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DEPARTMENT OF BIOMEDICAL ENGINEERING

VII Semester

OBT357 BIOTECHNOLOGY IN HEALTH CARE

UNIT- 3 VACCINOLOGY

3.3 Live attenuated and Killed Vaccines

- Live attenuated and killed (inactivated) vaccines are two cornerstone approaches in conventional vaccine development, each leveraging distinct mechanisms to stimulate immunity. Below is a detailed yet concise comparison of these vaccine types, focusing on their principles, development, examples, advantages, disadvantages, and applications.

3.3.1 Live attenuated Vaccine:

- **Principle**: These vaccines use a weakened (attenuated) form of the live pathogen (virus or bacterium) that can still replicate in the host but does not cause severe disease. The replication mimics a natural infection, triggering a robust immune response involving both humoral (antibody-mediated) and cellular immunity.
- **Development Process**:
  - Pathogens are attenuated by repeatedly growing them under suboptimal conditions (e.g., in non-human cell cultures, at lower temperatures, or through genetic modification) until they lose virulence.
  - Extensive testing ensures the attenuated strain is safe, stable, and unlikely to revert to a virulent form.
  - The process often takes years to balance attenuation with immunogenicity.
- **Examples**:

- ❖ Measles, mumps, and rubella (MMR) vaccine
- ❖ Oral polio vaccine (OPV, Sabin)
- ❖ Yellow fever vaccine
- ❖ Varicella (chickenpox) vaccine
- ❖ Bacillus Calmette-Guérin (BCG) vaccine for tuberculosis

❒ **Advantages**:

- ❖ **Strong, long-lasting immunity**: Mimics natural infection, stimulating both B-cell (antibody) and T-cell responses, often conferring lifelong or long-term protection with one or two doses.
- ❖ **Broad immune response**: Engages multiple arms of the immune system, including mucosal immunity (e.g., OPV protects at the gut level).
- ❖ **Cost-effective for mass campaigns**: Often requires fewer doses, reducing administration costs.

❒ **Disadvantages**:

- ❖ **Safety concerns**: Rare risk of reversion to virulence (e.g., vaccine-derived poliovirus in OPV, occurring in ~1 in 2.5 million doses).
- ❖ **Not suitable for immunocompromised individuals**: Live pathogens can cause disease in those with weakened immune systems (e.g., HIV patients, transplant recipients).
- ❖ **Cold-chain requirements**: Must be stored at specific temperatures to maintain viability, posing logistical challenges in resource-limited settings.

❒ **Applications**:

- ❖ Ideal for healthy populations and diseases requiring strong, long-term immunity.
- ❖ Widely used in global immunization programs (e.g., MMR, OPV in polio eradication campaigns).

3.3.2 Killed Vaccines:

- Killed (inactivated) vaccines are a type of conventional vaccine that uses a whole virus or bacteria that has been inactivated, or **killed**, by heat, chemicals, or radiation.
- These vaccines stimulate the immune system to produce antibodies and immune cells that recognize and fight the specific pathogen without causing illness.
- Examples include vaccines for polio (Salk vaccine), hepatitis A, rabies, influenza, and certain COVID-19 vaccines like CoronaVac and Covaxin.
- The dead pathogen cannot replicate or cause disease, but its structure remains intact enough for the immune system to recognize it and mount a response. Because the pathogen is not alive, the immune response is generally weaker than with a live vaccine. For this reason, multiple doses, or "booster shots," are often required to build and maintain long-lasting immunity.

3.3.2.1 Manufacturing of Killed Vaccines:

- The production of inactivated (killed) vaccines involves carefully controlled steps to make the pathogen non-infectious while preserving its ability to trigger an immune response.

Steps in Killed Vaccine Manufacturing:

1. **Pathogen Cultivation**:
    - **Virus or Bacteria Growth**: The target pathogen (virus or bacteria) is grown in large quantities. Viruses are typically cultured in cell lines (e.g., Vero cells for polio or influenza), while bacteria may be grown in nutrient-rich media.
    - **Conditions**: This step occurs in bioreactors under controlled conditions (temperature, pH, oxygen levels) to maximize pathogen yield. For example, influenza viruses are often grown in embryonated chicken eggs or cell cultures.
    - **Challenges**: Culturing pathogens is time-intensive and requires sterile environments to prevent contamination. Some pathogens, like hepatitis A, grow slowly, increasing production time.

2. **Harvesting**:

   - The pathogen is separated from the growth medium via centrifugation or filtration. For viruses grown in cells, the cells are lysed to release viral particles.
   - **Yield Optimization**: Careful handling ensures maximum recovery of intact pathogens for downstream processing.

3. **Inactivation**:

   - **Methods**: The pathogen is killed or inactivated using chemical agents (e.g., formaldehyde, beta-propiolactone), heat, or radiation. The goal is to destroy the pathogen's ability to replicate or cause disease while preserving antigenic structures.
   - **Common Agents**:
     - Formaldehyde: Used for polio and hepatitis A vaccines; it cross-links proteins to inactivate the pathogen.
     - Beta-propiolactone (BPL): Often used for rabies and influenza vaccines; it's less harsh on antigens.
   - **Validation**: Inactivation is rigorously tested to confirm no viable pathogens remain. For example, samples are cultured to ensure no replication occurs.
   - **Risk Management**: Incomplete inactivation could leave infectious particles, so multiple inactivation cycles and quality checks are standard.

4. **Purification**:

   - The inactivated pathogen is purified to remove impurities like cell debris, culture media, or inactivating agents. Techniques include ultracentrifugation, chromatography, or filtration.
   - **Goal**: Ensure the final product contains only the desired antigens and is free of contaminants that could cause side effects.

5. **Formulation**:

- **Adjuvants**: Aluminum salts (e.g., aluminum hydroxide) or other adjuvants are often added to enhance the immune response, as inactivated vaccines tend to be less immunogenic than live vaccines.
- **Stabilizers**: Excipients like sugars or gelatin are added to maintain vaccine stability during storage and transport.
- **Preservatives**: In some cases (e.g., multi-dose vials), preservatives like thiomersal are included to prevent bacterial contamination.
- **Dosage Adjustment**: The antigen concentration is standardized to ensure consistent immunogenicity.

6. **Quality Control and Testing**:

- **Sterility**: The vaccine is tested for bacterial, fungal, or viral contamination.
- **Potency**: Animal or in vitro tests confirm the vaccine induces an immune response.
- **Safety**: Tests ensure no residual live pathogens or toxic byproducts remain.
- **Stability**: The vaccine is tested under various conditions (e.g., temperature, pH) to confirm shelf life.

7. **Filling and Packaging**:

- The vaccine is filled into vials or syringes under sterile conditions. Multi-dose or single-dose formats are prepared based on intended use.
- **Lyophilization**: Some inactivated vaccines (e.g., rabies) are freeze-dried for stability, requiring reconstitution before use.
- **Labeling**: Vials are labeled with batch numbers, expiration dates, and storage instructions (e.g., 2–8°C for most inactivated vaccines).

8. **Regulatory Approval and Batch Release**:

- Each batch undergoes review by regulatory bodies (e.g., FDA, WHO, or local agencies) to ensure compliance with Good Manufacturing Practices (GMP).

- Independent testing by national control laboratories may be required before distribution.

Manufacturing Challenges:

- **Time and Cost**: Culturing and inactivating pathogens is resource-intensive compared to newer platforms like mRNA or subunit vaccines.
- **Scalability**: Large-scale production requires significant infrastructure, including bioreactors and cleanrooms.
- **Safety Risks**: Handling live pathogens before inactivation poses risks to workers, requiring stringent biosafety measures (e.g., BSL-2 or BSL-3 facilities).
- **Antigen Preservation**: Inactivation must balance pathogen destruction with preservation of immunogenic epitopes, which can be technically challenging.

Advantages and Disadvantages

- **Advantages:** They are very safe because they cannot cause the disease they are meant to prevent. They are also generally more stable than live vaccines, making them easier to transport and store.
- **Disadvantages:** They typically require multiple doses to achieve full immunity and the immune response may not be as long-lasting as that from live attenuated vaccines.

Feature	**Killed/Inactivated Vaccine**	**Live Attenuated Vaccine**
Pathogen Status	Dead/inactive	Live, weakened
Risk of Reversion	None	Present
Safety	Very high	Lower (esp. for immunocompromised)
Immune Response	Weaker, mainly humoral	Strong, humoral & cellular
Doses Needed	Multiple	Usually 1-2

Feature	**Killed/Inactivated Vaccine**	**Live Attenuated Vaccine**
Storage	More stable	Less stable
Examples	Polio (IPV), rabies, hepatitis A, flu	MMR, yellow fever, oral polio

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