

Preclinical Screening Methods

Immunomodulators – Preclinical Screening Methods

- **Immunomodulators** are substances that modify the immune system.
- **They can either:**
 - Enhance immune response (immunostimulants)
 - Suppress immune response (immunosuppressants)
- **The immune system consists of:**
 - Innate immunity (first line defense: macrophages, neutrophils)
 - Adaptive immunity (specific response: T-cells, B-cells, antibodies)
- Preclinical screening evaluates how a drug affects these immune components.

Objectives of Screening

- To evaluate effect on immune response (increase or decrease)
- Studying mechanism (cell-mediated or humoral immunity)
- To assess cytokine production
- To detect toxicity (immune suppression or overactivation)

In Vivo Methods (Animal Models)

A. Delayed Type Hypersensitivity (DTH) Response

- **Principle:** Measures cell-mediated immunity (T-cell function)
- **Method:**
 - Sensitize animals with antigen (e.g., sheep red blood cells)
 - Later challenge in footpad
 - Measure swelling (edema)
- **Observation:**
 - Increased swelling = immune stimulation
 - Decreased swelling = immunosuppression

B. Humoral Antibody Response (Hemagglutination Assay)

- **Principle:** Measures of antibody production by B-cells
- **Method:**
 - Inject antigen (SRBCs)
 - Collect blood serum

- Measure antibody titer

- **Observation:** Increased antibody levels = immunostimulant effect

C. Carbon Clearance Test

- **Principle:** Measures phagocytic activity of macrophages

- **Method:**

- Inject carbon particles intravenously
- Measure rate of clearance from blood

- **Observation:** Faster clearance = enhanced immune activity

D. Cyclophosphamide-Induced Immunosuppression Model

- **Principle:** Cyclophosphamide suppresses immune system

- **Method:** Administer cyclophosphamide + test drug

- **Observation:** Restoration of immune function = immunostimulant effect

E. Total and Differential Leukocyte Count

- **Method:** Count white blood cells (WBCs)

- **Observation:**

- Increase = immune stimulation
- Decrease = immunosuppression

In Vitro Methods

1. Lymphocyte Proliferation Assay

- **Principle:** Measures T-cell and B-cell activation

- **Method:** Stimulate lymphocytes with mitogens

- **Observation:** Increased proliferation = immunostimulation

2. Cytokine Assays

- **Measure immune signaling molecules:**

- IL-2, IL-6, TNF- α

- Increased levels indicate immune activation

3. Phagocytosis Assay

- Measures ability of macrophages to engulf particles

Other Alternative Models

- **Cell cultures:** Immune cells (macrophages, lymphocytes)
- **Zebrafish models:** Immune response studies

Parameters Measured

- Antibody levels
- Cell-mediated immunity
- Cytokine production
- Phagocytic activity

Toxicological Considerations

- Overstimulation → autoimmune disorders
- Suppression → increased infection risk
- Cytokine storm (excess immune activation)

Immunosuppressants – Preclinical Screening Methods

- **Immunosuppressants** are drugs that reduce or inhibit the immune response.
- **They are used in:**
 - Organ transplantation (to prevent rejection)
 - Autoimmune diseases (e.g., rheumatoid arthritis)
- **They mainly act by:**
 - Inhibiting T-cell activation
 - Reducing Cytokine Production
 - Suppressing antibody formation

Objectives of Screening

- To evaluate suppression of immune response
- To assess effect on T-cells and B-cells
- To prevent graft rejection
- To evaluate safety (infection risk)

In Vivo Methods (Animal Models)

A. Skin Graft Rejection Model

- **Principle:** Measures immune rejection of transplanted tissue
- **Method:** Transplant skin from donor to recipient animal
- **Observation:** Prolonged graft survival = immunosuppressive effect

B. Delayed Type Hypersensitivity (DTH)

- Same method as above

- **Observation:** Reduced swelling = immunosuppression

C. Antibody Production Assay

- **Method:** Measure antibody response to antigen
- **Observation:** Reduced antibody levels = immunosuppressive activity

D. Lymphoid Organ Weight

- **Organs:**
 - Spleen
 - Thymus
- **Observation:** Reduced weight = immunosuppression

E. Autoimmune Disease Models

- **Example:** Collagen-induced arthritis
- **Observation:** Reduced disease severity = immunosuppressive effect

In Vitro Methods

1. T-cell Proliferation Assay

- Measures inhibition of T-cell growth

2. Cytokine Inhibition Assay

- **Measure reduction in:**
 - IL-2 (important for T-cell activation)

3. Mixed Lymphocyte Reaction (MLR)

- **Principle:** Measures of immune response between different individuals
- **Observation:** Reduced reaction = immunosuppressive effect

Other Alternative Models

- **Cell cultures:** T-cells, dendritic cells
- **Organoid models:** Immune system simulation

Parameters Measured

- T-cell activity
- Antibody production
- Graft survival time
- Cytokine levels

Toxicological Considerations

- Increased susceptibility to infections
- Risk of cancer (due to immune suppression)
- Organ toxicity (kidney, liver)

Immunostimulants – Preclinical Screening Methods

- Immunostimulants are substances that **enhance or activate the immune system**.
- **They are used in:**
 - Immunodeficiency conditions
 - Infections
 - Cancer therapy (to boost immune response)
- **They act by:**
 - Stimulating immune cells (T-cells, B-cells, macrophages)
 - Increasing antibody production
 - Enhancing cytokine release

Objectives of Screening

- To evaluate enhancement of immune response
- To assess activation of innate and adaptive immunity
- To study cytokine production
- To identify mechanism of action
- To ensure no excessive immune activation (safety)

In Vivo Methods (Animal Models)

A. Carbon Clearance Test (Phagocytic Index)

- **Principle:** Measures activity of macrophages (innate immunity)
- **Method:**
 - Inject carbon ink intravenously
 - Measure rate of removal from blood
- **Observation:** Faster clearance = increased phagocytic activity = immunostimulation

B. Delayed Type Hypersensitivity (DTH) Response

- **Principle:** Evaluates T-cell mediated immunity
- **Method:**
 - Sensitize animal with antigen (e.g., SRBC)



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