

# 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  $\kappa$ , then  $\lambda$ ) 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. 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- $\gamma$ ).
  - 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                               |
|------------------------------------|-------------------------------------------------|----------------------------------------------------------|
| <b>Allogeneic Antigen Source</b>   | Genetically disparate donor tissue              | Semi-allogeneic fetus (50% paternal genome)              |
| <b>Evolutionary History</b>        | Artificial, modern medical intervention         | Conserved, selected biological process                   |
| <b>Classical MHC Expression</b>    | Donor organ expresses classical HLA-A and HLA-B | Fetal extravillous trophoblasts lack HLA-A and HLA-B     |
| <b>Predominant Allorecognition</b> | Direct and indirect allorecognition pathways    | Exclusively indirect allorecognition                     |
| <b>Local Checkpoint Regulation</b> | Requires lifelong synthetic immunosuppression   | Endogenous placental PD-L1 expression and decidual Tregs |
| <b>Clinical Outcome</b>            | 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.

# 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.

# Practice Multiple-Choice Questions

## Question 1

What physiological parameter acts as the primary chemical driver of minute-to-minute ventilation under resting conditions?

- A) Serum bicarbonate concentrations measured at renal tubular interfaces
- B) Arterial oxygen saturation ( $SaO_2$ ) dropping below 90%
- C) Systemic blood pressure fluctuations detected by carotid baroreceptors
- D) Changes in arterial  $PaCO_2$  and dissolved hydrogen ion concentration

## Question 2

In spirometry, how is clinically significant bronchodilator reversibility demonstrated in an asthmatic patient?

- A) The baseline FVC drops by 30% after inhaling hypertonic saline.
- B) The patient's resting tidal volume doubles within 60 seconds of exercise.
- C) The forced expiratory volume in 1 second ( $FEV_1$ ) increases markedly following administration of a short-acting  $\beta_2$ -agonist.
- D) The total lung capacity decreases to match age-predicted normal averages.

## Question 3

Why do intact pollen grains typically cause allergic rhinitis rather than lower-airway asthma exacerbations?

- A) Intact pollen grains ( $\sim 20\text{--}35\ \mu\text{m}$ ) are filtered by the nasal mucosa, whereas sub-micronic ruptured starch fragments ( $< 5\ \mu\text{m}$ ) penetrate the lower conducting airways.
- B) Intact pollen grains lack cell-surface proteins capable of binding human immunoglobulin E.
- C) Bronchial smooth muscle cells express neutralizing IgG4 antibodies that destroy whole pollen grains on contact.
- D) Alveolar macrophages phagocytose large pollen grains before they can trigger mast cell degranulation.

### Question 7

How does structural stabilization of the RSV Fusion (F) protein in its pre-fusion ( $F_{pre}$ ) conformation improve modern vaccine efficacy?

- A) It converts the glycoprotein into a pure polysaccharide that bypasses T-cell help.
- B) It preserves the specific structural epitopes targeted by highly potent neutralizing antibodies.
- C) It allows the antigen to pass intact through the blood-brain barrier.
- D) It forces the viral particle to undergo spontaneous lysis prior to injection.

### Question 8

What is the primary immunological mechanism of action of passive immunization with the monoclonal antibody nirsevimab?

- A) It stimulates long-term endogenous plasma cell differentiation in infant bone marrow.
- B) It provides immediate, pre-formed neutralizing IgG antibodies that block RSV entry in infants during their first viral season.
- C) It acts as an adjuvant by activating TLR9 receptors on neonatal dendritic cells.
- D) It permanently downregulates expression of the human neonatal Fc receptor (FcRn).

### Question 9

In the context of annual influenza vaccine updates, why are new formulations typically approved without repeating large-scale Phase 3 clinical efficacy trials?

- A) The influenza virus never alters its surface hemagglutinin and neuraminidase proteins.
- B) Regulatory agencies rely on a validated surrogate correlate of protection (serum hemagglutination inhibition titer  $\geq 1:40$ ).
- C) Modern influenza vaccines contain live virulent avian strains that bypass immune screening.
- D) Influenza vaccines are classified as over-the-counter dietary supplements.

## Answer Key & Detailed Explanations

1. **B** — In preclinical gnotobiotic trials, transferring stool from human non-responders into germ-free mice rendered them refractory to checkpoint inhibitors, whereas stool from responders rescued antitumor efficacy, providing causal proof of microbiota-mediated modulation.
2. **D** — Following clonal expansion, ~95% of the burst comprises short-lived terminal effectors (TEs) that undergo apoptotic contraction, while ~5% are memory precursor effector cells (MPECs) that survive to establish long-term memory.
3. **A** — TCR transgenic mice express a single monoclonal TCR across virtually all CD8+ T cells, altering normal repertoire development; transferring these cells into non-transgenic recipients allows evaluation at physiological precursor numbers within an intact, polyclonal immune system.
4. **B** — In vitro-primed CD8+ T cells adoptively transferred into germ-free mice successfully seeded the inflamed epidermis and formed  $T_{RM}$  cells, but circulating memory T cells in the spleen failed to survive, demonstrating that the systemic circulating memory pool depends on the microbiota.
5. **C** — Introducing conventional commensal microbes to germ-free mice for 7 days reconstituted a functional microbiota and rescued the splenic microenvironment's capacity to support circulating memory CD8+ T-cell survival.
6. **A** — Exposing activated CD8+ T cells to butyrate biased their differentiation toward memory precursor effector cells (MPECs), downregulating terminal effector markers and enhancing survival after transfer.
7. **C** — Extracellular flux analysis demonstrated that butyrate-treated T cells exhibited elevated baseline and maximal Oxygen Consumption Rates (OCR), indicating enhanced mitochondrial respiratory capacity and reliance on oxidative phosphorylation.
8. **B** — Stable isotope tracing with  $^{13}\text{C}_6$ -glucose revealed that while glycolytic flux to pyruvate proceeded normally, glucose-derived carbons did not enter the mitochondrial Krebs cycle in butyrate-treated cells.
9. **A** — Tracing with  $^{13}\text{C}_4$ -butyrate demonstrated that labeled carbons directly entered the Krebs cycle as acetyl-CoA, proving that butyrate serves as an alternative fuel substrate powering mitochondrial respiration in memory precursors.
10. **B** — Mammalian host cells lack the cellulases and enzymes required to break down complex dietary plant fibers; generation of bioactive SCFAs like butyrate requires fermentation by anaerobic commensal bacteria.