Antigen sampling and presentation

- What is an antigen
- How antigens are sampled when they enter the body
- How do B and T lymphocytes recognize antigens
- How are antigens presented on the cell surface of APCs for recognition by T cells.
- What is the role of MHC and CD1 molecules as antigen display molecules
How are antigens recognized by the immune system?

- Antigen refers to any substance that is recognized by T or B lymphocytes via their cell surface receptors and/or antibodies secreted by B cells.

- Antigens can be:
  - proteins & glycoproteins
  - polysaccharides
  - lipoproteins & lipopolysaccharides
  - nucleic acids
  - chemical compounds (nicotine, heavy metals, narcotics).

- Self antigens refer to all antigens that are normal constituents of the body.

- Non-self antigens refer to all antigens that are not normal constituents of the body.

- Immunogens refer to antigens that are able to stimulate a specific adaptive immune response when introduced into the body. Not all antigens are immunogens.

- To be immunogenic, antigens must fulfill certain criteria

- Hapten are small molecules that need a carrier to be immunogenic.
What is an epitope

- Antigens are generally larger than the binding sites of the B cell or T cell's antigen receptors that specifically recognize and bind them.

- The particular parts of the antigen that are specifically recognized by an antigen receptor is called **epitope** or **antigenic determinant**.

- **B cell epitopes** are either:
  - conformational corresponding to surface exposed regions of native antigen.
  - linear corresponding to surface exposed sequences of the antigen.
  - epitopes that are preferentially recognized within a given individual are called **dominant epitopes**.

- **T cell epitopes** are:
  - peptides, bound to MHC molecules & recognized by $\alpha\beta$ TCR (T cell receptors).
  - lipids or sugars bound to CD1 molecules & recognized by $\gamma\delta$ T cell receptors.

- Native antigens have to be processed into fragments so that internal motifs become accessible to MHC molecules.
Antigen sampling

- Since foreign antigens and microorganisms can enter the body at multiple sites, antigen-sampling cells are strategically located at all the possible sites of antigen entry:
  - skin
  - mucosal surfaces
  - the lymph nodes for antigens that escape sampling in skin or mucosal surfaces and find their way from the tissue spaces to the lymph.
  - in the spleen for antigens that gain access to the blood, for instance following bites by insects

- the resident parenchymal antigen-sampling cells.
  - These cells take up antigen and deliver it intact to underlying APCs.
  - Example: the epithelial M cells concentrated MALT.

- the migratory hematopoietic antigen-sampling cells.
  - These cells take up antigen and transport it to distant lymphoid organs.
  - APCs have competence for antigen processing and presentation
Antigen sampling in skin

- The skin harbors two populations of antigen-sampling cells:
  - **Langerhans cells**, which reside within the epidermis
  - **Dermal dendritic cells**, which reside within the dermis.
Antigen sampling in skin

- a unique population of immature DCs characterized by
  - the presence of large granules
    - Birbek granules
  - E-cadherin
  - langerin
  - CD1a,
  - lack DC-SIGN & mannose receptor
  - recruited from peripheral blood as `DC precursors & differentiate in the epidermis into resident LCs
  - LCs eventually pick up antigens (including apoptotic skin cells) and migrate by the afferent lymphatics to the regional lymph nodes,
Dermal dendritic cells are immature DCs
- express different markers as LCs
- lack Birbeck granules and langerin.
- express DC-SIGN & mannose receptor
- express high levels of DEC-205
Immature DCs occur in all tissues where antigen may enter into the body.

- High capacity for antigen uptake but a limited capacity for antigen presentation.

Immature DCs express a large number of receptors:

- **Chemokine receptors** (CCR1, CCR2, CCR5, CCR6)

- **Endocytic receptors** by which they can capture antigens.
  - Non-opsonic receptors (DC-SIGN, DEC-205, mannose receptor) specific for microbial products.
  - Integrin receptors, which are involved in the uptake of apoptotic bodies.
  - Opsonic receptors: Fc & complement receptors.

- **Sensing receptors** by which they can integrate information about the antigen.
  - TLRs
  - Cytokine receptors

**Antigen sampling cells: Dendritic cells**
Mature DCs are exclusively found in the T cell areas of secondary lymphoid organs.

They are characterized by a high propensity for antigen presentation and T cell activation but a reduced ability to capture antigens.

Mature DCs express the following:

- **Chemokine receptor** CCR7, so that they can leave non lymphoid peripheral tissues and migrate to the T cell areas of the secondary lymphoid organs where ligands for CCR7 are constitutively expressed.
- **Adhesion molecules** (CD48, CD58) required for interacting with T cells.
- **Cell surface MHC class II** molecules required for antigen presentation.
- **Co-stimulatory molecules** (CD80, CD86, CD40) required for activation of reactive T cells.

By contrast, they no longer express endocytic receptors.
Antigen Recognition

- Two main strategies have been developed by the adaptive immune system to recognize antigens:
  - Immunoglobulins (Igs), expressed by the B lymphocytes.
  - T-cell receptors (TCRs), expressed by the T lymphocytes.

- Antigen recognition by B cells clearly differs from antigen recognition by T cells:
  - B cells recognize intact antigens.
  - T cells recognize antigen fragments exposed on the surfaces of host cells by antigen presenting molecules.
Antigen Recognition by B lymphocytes

- **Thymus dependent (TD).**
  - TD antigens refer to antigens against which antigen-specific B cells cannot produce antibodies without the cognate help of antigen-specific CD4+ T cells (CD40/CD40L).
  - Most TD antigens are oligovalent protein antigens that bind specifically to the BCR.

- **Thymus independent type 1 (TI-1).**
  - TI-1 antigens are polyclonal, non-antigen specific activators of B cells that engage co-stimulatory receptors on B cells but not the BCRs.
  - Prototype: lipopolysaccharide (LPS) from gram-negative bacterial cell wall which binds to TLR4..

- **Thymus independent type 2 (TI-2).**
  - TI-2 antigens are high Mr molecules with repetitive epitopes that cause extensive cross linking of BCRs and stimulate B cell proliferation and differentiation without the cognate help of T lymphocytes.
  - Prototype high molecular weight polysaccharide such as the dextran B512 (Dx), LPS.
Antigen Recognition by T lymphocytes

- T cells detect antigens via T-cell receptors (TCRs) that recognize antigen when presented as short fragments bound to antigen-presenting molecules on the surface of antigen-presenting cells (APCs).

- T cells exist as two main populations which have their own antigen recognition strategy.
  - T cells bearing αβ TCRs recognize peptides of protein antigens in association with MHC molecules
    - CD4⁺ T helper cells recognize peptides of 10-20 amino acids presented by MHC class II molecules
    - CD8⁺ T cells recognize peptides of 8 to 10 amino acids presented by MHC class I molecule.
  - T cells bearing γδ TCRs recognize small phosphorylated molecules and lipidic bacterial antigens bound to evolutionary conserved and nonpolymorphic class I-like antigen-presenting molecules known as CD1 molecules.

[Diagram showing antigen presentation and T-cell recognition]
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Antigen Presentation

- **What is antigen presentation?**
  - Antigen presentation is the process by which cells display antigen in the form of short fragments bound to antigen-presenting molecules on their cell surface for recognition by T lymphocytes.

- **Antigen presentation involves two broad steps:**
  - **antigen processing**: the intracellular degradation of antigens.
  - **intracellular loading** of resulting antigen fragments onto intracellular MHC molecules followed by the transport and display of these complexes to the cell surface.

- **What is antigen presentation for?**
  - Antigen presentation is required for:
    - initiation of T cell-mediated immune responses.
    - induction of T cell tolerance.
    - triggering of T cell effector functions.
  - The outcome of antigen presentation is determined by:
    - the nature of the antigen
    - the type of cells presenting the antigen,
    - the antigen-presenting molecules these cells express
    - the origin of antigen to be presented.
Antigen Presentation: The molecules

- Antigen presenting molecules are glycoproteins which display antigens on the surface of cells for recognition by T cells.

- MHC molecules have specifically evolved to bind breakdown products of protein antigens. They comprise two main types of molecules.
  - MHC class I molecules are expressed on almost all cells and present peptides to MHC-class I restricted CD8+ T cells.
  - MHC class II molecules are normally expressed on a limited number of cells (dendritic cells, B cells, macrophages) and present peptides to MHC-class II restricted CD4+ T cells.

- CD1 molecules are expressed on a limited number of cell types and have specifically evolved to bind breakdown products of lipid and glycolipid antigens.
Antigen Presentation: The molecules

- The HLA complex is located on the short arm of chromosome 6 and covers almost 4 Mb.
  - It is the most gene-dense region and contains over 220 identified genes, of which: more than 40 encode MHC molecules.
  - About half are MHC-unrelated genes with immunological functions.
  - Many pseudogenes or genes with unknown function.

- The human class I region spans 2 Mb. It contains:
  - HLA-A coding for the α chain of "classical" MHC I molecules
  - HLA-B
  - HLA-C
  - A number of class Ib coding for the α chain of "non-classical" MHC class I molecules.
  - Pseudogenes such as HLA-H, -J, -K and -L,
  - MHC-unrelated genes with unknown functions.
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The human class II region spans ~ 750 kb. It contains:
- Three groups of "classical" class II genes, called HLA-DR, -DP and -DQ.
- Two pairs of "nonclassical" class II genes, called HLA-DM and HLA-DO.
- A series of genes that play major role into the class I antigen presentation pathway.
- Pseudogenes such as DPA2, DPB2, DQA2 and DQB2 genes,
- A dense cluster of non-immune genes located at its centromeric end and termed the extended class II region.
the two outermost domains (α1 and α2 in MHC class I; α1 and β1 in MHC class II) are MHC-unique domains, each composed of:
- four-stranded β sheet
- a single α-helix

- MHC domains fold together to form a narrow groove or cleft consisting of an eight-stranded β-pleated sheet topped by two α helices, oriented roughly parallel to each other.
- This groove forms the peptide binding site where peptides are bound and presented to T cells.
- In both MHC classes, the peptide binding "MHC superdomain" is supported by a pair of immunoglobulin-like domains, corresponding to α3 and β2m in MHC class I and α2 and β2 in MHC class II.
Antigen Presentation: The molecules

- MHC expression is co-dominant

- MHC class I
  - consist of an \(\alpha\) chain and \(\beta_2\) microglobulin
  - Up to 6 different MHC class I molecules can be expressed on the same cell

- MHC class II
  - consist of an \(\alpha\) and a \(\beta\) chain
  - \(\alpha\) and \(\beta\) chain can pair either in cis (both from the same chromosome) or in trans (one from each chromosome) association.
Which cells present antigen?

- All cells except red blood cells express MHC class I molecules, can present antigens and can be considered as antigen-presenting cells.

- One restricts however the term antigen presenting cells to those cells that play a role in antibody responses.

- **Professional antigen-presenting cells** refer to cells that
  - present antigens at their cell surface for initiating primary T cell responses.
  - deliver a costimulatory signal, a necessary condition for activating naïve CD4+ T cells and CD8+ T cells and promoting their differentiation into effector T cells.
  - Dendritic cells (DCs) are the best professional APCs, since they are the only APCs able to present antigens to naïve T cells.

- **Non-professional antigen-presenting cells** refer to cells that do occasionally present antigens along with costimulatory molecules, but whose primary function is different.

- **Target cells** refer to cells that present antigens in the context of MHC class I molecules in order to activate cytotoxic effector T cells and eventually be killed by them.
  - infected or malignant cells.
What is required?

- reside in or able to enter the T cell areas of lymphoid organs where naive CD4 T cells are confined
- are endocytically active in order to acquire exogenous antigens
- express MHC class II molecules (signal 1)
- express co-stimulatory molecules to activate naive CD4 T cells (signal 2)
DCs polarize the T helper cell response

Microbes

- Microbes
- Tissue factors

**Microbes**

- IFN-γ
- IFN-α
- IL-18

**Tissue factors**

- PGE2
- Histamine
- TSLP (thymic stromal lymphopoietin)
- IL-10
- TGF-β

**Th1**

- IFN-γ
- TNF-β

**Th2**

- IL-4
- IL-5
- IL-13

**Th3/Treg**

- TGF-β
- IL-10
How do cells present antigen?

- Cells have evolved three pathways for presenting antigen.
  - The **endogenous pathway** allows cells to present endogenously synthesized proteins on MHC class I molecules for recognition by CD8$^+$ T cells.
  - The **exogenous pathway** allows cells to present internalized exogenous antigens on MHC class II molecules for recognition by CD4$^+$ T cells.
  - The **cross pathway** allows cells to present exogenous antigens on MHC class I molecules for recognition by CD8$^+$ T cells.
The MHC I presentation pathway

- MHC class I biosynthesis starts by the separate translation of the α and β2m chains in the RER.

- Newly synthesized class I heavy chains associate with calnexin, an ER transmembrane chaperone protein that protects and stabilizes partly folded heavy chains until they assemble with β2m.

- When β2m binds to the heavy chain, calnexin is replaced with the "peptide-loading complex" including:
  - calreticulin
  - ERp57
  - tapasin
The MHC II presentation pathway

- **The endocytic protein-processing pathway**
  - Peptides that bind MHC class II molecules are generated in the endocytic pathway. Following internalization, the antigen is enclosed in an endosome that converts to an early endosome, and then to a late endosome, in which the antigen unfolds due to the low pH.
  - Their fusion with lysosomes creates a highly degradative environment which allows the denaturation and processing of endocytosed antigens into short antigenic peptides.

- **The biosynthetic pathway of MHC class II molecules**
  - MHC II molecules are made up of two transmembrane chains (α and β) synthesized and assembled in the ER with a third protein called the invariant chain (li). The li chain prevents the loading of peptides on class II molecules.
  - The αβ/li trimeric complexes are transported from the ER to the Golgi complex and then sent to the endocytic compartments. In these compartments, li is degraded, leaving MHC II molecules free to acquire peptide from endocytosed antigens.
The cross presentation pathway

- The cross presentation pathway is the process by which DCs present internalized exogenous antigens on MHC class I molecules for recognition by CD8+ T cells.
- It allows these cells to generate cytotoxic CD8+ T cells against virus, when DCs are not the target of virus and thus do not express viral antigens.
- The cross-presentation pathway requires the encounter between:
  - MHC I molecules generated in the endoplasmic reticulum (ER)
  - Peptides derived from in endocytosed antigens.
- This pathway requires
  - exogenous antigens to be re-routed for loading on MHC I molecules, despite their initial sequestration in the endocytic compartments.
MHC are antigen-presenting molecules that present protein antigens to T lymphocytes.

- MHC class I molecules present peptides (from cytosolic proteins) to CD8⁺ T cells.
- MHC class II molecules present peptides (from endocytosed proteins) to CD4⁺ T cells.

In any individual, the set of MHC molecules are sufficient to alert T cells to all possible infection.

- **MHC polygeny**: more than one MHC molecule of each class are expressed in cells
- **MHC polymorphism**: each MHC molecule exist as multiple variants so in heterozygote individuals, each MHC molecule exist as two variants.
- **Binding properties of MHC molecules**: each MHC variant is capable of binding a diverse set of peptides.

The MHC I or endogenous pathway allows cells to exhibit a representative fraction of their cytosolic content for inspection by MHC I-restricted cytotoxic CD8⁺ T cells.

- Cytosolic antigens are degraded by proteasome. Peptides are transported to the ER by TAP.
- MHC I molecules acquire peptides in the ER while they are synthesized.

The cross-presentation pathway is done by DCs. It allows DCs to initiate CD8⁺ T cell responses against viruses that infect cells other than DCs.

The MHC II or exogenous pathway allows DCs, B cells and macrophages to exhibit a representative fraction of their extracellular environment for inspection by MHC II-restricted helper CD4⁺ T cells.

- Endocytosed antigens are degraded along the endocytic pathway
- MHC II molecules acquire peptides in lysosome/late endosome on their way to the plasma membrane.
Learning objectives

- Explain the differences between professional and non-professional APCs
- Describe how antigens are sampled in the skin, at mucosal surfaces and from blood
- Describe how antigens are processed for presentation to helper CD4 T lymphocytes
- Describe how antigens are processed for presentation to cytotoxic CD8 T lymphocytes
- Explain the differences between the MHC class I and class II pathways.
- Identify the signals that up regulate the co-stimulatory molecules on professional APCs
- Describe the properties of the different dendritic cell sub-types.
- Describe the properties of immature and mature dendritic cells.