Cytotoxic Cells: CD8^{+} CTLs, NK cells, CD4+ killers

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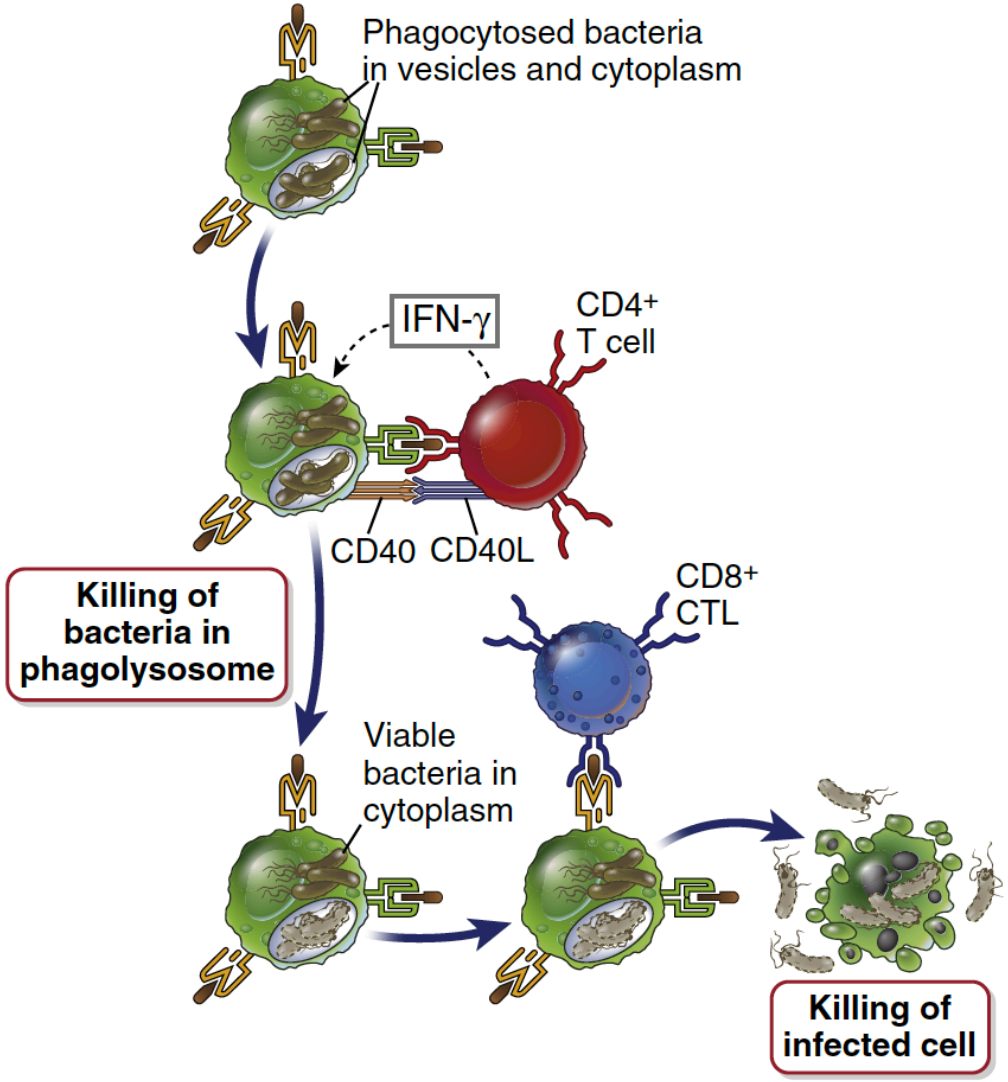
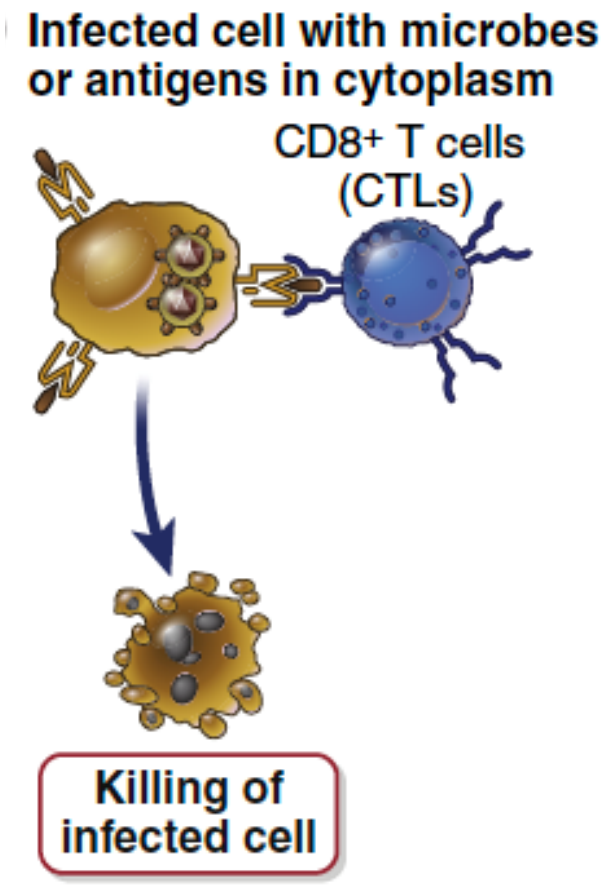
Lecture outline

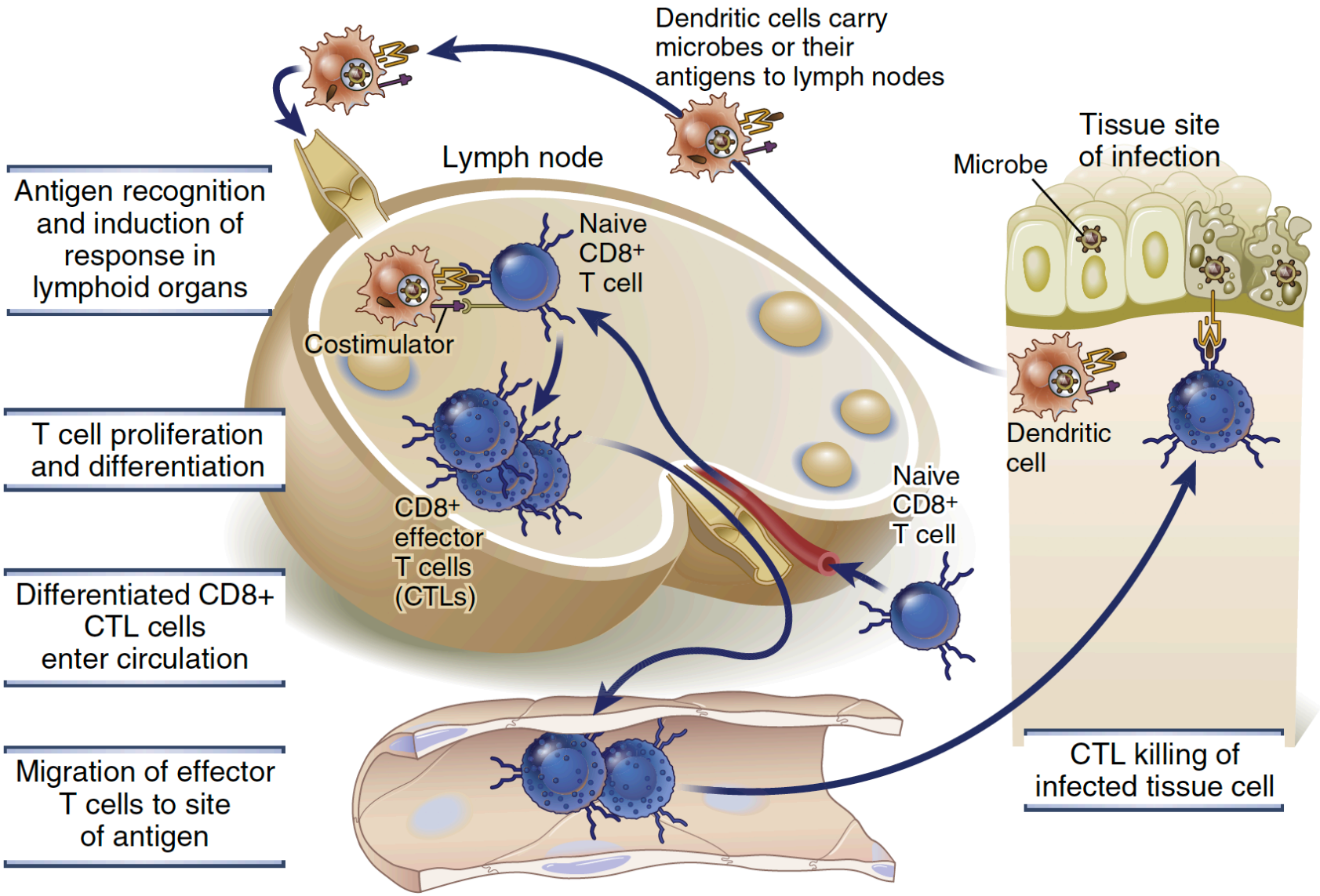
- Overview CD8+ T cell mediated immunity
- CD8+ T cell activation and differentiation into CTLs
- CTL effector function
- CTL function and dysfunction in human diseases
- Viral evasion of CTLs
- NK cell overview
- NK cell activation and inhibition

Role of CD8 T cells in eradicating infection

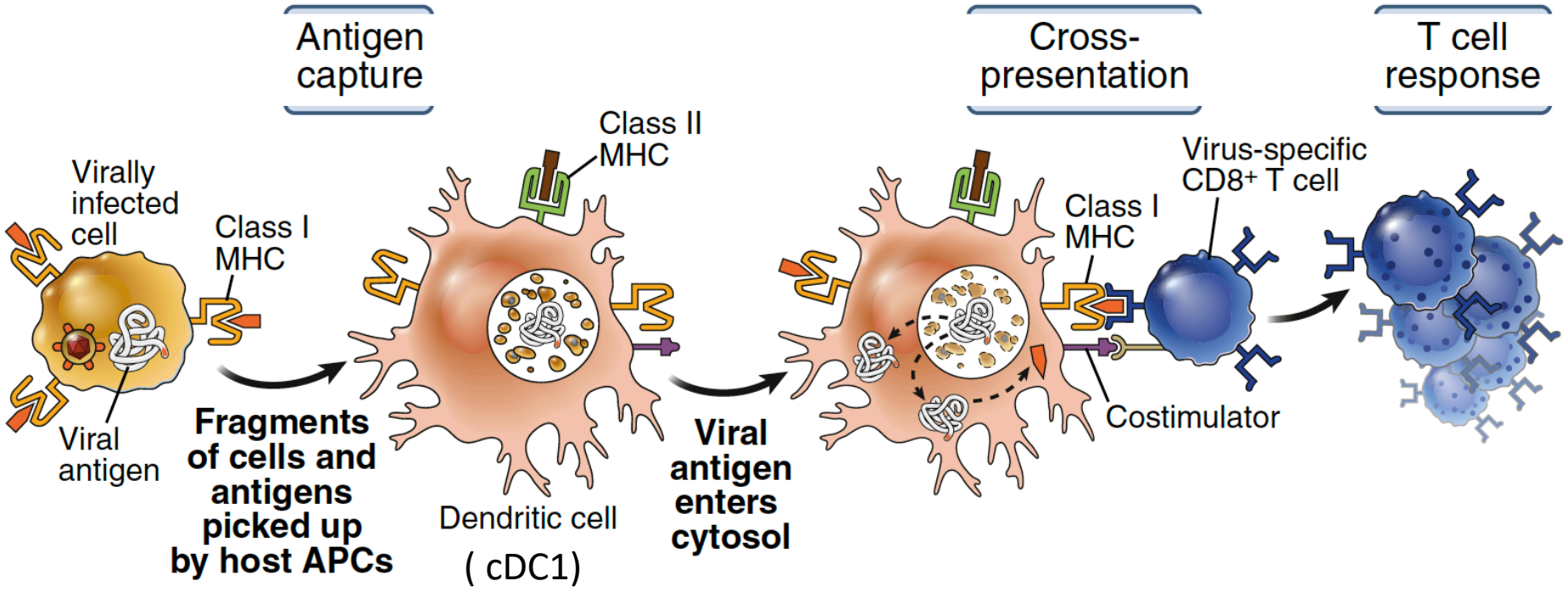




Induction and effector phases of CD8+ T cell responses

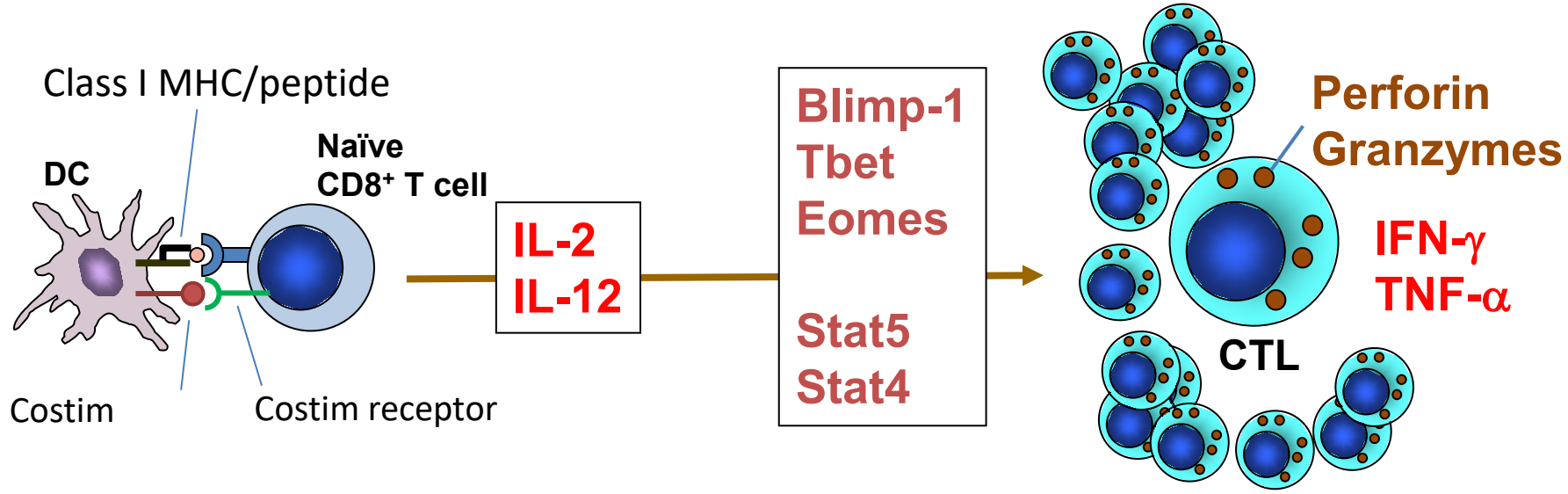


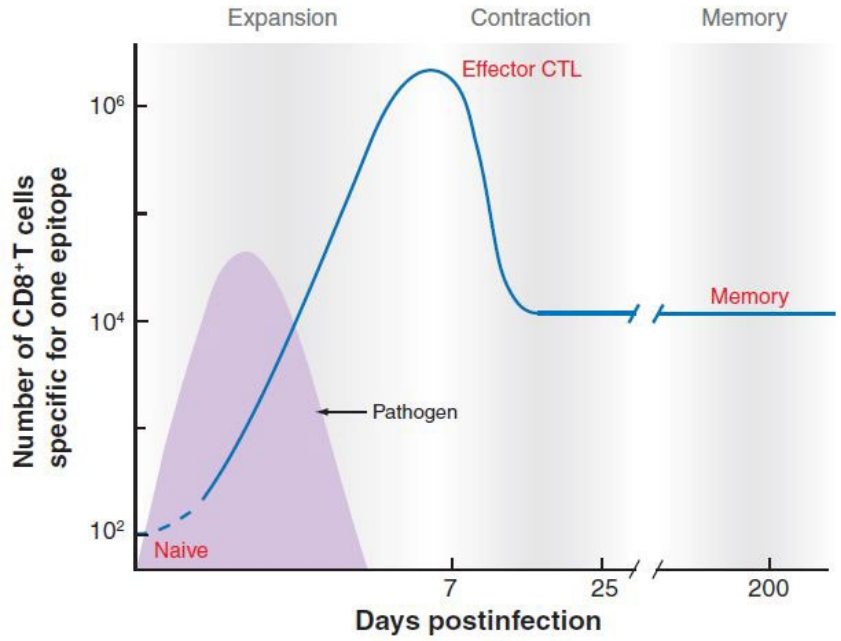
How do naive T cells specific for a virus that does not infect DCs get activated?



Cross presentation: *Proteins taken into cell via endocytosis/phagocytosis are transported to cytosol where they enter the class I MHC processing pathway*

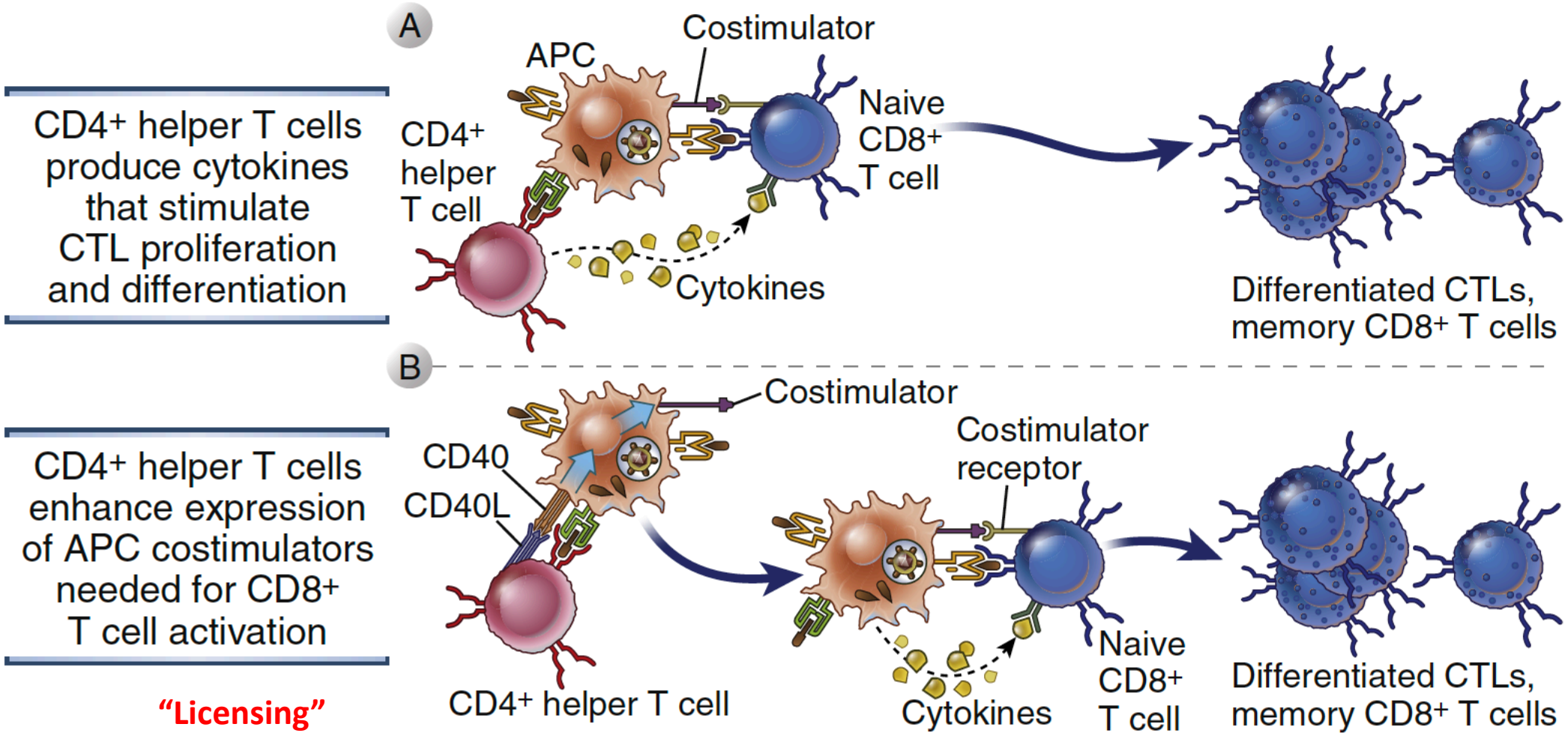
Effector CD8+ T Cell Differentiation and Expansion





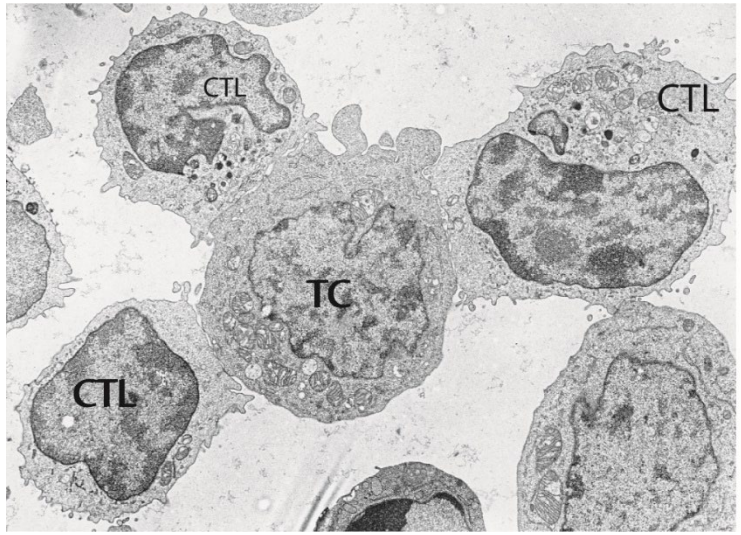
- **Prior to infection:** 1 in 100,000 naïve CD8+ T cells specific for any viral peptide
- **After antigen stimulation**: 15–20 divisions, 50,000-fold expansion
- **After infection is resolved:** 90%–95% CTL undergo apoptosis
- **For up to 75 years:** long-lived population of memory cells remain

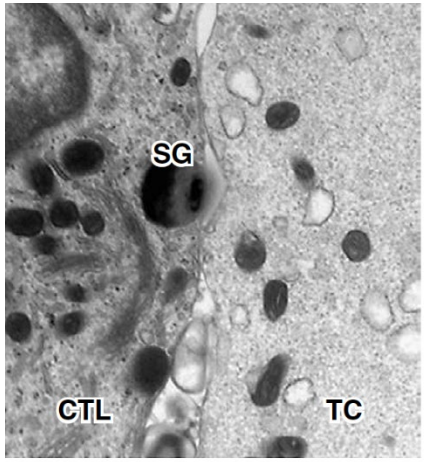
Role of helper T cells in the differentiation of CD8+ T lymphocytes

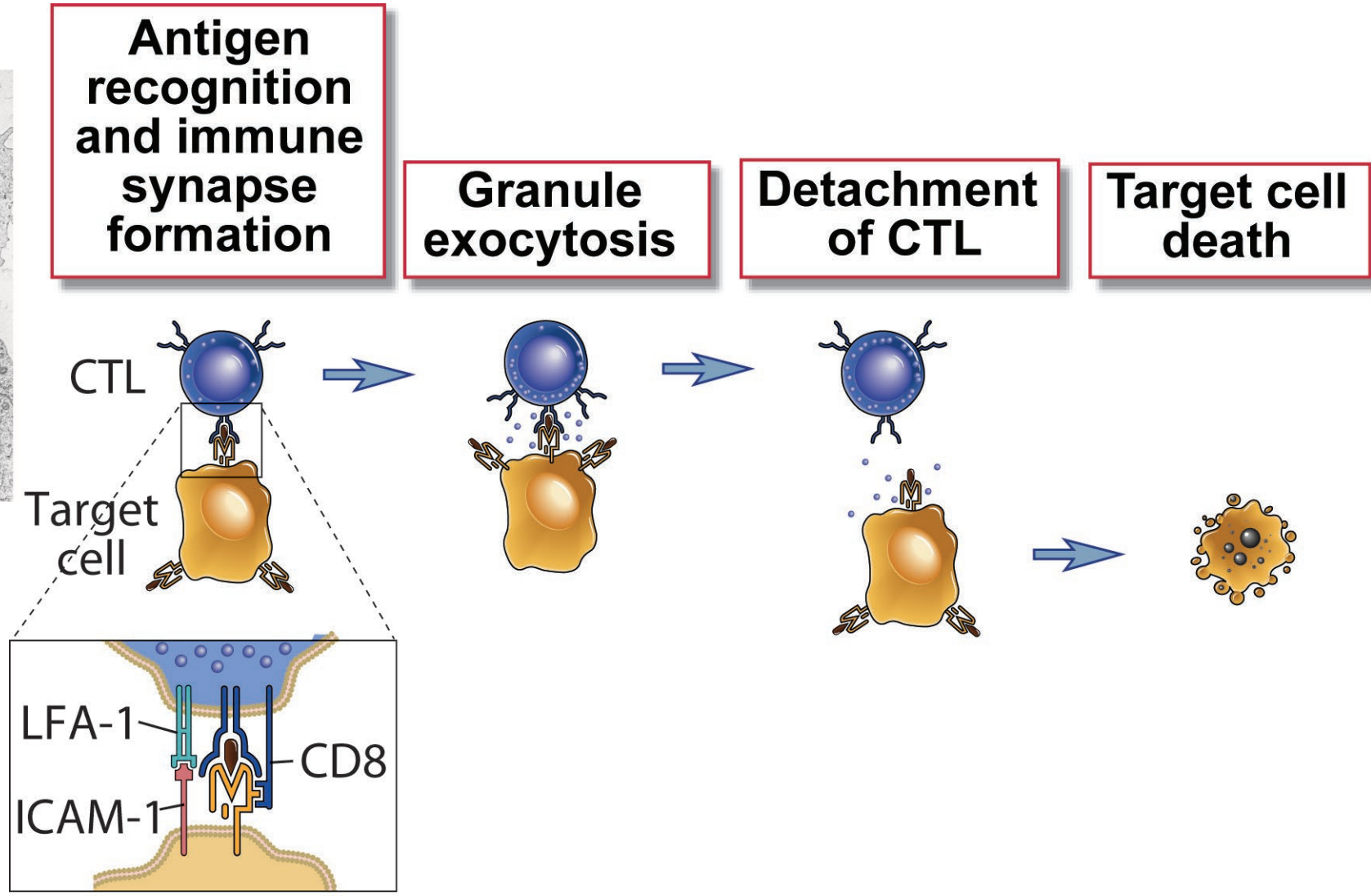


Major role of CD4+ T cells in CD8+ T cell response is the generation of memory CD8+ T cells

Steps in cytotoxic T lymphocyte–mediated lysis of target cells



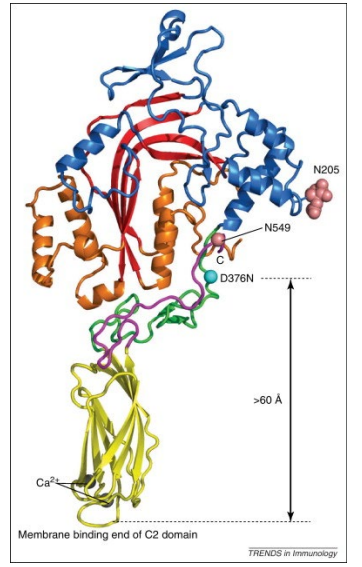




CTL Granule Proteins: PERFORIN and GRANZYMES

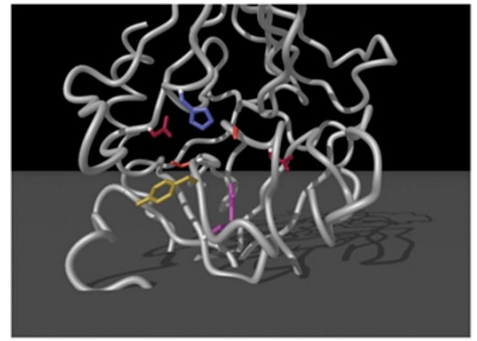
PERFORIN:

- Pore forming/membrane disruptive protein
- Works on cholesterol rich membranes (e.g. mammalian but not microbial cells)
- Homologous to Complement C9
- Required for delivery of granzymes into target cells

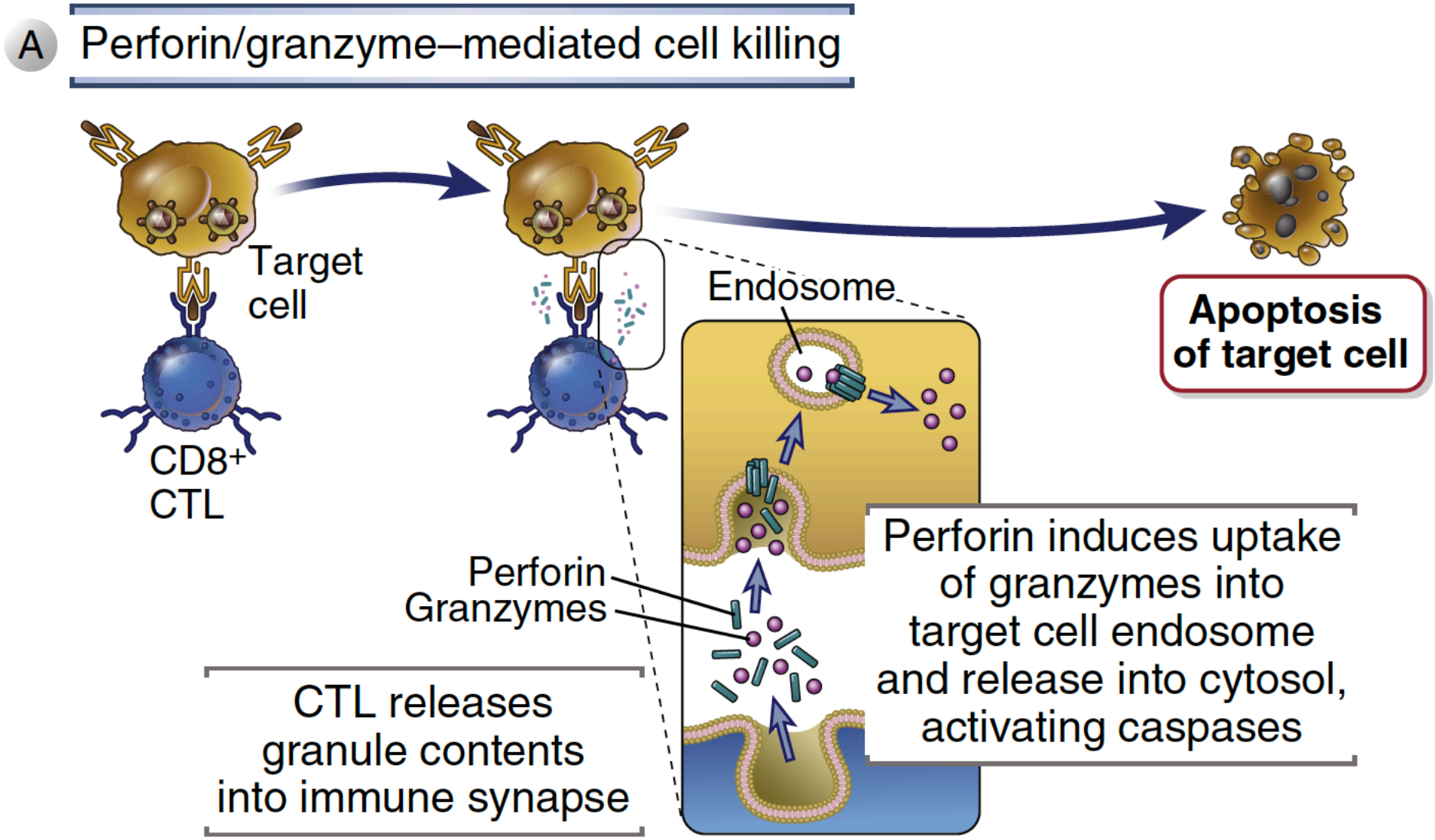


GRANZYMES

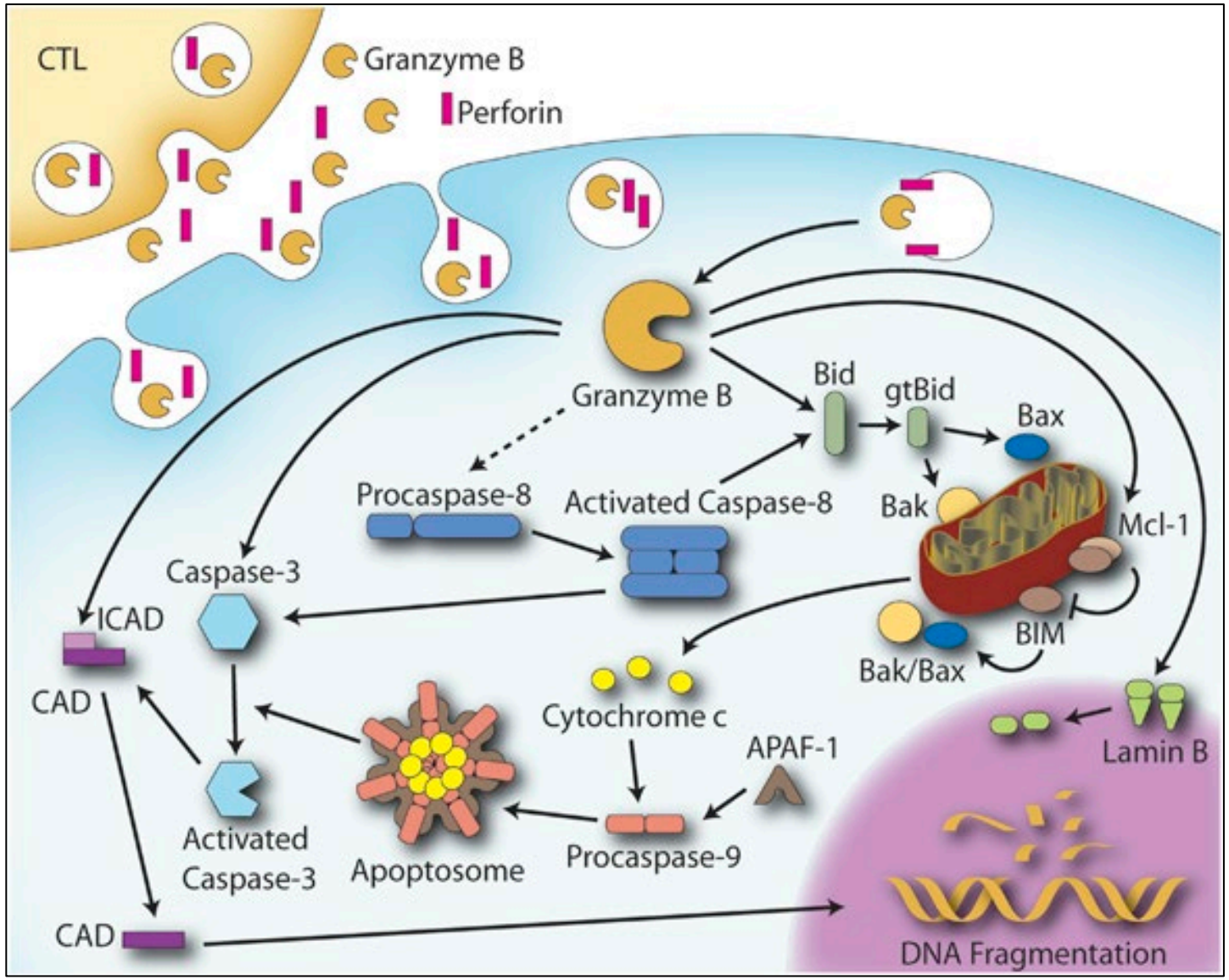
- Serine proteases that cleave a variety of substrates, including caspases.
- 5 human Gzms
- Gzms activate target cell apoptosis through *caspase-dependent* and *independent* pathways
- GzmB has the strongest pro-apoptotic function; most clearly implicated in CTL and NK induced apoptosis



Mechanisms of cytotoxic T lymphocyte–mediated killing of target cells



Granzyme B Delivery Cell Death



CAD= Caspase-activated DNase

CTLs are Serial Killers

One CTL can sequentially kill several target cells

The CTL may protect itself by cathepsins that degrade released perforin that binds to the CTL membrane

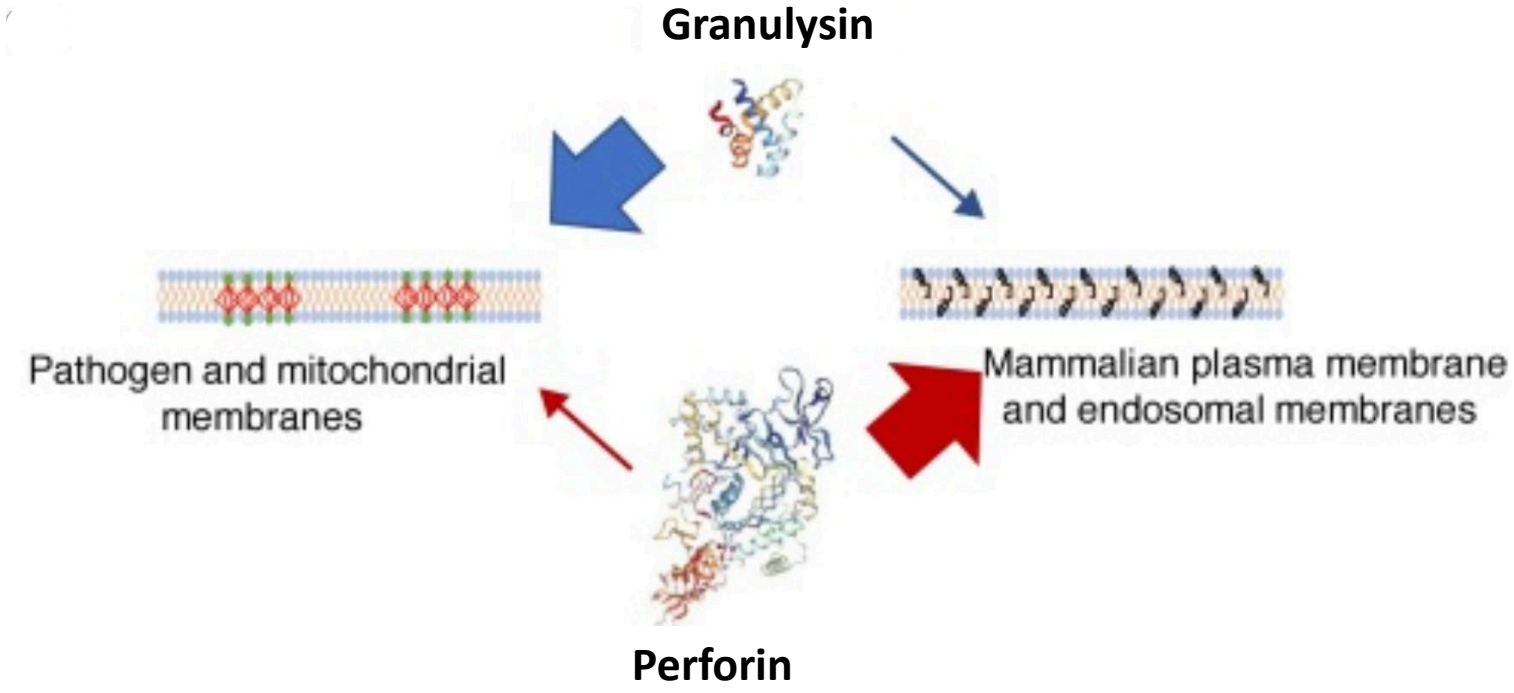
Perforin molecules that diffuse away are inhibited by plasma lipids

The formation of an immune synapse between a CTL and target limit bystander cell damage.

Bystander cells (e.g. antigen presenting cells) may be protected from death by expressing specific and irreversible granzyme inhibitors (serpins).

CTL Granule Proteins: Granulysin

- Lipid-binding, cationic peptide
- Works on cholesterol-poor membranes on microbes
- Preferentially lyses microbial cells
- Has alarmin/pro-inflammatory properties



Farokh Dotiwala and Judy Lieberman,*Curr Opin Immunol. 2019 Oct; 60: 19–29.

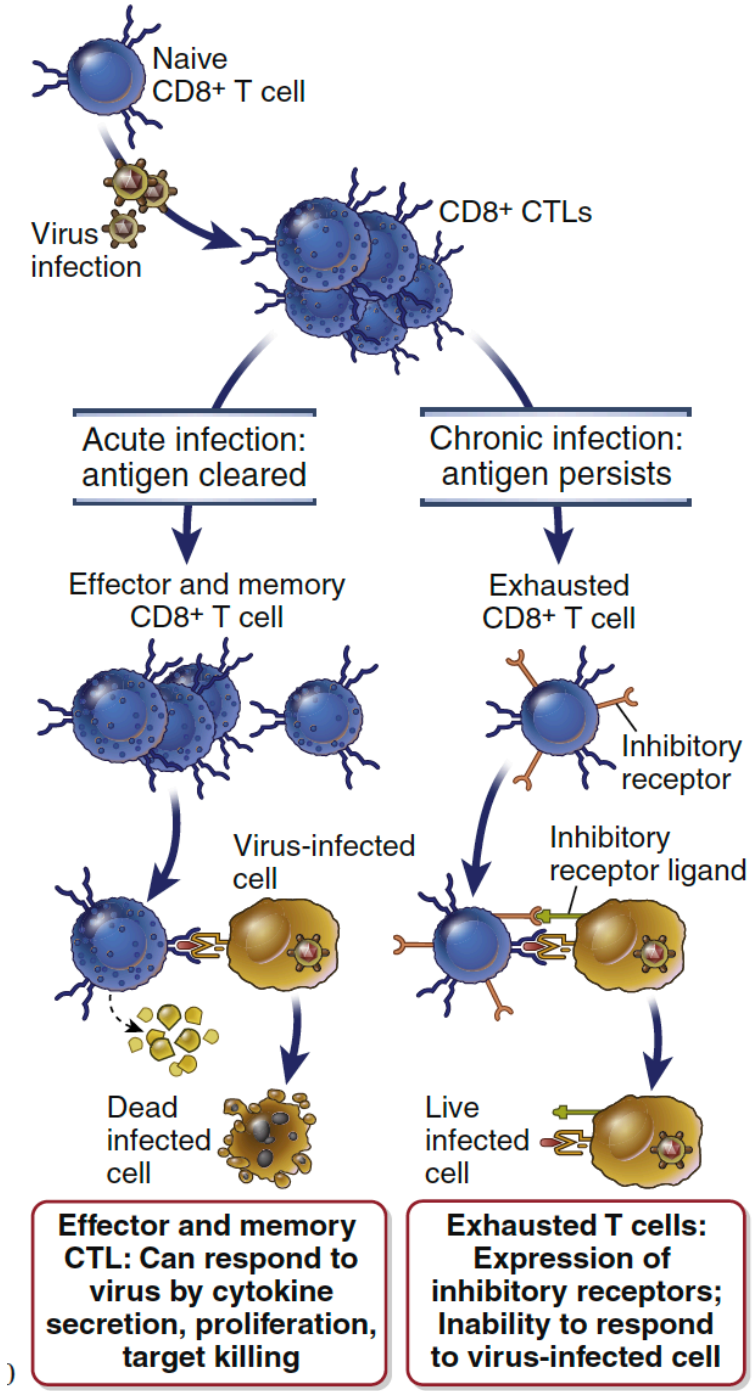
Clinical Evidence for Roles of $CD8^+$ T cells

- CD8 T cell deficiency caused by homozygous mutations in *CD8*, *TAP1*, *TAP2, DOCK8*
  - Chronic sinopulmonary infections
  - Severe cutaneous HSV and HPV infections

- Exhausted $CD8^+$ T cells in cancer patients
  - Block PD-1, revive CTL, enhanced anti-tumor immunity and anti-viral responses

- A functional CTL (CD8+) response is required to clear the infection and
  - COVID-19 severity is increased as the CD8+ response becomes somehow diminished or exhausted

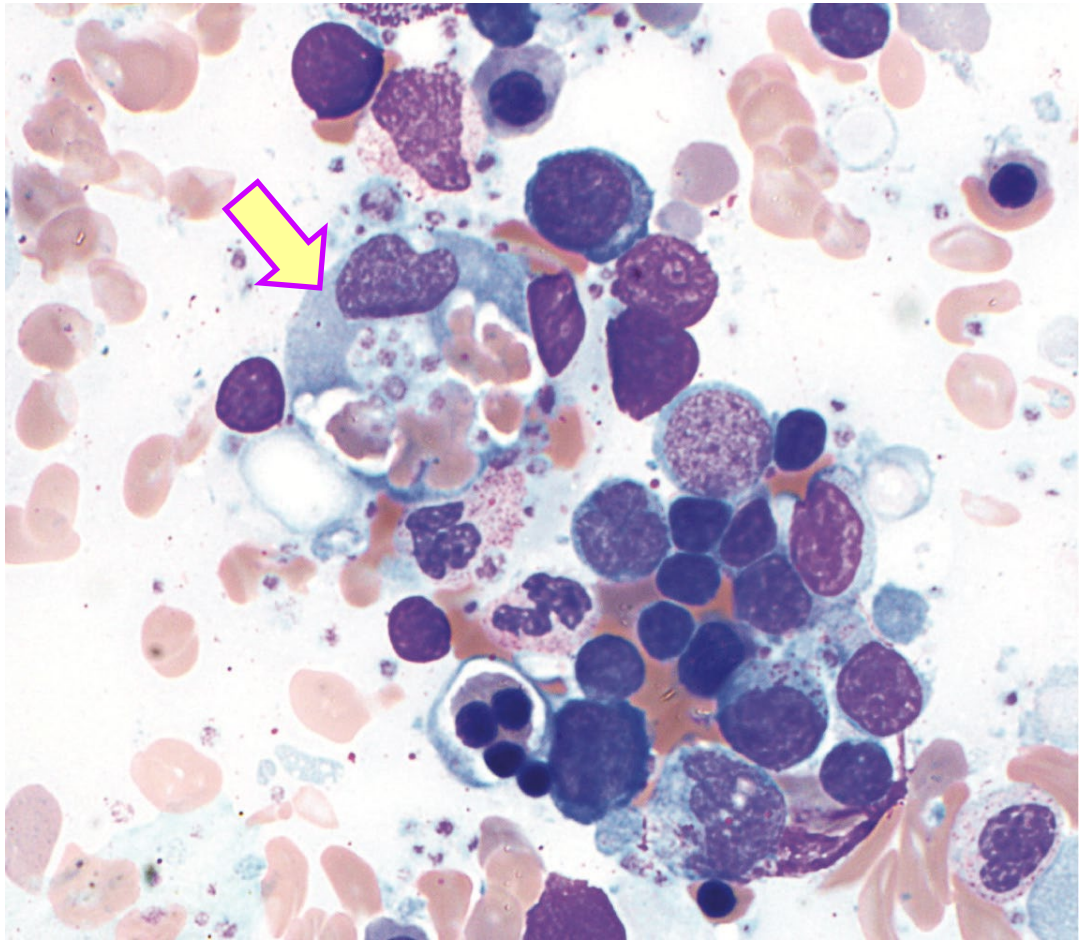
T cell exhaustion

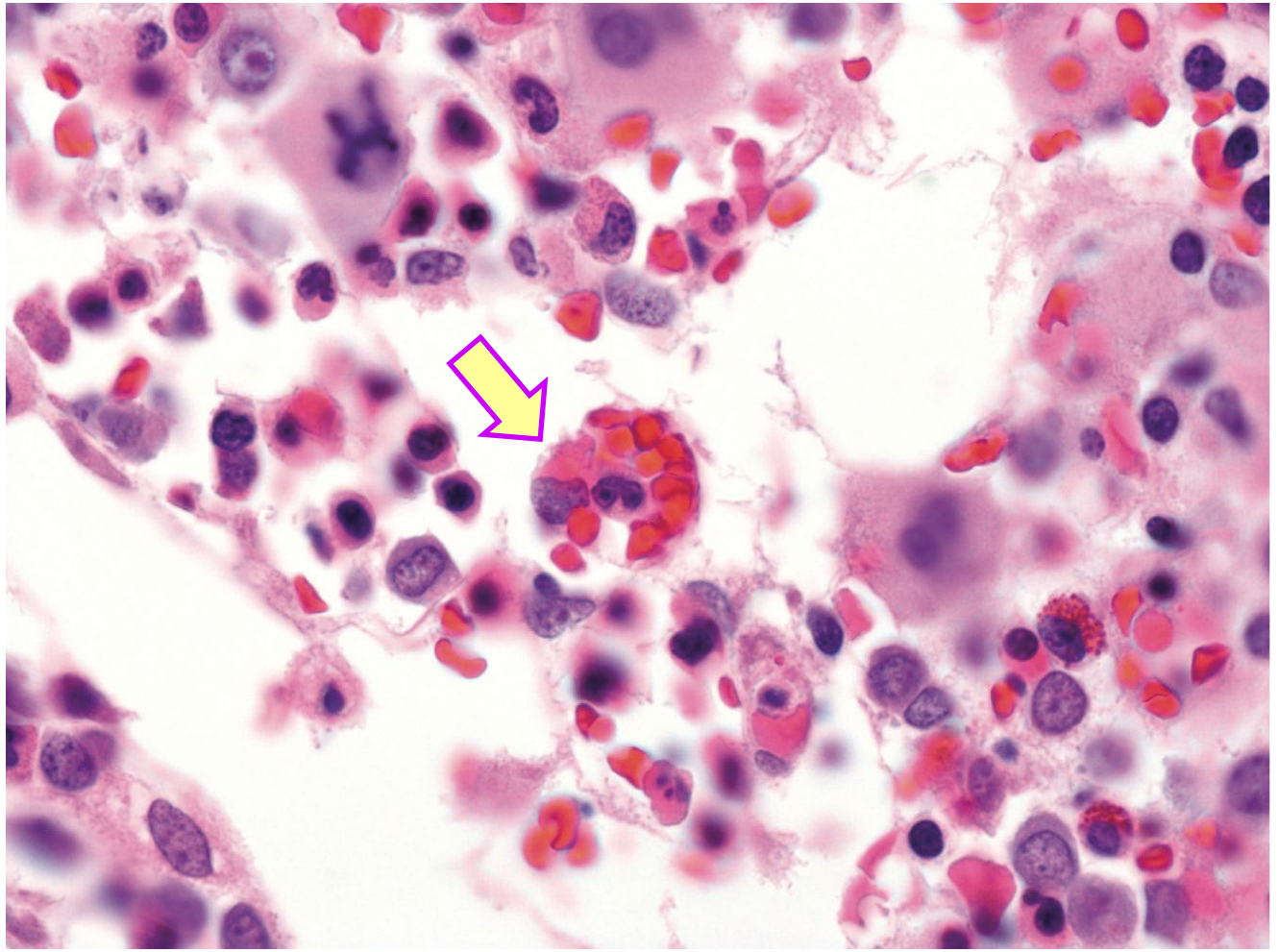


Role of CTL/Perforin in Diseases

- Perforin plays a permissive role of in malaria: attack on antigen-bearing brain endothelial cells
- Perforin plays a key role in the autoimmune destruction of insulin-producing β cells in the pancreatic Islets leading to Type 1 diabetes mellitus
- CTL are major contributors to:
  - Viral myocarditis and dilated cardiomyopathy
  - Allograft rejection
  - Liver injury in acute viral hepatitis

Hemophagocytic lymphohistiocytosis





Hanny Al-Samkari , Nancy Berliner. Annu Rev Pathol 2018 Jan 24;13:27-49.

What Happens Without CTL of NK Killing Function?

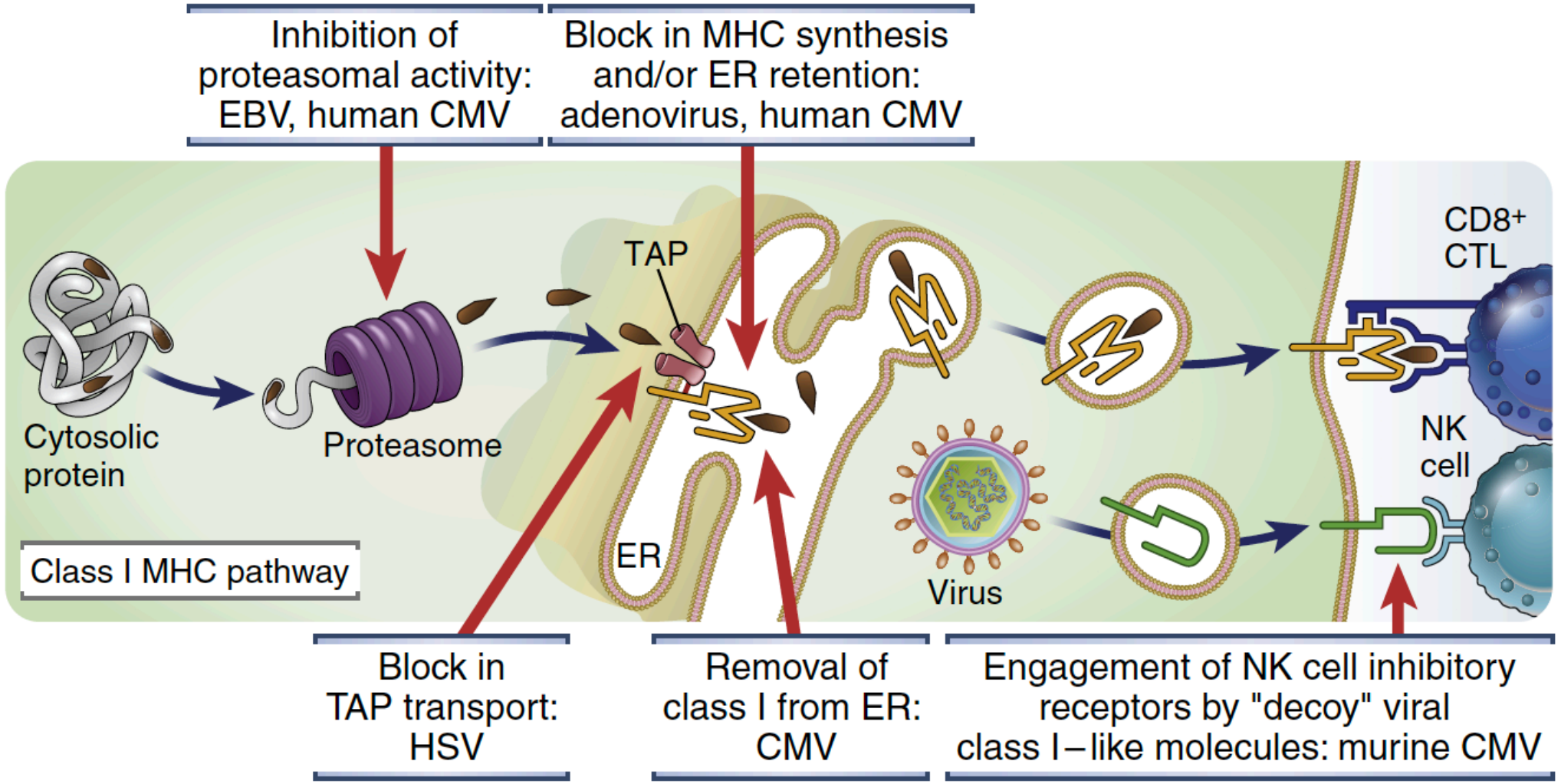
•**Familial hemophagocytic lymphohistiocytosis** (HLH):mutations in perforin gene or other genes critical for degranulation of cytotoxic granules

•NK cells and CTL can be activated by infected cells to secrete interferon-γ, but cannot kill the infected cells, so excess interferon-γ keeps getting produced

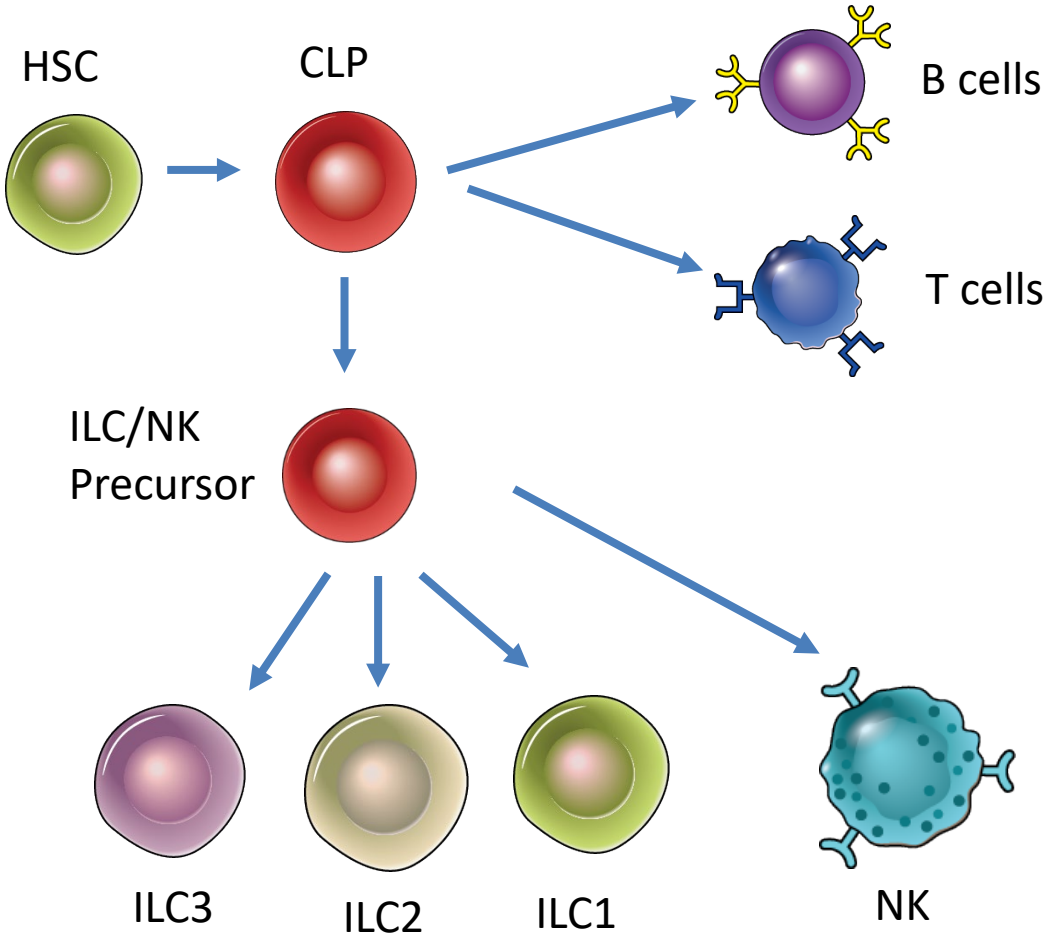
•Uncontrolled activation and proliferation of CD4+ and CD8+ T cells, cytokine storm, macrophage activation and proliferation, pancytopenia, and anemia.

•Activated macrophages in the spleen and bone marrow are intensely phagocytic, removing erythrocytes, leukocytes, and platelets from the circulation

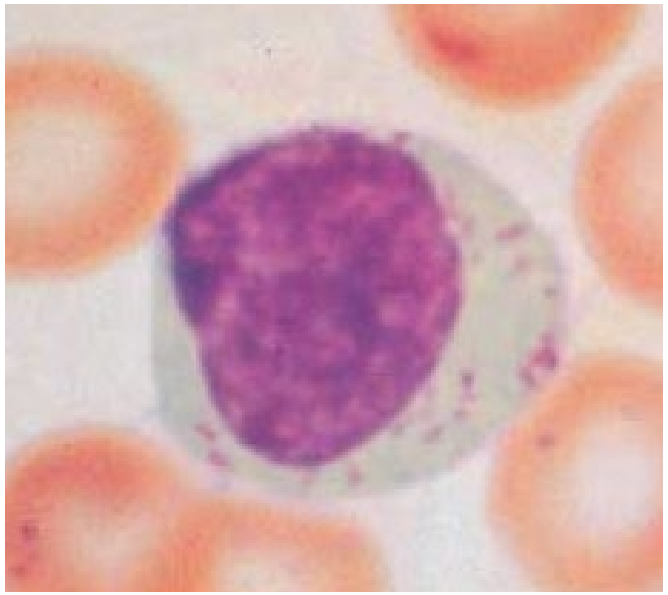
Viral evasion of CTL surveillance



Natural Killer (NK) Cells



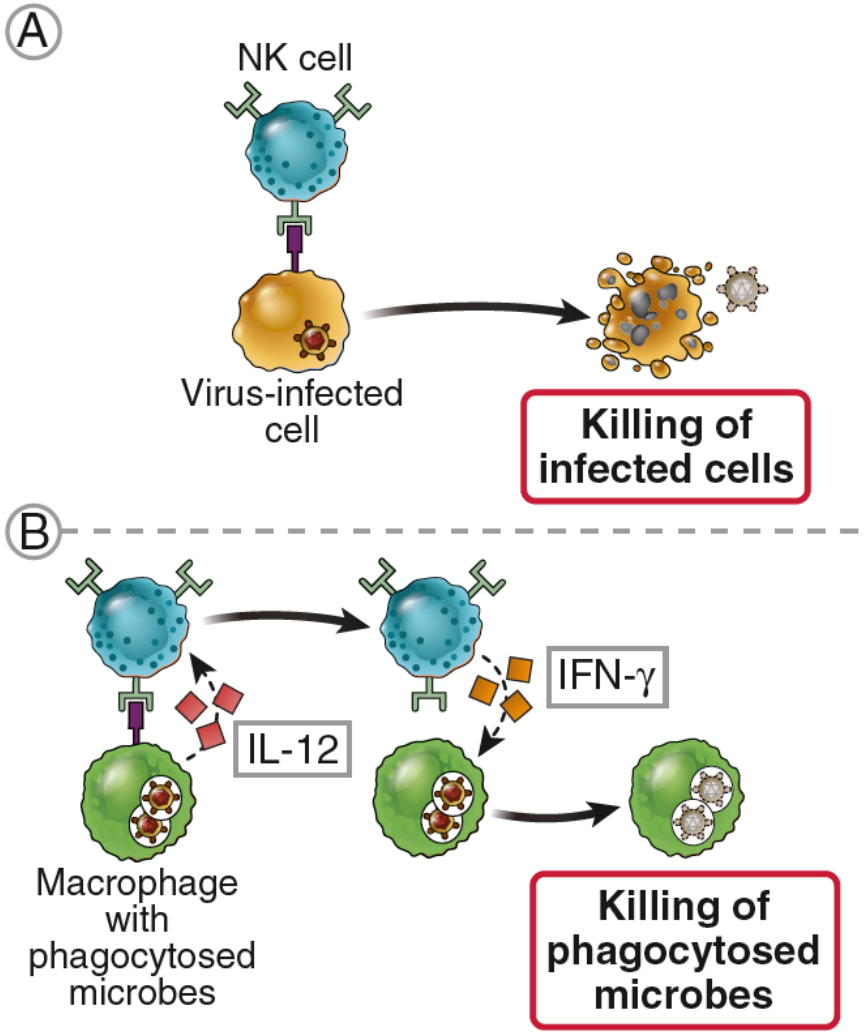
CD3-,CD56+, (Nkp46+)



5-10 % of blood lymphoid cells

Like other ILCs, NK do not have highly diverse clonally distributed antigen receptors

NK Cell Function



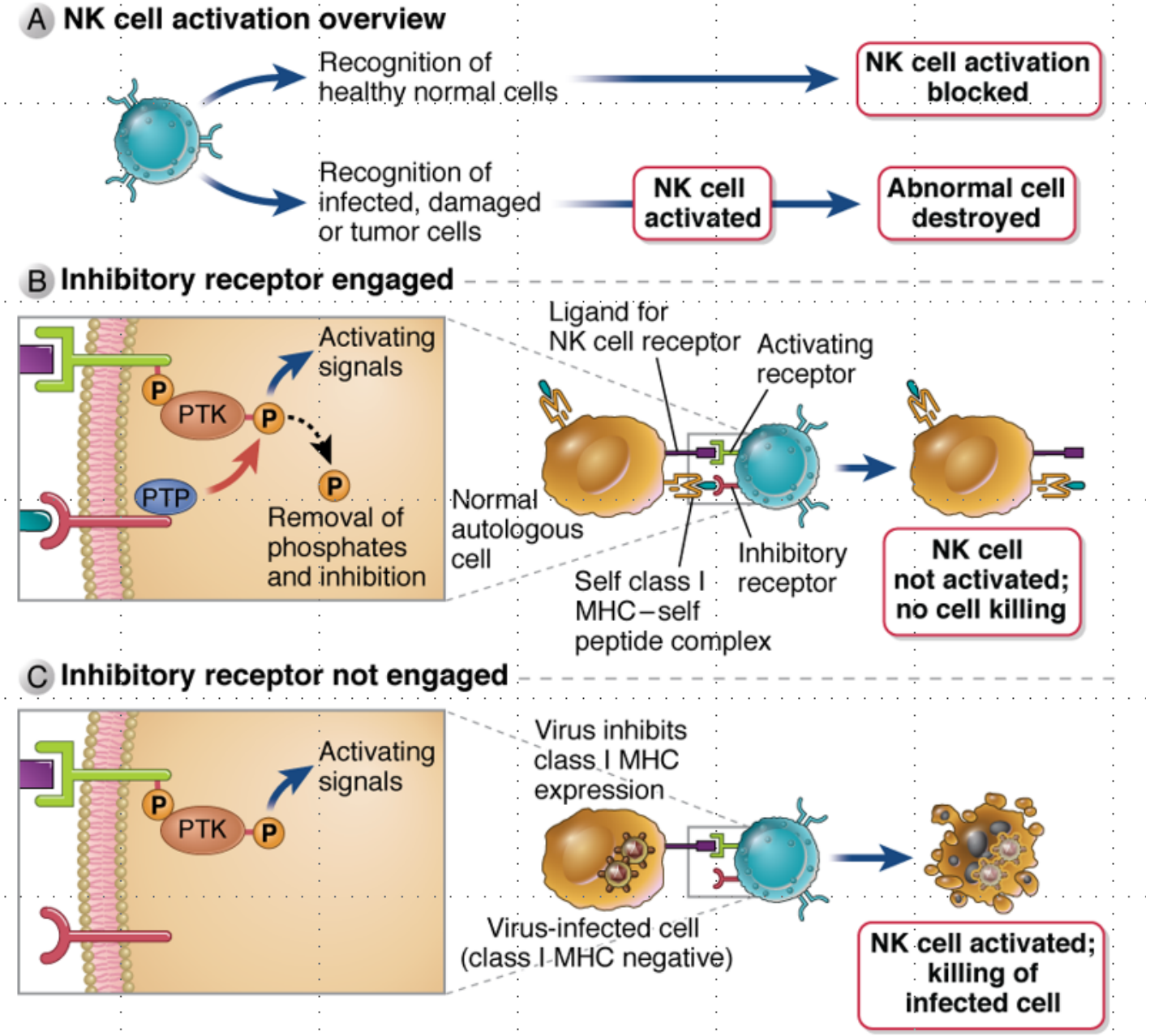
- Functions of NK cells are similar to those of CD8+ cytotoxic T lymphocytes (CTLs)
- NK activation by infected cells results in release of perforin, granzymes which kill the target cell, and secretion of interferon γ, which activates macrophages.

NK Activation: Balance of Inhibitory and Activating Receptors

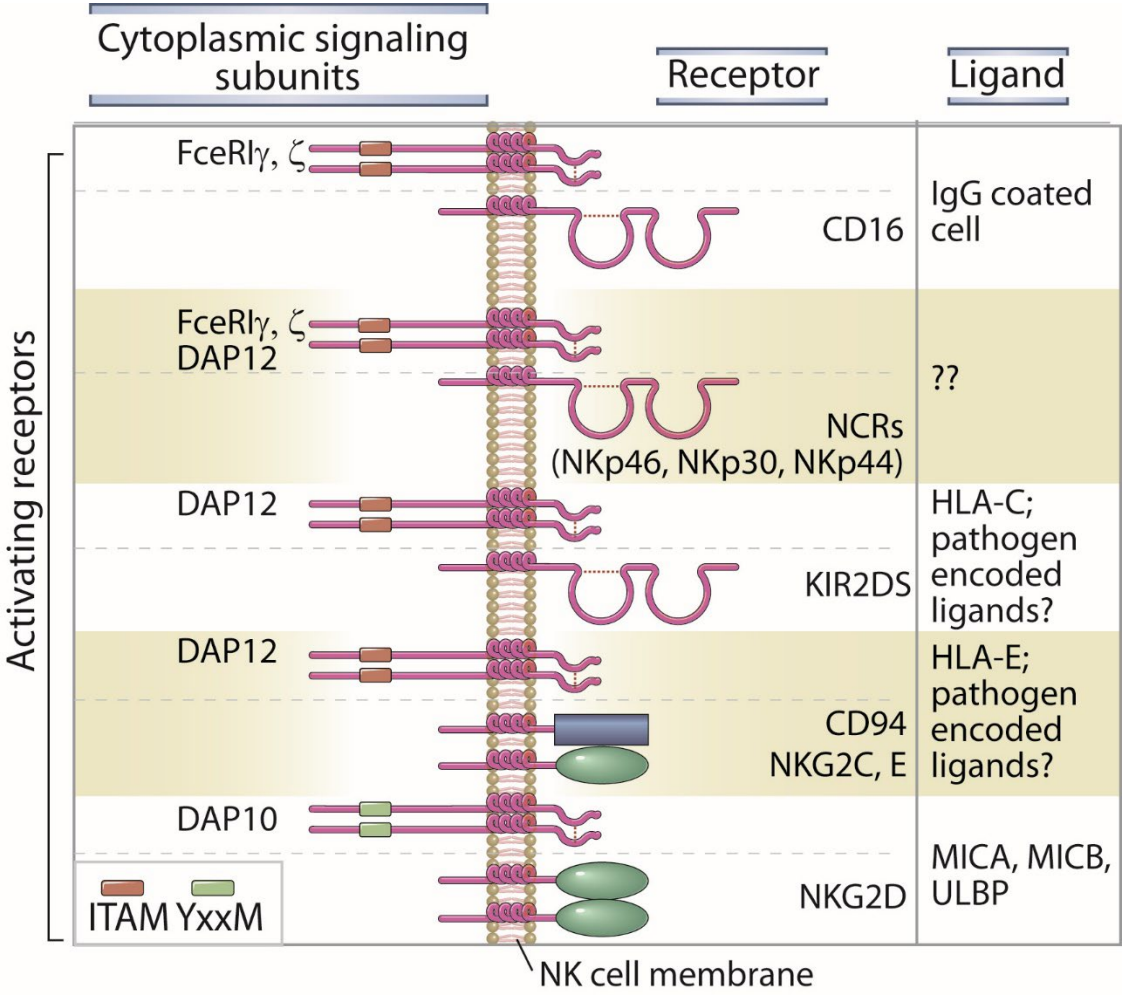
Inhibitory receptors:

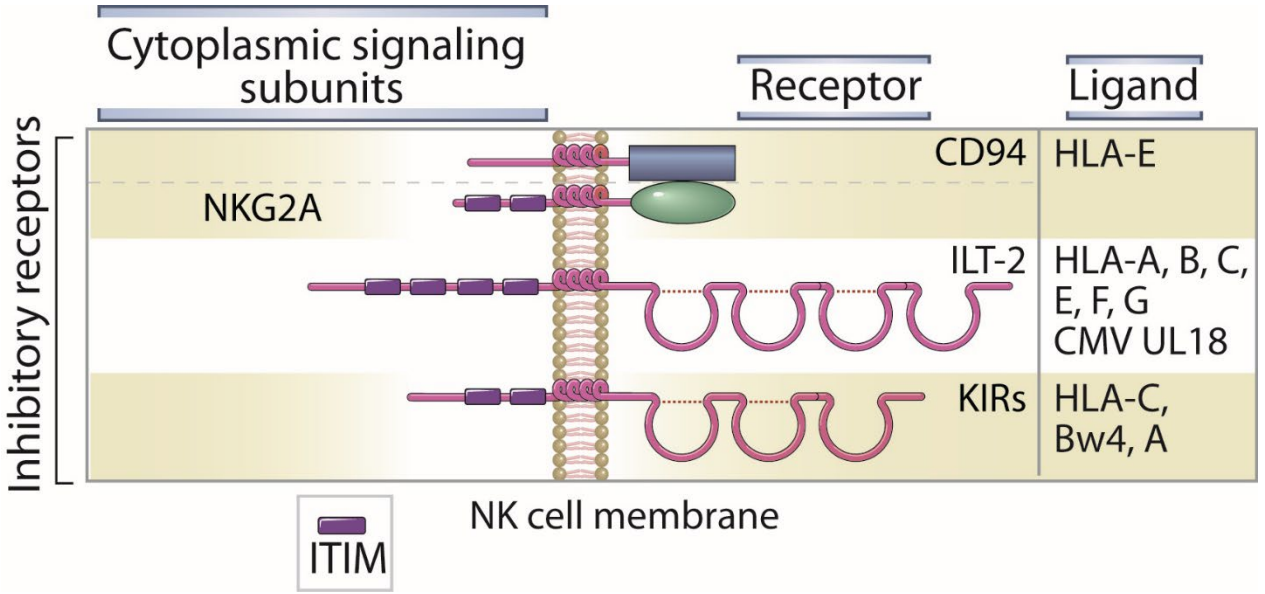
Recognize Class I MHC proteins (markers of normal self expressed by all nucleated cells)

Signal via ITIM motifs

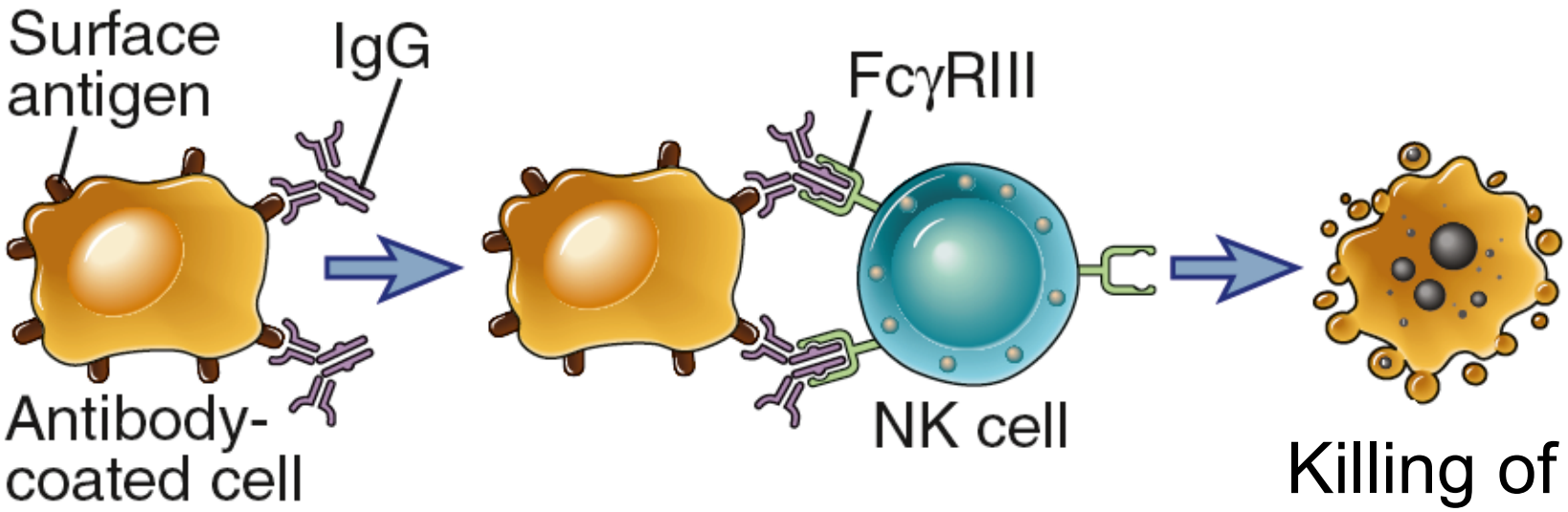


NK Cell Activating and Inhibitory Receptors





Antibody-dependent cellular cytotoxicity (ADCC)



Clinical Evidence of Role of NK cell in Defense and Disease

Evidence that NK cells are important in viral immunity in humans and mice

- Severe Epstein-Barr virus infection in rare patients lacking NK cell function (*MCM4, GATA2, IRF8* mutatoons)

- Severe herpesvirus infections in rare patients without Natural Killer cells.

- Natural Killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo.

NK cells in Hematopoietic Stem Cell Transplantation

- Detrimental effects –grafted allogeneic stem cell rejection by host NK cells

- Beneficial effects - in leukemia patients given an allogeneic hematopoietic stem cell graft ---- graft vs. leukemia activity – arising donor NK cells kill residual host leukemia cells