

Corso di Immunologia - III anno
Prof. Paolini

Lezione 31/10/2025

"I linfociti T citotossici"

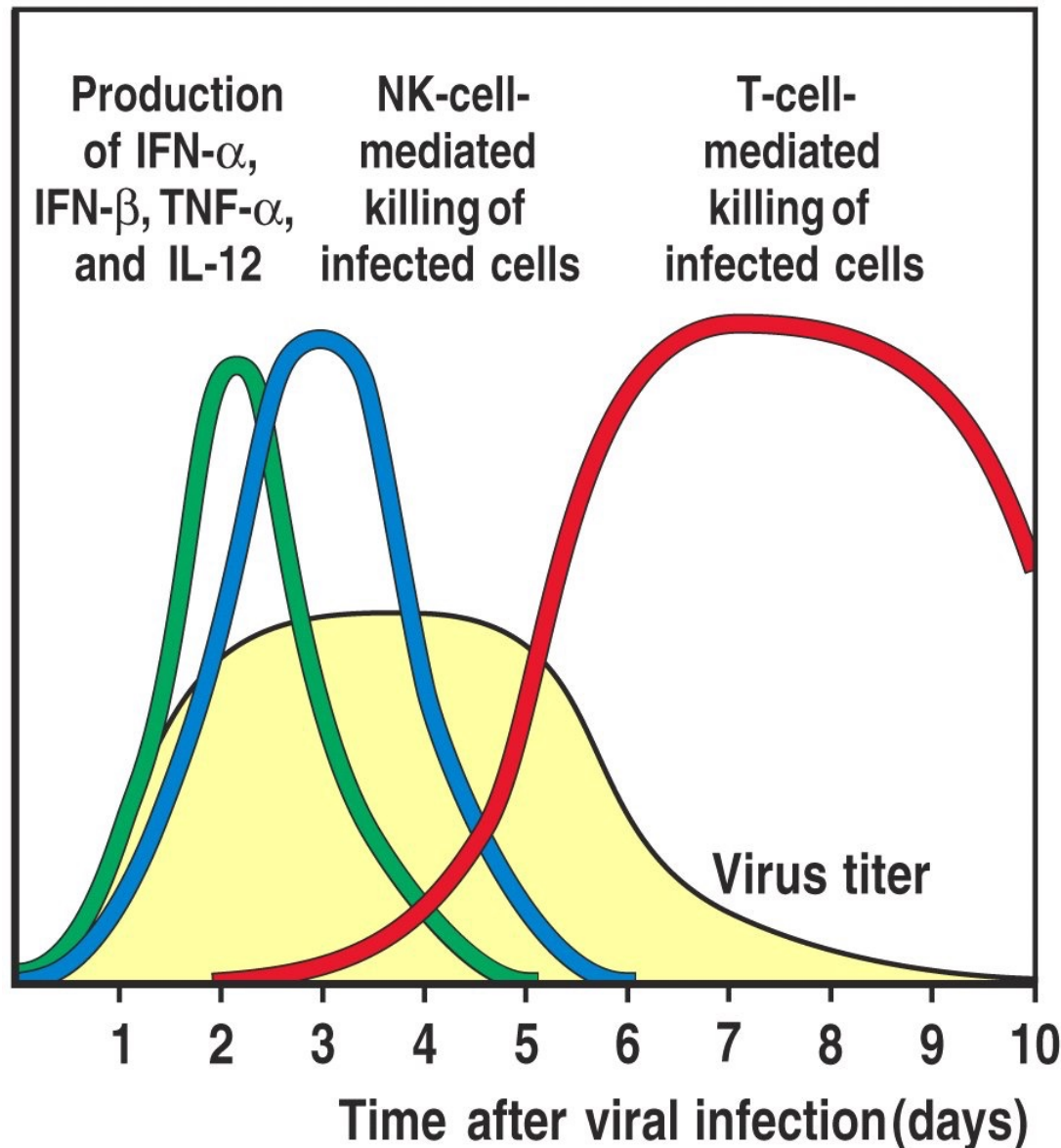
Il materiale presente in questo documento viene distribuito esclusivamente ad uso interno e per scopi didattici.

Lymphocyte cytotoxicity

- Effector mechanism of cell-mediated immunity
- Directed against:
 - ❖ Infected cells (intracellular pathogens)
 - ❖ Tumor cells
 - ❖ Allogeneic cells
- 2 major lymphocyte populations:

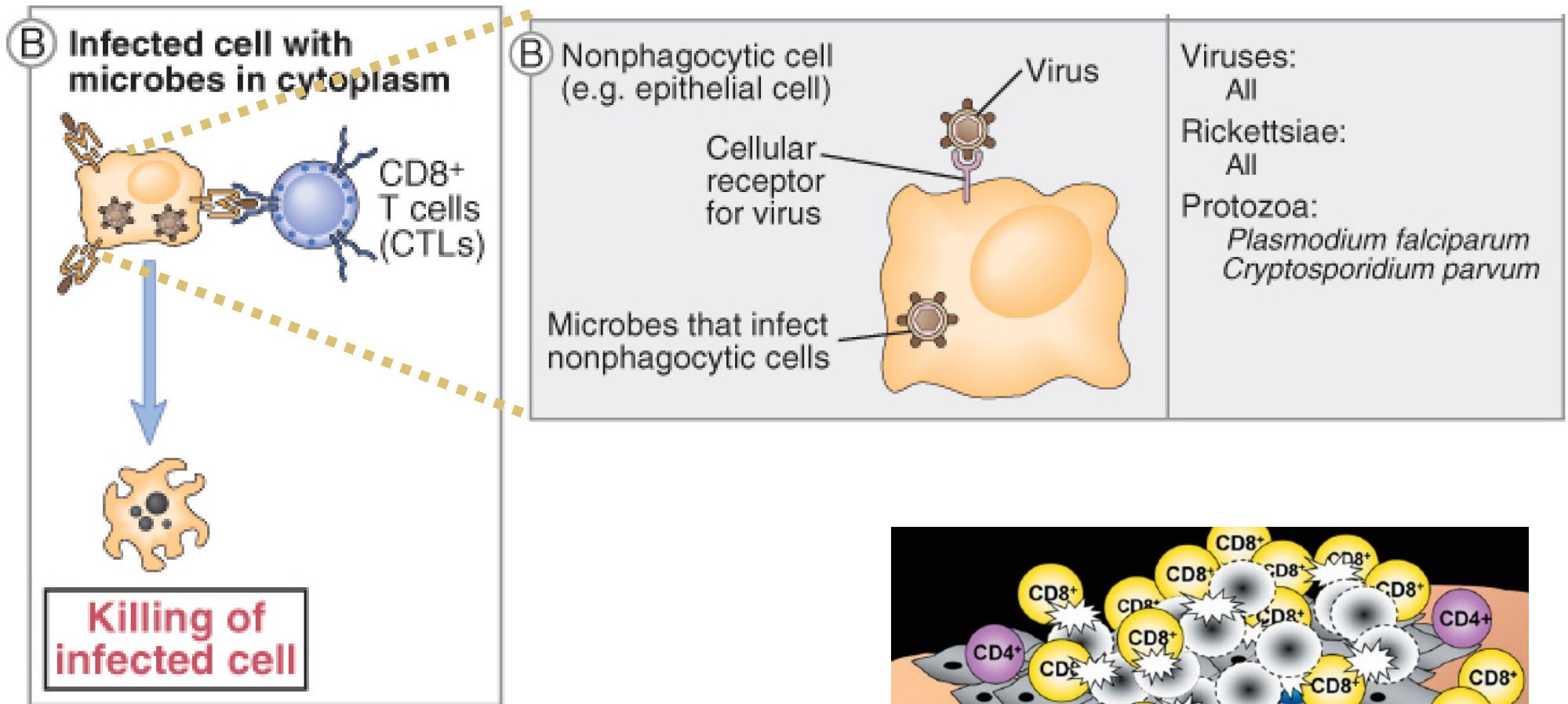
□ CD8+ T lymphocytes	CTL
□ Natural Killer cells	NK
- Different mechanisms for target cell recognition
- Same lytic mechanisms

Why do exist different cytotoxic lymphocyte populations?

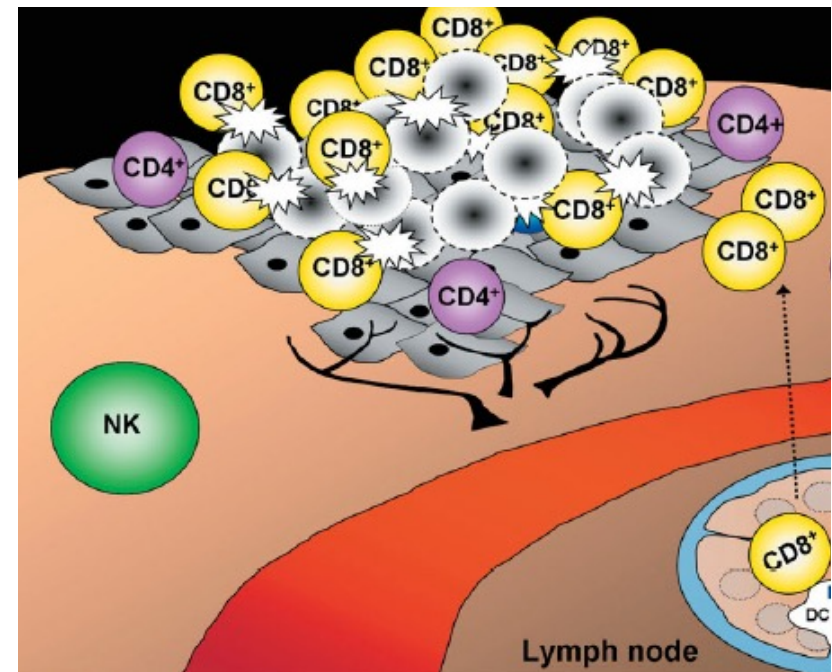


Complementary strategies in the defense against intracellular pathogens!

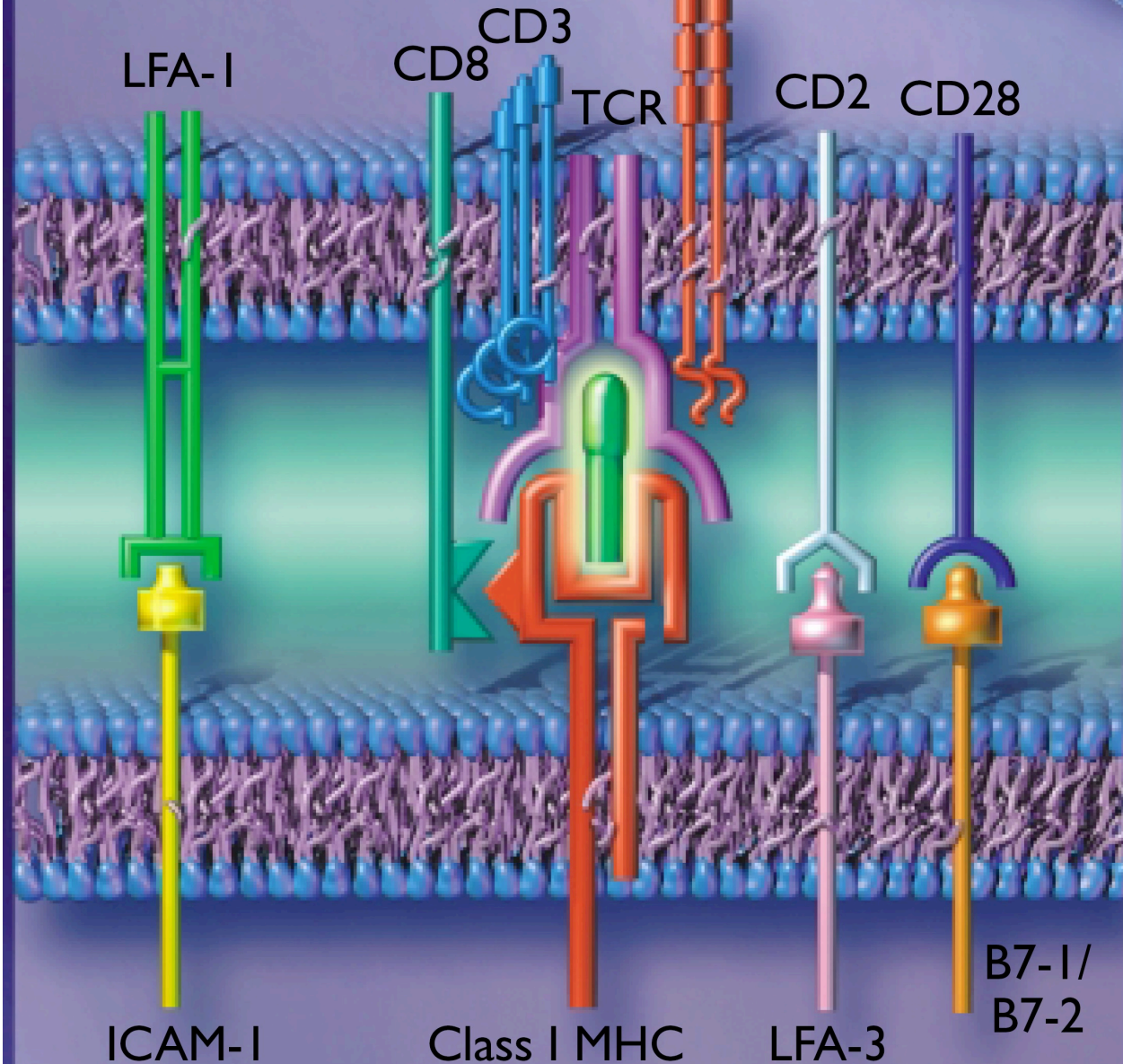
CTL participate to the response against intracellular pathogens



...and tumor cells



CD8⁺ T lymphocyte



Class I MHC-expressing cell

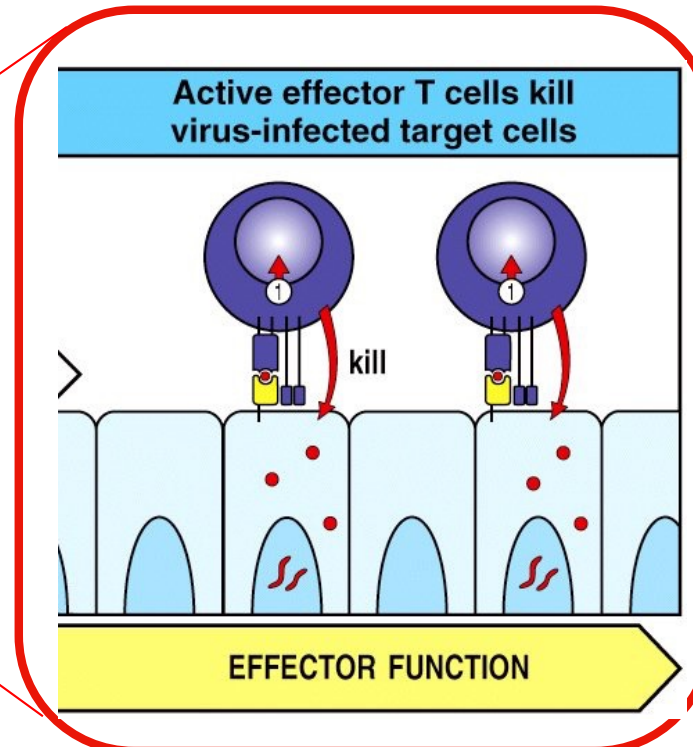
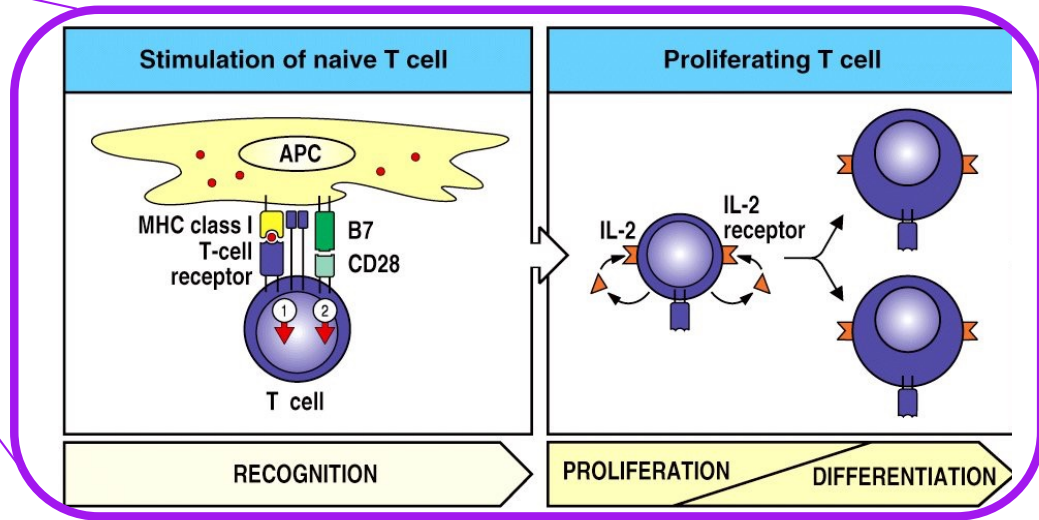
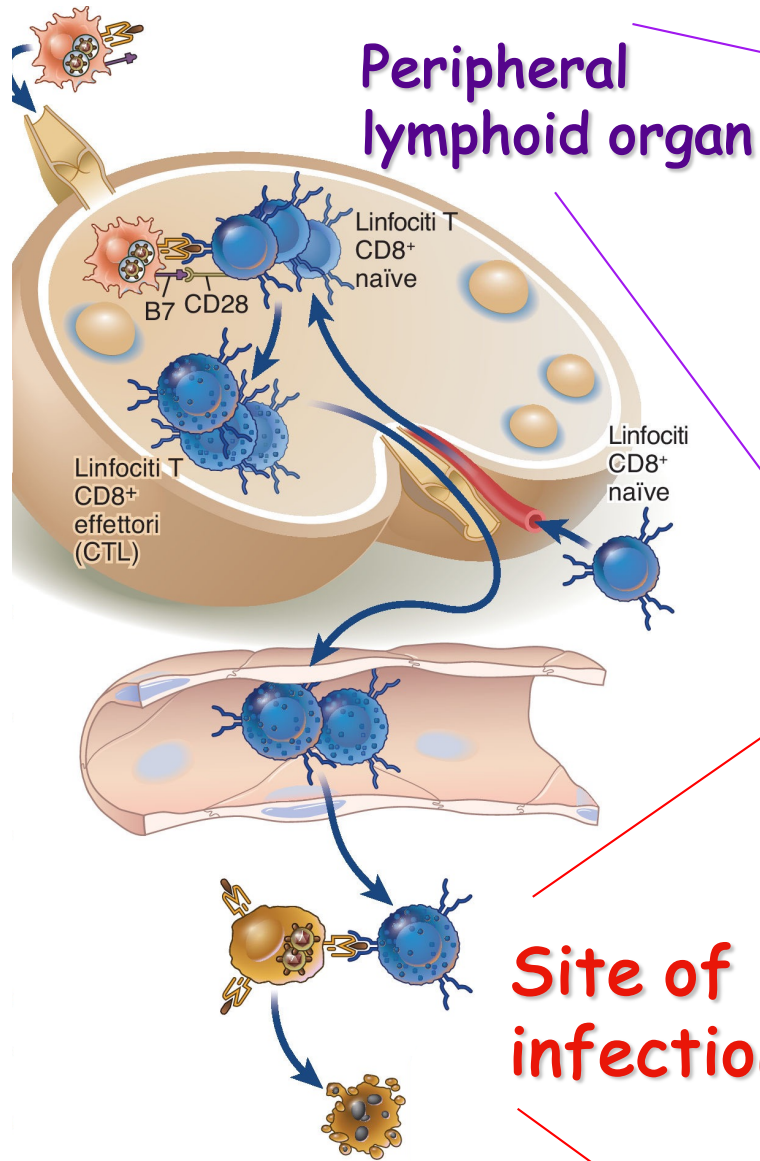
**CD8 T cell
recognition is**

specific

and

**MHC class I-
restricted**

Differentiation of pre-CTL to effector cells

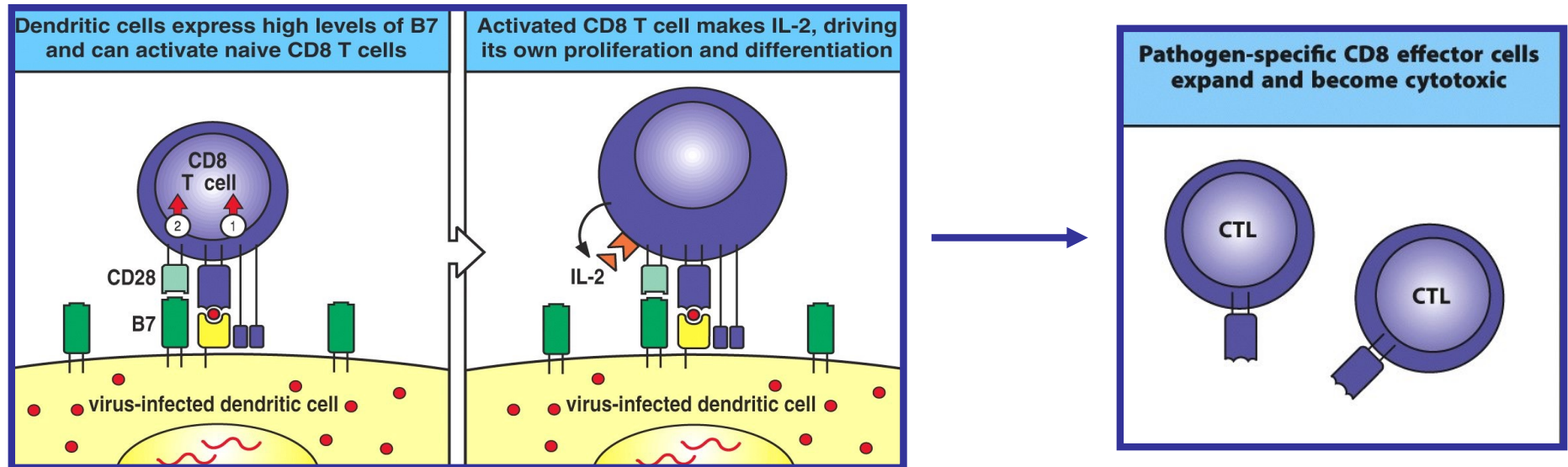


CD8 T CELLS MATURE IN THE THYMUS
AND ARE **PRE-CTLs**,
ABLE TO RECOGNIZE ANTIGENS BUT NOT TO KILL
INFECTED TARGET CELLS

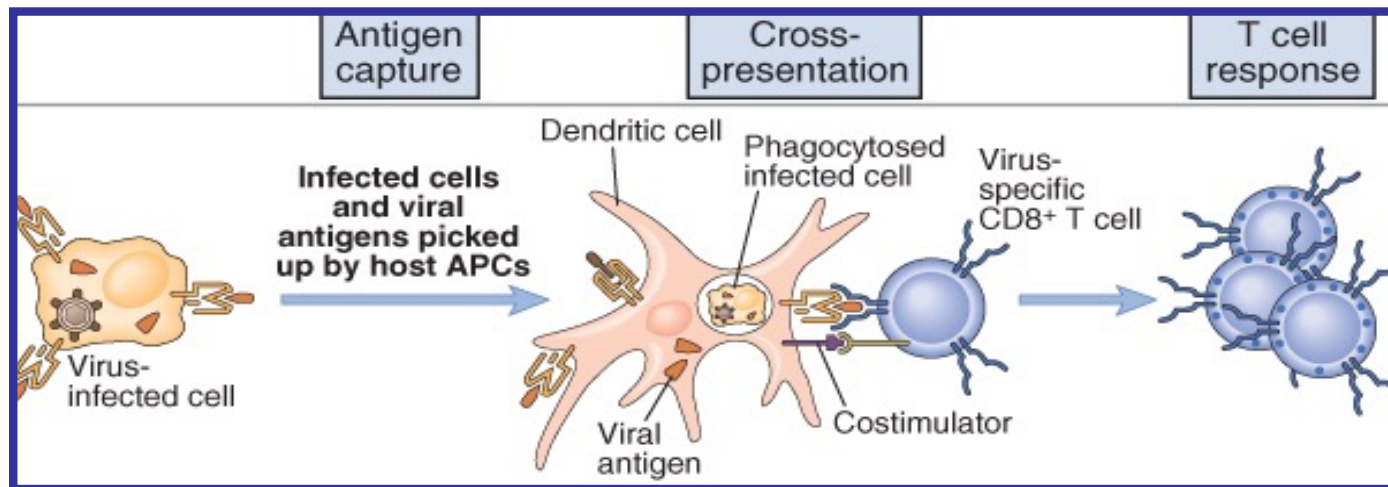
HOW ARE THEY ACTIVATED AND HOW DO THEY
BECOME **CTLs**?

Different mechanisms for CTL differentiation

I° Naive CD8⁺ lymphocytes are activated by a dendritic cell infected by an intracellular pathogen.

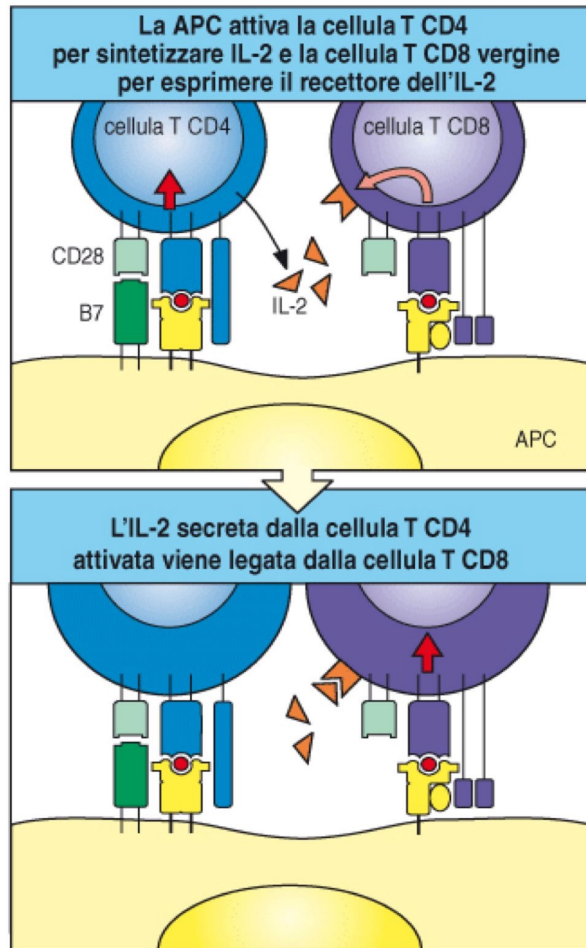


... or from an uninfected dendritic cell that cross-presents viral antigens



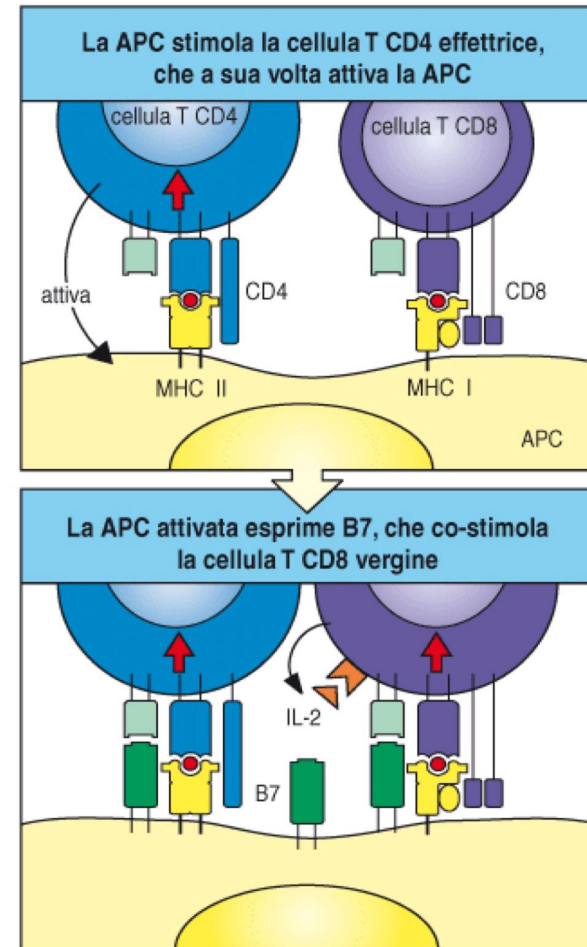
Alternative mechanisms requires help from CD4+ T cells

II°



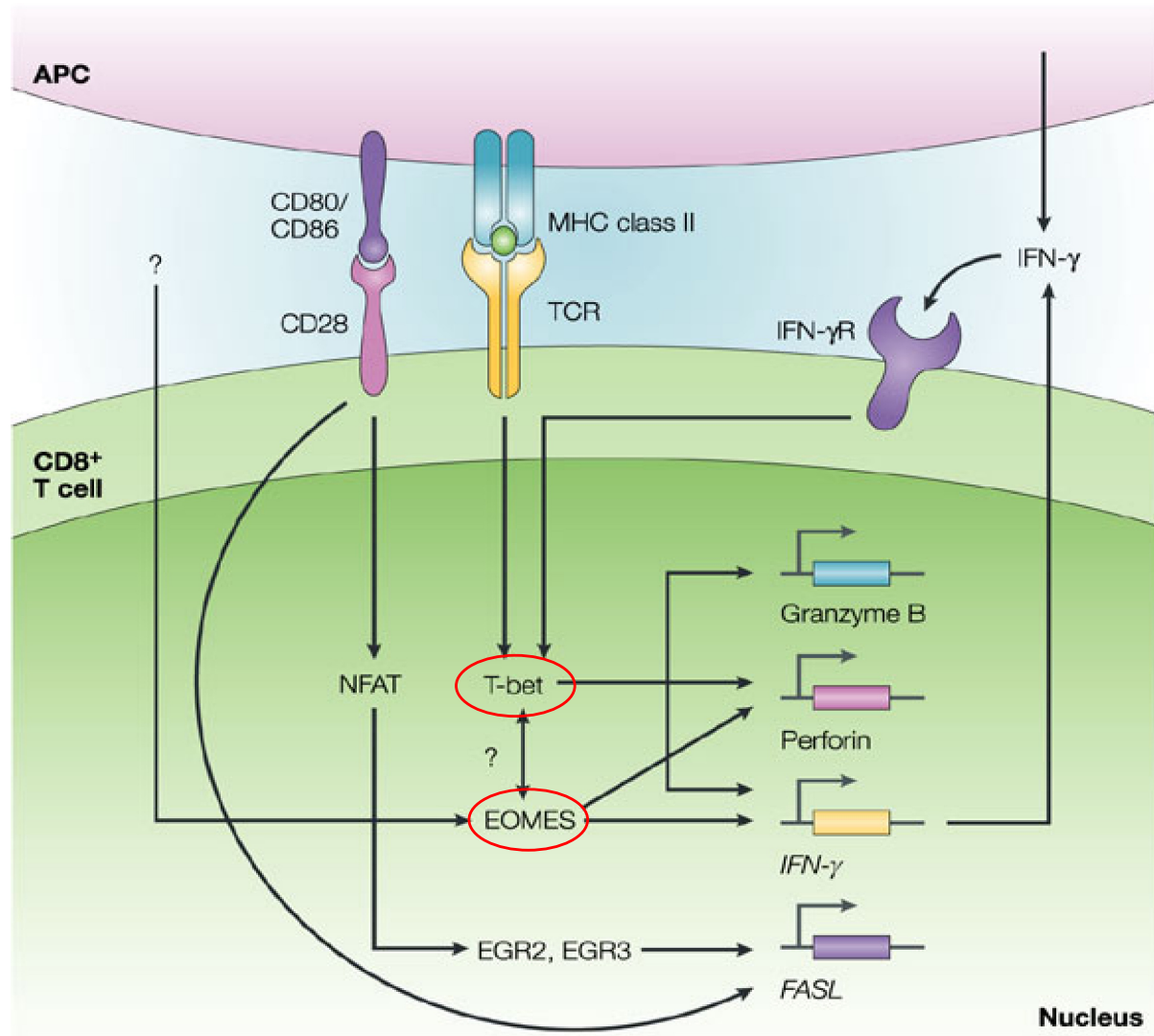
CD4 produces IL-2
which activates CD8

III°



CD4 activates DC to express
ligands that induce CD8 to produce
IL-2 driving its own proliferation

Cytotoxic mediators are acquired during differentiation: CTLs acquire the ability to kill!!



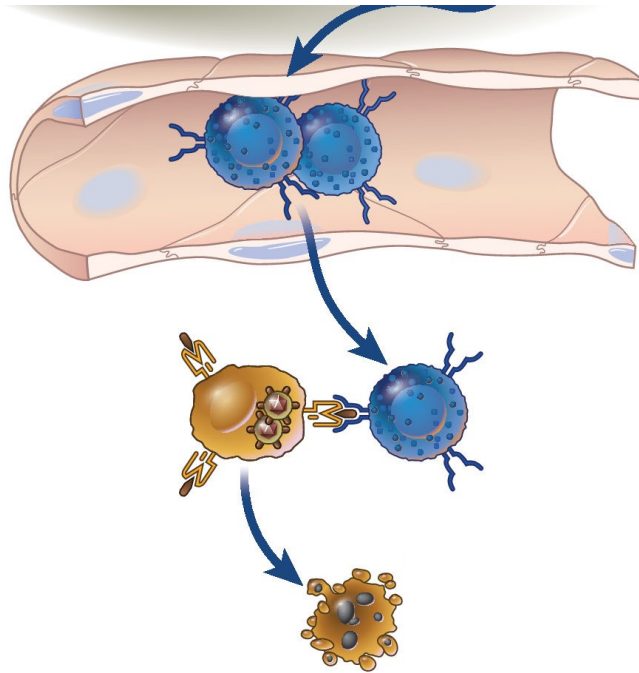
The synthesis of mediators is induced:

- Cytotoxic granules (perforin and granzymes)
- Cytokines (IFN γ e TNF β)
- Expression of FasL

EOMES = Eomesodermin

EGR2 = early growth response 2

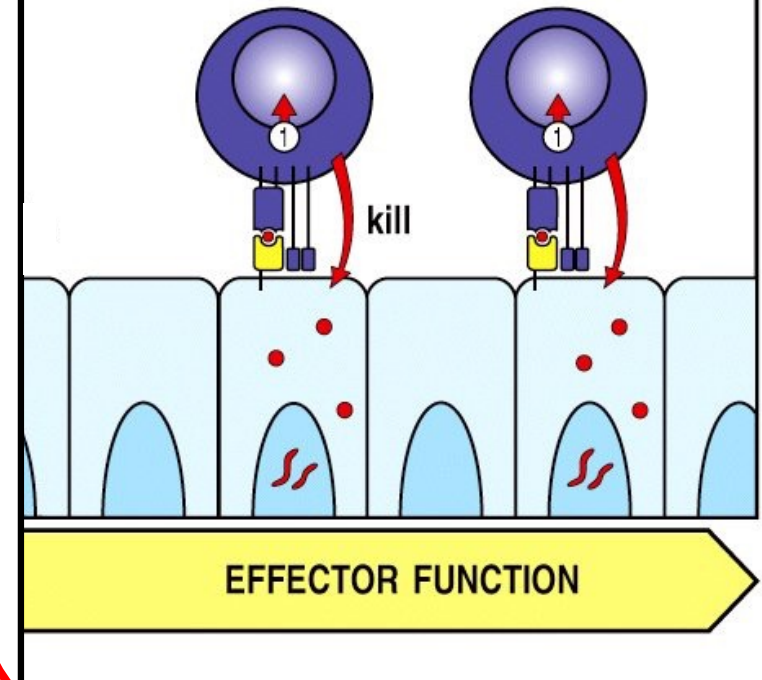
CTLs migrate to the site of infection...



.... where they are activated by antigen recognition and kill the infected cell!

Site of infection

Active effector T cells kill virus-infected target cells



MECHANISMS OF CTL-MEDIATED LYSIS

CYTOTOXICITY DEPENDENT ON:

Direct contact:

Secretory Mechanism

- Calcium-Dependent
- Granule Exocytosis

Non secretory mechanism

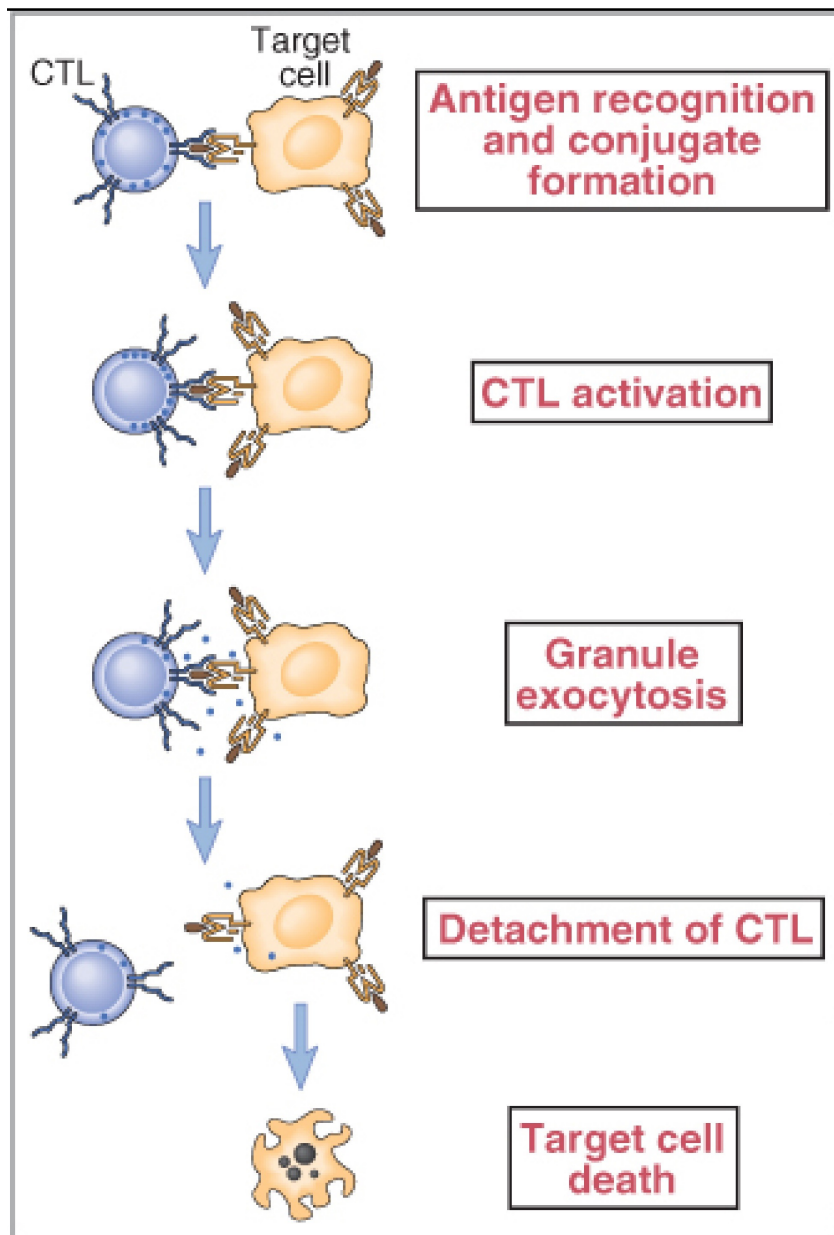
- Calcium-Independent
- Fas-FAsL

Cytokine secretion: $\text{IFN}\gamma$ $\text{TNF } \alpha/\beta$

TARGET CELL DEATH

- Osmotic lysis
- Apoptosis

1° CELL-CONTACT-DEPENDENT CYTOTOXICITY: SECRETORY MECHANISM



**Cytotoxic
T-Lymphocyte
Killing Target**

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Quill Graphics
Charlottesville, VA USA

Antigen recognition augments LFA-1 affinity & avidity: Formation of a stable effector/target conjugate

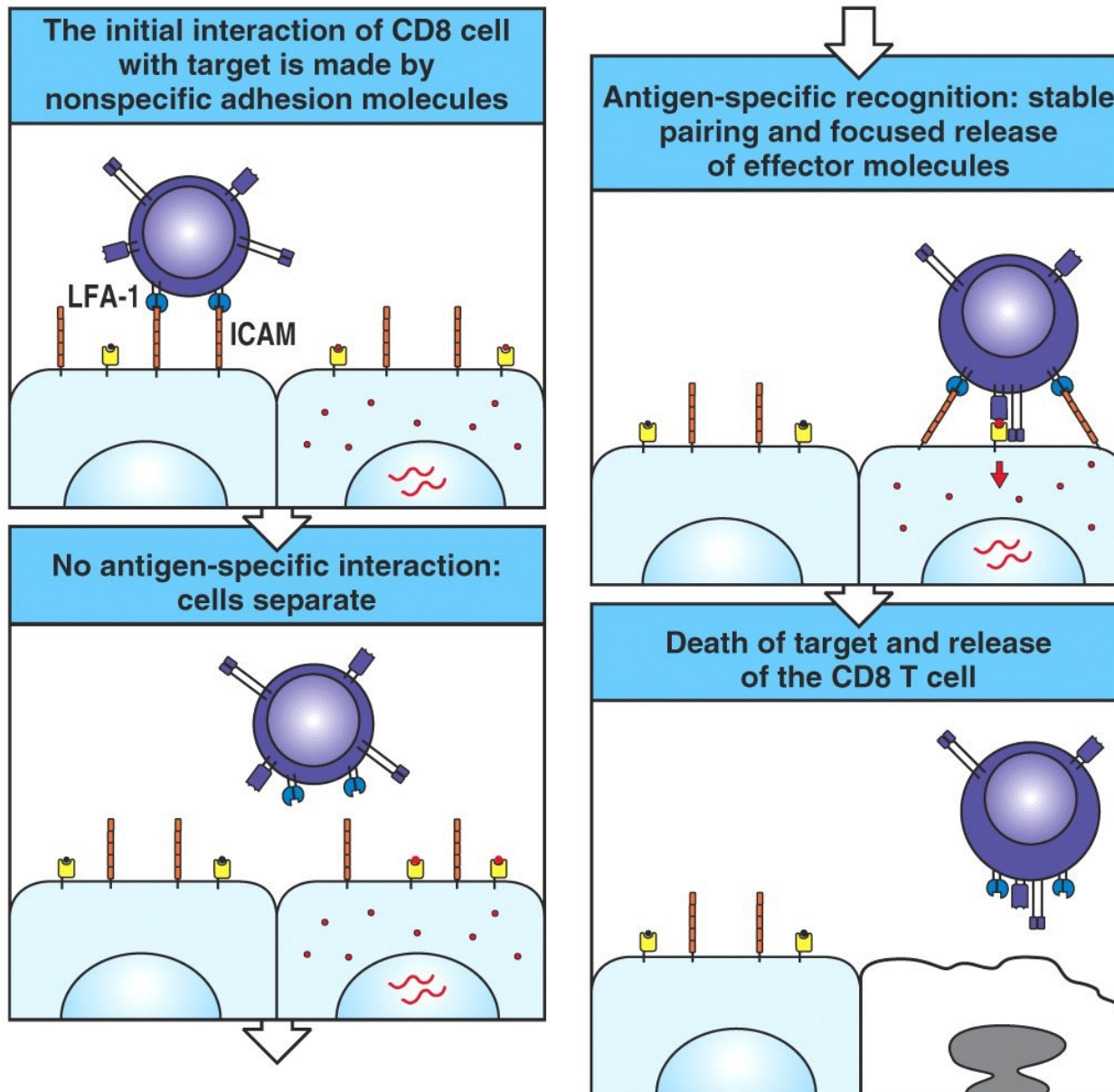
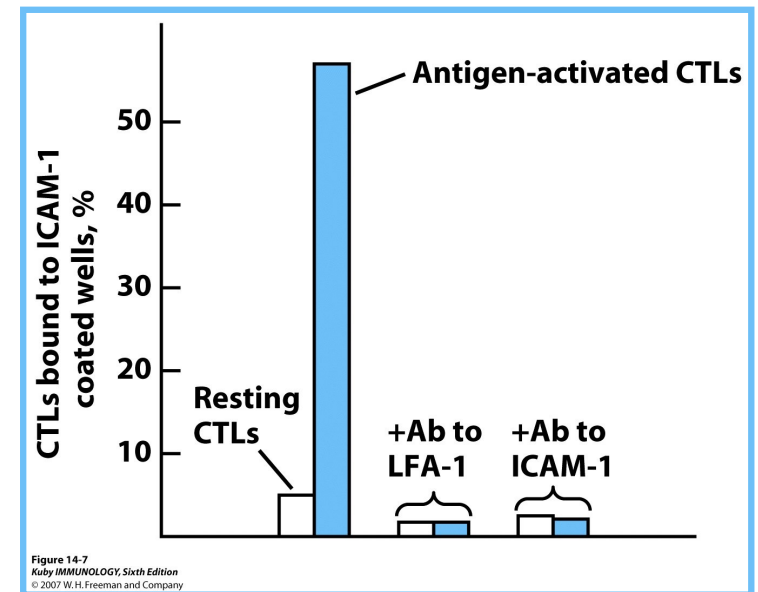
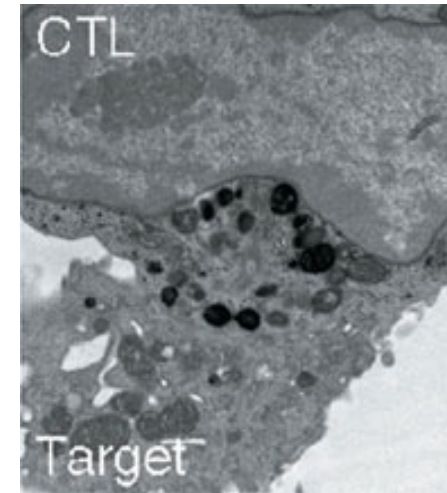
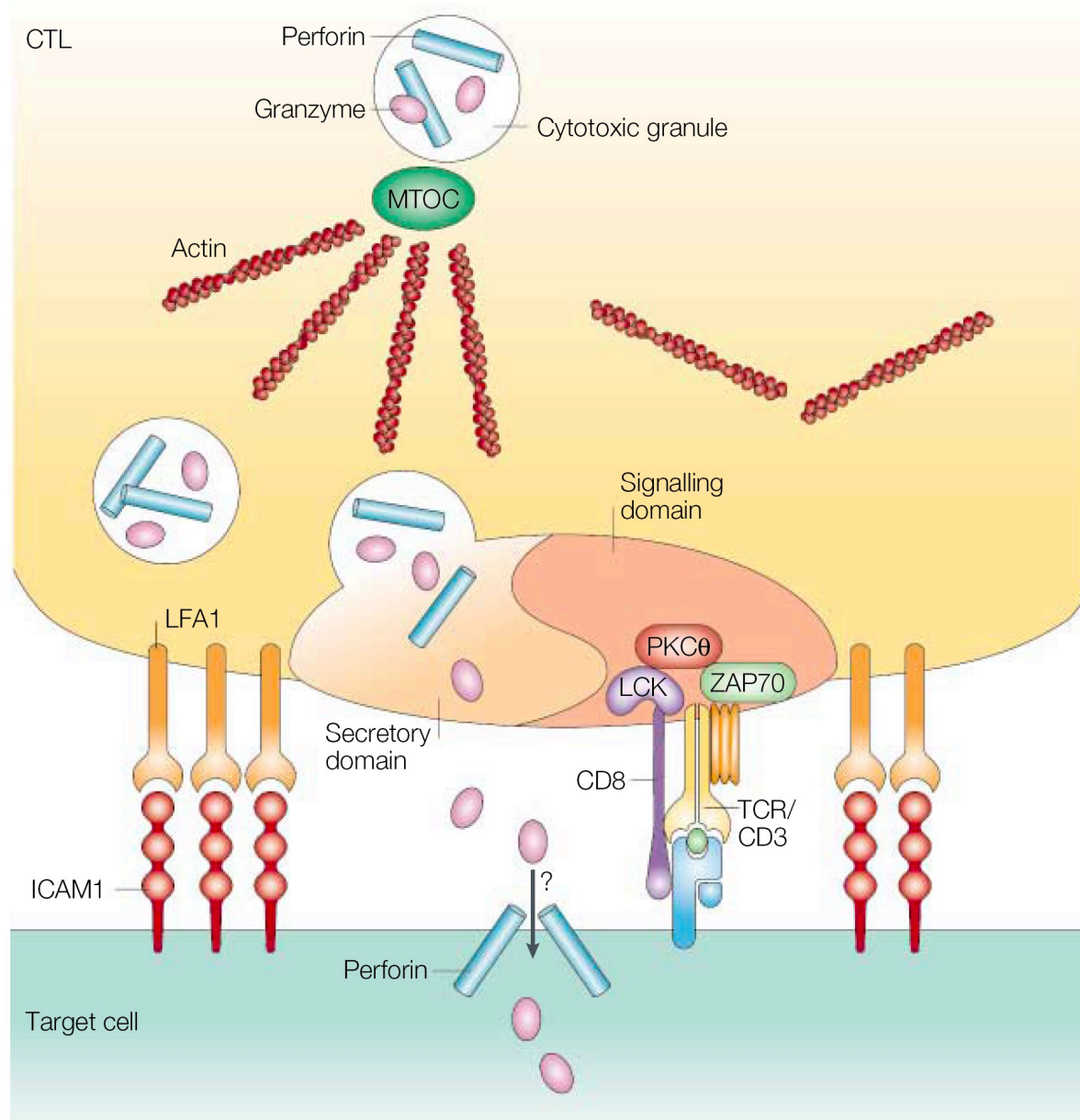


Figure 8-28 Immunobiology, 6/e. (© Garland Science 2005)

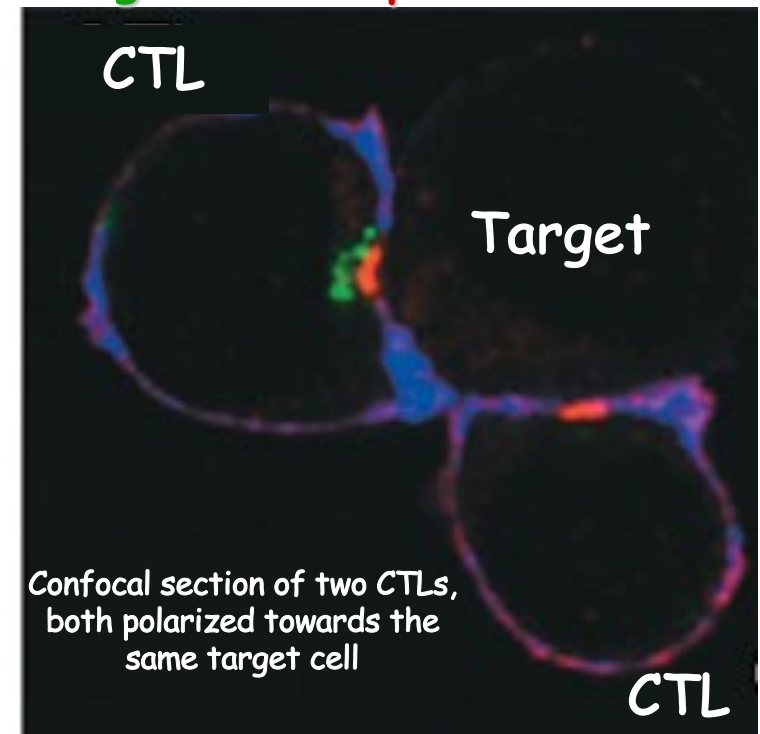


The secretory synapse:

Immunological synapse formed by CTL and target cell



granules p56Lck actin



The different phases of the secretory mechanism

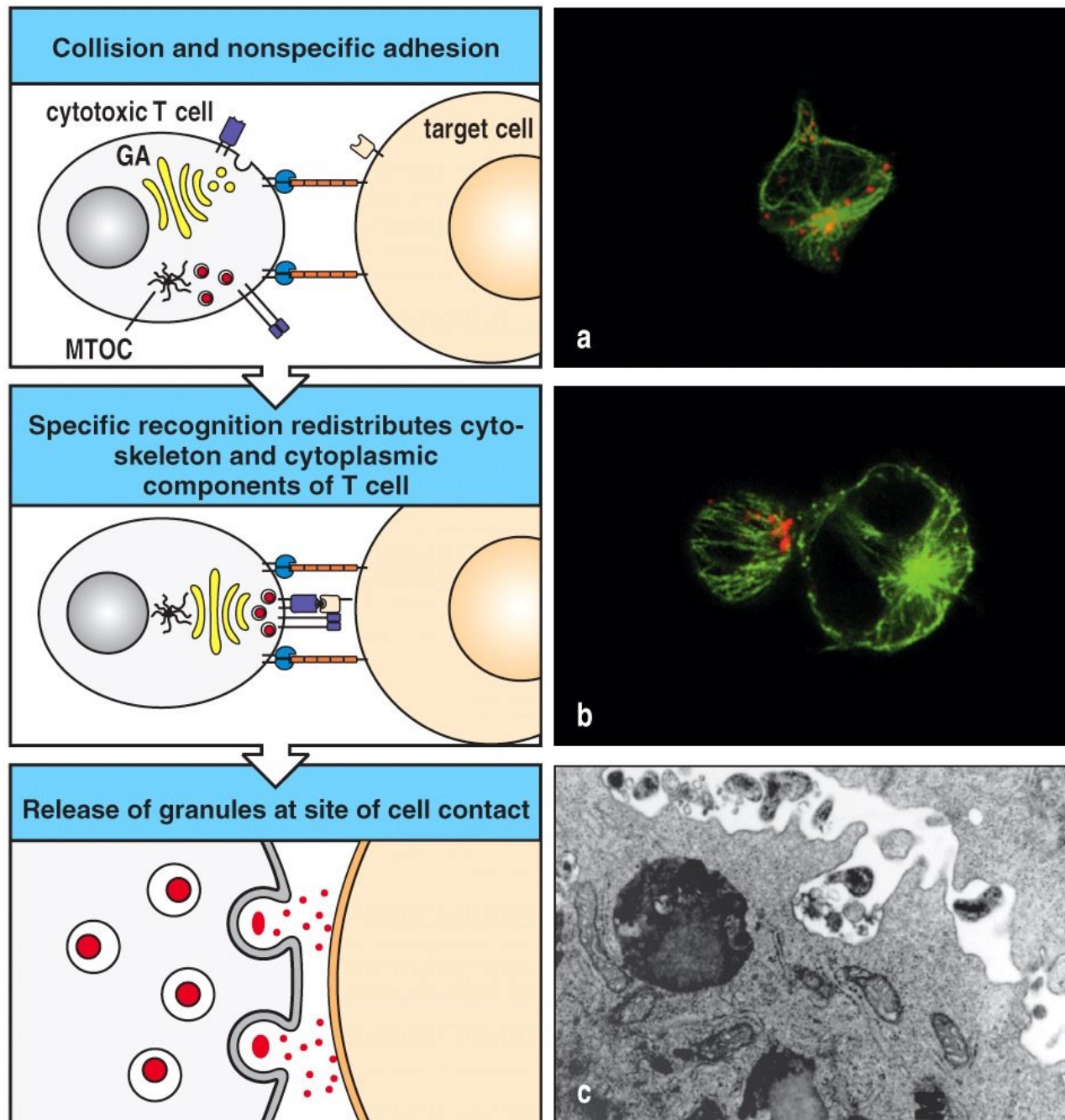
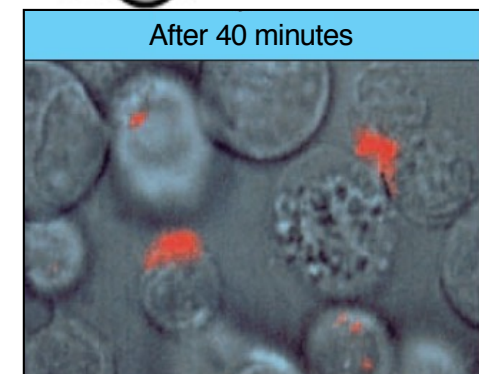
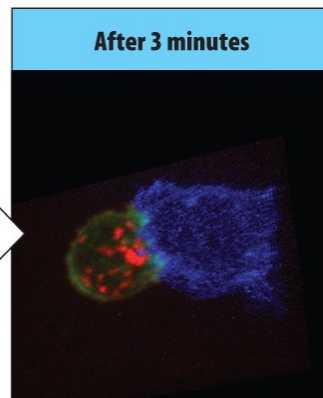
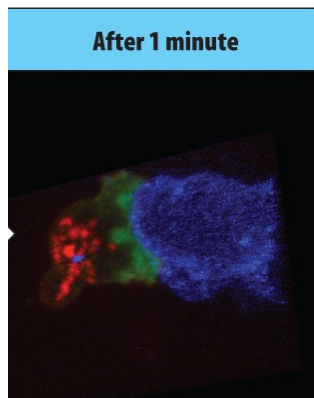
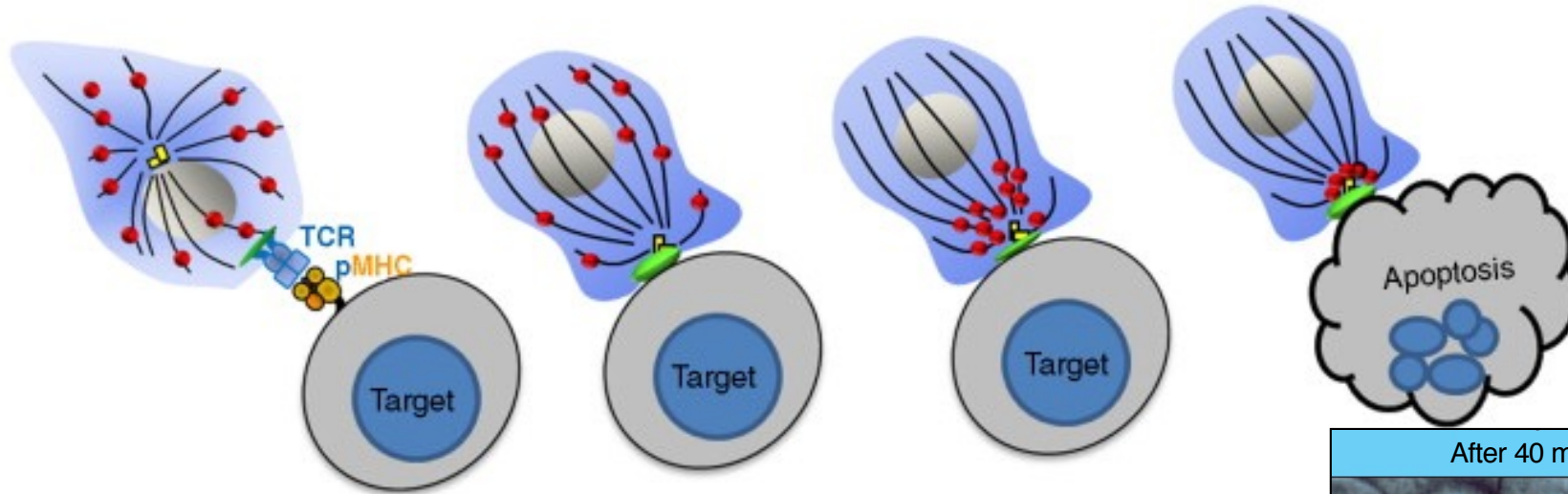
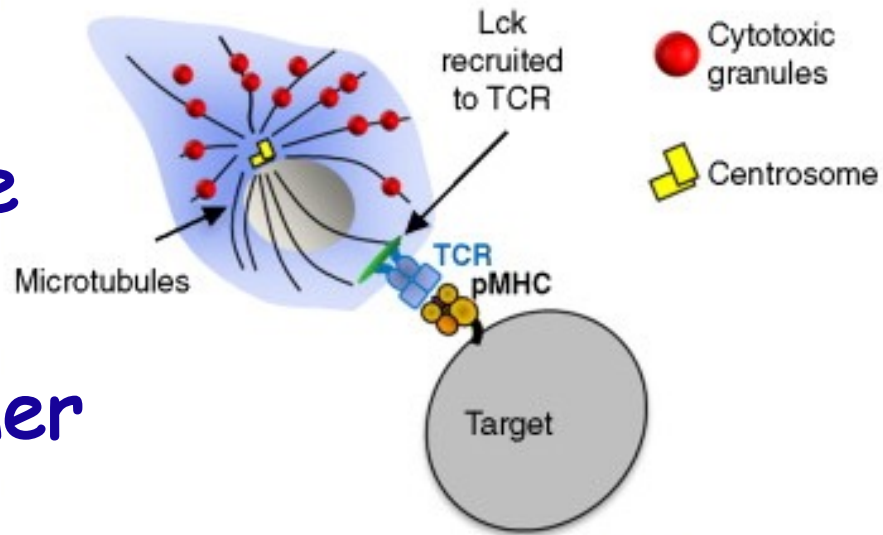
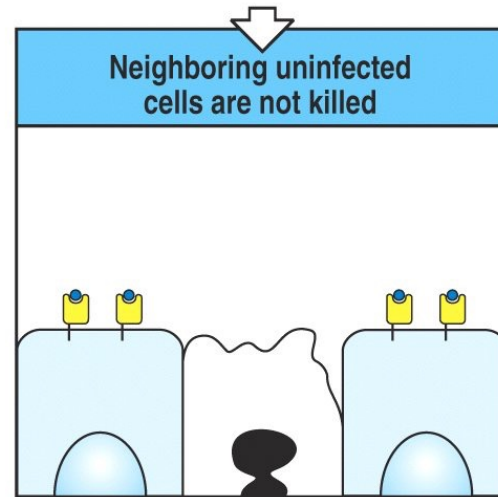
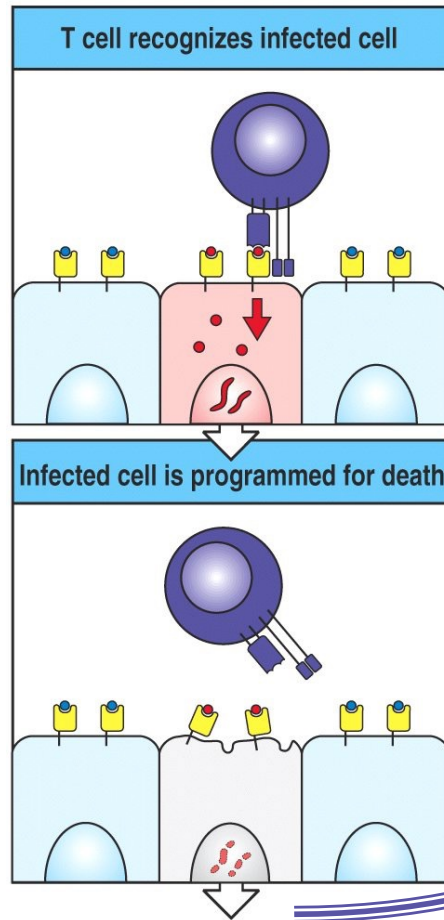


Figure 8-29 Immunobiology, 6/e. (© Garland Science 2005)

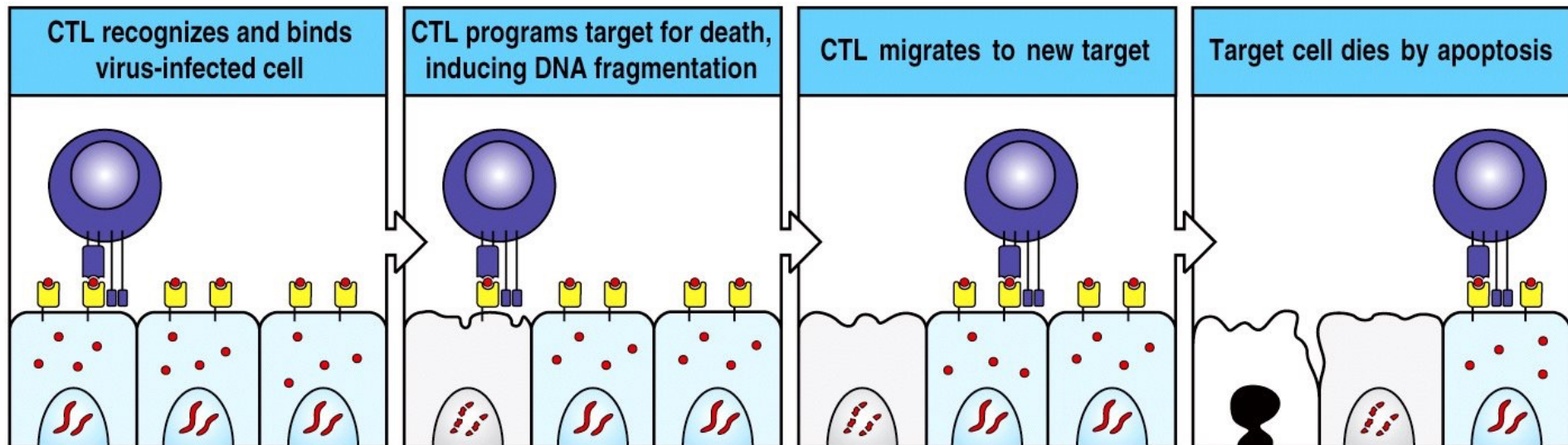
The granules are
secreted in a
POLARIZED manner





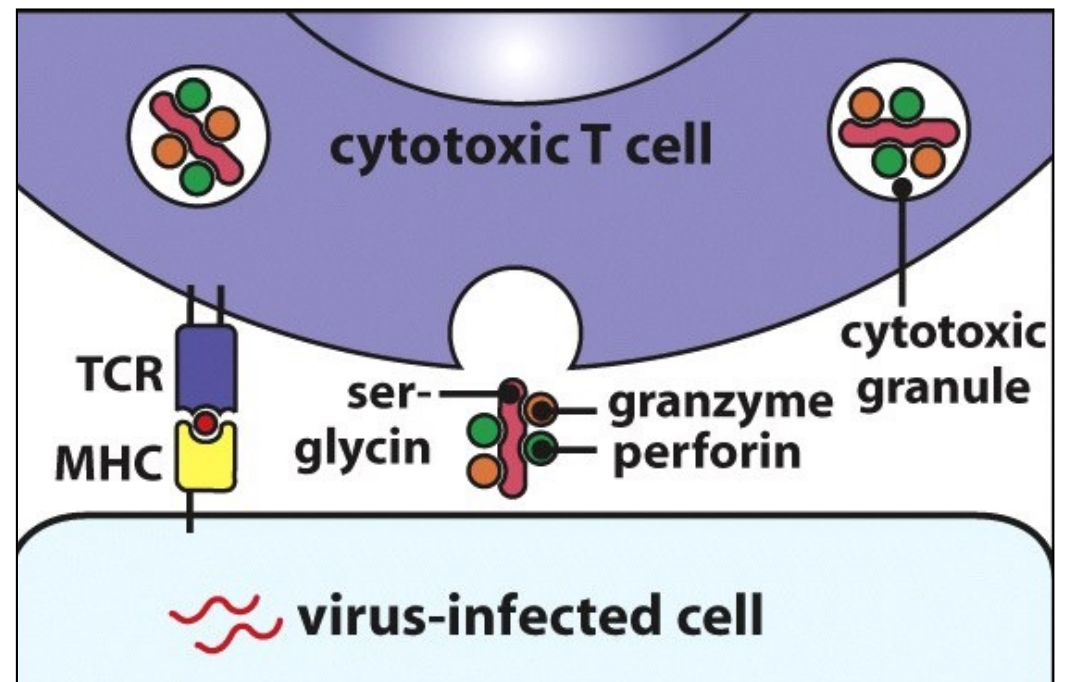
CTL exerts a selective cytotoxic activity.....

..... acting as a serial killer

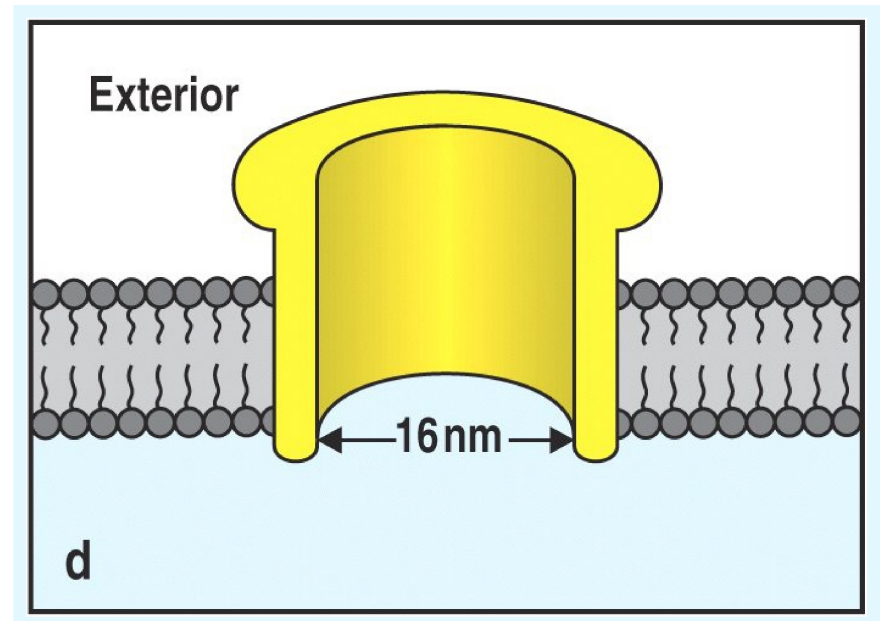
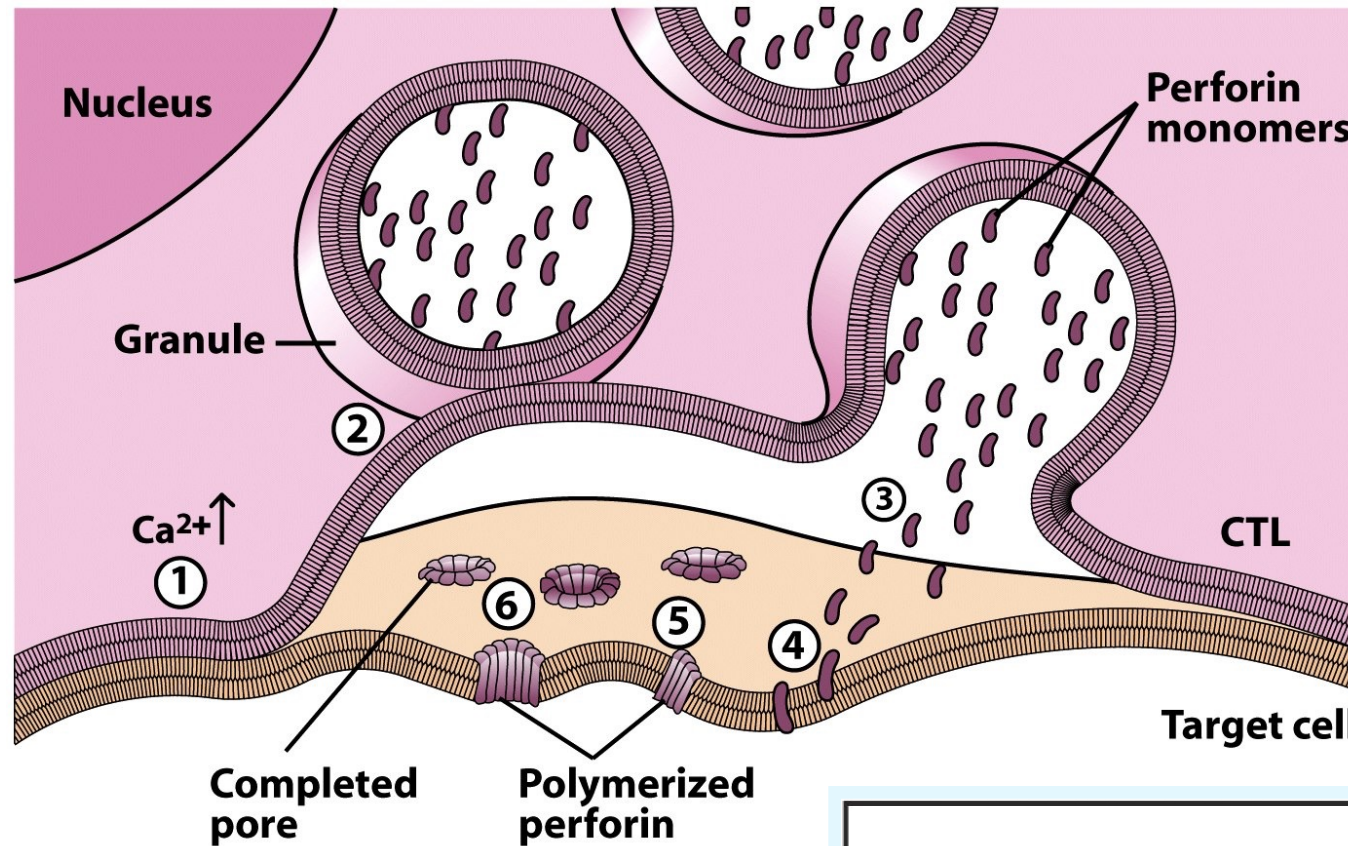


What's in CTL cytotoxic granules?

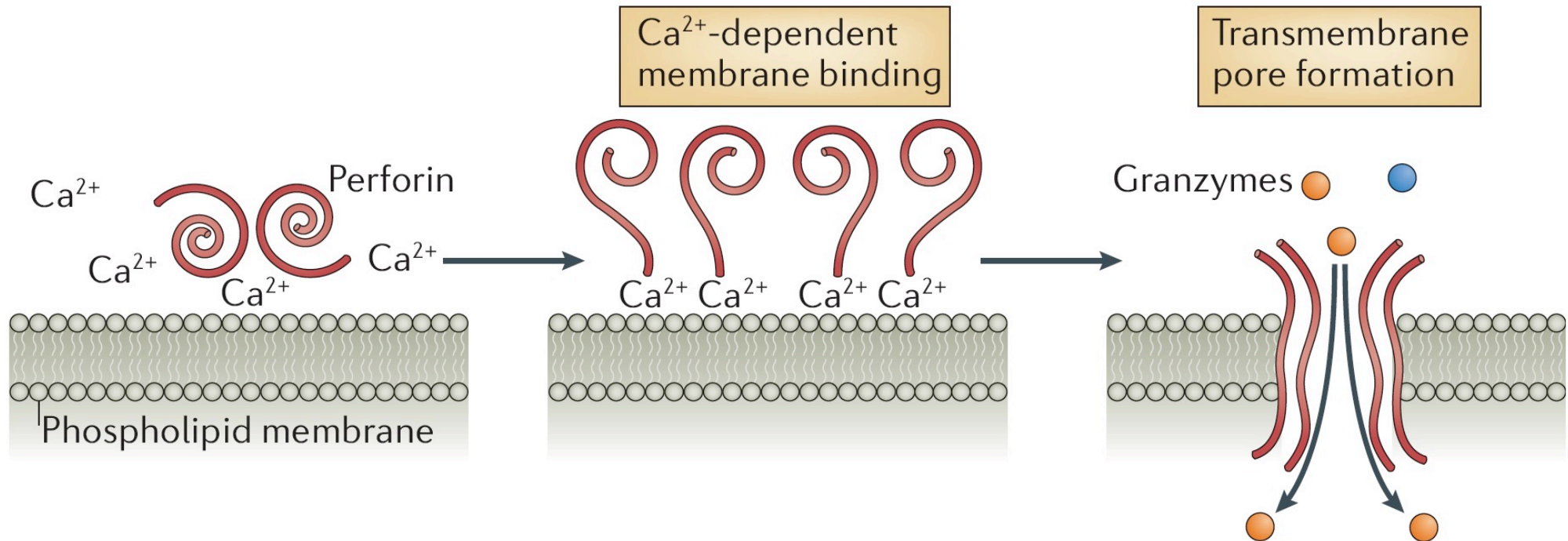
Protein in granules of cytotoxic T cells	Actions on target cells
Perforin	Aids in delivering contents of granules into the cytoplasm of target cell
Granzymes	Serine proteases, which activate apoptosis once in the cytoplasm of the target cell
Granulysin	Has antimicrobial actions and can induce apoptosis
Proteoglycans	a structural role and a protective function



Perforin polymerization creates pores on target cell membrane



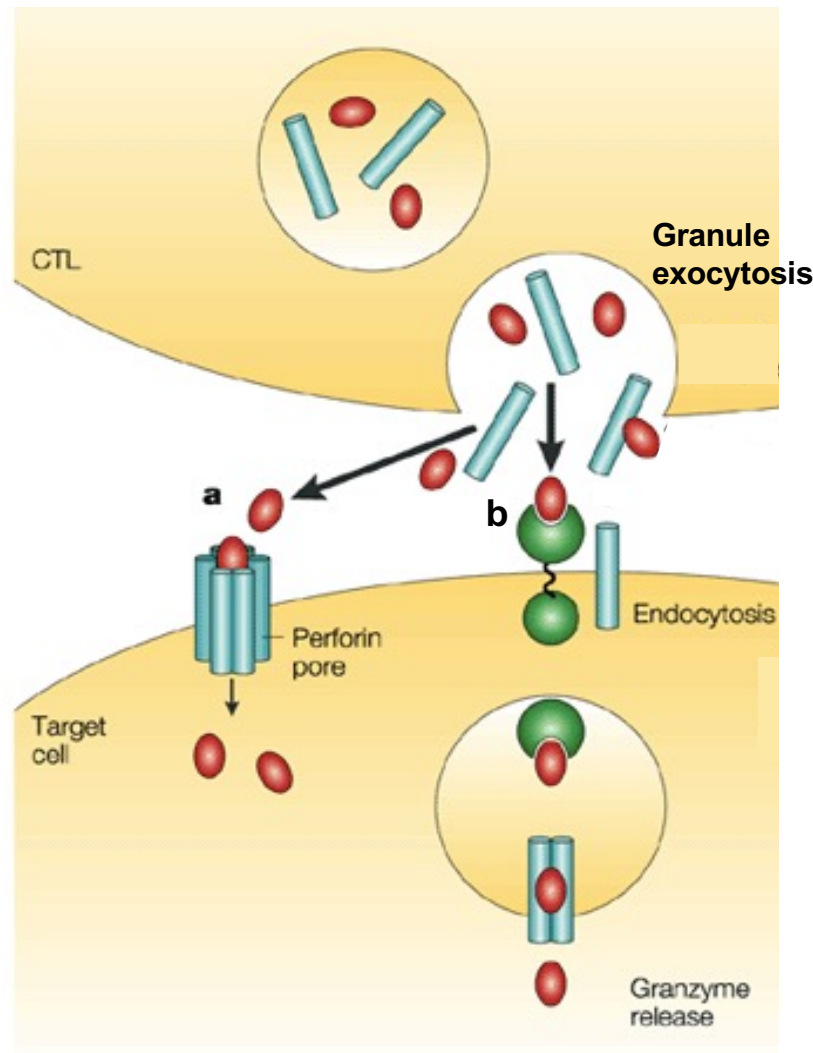
Model of perforin and granzyme synergy in target-cell death



Perforin polymerizes into transmembrane pores (Ca^{++} - and pH-dependent), through which granzymes might diffuse into the cell

Models of granzyme entry into the target cell

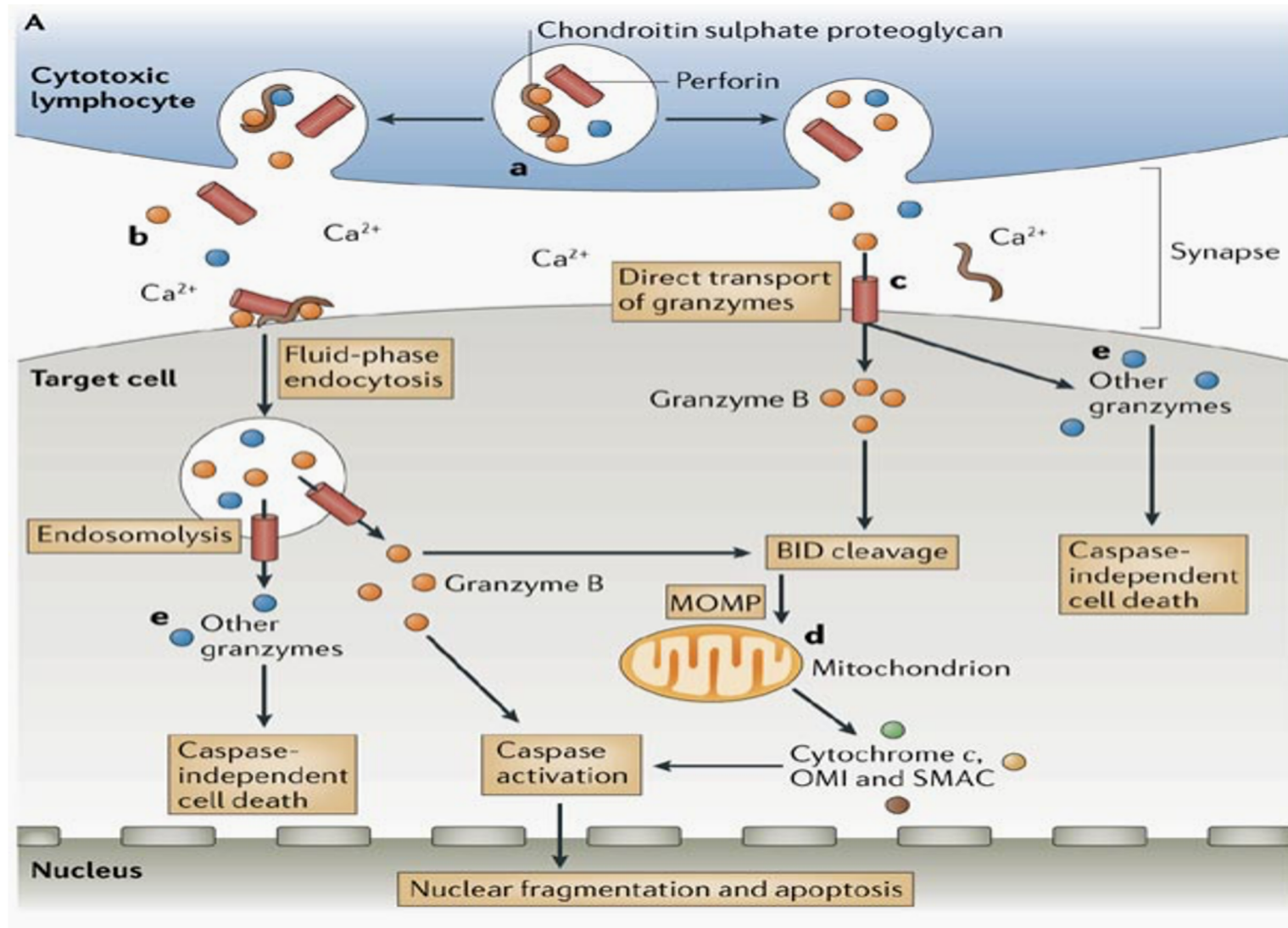
Perforin allows the diffusion of granzymes into the cytoplasm of the target cell by creating pores on its plasma membrane



Granzymes are recognized by specific receptors and internalized along with perforin.

Inside endosomes, perforin allows granzymes to escape into the cytosol.

Perforin-Granzyme mediated target-cell death

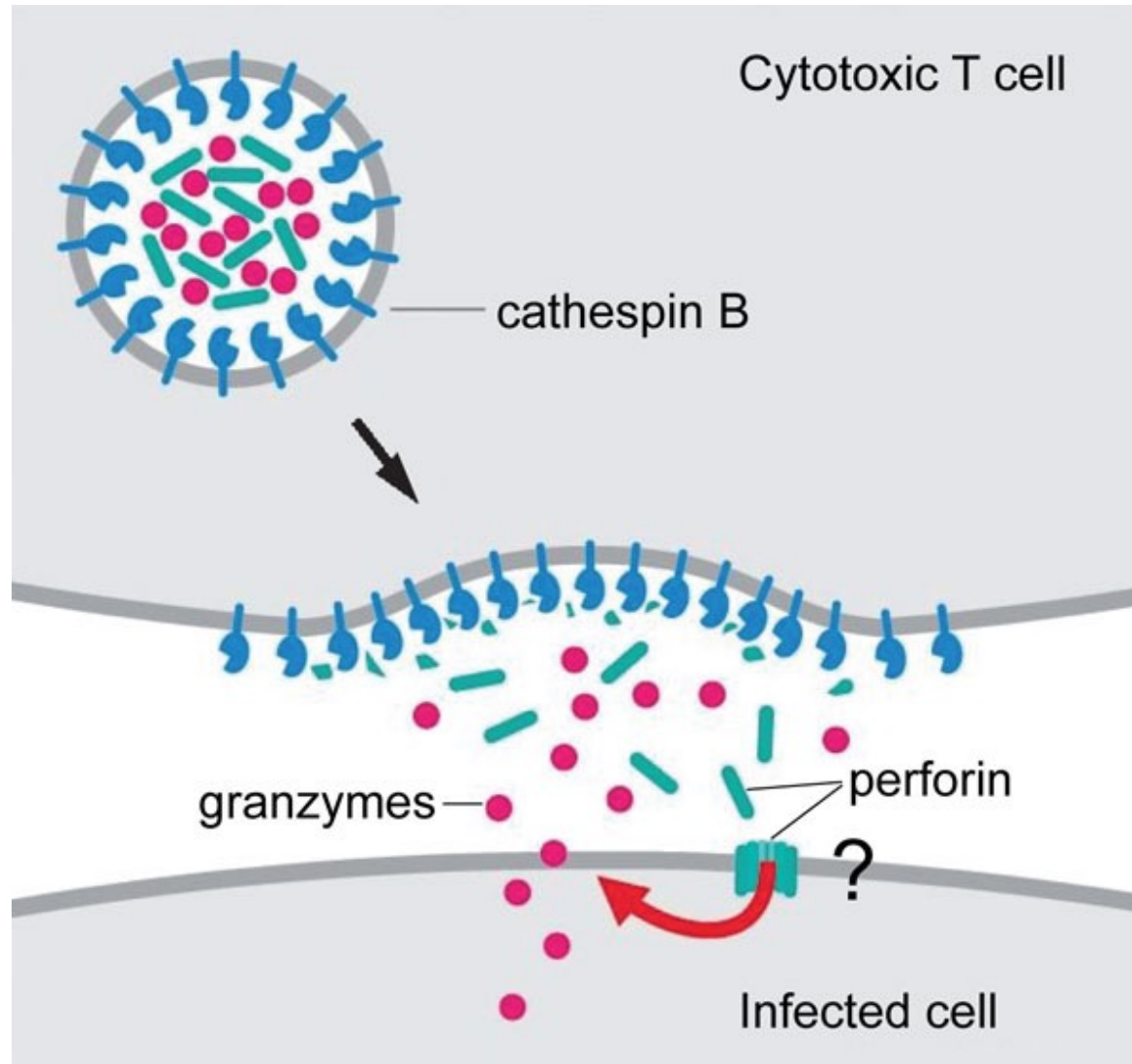


Perforin induces pores and osmotic death!!

Granzyme B activates caspases and causes apoptotic death!!!

Granzyme A releases an endonuclease that cuts DNA and induces apoptosis

Why isn't the CTL killed?



Codice OPIS assegnato al corso integrato tenuto da:

Prof. ROSSELLA PAOLINI

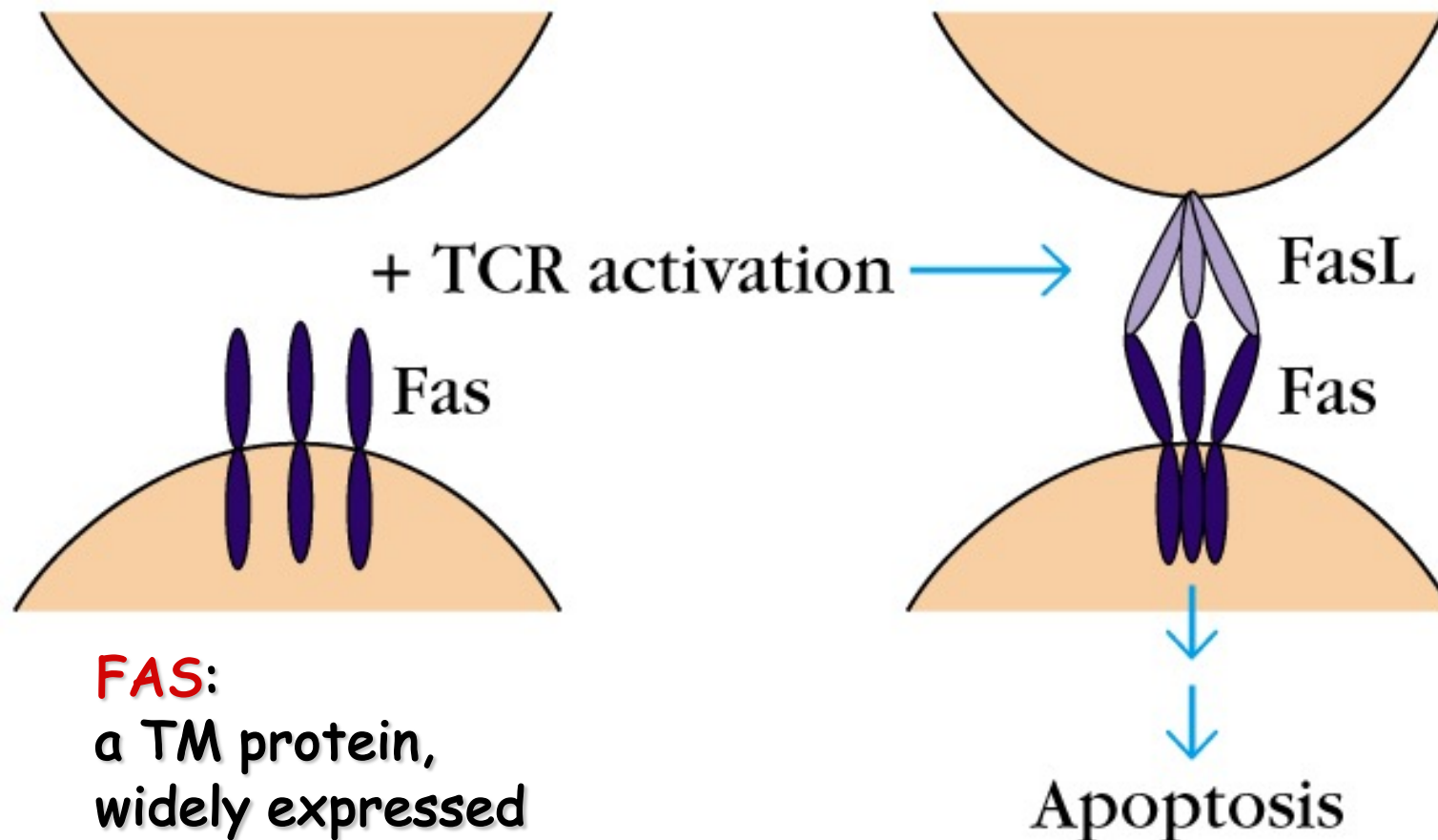
Biotechnologie (29887)

Modulo	Insegnamento	Codice OPIS
IMMUNOLOGIA I (1051487_2)	IMMUNOLOGIA (10514879)	1BH7HMGQ
IMMUNOLOGIA II (1051487_1)	IMMUNOLOGIA (10514879)	MH4E21B4

2° CELL-CONTACT DEPENDENT CTL CYTOTOXICITY: NON-SECRETORY MECHANISM (FAS/FASL)

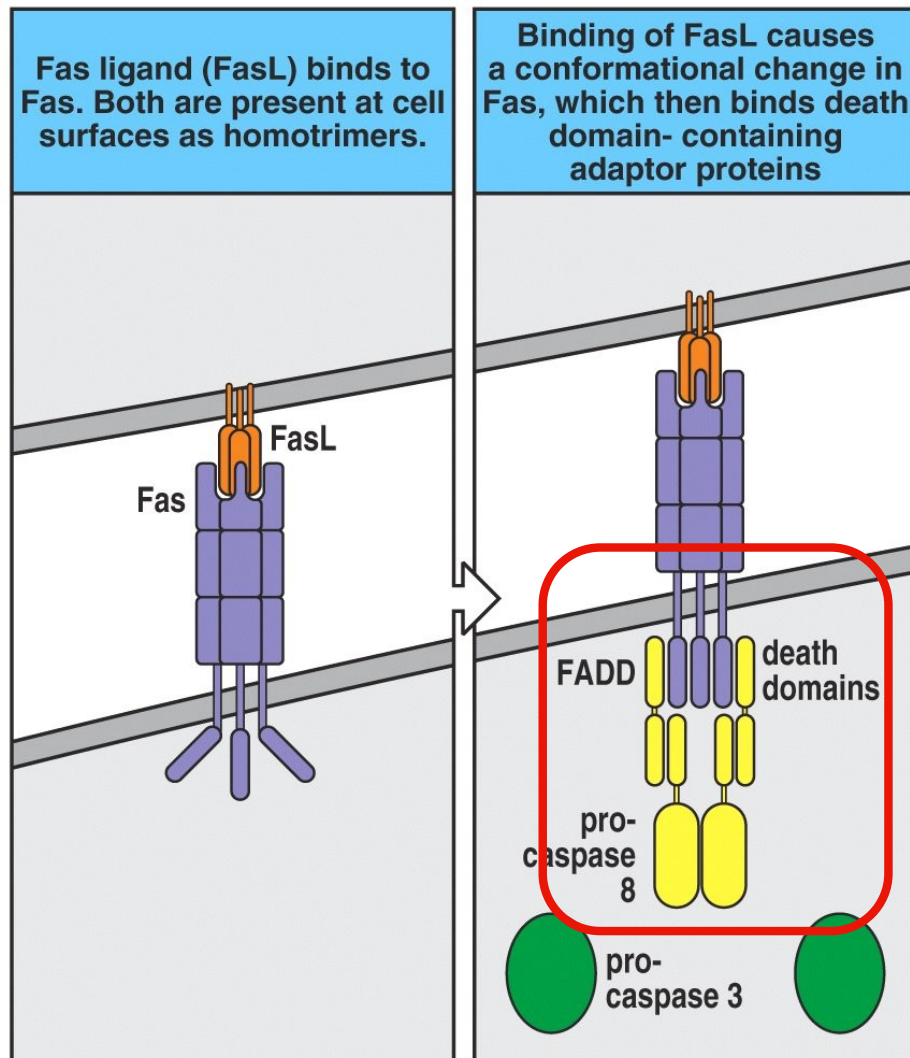
FAS ligand (FASL):

- a TM protein induced on activated T cells (CD8, Th1) and released from cell surface in soluble form



FAS engagement initiates a cascade of caspases

1. Caspase 8



FAS belongs to the death receptor family (with TNF-RI), and expresses a death domain (DD)

Trimeric FAS
FADD
Caspase 8

DISC complex

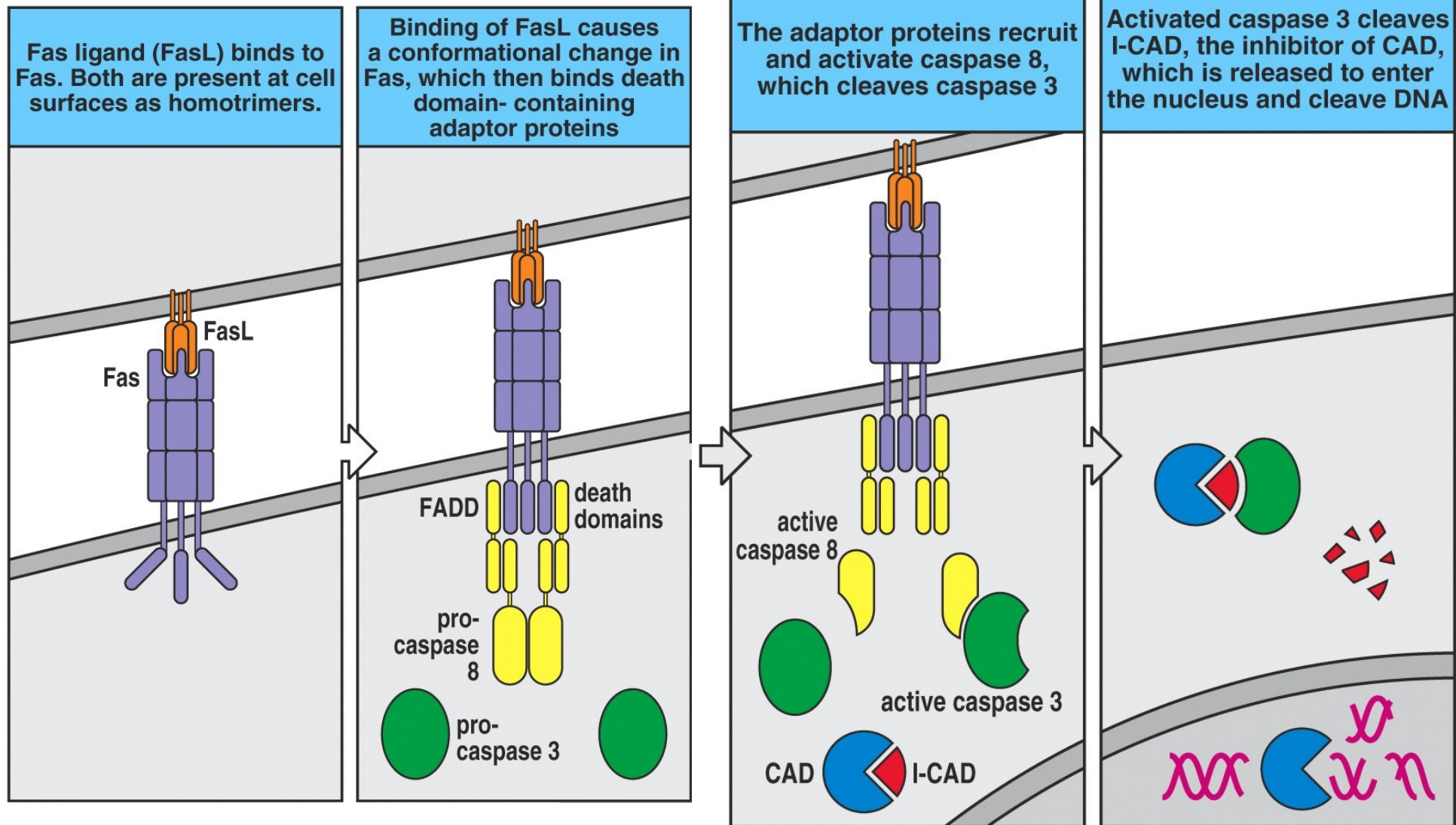
Figure 6-24 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

FAS engagement initiates a cascade of caspases

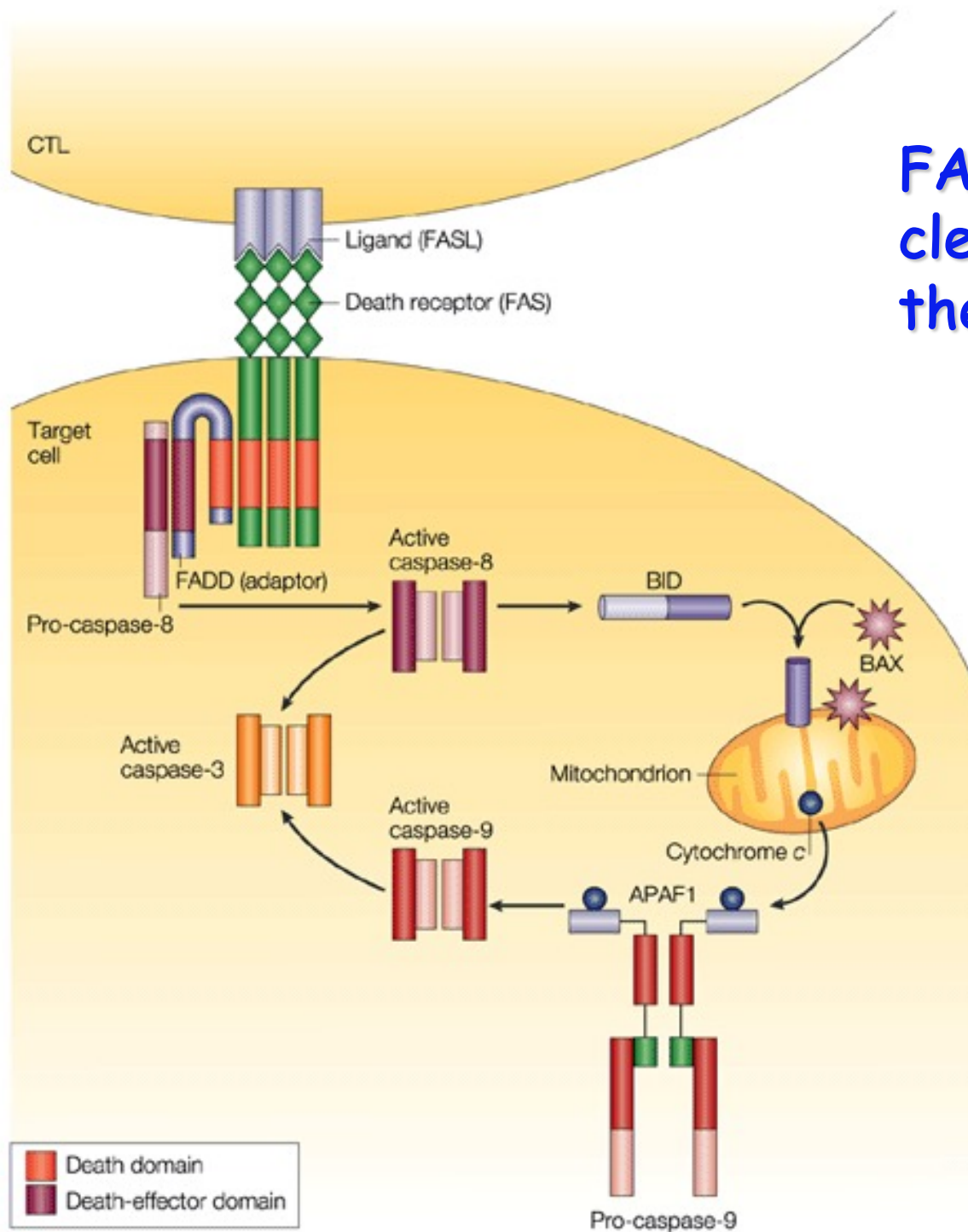
1. Caspase 8



2. Caspase 3

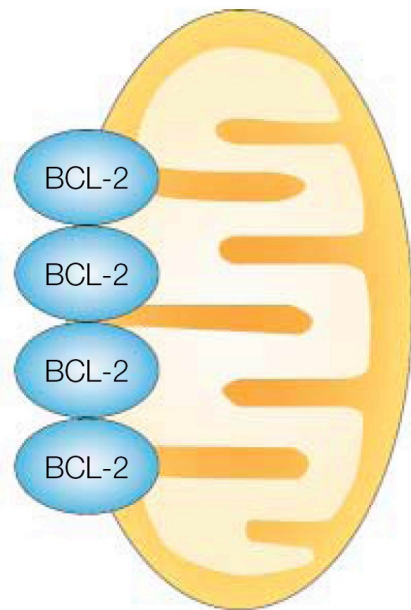


Multiple caspase 3 substrates lead to:
cell shrinkage, and membrane blebbing
DNA degradation, and block of DNA repair

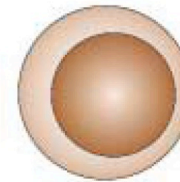


FAS-activated caspase 8 cleaves **BID**, a member of the BCL-2 family

Presence of trophic factor



No mitochondrial damage

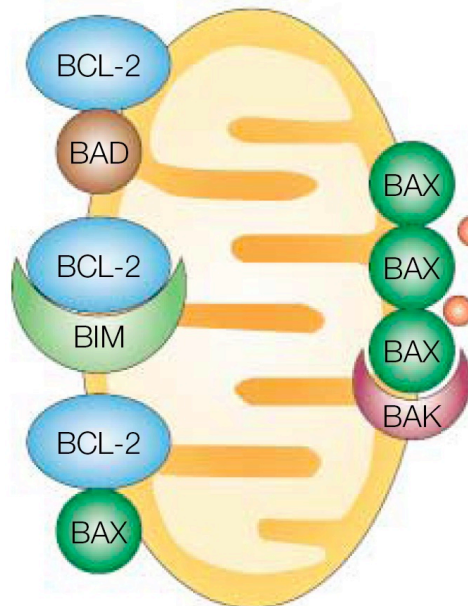


Apoptosis prevented

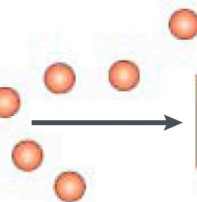
Proapoptotic and antiapoptotic members of BCL-2 family regulate cell survival

BCL-2
BCL-XL
(anti-apoptotic)

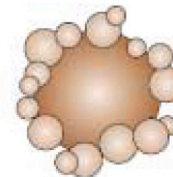
Absence of trophic factor



Mitochondrial damage



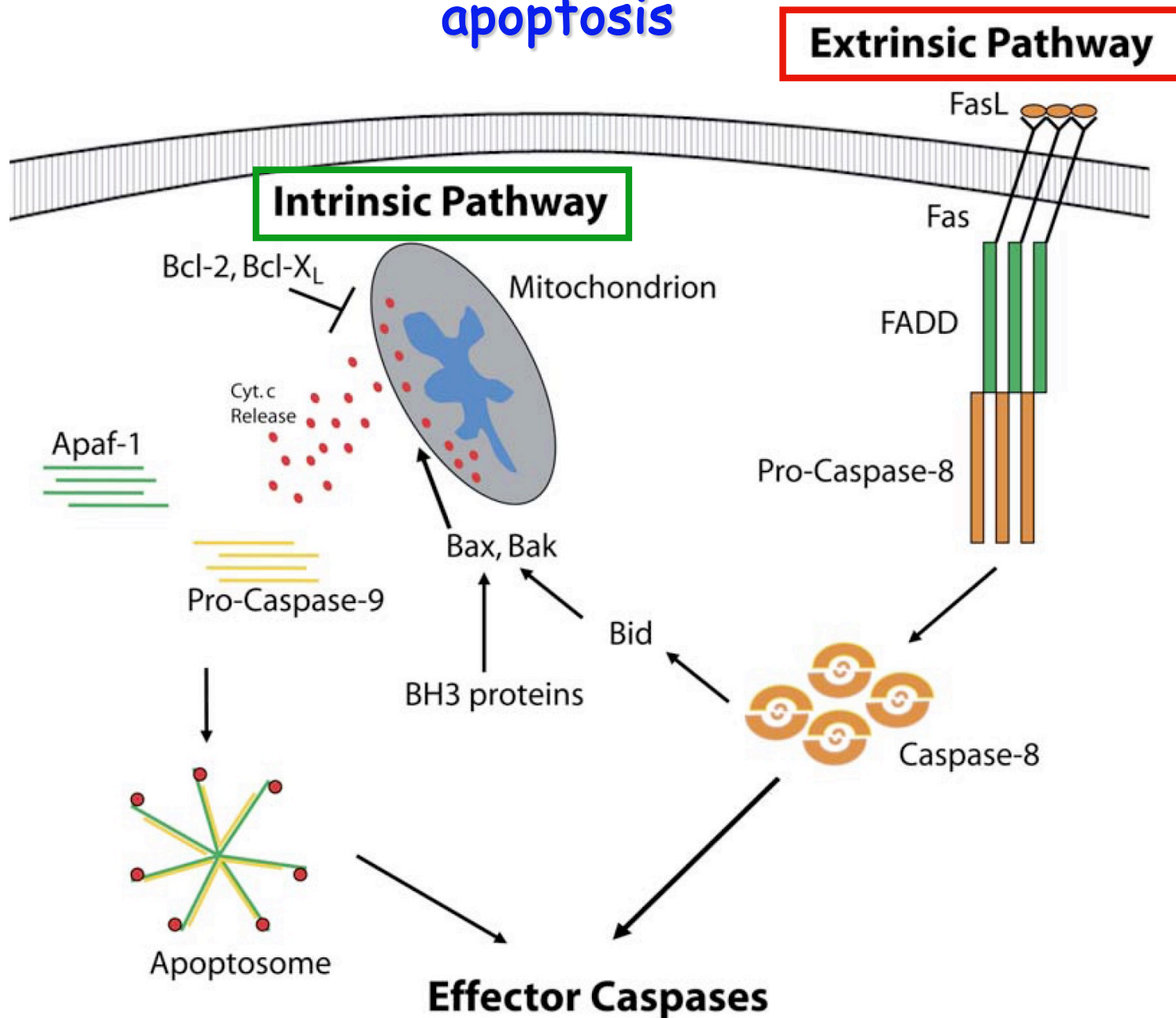
Cytochrome c release



Apoptosis

Bax, Bad
Bim, Bak
(pro-apoptotic)

Cooperation between the **extrinsic** (death receptor-mediated) and the **intrinsic** (mitochondrion-mediated) pathways to cell apoptosis

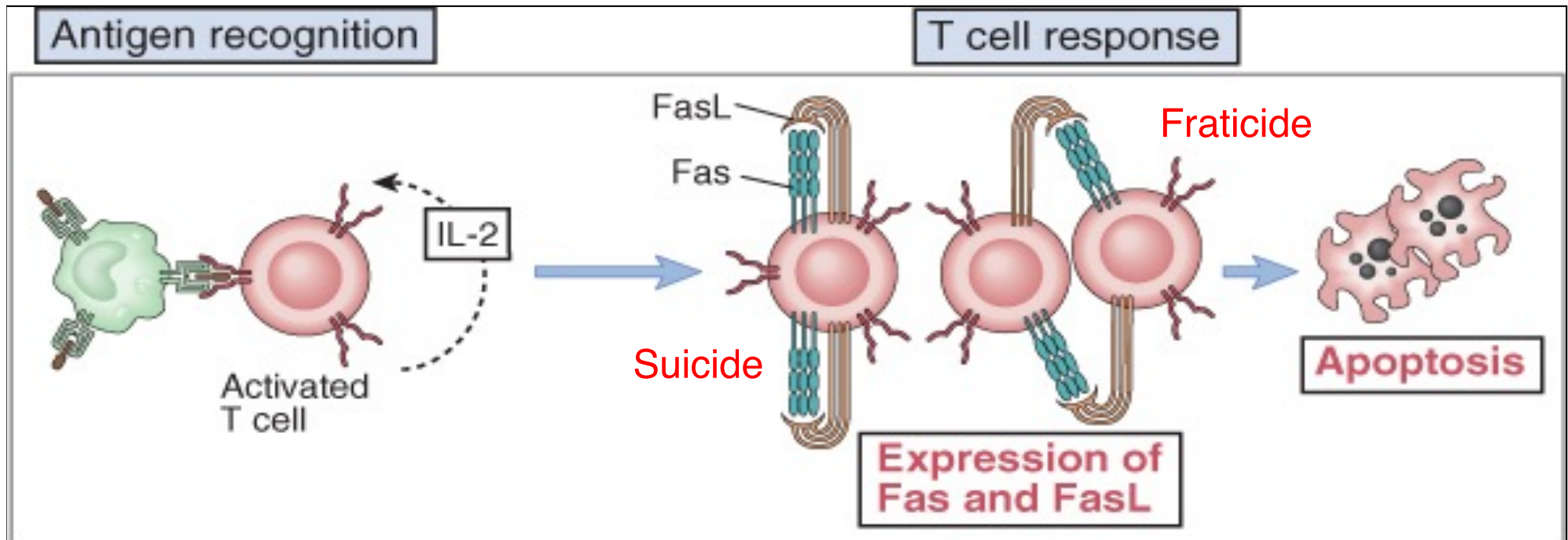


FAS-MEDIATED CYTOTOXICITY:

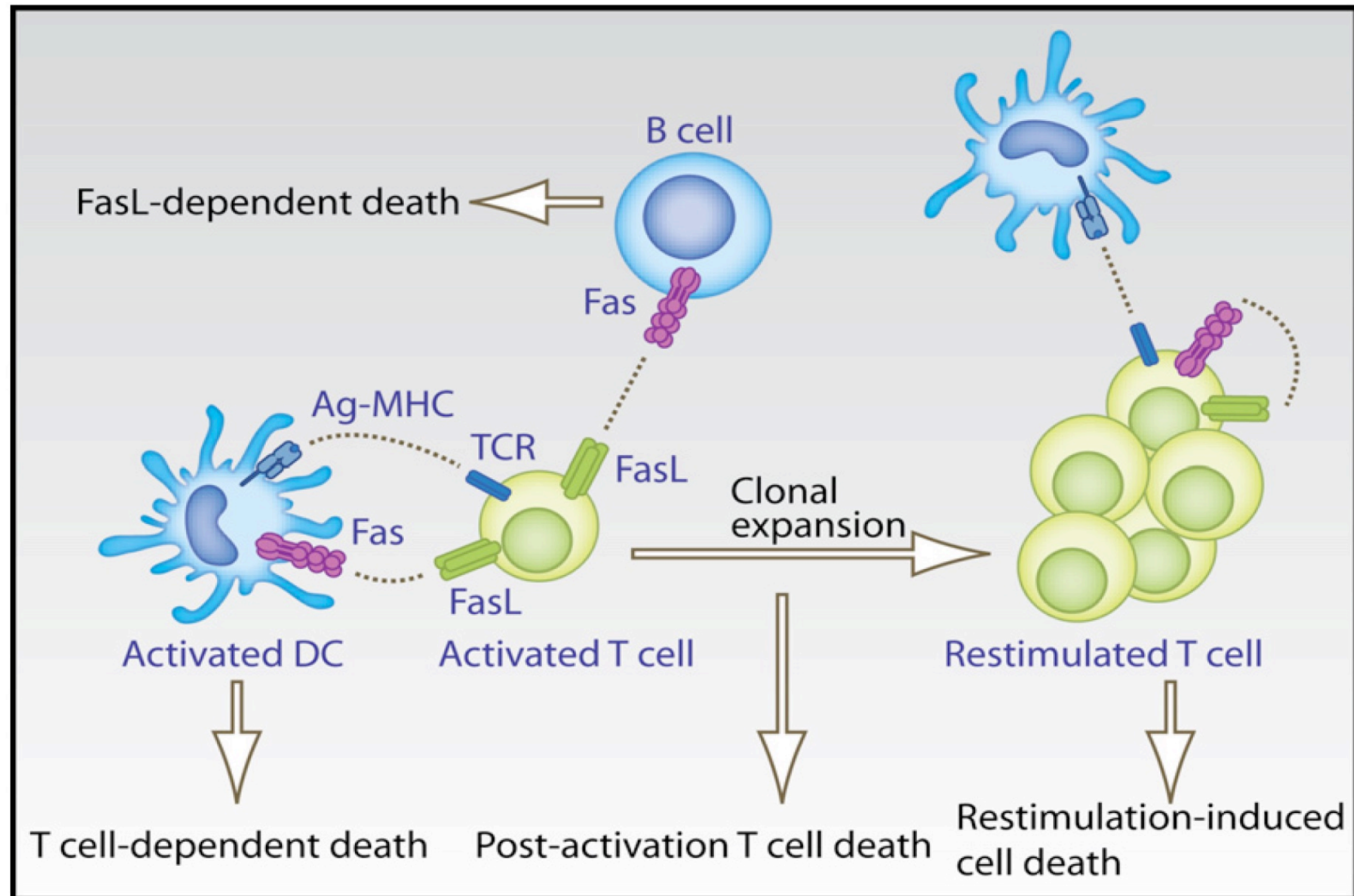
- Mechanism of immune response regulation
- Mechanism used by tumors to evade immunological surveillance
- Defense mechanism of immunologically privileged organs

NEGATIVE SIGNALS REGULATING T LYMPHOCYTE ACTIVATION

FasL-induced T cell death



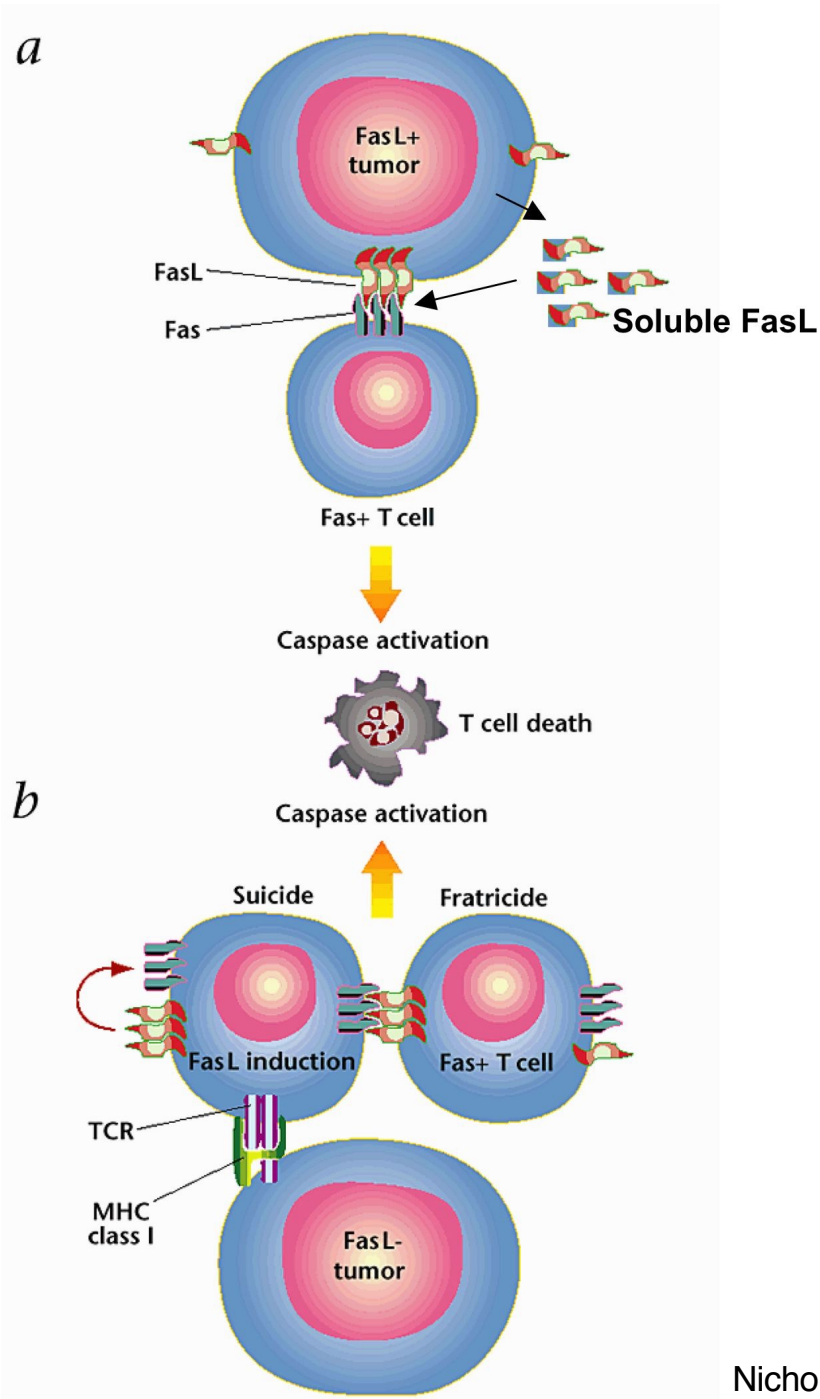
FAS/FASL: a homeostatic mechanism of the immune system



FasL-induced T cell death upon recognition of tumor cells

Some tumors evade the immune response by expressing Fas ligand (FasL) and a soluble isoform of FasL

Activated T cells can kill themselves ('suicide') and each other ('fratricide') through the same caspase-based mechanism

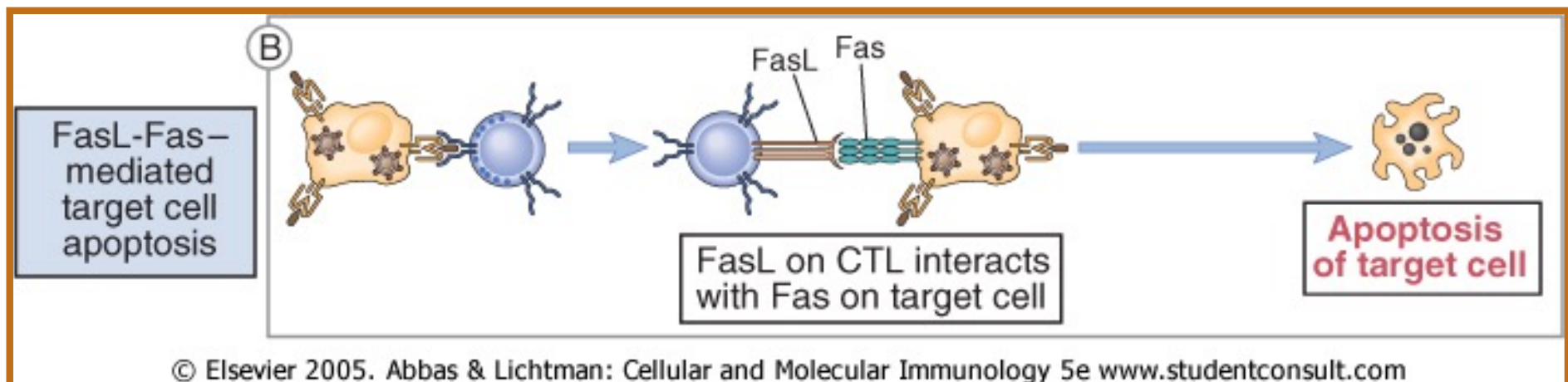
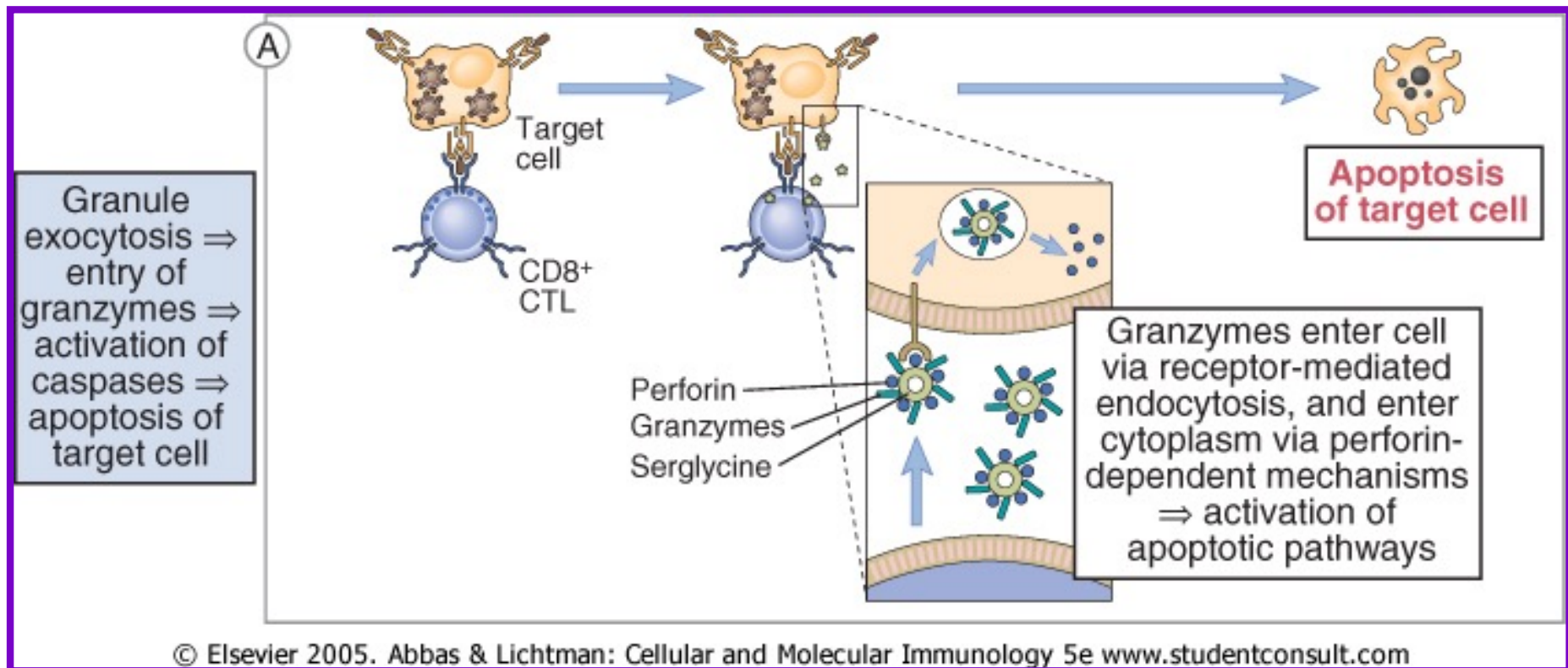


**Cells from "immunologically privileged organs"
express Fas ligand**

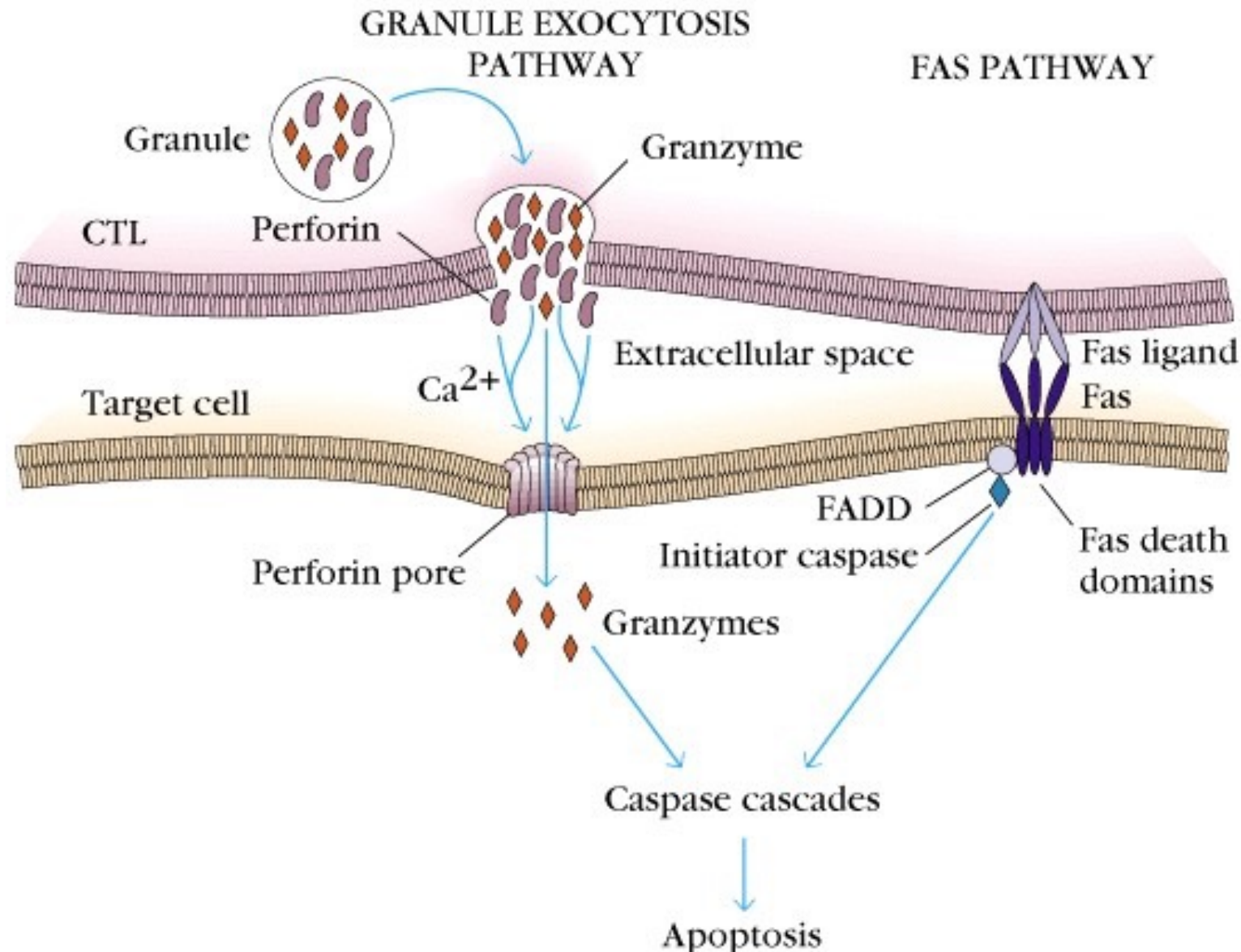
Immunologically privileged sites
Brain
Eye
Testis
Uterus (fetus)
Hamster cheek pouch

Figure 13-12 Immunobiology, 6/e. (© Garland Science 2005)

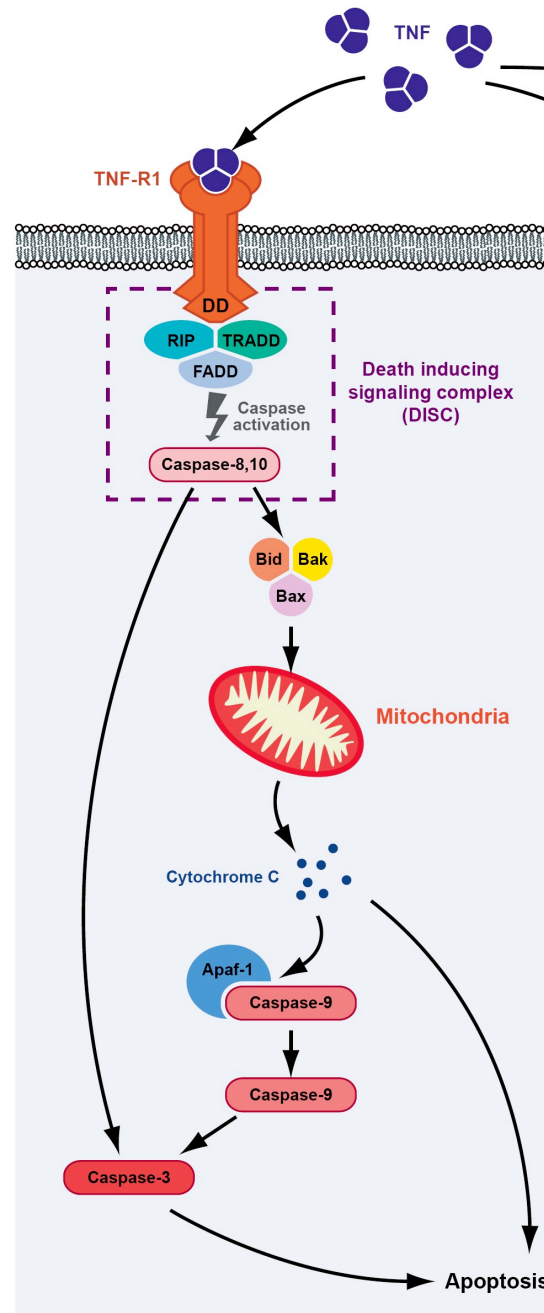
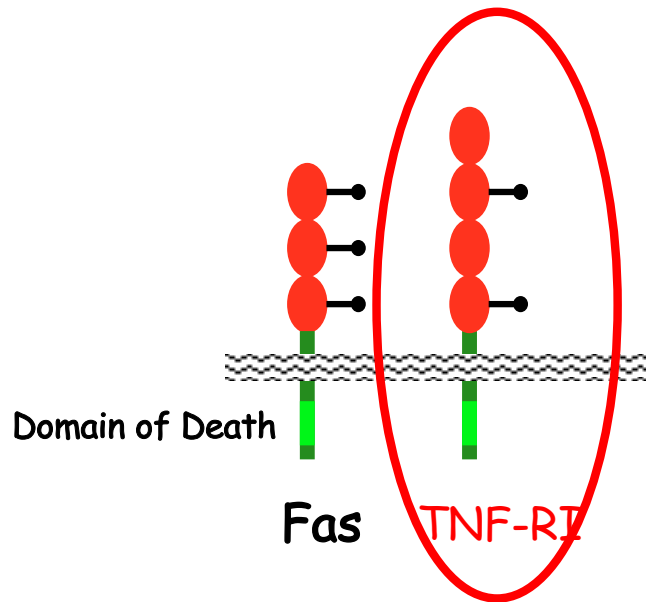
Two major lytic mechanisms of lymphocytes



Cooperation between the granule-dependent and the FAS-mediated mechanism of cytotoxicity



3° CTL also produce *CYTOTOXIC* cytokines



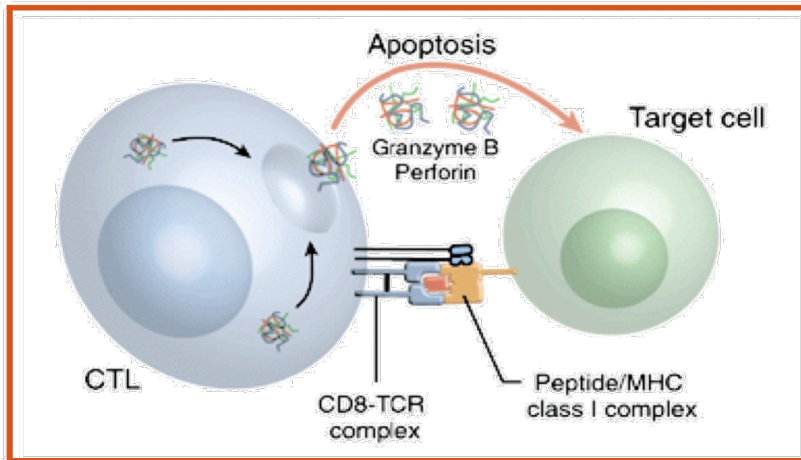
Tumor **N**ecrosis **F**actor

TNF α

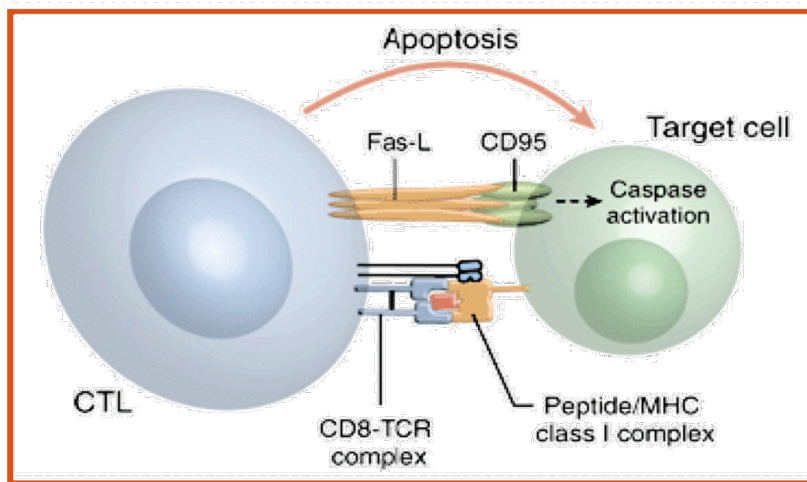
TNF β (LT)

that can induce
apoptosis (through
TNF-RI)

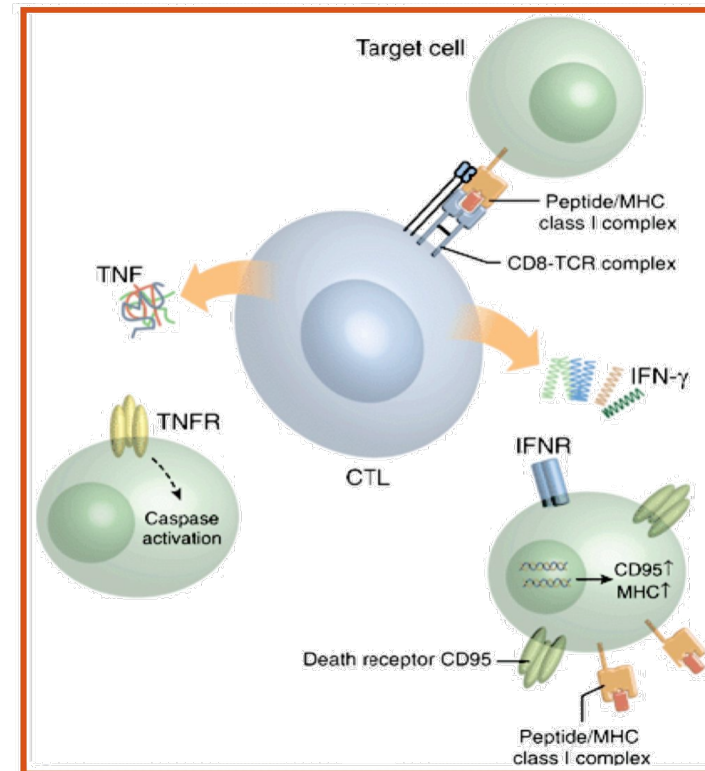
CTL-mediated cytotoxicity



Direct killing by release of granzyme B and perforin into the intercellular space between CTL and target cell

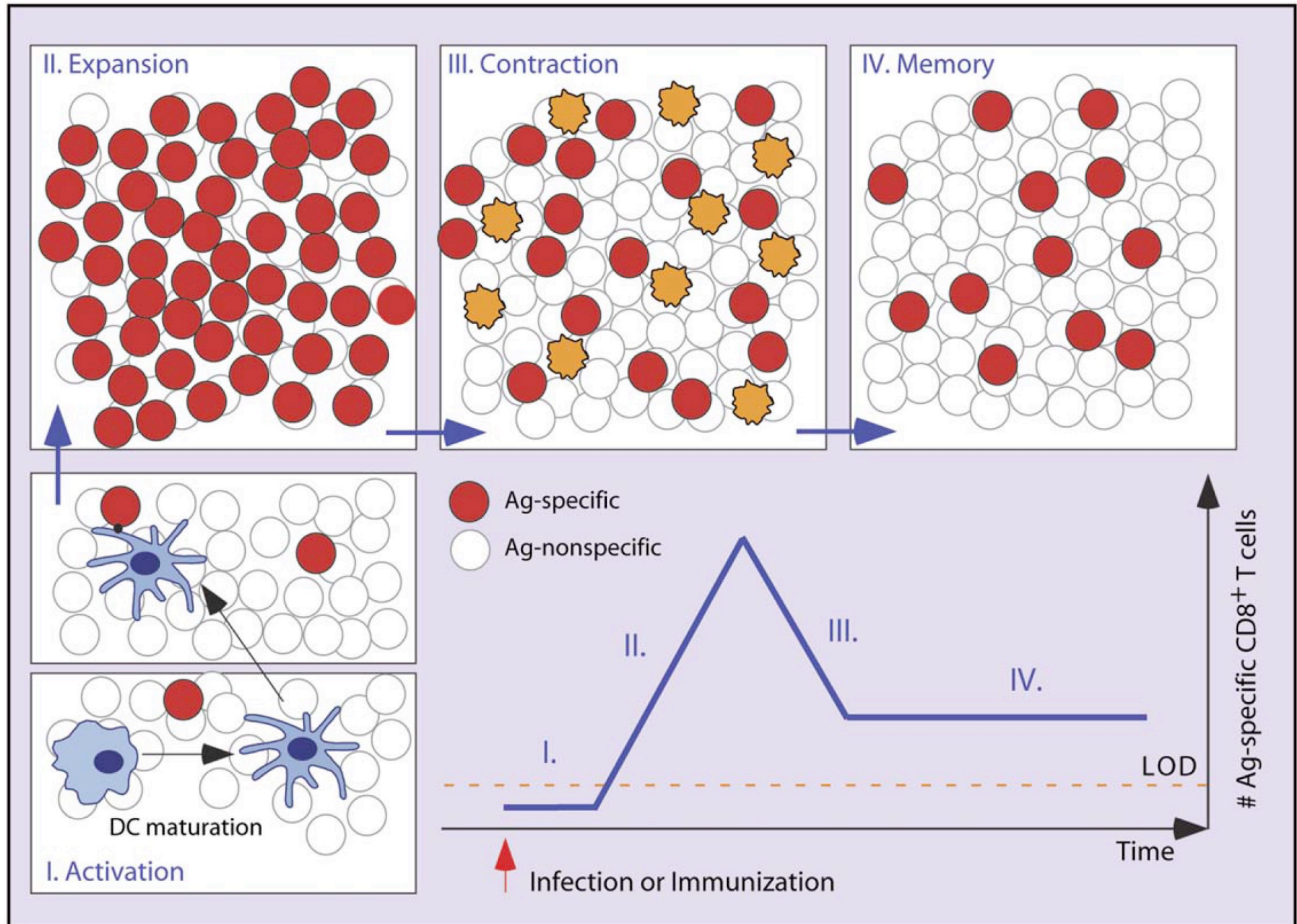


Induction of apoptosis in target cells via death receptor triggering

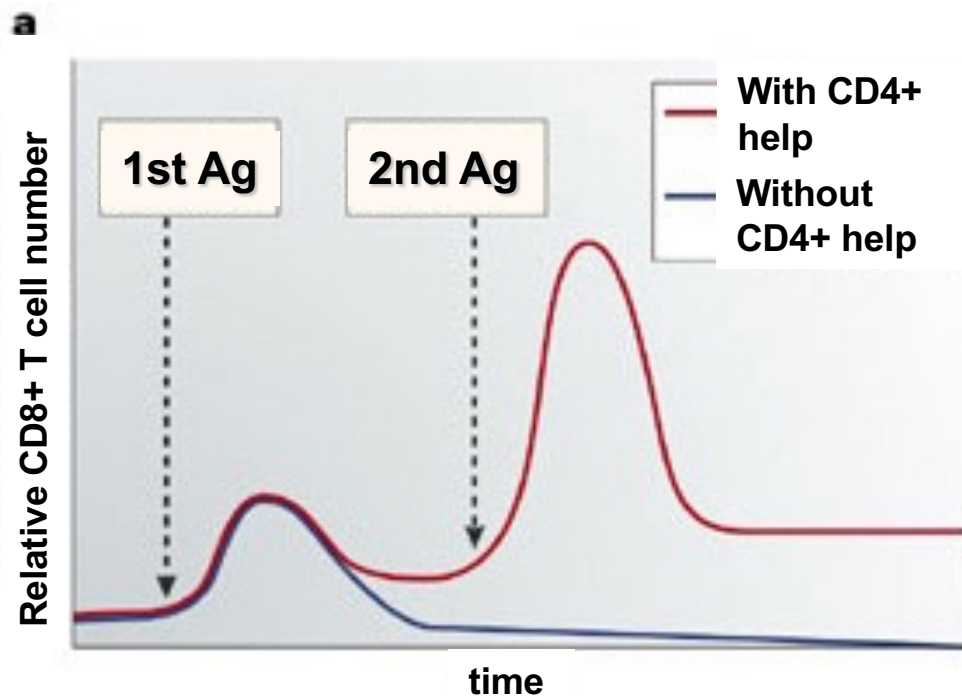


Indirect killing of target cells by release of Tumor Necrosis Factor-alpha and IFN-gamma

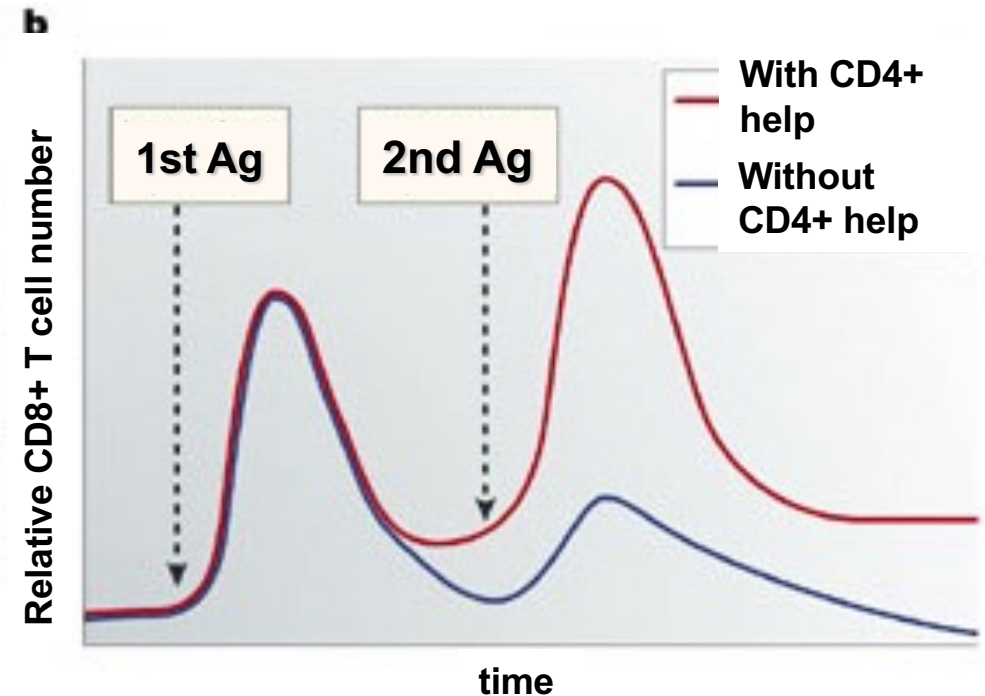
Naive-to-memory progression after acute infection or vaccination



The development of memory cells requires CD4+ T-cell help (cytokines, etc.)

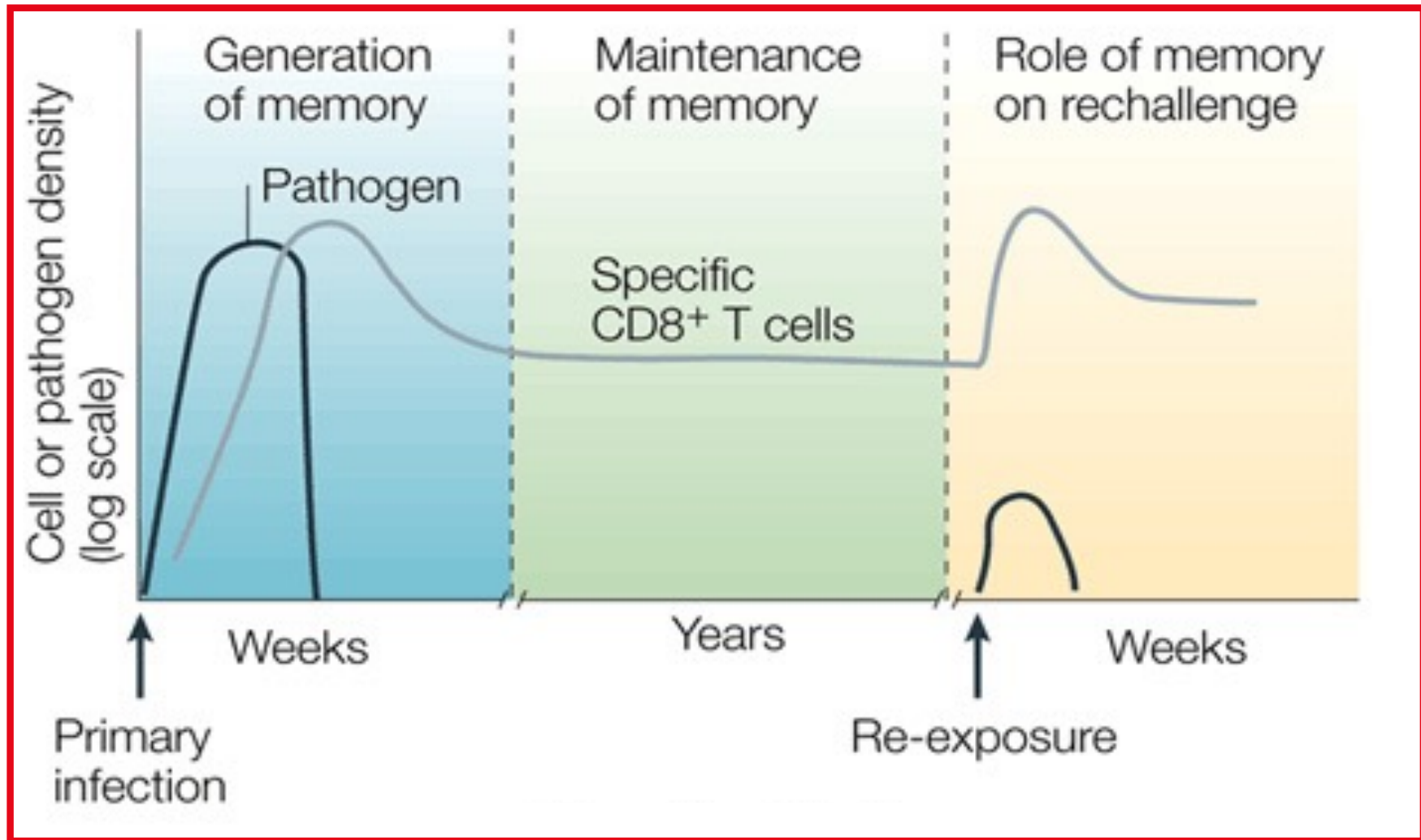


**Cellular antigen
(weakly immunogenic)**

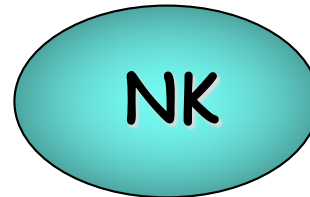
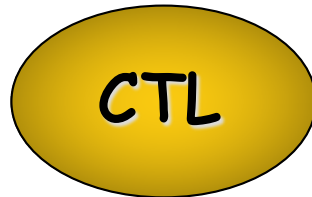


**Viral or bacterial antigen
(strongly immunogenic)**

Memory CTL are less dependent on CD4+ T help



Two types of cytotoxic lymphocytes



- Different recognition strategies
- Distinct activation mechanisms
- Common lytic mechanisms

They use **complementary** defense strategies against intracellular pathogens and tumor cells!