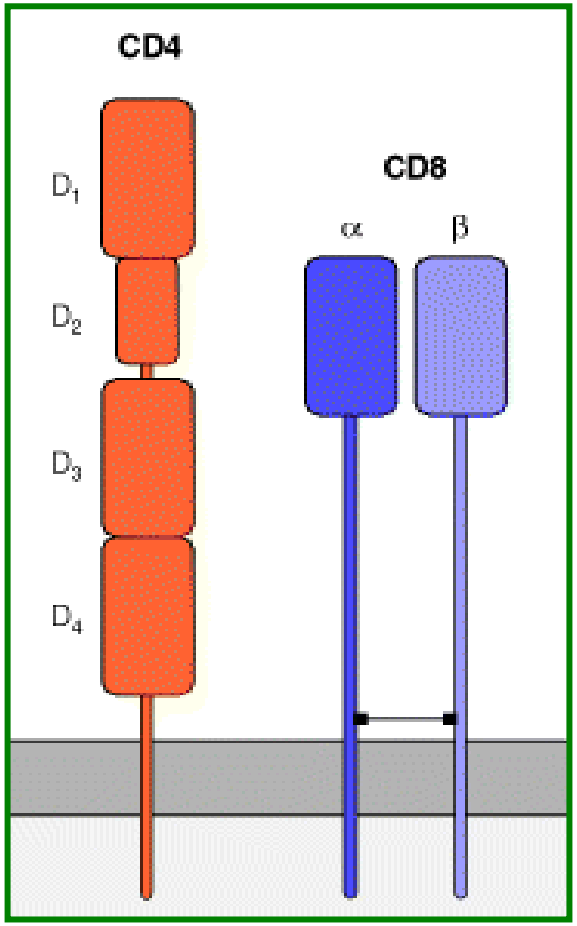
CD3:

• When the TCR recognizes antigen, these associated proteins transduce the signals that lead to T cell activation



2: CD4 and CD8 Co-receptors

- The signal from the T cell complex is enhanced by simultaneous binding of the MHC molecules by a specific coreceptor
- On helper T cells, this coreceptor is CD4 that exclusively binds the class II MHC
- On cytotoxic T cells, this coreceptor is CD8 that is specific for class I MHC.

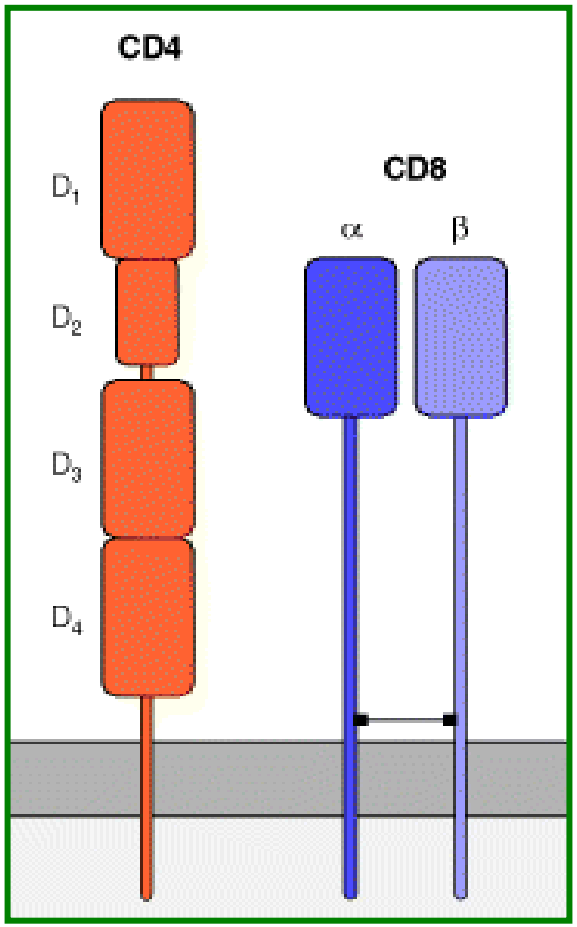


Structure of CD4 and CD8

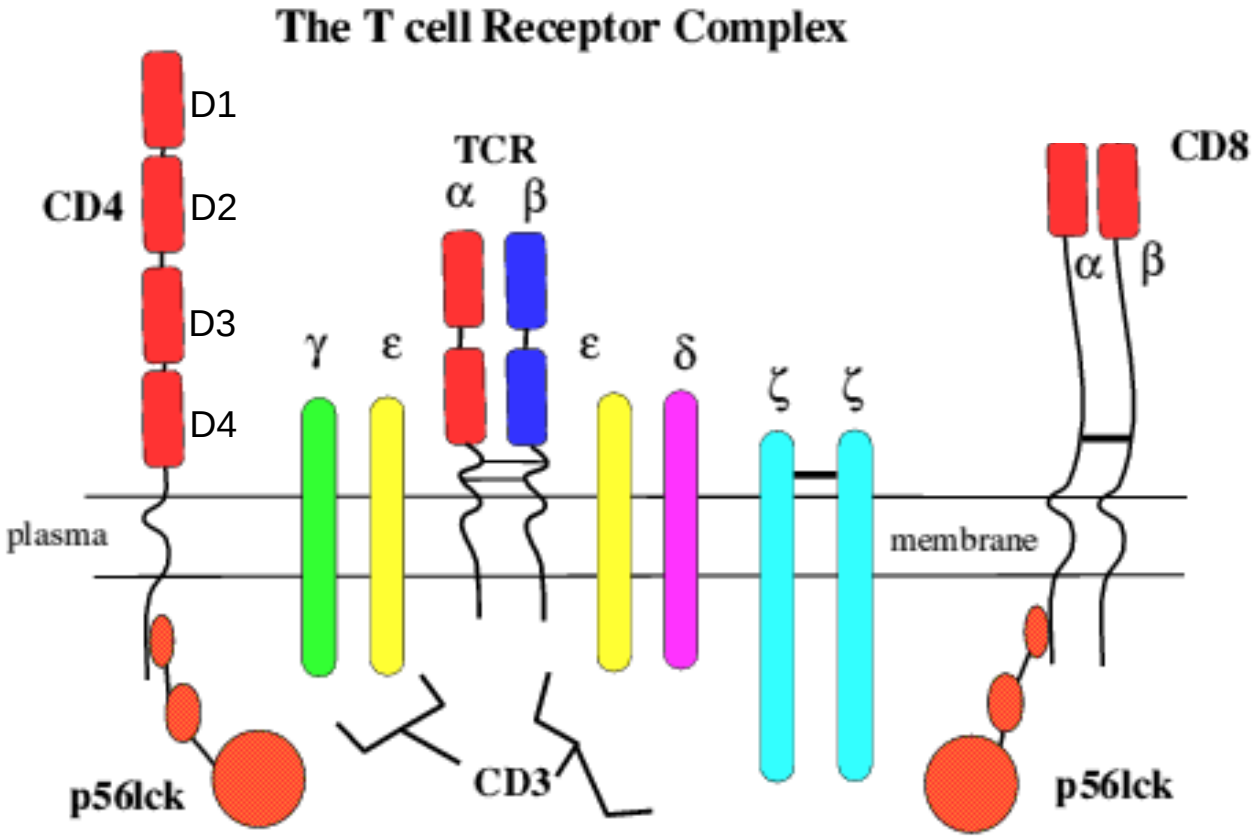
- CD4 and CD8 are transmembrane glycoprotein members of the Ig superfamily, with similar functions but different structures.

- **CD4** is expressed as a monomer on the surface of peripheral T cells and thymocytes and is also present on mononuclear phagocytes and some dendritic cells

- **CD8** molecules exist as disulfide-linked heterodimers composed of two related chains called CD8α and CD8β



Structures of CD4 and CD8 co-receptors and TCR/CD3 Complex



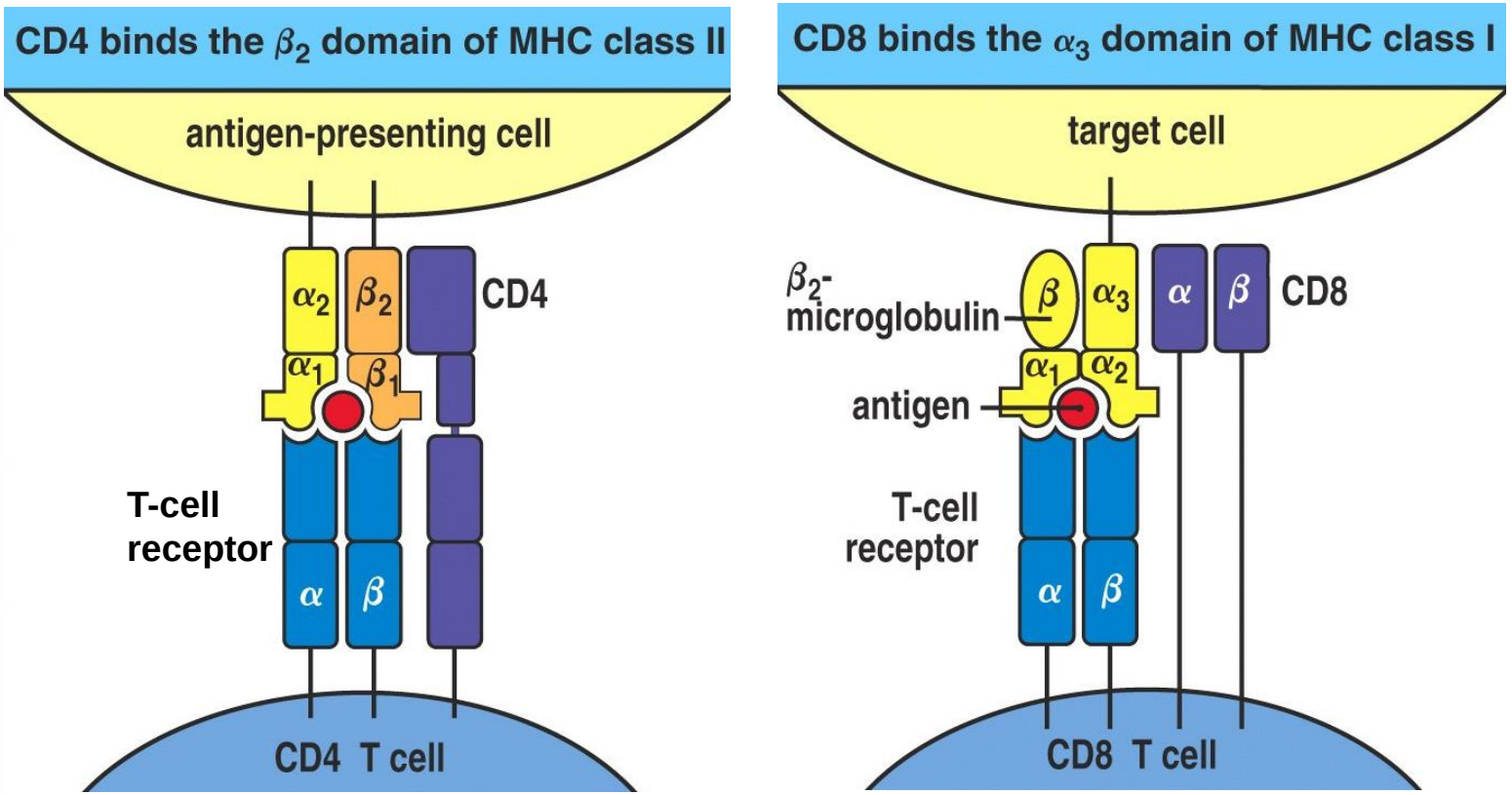
Functions of CD4 and CD8

The selective binding of CD4 to class II MHC molecules and of CD8 to class I MHC molecules ensures that $CD4^+$ T cells respond to class II-associated peptide antigens and that $CD8^+$ T cells respond to class I-associated peptides

a:- CD4 and CD8 participate in the early signal transduction events that occur after T cell recognition of Ag peptide-MHC complexes on APC

b:- CD4 and CD8 promote the adhesion of MHC-restricted T cells to APCs or target cells expressing Ag peptide–MHC complexes

CD4 and CD8 with their Ligands



The two most membrane distal domains in CD4 are thought to bind Class II MHC β2 domain. CD8 binds to the α3 region of Class I MHC. The cytoplasmic tails of both CD4 and CD8 associate with a cytoplasmic tyrosine kinase, Lck, to initiate signal transduction.

3: Costimulatory Molecules

(Co-signalling Molecules)

- A general property of naive T and B cells is that they need two distinct extracellular signals to initiate their proliferation and differentiation into effector cells

Two-signal model **

- The first signal is provided by antigen binding to the antigen receptor and is responsible for ensuring the specificity of the subsequent immune response

- The second signal is provided by costimulators because they function together with antigen to stimulate T/B cells. In the absence of costimulation, T/B cells that encounter antigens either fail to respond and die by apoptosis or enter a state of unresponsiveness called anergy

T cell Activation

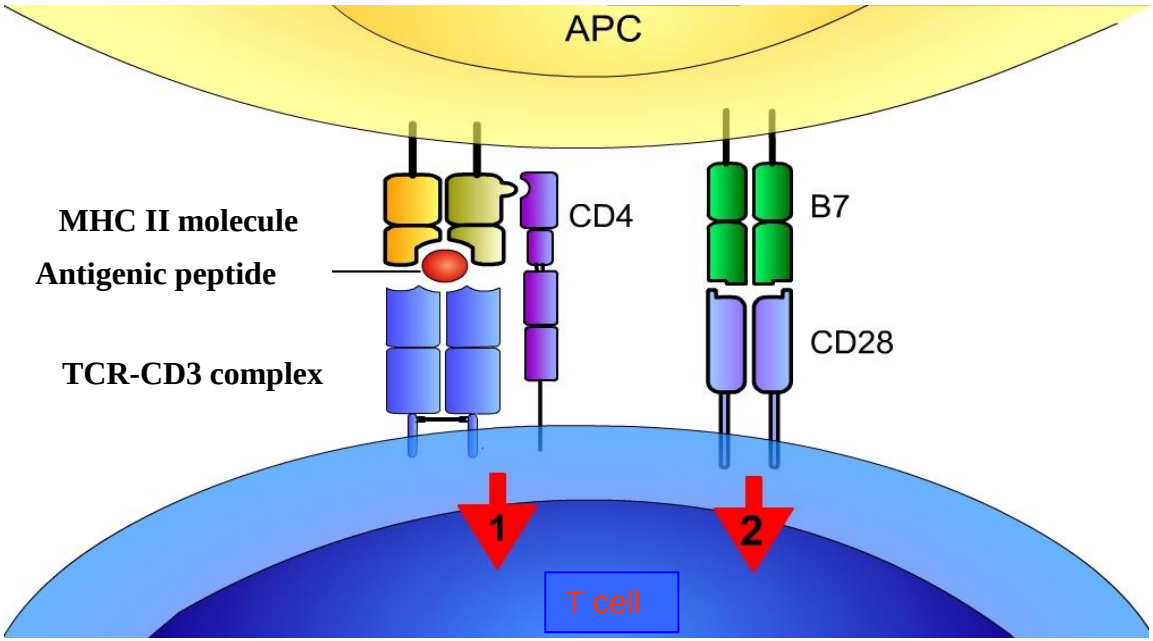
T cells require two signals to become fully activated

- 1st signal, which is antigen-specific, is that TCR recognizes a peptide presented by MHC on APC (and CD4 or CD8 recognizes MHC class II or I molecules), which passes the signal into T cell via CD3. This ensures that only a T cell with a TCR specific to that peptide is activated

- 2nd signal, the co-stimulatory signal, is antigen nonspecific and is provided by the interaction between costimulatory molecules expressed on the membrane of APC and the T cell. One of the best characterized costimulatory molecules expressed by T cells is **CD28**, which interacts with **CD80 (B7-1) and CD86 (B7-2)** on the membrane of APC. The second signal licenses the T cell to respond to an antigen. Without costimulatory signals may lead to T cell anergy, T cell deletion or the development of immune tolerance

Initial activation of T cell requires two signals:
Specific and costimulatory signals

- The signals delivered by the TCR are insufficient to fully activate T cells
- T cell activation requires the delivery of both the TCR signals and a second set of signals generated by costimulators



Major Co-signalling Molecules on T cells

a:- CD28 and CTLA-4 (cytotoxic T lymphocyte antigen-4)

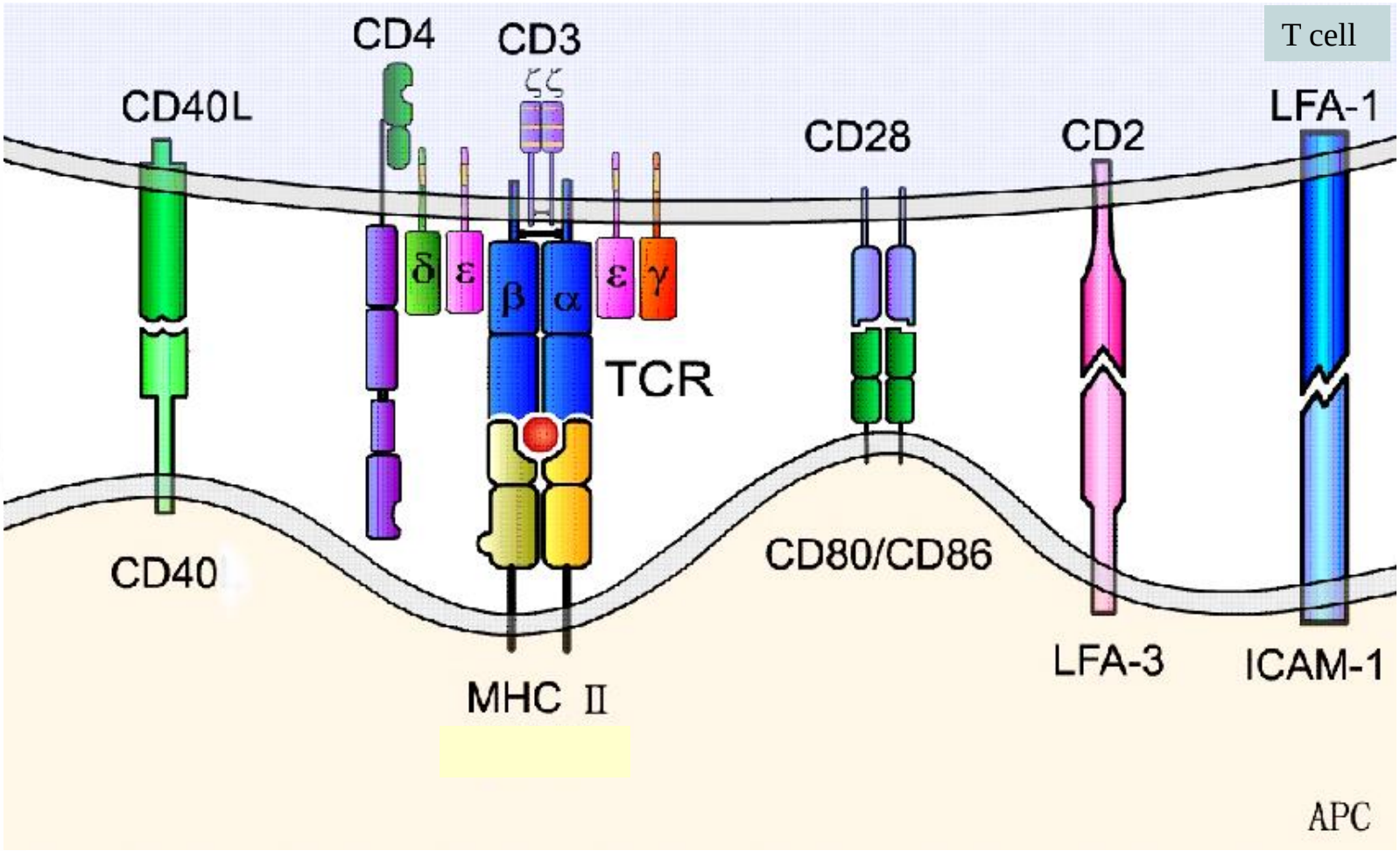
- Have high homology in the structures. Both can bind to the ligands CD80 (B7.1) and CD86 (B7.2) on APC
- Binding of CD28/CD80 transmits a stimulatory signal whereas CTLA4 transmits an inhibitory signal for activation of T cells

b:- CD40L (CD154): The CD40L binds to the B cell's CD40 receptor, causing resting B cell activation and TD-Ag B cell response

c:- LFA-1 (lymphocyte function associated antigen-1): binds to ICAM-1 on APC to enhance cell-mediated immune responses

d:- LFA-2 (CD2): bind to LFA-3 (CD58), CD59 and CD48 on APC which enhances T cell activation

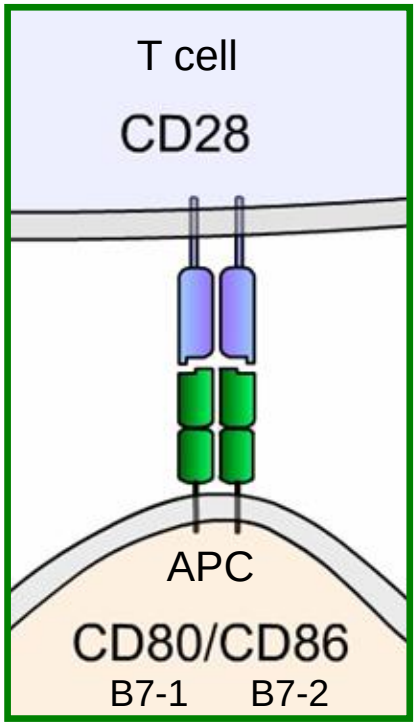
Major co-stimulatory molecules between T cell and APC



Major Costimulatory Molecules on T cells

a:- CD28 * *

- One of the molecules expressed on T cells that provide costimulatory signals, which are required for T cell activation.
- CD28 is expressed on more than 90% of $CD4^+$ T cells and on 50% of $CD8^+$ T cells in humans.
- CD28 is the receptor of B7-1 (CD80) and B7-2 (CD86).
- Binding of B7 molecules on APCs to CD28 delivers signals to the T cells that induce the expression of anti-apoptotic proteins, stimulate production of growth factors and other cytokines, and promote T cell proliferation and differentiation.
- Thus, CD28 is the principal receptor for delivering second signals for T cell activation

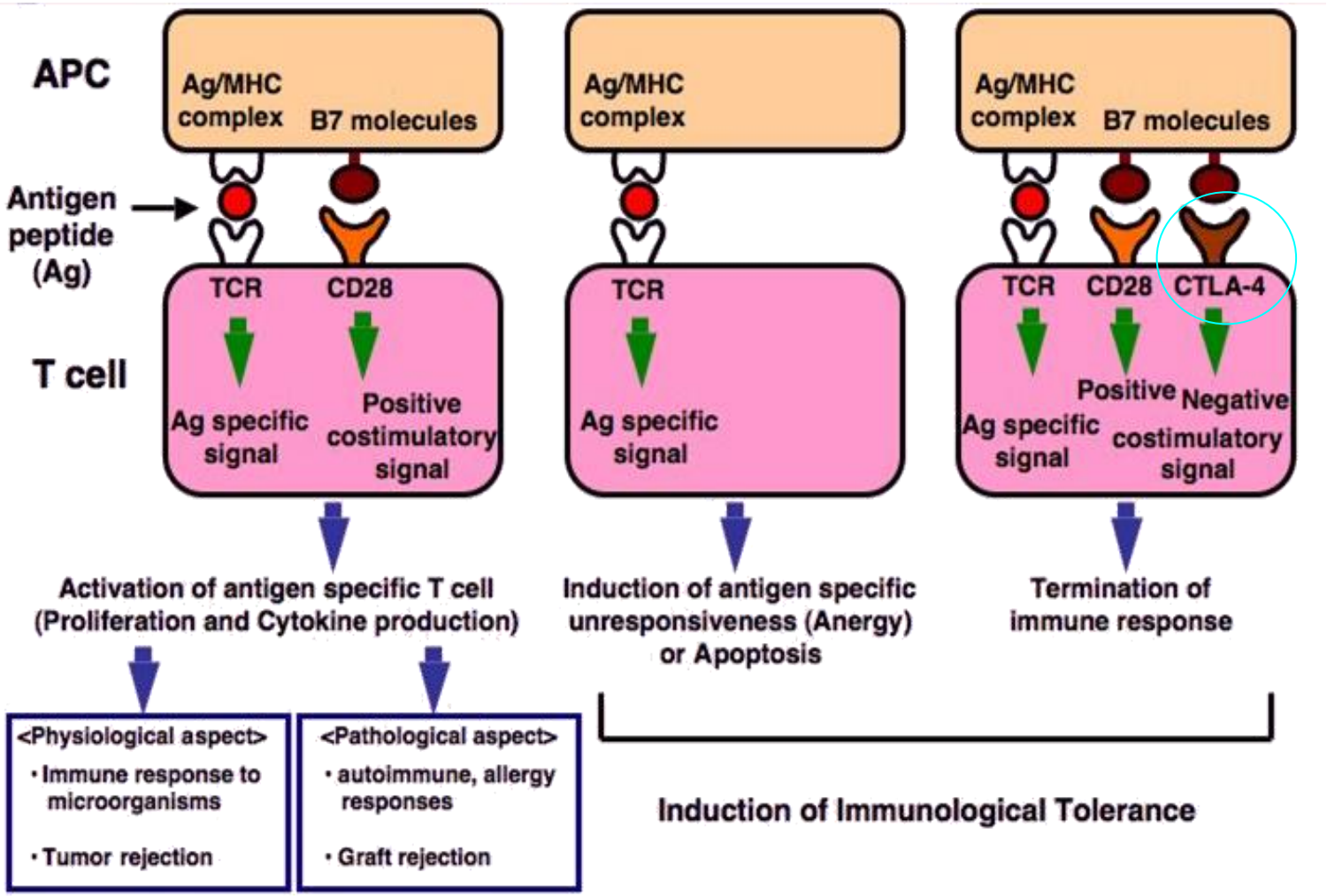


CD28 is a homodimer of two chains with Ig domains.

b:- CTLA-4 (cytotoxic T lymphocyte antigen-4, CD152)**

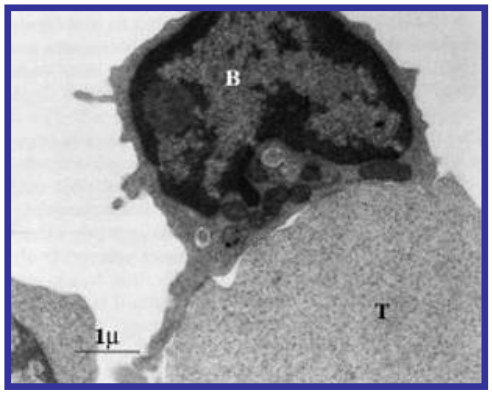
- CD152 is structurally homologous to CD28. Both can bind to the ligands CD80 (B7-1) and CD86 (B7-2) on APC
- CD152 is expressed on recently activated $CD4^+$ and $CD8^+$ T cells
- Its function is to inhibit T cell activation by counteracting signals delivered by CD28
- Binding of CD28/B7 transmits a stimulatory signal whereas CD152/B7 transmits an inhibitory signal for activation of T cells .
- Thus, CTLA-4 is involved in terminating T cell responses

To be or not to be: Immunological tolerance by T cell costimulatory signals



c:- CD40L (CD154) **

- The CD40L is expressed on activated T cells.
- CD40L binds to CD40 on B lymphocytes, macrophages, dendritic cells, and endothelial cells and activates these cells.
- CD40L is an important mediator of many of the effector functions of helper T cells, such as the stimulation of B cells to produce antibodies and the activation of macrophages to destroy phagocytosed microbes.



4: Mitogen Receptors

- T cells express some mitogen receptors such as phytohaemagglutinin (PHA) , concanavalin A (ConA) and pokeweed mitogen (PWM) receptor. Binding of such receptors on rest cells to their ligands drive these cells become into lymphoblasts

5: Cytokine Receptors

- Various stages of T cells express numerous cytokine receptors such as IL-1R, IL-2R, IL-4R etc. Binding of cytokines to their receptors induces or enhances activation, proliferation and differentiation of T cells

6：Molecules for Apoptosis and Homing

- Molecules relevant to apoptosis: such as Fas (CD95), a member 6 of TNF receptor superfamily (TNFRSf6), expressed by thymocytes, activated T, B cells, NK cells and fibroblasts etc. Binding to its ligand (FasL) activates caspase 8, 10, 3, 6, and 7, causing apoptosis of target cells

- Molecules relevant to homing: such as LFA-1 (ICAM-1 receptor), L-selectin and CD44 (E-selectin and L-selectin ligand) etc

- Lymphocyte homing receptors are cell adhesion molecules which target addressin expressed lymphoid high endothelial venule. These molecules and their ligands participate in homing of lymphocytes to lymphoid organs

IV: T Cell Subsets and Functions

The T cell population is heterogeneous with respect to both functional capabilities and cell surface phenotypes

1: $\alpha\beta$TCR T cell and $\gamma\delta$TCR T cells

2: Naïve T cells, Effector T cells, Memory T cells

3: $CD4^+$ Th cells, $CD8^+$ CTL cells and regulatory T cells

1: Comparison of αβ TCR and γδ TCR T cells

Characters		TCRαβ T cell	TCRγδ T cell
TCR Variety		High	Low
Distribution	Peripheral blood	60-70%	5-15%
	Organs	Peripheral lymphoid organs	mucosa
Phenotype	$CD3^+CD2^+$	100%	100%
	$CD4^+CD8^-$	60-65%	<1%
	$CD4^-CD8^+$	30-35%	20-50%
	$CD4^-CD8^-$	<5%	>50%
Ag recognition		8-17 aa	peptide, HSP, lipid, polysaccharide
MHC restriction		Classic MHC	MHC-like molecules
Help cells		Th	
Cytotoxic cells		Tc	Tc

2: Naïve T cells, Effect T cells, Memory T cells

Naïve T cell

- Matured T cell (naïve meaning they have never been exposed to the antigen to which they can respond)
- Highly expressing CD45RA**
- After the naïve T cell encounters an antigen, it becomes activated and begins to proliferate into many clones or daughter cells (effector T cells and memory T cells)

Effector T cell

- Terminal T cell.
- After stimulation with antigen, the cells eventually play the roles through clonal expansion and differentiation
- The cells highly express CD25 (IL-2R), adhesion molecules, membrane FasL and CD45RO**

2: Naïve T cells, Effect T cells, Memory T cells

Memory T cell

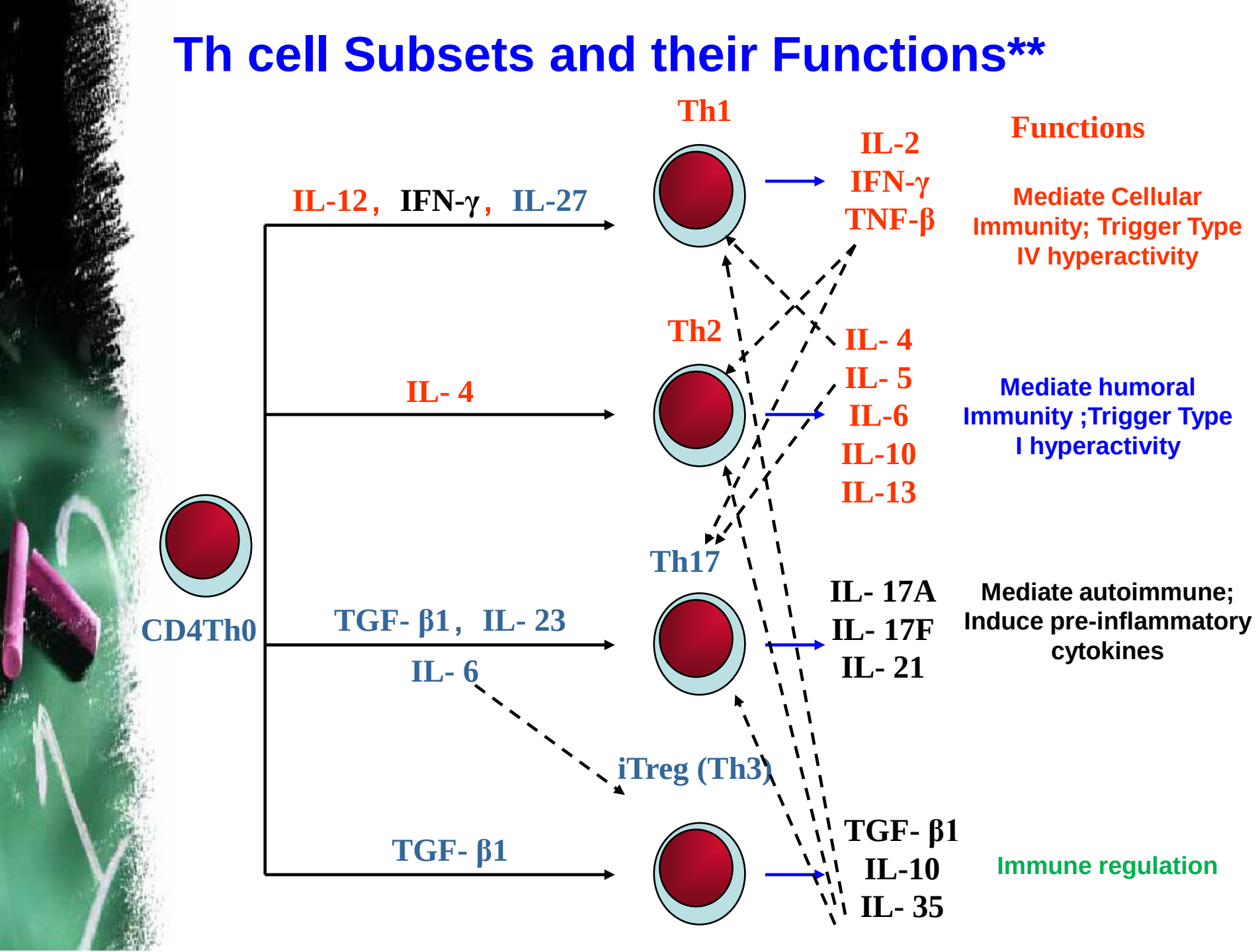
- A subset of antigen-specific T cells that survive in an inactive state in the host for a long-term until they re-encounter the same antigen and reactivate
- These cells express CD45RO**
- These cells quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections

3: CD4+ T cells, CD8+ T cells and regulatory T cells

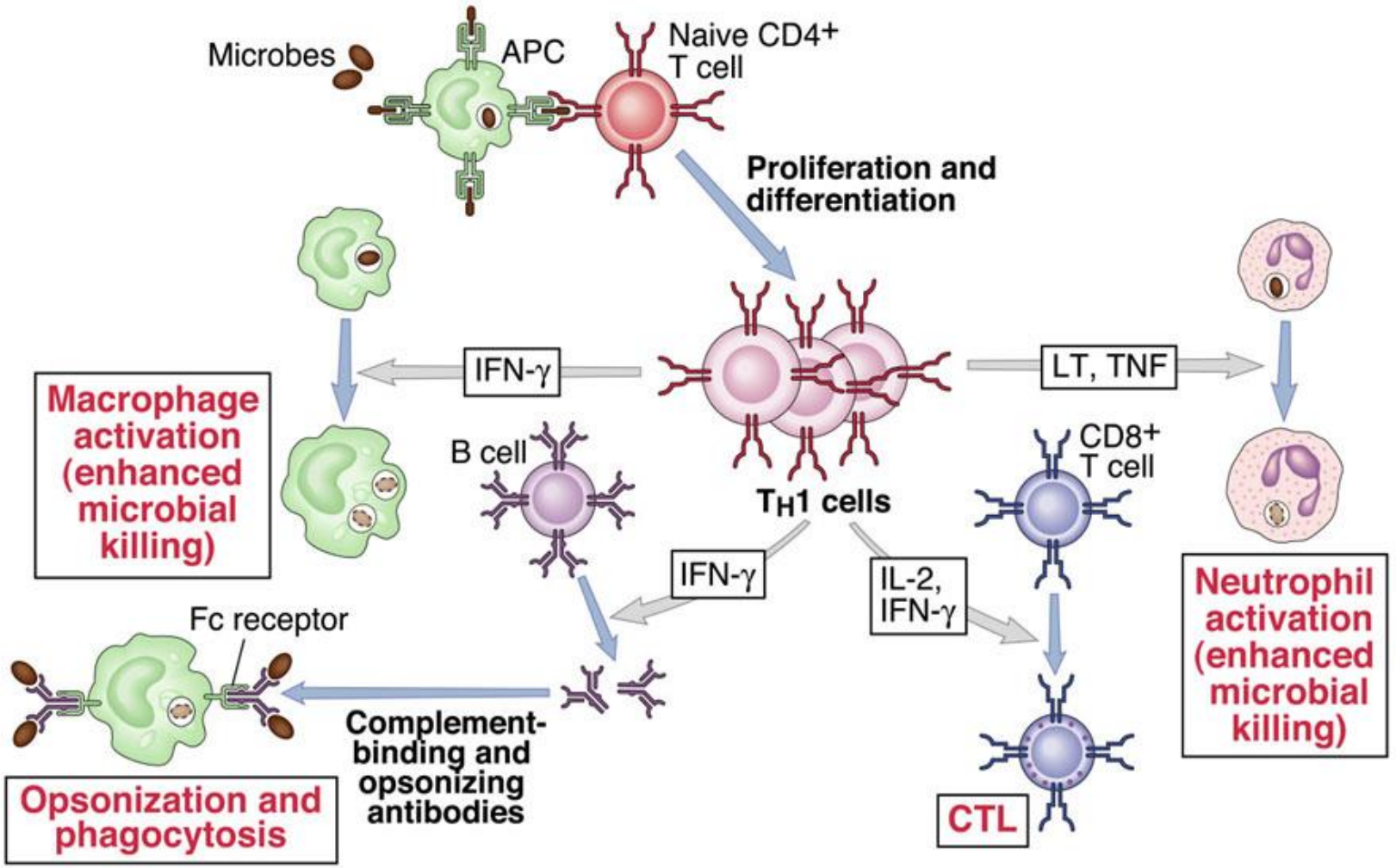
CD4+ T cells

- CD phenotypes: CD3+, CD4+, CD8-
- Majority is αβTCR T cells
- Antigen recognition restricted by MHC-II molecules
- Majority of CD4+ T cells are T helper cells (Th)
- Th cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of APCs
- Once activated, they divide rapidly and secrete cytokines that regulate or assist in the immune response
- Depending on the cytokine signals received, these cells can differentiate into one of several subtypes, including Th1, Th2, Th3 and Th17, which secrete different cytokines to facilitate a different type of immune response

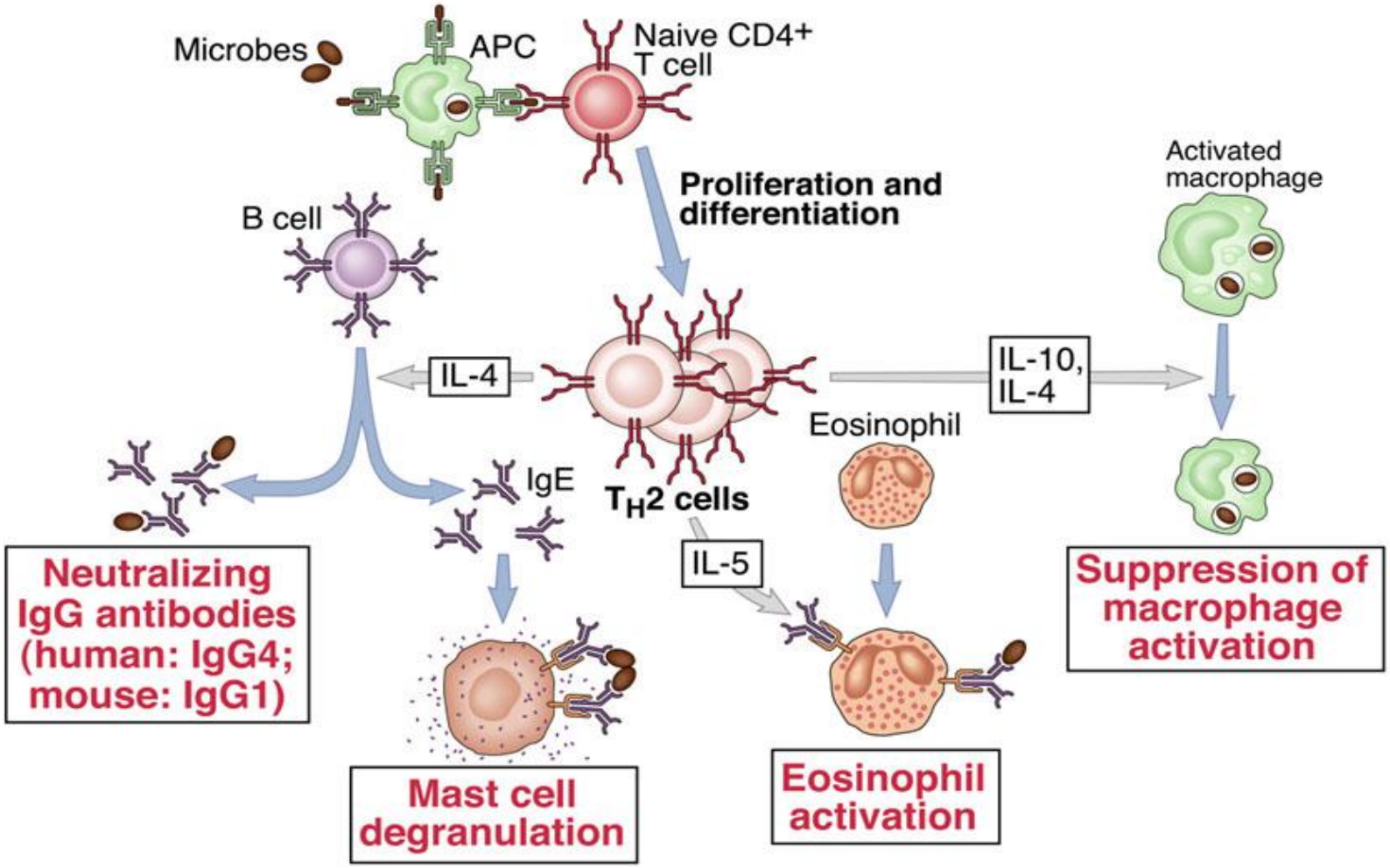
Th cell Subsets and their Functions**



Function of CD4+ Th1 Cells



Function of CD4+ Th2 Cell

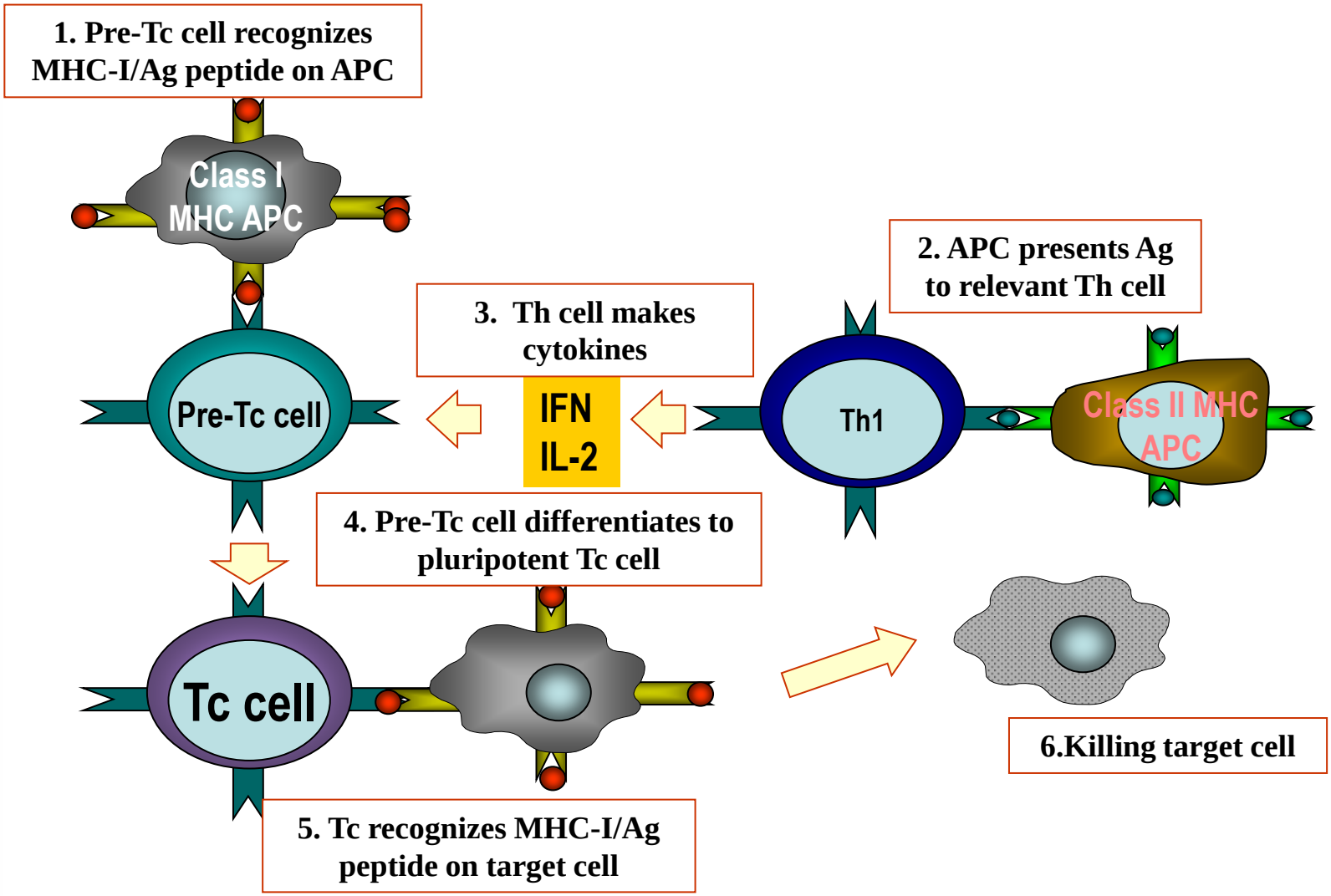


3: $CD4^+$ T cells, $CD8^+$ T cells and regulatory T cells

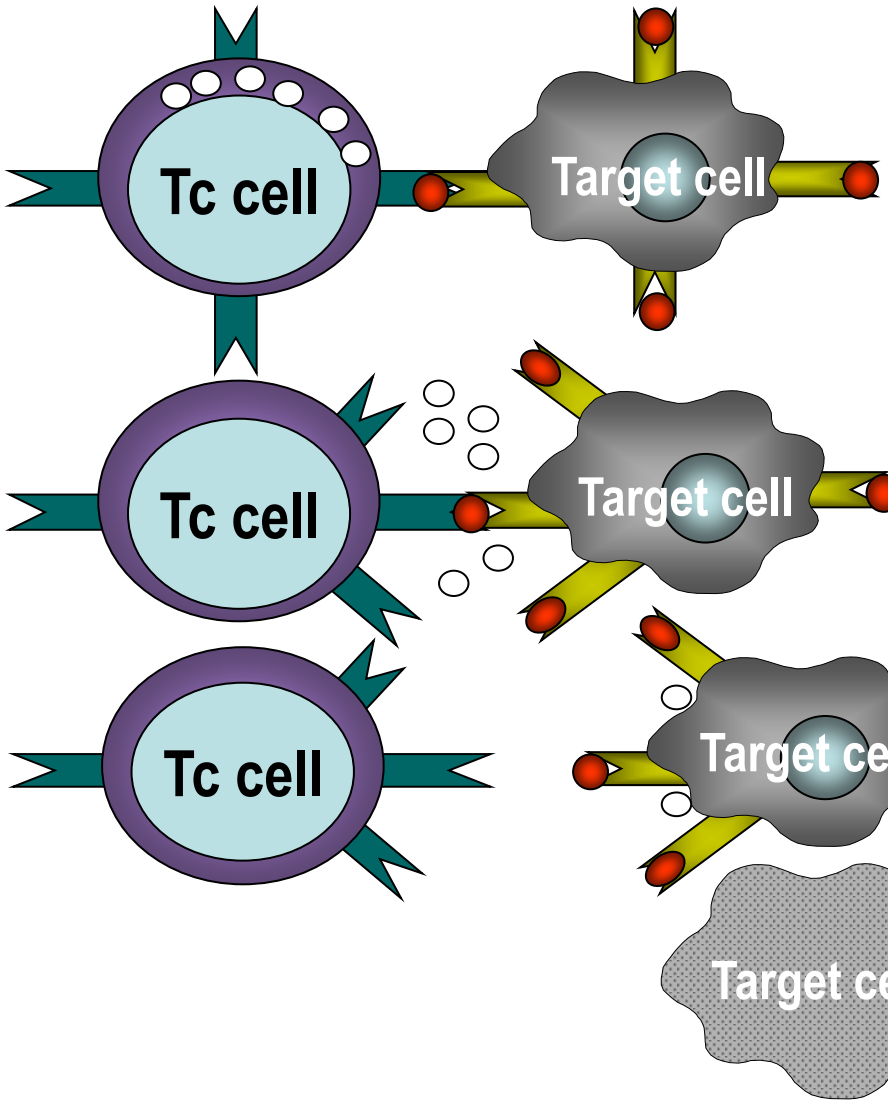
$CD8^+$ T cell**

- CD phenotypes: $CD3^+$, $CD4^-$, $CD8^+$
- Have both TCR: $\alpha\beta$ TCR and $\gamma\delta$ TCR T cells
- Antigen recognition restricted by MHC-Ⅰ molecules
- Majority of $CD8^+$ T cells are cytotoxic T cells (Tc cells, or CTLs)
- Tc cells destroy virally infected cells and tumor cells, and are also implicated in transplant rejection
- These cells recognize their targets by binding to antigen associated with MHC class I molecules, which is present on the surface of nearly every cell of the body
- Tc cell proliferation is dependent on repeated encounters with Ag. Each cell that is stimulated by Ag divides and progressively differentiates into effector Tc cells then memory Tc cells

Development and Function of Tc Cell



Tc-mediated killing



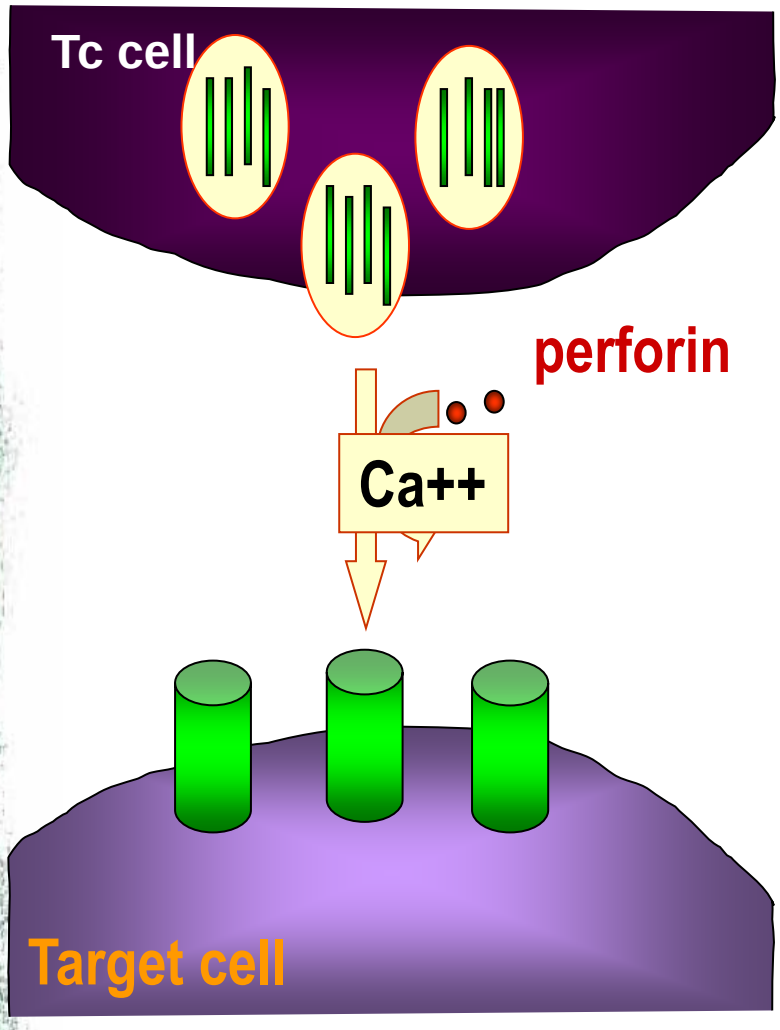
1. Tc recognizes Ag on target cell

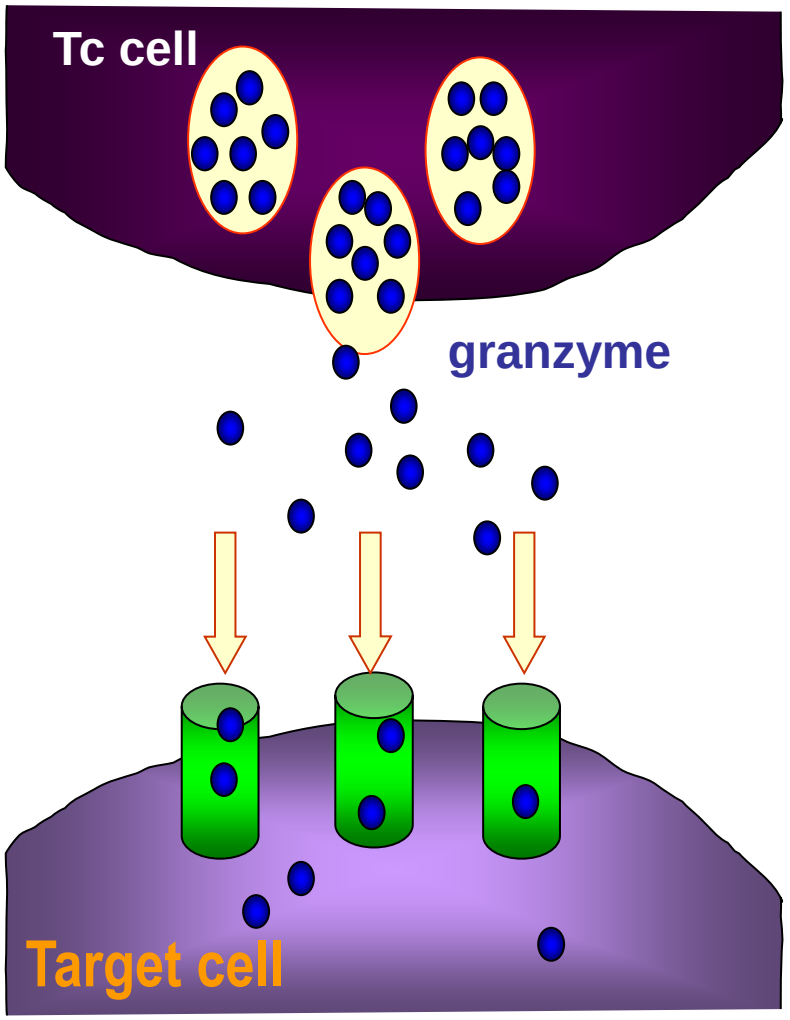
2. Tc releases perforin or granzyme

3. Tc detaches from target cell

4. Apoptosis of target cell

Tc-mediated killing





3: $CD4^+$ T cells, $CD8^+$ T cells and regulatory T cells

Regulatory T cells (Treg cells)

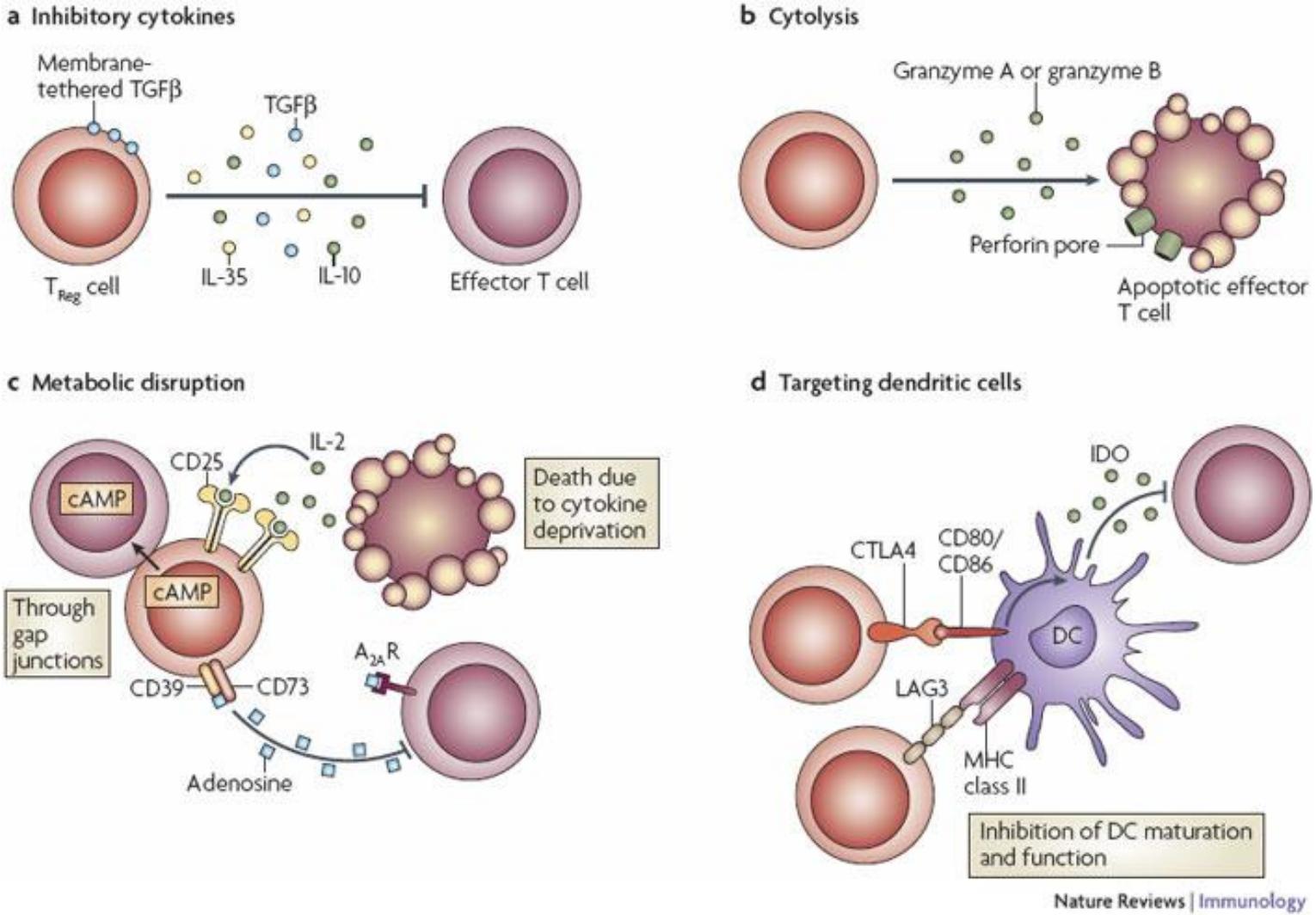
- Treg cells are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus

- Two major classes of Treg cells have been described, including the naturally occurring Treg cells and the adaptive Treg cells

- Naturally occurring Treg cells (also known as $CD4^+CD25^+FoxP3^+$ Treg cells) arise in the thymus, whereas the adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response

- Possible mechanisms: directly contact with target cells or through immunoregulatory cytokines such as IL-10 or TGF-β

How does Treg work



V: T Cell-mediated immune responses

- **Immune cells exert its effect to clear the role of foreign antigenic events, normally refers to T cells ($CD4^+$ Th cells and $CD8^+$ CTL cells, but not B cells) and APC. In this point of view, also called cell-mediated immunity or immune response (CMI)**

- **In clinic, it may include:**

a: - Delayed type hypersensitivity (DTH reaction)

b:- Response to infection against intracellular bacterium

c:- Anti-tumor immunity

d:- Transplant rejection

e:- Some autoimmune diseases

f:- Certain drug-induced hypersencitivities

1: Activation and Differentiation of CD4⁺Th1 cells

- Interaction of $CD4^+$ Th0 cells and APC provides two-signal stimulation which activate $CD4^+$ Th0 cells. Under action of IL-12 IFN-γ cytokines, these cells further proliferate and differentiate into $CD4^+$ Th1 effector cells under action of IL-12 IFN-γ cytokine, mainly under the effect of proliferation and differentiation effects of $CD4^+$ Th1 cells (inflammatory $CD4^+$ T cells)

- Once CD4+ Th1 cells encounter and react with APC, these cells release IL-2, IFN-γ and TNF-α/β, etc

- These mediators result in chronic inflammation characterized with accumulation and infiltration of lymphocytes and monocyte-macrophage

- IFN-γ from activated $CD4^+$ Th1 cells further activate macrophages, enhance inflammation and clean antigen/pathogen

2: Activation of $CD8^+$ CTL (cytotoxic T lymphocyte)

- $CD8^+$ CTL cells bind to Ag peptide-MHC molecules on the surface of APC via surface TCR-CD3 complex and CD8 molecules, obtain the first signal of activation

- Acting of APC surface costimulatory molecules with their receptors on $CD8^+$ CTL cells provide the second activation signal, such as B7:CD28, etc

- In synergy of IL-12, IL-2 and IFN-γ, activated $CD8^+$ CTL cells proliferate and differentiate into effetor $CT8^+$ CTL, with killing effect. Activated $CD4^+$ Th1 cells might enhance this process via releasing IL-2 and IFN-γ

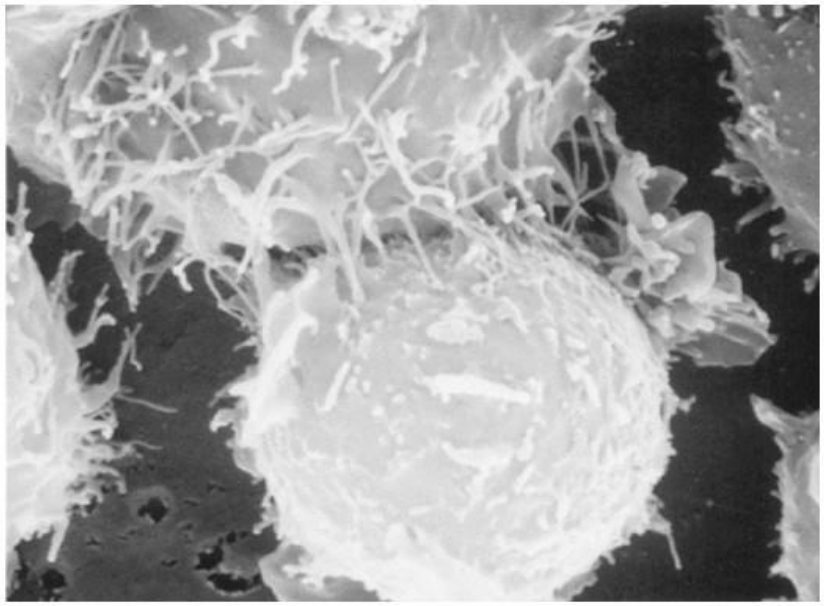
3: Substances relevant to $CD8^+$ CTL killing

- **Perforin: lysis of target cells**
- **Serine protease: activation of target apoptotic pathway of apoptosis**
- **Expression of FasL: binding to Fas molecules on the target cell promotes signal of apoptosis in the target cells**
- **Secrete TNF-α: which binds to the TNFR-I on the target cell and promotes signal of apoptosis in target cells**
- **All these remove tumour- and virus-infected cells**

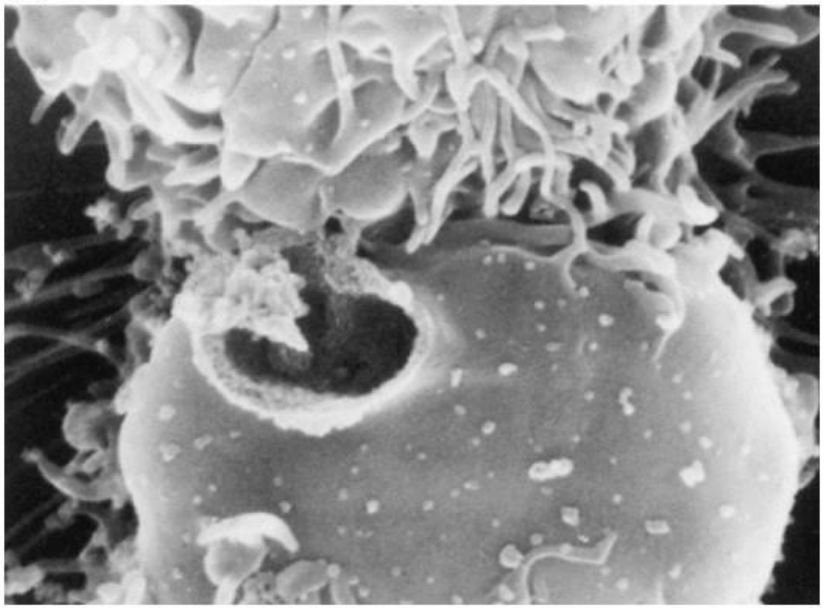
Perforin
proteases

Killing of target cells by $CD8^+$ CTL

(a)

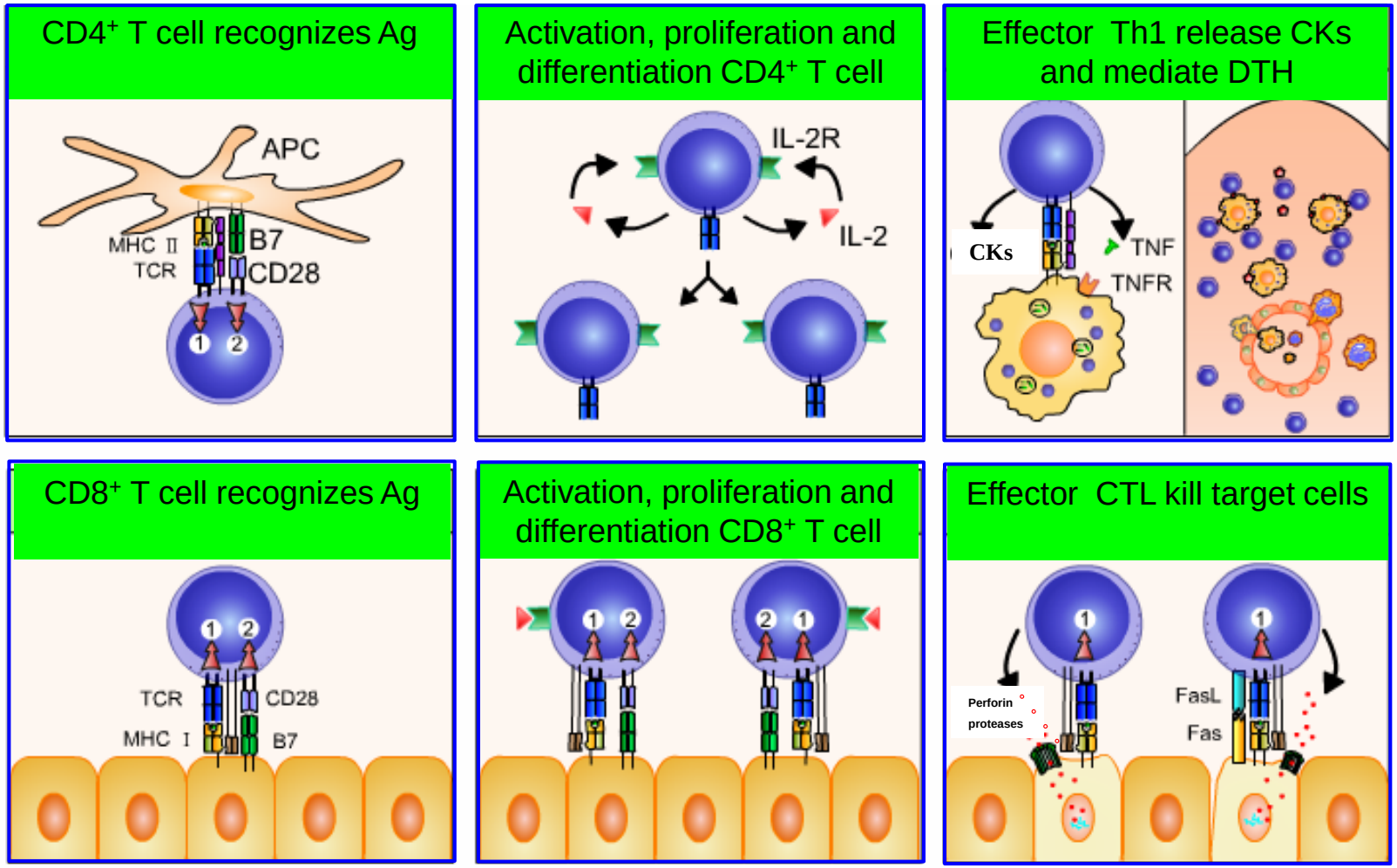


(b)



4: Phases of T cell-mediated immune response

- **It could be divided into 3 phases: 1) recognition 2) activation, proliferation and differentiation and 3) effector phase**



CKs: cytokines

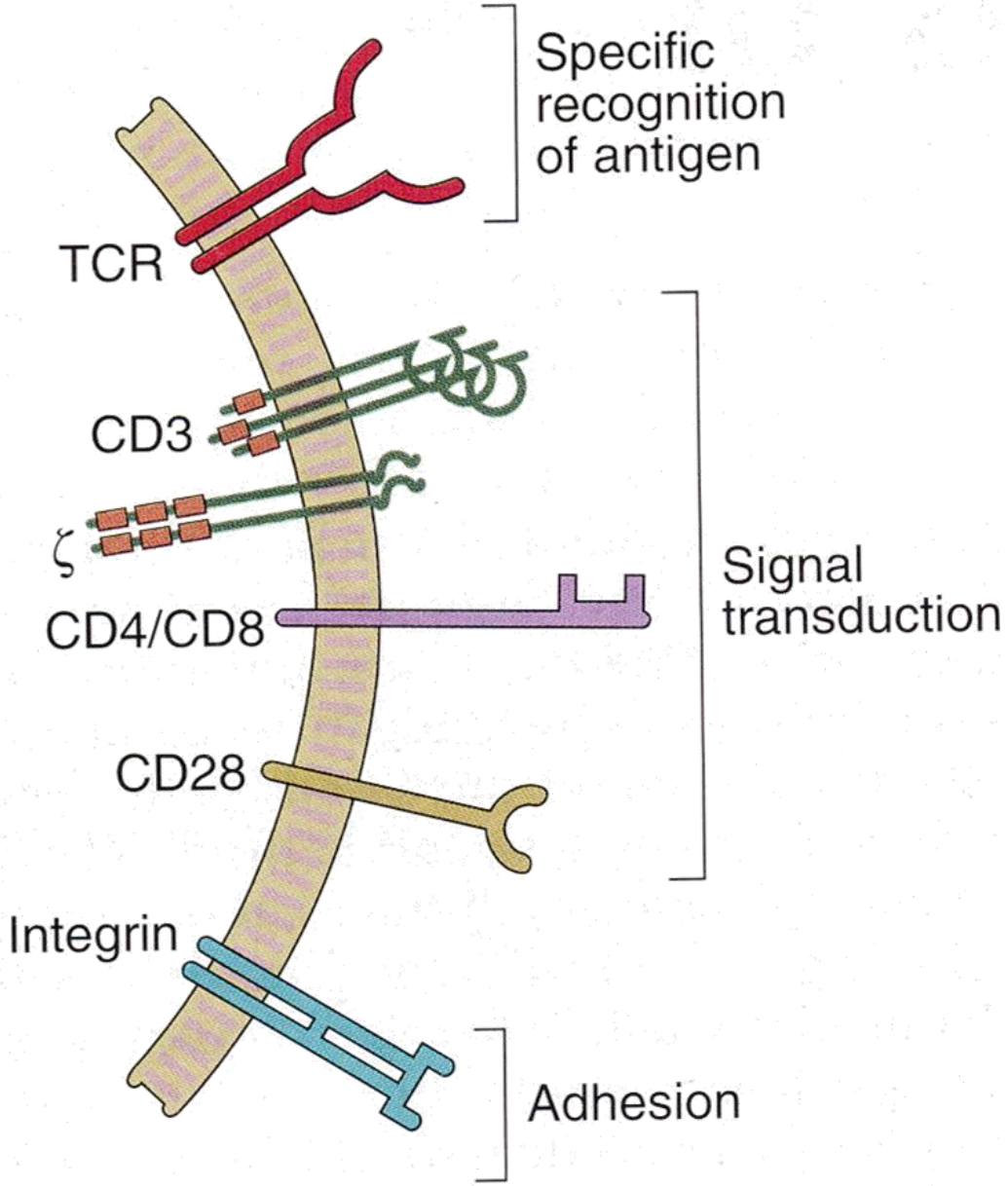
5: Features of T cell-mediated killing

- **Specifically killing of the target cells (antigen specificity)**
- **The killing is restricted by the MHC-I molecules**
- **Must directly contact with the target cells**
- **One effector CD8+ CTL cell can continuously kill a number of target cells**

Summary

- T cell development, maturation and differentiation
- Activation: Two signals
- Surface molecules: TCR and others….
- Subtypes of T cells and their functions
- T-cell-mediated Immune responses

Summary of principal functions of surface molecules on T cells



Learning Objectives

- Master how T cell developments in Thymus
- Master the major molecules expressed on T cell surface and their roles and the major characters of various subgroups of T cells
- Master the functions of CD4 and CD8 T cells
- Master T-cell-mediated immune responses

Further readings

- Medical Immunology, by Yunqing An and Zhi Yao. 2013-12. ISBN: 978-7-5659-0750-0.
- Immunology, 7th Edition, by David Male, Jonathan Brostoff, David Roth and Ivan Roitt. 2006-05-09. ISBN: 97803233992.
- **http://immuneweb.xxmc.edu.cn/**
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