Cell Mediated Immunity

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Activation of CD8 T cell

- **CONDITION # 1:** An antigen **MUST be MEMBRANE BOUND** (e.g. on APC)
- **CONDITION # 2:** An antigen **MUST be PROCESSED** (e.g. by APC)
- **CONDITION # 3:** The processed antigen **must associate with MHC-I** present on the membrane of cells (e.g. on APC)
- **CONDITION # 4:** Activation of Tc cells **require at least 2 signals**:

  First, is the **primary signal** that is antigen-specific (delivered through TCR/CD complex), and

  Second, **co-stimulatory signal** (through molecular contacts between Tc cells and APC and/or cytokines)

Activation of CD8 T cell

Cytotoxic T cell responses can be thought of as occurring in five stages:

- Activation of the naïve Tc cell by a licensed DC (or any APC) in a secondary lymphoid tissue ;
- Proliferation (division) and differentiation of the activated Tc cell into daughter cells called *pre-CTLs or T-blast cells* (blastogenesis);
- Differentiation of a pre-CTL in an inflammatory site into an *"armed" CTL*;
- Activation of the armed CTL by encounter with specific non-self peptide presented by MHC class I on a target cell; and
- CTL-mediated destruction of the target cell as well as other cells displaying the identical pMHC.

Th1 cells also helps in co-activation of Tc cells simultaneously by secreting cytokines such as INF-γ and IL-2, IL-12

Activation of CD8 T cell

- In the absence of antigen, naive T cells in the LNs engage in what appears to be a random walk in the T cell area, which is actually their wandering on the fibroblastic reticular network.
- During an infection, naive $CD8^+$ T cells are primed by antigen-presenting cells (APCs) in secondary lymphoid organs such as lymph nodes (LN) and spleen.
- The naive $CD8^+$ T cells first contact the antigen-bearing DCs in the subcapsular sinus region or the interfollicular region of the draining LN.
- Shortly after infection the $CD8^+$ T cells and DCs are quickly enriched in the peripheral regions of the nodes.
- (Although the first and major cell population infected by pathogen is $CD169^+$ macrophages lining the subcapsular sinus, but instead of these antigen-rich macrophages, naive $CD8^+$ T cells favor the DC population to deliver the antigen first to start their differentiation to effector cells).
- At this peripheral site of the LNs, initial antigen-specific contacts between the $CD8^+$ T cells and DCs are formed that lead to $CD8^+$ T cell activation and expansion.
- The chemokine-chemokine receptor (XCL1-XCR1) interactions maximize the recruitment of both antigen-specific naive $CD8^+$ T cells and DCs during priming

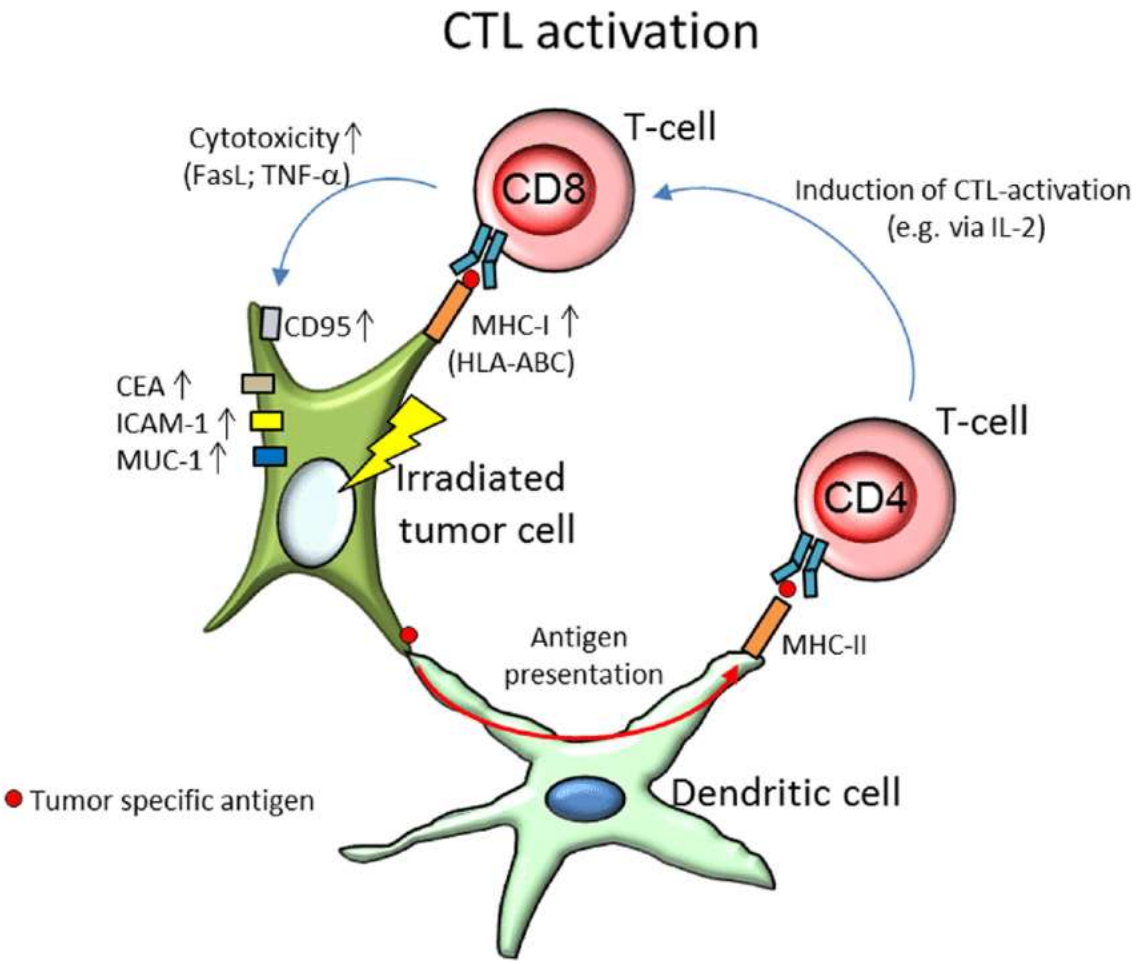
Activation of CD8 T cell

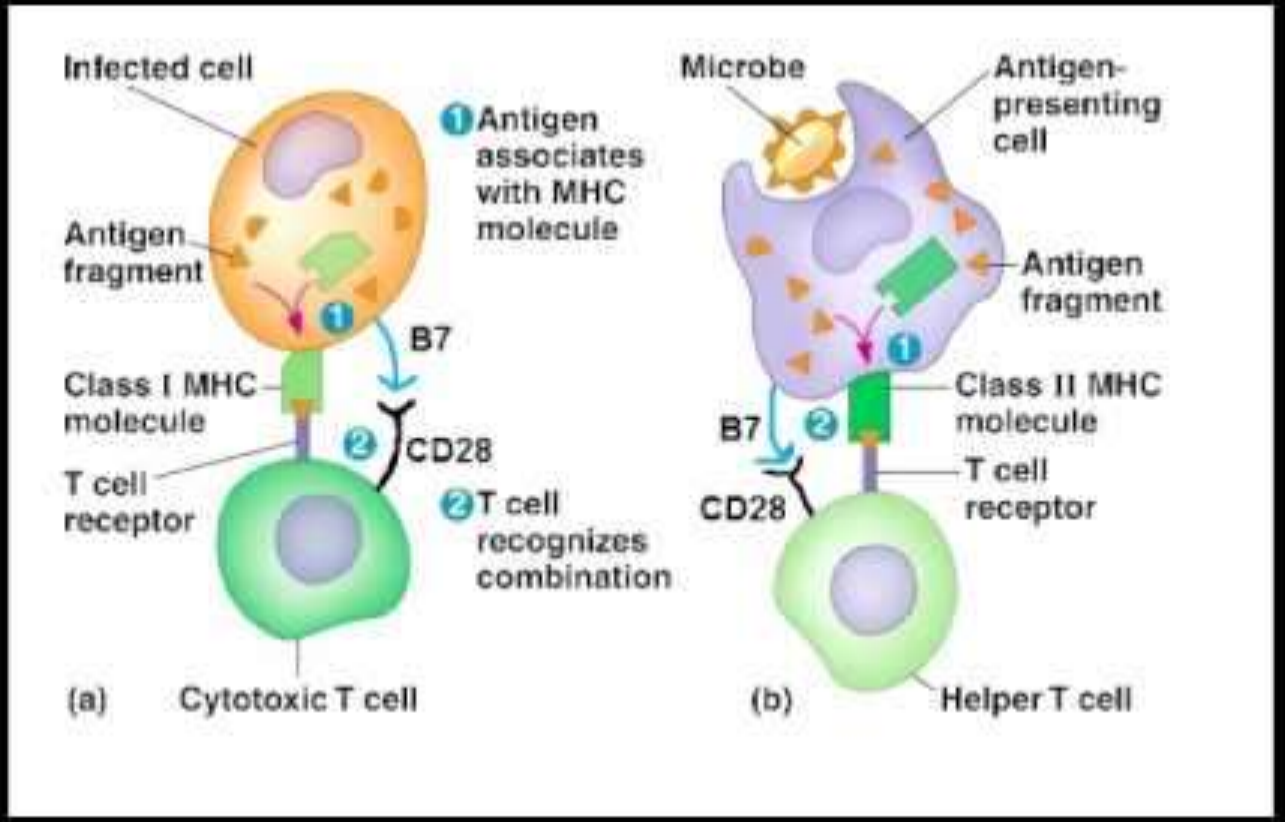
- To achieve maximal expansion, $CD8^+$ T cells need to integrate multiple signals, including the TCR, costimulatory signals, and inflammatory cytokines, such as IL-12, IL-2 and type -I IFN.
- At the peak of the primary response to pathogen, the population of antigen-induced $CD8^+$ effector T cells is phenotypically and functionally heterogeneous.
- The short-lived effector cells (SLEC) form the bulk of the population but will mostly die off when infection is cleared, whereas memory precursor effector cells (MPEC) that may have received less stimulation survive and contribute preferentially to the memory population.
- (Calculations imply that a naive $CD8^+$ T cell may go through as many as 19 cell divisions in the week after pathogen stimulation, representing a potential 500,000-fold expansion! The rate of maximal $CD8^+$ cell division is usually 4–6 hr).
- IL-12, play a key role in terminal differentiation of $CD8^+$ effector T cells.
- IL-2 promotes SLEC differentiation, whereas in the absence or decreased amount of IL-2, $CD8^+$ T cells exhibit defective effector function and preferentially become $CD62L^{hi}$ memory cells

Activation of CD8 T cell

- Shortly after recognition of antigen on DCs in the central lymphoid organs, activated T cells enter peripheral tissues.
- $CD4^+$ T cell help in activating the APC is required for the generation of the $CD8^+$ primary response to non-inflammatory antigens and certain viral infections, but not for all.
- Thus even after migration from the l lymphoid organs where the response initiated, $CD8^+$ T cell interactions with helper T cells and with specialized APCs continue at peripheral sites of infection.

Activation of CD8 T cell



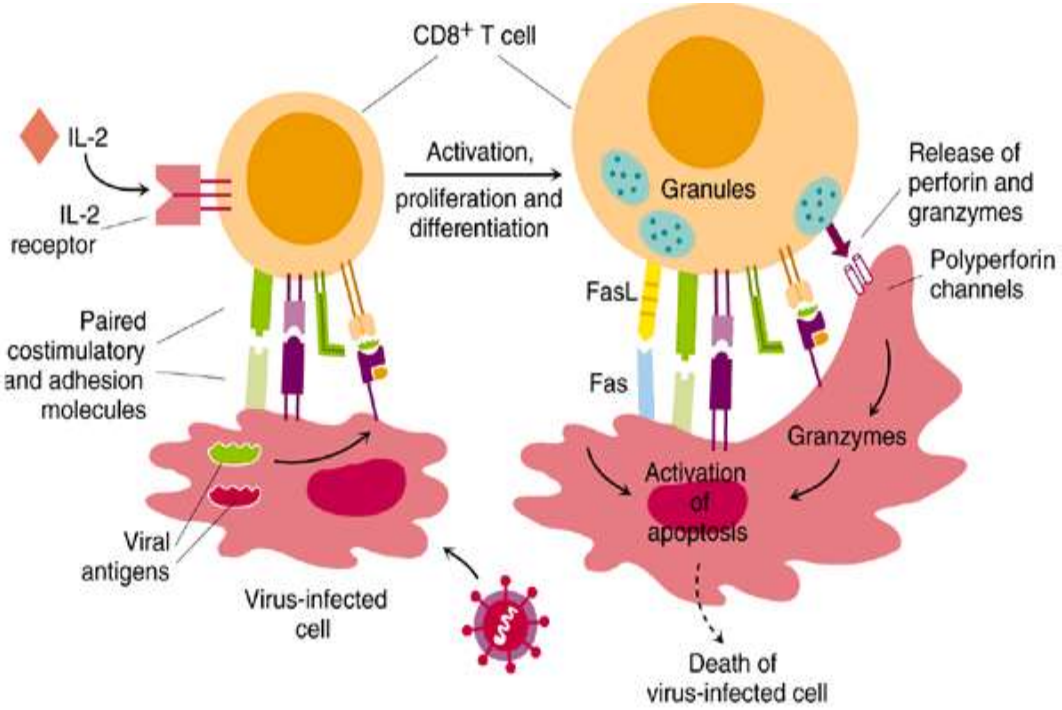

Infected cell
1 Antigen associates with MHC molecule
Antigen fragment
B7
Class I MHC molecule
2 CD28
T cell receptor
2 T cell recognizes combination
(a)
Cytotoxic T cell
Microbe
Antigen-presenting cell
Antigen fragment
Class II MHC molecule
B7
2
T cell receptor
CD28
(b)
Helper T cell

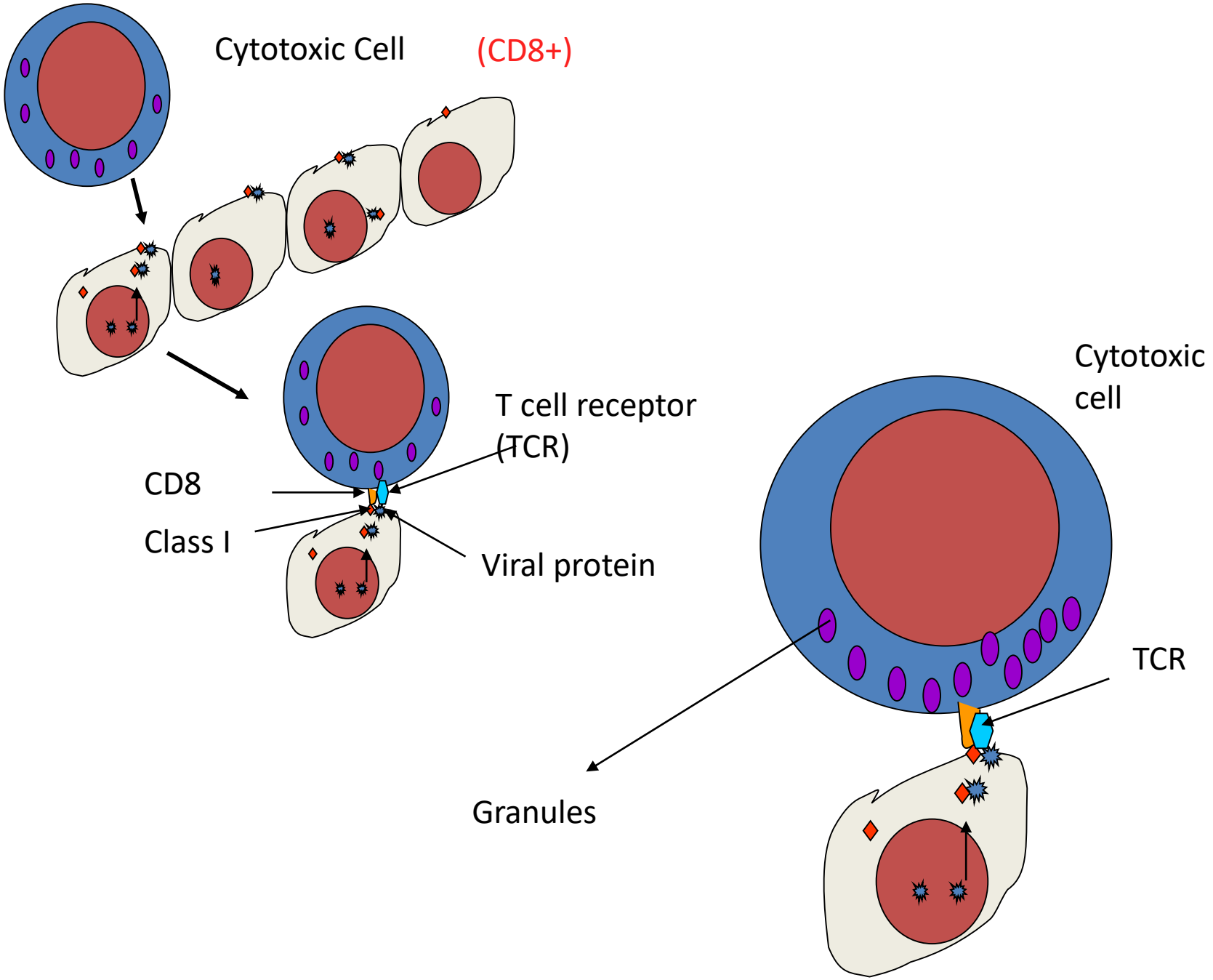
Tc cell cytoxicity - CMI

T-Cell Cytoxicity

- Definition - cytotoxicity involving direct contact between CTLs and target cells, resulting in target cell lysis or apoptosis

- Mechanisms
  - TCR on CD8+ CTL binds to Ag-MHC Class I complexes on target cell
  - CTL activation results in release of granules containing perforin and granzymes
  - Perforin can mediate pore formation, target cell lysis
  - Granzymes together with Fas-Fas ligand interaction trigger apoptosis of target cell (programmed cell death)
  - Cytokines released(IFN-γ, TNF-α) may also be cytotoxic




Cytotoxic Cell
(CD8+)
T cell receptor
(TCR)
CD8
Class I
Viral protein
Cytotoxic
cell
TCR
Granules

The Perforin-Granyzyme Pathway

- The principal mechanism through which cytotoxic T cells act is by the calcium-dependent release of specialized lytic granules upon recognition of antigen on the surface of a target cell. These granules are modified lysosomes that contain at least two distinct classes of cytotoxic effector proteins that are expressed selectively in Tc cells.
- One of these cytotoxic proteins, known as perforin, polymerizes to form transmembrane pores in target cell membranes.
- The other class of cytotoxic proteins comprises at least three proteases called granzymes, which belong to the family of serine proteases enzymes.
- On release from the granule, perforin forms a cylindrical structure (MAC) that is lipophilic on the outside and hydrophilic down with a a hollow center of 16 nm inner diameter.
- The granzymes move into the target cell through these pores.
- The granzymes activate an enzyme cascade, in the target cell. Granzyme B cleave the ubiquitous cellular enzyme CPP-32, which is a caspase and activates a nuclease, called caspase-activated deoxyribonuclease (CAD). This enzyme is believed to be the final effector of DNA degradation in apoptosis.

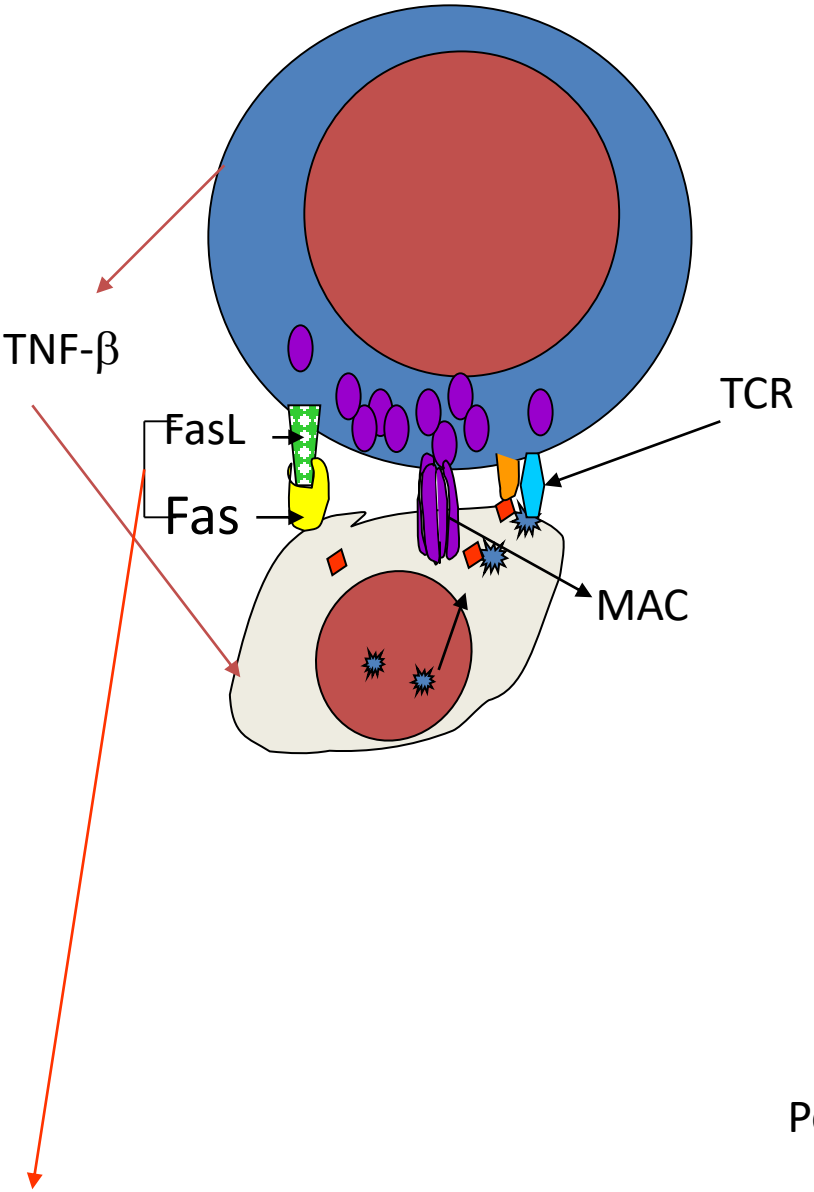
Other pathways of Tc cells

Fas-FasL pathway

- This mechanism involves the binding of Fas in the target cell membrane by the Fas ligand, which is present in the membranes of activated cytotoxic T cells
- Ligation of Fas leads to activation of caspases, which induce apoptosis in the target cell

Cytokines secretion pathway

- Most cytotoxic CD8 T cells also release the cytokines IFN-γ, TNF-α, and TNF-β, which contribute to host defense in several ways
- IFN-γ directly inhibits viral replication, induces the increased expression of MHC class I on target cells, and activate macrophages.
- TNF-α or TNF-β can synergize with IFN-γ in macrophage activation, and in killing some target cells through their interaction with TNFR-I.

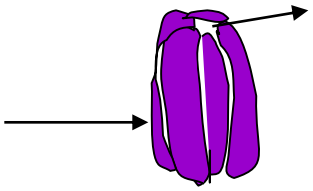


Cytotoxicity by three mechanisms:

1) **Granzymes/perforins pathway**
2) **Fas-Fas ligand pathway**
3) **TNF pathway**

Perforins are found in granules. They polymerize within target cells to form large circular complex (Membrane attack complex (**MAC**) with a "hole" in the center. Granzymes enter through this hole of MAC and trigger apoptotic cell death

Perforins

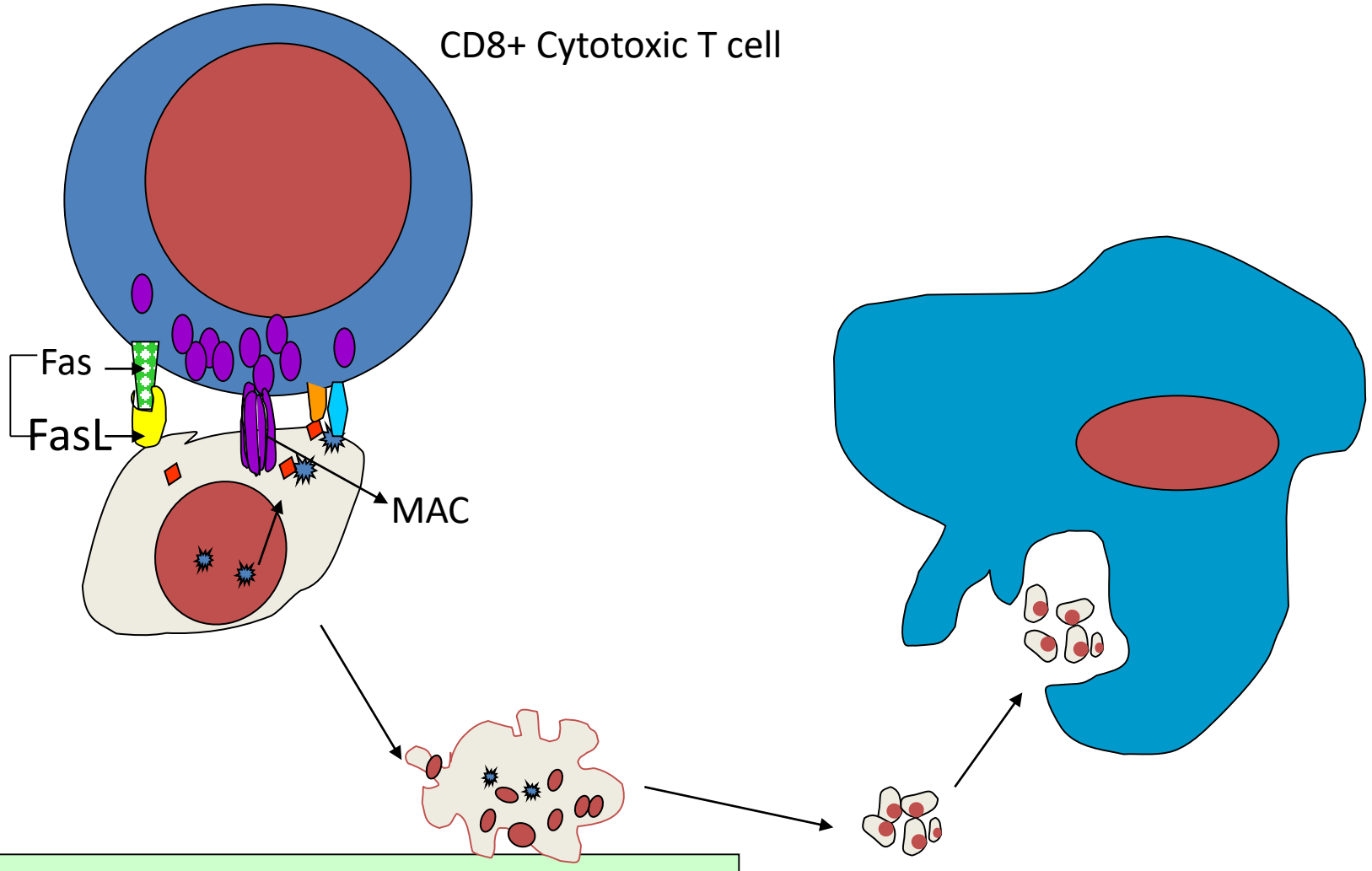


Granzymes enter Through this perforin hole

Fas-FasLigand interaction leads to apoptotic deathof target cells.

CMI - Apoptosis (programmed cell death)

In the cytoplasm of the target cell, the energy-dependent binding of cytochrome-C to apoptotic protease-activating factor (Apaf-1) triggers the caspase cascade resulting in degradation of vital cellular proteins and genetic materials. This leads to the formation of apoptotic bodies, which are taken up by surrounding phagocytes without causing inflammation.



Target cell begins to lose shape (membrane blebbing, nucleus disintegerates, “chopping” of DNA

Dying cell (Apoptotic Bodies) are engulfed by macrophages

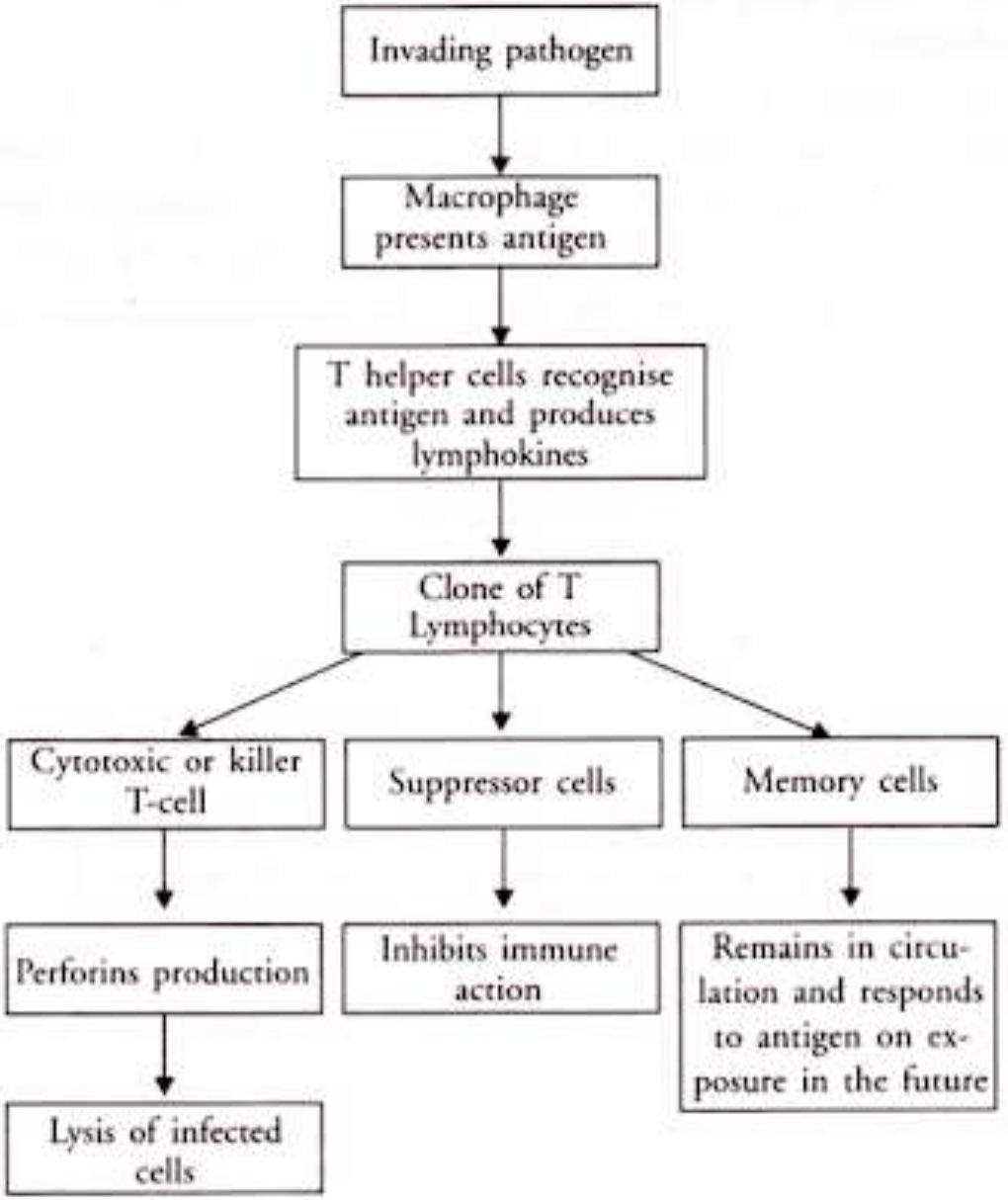


Fig. 11 Flow chart of cell mediated immune response.