

Lecture 1.1 Antigen Processing and Presentation Pathways

1. Overview of Antigen Presentation Pathways

Core Principles

- **CD8+ T Cells:** Express the CD8 co-receptor and recognize antigenic peptides presented by **MHC Class I** molecules.
- **CD4+ T Cells:** Express the CD4 co-receptor and recognize antigenic peptides presented by **MHC Class II** molecules.
- **Origin of Antigens:**
 - **MHC Class I:** Presents peptides derived from endogenous, **cytosolic proteins** (providing a real-time snapshot of the intracellular proteome).
 - **MHC Class II:** Presents peptides derived from **exogenous proteins** captured and internalized into the endosomal/lysosomal compartment.
- **Cross-Presentation:** The functional exception where specialized antigen-presenting cells (APCs), notably dendritic cells (DCs), route internalized exogenous antigens into the cytosolic MHC Class I pathway to prime CD8+ T cells.
- **Constitutive Presentation:**
 - All nucleated cells continuously express MHC Class I loaded with endogenous self-peptides, maintaining immune tolerance under healthy baseline conditions.
 - Infection or malignant transformation alters the peptide repertoire, generating foreign or altered-self peptides that activate CD8+ T cells.

2. The Ubiquitin-Proteasome System (UPS)

Cytosolic protein degradation relies on the coordinated action of the ubiquitin-proteasome system.

Ubiquitination Cascade

Ubiquitin is a small, highly conserved 76-amino-acid regulatory protein present ubiquitously in the eukaryotic cytosol. Conjugation requires three enzymatic steps:

1. **E1 (Ubiquitin-Activating Enzyme):** Uses ATP to activate ubiquitin, forming a high-energy thioester bond.
2. **E2 (Ubiquitin-Conjugating Enzyme):** Acts as a transient carrier, accepting activated ubiquitin from E1.
3. **E3 (Ubiquitin Ligase):** Imparts substrate specificity. With over 600 distinct E3 ligases in the human genome, each binds specific target proteins and facilitates the transfer of ubiquitin from E2 to a target lysine residue via its C-terminal glycine.

Lecture 1.2 Immunological Tolerance

1. Principles and Conceptual Overview of Tolerance

A. Definition and Scope

- **Immunological Tolerance:** The state of unresponsiveness of the immune system to specific antigens to prevent host tissue pathology and maintain physiological homeostasis.
- **Self-Tolerance vs. Environmental Tolerance:** Self-tolerance eliminates or silences lymphocytes recognizing endogenous self-antigens. Environmental/mucosal tolerance suppresses harmful inflammatory reactions against non-pathogenic foreign antigens (e.g., dietary proteins, commensal microbiota, and allogeneic fetal tissues).
- **Multi-Layered Defense:** Tolerance is organized in serial anatomical barriers. Central tolerance acts first during lymphocyte ontogeny in primary lymphoid organs; lymphocytes that escape are subjected to peripheral tolerance mechanisms that restrain activation and effector function in secondary lymphoid organs and non-lymphoid tissues.

B. Central vs. Peripheral Tolerance

- **Central Tolerance:** Occurs within primary generative lymphoid organs (bone marrow for B cells, thymus for T cells) during early differentiation stages.
- **Peripheral Tolerance:** Operates in the periphery after mature or immature lymphocytes migrate from primary lymphoid organs into blood, lymph nodes, spleen, and mucosal tissues.

2. Central B Cell Tolerance Mechanisms

During assembly and testing of the B-cell receptor (BCR) in the bone marrow, developing immature B cells encounter self-antigens in their microenvironment:

- **Clonal Deletion:** High-affinity cross-linking of BCRs by multivalent, membrane-bound self-antigens triggers apoptotic elimination mediated by pro-apoptotic Bcl-2 family members such as Bim.
- **Receptor Editing:** When an immature B cell binds strongly to a multivalent self-antigen, it can maintain the expression of RAG-1 and RAG-2 recombinases. This allows secondary recombination events at light-chain gene loci (first κ , then λ) to alter antigen specificity away from self-reactivity before central exit.
- **Anergy:** Exposure to soluble, monomeric, or low-valency self-antigens that produce weak BCR cross-linking induces anergy—a biochemically unresponsive state marked by downregulated surface IgM and impaired downstream activation.
- **Clonal Ignorance:** B cells that recognize self-antigens present at very low concentrations or sequestered from the bone marrow pool ignore the antigen, exiting into the periphery functionally naive.

B. Developmental Fates in the Thymus

- **Negative Selection (Clonal Deletion):** High-affinity interaction between a TCR and self-peptide:MHC complexes triggers Bim-dependent apoptosis.
- **Thymic Regulatory T Cell (tTreg) Generation:** A subset of CD4⁺ thymocytes recognizing self-peptide:MHC with intermediate-to-high affinity upregulates the lineage-defining transcription factor FOXP3, committing to an immunosuppressive regulatory phenotype rather than undergoing deletion.

4. Peripheral Tolerance Mechanisms

Immature or mature lymphocytes that escape central tolerance encounter multiple peripheral checkpoints:

A. Peripheral Deletion

- **B Cells:** Immature B cells entering the spleen require B-cell activating factor (BAFF) from follicular dendritic cells. Anergic or self-reactive B cells compete poorly for limited BAFF and undergo apoptosis. Additionally, B cells that acquire de novo autoreactivity through somatic hypermutation in germinal centers die by neglect if they fail to receive cognate T-follicular helper (T_{FH}) survival signals.
- **T Cells:** Peripheral T cells encountering high-affinity self-antigen without appropriate inflammatory context undergo activation-induced cell death via intrinsic (mitochondrial Bim/Bcl-2) or extrinsic (Fas/FasL) apoptotic pathways.

B. Regulatory T Cells (Tregs) and Diversion

- **Peripherally Induced Tregs (pTregs):** Naive CD4⁺ T cells stimulated by sub-immunogenic antigen concentrations in the presence of transforming growth factor-beta ($TGF-\beta$), retinoic acid, and short-chain fatty acids (SCFAs) upregulate FOXP3.
- **Functional Roles:** Essential for mucosal homeostasis in the gut, maintenance of the maternal-fetal interface during pregnancy, and resolution of inflammatory responses.
- **IPEX Syndrome:** Hemizygous mutations in *FOXP3* lead to Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, a severe, systemic autoimmune syndrome manifesting in infancy.

C. Anergy vs. T-Cell Exhaustion

- **Anergy:** A biochemically uncoupled state induced when a naive T cell receives TCR engagement (**Signal 1**) in the absence of CD28-mediated co-stimulation (**Signal 2**, via CD80/CD86 on mature APCs).
 - Results in defective IL-2 gene transcription, blocked cell-cycle entry, and metabolic quiescence.
 - Can be overcome if strong inflammatory signals subsequently upregulate co-stimulatory ligands on local APCs.

B. Anatomy of the Maternal-Fetal Interface

- **Placenta:** A transient organ derived entirely from fetal embryonic tissue. All placental cells express the fetal genome, containing 50% non-maternal (paternal) genetic material.
- **Decidua:** The specialized, hormonally modified mucosal lining of the pregnant uterus derived exclusively from maternal tissue.
- **Trophoblasts:** The primary epithelial cells of the placenta. Extravillous trophoblasts (EVTs) physically invade the maternal decidua to anchor the placenta and interact with maternal cells.
- **Decidual Leukocyte Composition:** Single-cell RNA sequencing (scRNA-seq) demonstrates that maternal decidual natural killer (dNK) cells represent a predominant, transcriptionally distinct immune population at the implantation site.

C. Spiral Artery Remodeling

- **Physiological Goal:** In the non-pregnant uterus, spiral arteries are narrow, high-resistance vessels. During early pregnancy, these vessels must transform into wide, dilated, low-resistance conduits to ensure adequate blood flow and nutrient-waste exchange.
- **Mechanism of Action:**
 - Maternal dNK cells home to and surround the decidual spiral arteries.
 - dNK cells secrete pro-angiogenic factors (such as angiopoietins) and interferon-gamma (IFN- γ).
 - Secreted factors degrade the vascular smooth muscle layer and remodel the surrounding extracellular matrix (ECM), allowing the arterial lumen to widen.
 - In vitro co-culture assays show that EVT alone do not degrade vascular smooth muscle; dNK cell activation by EVTs is required to release the necessary matrix-degrading soluble mediators.

D. Pathological Consequences of Vascular Dysregulation

- **Shallow Trophoblast Invasion and Under-Remodeling:**
 - *Etiology:* Insufficient EVT invasion and inadequate dNK activation leave spiral arteries narrow and constricted.
 - *Clinical Manifestations:* Reduced placental perfusion causes **Fetal Growth Restriction (FGR)** (infant small for gestational age) and maternal **Preeclampsia** (a systemic syndrome characterized by gestational hypertension, proteinuria, and endothelial damage that may necessitate preterm delivery).
- **Excessive Trophoblast Invasion (Placenta Accreta):**
 - *Etiology:* Unrestrained EVT invasion penetrates beyond the decidua into the deep muscular myometrium.
 - *Clinical Manifestations:* Hyper-vascularization leads to elevated fetal birth weight; upon delivery, the placenta cannot detach cleanly, causing catastrophic postpartum hemorrhage that often requires emergency hysterectomy.

5. Comparative Paradigm: Transplantation vs. Pregnancy

Feature	Solid Organ Allograft	Successful Human Pregnancy
Allogeneic Antigen Source	Genetically disparate donor tissue	Semi-allogeneic fetus (50% paternal genome)
Evolutionary History	Artificial, modern medical intervention	Conserved, selected biological process
Classical MHC Expression	Donor organ expresses classical HLA-A and HLA-B	Fetal extravillous trophoblasts lack HLA-A and HLA-B
Predominant Allorecognition	Direct and indirect allorecognition pathways	Exclusively indirect allorecognition
Local Checkpoint Regulation	Requires lifelong synthetic immunosuppression	Endogenous placental PD-L1 expression and decidual Tregs
Clinical Outcome	Inevitable rejection without drug maintenance	Complete gestation without foreign graft rejection

6. Hematopoietic Stem Cell Transplantation (HSCT) and Graft-versus-Host Disease (GvHD)

- **Pathophysiological Inversion:** Unlike solid organ transplantation (where the host immune system attacks a donor graft), allogeneic HSCT introduces a functional donor immune system into an immunocompromised recipient.
- **Graft-versus-Host Disease (GvHD):** Mature donor T cells within the stem cell graft recognize recipient tissues as foreign, initiating a systemic attack against host organs.
 - *Primary Target Organs:* Epithelial tissues of the skin (maculopapular rash, desquamation), gastrointestinal tract (mucosal ulceration, severe diarrhea), and liver (hyperbilirubinemia, cholestatic jaundice).
- **Clinical Imperative for HLA Matching:** Meticulous, high-resolution HLA matching is mandatory in allogeneic HSCT to prevent fatal GvHD.
- **Pre-Transplant Mixed Lymphocyte Reaction (MLR):** An in vitro functional compatibility assay where donor T cells are cultured with recipient APCs. Proliferation and cytotoxicity of donor T cells in vitro indicate high in vivo risk for GvHD, precluding transplantation.

Lecture 4.1: Vaccine Immunology, Design Principles, and Translational Challenges

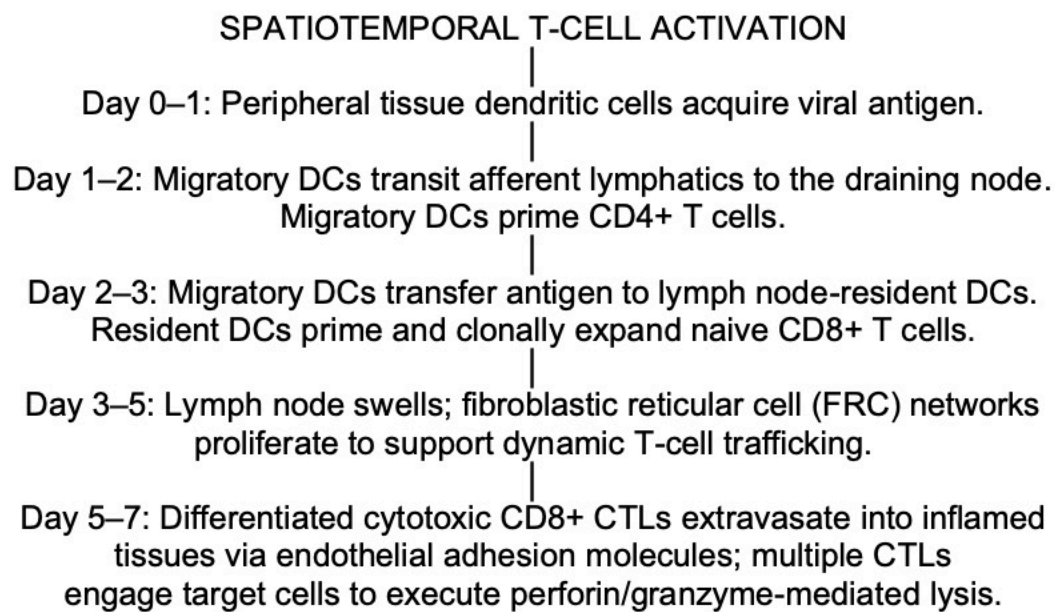
1. Epidemiological Concepts and Herd Immunity

- **Global Burden and Inequity:** Infectious diseases disproportionately impact low- and middle-income regions, notably Sub-Saharan Africa. Uncontrolled transmission can drive exponential case growth within naive populations.
- **Basic Reproduction Number (R_0):**
 - *Definition:* The average number of secondary infectious cases generated by a single index case in a completely susceptible population.
 - *Clinical Utility:* Quantifies contagiousness, guides public health resource distribution, and determines the target vaccination coverage needed to halt transmission.
- **Herd Immunity Threshold (H_I):**
 - *Mathematical Derivation:* $H_I = 1 - \frac{1}{R_0}$
 - *Pathogen Comparisons:*
 - **Influenza:** Relatively low R_0 (~1.3–1.8), requiring lower herd immunity thresholds (~30–45%).
 - **SARS-CoV-2 (Ancestral):** $R_0 \approx 3$, setting an initial herd immunity threshold of approximately 67%. (Without immunity, an infection fatality rate of ~0.2% would translate to roughly 30 million deaths globally).
 - **Measles:** Exceptionally high R_0 (~12–18) due to airborne stability (aerosols remain infectious in enclosed airspaces for up to 2 hours), requiring a herd immunity threshold of $\geq 95\%$. Declines in vaccine coverage rapidly precipitate community outbreaks.
- **Community-Wide Benefits:** Vaccination establishes population-level protection for individuals who cannot mount protective responses or cannot be vaccinated, including neonates under 6 months, patients undergoing cytotoxic cancer chemotherapy, and individuals with primary immunodeficiencies.

2. Paradigm Shifts in Vaccine Design: Empirical vs. Rational Approaches

- **Empirical Vaccine Design (Historical Jenner/Pasteur Era):**
 - Relied on isolating the whole pathogen, weakening it via serial passaging (attenuation), killing it chemically/thermally, or purifying simple secreted proteins (e.g., formaldehyde-inactivated tetanus toxoid).
- **Rational Vaccine Design (Modern Era):**
 - Incorporates structural biology, recombinant DNA technology, bioinformatic modeling, targeted adjuvant formulations, and stabilized conformational antigen engineering.
 - Focuses on presenting structurally intact, neutralizing epitopes rather than crude pathogen preparations.

8. Adaptive Antiviral Immunity: Spatiotemporal Coordination



A. Dendritic Cell Division of Labor

- **Antigen Hand-Off in Lymph Nodes:**
 - Tissue-migratory dendritic cells that encounter viral antigens in infected skin or mucosal tissues migrate via afferent lymphatics into the draining lymph node to directly prime naive **CD4+ helper T cells**.
 - These migratory DCs transfer processed viral antigens to **lymph node-resident dendritic cells** via cross-presentation, which then prime naive **CD8+ cytotoxic T cells**.

B. CD4+ T-Cell Help and CD8+ T-Cell Memory

- While some high-affinity primary CD8+ T-cell responses can expand without CD4+ helper T cells, CD4+ T-cell help is required to generate robust, durable **memory CD8+ T cells**.
- *Signaling Circuit:* CD4+ T cells express **CD40 Ligand (CD40L)**, which engages **CD40** on dendritic cells, "licensing" the DC. Licensed DCs upregulate costimulatory ligands (e.g., CD70) that bind coreceptors such as **CD27** on CD8+ T cells, ensuring long-term survival, self-renewal capacity, and effective recall responsiveness.

C. Structural Dynamics of the Draining Lymph Node

- **Fibroblastic Reticular Cell (FRC) Networks:** Lymphoid organs are constructed around a structural meshwork of FRCs that guide lymphocyte migration and provide essential survival cytokines (such as IL-7).
- **Nodal Hypertrophy:** During an active infection, draining lymph nodes swell considerably. This enlargement is driven by both lymphocyte recruitment/trapping and the active proliferation of the FRC network, expanding the structural framework required to coordinate T-cell selection.

Lecture 8.2: Mucosal Immunology of the Gastrointestinal Tract

1. Structural, Anatomical, and Functional Overview of Mucosal Barriers

The Mucosal Dilemma: Area, Permeability, and Vulnerability

- **Mucosal Locations:** The body's primary barrier sites encompass the internal mucosal linings—the respiratory, gastrointestinal (GI), and urogenital tracts, alongside exocrine glands (e.g., mammary glands)—as well as the external cutaneous barrier (the skin).
- **Physiological Imperative:** Mucosal barriers must balance conflicting physiological requirements:
 - **Expansive Surface Area:** Facilitates nutrient uptake, water balance, and gas exchange.
 - **Thinness and Selective Permeability:** Essential for efficient molecular transport (e.g., nutrient absorption across enterocytes, gas diffusion in alveoli), but creates inherent vulnerability to pathogen invasion.
- **Global Health Burden:** Infectious agents frequently exploit these fragile surfaces through inhalation, ingestion, sexual contact, or mechanical breaches (e.g., trauma, insect vectors). Mucosal infections account for an estimated 10 million deaths annually worldwide (excluding pandemic peaks).

2. Gross and Microscopic Anatomy of the Gastrointestinal Tract

Gross Anatomy and Regional Specialization

- **The Stomach:** Creates a chemical barrier characterized by an acidic luminal pH (pH 1.5--2.0), hydrolyzing ingested foodstuffs and neutralizing ingested pathogens.
- **The Small Intestine:** The primary site of nutrient digestion and enzymatic absorption, subdivided into three segments:
 - *Duodenum:* Shortest, proximal segment receiving biliary and pancreatic secretions.
 - *Jejunum:* The middle, largest division where bulk nutrient absorption occurs.
 - *Ileum:* Distal division concluding at the ileocecal junction; houses specialized inductive lymphoid tissues (Peyer's patches).
- **The Large Intestine (Cecum, Colon, Rectum):** Concentrates undigested waste, reabsorbs water and electrolytes, and stores feces prior to defecation.