ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

EVALUATION OF ANTI-ALLERGIC ACTIVITY THROUGH *IN VITRO* AND *IN VIVO* MODELS

J. Shriya kumari, K. Akshitha Reddy, K. Shivanandu, K. Suresh, K. K. Vardhan Reddy, Dr. Divya Yada*

Malla Reddy College of Pharmacy, Secunderabad. Affiliated to Osmania University, Hyderabad-500100

*Corresponding author E- mail: yada.divya@gmail.com

ARTICLE INFO

Key words:

Allergy, *In-vitro, In-vivo*, IgE, Sensitization

Access this article online
Website:
https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Allergies represent a spectrum of hypersensitivity reactions triggered by the immune system's interaction with various allergens. The underlying mechanisms involve immunoglobulin E (IgE) production, mast cell degranulation, and cytokine-mediated inflammation, leading to diverse symptoms ranging from mild irritation to severe anaphylaxis. Research leveraging in vitro and in vivo animal models has advanced our understanding of allergic diseases, facilitating the development of novel therapeutic interventions. In vitro models, such as mast cell degranulation assays, epithelial cell studies, and cytokine profiling, allow detailed examination of cellular responses. In vivo models, including mouse sensitization and challenge frameworks, mimic systemic immune responses. These approaches collectively provide insights into allergic pathophysiology, enabling the design of targeted therapies, such as monoclonal antibodies and immune modulators, offering hope for the effective management of allergic conditions

INTRODUCTION:

Allergies are characterized as abnormal immune responses triggered by typically harmless substances, such as pollen, food proteins, or medications[1] .These conditions significantly impact global health, with rising prevalence due to environmental and lifestyle changes[13]. The allergic cascade involves a complex interplay of immune cells, including mast cells, eosinophils, and T-helper cells, orchestrated by cytokines like IL-4, IL-5, and IL-13[3]. The mechanisms underlying allergic reactions are divided into sensitization and effector phases, with IgE-mediated mast cell degranulation being a hallmark of immediate hypersensitivity[5]. The study of allergic diseases has greatly benefited from the use of in vitro and in vivo models [8]. *In vitro* systems, such as mast cell degranulation and dendritic cell assays, offer controlled Environments to study molecular mechanisms [36]. *In vivo* models, including murine asthma frameworks, provide insights into whole-organism responses to allergens, including airway hyper responsiveness and systemic anaphylaxis [14]. Furthermore, genetically modified animal models enable targeted exploration of specific immune pathways [32]. This survey diagrams the utility of these models in progressing our understanding of unfavorably susceptible disease instruments and highlights their part in creating helpful methodologies for conditions such as asthma, rhinitis, and atopic dermatitis [29].

WHAT IS MEAN BY ALLERGY?

Allergies are a sort of extreme touchiness response caused by the safe framework. There are many sorts of hypersensitivities. You can be unfavorably susceptible to natural allergens

(like dusts and clean mites), certain solutions (like anti-microbials) or certain nourishments. Different sorts of hypersensitivities can have distinctive side effects. The part of an allergist is to offer assistance understand your hypersensitivity side effects, and to decide if and how they're related to allergies.[33] [Fig.1]

Fig.1 allergy

WHAT IS THE CAUSE OF ALLERGY

Antibody called immunoglobulin E (IgE) for certain proteins show in allergens. When the IgE counter acting agent recognizes certain allergenic proteins, this triggers a arrangement of safe responses that actuate sensitivity cells and cause the discharge of unfavorably susceptible chemicals (such as histamine, leukotrienes and prostaglandins). These Hypersensitivities are more often than not caused by having an allergic sort cells and chemicals are what cause different hypersensitivity side effects, counting hives, swelling, nasal blockage and wheezing.

TYPES OF ALLERGY

Seasonal/environmental allergies: The major causes of regular and natural sensitivities incorporate indoor and open air allergens. Symptoms of regular and natural sensitivities can incorporate: Runny nose, Nasal congestion, Sinus pressure, Itchy and/or watery eyes, Wheezing and shortness of breath, particularly in those with a history of asthma

Food allergies: Nourishment sensitivities are a sort of extreme touchiness response to certain nourishments. Nourishment hypersensitivities have gotten to be more common in later decades, particularly among children. The commonplace side effects of nourishment hypersensitivity happen inside a few hours of eating the nourishment and can include: Hives, Swelling of the lips, tongue and throat, Gastrointestinal disturbed and diarrhoea, Nausea, Vomiting. A serious unfavorably susceptible response including different frameworks is called anaphylaxis. [Fig 2]

Fig.2 food allergy

Drug allergies: Sedate hypersensitivities are a portion of a range of touchiness responses to solutions and drugs. There is a range of extreme touchiness responses to drugs. A few responses happen quickly after taking a medicine (immediate-type hypersensitivity).

The side effects of an immediate-type medicate sensitivity ordinarily happen inside a few hours of taking a medicine, and they incorporate: Hives, Swelling of the lips, confront, tongue or throat, Difficulty breathing or wheezing. In a few extreme cases, moo blood weight [34]

ALLERGY TREATING ANIMAL MODELS: Animal models play a crucial role in understanding the mechanisms underlying allergic diseases and testing potential treatments before clinical application in humans. Both in vitro (outside the living organism) and in vivo (within the living organism) animal models are commonly used in allergy research. Here's an overview of how each model is used in allergy treatments [fig.3]

In Vitro Animal Models for Allergy Treatment: In vitro models use isolated cells or tissues to study specific aspects of allergic responses in a controlled environment. These models can help researchers understand the cellular and molecular mechanisms of allergy without the complexity of an entire living organism.

Human Mast Cell Degranulation Assays

Purpose: Mast cells are key players in allergic reactions. In vitro models involve isolating mast cells from animals (or human sources) and exposing them to allergens. The degranulation (release of histamine and other mediators) is monitored to assess the effects of potential treatments.

Applications: Testing anti-histamines, mast cell stabilizers, or new immune modulating drugs. **Example:** Rat or mouse mast cells treated with allergens (like ovalbumin) to study degranulation and mediator release.

Dendritic Cell and T-cell Interaction Model **Purpose:** Dendritic cells (DCs) are antigen-presenting cells that initiate allergic immune responses. In vitro models using isolated DCs and T-cells can be used to study the activation of T-helper 2 (Th2) cells, which are central to allergic reactions.

Applications: Identifying potential immunotherapies that block DC activation or the Th2 response. **Example:** Co-culturing mouse bone marrow-derived dendritic cells with T-helper cells to study how allergens or treatments modulate T-cell responses.

Epithelial Cell Models: Purpose: Respiratory epithelial cells play a role in the initiation of allergic responses in asthma and rhinitis. In vitro models using bronchial epithelial cells exposed to allergens (e.g., house dust mites, pollen) can assess the inflammatory response

Applications: Testing anti-inflammatory or barrier-enhancing drugs that could prevent allergen-induced airway hyper responsiveness. **Example:** Exposing human bronchial epithelial cells (from animal models or human sources) to allergens to study cytokine release and epithelial damage

Cytokine and Immune Mediator Profiling

Purpose: Many allergic diseases are driven by the release of specific cytokines (e.g., IL-4, IL-5, IL-13). In vitro models of immune cell cultures (e.g., Th2 cells, eosinophils) can help to measure the effect of potential treatments on cytokine production **Applications:** Screening drugs or biologicals (e.g., monoclonal antibodies targeting IL-5 or IL-13). **Example:** Mouse T-helper cells treated with allergens to examine the effects of immune-modulatory drugs on cytokine secretion

***In Vivo* Animal Models for Allergy Treatment:** *In vivo* models involve the use of whole animals to study how allergies develop and respond to treatments in the context of a functioning immune system. These models are critical for studying systemic effects, efficacy, and safety.

Sensitization and Challenge Models **Purpose**: These are the most commonly used in vivo models for allergy research. Animals (typically mice or rats) are sensitized to an allergen (e.g., ovalbumin, house dust mites) and then challenged with the same allergen to provoke an allergic response.

Applications: Testing the efficacy of treatments for allergic diseases like asthma, rhinitis, or anaphylaxis. **Example:** The ovalbumin (OVA)-induced asthma modelin mice, where animals are sensitized to OVA and then exposed to aerosolized OVA to mimic asthma-like responses (e.g., airway inflammation, hyper responsiveness)

Mouse Models of Asthma Purpose: Asthma is a common allergic disease characterized by inflammation of the airways. Mouse models are widely used to study allergic asthma because they mimic many of the features of human asthma, such as eosinophilic inflammation, airway hyperresponsiveness, and mucus production. **Applications:** Testing corticosteroids, bronchodilators, biologic therapies (e.g., anti IL-5, anti-IL-4), and new immune modulators. **Example:** The OVA asthma model involves sensitizing and challenging mice with OVA and then evaluating treatments on airway inflammation, IgE levels, and lung Function.

© Journal of Global Trends in Pharmaceutical Sciences

Anaphylaxis Models Purpose: Anaphylaxis is a severe, systemic allergic reaction. In vivo models of anaphylaxis are typically used to study the mechanisms of acute allergic reactions and evaluate emergency treatments, including epinephrine and other drugs. **Applications**: Testing drugs that could prevent or treat anaphylactic reactions. **Example**: Mouse models using IgE sensitization followed by allergen challenge, monitoring parameters like blood pressure, respiratory function, and survival rates.

Eosinophilic Inflammation Models Purpose: Eosinophils are important in many allergic responses, particularly in asthma and rhinitis. These models are designed to simulate eosinophil-driven inflammation, a key feature of allergic diseases. **Applications**: Testing treatments that target eosinophils, such as monoclonal antibodies or small molecule inhibitors. **Example**: Mouse models with eosinophilic inflammation induced by allergens like ragweed or by genetic modification (e.g., eosinophil-specific knockout mice).

Skin Allergy Models (e.g., Atopic Dermatitis) **Purpose:** Atopic dermatitis is a chronic allergic skin condition. Mouse models can be used to study the pathogenesis of atopic dermatitis, including skin barrier dysfunction, immune cell infiltration, and cytokine production. **Applications**: Testing therapies for topical or systemic treatments of skin allergies, including corticosteroids, calcineurin inhibitors, or biologic therapies. **Example:** A mouse model of atopic dermatitis (using DNCB sensitization or genetic models) to study skin inflammation, itching, and potential therapeutic agents.

Genetically Modified Animal Models Purpose: Genetically modified animals, such as knockout mice or transgenic mice, are used to study the role of specific genes in allergy development. These models can help identify potential therapeutic targets and mechanisms. **Applications:** Investigating immune system components like IL-4, IL-13, or IgE in allergic responses and testing specific genetic targets for therapy. **Example:** IL-4 or IL-13 knockout mice to study the role of these cytokines in allergic inflammation and test related therapies.[35]

IN VITRO ANIMAL MODELS

●**Human Mast Cell Degranulation Assays:** Mast cells play a pivotal role in allergic reactions by releasing mediators such as histamine, tryptase, and cytokines during degranulation, contributing to inflammation and hypersensitivity. Human Mast Cell Degranulation Assays are widely used to evaluate mast cell activation, to test the effects of potential anti-allergic drugs, and to study the mechanisms behind allergic responses.

Principle of Human Mast Cell Deregulation Assay: The principle behind the Human Mast Cell Degranulation Assay is based on detecting the release of mediators from mast cells following their activation. Mast cell degranulation occurs when these cells release pre-stored granule contents in response to various stimuli, such as allergens, immunological signals, or pharmacological agents.[Fig.4] IgE-mediated Activation: Mast cells are sensitized by IgE antibodies, which bind to high affinity receptors called FcεRI on the cell surface. When an allergen cross-links these IgE molecules, it triggers mast cell activation and degranulation. **Mediator Release**: During degranulation, mast cells release various bioactive substances, including histamine, proteases (like tryptase), cytokines (e.g., IL-4, IL-5), leukotrienes, and prostaglandins. Assay Purpose: By measuring the release of these mediators (such as histamine, tryptase, or β-hexosaminidase), researchers can assess the extent of mast cell activation and the effects of different stimuli or drugs that inhibit or enhance degranulation.

Procedure of Human Mast Cell Degranulation Assay: The following is a typical procedure for conducting a human mast cell degranulation assay:

© Journal of Global Trends in Pharmaceutical Sciences

Isolation and Culturing of Mast Cells: Human Mast Cells can be isolated from bone marrow, umbilical cord blood, or peripheral blood progenitors, and cultured in the presence of cytokines (e.g., IL-3 and stem cell factor) to induce differentiation into mature mast cells. Alternatively, mast cell lines such as HMC-1 or KU-812 can be used for convenience. Sensitization of Mast Cells (if using allergen-based activation): Mast cells are incubated with IgE antibodies specific to a given allergen (e.g. ovalbumin, house dust mites). The IgE binds to the FcεRI receptors on the mast cells. This sensitization step ensures that mast cells are primed for an allergen-induced activation.

Activation of Mast Cells: After sensitization, mast cells are exposed to allergen (e.g., ovalbumin), which binds to the IgE molecules, causing cross-linking of the FcεRI receptors. This triggers intracellular signaling pathways that initiate degranulation. In some assays, mast cells may be activated directly using calcium ionophores (e.g., A23187) or compound 48/80, which bypass the IgE-mediated activation pathway and induce degranulation by increasing intracellular calcium levels.

Collection of Supernatant: After a defined period of activation (usually 30-60 minutes), the supernatant (containing the released mediators) is collected. The mast cells themselves can be harvested for further analysis or to measure cellular changes.

Detection of Deregulation: The released mediators are detected by: Histamine Release Assay: Histamine is measured in the supernatant using fluorometric or colorimetric assays, such as the O-phthalaldehyde reaction. β-hexosaminidase Release Assay: This enzyme is stored in mast cell granules and is released during degranulation. Its activity can be quantified colorimetrically or fluorometrically.

Tryptase Assay: Tryptase is another enzyme released by mast cells and can be measured by ELISA or Western blotting.

Flow Cytometry: Flow cytometry can detect changes in the expression of surface markers (e.g., CD63, which is upregulated during degranulation) or intracellular calcium levels to assess degranulation.

Data Analysis: the results are expressed as percent degranulation, which is calculated by comparing the amount of mediator released to the total amount of mediator in the cells before activation. This provides a quantitative measure of mast cell activation.

Mechanism Behind Mast Cell Degranulation: Mast cell degranulation is triggered by multiple steps involving intricate cellular signalling mechanisms. Here's an overview of the key stages:

IgE Sensitization: Mast cells express the high-affinity receptor FcεRI on their surface. During initial exposure to an allergen, IgE antibodies are produced and bind to these receptors. When an allergen re-exposes the individual, the allergen binds to the IgE on FcεRI, cross-linking multiple IgE molecules. This triggers degranulation.

Activation of Intracellular Signalling Pathways: The cross-linking of FcεRI activates a cascade of intracellular signalling events: Src-family kinases (e.g., Lyn) are activated. This leads to the phosphorylation of the Immunoreceptor Tyrosine-based Activation Motif (ITAMs) on the receptor, recruiting and activating Syk kinase.

 Activation of Syk kinase leads to the production of inositol trisphosphate (IP3) and diacylglycerol (DAG), both of which are essential for calcium mobilization and activation of other signalling pathways.

Calcium Influx and Granule Fusion: The production of IP3 triggers the release of calcium ions from intracellular stores in the endoplasmic reticulum. DAG activates protein kinase C (PKC), further promoting granule fusion with the plasma membrane. o The elevated intracellular calcium levels trigger the exocytosis of mast cell granules

© Journal of Global Trends in Pharmaceutical Sciences

containing mediators (histamine, cytokines, proteases, etc.).

Mediator Release: o Granule fusion results in the release of bioactive molecules into the extracellular environment, including: Histamine: Increases vascular permeability, leading to edema and inflammation. Proteases(e.g., tryptase): Contribute to tissue remodelling and inflammation. Cytokines(e.g., IL-4, IL-5, TNF-α): Recruit and activate other immune cells, amplifying the allergic response. Leukotrienes and prostaglandins: Lipid mediators that contribute to bronchoconstriction and inflammation.

Feedback Regulation: Inflammatory mediators released during degranulation can act as feedback signals, perpetuating and amplifying the allergic response. Additionally, mast cells can re sensitize over time, making subsequent allergen exposures more potent. [1] [2] [3] [4] [5]

Dendritic Cell and T-cell Interaction Models: Dendritic cells (DCs) and T cells are central players in the initiation and regulation of immune responses, including in allergic reactions. Dendritic cells act as antigen presenting cells (APCs) that capture, process, and present antigens to T cells, leading to T cell activation and differentiation. The interaction between DCs and T cells is crucial for determining the type of immune response that is mounted, whether it's a Th1, Th2, or regulatory T cell (Treg) response. Understanding the mechanisms behind this interaction is key to developing targeted therapies for allergic diseases, autoimmunity, and immunotherapies. Here is a detailed breakdown of the principle, procedure, and mechanism behind Dendritic Cell and T-cell Interaction Models used to study immune responses, particularly in the context of allergies.

Principle of Dendritic Cell and T-cell Interaction Models: The principle behind DC and T-cell interaction models is based on the capacity of DCs to present antigens to T cells and activate them through a complex signalling process. These interactions are essential for the initiation of both innate and adaptive immune responses. Dendritic cells act as professional antigen-presenting cells that capture and process foreign Antigens. They then migrate to lymph nodes where they present processed antigens on their surface using MHC molecules (Major Histocompatibility Complex). Tcells, which express T cell receptors (TCRs), recognize the antigen-MHC complexes presented on DCs. This recognition, combined with co-stimulatory signals provided by the DCs, leads to T cell activation, proliferation, and differentiation. In the context of allergy, DCs can influence T-cell differentiation, leading to a Th2 immune response, which is a hallmark of allergic diseases (e.g., asthma, rhinitis, and atopic dermatitis). These models are typically used to investigate the impact of different allergens, cytokines, or therapeutic interventions on the activation and differentiation of T cells.

Procedure of Dendritic Cell and T-cell Interaction Models: The procedure for studying DC and T-cell interactions in vitro generally involves the following steps:

1. Isolation and Culturing of Dendritic Cells: Human or murine DCs can be isolated from peripheral blood, bone marrow, or other tissues. Bone marrow-derived dendritic cells (BMDCs) are often cultured by differentiating bone marrow cells in the presence of cytokines such as GM-CSF (Granulocyte-macrophage colony stimulating factor) and IL-4 to promote DC differentiation. Plasmacytoid dendritic cells (pDCs) or monocyte-derived dendritic cells (moDCs) can also be differentiated from monocytes (isolated from human blood) using cytokines like GM-CSF and IL-4.

2. Priming of Dendritic Cells with Antigens: Dendritic cells are exposed to allergen-derived antigens (such as ovalbumin in mouse models or house dust mite extract in human models) or model antigens like fluorescent labelled ovalbumin (OVA). The antigen is taken up by DCs via endocytosis or phagocytosis. Once internalized, the antigen is processed and presented on the surface in association with

© Journal of Global Trends in Pharmaceutical Sciences

MHC class I (for CD8+ T cells) or MHC class II (for CD4+ T cells) molecules.

3. T-cell Isolation and Activation: Naïve Tcells are isolated from peripheral blood or lymphoid tissues using methods like magnetic-activated cell sorting (MACS) or flow cytometry. The Tcells are typically labelled with a fluorescent marker (e.g., CFSE for proliferation tracking). Tcells are then co-cultured with antigen-pulsed dendritic cells in a T cell/DC co-culture system.

4. Co-culture and Stimulation: Tcells are stimulated by the antigen presented by DCs in the co-culture system. DCs provide the necessary signal 1 (antigen-MHC presentation) and signal 2 (co-stimulatory signals such as CD80, CD86). In some experimental setups, the T cells are polarized by adding specific cytokines to drive differentiation into distinct T-helper subsets, such as Th1, Th2. The polarization can be induced by adding IL-4 and anti-IFN-γ. Th1polarization can be induced by adding IL-12 and anti-IL-4.

o Treg induction can be achieved by adding TGF-β and IL-2

5. Analysis of T-cell Responses: Proliferation: T cell proliferation is often assessed using a CFSE dilution assay (cell tracing via fluorescence) or [3H] thymidine incorporation. Cytokine Production: After a defined period (e.g., 72 hours), the supernatant is collected, and cytokine levels (e.g., IL-4, IL-5, IL-13 for Th2; IFN-γ for Th1) are measured using ELISA or Cytometric Bead Arrays (CBA). Flow Cytometry: Surface markers (e.g., CD4, CD8, CD69, CD25, CD62L, CD44) and intracellular cytokines (e.g., IFN-γ, IL-4, IL-13) can be measured by flow cytometry after fixation and permeabilization. T-cell Differentiation: T cells can be analysed for their differentiation status by examining the Expression of transcription factors like T-bet (for Th1), GATA-3 (for Th2), and RORγt (for The17) using real-time PCR, Western blotting, or flow cytometry.

6. Data Interpretation: Cytokine profiles and T-cell proliferation are analysed to assess the functional outcome of T cell activation and differentiation. The effects of different drugs, cytokines, or genetic modifications (e.g., cytokine neutralization, receptor blocking) on the DC-T cell interaction can also be assessed.

Mechanism of Dendritic Cell and T-cell Interaction: The interaction between DCs and T cells is a multi-step process, involving antigen uptake, processing, and presentation, followed by T-cell activation and differentiation: Antigen Capture and Presentation: DCs capture antigens through receptors such as mannose receptors, dectin-1, and DC-SIGN, or via phagocytosis. Once the antigen is processed, it is presented on MHC molecules on the DC surface. MHC Class presents endogenous antigens to CD8+ cytotoxic T cells, and MHC Class II presents exogenous antigens to CD4+ helper T cells.

T-cell Activation: The interaction between the TCR on T cells and the antigen-MHC complex on DCs provides the primary signal (signal 1) for T cell activation. Co-stimulatory molecules (such as CD80 and CD86) on DCs engage CD28 on T cells to provide signal 2, which is required for full T-cell activation. This interaction induces NF-κB, MAPK, and PI3K signalling pathways in T cells, resulting in T cell proliferation and cytokine production.

Cytokine Signalling and T-cell Differentiation: The cytokine environment in which the DC-T cell interaction occurs is crucial for determining the type of T-helper cell that will be generated: IL-4promotes differentiation into Th2 cells (critical for allergic responses). IL-12induces differentiation into Th1 cells (important in responses against intracellular pathogens).

 TGF-βandIL-6 promote differentiation into Th17 cells (involved in autoimmune responses). TGF-βandIL-2 induce regulatory T cells (Tregs), which suppress immune responses.

Effector Functions of T cells: Th2cells produce cytokines such as IL-4, IL-5, and IL-13, which are associated with allergic inflammation, eosinophil recruitment, and IgE class switching.

© Journal of Global Trends in Pharmaceutical Sciences

o Th1cells produce IFN-γ, promoting cell-mediated immunity, and are involved in responses against viral and intracellular bacterial infections. o Treg cells produce IL-10 and TGF-β, which inhibit the immune response to maintain tolerance. [6]

Epithelial Cell Models: Epithelial cells serve as a critical interface between the external environment and the body. In various models of immunology, especially in the context of allergic diseases, inflammation, and immune responses, epithelial cells play a central role in maintaining barrier function, initiating immune responses, and regulating local tissue Inflammation. The use of epithelial cell models is crucial for understanding how these cells interact with pathogens, allergens, or therapeutic agents. In this context, epithelial cell models are extensively employed to study various aspects of immune system activation, epithelial dysfunction in allergic diseases, and drug development. Below is an explanation of the principle, procedure, and mechanism behind epithelial cell models.

Principle of Epithelial Cell Models: The principle behind epithelial cell models is based on the ability of epithelial cells to form a physical barrier and actively participate in immune responses when various stimuli, such as allergens, pathogens, or inflammatory mediators. These models are designed to mimic the natural behaviour of epithelial cells in the body, allowing researchers to study their responses in a controlled environment.

1. Barrier Function: Epithelial cells form tight junctions and apical-basal polarity, which are essential for the physical barrier function. This is particularly important in models studying diseases like asthma, where the epithelial barrier is compromised.

2. Immune Modulation: Epithelial cells secrete cytokines, chemokines, and antimicrobial peptides in response to various stimuli, acting as a "first responder" in immune defence. These cells can interact with innate immune cells like dendritic cells, mast cells, and eosinophils.

3. Inflammatory Response: Upon stimulation (e.g., with allergens, cytokines like IL-13, or pathogen-associated molecular patterns like LPS), epithelial cells can release mediators that recruit immune cells and amplify inflammation, as seen in diseases like asthma, rhinitis, or inflammatory bowel disease.

Procedure of Epithelial Cell Models: The procedure for conducting experiments using epithelial cell models generally involves several key steps, including cell culture, treatment with stimulants (e.g., allergens, cytokines), and analysis of cell behaviour and mediator release. Below is an outline of the general procedure:

1. Isolation and Culture of Epithelial Cells:

Cell Lines: Human or animal epithelial cell lines are commonly used for in vitro experiments. These include:

o BEAS-2B(human bronchial epithelial cell line)

o A549(human alveolar epithelial cell line)

o Calu-3(human lung epithelial cell line)

o Caco-2(human intestinal epithelial cell line)

o NHEK(Normal Human Epidermal Keratinocytes) for skin-related studies.

 Primary Epithelial Cells: In some cases, primary epithelial cells derived from human or animal tissues (e.g., lung, intestine, skin) are cultured to provide a more physiologically relevant model. Primary cells may be more challenging to maintain but offer a closer representation of natural tissue responses.

2. Formation of Epithelial Monolayers: For many experiments, monolayers of epithelial cells are cultured on transwell inserts to create a polarized, functional epithelial layer with tight junctions.

 Tight junctions between epithelial cells are formed during culture and can be assessed by measuring transepithelial electrical resistance (TEER) or by using fluorescent tracers (e.g., FITC-dextran) to assess barrier integrity. Air-liquid interface models (for respiratory epithelial cells) or side-by-side cultures (for gut or skin cells) may be used to mimic more realistic tissue architecture and conditions. [Fig.5]

3. **Stimulation of Epithelial Cells:** Epithelial cells can be exposed to various stimuli,

© Journal of Global Trends in Pharmaceutical Sciences

including: o Allergens: E.g., ovalbumin for asthma models or house dust mites for allergic rhinitis. Cytokines: E.g., IL-13, IL-4, or TNF-α to simulate inflammatory responses. Pathogensor PAMPs: E.g., LPS (lipopolysaccharide) for bacterial stimulation.

o Chemical agents: E.g., cigarette smoke, pollutants, or toxins to mimic environmental exposures. The epithelial cells can be treated with these stimuli at different concentrations and time points to assess their responses (e.g., cytokine production, changes in barrier function).

4. Measurement of Epithelial Cell Responses: Cytokine Production: After stimulation, the supernatant is collected, and cytokines (e.g., IL-6, IL-8, TNF-α, IL-33) are measured using ELISA or Cytometric Bead Arrays (CBA). Gene Expression: Changes in gene expression related to inflammation, barrier function, or epithelial cell activation are measured using RT-qPCR, Western blotting, or RNA sequencing Barrier Integrity: TEER is measured to assess the integrity of the epithelial barrier. Fluorescent tracers can also be used to assess permeability. Cell Proliferation and Viability: MTT assay, AlamarBlue, or propidium iodide staining are commonly used to assess cell viability and proliferation after treatment.

5. Co-culture with Immune Cells: In many models, epithelial cells are co-cultured with immune cells (e.g., dendritic cells, T cells, mast cells) to study the cross-talk between epithelial cells and immune system components. Immune cell activation or cytokine production can be assessed in response to signals provided by the epithelial cells.

Mechanism of Epithelial Cell Models The mechanism behind epithelial cell models involves several key biological processes that contribute to the immune response, inflammation, and barrier dysfunction:

Epithelial Barrier Function: Epithelial cells are tightly joined by tight junctions, adherent junctions, and desmosomes, which maintain the integrity of the epithelial barrier. These junctions regulate the paracellular passage of ions, small molecules, and pathogens.

o Disruption of these junctions can lead to increased epithelial permeability, a hallmark of many inflammatory diseases, including asthma, allergic rhinitis, eczema, and inflammatory bowel disease (IBD).

Activation of Inflammatory Pathways: When epithelial cells are exposed to allergens, pathogens, or pro-inflammatory cytokines (like IL-13 or TNF-α), they release various pro-inflammatory mediators such as IL-6, IL-8, CCL2, TNF-α, and IL-33.

o IL-13is acritical cytokine in allergic diseases that induces epithelial cell activation, mucus hyper production, and disruption of tight junctions. It also promotes the differentiation of Th2 cells, a key feature of allergic inflammation. o NF-κBand MAPK pathways are commonly activated in epithelial cells upon exposure to inflammatory stimuli, leading to the production of pro-inflammatory cytokines and chemokines.

Epithelial-Immune Cell Interactions: Epithelial cells interact with immune cells such as mast cells, dendritic cells, T cells, and eosinophils, which are recruited to the site of inflammation. o Epithelial cells can act as "first responders" to allergens or pathogens by expressing pattern recognition receptors (PRRs) such as TLR4 (Toll-like receptor 4) for LPS or TLR2 for other microbial components. These receptors activate downstream signalling pathways, including NF-κB, leading to the release of pro-inflammatory cytokines. o Epithelial cells can also induce Th2 differentiation by releasing IL-25, IL-33, or TSLP (Thymic Stromal Lymphopoietin), which are important in promoting allergic inflammation.

Mucus Production and Hypersecretion:

o In response to inflammatory signals like IL-13, epithelial cells in the airway can undergo goblet cell metaplasia, leading to mucus hypersecretion. This is a key feature of diseases like asthma and chronic obstructive pulmonary disease (COPD). Epithelial cells also produce mucins, such as MUC5AC, which contribute to mucus

© Journal of Global Trends in Pharmaceutical Sciences

production and are commonly upregulated in allergic asthma models.

Apoptosis and Repair: In response to stress or damage, epithelial cells may undergo apoptosis, a form of programmed cell death. This process is tightly regulated to prevent excessive inflammation or tissue damage. After injury, epithelial cells play a key role in tissue repair through the activation of pathways like TGF-β signalling, which promotes epithelial regeneration and wound healing. [7]

Cytokine and Immune Mediator Profiling

Cytokine and immune mediator profiling is an essential technique in allergy research to evaluate the production of key signalling molecules that drive allergic responses. This method allows researchers to understand the immune microenvironment and test potential therapeutic interventions.

Principle: The principle behind cytokine and immune mediator profiling is the detection and quantification of specific cytokines and mediators released by immune cells in response to allergens or therapeutic agents. These mediators, such as IL-4, IL-5, IL-13, and others, are central to allergic inflammation and immune regulation.

Key Concepts:

1. Cytokines in Allergy: Th2 cytokines (IL-4, IL-5, IL-13) are critical in promoting IgE production, eosinophil activation, and mucus secretion.

2. Mediator Profiling: Tracks inflammatory proteins (e.g., histamine, prostaglandins) and their modulation by treatments.

3. Analytical Tools: Uses ELISA, Luminex, flow cytometry, or transcriptomic for cytokine detection and quantification.

Procedure

Step 1: Cell Preparation

1. Cell Isolation: Collect immune cells from blood (PBMCs), bone marrow, or tissues.

Use density gradient centrifugation (e.g., Ficoll) to isolate mononuclear cells or magnetic-activated cell sorting (MACS) for specific subsets (e.g., Th2 cells, eosinophils).

2. Cell Culture: Plate cells in appropriate culture medium (e.g., RPMI-1640 with 10% FBS). Maintain cells at 37°C in a 5% CO_2 incubator.

Step 2: Stimulation Allergen Stimulation: Add allergens like house dust mite extract, ovalbumin, or specific antigens to culture.

2. Cytokine Stimulation: Use recombinant cytokines (e.g., IL-4, IL-13) to polarize immune cells.

3. Therapeutic Testing:

Include test compounds (e.g., monoclonal antibodies or small molecules) to observe their effect on cytokine production.

Step 3: Supernatant Collection

After 24–72 hours of stimulation, collect the culture supernatant. Centrifuge to remove cellular debris and store at -80°C until analysis.

Step 4: Detection and Quantification

1. ELISA (Enzyme-Linked Immunosorbent Assay): Quantifies individual cytokines (e.g., IL-4, IL-5, IL-13) using specific antibodies and colorimetric/fluorescent readouts. Advantage: High sensitivity and specificity.

2. Luminex or Cytometric Bead Arrays (CBA): Allows simultaneous detection of multiple cytokines using fluorescent beads. Advantage: Multiplex capability for comprehensive profiling.

3. qPCR/RT-PCR: Measures mRNA levels of cytokines and mediators to study transcriptional regulation.

4. Flow Cytometry:

Detects intracellular cytokines (e.g., IFN-γ, IL-4) after fixation and permeabilization. Use surface markers (e.g., CD4 for T cells) for subset-specific cytokine analysis.

5. Mass Spectrometry: Quantifies lipid mediators (e.g., prostaglandins, leukotrienes).

Step 5: Data Analysis Normalize cytokine levels against controls. Compare treated versus untreated groups for evaluating therapeutic efficacy.

Mechanism

1. Cytokine Production Pathways 1. Allergen-Induced Cytokines:

© Journal of Global Trends in Pharmaceutical Sciences

Allergens activate dendritic cells to polarize naïve T cells into Th2 cells.
Th2 cells produce cytokines like IL-4 (stimulates IgE production), IL-5 (eosinophil recruitment), and IL-13 (mucus hypersecretion).
2. Eosinophil Activation: IL-5 recruits eosinophils, promoting tissue damage and inflammation.
3. Mast Cell Mediators: IgE-bound mast cells release histamine, prostaglandins, and leukotrienes upon allergen exposure.

2. Therapeutic Mechanisms

1. Biologics: Monoclonal antibodies (e.g., dupilumab) block IL-4Rα, inhibiting both IL-4 and IL-13 signalling
2. Small Molecules: Inhibitors target signalling pathways like JAK-STAT to reduce cytokine production.
3. Immunotherapy: Reduces Th2 responses and shifts towards Treg-mediated tolerance.[8] [9] [10]

IN VIVO ANIMAL MODELS

Sensitization and Challenge Models
Sensitization and challenge models are widely used in vivo systems in allergy research to mimic the initiation and progression of allergic diseases. These models are critical for studying immune responses, understanding disease mechanisms, and evaluating potential therapies for allergic conditions such as asthma, rhinitis, and food allergies.

Principle The principle of sensitization and challenge models is based on the two-phase immune response to allergens: [fig.6]

1.Sensitization Phase: The immune system is exposed to an allergen (e.g., ovalbumin, house dust mites) to elicit an allergic sensitization.
This involves dendritic cell activation, T-helper 2 (Th2) cell polarization, and allergen-specific IgE production.

2.Challenge Phase: Re-exposure to the allergen triggers an allergic reaction mediated by mast cells, basophils, eosinophils, and Th2 cells. The response mimics human allergic symptoms such as airway inflammation, hyperresponsiveness, and mucus secretion.

Procedure Step 1: Animal Selection

Commonly used species:
Mice: Most frequently used due to genetic tractability and immune system similarity.
Rats: Preferred for studying airway hyperresponsiveness.
Other models include guinea pigs and rabbits for specific allergic conditions.

Step 2: Sensitization

1. Allergen Selection: Protein allergens: Ovalbumin (OVA), house dust mite (HDM), peanut proteins. Adjuvants (optional): Aluminium hydroxide (alum) enhances immune responses.

2. Allergen Administration:
Routes: **Intraperitoneal** (i.p.): Used with alum to enhance sensitization.
Subcutaneous (s.c.): Preferred for skin sensitization models.
Intranasal or inhalation: Mimics natural allergen exposure for respiratory allergies.
Dosage and Duration: Typical protocols involve 2–3 sensitization doses over 1–2 weeks.

3. Outcome: Induction of allergen-specific IgE and Th2 polarization.

Step 3: Challenge

1. **Allergen Re-exposure:** Administer the allergen via inhalation, intranasal, or oral routes, depending on the allergic condition being modelled Frequency and duration: Acute models: Single or short-term allergen exposure. Chronic models: Repeated allergen challenges over weeks to months.
2. **Assessment of Allergic Response:** Time post-challenge varies: Immediate reactions (minutes to hours) or late-phase responses (24–48 hours).

Step 4: Evaluation of Allergic Responses

1. Immune Responses: Measure allergen-specific IgE, cytokines (IL-4, IL-5, IL-13), and eosinophil levels.

2. Pathophysiological Changes:
Asthma Models: Assess airway hyperresponsiveness using plethysmography.
Analyse lung histology for eosinophilic infiltration and mucus production.

© Journal of Global Trends in Pharmaceutical Sciences

Fig.3 anti allergic models list

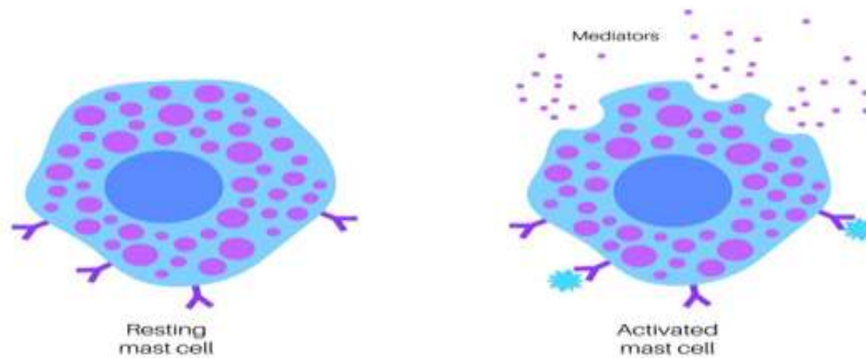

Fig.4 Mast cell activation by IgE antibody

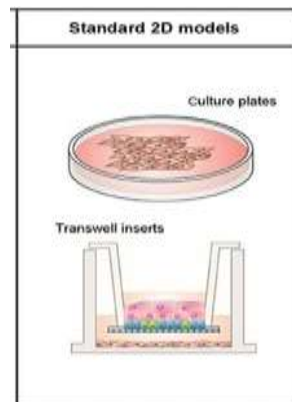

Fig.5 transwell inserts

© Journal of Global Trends in Pharmaceutical Sciences

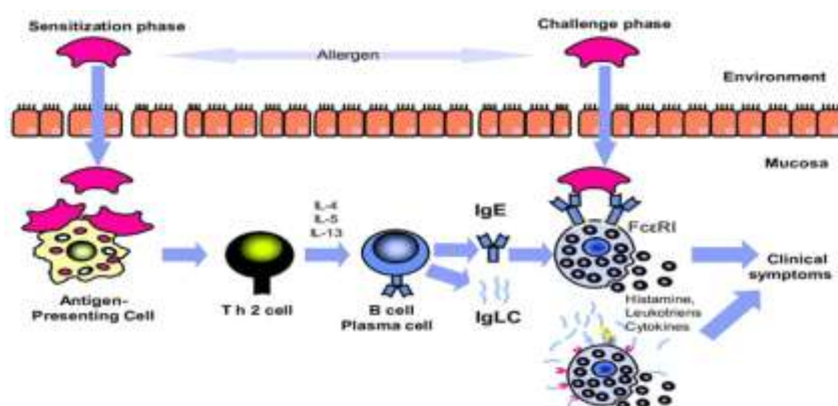

Fig.6 demonstration of sensitization and challenge phase

Food Allergy Models: Observe symptoms like diarrhoea or anaphylaxis. Monitor mast cell degranulation markers (histamine, β-hexosaminidase).

3. **Therapeutic Testing:** Administer drugs (e.g., corticosteroids, anti-IL-5 biologics) before or after allergen challenges to evaluate efficacy.

Mechanism

1. **Sensitization Phase**

***Allergen Uptake**: Dendritic cells capture the allergen and migrate to lymph nodes.

***Th2 Polarization:** Dendritic cells present the allergen to naïve CD4+ T cells via MHC class II. IL-4 drives Th2 differentiation.

***IgE Production:** Th2 cells produce IL-4 and IL-13, promoting class-switching in B cells to produce allergen-specific IgE. IgE binds to FcεRI on mast cells, priming them for the challenge phase.

2. **Challenge Phase**

***Allergen Recognition:** Re-exposure leads to allergen binding to IgE on mast cells.

***Mast Cell Degranulation:** Release of histamine, leukotrienes, prostaglandins, and cytokines causes acute allergic symptoms.

***Late-Phase Response:** Eosinophils, basophils, and Th2 cells infiltrate tissues, releasing additional mediators (IL-5, IL-13).

This prolongs inflammation and causes tissue damage (e.g., airway remodelling in asthma).[11] [12] [13]

Mouse Models of Asthma Mouse models of asthma are widely used to study the pathogenesis, immune responses, and therapeutic strategies for asthma. These models replicate key features of human asthma, such as airway inflammation, eosinophilia, mucus hypersecretion, and airway hyperresponsiveness (AHR).

Principle The principle of mouse models of asthma is to simulate human asthma by exposing mice to allergens, inducing sensitization, and subsequent airway challenges. This leads to immune responses dominated by Th2 cells, resulting in airway inflammation and remodelling. Key Features of the Models: Sensitization: Initiates an adaptive immune response to allergens. Challenge: Triggers acute and chronic asthma-like symptoms. Assessment: Measures inflammatory and physiological changes, including cytokine production, airway responsiveness, and histological changes.

Procedure

Step 1: Selection of Mice Strain: BALB/c mice are commonly used because they exhibit strong Th2-skewed responses. C57BL/6 mice are also used for Th1/Th17 studies. Age and Sex: Typically, 6–8-week-old mice are preferred. Both sexes can be used, depending on the study objective.

Step 2: Allergen Sensitization

1. Allergen Preparation: Common allergens: Ovalbumin (OVA), house dust mites (HDM), cockroach extract, or fungal spores. Adjuvant: Aluminium

© Journal of Global Trends in Pharmaceutical Sciences

hydroxide (alum) is often used to enhance sensitization.
2. Route of Administration: Intraperitoneal (i.p.) injection with allergen + alum. Alternatives: Intranasal or subcutaneous routes.
3. Frequency:

Sensitization typically involves two or more allergen doses over 7–14 days.

Step 3: Airway Challenge

1. Allergen Re-exposure: Mice are challenged with the allergen (e.g., via intranasal or aerosolized exposure). Frequency: Daily or every other day for 1–2 weeks, depending on the model.
2. Control Group: Use a phosphate-buffered saline (PBS)-challenged group for comparison.

Step 4: Assessment of Asthma Features

1. Airway Hyperresponsiveness (AHR): Measure airway resistance using invasive plethysmography or non-invasive whole-body plethysmography after methacholine exposure.
2. Cytokine and Immune Cell Analysis: Collect bronchoalveolar lavage fluid (BALF) to measure: Immune cells (eosinophils, neutrophils, macrophages, and lymphocytes). Cytokines (e.g., IL-4, IL-5, IL-13) via ELISA or multiplex assays.
3. Histological Analysis: Examine lung tissue for: Eosinophil infiltration. Goblet cell hyperplasia (mucus production). Subepithelial fibrosis (airway remodelling).

Step 5: Therapeutic Interventions

1. Administer drugs such as: Corticosteroids: Reduce inflammation.

Biologics: Target IL-5, IL-4, or IL-13 pathways. Bronchodilators: Relax airway smooth muscles. Evaluate their effect on asthma symptoms and inflammatory markers.

Mechanism

1. Sensitization Phase

1. Antigen Presentation: Dendritic cells take up allergens and migrate to lymph nodes.
2. Th2 Polarization: Allergen presentation by dendritic cells leads to naïve CD4+ T cell differentiation into Th2 cells.
3. IgE Production:

Th2 cytokines (IL-4, IL-13) stimulate B cells to produce allergen-specific IgE

IgE binds to FcεRI on mast cells, sensitizing them for the challenge phase.

2. Challenge Phase

1. Allergen Recognition: Re-exposure to allergens cross-links IgE on mast cells.
2. Mediator Release: Mast cells degranulate, releasing histamine, leukotrienes, and prostaglandins.
3. Inflammatory Cascade: Recruitment of eosinophils, Th2 cells, and other immune cells amplifies inflammation.IL-5 drives eosinophil recruitment. IL-13 induces mucus hypersecretion and airway remodelling.
4. Airway Hyperresponsiveness (AHR) Mediator-induced smooth muscle contraction narrows airways. Persistent inflammation exacerbates bronchial sensitivity to stimuli like methacholine. [14] [15] [16]

●Anaphylaxis Models

Anaphylaxis models are in vivo systems designed to mimic the severe, systemic allergic reactions observed in humans. These models are essential for understanding the underlying mechanisms of anaphylaxis, studying its progression, and testing emergency treatments or preventive therapies.

Principle: The principle of anaphylaxis models is based on triggering hypersensitivity reactions through allergen-specific IgE-mediated mast cell degranulation or IgG-mediated immune complex formation. These reactions lead to the release of inflammatory mediators, causing symptoms such as hypotension, airway obstruction, and, in severe cases, death.

Types of Anaphylaxis Models: Passive Anaphylaxis: Involves the transfer of allergen-specific IgE or IgG antibodies into the animal

© Journal of Global Trends in Pharmaceutical Sciences

before allergen challenge. Used to isolate the effects of pre-formed antibodies. Active Anaphylaxis: Involves immunizing the animal with an allergen to elicit endogenous IgE or IgG production. Mimics the natural sensitization process.

Procedure

Step 1: Animal Selection Species: Mice are the most commonly used due to their well-characterized immune system and availability of transgenic model Guinea pigs are also used, as they closely mimic human anaphylaxis symptoms. Strain: BALB/c and C57BL/6 mice are frequently chosen.

Step 2: Sensitization (Active Anaphylaxis)

1. Allergen Administration: Allergen examples: Ovalbumin (OVA), house dust mite extract, or food allergens (e.g., peanuts). Route: Intraperitoneal (i.p.) injection with an adjuvant like alum. Subcutaneous or oral administration, depending on the type of allergy.
2. Timeline: Sensitization typically occurs over 1–2 weeks with multiple doses of the allergen.

Step 3: Antibody Transfer (Passive Anaphylaxis) IgE Transfer: Inject purified allergen-specific IgE or polyclonal IgE antibodies intravenously or intraperitoneally. Typically performed 24 hours before allergen challenge.

1. IgG Transfer: Inject allergen-specific IgG to evaluate IgG-mediated anaphylaxis pathways.

Step 4: Allergen Challenge

1. Allergen Re-exposure: Administer the allergen intraperitoneally, intravenously, or via aerosolization.
2. Timing: Immediate hypersensitivity reactions occur within minutes post-challenge.
3. Dosage: Use a dose that reliably elicits symptoms without causing excessive mortality in untreated animals.

Step 5: Monitoring and Assessment

1. Physiological Parameters: Measure changes in blood pressure, body temperature, and respiratory rate.

Hypotension and hypothermia are primary indicators of systemic anaphylaxis.

2. Biochemical Markers:

Quantify serum levels of histamine, tryptase, and leukotrienes. Cytokines like TNF-α and IL-6 can be measured using ELISA.

3. Survival Analysis: Observe animals over a defined period to assess mortality and recovery.

Step 6: Therapeutic Testing Administer emergency treatments such as: Epinephrine: Standard for reversing hypotension and bronchoconstriction. Anti-histamines: Block histamine effects. Biologics: Monoclonal antibodies targeting IgE, mast cell receptors, or cytokines.

Mechanism

1. **Allergen-Specific IgE and Mast Cell Activation (Classic Pathway)**
   1. Sensitization: Allergen-specific IgE binds to high-affinity FcεRI receptors on mast cells and basophils.
   2. Allergen Challenge:

Cross-linking of IgE on mast cells triggers rapid degranulation. Mediator Release: Histamine, leukotrienes, prostaglandins, and cytokines are released.

Effects: Histamine: Causes vasodilation, increased vascular permeability, and smooth muscle contraction. Leukotrienes and Prostaglandins: Exacerbate airway constriction and inflammation.

2. **IgG and FcγRIII-Mediated Pathway**

© Journal of Global Trends in Pharmaceutical Sciences

1. Immune Complex Formation: Allergen-specific IgG binds allergens, forming immune complexes.
2. FcγRIII Activation: Activates neutrophils and macrophages, causing systemic inflammation.
3. **Cytokine Release:** TNF-α and IL-6 contribute to vascular leakage and shock.
4. **Downstream Effects**

1. Hypotension: Increased vascular permeability causes fluid leakage, reducing blood volume.
2. Bronchoconstriction: Mediator-induced smooth muscle contraction in airways leads to respiratory distress.
3. Hypothermia: Mediator release affects thermoregulation in severe reactions.[17] [18] [19][20]

Eosinophilic Inflammation Models: Eosinophilic inflammation models are commonly used in allergy research to study diseases like asthma, eosinophilic esophagitis, and allergic rhinitis. These models simulate the recruitment and activation of eosinophils, which are key mediators of allergic inflammation.

Principle: The principle of eosinophilic inflammation models lies in the induction of allergic inflammation through exposure to allergens or cytokines. These stimuli recruit eosinophils to tissues and activate them to release toxic granules, lipid mediators, and cytokines, resulting in tissue damage and inflammation.

Key Features:

1. Eosinophil Recruitment: Driven by Th2 cytokines (e.g., IL-5, IL-13) and chemokines (e.g., eotaxin).
2. Eosinophil Activation Activated eosinophils release inflammatory mediators like major basic protein (MBP) and eosinophil peroxidase (EPO).

Procedure Step 1: Animal Selection: Species: Mice are most commonly used due to genetic tools (e.g., IL-5 transgenic or eosinophil-deficient mice). Guinea pigs and rats are also used for airway inflammation studies. Strain: BALB/c mice (Th2-skewed response) or C57BL/6 mice (moderate eosinophilic response).

Step 2: Sensitization: Allergen Administration: Common allergens include: Ovalbumin (OVA): Widely used in asthma models. House dust mite (HDM): Mimics natural allergens. Ragweed or fungal spores for seasonal allergies. Route: Intraperitoneal (i.p.) injection with or without alum adjuvant. Intranasal (i.n.) or aerosolized administration for respiratory models. Timing: Sensitization is usually done over 1–2 weeks with 2–3 doses. Cytokine Stimulation (alternative approach): Direct administration of IL-5 or eotaxin can bypass sensitization to recruit eosinophils directly.

Step 3: Challenge Allergen Re-exposure: Administer allergens intranasally, by inhalation, or via subcutaneous injection.

1. Dose and Frequency: Acute models: Single or short-term allergen exposure. Chronic models: Repeated challenges over weeks to months.

Step 4: Assessment of Eosinophilic Inflammation Broncho alveolar Lavage Fluid (BALF) Analysis: Collect BALF to quantify eosinophils and other immune cells. Measure cytokines (e.g., IL-4, IL-5, IL-13) and chemokines (e.g., exotoxin).

1. Tissue Analysis: Perform histology on lungs, oesophagus, or skin to assess eosinophilic infiltration. Stain eosinophils using haematoxylin and eosin (H&E) or specific markers (e.g., MBP).
2. Molecular Analysis: Use ELISA or PCR to measure cytokines, chemokines, and other markers of eosinophilic inflammation.

Step 5: Therapeutic Testing Administer therapeutic agents such as: Biologics: Anti-IL-

© Journal of Global Trends in Pharmaceutical Sciences

5 (mepolizumab), anti-IL-13 (tralokinumab). Small Molecules: Chemokine receptor antagonists (e.g., CCR3 inhibitors). Steroids: Reduce eosinophil recruitment and activation. Evaluate their impact on eosinophil levels and inflammation severity.

Mechanism: Recruitment of Eosinophils

1. Th2 Cytokine Production: Allergens activate dendritic cells to prime Th2 cells, leading to IL-5 and IL-13 production. IL-5 promotes eosinophil maturation and release from the bone marrow.
2. **Chemokine Signalling**: Eotaxin (CCL11) binds CCR3 receptors on eosinophils, driving their migration to inflamed tissues.
3. **Eosinophil Activation**

1. Adhesion and Infiltration: Eosinophils adhere to vascular endothelium and transmigrate into tissues.
2. Mediator Release: Cytotoxic Granules: Major basic protein (MBP): Damages epithelial cells.

Eosinophil cationic protein (ECP): Induces tissue remodelling. Cytokines and Chemokines: IL-13 amplifies mucus secretion. TNF-α recruits additional inflammatory cells. Lipid Mediators: Leukotrienes (e.g., LTC4) contribute to bronchoconstriction and inflammation. Tissue Damage and Remodelling Chronic inflammation leads to fibrosis, epithelial barrier dysfunction, and airway remodeling (e.g., thickened smooth muscle). [21] [22] [23] [24]

Skin Allergy Models: Skin allergy models are essential tools to study allergic conditions such as atopic dermatitis (AD), contact dermatitis, and other cutaneous hypersensitivities. These models mimic immune and inflammatory responses in the skin and are used to investigate pathogenesis and evaluate potential treatments.

Principle: The principle of skin allergy models lies in inducing allergic skin inflammation through exposure to allergens, irritants, or sensitizers. These agents disrupt the skin barrier, trigger immune cell recruitment, and promote the release of inflammatory mediators.

Key Features:

1. Barrier Dysfunction: Allergens compromise the epidermal barrier, allowing immune activation.
2. Immune Responses: Allergens trigger dendritic cell activation, leading to Th2 or Th1 immune responses.
   3.Chronic Inflammation: Repeated allergen exposure results in chronic inflammation, resembling human skin allergies.

Procedure Step 1: Animal Selection Species: Mice are the most commonly used models due to genetic tools and similarities to human immune responses. Rats, guinea pigs, and pigs are used in specific cases. Strains: BALB/c mice (Th2-skewed responses) for atopic dermatitis models. C57BL/6 mice for Th1/Th17 inflammation studies.

Step 2: Induction of Skin Allergy

1. Allergens/Sensitizers: Common agents: Atopic Dermatitis Models: House dust mites (HDM), Staphylococcus aureus-derived antigens. Ovalbumin (OVA) as a protein allergen. Contact Dermatitis Models: Dinitrochlorobenzene (DNCB), oxazolone, or urushiol (poison ivy component).
2. Application Method: Topical: Apply allergens directly to the shaved or tape-stripped skin. Intradermal Injection: Inject allergens to induce localized inflammation.
3. Use of Adjuvants: Aluminium hydroxide (alum) or lipopolysaccharide (LPS) may be added to enhance immune activation.

Step 3: Chronic Inflammation (Optional) Repeated exposure to allergens at regular

© Journal of Global Trends in Pharmaceutical Sciences

intervals (e.g., twice weekly for 3–4 weeks) mimics chronic allergic inflammation.

Step4: Assessment of Skin Allergy

1. Clinical Observations: Measure skin thickness, redness, oedema, and lesion severity. Use scoring systems such as the Eczema Area and Severity Index (EASI) for atopic dermatitis.
2. Histological Analysis: Examine skin biopsies for: Epidermal hyperplasia. Immune cell infiltration (eosinophils, T cells, mast cells). Collagen deposition and fibrosis.
3. Cytokine and Chemokine Measurement: Collect skin tissue or serum to measure: Th2 cytokines (IL-4, IL-13). Pro-inflammatory cytokines (IL-17, TNF-α). Chemokines (e.g., CCL17, CCL22). Barrier Function Tests: Trans epidermal Water Loss (TEWL): Measures skin barrier integrity. Dye Penetration Assay: Visualizes barrier disruption.

Step 5: Therapeutic Interventions Administer treatments like: Topical Agents: Corticosteroids, calcineurin inhibitors (e.g., tacrolimus). Biologics: Anti-IL-4/IL-13 (e.g., dupilumab) or anti-IL-17 therapies. Small Molecules: JAK inhibitors or PDE4 inhibitors. Monitor reduction in inflammation, lesion size, and cytokine levels.

Mechanism

1. **Allergen-Induced Immune Activation** Barrier Disruption: Allergens compromise tight junctions and corneocytes in the stratum corneum. This allows allergens to penetrate and activate keratinocytes.

2. Keratinocyte Signalling**:** Activated keratinocytes release thymic stromal lymphopoietin (TSLP), IL-33, and IL-25, recruiting immune cells.

2.**Dendritic Cell Activation and T-Cell Polarization** Antigen Presentation: Langerhans cells and dermal dendritic cells capture allergens and migrate to lymph nodes.

1. Th2 Dominance: Naïve T cells differentiate into Th2 cells under the influence of IL-4 and IL-13.

Th2 cells produce cytokines that drive IgE production and eosinophil recruitment.

2. **Inflammatory Mediator Release**
1. Mast Cell Degranulation: gE binds to FcεRI on mast cells. Allergen cross-linking triggers histamine and prostaglandin release. Eosinophil Activation: IL-5 recruits eosinophils, which release toxic granules (e.g., major basic protein, eosinophil peroxidase).
2. Chronic Inflammation: Persistent Th2/Th1 responses promote fibrosis, epidermal hyperplasia, and lichenification.
1. **Late-Phase and Chronic Responses:** Th17 and Th1 Contribution: IL-17 and IFN-γ amplify inflammation, disrupt barrier function, and sustain chronic lesions.
2. Tissue Remodelling: Fibroblast activation by TGF-β contributes to dermal thickening and scarring.[25] [26] [27] [28]

Genetically Modified Animal Models: Genetically modified (GM) animal models are essential tools for studying allergic diseases, enabling precise dissection of molecular and cellular mechanisms. These models are created by altering specific genes associated with allergic responses, such as those involved in cytokine signalling, antibody production, or immune cell activation.

Principle The principle of genetically modified animal models is based on manipulating specific genes to either study their roles in allergy development or assess therapeutic interventions targeting those pathways.

© Journal of Global Trends in Pharmaceutical Sciences

Key Approaches: Gene Knockout (KO): Eliminating specific genes to understand their contribution to allergic responses (e.g., IL-4 KO for Th2 immunity). Transgenic (TG) Models: Introducing foreign or overexpressing specific genes (e.g., IL-5 TG mice for eosinophilia studies).

1. Conditional Knockouts (cKO): Gene deletion in specific tissues or cells using Cre-loxP systems (e.g., mast cell-specific FcεRI deletion).
2. Humanized Models: Replacing mouse genes with human genes (e.g., human IgE receptor FcεRI) to test therapies.

Procedure Step 1: Generation of Genetically Modified Animals Gene Knockout (KO): CRISPR-Cas9: Target-specific DNA sequences are edited using CRISPR for rapid KO generation. Example: IL-4 KO mice for studying Th2-driven allergies. Homologous Recombination: Embryonic stem (ES) cells are genetically modified and injected into blastocysts. Example: FcεRI KO mice to study IgE-mediated mast cell activation.

1. Transgenic (TG) Animals: A foreign gene is inserted into the genome using pronuclear injection or lentiviral vectors. Example: IL-5 TG mice for studying eosinophilic inflammation.
2. Conditional Knockouts (cKO): Cre-loxP System: LoxP sites flank the target gene, and tissue-specific Cre recombinase deletes it. Example: T-cell-specific IL-13 KO to study airway inflammation.
3. Humanized Models: Replace mouse genes with human counterparts (e.g., human FcεRIα in mast cells).

Step 2: Breeding and Validation Breeding strategies ensure homozygosity or maintenance of desired traits Validation: PCR or Southern blot for gene modification. qPCR or Western blot to confirm target gene expression.

Step 3: Experimental Protocols Sensitization and Challenge: use allergens like ovalbumin (OVA), house dust mites (HDM), or food allergens Routes: Intraperitoneal (i.p.), intranasal, or oral administration.

1. Therapeutic Testing: Administer biologics, small molecules, or immunotherapies targeting the altered gene pathway.

Step 4: Data Collection

1. Immune Response Analysis: Measure cytokine production (e.g., IL-4, IL-13). Assess immune cell populations using flow cytometry.
2. Physiological and Histological Assessment: Examine tissue inflammation, fibrosis, and immune infiltration.
3. Behavioral Analysis:
4. Monitor clinical symptoms like scratching, airway resistance, or anaphylaxis severity.

Mechanism

1. Role of Genetically Modified Genes in Allergies

1. Cytokine Pathways: IL-4 and IL-13 are critical for Th2 differentiation, IgE class switching, and mucus hypersecretion. Example: IL-4 KO mice show reduced IgE levels and Th2 responses.
2. IgE and Mast Cell Function FcεRI KO mice lack IgE receptor signaling, preventing mast cell degranulation.
3. Eosinophil Recruitment: IL-5 TG mice exhibit increased eosinophil production and tissue infiltration.

2. Modeling Human Allergies

1. Humanized FcεRI Models: Mimic human IgE interactions for testing anti-IgE therapies (e.g., omalizumab).
2. T-cell-Specific Modifications: Conditional KO models dissect Th1/Th2 balance in airway inflammation.
3. Dendritic Cell Pathways: TSLP-overexpressing mice study epithelial-

© Journal of Global Trends in Pharmaceutical Sciences

immune interactions in atopic dermatitis.

4. Testing Therapies in Modified Models Targeting specific pathways provides insights into therapeutic mechanisms: Anti-IL-5 biologics in eosinophilic models. Anti-IL-4Rα therapies (e.g., dupilumab) in Th2-skewed models. [29] [30] [31] [32]

CONCLUSION:

Both in vitro and in vivo animal models are indispensable tools for advancing allergy research. In vitro models are useful for studying cellular and molecular responses to allergens in a controlled setting, allowing for the testing of specific compounds or biological agents. Meanwhile, in vivo models enable researchers to examine the broader physiological and immune system responses, providing more comprehensive data on the efficacy and safety of potential treatments. Together, these models provide valuable insights that can lead to better therapies for allergic diseases in humans

1. REFERENCES:

1. Galli, S. J., Tsai, M., & Piliponsky, A. M. (2008). The development of allergic inflammation. Nature, 454(7203), 445–454.

2. Brown, M. A., & Britten, K. L. (2013). Mast Cells and Their Role in the Pathogenesis of Allergic Diseases. In Allergy: Principles and Practice (7th ed., pp. 881-896

3. Kraft, S., & Kinet, J. P. (2007). New developments in FcεRI regulation and signaling. Nature Reviews Immunology, 7(5), 365–378.

4. Schwartz, L. B. (2003). Mast cell tryptase. Methods in Enzymology, 370, 105 123.

5. Oettgen, H. C. (2009). Mechanisms of mast cell-mediated allergic disease. Nature Reviews Immunology, 9(6), 473–482.

6. Banchereau, J., & Steinman, R. M. (1998). Dend

7. Lambrecht, B. N., & Hammad,H. (2014). The airway epithelium in asthma. Nature Medicine

8. Galli, S. J., & Tsai, M. (2008). "The development of allergic inflammation." Nature, 454(7203), 445–454.

9. Kraft, S., & Kinet, J. P. (2007). "New developments in FcεRI regulation and signaling." Nature Reviews Immunology, 7(5), 65–378.

10. Oettgen, H. C. (2009). "Mechanisms of mast cell-mediated allergic disease." Nature Reviews Immunology, 9(6), 473–482.

11. Galli, S. J., & Tsai, M. (2008). "The development of allergic inflammation." Nature, 454(7203), 445–454.

12. Kraft, S., & Kinet, J. P. (2007). "New developments in FcεRI regulation and signaling." Nature Reviews Immunology, 7(5), 365–378.

13. Lambrecht, B. N., & Hammad, H. (2015). "The immunology of the allergy epidemic and the hygiene hypothesis." Nature Immunology, 16(6), 364–370.

14. Holgate, S. T., & Polosa, R. (2008). "The mechanisms, diagnosis, and management of severe asthma in adults." The Lancet, 368(9537), 780–793.

15. Galli, S. J., & Tsai, M. (2008). "IgE and mast cells in allergic disease." Nature Medicine, 18(5), 693–704.

16. Lambrecht, B. N., & Hammad, H. (2015). "The immunology of the allergy epidemic and the hygiene hypothesis." Nature Immunology, 16(6), 364–370.

17. Galli, S. J., & Tsai, M. (2008). "IgE and mast cells in allergic disease." Nature Medicine, 18(5), 693–704.

18. Kita, H. (2011). "Eosinophils: Multifaceted biological properties and roles in

© Journal of Global Trends in Pharmaceutical Sciences

health and disease." Immunological Reviews, 242(1), 161–177.

19. Oettgen, H. C. (2009). "Mechanisms of mast cell-mediated allergic disease." Nature Reviews Immunology, 9(6), 473–482.
20. Stone, S. F., et al. (2010). "Anaphylaxis: Pathophysiology, diagnosis, and treatment." Immunology and Allergy Clinics of North America, 30(2), 275–293.

21. Fulkerson, P. C., & Rothenberg, M. E. (2013). "Targeting eosinophils in allergy, inflammation, and beyond." Nature Reviews Drug Discovery, 12(2), 117–129.
22. Jacobsen, E. A., et al. (2012). "Eosinophils: Regulators of innate and adaptive immunity, and contributors to the resolution of inflammation." Immunological Reviews, 242(1), 81–90.

23. Yamaguchi, Y., et al. (1991). "Interleukin-5 regulates eosinophil function in asthma and other diseases." Immunology Today, 12(11), 404–407.
24. Lambrecht, B. N., & Hammad, H. (2015). "The immunology of asthma." Nature Immunology, 16(1), 45–56.
25. Nomura, T., et al. (2003). "Role of interleukin-4 and interleukin-13 in skin diseases and barrier dysfunction." The Journal of Allergy and Clinical Immunology, 113(4), 556–562.
26. Nakajima, S., & Kitoh, A. (2015). "The role of Th2 cytokines and the TSLP-OX40L pathway in atopic dermatitis." Immunological Reviews, 274(1), 296–306.
27. Kim, B. E., et al. (2013). "Atopic dermatitis: The skin barrier and beyond." Allergy, 68(1), 47–55.
28. Werfel, T., et al. (2016). "Role of immune cells and keratinocytes in atopic dermatitis." Journal of Allergy and Clinical Immunology, 138(2), 336–349.

29. Finkelman, F. D., et al. (1999). "Cytokines and their role in allergic diseases." Journal of Allergy and Clinical Immunology, 103(2), 101–112.

30. Schwartz, L. B., & Austen, K. F. (1980). "Fc receptors on mast cells and basophils." The Journal of Clinical Investigation, 65(1), 2–13.
31. Munitz, A., et al. (2009). "IL-5 and eosinophils: A comprehensive review of their role in allergic diseases." Clinical and Experimental Allergy, 39(1), 12–20.

32. Ebright, M. I., et al. (2016). "Humanized mouse models for IgE and mast cell research." Journal of Immunology Research, 2016, 8423231.

33. American Academy of Allergy, Asthma & Immunology (AAAAI) https://www.aaaai.org

34. Mayo Clinic [Allergy Symptoms and Causes](https://www.mayoclinic.org)"

35. Nature Reviews Immunology** * Articles on the role of animal models in understanding allergies and immune responses. * [Nature Reviews Immunol](https://www.nature.com/nri)"

36. Schwartz, L. B. (2003). Mast cell tryptase. Methods in Enzymology, 370, 105-123.

© Journal of Global Trends in Pharmaceutical Sciences