"How T cells see the world"

Antigen Presentation

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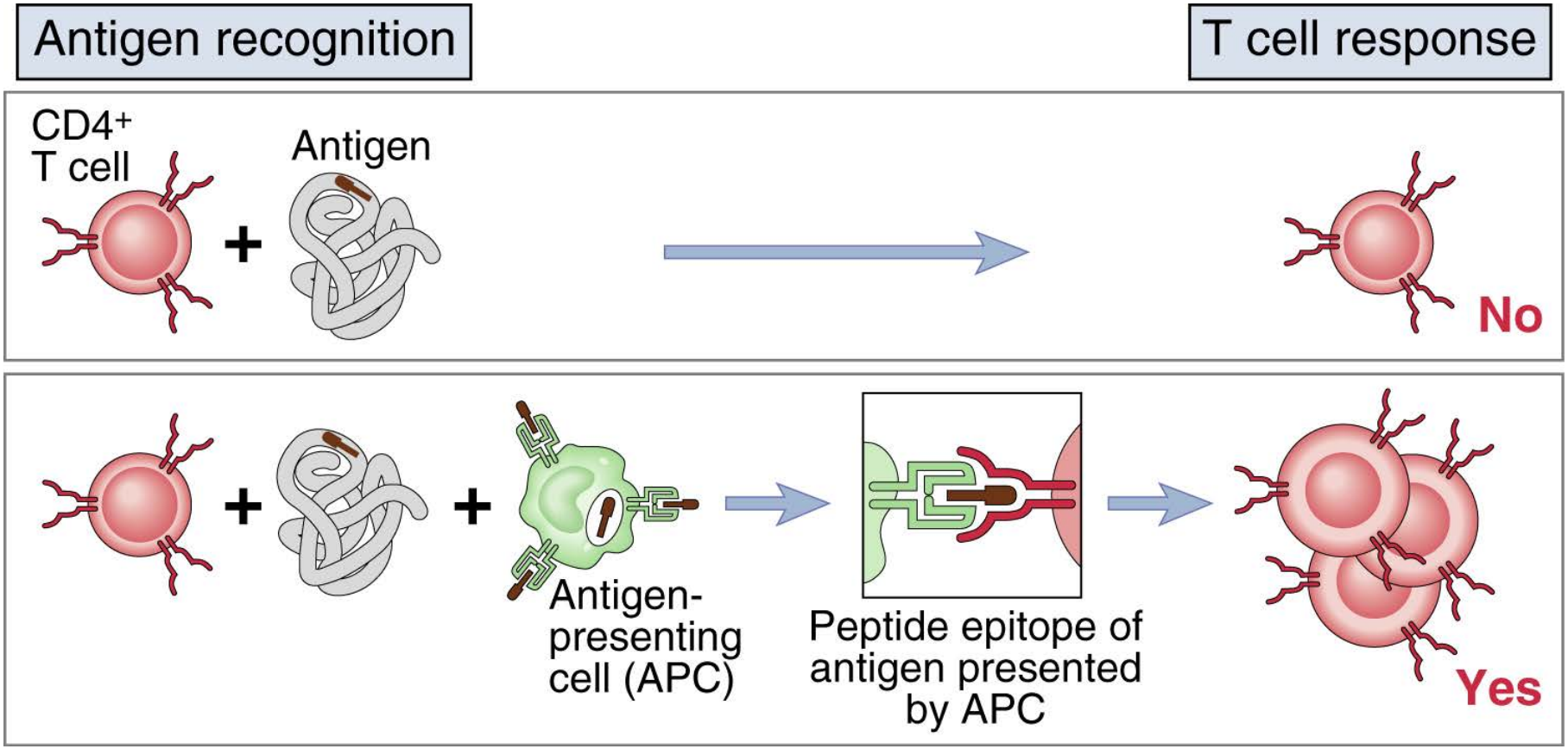
Lecture outline

- Capture of antigens from sites of entry and display of antigens to T cells
- Function of MHC molecules as the peptide display molecules of adaptive immunity
- Recognition of protein antigens by different classes of T cells (helper vs cytotoxic)

What do T cells see?



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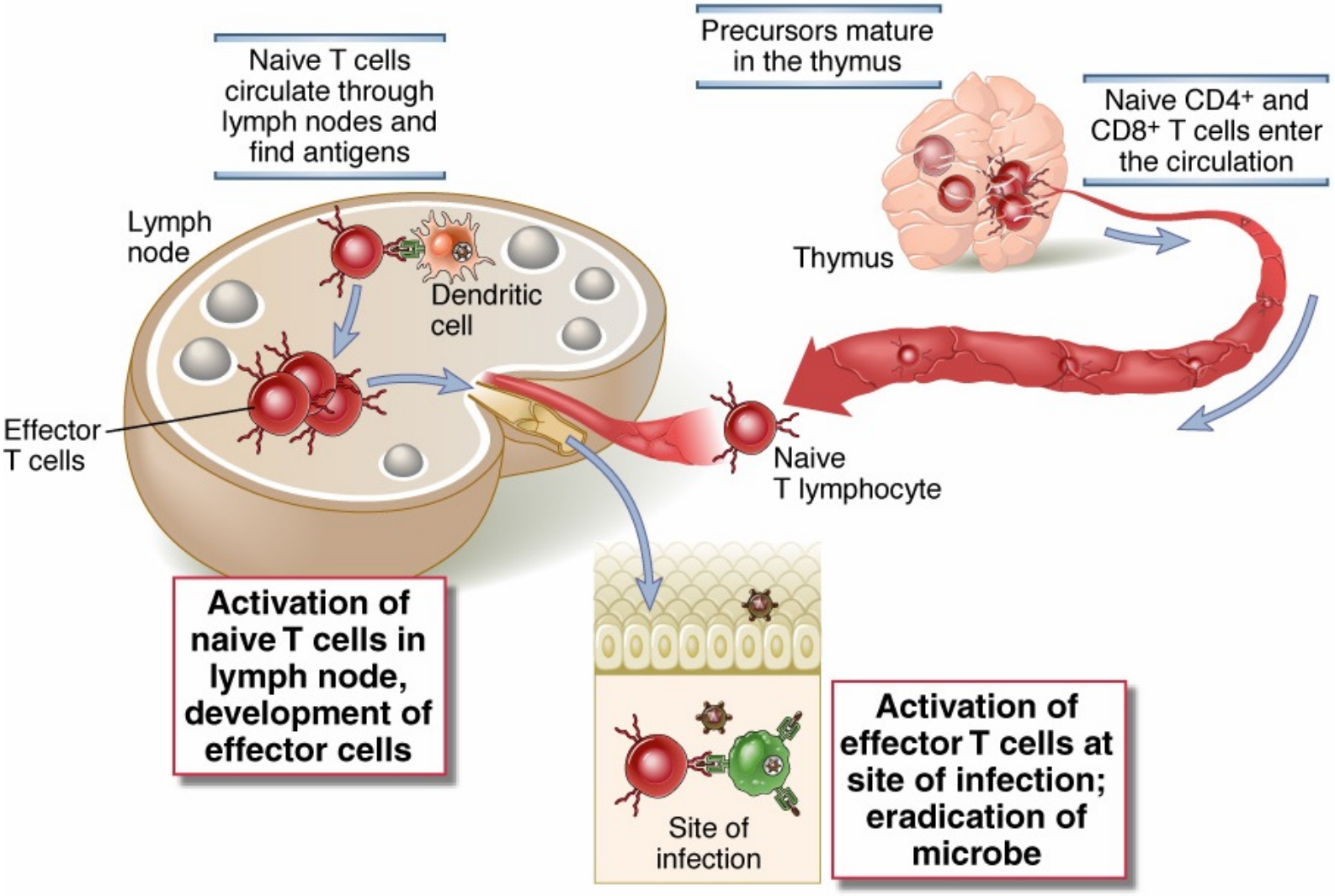
The life history of T lymphocytes



Abbas, Lichtman and Pillai. *Cellular and Molecular Immunology, 7th edition, 2011*

The challenge for lymphocytes

- Very few lymphocytes in the body are specific for any one microbe (or antigen)
  - Specificity and diversity of antigen receptors: the immune system recognizes and distinguishes between 10^6 - 10^9 antigens; therefore, few lymphocytes with the same receptors

The challenge for lymphocytes

- Very few lymphocytes in the body are specific for any one microbe (or antigen)
  - Specificity and diversity of antigen receptors: the immune system recognizes and distinguishes between 10^6 - 10^9 antigens
- **Lymphocytes must be able to locate microbes that enter and reside anywhere in the body**
  - **Usual routes of entry are through epithelia, but infections may take hold anywhere**

The challenge for lymphocytes

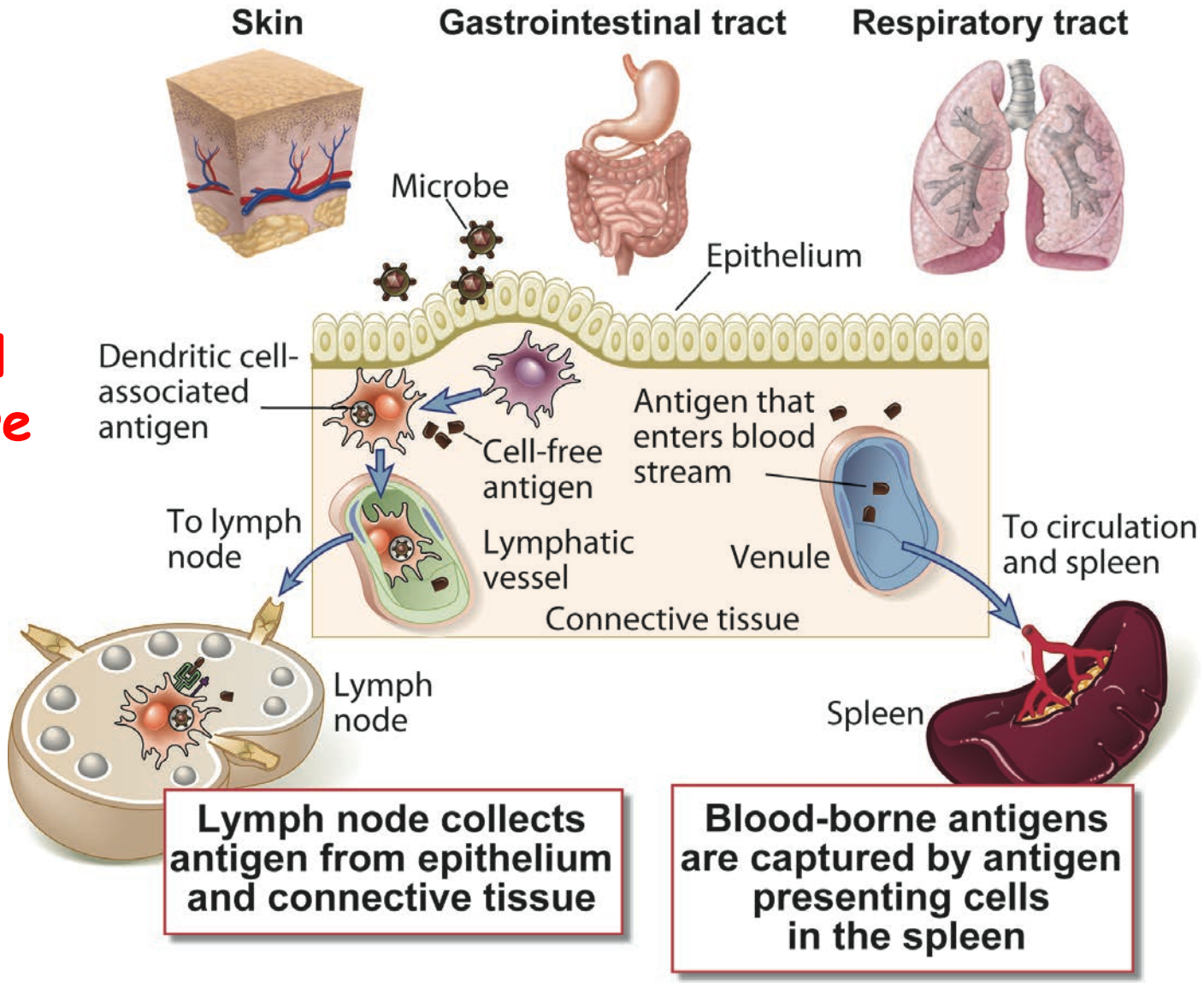
- Very few lymphocytes in the body are specific for any one microbe (or antigen)
- Lymphocytes must be able to locate microbes that enter anywhere in the body
- **Lymphocytes must respond to each microbe in ways that are able to eradicate that microbe; best exemplified by T cells**
  - Extracellular microbes: antibodies; destruction in phagocytes (need helper T cells)
  - Intracellular microbes: killing of infected cells (need CTLs)
  - How do T cells distinguish antigens in different cellular locations?

Capture of antigens

Sites of antigen entry

Sites of initial antigen capture

Sites of antigen collection and capture



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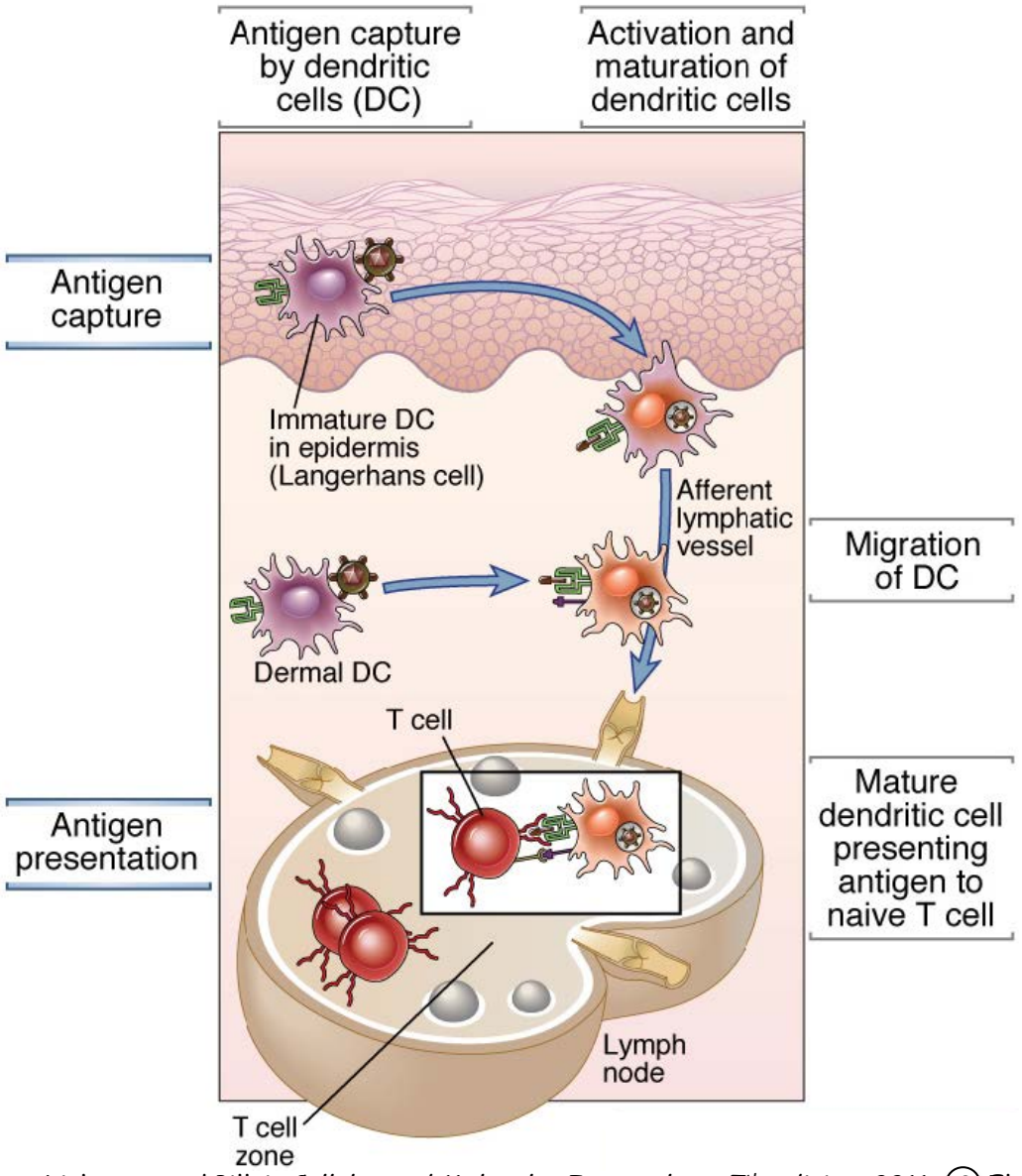
Capture and presentation of antigens by dendritic cells



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Sites of microbe entry: *skin, GI tract, airways (organs with continuous epithelia, populated with dendritic cells). Less often -- colonized tissues, blood*

Sites of lymphocyte activation: *peripheral lymphoid organs (lymph nodes, spleen), mucosal and cutaneous lymphoid tissues*

Antigens and T cells come together in the same organs

Dendritic cell subsets

- **Conventional**: CD11c+, role in presentation of most antigens
- **Plasmacytoid**: source of type I IFN

- **Immature**: in tissues; role in presentation of self antigen and maintenance of tolerance
- **Mature**: activated by TLR and other signals; role in T cell activation

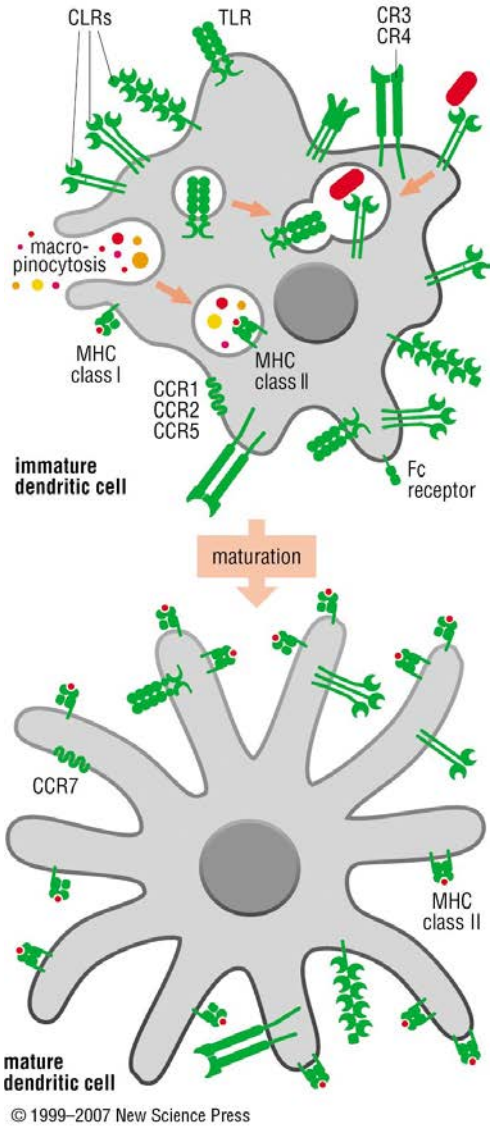
- Many other subsets described

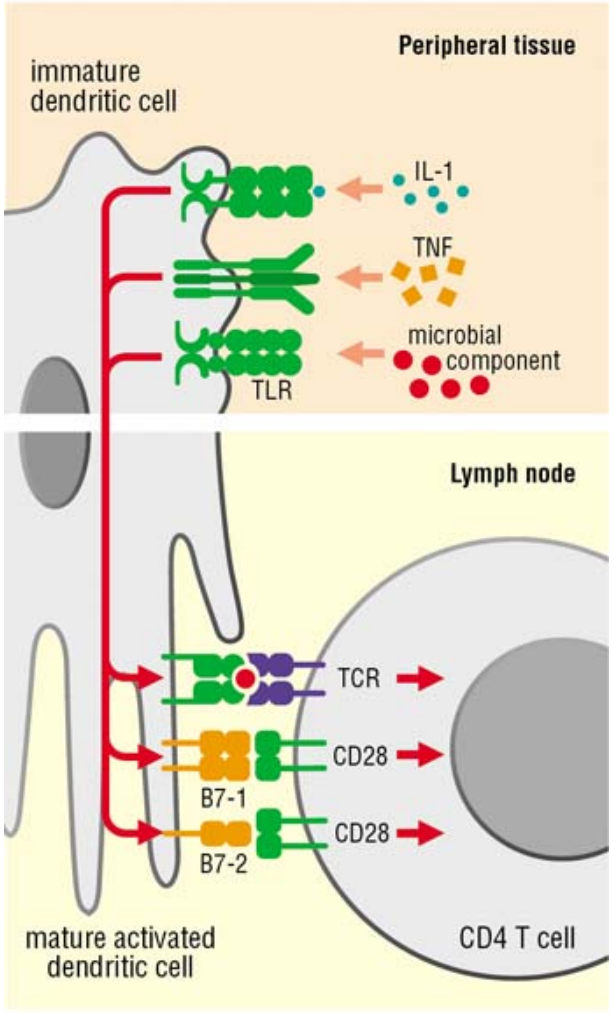
Maturation of Dendritic Cells

From **Immunity: The Immune Response in Infectious and Inflammatory Disease** by DeFranco, Locksley and Robertson

Class II MHC molecules

Number	T ½
10^6	~10 hr
7×10^6	>100 hr





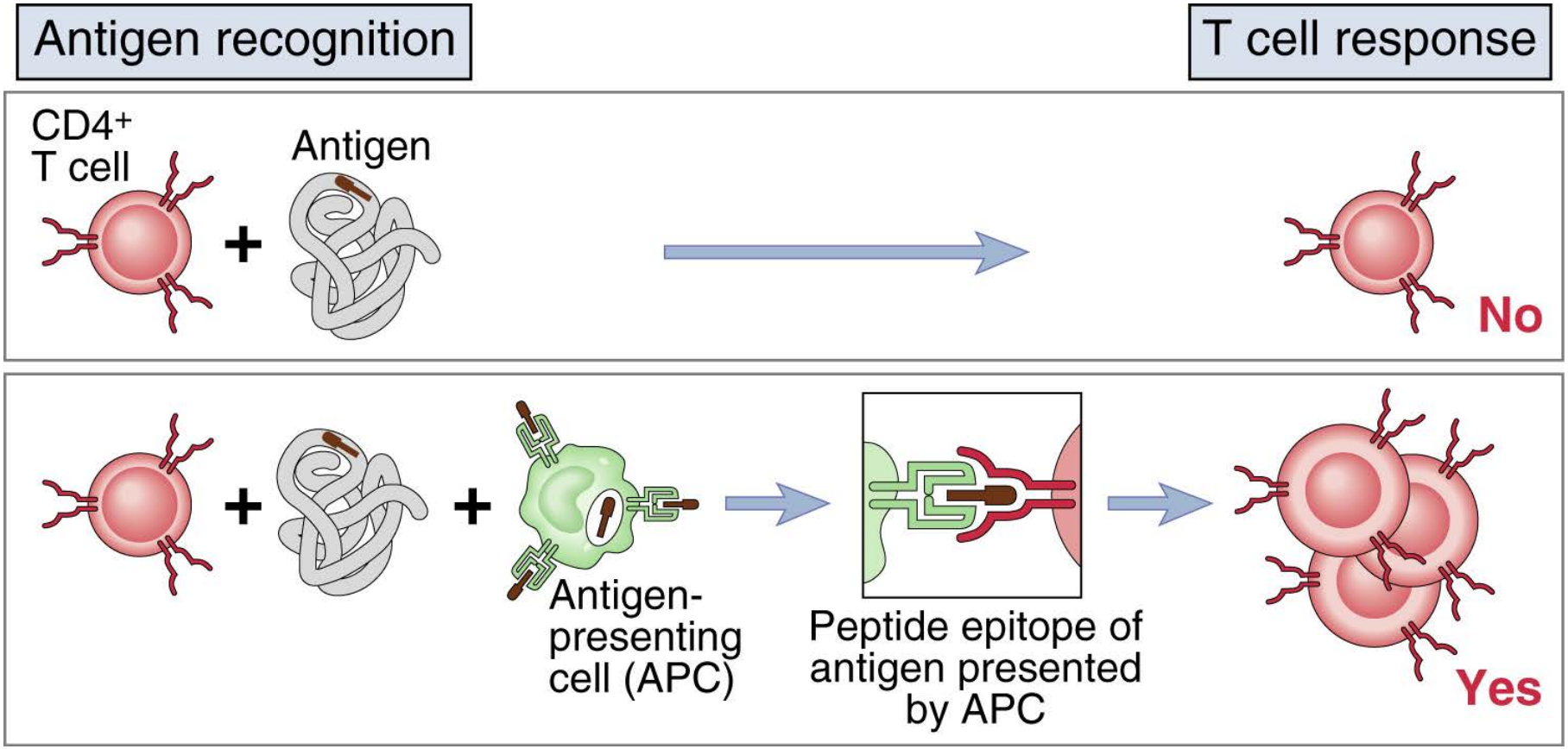
Why are dendritic cells the most efficient APCs for initiating immune responses?

- **Location:** at sites of microbe entry (epithelia), tissues
- **Receptors for capturing and reacting to microbes:** Toll-like receptors, other receptors
- **Migration to T cell zones of lymphoid organs**
  - Role of CCR7
  - Co-localize with naïve T cells
- **Maturation during migration:** Conversion from cells designed for antigen capture into cells for antigen presentation and T cell activation

- **Practical application:** dendritic cell-based vaccines for tumors

Take home messages

What do T cells see?



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What do T cells see?

- All functions of T cells are mediated by interactions with other cells
  - Helper T cells "help" B cells to make antibodies and "help" macrophages to destroy what they have eaten
  - Cytotoxic (killer) T lymphocytes kill infected cells

- How does the immune system ensure that T cells see only antigens on other cells?

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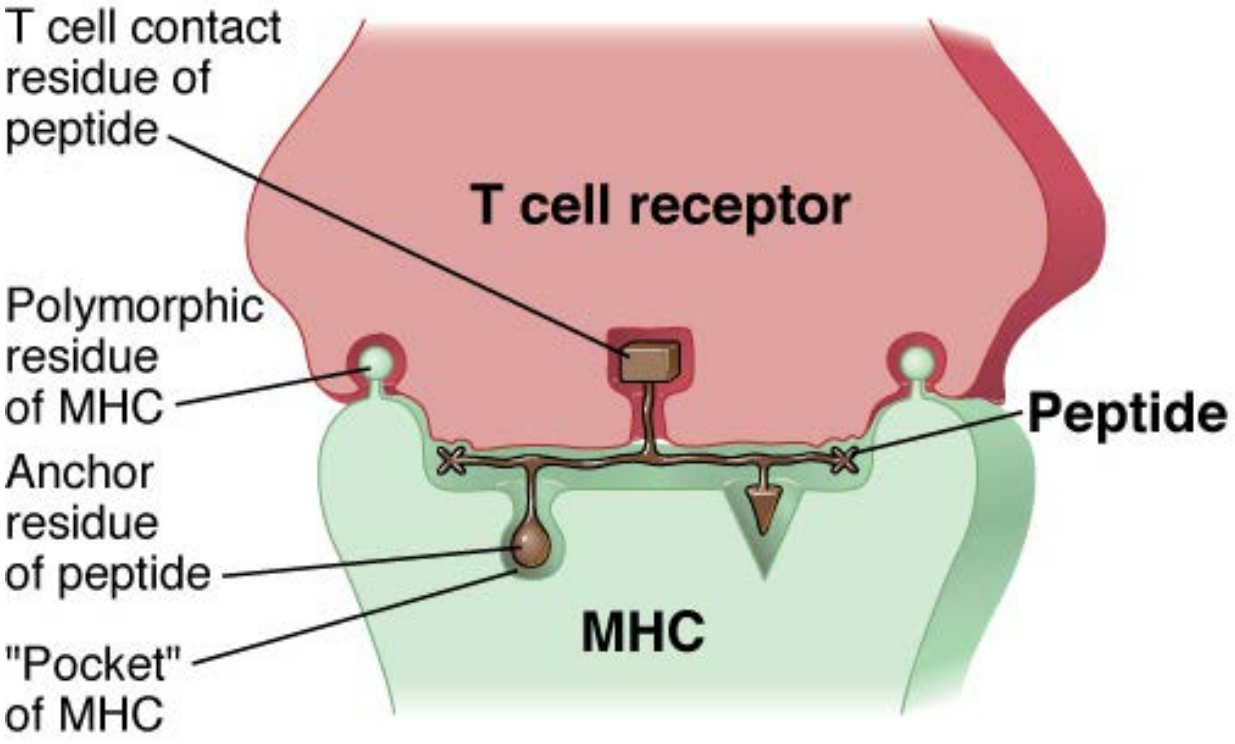
- To ensure cellular communications, T cells see antigens NOT in the circulation but only when displayed by molecules on the surface of other cells
  - These molecules are HLA (generic name: MHC) and the cells displaying the antigen are APCs

Take home messages

A model of T cell recognition of peptide displayed by an MHC molecule



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Human MHC = HLA

Because MHC molecules are on cells and can display only peptides, T lymphocytes can recognize only cell-associated protein antigens

What is the MHC?

- A genetic locus discovered on the basis of transplantation (major histocompatibility complex)
  - Different individuals express products of different MHC alleles and reject grafts from one another
  - Human MHC: HLA (human leukocyte antigens)
- MHC molecules are the peptide display molecules of the immune system

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- MHC molecules are the peptide display molecules of the immune system
- **Different alleles of MHC molecules bind and display distinct but overlapping sets of peptides**
  - **Determines which protein antigens are recognized in different individuals**
  - **MHC genes are highly polymorphic (>7500 alleles [variants] in the population); the MHC molecules in the population can display many different peptides**

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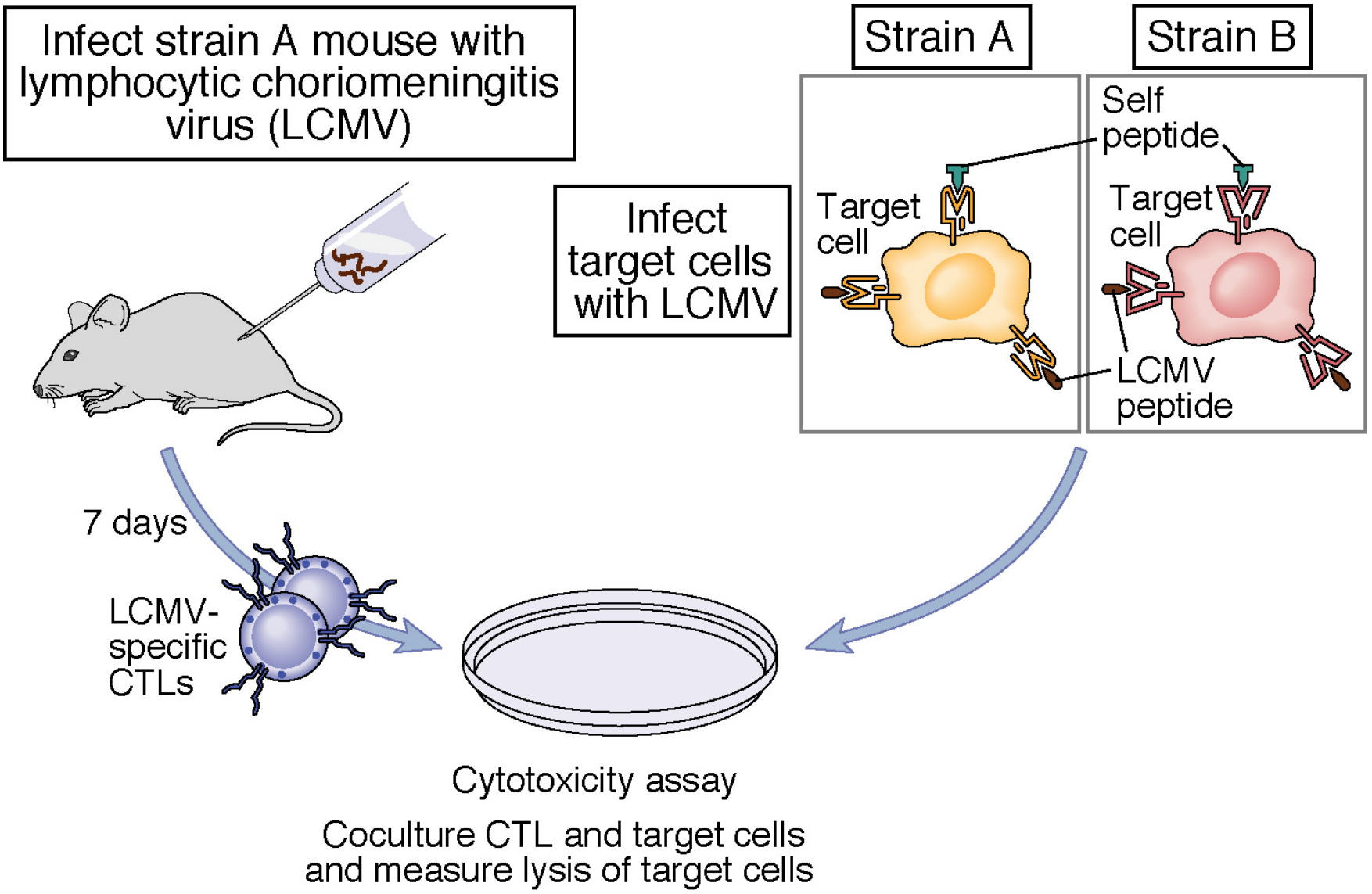
- **MHC molecules determine how antigens in different cellular compartments are recognized by different classes of T cells (CD4+ and CD8+)**

MHC-restricted antigen recognition by T cells

- Any T cell can recognize an antigen on an APC only if that antigen is displayed by MHC molecules
  - Antigen receptors of T cells have dual specificities: 1. for peptide antigen (responsible for specificity of immune response) and 2. for MHC molecules (responsible for MHC restriction)
  - During maturation in the thymus, T cells whose antigen receptors see MHC are selected to survive and mature; therefore, mature T cells are "MHC-restricted"

T cell Recognition of Virus is Genetically Controlled



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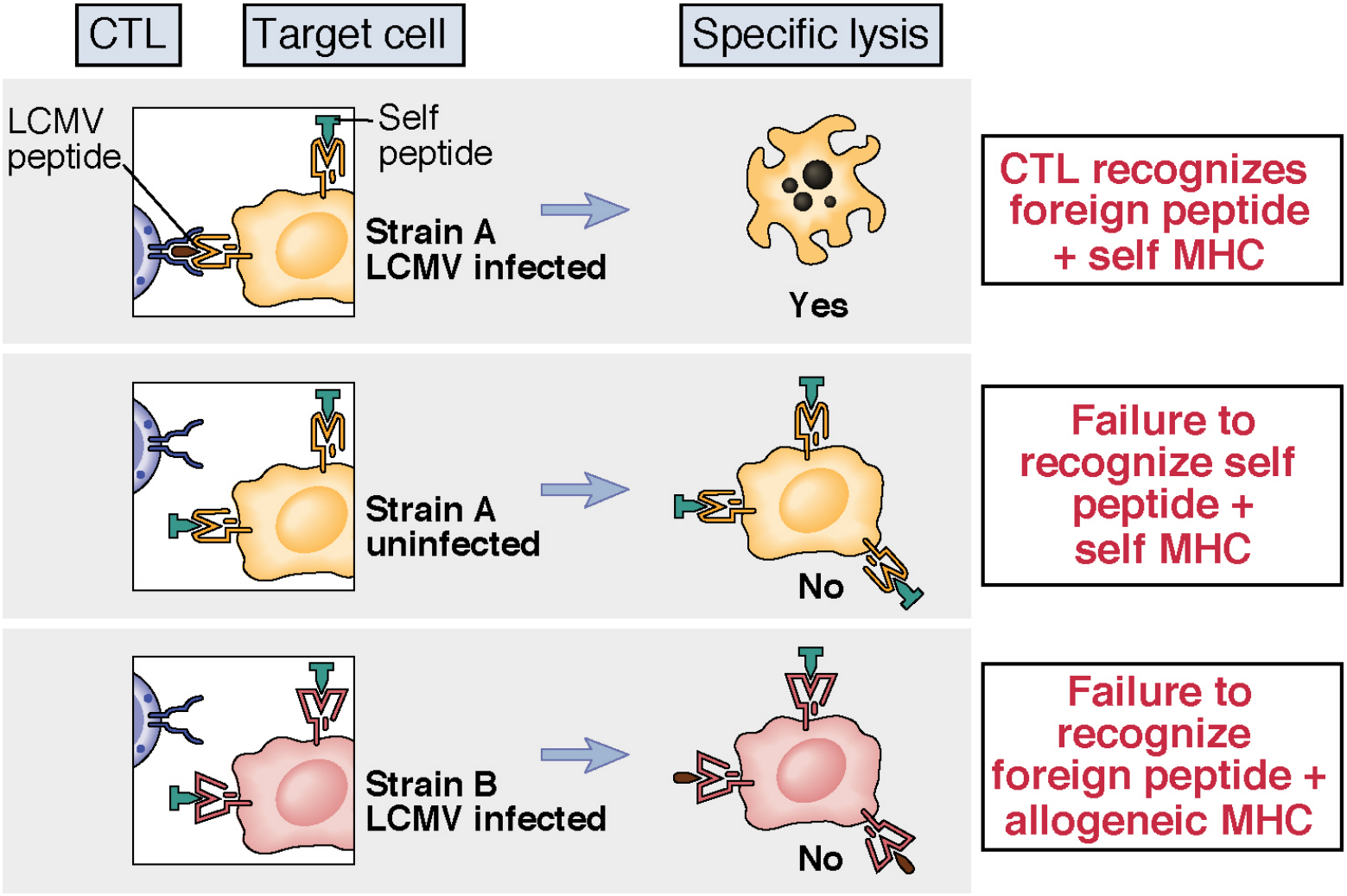
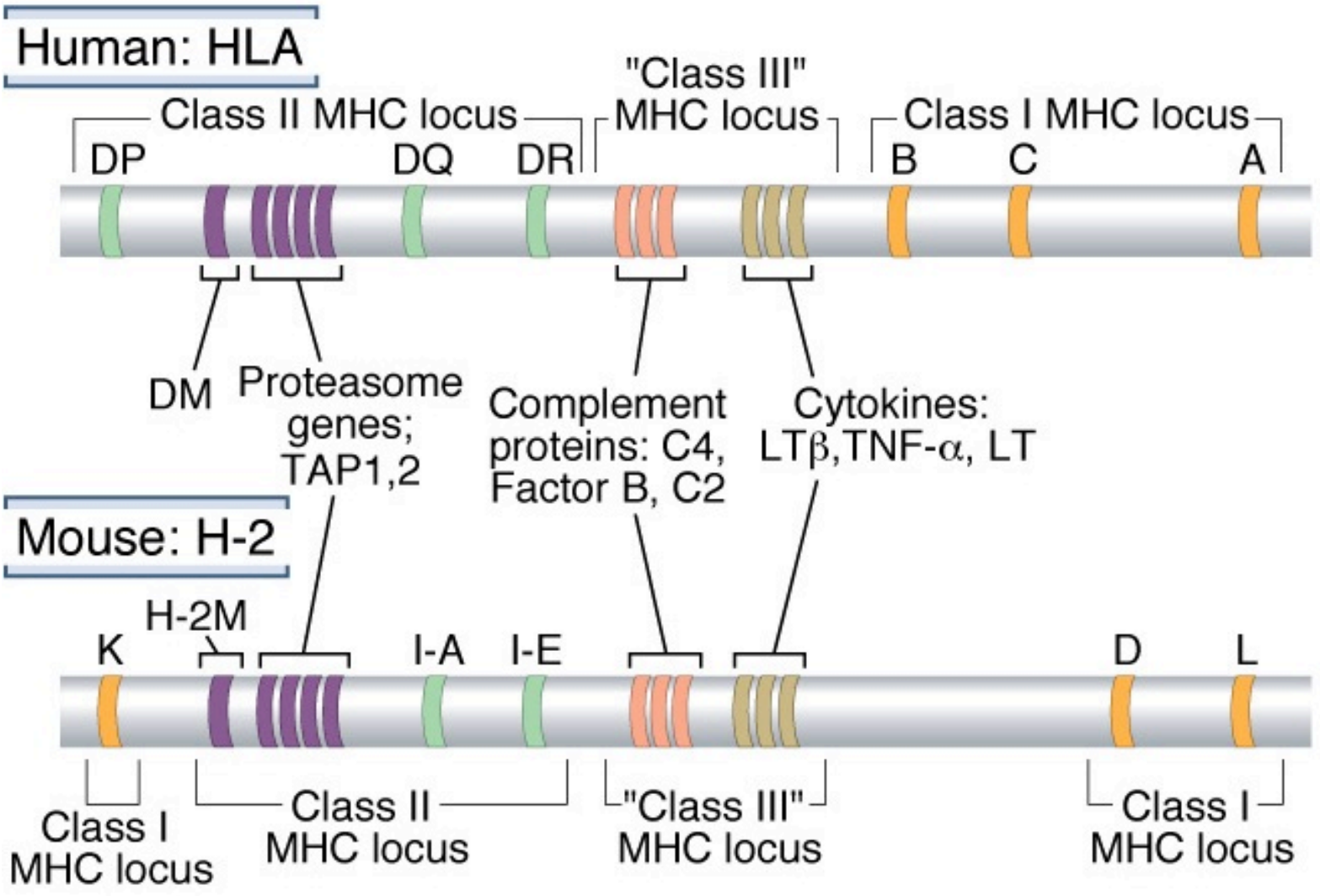
Self MHC Restriction of T cells



The genes that differ between strains A and B and control T cell recognition were found to map to a locus called the MHC

The genes of the MHC locus

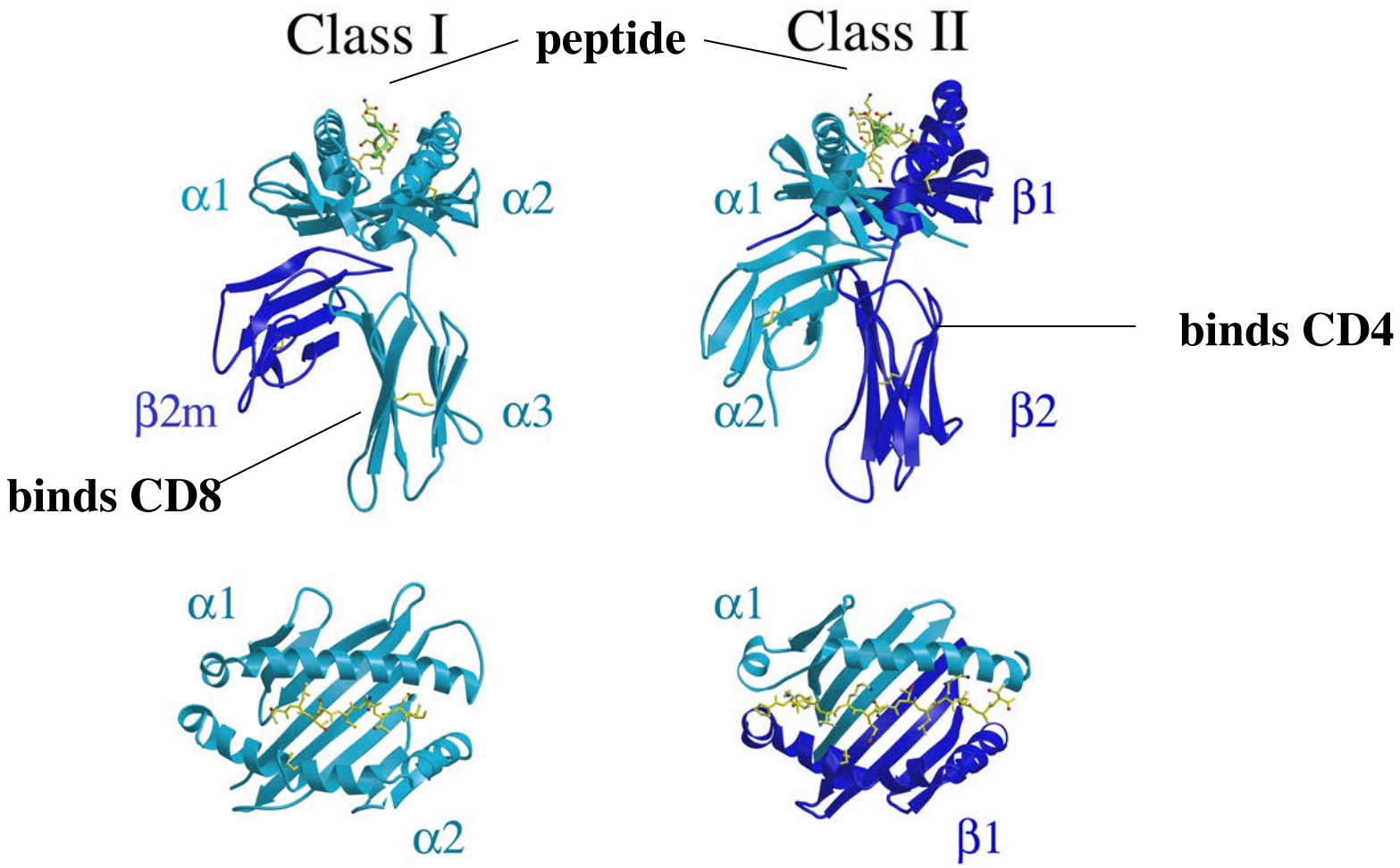


Genes in the MHC locus encode most of the proteins that form the machinery of antigen processing and presentation

Some important properties of MHC molecules

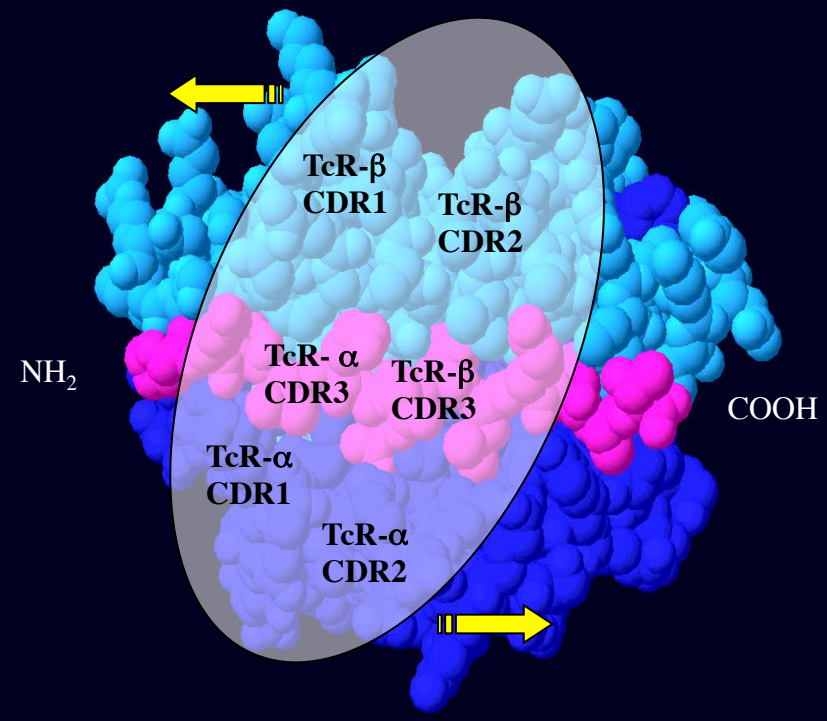
- MHC molecules are the immune system's mechanism for displaying peptide antigens to T lymphocytes:
  - Highly polymorphic genes: large number of alleles in the population
  - Co-dominantly expressed: each cell has six class I molecules (3 from each parent) and 10-20 class II molecules (3 from each parent + some hybrid molecules)
  - Class I MHC molecules are expressed on all nucleated cells
  - Class II MHC molecules are expressed on few cells types (specialized APCs, e.g. dendritic cells; B lymphocytes, macrophages)
  - Stable expression of MHC molecules on cell surfaces requires the peptide cargo
  - MHC molecules present foreign and self peptides
  - Expression of Class II MHC molecules, in particular, is up-regulated by activation of the innate immune response (IFNs, etc.)

MHC Structures



All MHC molecules have a similar basic structure: the cleft at the N-terminal region binds peptide antigens and is recognized by T cell receptors and the membrane-proximal domain binds CD4 or CD8.

The typical "footprint" of T cell receptor (TcR) when bound to MHC-peptide

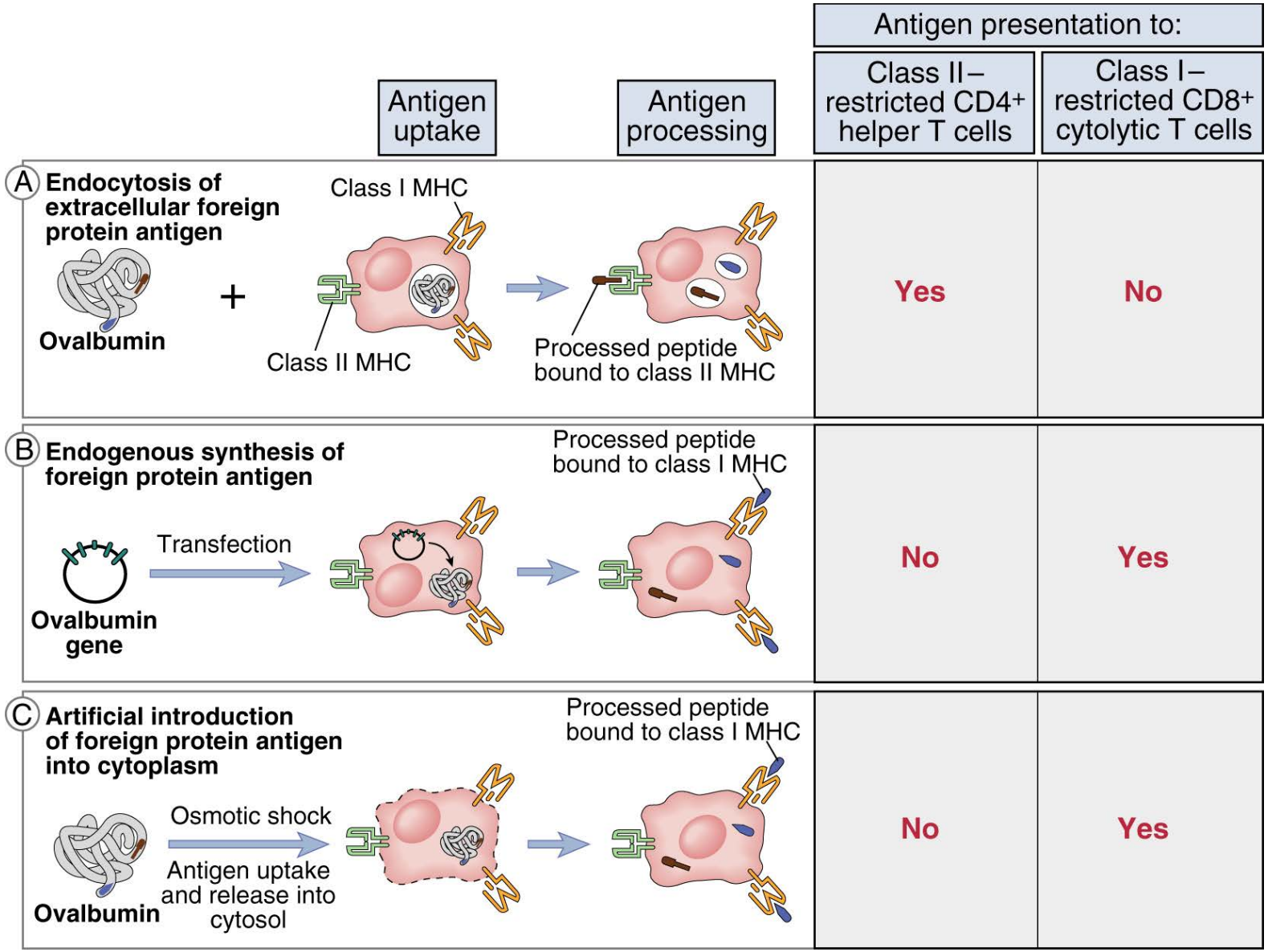


Leddon and Sant, Curr. Opin. Organ Transplant. 2010

Antigen processing

- Conversion of native antigen (large globular protein) into peptides capable of binding to MHC molecules
- Occurs in cellular compartments where MHC molecules are synthesized and assembled
  - Determines how antigen in different cellular compartments generates peptides that are displayed by class I or class II MHC molecules

Presentation of Extracellular and Cytosolic Antigens



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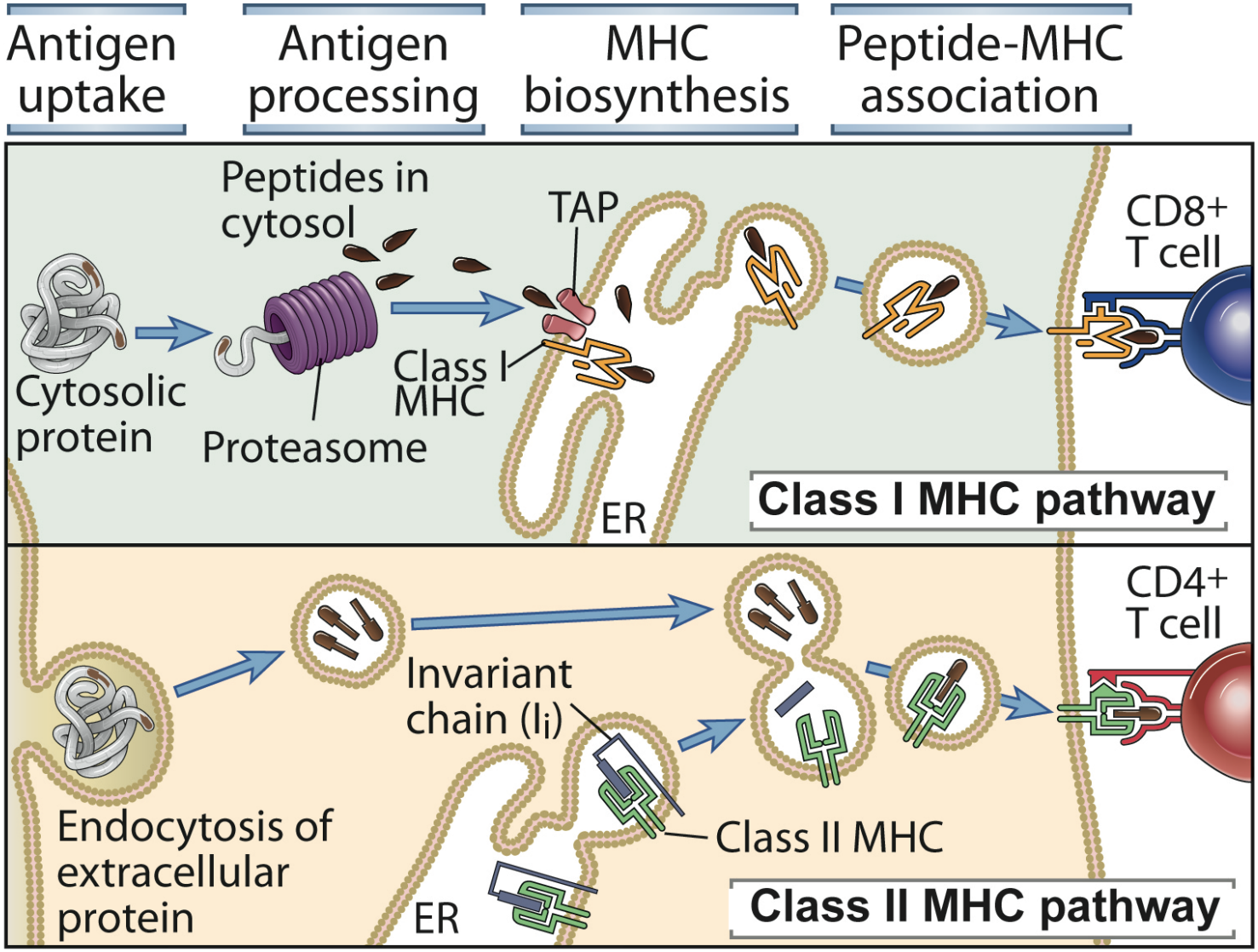
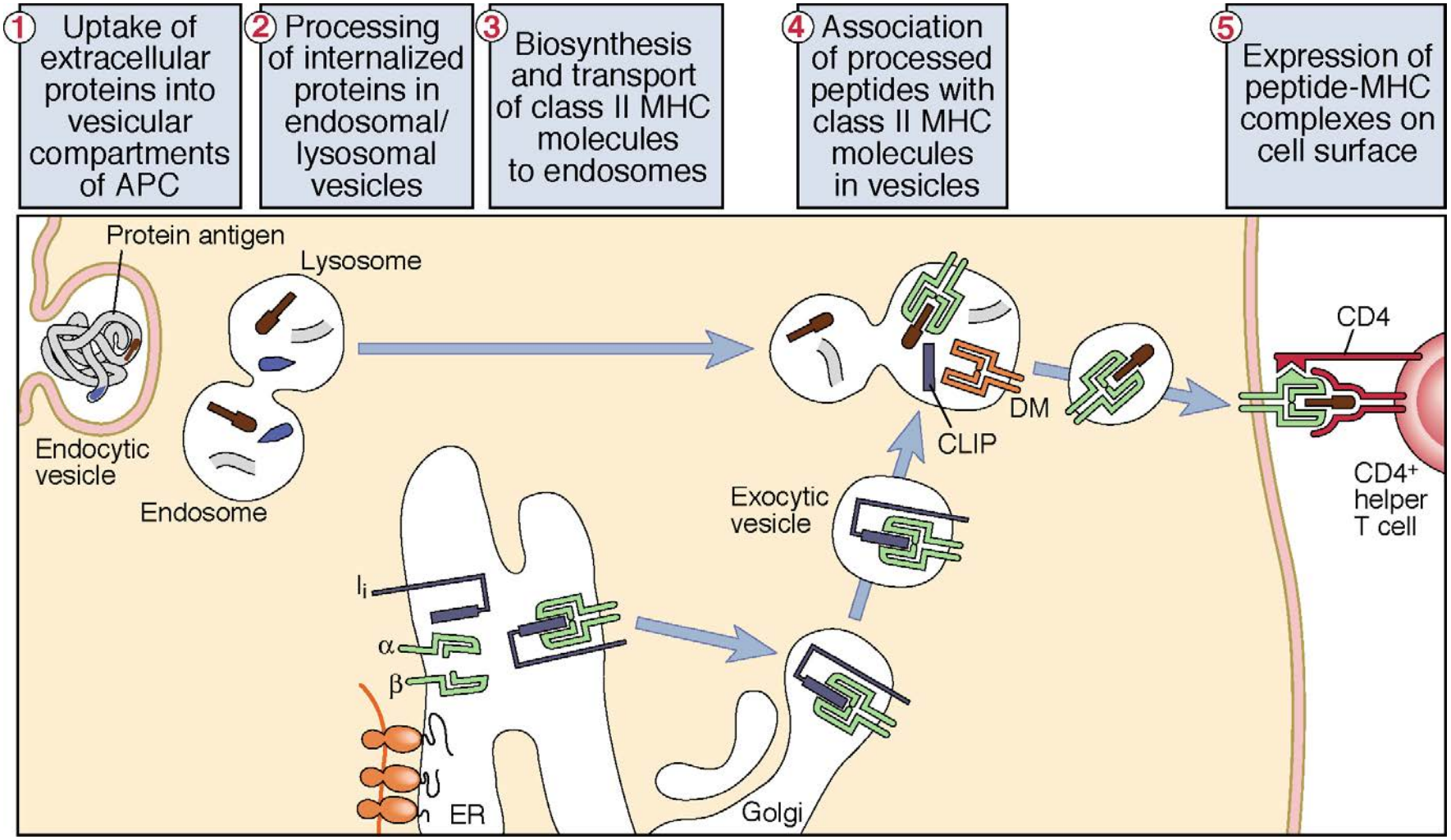
Pathways of antigen processing



Protein antigen in cytosol (cytoplasm) --> class I MHC -- CTLs
Protein antigen in vesicles --> class II MHC --> helper T cells

The class II MHC pathway of processing of internalized vesicular protein antigens



Endocytosed proteins are cleaved into peptides in vesicles; class II MHC molecules are available to bind the peptides in the same vesicles

Class II MHC pathway of presentation of vesicular peptide antigens

- Helper T cells need to help macrophages and B cells that have encountered (and ingested) microbes
- Proteins ingested into endosomes/lysosomes (vesicles) are processed and their peptides are presented in association with class II MHC molecules
- Most vesicular peptides are derived from extracellular proteins that are ingested into vesicles
- Class II MHC is expressed only on specialized cells (e.g. B cells, macrophages) that are capable of ingesting microbes and antigens into vesicles

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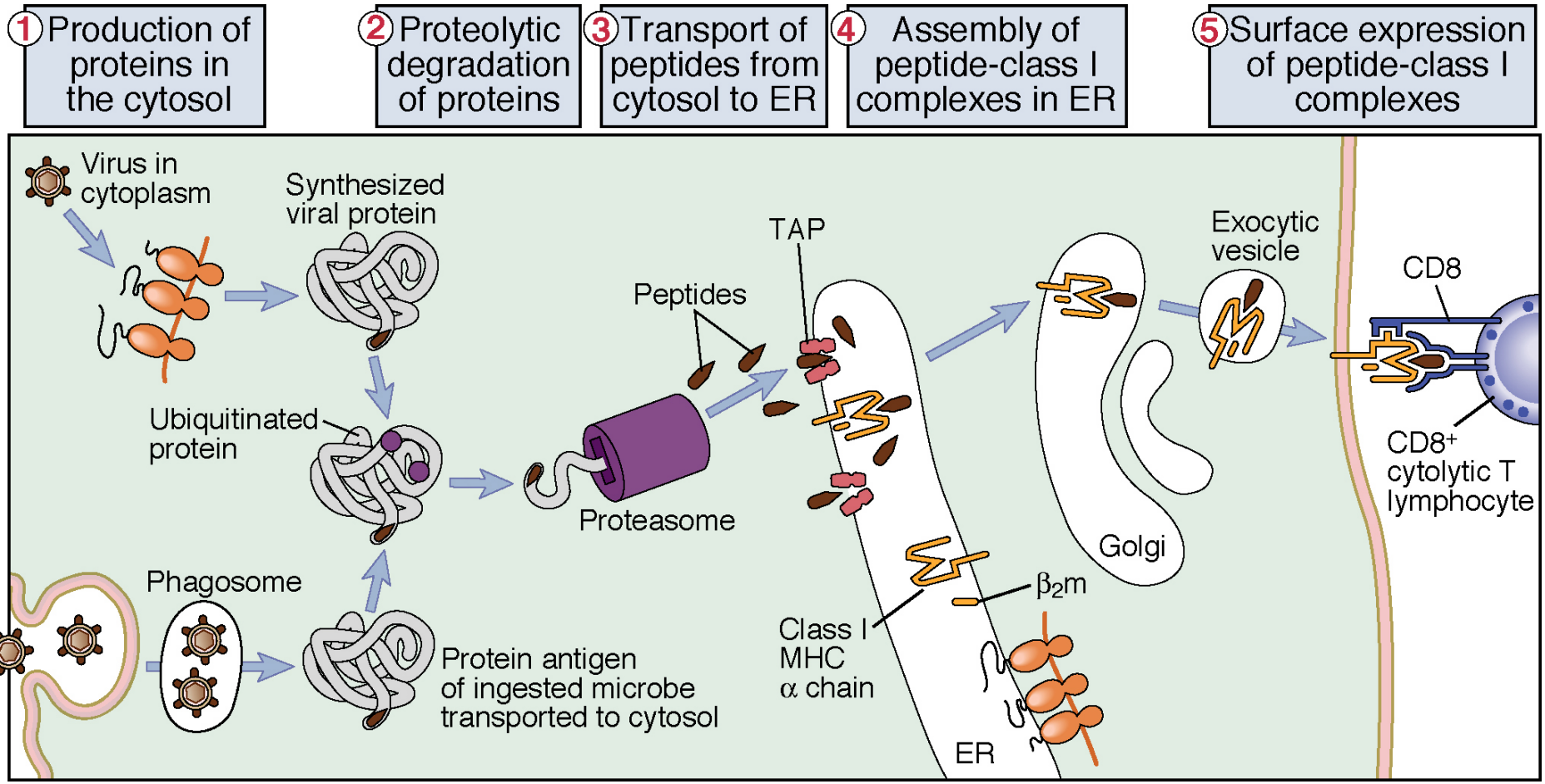
- Class II MHC is expressed only on specialized cells (e.g. B cells, macrophages) that are capable of ingesting microbes and antigens into vesicles
- **CD4 binds to class II MHC; therefore, CD4+ T cells recognize class II-displayed peptides**
- **CD4+ T cells are helper cells that activate B lymphocytes and macrophages**
- **Antibodies (products of activated B cells) and activated macrophages combat extracellular microbes**

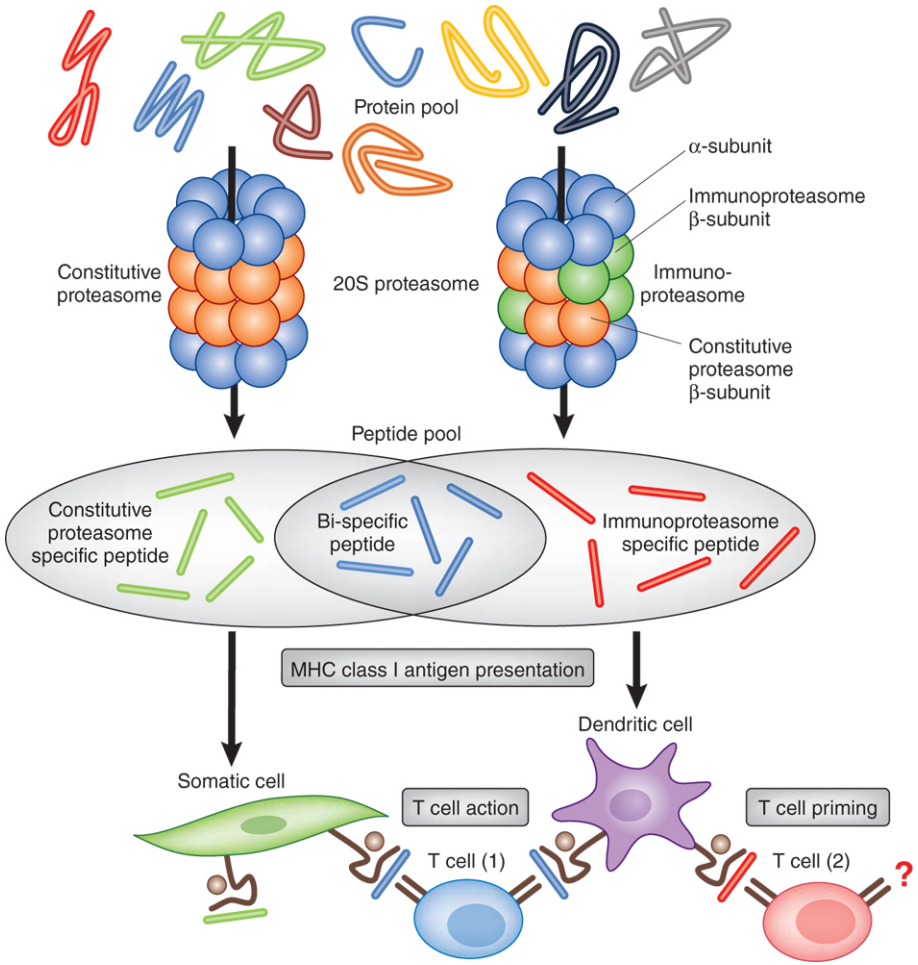
The class I MHC pathway of processing of endogenous cytosolic protein antigens



Cytoplasmic peptides are actively transported into the ER; class I MHC molecules are available to bind peptides in the ER

The Immunoproteasome: Interferon g induces new subunit proteases to alter peptides presented

Spaapen and Neefjes, Nat. Immunol. 2012



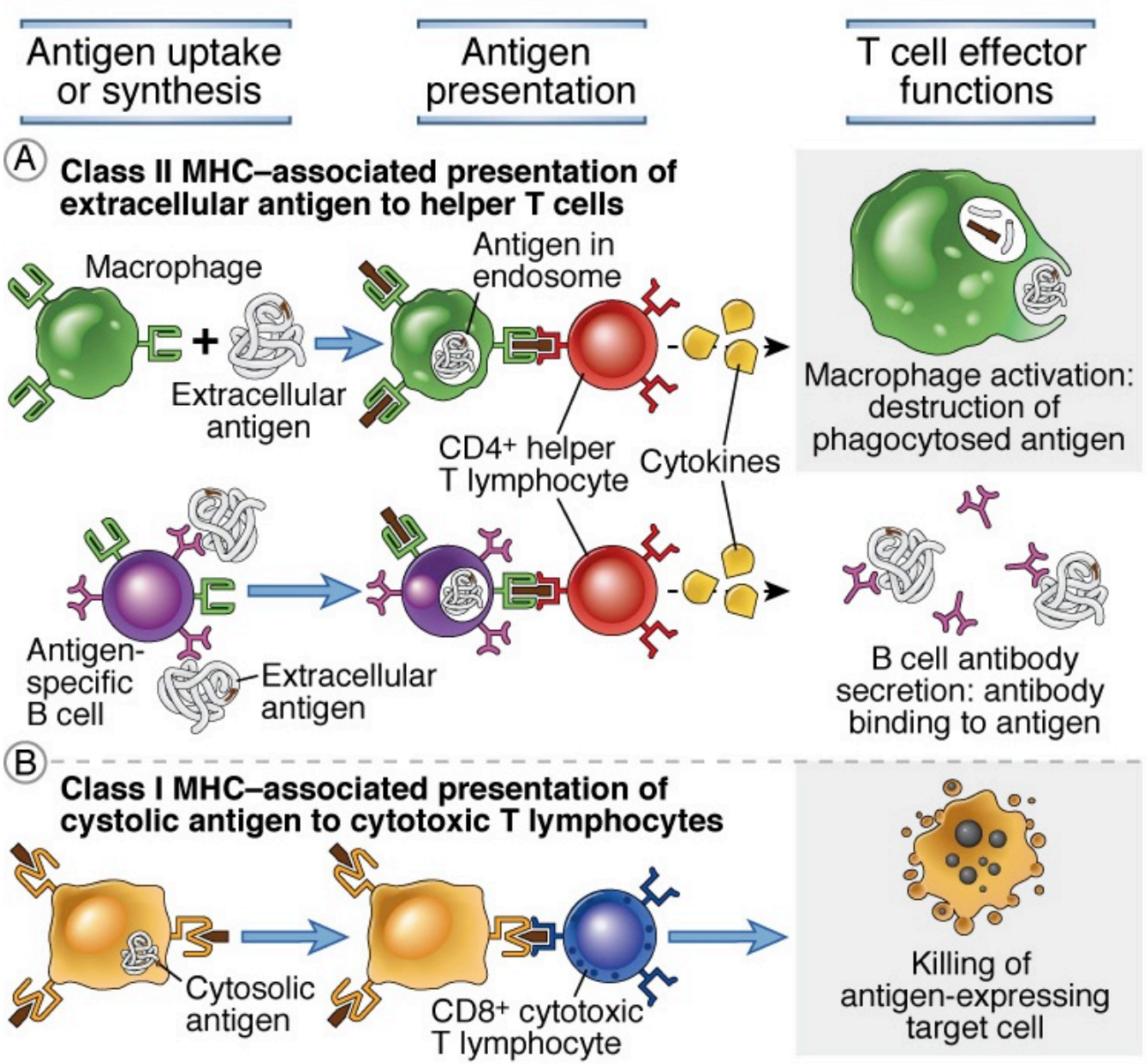
Class I MHC pathway of presentation of cytosolic peptide antigens

- Cytotoxic T lymphocytes need to kill cells containing cytoplasmic microbes, and tumor cells (which contain tumor antigens in the cytoplasm)
- Cytosolic proteins are processed into peptides that are presented in association with class I molecules
- Most cytosolic peptides are derived from endogenously synthesized (e.g. viral, tumor) proteins
- All nucleated cells (which are capable of being infected by viruses or transformed) express class I

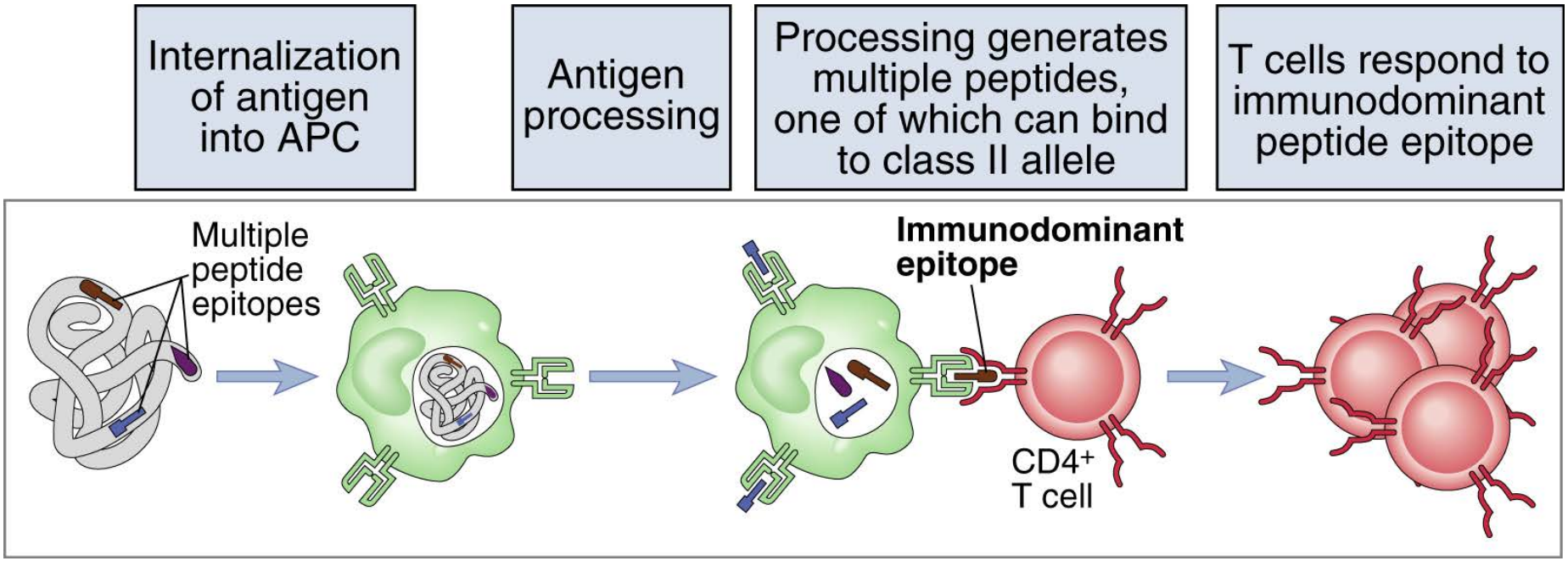
Class I MHC pathway of presentation of cytosolic peptide antigens

- Cytosolic proteins are processed into peptides that are presented in association with class I molecules
- Most cytosolic peptides are derived from endogenously synthesized (e.g. viral, tumor) proteins; all nucleated cells (which are capable of being infected by viruses or transformed) express class I
- **CD8 binds to class I MHC; therefore, CD8+ T cells recognize class I-displayed peptides**
- **CD8+ T cells are cytotoxic cells that kill any nucleated cells that harbor infections (thus eliminating reservoirs of infection) or are transformed**

How class I- and class II-associated antigen presentation influences the nature of the T cell response



Immunodominance of Peptide Epitopes



Abbas & Lichtman. Cellular and Molecular Immunology, 5th ed. W. B. Saunders 2003

"Determinant Selection" - MHC alleles select best binding peptides and thereby select which determinants will be immunogenic in an individual.

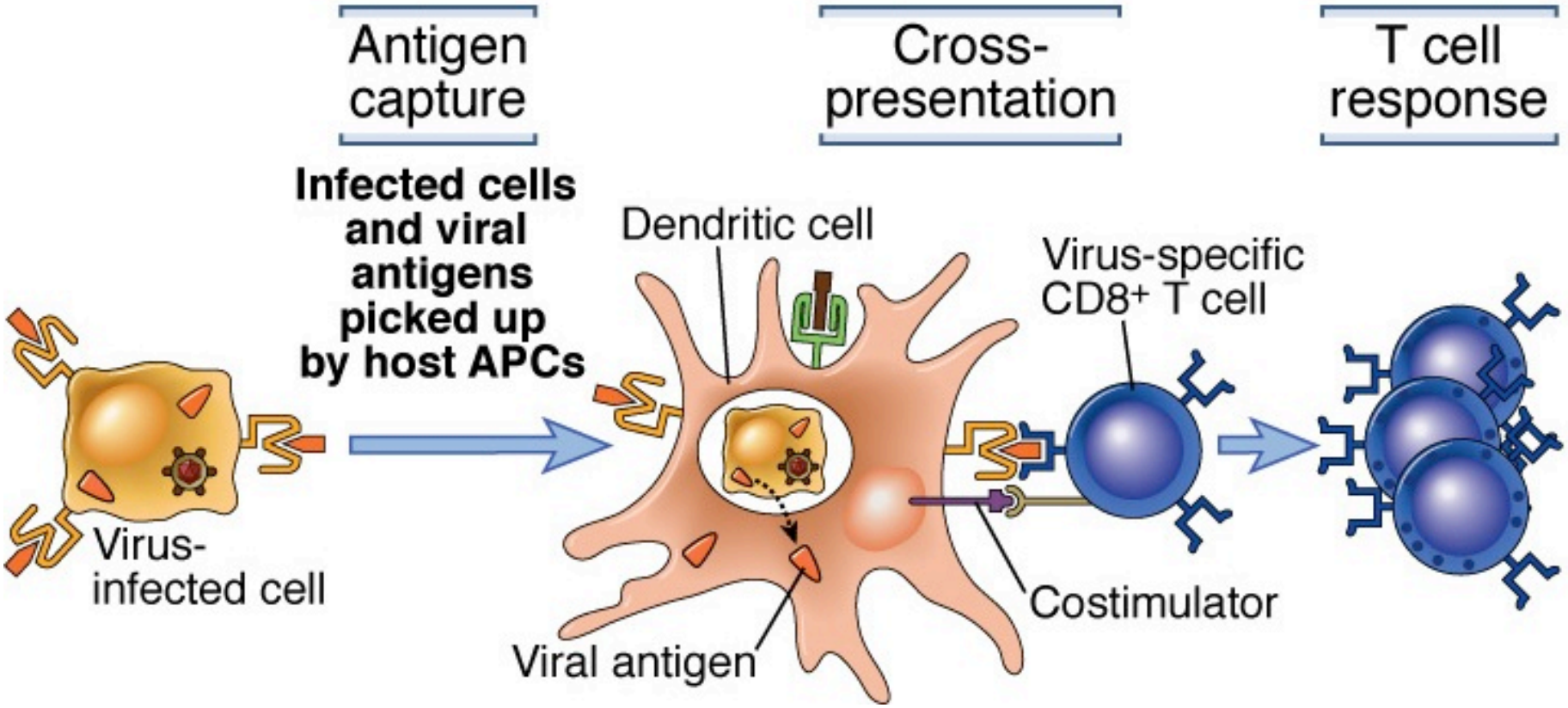
The Problem for CD8 T cells

- Viruses and tumors may be present in any nucleated cells; therefore, the immune system has to be able to generate CTL responses (class I-restricted) to any nucleated cell

- Only some APCs, particularly DCs, are able to initiate the responses of naïve T cells

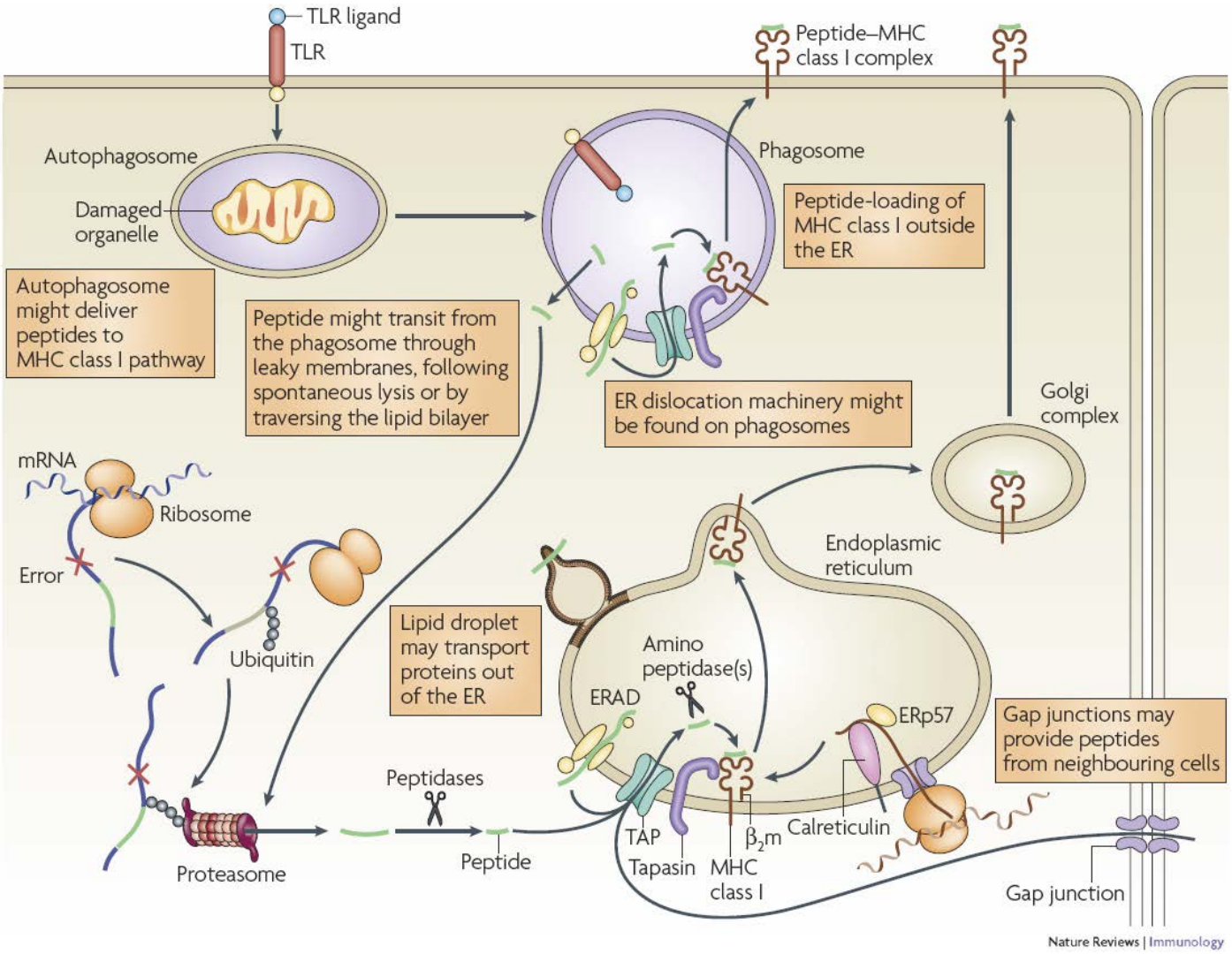
- How are antigens from virus-infected or neoplastic non-APC cell types "transferred" to APCs?

Cross presentation of microbial antigens from infected cells to CD8+ T cells



Antigens of viruses or tumors that are produced in cells other than APCs have to elicit CTL responses, which usually requires DCs; antigen-producing cell is phagocytosed by DCs, and phagocytosed antigen enters the class I pathway (exception to the rule!)

Potential Mechanisms for Cross Presentation



Multiple mechanisms may allow vesicular proteins to get to cytosol or to Class I MHC molecules.

Functions of APCs

- Capture antigens and take them to the "correct" anatomic site
  - Antigens are concentrated in peripheral lymphoid organs, through which naïve lymphocytes circulate
- Display antigens in a form that can be recognized by specific lymphocytes
  - For T cells: MHC-associated peptides (cytosolic peptides to class I, vesicular peptides to class II)
  - For B cells: native antigens
- Provide "second signals" for T cell activation

APCs and self antigens

- Normally, APCs are constantly presenting self antigens
  - MHC molecules do not distinguish self from foreign
- If MHC molecules are bathed in self peptides, how can they ever be free to present microbial peptides?
  - Very few peptides (complexed with MHC) are enough to activate specific T cells
  - Microbes induce "second signals" on APCs

- *If self peptides are always being displayed, why do we not react against our own antigens?*

How do T lymphocytes meet their challenges?

- Very few lymphocytes in the body are specific for any one microbe (or antigen)
- Lymphocytes must be able to locate and respond to microbes that enter anywhere in the body
  - Antigens are transported to and concentrated in the lymphoid organs through which naïve T cells constantly circulate, increasing likelihood of encounter
  - Lymphoid organs, which are specialized to initiate immune responses, drain all tissue sites

How do T lymphocytes meet their challenges?

- Very few lymphocytes in the body are specific for any one microbe (or antigen)
- Lymphocytes must be able to locate and respond to microbes that enter anywhere in the body
- **Lymphocytes must respond to each microbe in ways that are best able to eradicate that microbe**
  - Antigens of endogenous and extracellular microbes are displayed to different subsets of T cells by class I and class II MHC molecules
  - Even the same microbe may be recognized by CD4+ or CD8+ T cells depending on its cellular location