BIO-479 Exercise 8

Lecture 12 Recapitulation

Passive Immunization Mechanisms and Risks

- A patient is admitted to the emergency room with a suspected botulism infection. The treatment protocol requires the immediate administration of a horse-derived antitoxin. Which of the following statements is correct?
- A. The administration of the antitoxin will trigger the patient's adaptive immune system to produce high-affinity IgG antibodies against the toxin, providing long-term protection against recurrence.
- B. Because the antibodies are derived from a different species (xenogeneic), the patient is at risk of mounting an anti-allotype response against the subtle amino acid differences in the constant regions of the light and heavy chains.
- C. The therapeutic mechanism relies primarily on neutralization and opsonization; however, the patient risks developing an anti-isotype response against the foreign constant determinants of the equine antibodies, which could lead to serum sickness or anaphylaxis.
- D. If pooled human immunoglobulin were used instead of horse antitoxin, the risk of an immune reaction would be completely eliminated.
- E. The passive transfer of antibodies recruits Natural Killer (NK) cells to destroy the free toxin molecules via Antibody-Dependent Cellular Cytotoxicity (ADCC) without the need for phagocytosis.

Conjugate Vaccine Immunology

- You decide to create a conjugate vaccine by linking the capsular polysaccharide to a tetanus toxoid carrier protein. Which of the following accurately describes the cellular interactions required to generate high-affinity IgG antibodies and immunological memory with this platform?
- A. The B cell receptor (BCR) binds the polysaccharide epitope, the complex is internalized, and peptides derived from the protein carrier are presented on MHC Class II to activate CD4+ Helper T cells.
- B. The polysaccharide component binds directly to MHC Class II molecules on Dendritic Cells, while the protein carrier serves as an adjuvant to trigger Toll-Like Receptors (TLRs).
- C. The B cell recognizes the protein carrier via its BCR, while the T cell provides help by recognizing the polysaccharide antigen presented on the B cell's MHC Class II.
- D. Conjugation converts the polysaccharide into a T-cell independent antigen, allowing it to cross-link BCRs extensively and induce isotype switching without T-cell help.
- E. The protein carrier is required primarily to increase the molecular size of the vaccine, preventing rapid renal clearance, but does not participate directly in the immunological synapse.

Live Attenuated vs. Inactivated Vaccine Profiles

- When comparing the immunological profiles of live attenuated vaccines versus inactivated vaccines, which statement represents a valid immunological advantage of the live attenuated platform?

•A. Live attenuated vaccines are safer for immunocompromised hosts because the attenuation process involves the permanent deletion of all replication genes.

•B. Inactivated vaccines are superior at inducing CD8+ T-cell responses because the high antigen load is immediately available for cross-presentation.

•C. Live attenuated vaccines replicate intracellularly, directing antigens into the endogenous processing pathway for presentation on MHC Class I, thereby effectively priming CD8+ Cytotoxic T Lymphocytes (CTLs).

•D. Live vaccines are generated solely through "rational design" (gene engineering), whereas inactivated vaccines rely on "empirical" methods (passage in animal cells), making live vaccines inherently more stable.

•E. Unlike inactivated vaccines, live vaccines trigger B-cell responses exclusively and fail to induce significant Helper T-cell (CD4+) pathways due to viral interference.

DNA Vaccines

- According to the proposed model of immune priming, how are **CD8+ T-cell responses** generated against the encoded antigen for DNA vaccines?
- A. The injected DNA integrates into the genome of the CD8+ T cells, causing them to express the antigen on their own surface and undergo auto-activation.
- B. Muscle cells (myocytes) are transfected and express the protein, which is then shed and taken up by Antigen Presenting Cells (APCs) for cross-presentation on MHC Class I.
- C. The DNA plasmid acts as a PAMP (Pathogen-Associated Molecular Pattern) that binds TLR9 inside the CD8+ T cell, bypassing the need for peptide presentation.
- D. DNA vaccines are incapable of inducing CD8+ T-cell responses; they exclusively drive humoral immunity by secreting the protein into the blood to bind B cells.
- E. The DNA directly transfects the draining lymph nodes' B cells, which present the antigen to CD8+ T cells via MHC Class II molecules

Specificity of Pattern Recognition Receptors (PRRs) in Vaccine Design

- You are developing a novel synthetic vaccine against an RNA virus. To mimic the natural viral infection and stimulate a strong Th1/CD8+ T-cell response, you decide to incorporate an adjuvant specifically designed to activate endosomal Pattern Recognition Receptors (PRRs) that recognize viral genetic material. Which adjuvant candidate targets the correct receptor?
- A. A synthetic double-stranded RNA (dsRNA) analogue, designed to bind TLR3.
- B. Purified Flagellin, designed to bind TLR5.
- C. Peptidoglycan fragments, designed to bind TLR2.
- D. Lipopolysaccharide (LPS) derivatives, designed to bind TLR4.
- E. Muramyl dipeptide (MDP), designed to bind surface C-type lectin receptors.

Mechanisms of Adjuvant Action

- When comparing Alum (Aluminum hydroxide) to Immune Stimulatory Complexes (ISCOMs), which of the following statements accurately characterizes the distinct immunological advantage of ISCOMs over Alum?
- A. Alum actively stimulates C-type lectin receptors to induce inflammatory cytokines, whereas ISCOMs rely solely on the "depot effect" (delayed release).
- B. Alum is an oil-in-water emulsion that activates NOD-like receptors, whereas ISCOMs are mineral salts that strictly enhance phagocytosis.
- C. ISCOMs contain matrix proteins that deliver antigen into the cytosol, allowing for processing via the endogenous pathway and the induction of cytotoxic T cells (CTLs), whereas Alum primarily enhances macrophage uptake and humoral immunity.
- D. ISCOMs are derived from *Bordetella pertussis* and function by neutralizing toxins, whereas Alum functions by recruiting NK cells for Antibody-Dependent Cellular Cytotoxicity (ADCC).
- E. Alum induces strong co-stimulation by binding TLR9, whereas ISCOMs prevent antigen degradation without activating APCs.

Antigen Dissemination and DC Activation

- Which statement correctly describes the dissemination pattern and cellular logic for a non-live (subunit) vaccine injected intramuscularly?
- A. Because the vaccine cannot replicate, it disseminates systemically to launch multiple foci of infection, making the specific site of injection irrelevant to the immune response.
- B. The antigen is usually retained locally (limited in time and space); therefore, the vaccine relies on the high density of DCs in the muscle/skin to uptake the antigen and migrate via afferent lymphatic vessels to the draining lymph node.
- C. The vaccine passively diffuses directly into the efferent lymphatic vessels, bypassing the need for peripheral Dendritic Cells, and binds directly to naive B cells in the spleen.
- D. The adjuvant triggers the muscle cells (myocytes) to express MHC Class II, allowing the muscle tissue itself to present the antigen directly to circulating CD4+ T cells.
- E. The injection triggers immediate apoptosis of local DCs, which release the antigen in the form of exosomes to be picked up by liver macrophages (Kupffer cells).

Incubation Period and Vaccine Strategy

- A pathogen like the Influenza virus has a short incubation period (1-2 days), whereas Poliovirus has a longer incubation period (>3 days) before invading neurons. How does this difference dictate the specific immunological goal of the respective vaccines?
- A. Influenza vaccines must rely on T-cell memory to kill infected cells, as antibodies are ineffective against rapid replicators.
- B. Polio vaccines must maintain high levels of circulating antibodies constantly, because the memory response is too slow to prevent neuronal invasion.
- C. Influenza vaccines must maintain high levels of pre-existing neutralizing antibodies, because the virus replicates and causes disease faster than a memory B-cell response can be reactivated.
- D. Both vaccines function identically by establishing a latent reservoir of antigen that trickles out over time to keep the immune system active.
- E. Polio vaccines target the innate immune system to clear the virus within hours, rendering the incubation period irrelevant.

Mechanism of Disease Prevention vs. Infection

- Which statement accurately describes critical distinction in how most licensed vaccines (e.g., Polio, Measles) function relative to mucosal infection?

•A. Current vaccines effectively sterilize the mucosa, preventing the pathogen from ever adhering to or penetrating the epithelium.

•B. Vaccination typically does not prevent local infection at the mucosal entry site; instead, it limits systemic propagation and spread, thereby preventing clinical disease.

•C. Systemic immunization leads to such high IgA titers in the respiratory and gut mucosa that the "portal of entry" is blocked.

•D. The primary goal of these vaccines is to induce tolerance, allowing the pathogen to replicate locally without triggering a damaging inflammatory response (disease).

•E. Vaccines for mucosal pathogens work exclusively by inducing CD8+ T cells to destroy the epithelial barrier, removing the site of infection.

Strategic Considerations for Infant Immunization

- When designing a vaccination schedule for newborns, why are multiple boosters often required, or why is the initiation of certain vaccines (like Measles) delayed until after 12 months?
- A. Infants are born with a completely tolerant immune system that cannot recognize foreign antigens until age 1.
- B. High levels of maternal antibodies (IgG) transferred transplacentally can bind and neutralize the vaccine antigen, reducing its efficacy in priming the infant's own immune system.
- C. The infant's bone marrow does not produce B cells until 6 months of age, making earlier vaccination biologically impossible.
- D. Vaccines administered to infants induce a "hyper-virulent" state because their innate immune system overreacts to adjuvants.
- E. Infant Dendritic Cells lack the specific Pattern Recognition Receptors (PRRs) necessary to detect vaccine antigens.

Mechanism of Systemic RNA-Lipoplex (RNA-LPX) Vaccines

- The lecture presents a therapeutic vaccination strategy using RNA-lipoplexes (RNA-LPX). Unlike traditional chemotherapy which targets dividing cells, or prophylactic vaccines which induce antibodies, how does this specific platform mechanically bridge innate and adaptive immunity to treat existing tumors?

•A. The nanoparticles are targeted to fuse specifically with tumor cells, where the RNA encodes a suicide gene (toxin) that induces immediate apoptosis of the cancer mass.
•B. The lipoplexes target Dendritic Cells (DCs) in lymphoid tissues, where the RNA serves a dual function: it acts as a ligand for TLR7 (inducing Type I Interferons) and serves as the template for tumor antigen translation and presentation on MHC molecules.
•C. The RNA integrates into the genome of the patient's B cells, converting them into plasma cells that permanently secrete high-affinity monoclonal antibodies against surface tumor markers (e.g., NY-ESO-1).
•D. The primary mechanism is the blockade of immune checkpoints (like PD-1/PD-L1) on the surface of T cells; the RNA component is non-coding and serves strictly as a structural scaffold for the liposome.
•E. The RNA-LPX complex functions as a "sponge" to absorb immunosuppressive cytokines (like IL-10) from the tumor microenvironment, thereby reversing T-cell exhaustion without delivering antigens.

Classification and Immunogenicity of Tumor Antigens

- Designing a cancer vaccine requires selecting an appropriate target antigen. Slide 51 categorizes various tumor antigens, including Cancer-Testis antigens (e.g., NY-ESO-1, MAGE). What is the specific immunological rationale for targeting these antigens?
- A. They are products of oncogenic viruses (like HPV E6/E7); therefore, the immune system recognizes them as completely foreign viral pathogen-associated molecular patterns (PAMPs).
- B. They are mutated "passenger" genes that accumulate randomly; while unique to the tumor, they are highly unstable and frequently lost during tumor progression.
- C. They are aberrantly expressed self-proteins; normally restricted to germ cells (which lack MHC expression), their expression in tumor cells makes them visible to the immune system as "foreign" without risking autoimmunity in somatic tissues.
- D. They are ubiquitous metabolic enzymes found in all rapidly dividing cells; targeting them induces a broad immune response against both the tumor and the healthy bone marrow (a manageable side effect).
- E. They are peptide fragments derived from the tumor stroma (blood vessels), allowing the vaccine to starve the tumor by destroying its vascular supply via CD4+ T-cell help.

Quantification of Antigen-Specific T-Cell Responses

- In the clinical study of the RNA-LPX vaccine, researchers demonstrated a significant expansion of NY-ESO-1 specific CD8+ T cells (from 0% to 0.82% of the population). According to Slide 55, which specific methodological tool is required to identify and quantify these specific T cells via Flow Cytometry?

- A. Peptide:MHC Tetramers: Recombinant MHC molecules refolded with the specific peptide and linked to a fluorochrome via streptavidin.
- B. ELISA (Enzyme-Linked Immunosorbent Assay): Using plate-bound tumor antigens to capture and colorimetrically quantify circulating antibodies.
- C. Intracellular Cytokine Staining: Permeabilizing the cells to detect total IFN-gamma production in the entire CD8+ population after non-specific stimulation.
- D. Standard Immunophenotyping: Using only anti-CD3 and anti-CD8 antibodies to measure the total increase in the cytotoxic T-cell compartment size.
- E. Western Blotting: Lysing the T cells and probing for the presence of the T-Cell Receptor (TCR) protein using a generic anti-TCR antibody.

- You are leading a global health task force to eradicate a newly emerged pathogen, *Virus X*.
- **Pathogen Profile:** *Virus X* is an encapsulated RNA virus with a moderate incubation period (5 days).
- **Target Population:** It causes severe systemic disease primarily in infants (<6 months) and the elderly.
- **Immunology:** The viral capsule prevents phagocytosis, and the virus requires a cellular reservoir to replicate.
- Based on the principles of vaccination covered in this lecture, which of the following vaccine strategies represents the most scientifically sound approach to protect the vulnerable populations and achieve eradication?

- A. Develop a polysaccharide conjugate vaccine linking the viral capsule to a protein carrier; this is essential to convert the T-independent capsular antigen into a T-dependent one, enabling the generation of high-affinity IgG and immunological memory in infants.

- B. Deploy a live attenuated vaccine immediately for all age groups; since live vaccines mimic natural infection, they are the only platform capable of sterilizing the respiratory mucosa (blocking the "portal of entry" entirely) and are safe for immunocompromised elderly patients.

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•C. Use a passive immunization strategy with pooled human immunoglobulins for the entire population; this avoids the risk of anti-isotype responses seen with animal sera and provides the lifelong memory protection required for global eradication.

•D. Engineer a DNA vaccine encoding the viral capsule; this is the optimal choice for the elderly because DNA vaccines work by permanently integrating into the host genome, providing a constant source of antigen that bypasses the need for boosting.

•E. Create a purified protein subunit vaccine (using the viral surface protein) mixed with Alum; this platform is ideal because a single dose is sufficient to induce strong CD8+ Cytotoxic T-cell immunity, which is required to clear the intracellular viral reservoir.