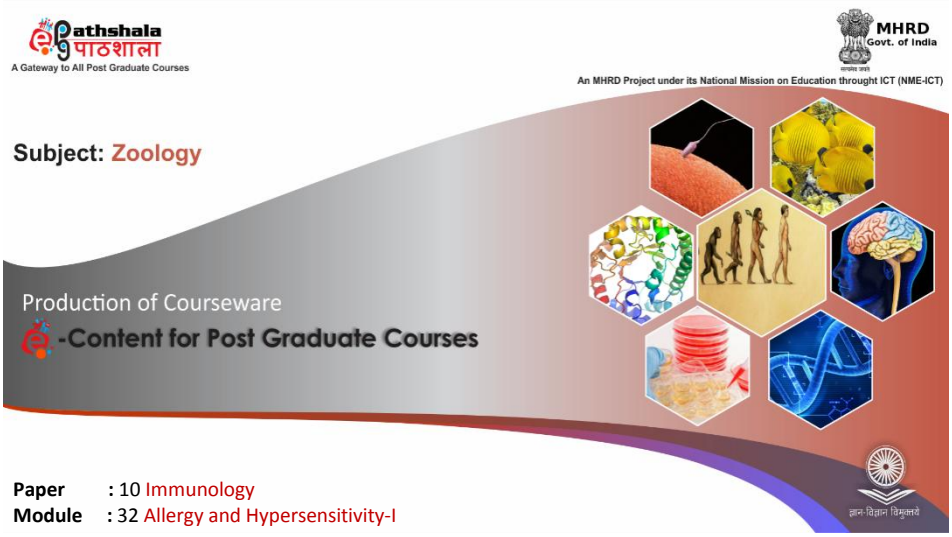

Paper : 10 Immunology
Module : 32 Allergy and Hypersensitivity-I

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Description of Module	
Subject Name	ZOOLOGY
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Keywords	Allergen, Allergy, Anaphylaxis, Atopy, Basophils, Hypersensitivity, Mast Cells, Degranulation, Systemic Anaphylaxis, Localized Anaphylaxis, Hay Fever, Asthma, Hyposensitization, RIST, RAST

Contents

1. Learning Outcomes

After studying this module, you shall be able to

- **Know:** What is allergy? How is it different from anaphylaxis? What are hypersensitive reactions?
- **Learn:** How dysregulated immune system or hypersensitivity affects us.
- **Identify:** The components and mechanisms that mediate such hypersensitive response.
- **Analyze:** The underlying beauty of our innate immune system in terms of its regulation.

2. Introduction

- The word 'allergy' originates from Greek word 'allos', meaning 'other or different', and 'ergia', meaning 'energy or action', in the sense of "change in reactivity or capacity to react".
- The concept of allergy was given by a Viennese Paediatrician, Von Pirquet in 1906 in a classic article entitled 'Allergie'.
- According to Pirquet, an antigen causes change in reactivity which can be protective i.e. immunity or harmful i.e. hypersensitivity.
- Richet and Portier proposed a different term 'anaphylaxis' in contrast to prophylaxis for the 'over-reaction' of immune system.
- A. F. Coca and R, A. Cooke proposed a general term 'Hypersensitivity' to resolve the contradiction between allergy and anaphylaxis.
- Hypersensitivity is a general term for inappropriate, deleterious immune response raised against an allergen. Gell and Coomb proposed a classification in 1963 according to which different hypersensitive reactions can be classified in four types.
- The classification is based on the type of immune branch involved i.e. humoral or cell-mediated (type I, II and III within humoral branch and type IV in cell-mediated branch); time taken to show symptoms i.e. immediate or delayed (type I, II and III are immediate type and type IV is delayed type hypersensitivity); immune mechanisms, cells and mediator molecules involved.
- Type-I hypersensitivity is IgE based allergic reaction raised against allergens. It is a humoral immunity based immediate type hypersensitive reaction. Ex. Asthma.
- Type-II hypersensitivity is IgG mediated cytotoxic hypersensitivity which is also humoralimmunity based immediate type hypersensitive reaction. Ex. *Erythroblastosis fetalis*
- Type-III hypersensitivity is immune complex mediated, humoral, immediate type hypersensitivity. Ex. Rheumatoid arthritis.

- Type-IV hypersensitivity is cell-mediated hypersensitivity. It is cell mediated immunity based, delayed type hypersensitive reaction. Ex. Contact dermatitis.
- Basic components of type-I hypersensitivity are allergens, immune cell, Cytokines, IgE, Fc receptors for IgE and various mediator molecules. The degranulation of basophils and mast cells is the key point of type-I hypersensitivity.
- Clinical manifestation of type-I hypersensitivity includes systemic anaphylaxis and localized anaphylaxis i.e. allergic dermatitis, hay fever, asthma etc.
- Various factors regulate type-I hypersensitive response such as: dose and type of allergen, processing of allergen, genetic constitution of the individual and the relative ratio of T_H1 and T_H2 cells.
- Wheel and flare test, Radioimmunosorbent assay (RIST) and Radioallergosorbent assay (RAST) are common medical tests to detect the type-I hypersensitive individuals for a particular allergen.
- The treatment/ precaution involves avoidance of allergen, modulation of immunological response, stabilization of the triggering cells, antagonism of mediators andattacking chronic inflammation. Various drugs are available to cure type-I hypersensitive reactions now.

3. Concept of allergy and anaphylaxis

The allergy (from Greek word allos, meaning 'other or different', and ergia, meaning 'energy or action', in the sense of "change in reactivity or capacity to react") is a fundamental aspect of clinical immunology. The concept of allergy was given by a Viennese Paediatrician, Clemens Von Pirquet Freiher in 1906 in a classic article entitled 'Allergie'. According to the original idea of Von Pirquet, when an individual comes in contact with an antigen (e.g. pollens, germs, food particle etc.), a change in reactivity occurs. This change which was termed as 'allergy' by Von Pirquet, can induce a protective or harmful response. The protective response makes the individual immune to these antigens and hence called as 'immunity', which does not manifest any symptoms. On the other hand, there is a harmful response which shows symptoms or signs of contact with antigen, and is called as 'hypersensitivity'. The immunity and hypersensitivity can overlap and are result of same physiological response i.e. response of host immune system. A diagrammatic depiction of this idea is represented in Figure 1.

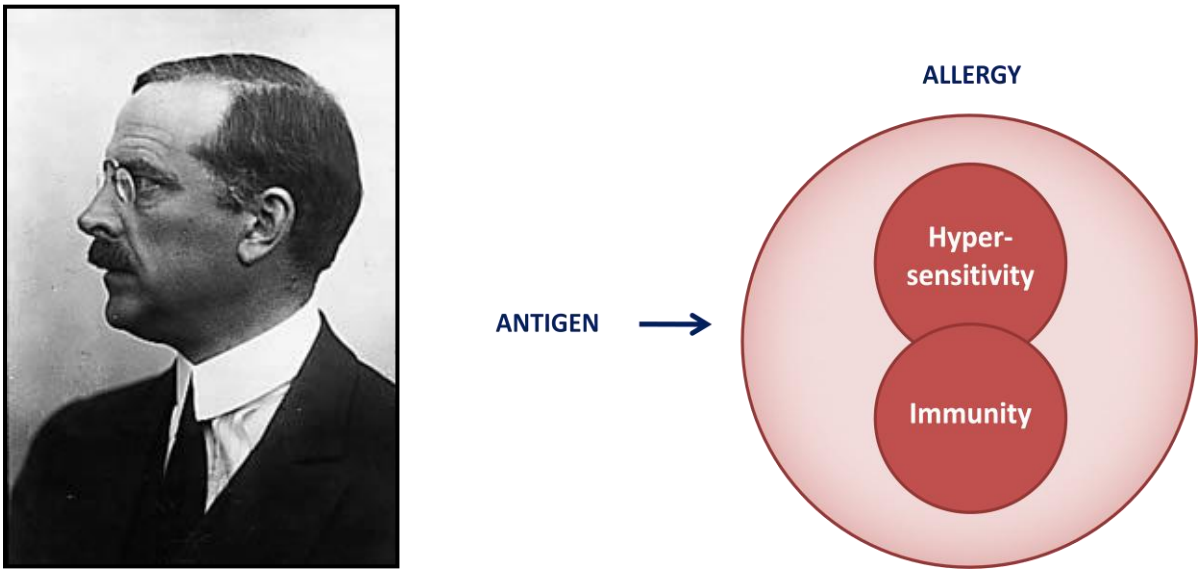


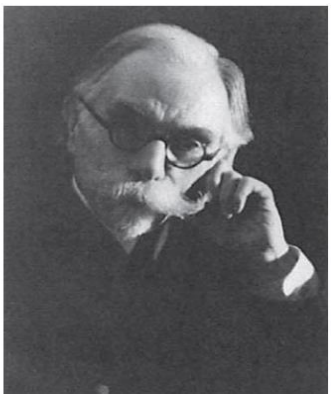
Figure 1: Clemens Von Pirquet Freiher (on left) and his idea of allergy (on right)
Source: https://en.wikipedia.org/wiki/Clemens_von_Pirquet

Unfortunately this new idea was not easily acceptable due to existing strength of idea of immunity. The revolutionary thought of Pirquet came at a time when immunology was establishing as science. The idea of immunity by notable scientists such as Pasteur, Ehrlich, Metchnikoff, Bordet, Behring etc., in nineteenth century hindered and delayed the acceptance of Von Pirquet's idea. Nobody could imagine at that time, that immune system can harm the host it protects.

The French Physiologists Charles Richet (1850-1935) and Paul Portier (1866-1962) rejected the term 'allergy' as they regarded it unnecessary new term in addition to 'anaphylaxis' that they already coined. These two scientists were suggested by Prince to study the toxic properties of Physalia ('Portuguese man of war' jelly fish) in the south sea in the laboratory aboard the Yacht 'Princesse Alice-II'. Richet and Portier continued their studies in Paris but due to unavailability of Physalia, they used Actinia. They experimented with the use of isolated jellyfish toxins as vaccines. They injected dogs with the purified toxins, followed by a booster. Instead of producing antibodies against the toxins, the dogs immediately showed vomiting, diarrhea, asphyxia, and even death. These dogs clearly "overreacted" to the antigen which was termed as anaphylaxis by Portier and Richet in contrast to prophylaxis, to describe this overreaction. Richet was awarded the Nobel Prize in Physiology or Medicine in 1913 in recognition of his work on anaphylaxis.

Von Pirquet and Richet's perspectives were different but since anaphylaxis was easier to understand, it gained more interest. The phenomenon of 'allergy' was much broader about the functioning of immune system and its involvement in diseases than 'anaphylaxis', an initial purely experimental phenomenon. During the search for active substance of anaphylaxis, it was realized that it's not only the effect of toxin but some principle in the organism itself is also involved. Sir Henry Dale (1875–1968) had major contribution in the elucidation of the same. He along with co-worker P.P. Laidlaw studied the physiological aspects of histamine and developed the concept of histamine shock as basis of anaphylaxis.


Charles Richet

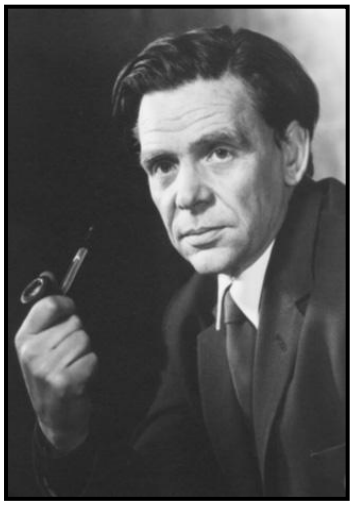

Paul Portier


Sir Henry Dale

Source: https://en.wikipedia.org

Arthur F. Coca and Robert A. Cooke published many articles in 1923-1926 to resolve the allergy and anaphylaxis contradictions. They preferred to employ the word "hypersensitiveness" as a general term. They divided hypersensitivity into normal type (i.e. contact dermatitis, serum sickness etc) and abnormal type (i.e. anaphylaxis, atopy etc.). Later medical community began to use 'allergy' for human hypersensitive reactions and 'anaphylaxis' for experimental animals.

There were no significant modifications in understanding of idea of 'allergy' or its use until 1963 when Philip George Houthem Gell (1914 –2001) and Robert Royston Amos (Robin) Coombs (1921-2006) classified hypersensitive reactions in their book 'Clinical aspects of Immunology'.



Philip George Houthem Gell

Robert Royston Amos (Robin) Coombs

Source: https://en.wikipedia.org

4. Hypersensitivity

The primary aim of an immune response is to remove the exogenous/ endogenous antigen by various mechanisms using effector molecules (Fig. 2). The body homeostasis is re-gained after a localized inflammation and removal of antigen without causing a damage to host tissue. But under certain circumstances, this beneficial inflammatory response can cause deleterious outcomes including host tissue injury, disease or even death. Such a harmful inappropriate immune response is termed as 'hypersensitive response/ reaction' or simply 'hypersensitivity'. The word 'hypersensitive' implies that a person is excessively/ more sensitive to an antigen as compared to normal response shown by other persons. Though it indicates towards an elevated/ increased immune response but it's not the case always. An inappropriate immune response is also considered as hypersensitivity besides a heightened immune response.

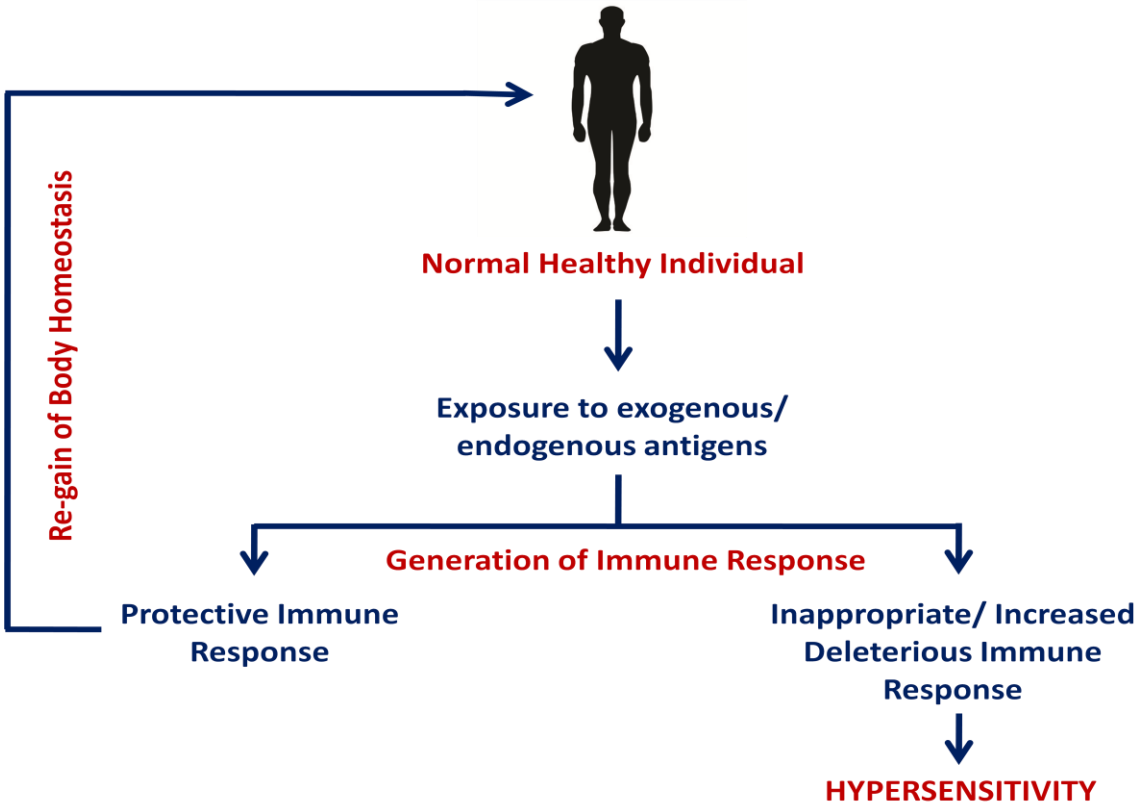


Figure 2: A generalized view to represent protective immune response and deleterious hypersensitive response Source: Author

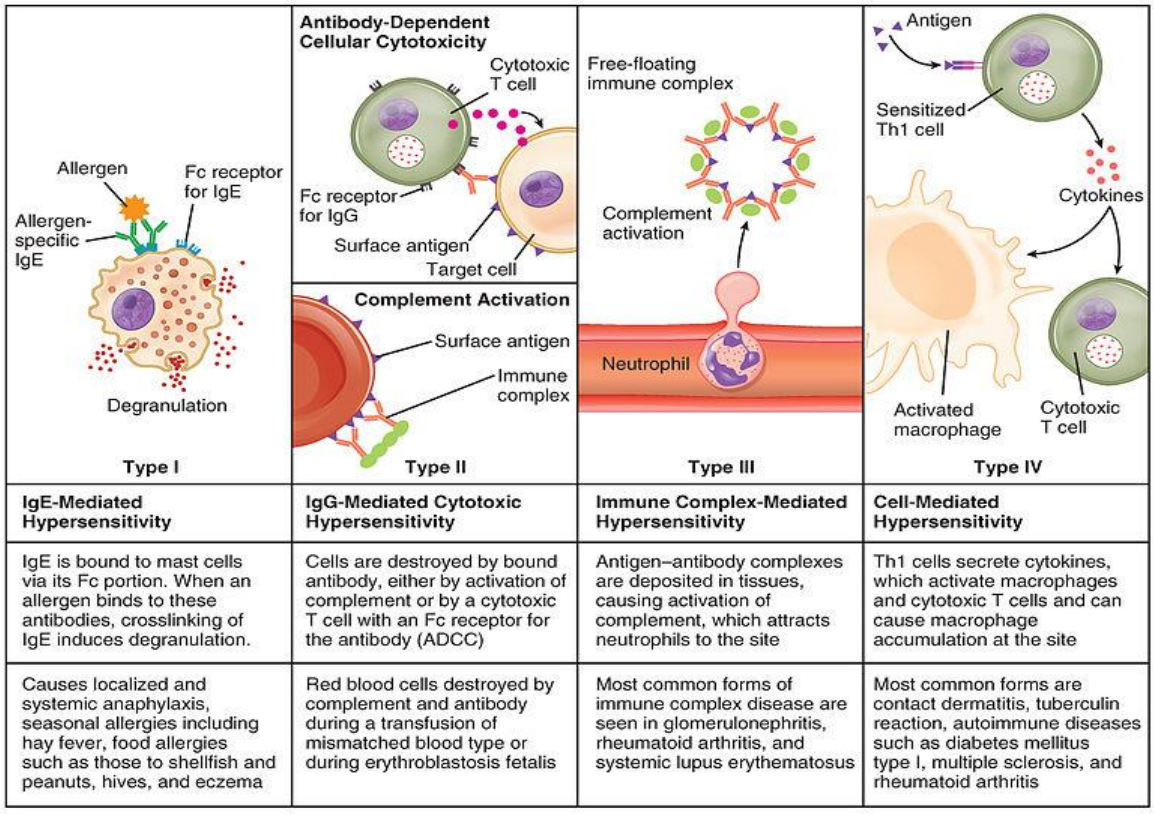
5. Immediate V/s Delayed Hypersensitivity

Based on the time taken to manifest the symptom, a hypersensitive reaction can be classified as immediate or delayed type. If the symptoms are observed within minutes or hours of antigen encounter in a sensitized recipient, the hypersensitive reaction is called as 'Immediate Hypersensitivity'. The anaphylactic reactions initiated by antibody or antigen-antibody complex (immune complex) i.e. humoral immunity come under this category.

Delayed type hypersensitivity (DTH) in contrast, named so because of delay in appearance of symptoms until several days after exposure. Hypersensitive reaction in the course of cell mediated immune response comes under this category.

6. Gell and Coomb's Classification of Hypersensitive Reactions

Based on the type of immune response involved i.e. humoral or cell-mediated and the effector molecules involved, different immune mechanisms are involved in generation of hypersensitive reactions. Accordingly, hypersensitive reactions can be categorized in different types. The British Immunologists, Philip George Houthem Gell (1914 –2001) and Robert Royston Amos (Robin) Coombs (1921-2006) proposed a classification scheme for hypersensitive reactions in their book 'Clinical aspects of Immunology'in 1963. They divided hypersensitive reactions as type I, type II, type III and type IV hypersensitive reactions. The classification is based on the type of immune branch involved i.e. humoral or cell-mediated (type I, II and III within humoral branch and type IV in cell-mediated branch); time taken to show symptoms i.e. immediate or delayed (type I, II and III are immediate type and type IV is delayed type hypersensitivity); immune mechanisms, cells and mediator molecules involved. A pictorial representation of different hypersensitive reaction types is shown in figure 3.



IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
IgE is bound to mast cells via its Fc portion. When an allergen binds to these antibodies, crosslinking of IgE induces degranulation.	Cells are destroyed by bound antibody, either by activation of complement or by a cytotoxic T cell with an Fc receptor for the antibody (ADCC)	Antigen–antibody complexes are deposited in tissues, causing activation of complement, which attracts neutrophils to the site	Th1 cells secrete cytokines, which activate macrophages and cytotoxic T cells and can cause macrophage accumulation at the site
Causes localized and systemic anaphylaxis, seasonal allergies including hay fever, food allergies such as those to shellfish and peanuts, hives, and eczema	Red blood cells destroyed by complement and antibody during a transfusion of mismatched blood type or during erythroblastosis fetalis	Most common forms of immune complex disease are seen in glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Most common forms are contact dermatitis, tuberculin reaction, autoimmune diseases such as diabetes mellitus type I, multiple sclerosis, and rheumatoid arthritis

Figure 3: An overview of hypersensitivity types as classified by Gell and Coombs
Source:
https://commons.wikimedia.org/wiki/File:2228_Immune_Hypersensitivity_new.jpg

7. Type-I Hypersensitivity

GENERAL ASPECTS

Type-I hypersensitivity is an immediate type hypersensitivity within the humoral branch of immunity. It is induced by antigens called as 'allergens' Type-I hypersensitivity is similar to normal humoral immune response except the fact that plasma cells secrete IgE antibody in response to activation of allergen-specific T_H-2 cells. These IgE antibodies bind with high affinity to Fc receptors on blood basophils and tissue mast cells. These IgE coated mast cells and basophils are called as sensitized cells. The exposure of same allergen later causes cross-linkage of these membrane bound IgE on sensitized mast cells and basophils, causing their degranulation. These granules release pharmacologically active mediator molecules e.g. histamines which primarily cause vasodilation and smooth muscle contraction in a localized manner or systemically. The clinical manifestation of type-I hypersensitivity includes systemic anaphylaxis or localized anaphylaxis including hay fever, hives, eczema, asthma, food allergy etc. A generalized type-I hypersensitive reaction is shown in figure 4.

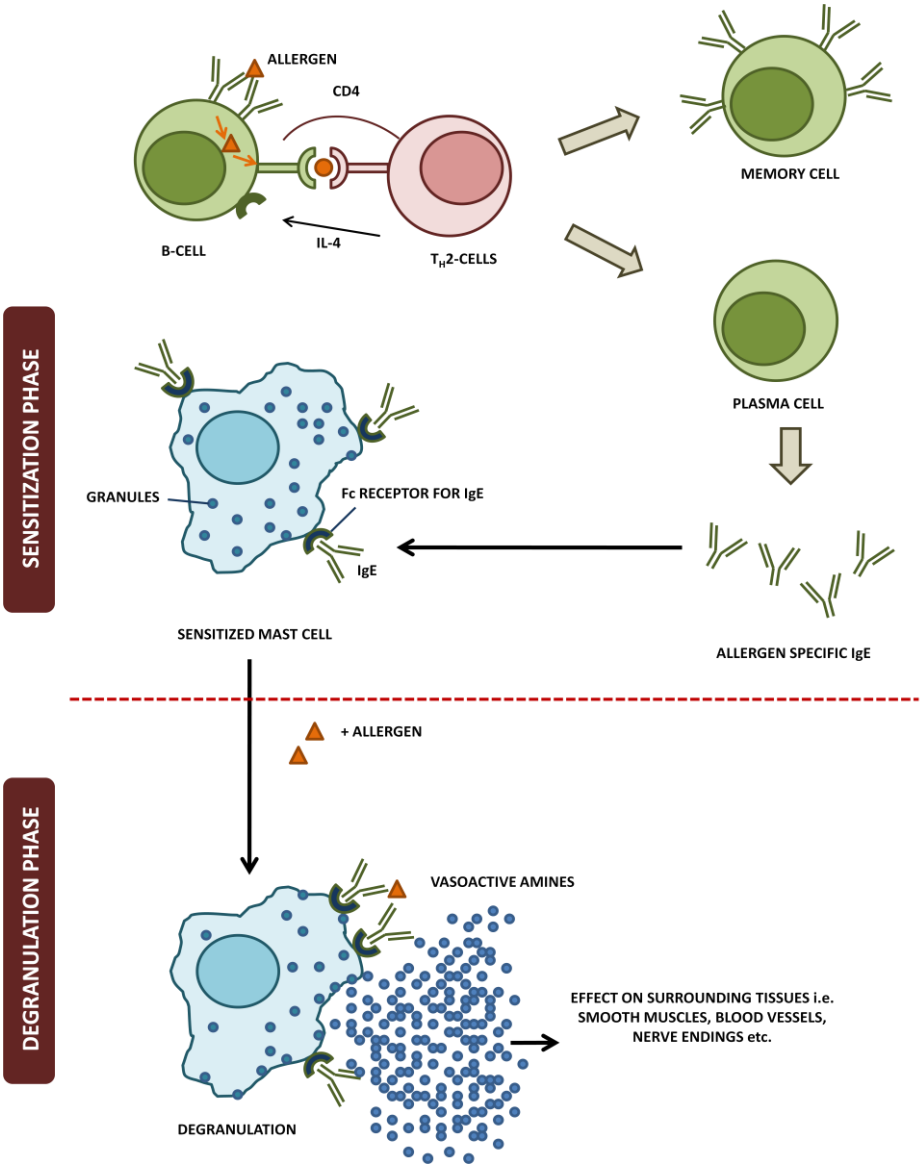


Figure 4: Generalized mechanism of type-I hypersensitive reaction. Sensitization phase involves exposure to allergen, activation of T_H2 cells, release of IgE from B cells and binding of these IgE with Fc receptor of mast cells. Degranulation phase involves release of mediators on re-exposure of same allergen and their further effects on surrounding tissues. Source: Author

COMPONENTS

ALLERGENS

The term allergen refers to non-parasitic environmental antigens which stimulate type-I hypersensitive response in allergic/ atopic individuals on repeated exposures. The

significantly high serum IgE level is mounted in humans against parasitic infections only. But the people who are genetically pre-disposed to development of IgE mediated immediate hypersensitivity (type-I) against common environmental antigens have this abnormality, called as 'atopy'. These individuals have regulatory defects for IgE because of which non-parasitic antigens also stimulate IgE production causing tissue damage and hypersensitivity.

The inherited atopy phenomenon is multigenic. The genetic cause behind the phenomenon of atopy has been mapped to various loci by linkage analysis which are:

i) On Chromosome 5, is present a locus that is linked to a region that codes cytokines IL-3, IL-4, IL-5, IL-9 and IL-13, granulocyte-monocyte colony-stimulating factor (GM-CSF). All these cytokines promote IgE production.

ii) On Chromosome 11, is present another locus that is linked to a region that encodes the β-chain of high affinity IgE receptor.

iii) On Chromosome 6, is present the MHC genes which have also been linked to IgE response.

Some of the common allergens are summarized in Table 1.

Common Allergens Causing Type-I Hypersensitivity	
Food Type	Examples
Foods	Eggs, Mushrooms, Milk, Peas, Beans, Seafood, Nuts etc.
Insect Products	Venom of Bees, Ants, Wasps, Cockroach Calyx, Dust Mites etc.
Drugs	Salicylates, Penicillin, Anesthetics, Sulfonamides etc.
Plant Pollens	Ragweed, Rye Grass, Birch Trees etc.
Proteins	Vaccines, Foreign Serum etc.
Others	Mold Spores, Latex, Animal Hair etc.

Table 1: Some of the common allergens known to cause type-I hypersensitive reactions

Most of the allergens are small sized, soluble in nature, glycosylated proteins or protein-bound substances with molecular weight 15,000 to 40,000. The allergenicity of an allergen

depends on its ability to evoke a TH2-mediated immune response which in turn depends on a number of factors such as:

i) Type of allergen
ii) Dose of allergen
iii) Sensitizing route
iv) Adjuvant
v) Recipient's genetic/ hereditary predisposition i.e. atopy

Value Addition: Why do some allergens (Ragweed pollens) induce potent allergic responses whereas other equally abundant allergens (Nettle pollens) do not.

It depends upon the various factors listed above such as sensitization routes, adjuvant and genetic constitution of host beside type and dose of allergen.

T_H2 CELLS

The type of immune response against a particular antigenic challenge is determined by the cytokine-secretion pattern of different subtypes of T_H cells. $CD4^+$ T_H cells exert their functions through cytokines, in autocrine or paracrine way. There are two subtypes of $CD4^+$ T_H cells i.e. T_H1 and T_H2 cells. These two subsets differ in the profile of cytokines they secrete. T_H2 cells secrete GM-CSF, IL-3, IL-4, IL-5, IL-10 and IL-13. The primary functions of T_H2 cells include IgE production, eosinophil and mast cell activation and differentiation and help to B cells. IL-4 secreted by T_H2 cells promote the class switching in B cells from IgM to IgE. This effect on IgE production meshes with the differentiation and activation of eosinophil by IL-5, because eosinophils possess Fcε receptors which bind IgE. The parasitic infections and allergic reactions the induce T_H2 responses leading to anti-parasitic and type-I hypersensitive reactions. The T_H2 cytokine profile is higher in allergic diseases and helminthic infections.

IgE

Immunoglobulin E (IgE) is named so in reference to E antigen of Ragweed pollen which is a potent inducer of this class of antibody. Though the amount of IgE in serum is very low but its potent biological activity made it to be identified. (Note: Atopic individuals have 10 times

more serum IgE level than normal ones). It was identified by Otto Carl Willey Prausnitz (1876-1963) and Heinz Kustner (1897-1963) in 1921 as a serum component responsible for allergic reaction. They injected serum from an allergic person to a non-allergic person intradermally. The later injection of appropriate antigen at the same site caused swelling and redness termed as wheel and flare reaction. This reaction is called as P-K reaction after the name of their discoverer Prausnitz and Kustner. It was the first evidence of biological activity of IgE, though the IgE was not known at that time.

The IgE was first identified by Kimishige and Teruka Ishizaka in 1966. They immunized the rabbit with serum of an allergic human individual to prepare anti-isotype antiserum. The serum of rabbit thus obtained was reacted with all the known antibodies at that time i.e. IgG, IgM, IgD and IgA. This way, each of the known anti-isotype antibodies was precipitated and removed from the rabbit antiserum. The remaining serum was still having an antibody which could block the P-K reaction. This new class of antibody was named as IgE.

IgE mediates two immune functions i.e. (i) allergic reaction or type-I hypersensitive reaction, and (ii) antibody dependent cell mediated cytotoxicity (ADCC) anti-parasitic defence. IgE has higher molecular weight (1,90,000) than IgG (1,50,000) due to additional constant region domain which replaces the hinge region of IgG.

Heinz Kustner Otto Carl Willey Prausnitz

Source: https://en.wikipedia.org

Properties of Human Serum Immunoglobulin E (IgE)	
Molecular Weight	**1,90,000**
Heavy Chain Component	**ε**
Light Chain Component	**κ or λ**
Molecular Formula	**ε2κ2 or ε2λ2**
Normal Serum Level (µg/ mL)	**0.1-0.4**
In-vivo Serum Half-Life (Days)	**2.5**
Primary Function	**Mast cell and eosinophil degranulation**

Table 2: Some of the properties of human immunoglobulin E (IgE)

TISSUE MAST CELLS AND BLOOD BASOPHILS

MAST CELLS

- Mast cells originate from $CD34^+$/ $CD117^+$/ $CD13^+$ multipotent hematopoietic progenitor cells (in humans) in bone marrow, from where they migrate to various tissues through blood where they mature.
- They were first identified by Dr Von Recklinghausen in 1863 as granular cells in the mesentery of the frog.
- Initially mast cells were named as “Mastzellen” by Dr Paul Ehrlich in 1878.
- Also known as ‘mastocyte’ or ‘labrocyte’.

- It was the discovery of histamine in 1910, slow-reacting substance of anaphylaxis (now leukotrienes) in 1938, and IgE in 1966 that provided initial insights into the role of Mast Cells in allergic reactions.
- A number of stimuli can activate Mast cells which then release a spectrum of mediators in discriminating manner. These mediators include histamine, proteases and other enzymes, growth factors, chemokines, cytokines, reactive oxygen and nitrogen species and arachidonic acid metabolites. These mediators perform their roles in allergic reactions.
- Mast cells adapt various phenotypes based on their microenvironment. This property is called as 'Mast Cell Heterogeneity'. Mast Cell Heterogeneity is decided on the basis of their location, content of proteases, histo-chemical staining, and reactivity to anti-allergic drugs.
- Rodents have two major subtypes of Mast cells i.e. mucosal type (MC_T) and connective tissue type (MC_{TC}). Whereas humans have three major subtypes of Mast cells on the basis of content of serine proteases: tryptase-only type (MC_T), chymase-only type (MC_C), or both tryptase- and chymase-positive type (MC_{TC}).
- The primary functions that Mast cells perform are: Maintenance of homeostasis, act as interface between innate and adaptive immunity, protective roles in various infections, role in allergic or type-I hypersensitive reactions etc.

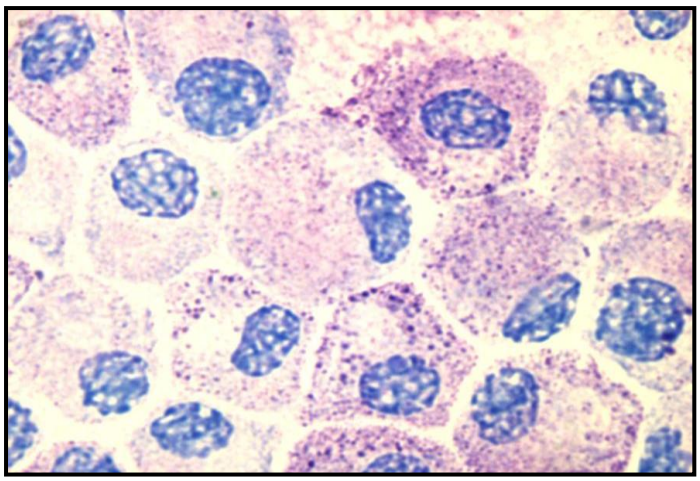


Figure 5: Cultured Mast cells at 1000X magnification, stained with Tol Blue.

Source: https://en.wikipedia.org/wiki/Mast_cell#/media/File:SMCpolyhydroxysmall.jpg

Property	Mucosal Mast Cell	Connective Tissue Mast Cell
Distribution	Gut and Lung	Most of the tissues, predominately in skin and intestinal submucosa
Abbreviation	MC_T	MC_{TC}
T-cell dependence	+	-
Differentiation favoured by	IL#	Fibroblast factor
FcεRI	2×10^5 / cell	3×10^4/ cell
Granule staining with alcian blue and safranine	Blue and brown	Blue
Granule ultra-structure	Scrolls	Lattices and Granules
Granule protease type	Tryptase	Tryptase and Chymase
Granule proteoglycan	Chondroitin sulfate	Heparin
Histamine release in degranulation	+	++
LTC4:PGD2 release in degranulation	25:1	1:40
Degranulation blockage by disodium cromoglycate/ theophylline	-	+

Table 3: Differences between mucosal (MC_T) and connective tissue mast cells (MC_{TC})

BASOPHILS

- Basophils are the non-phagocytic, granulocytic leucocytes that are found in most vertebrates.
- They are the least abundant population of granulocyte in peripheral blood comprising 0.5 -1% of total leucocytes.
- The granulated cytoplasm of basophils stains with basic dye and hence the name basophil.

- They were discovered by Paul Ehrlich in 1879 based on their unique microscope appearance on exposure to basic dyes.
- Basophil develops from basophil precursor cells which in turn originate from hematopoietic stem cell (HSC) - derived granulocyte-monocyte progenitor (GMP) cells in bone marrow.
- They have a short life span of around 1-2 days only.
- They contain a multi-lobed nucleus, some mitochondria, glycogen granules and membrane bound granules containing pharmacologically active mediator molecules (Fig. 6).
- The primary functions is release of histamine and leukotrienes in response to IgE mediated activation i.e. role in type-I hypersensitivity. Basophils have roles in inflammation and associated diseases such as Urticaria, Asthma etc. Also, they are involved in protection against parasitic infections.

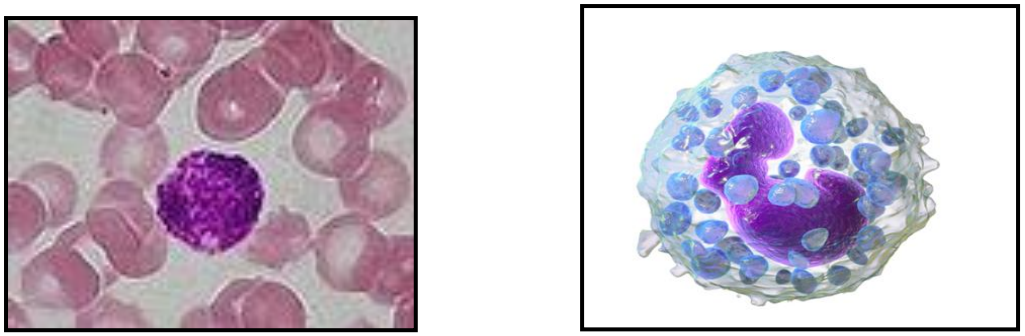


Figure 6: Basophil along with RBCs and diagrammatic view of a single basophil
Source: https://en.wikipedia.org/wiki/Basophil
https://en.wikipedia.org/wiki/White_blood_cell

F_C RECEPTORS

The biological activity of IgE depends upon its binding with its receptors. These receptors are specific for the Fc region of ε heavy chain of IgE antibody. Two types of Fcε receptors are there based on their difference in affinity for IgE, cellular distribution and their effects post-IgE binding i.e. FcεRI (Fig. 7) and FcεRII or CD23 (Fig. 8).

FcεRI

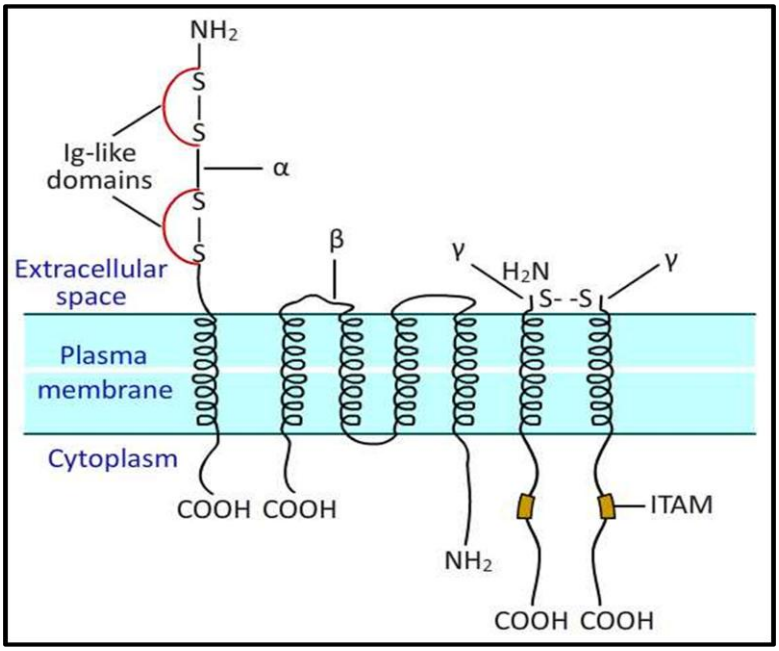
- Also called as high affinity IgE receptor because of its its capacity to bind IgE despite low serum concentration of IgE i.e. 1 x 10^{-7}M.
- K_d = 1-2 x 10^{-9}M
- A single human basophil contains 40,000-90,000 FcεRI.
- FcεRI is present primarily on cells such as Mast cells, Basophils, Langerhans cells, Monocytes.
- Structurally, an FcεRI is made up of four polypeptide chains i.e. one α, one β and two γ chains.

  α chain: The amino terminal of α chain lies in extracellular region whereas carboxylic one lies in cytoplasm. The extracellular region contains two 90 amino acid domains which resemble immunoglobulin fold structure. These immunoglobulin fold like structures bind with C_H3/ C_H3 and C_H4/C_H4 domains of IgE.

  β chain: The β chain has its amino and carboxylic terminal in cytoplasm only. It spans the plasma membrane four times. It acts as linker between α and γ chain.

  γ chain: Two identical γ chains are there which as linked together by di-sulfide linkage. Each γ chain possesses a conserved sequence called as immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. This ITAM motif interacts with tyrosine kinase to transduce an activation signal to cell.

- The allergens upon binding to IgE causes cross-linkage, leading to aggregation of FcεRI receptors. ITAM interacts with tyrosine kinase which further causes tyrosine phosphorylation and mast cell degranulation.



High Affinity IgE Receptor FcεRI

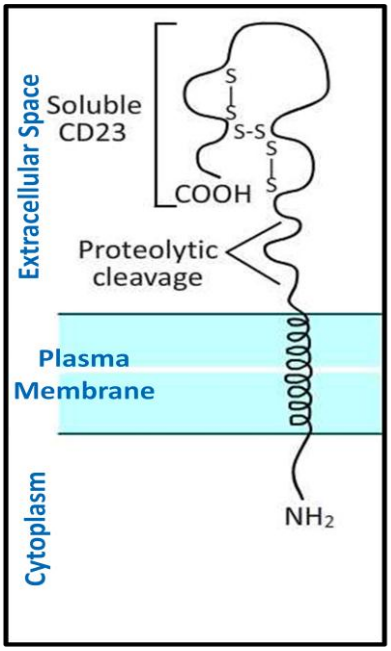
Figure 7: A Diagrammatic view of FcεRI receptor

Source: Author[Modified from Kuby's Immunology 4th ed. by R. A. Goldsby, T. J. Kindt and B. A. Osborne]

FcεRII

- Also called as low affinity IgE receptor
- Also termed as CD23
- K_d= 1 x 10^{-6}M
- Specific for C_H3/ C_H3 domain of IgE
- FcεRIIis present primarily on cells such as B cells, eosinophils, alveolar macrophages.
- Structurally, it is composed of a single polypeptide chain with its carboxylic terminal in extracellular region and amino terminal in cytoplasm. The extracellular domain contains disulfide linkages.
- The functions ofFcεRII include

  IgE transport across human intestinal epithelium, positive-feedback mechanism to enhance allergic sensitization (B cells), activation of alveolar macrophages and eosinophils.



Low Affinity IgE Receptor FcεRII (CD23)

Figure 8: A Diagrammatic view of FcεRII receptor
Source: Author [Modified from Kuby's Immunology 4th ed. by R. A. Goldsby, T. J. Kindt and B. A. Osborne]

8. Degranulation Process and It's Mechanism

Degranulation is simply a cellular process of releasing bioactive molecules from cytoplasmic granules/ secretory vesicles in cells like mast cells and granulocytes (Fig. 9). With respect to type-I hypersensitivity, we'll discuss the degranulation of mast cells and basophils primarily. Both these cell types show similar mechanism of degranulation with minor differences. For simplicity, let us discuss the general mechanism of degranulation irrespective of cell type involved. The binding of allergen to bound IgE on cell surface doesn't trigger the degranulation process. The initiation of degranulation requires cross-linkage of IgE receptors present on these cell surfaces. The significance of cross-linkage lies in the fact that monovalent allergen cannot initiate start degranulation because eof its inability to cross-link the bound IgE receptors. The experimental evidences have proved that IgE to allergen ratio of 2:1 or more can only induce degranulation. Different mechanisms can cross-link these IgE

receptors. IgE:allergen ratio lesser than this could not induce degranulation due to its inability of cross-linking.

Another important observation that emerged from these experiments was that the cross-linkage of two or more FcεRI is required for degranulation initiation with or without bound IgE. Various mechanisms including the ones that can bypass the need of allergen or IgE are shown in the figure 9 below:

- **Normal Mechanims**

A) Cross-linkage of cell bound IgE by allergen

- **Mechanisms that do not require allergen**

B) Cross-linkage of cell bound IgE by anti-isotype or anti-idiotype antibody

C) Cross-linkage of cell bound IgE by chemical

- **Mechanisms require neither allergen nor IgE**

D) Cross-linkage of cell bound IgE by anti-receptor antibody

- **Mechanisms require neither allergen nor IgE and not even cross-linkage of receptor**

E) Calcium ion influx by ionophore and increased permeability for calcium

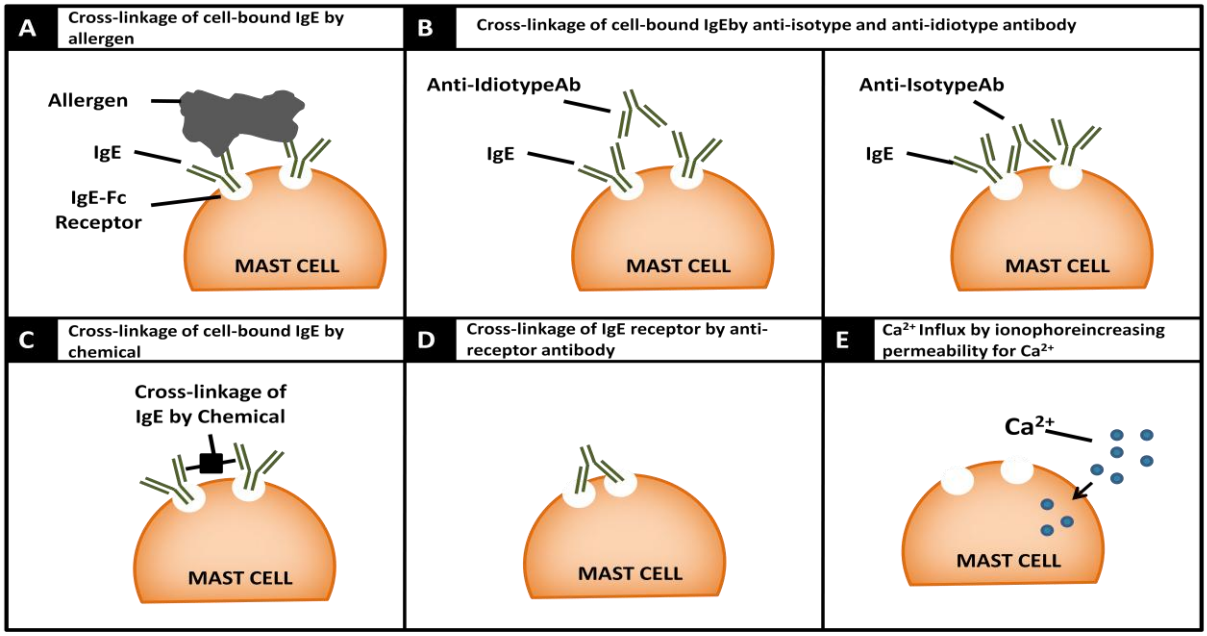


Figure 9: Possible mechanisms which can trigger the initiation of mast cell granulation.

Source: Author[Modified from Kuby's Immunology 4th ed. by R. A. Goldsby, T. J. Kindt and B. A. Osborne]

Biochemical Events in Activation and Degranulation of Mast Cells:

A series of well-coordinated intracellular events helps in activation and degranulation or release of mediators from mast cells (or basophils) (Fig. 10). Various protein and lipid kinases and phosphatases along with cytoskeletal re-arrangements are involved in these biochemical events. A sequential mechanism along with a pictorial depiction of the same is elaborated below:

1. Allergen cross-linkage of bound IgE (or by other mechanism listed above)
Aggregation of FcεRI
Activation of protein tyrosine kinase (PTKs), present on cytoplasmic domains of β and γ chains of FcεRI
PTKs phosphorylates tyrosines within ITAMs of γ subunit of FcεRI, residues on β subunit of FcεRI and Phospholipase C
Phospholipase C converts phosphatidyl-inositol-4,5 bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3)

⇩

2. DAG activates protein kinase C (PKC) which is necessary for microtubular assembly and fusion of granules with plasma membrane, along with Ca^{2+}. IP3 on the other hand is responsible for intracellular mobilization of Ca^{2+}

⇩

3. FcεRI cross-linkage also activates other enzymes that convert phosphatidylserine (PS) into phosphatidylethanolamine (PE)
PE is methylated to phosphatidylcholine (PC) by phospholipid methyl transferase enzymes I and II (PMTs I and II)

⇩

4. PC accumulation on exterior surface of plasma membrane causes an increase in Ca^{2+} influx

5

Ca^{2+} influx activates phospholipase A_2that breaks down PC into lysophosphatidylcholine (lysoPC) and arachidonic acid
Arachidonic acid is converted to leukotrienes and prostaglandin D_2, the mediators

6

FcεRI cross-linkage also activates the enzyme adenylate cyclise which causes a transient increase in cAMP within 15s. Later, cAMP is dropped by protein kinase C (PKC)

7

Protein kinases phophorylate the membrane proteins of granules that further changes the permeability of granules for water and Ca^{2+}
The resulted swelling in granules facilitates their fusion with plasma membrane and ultimately the degranulation/ release of mediators.

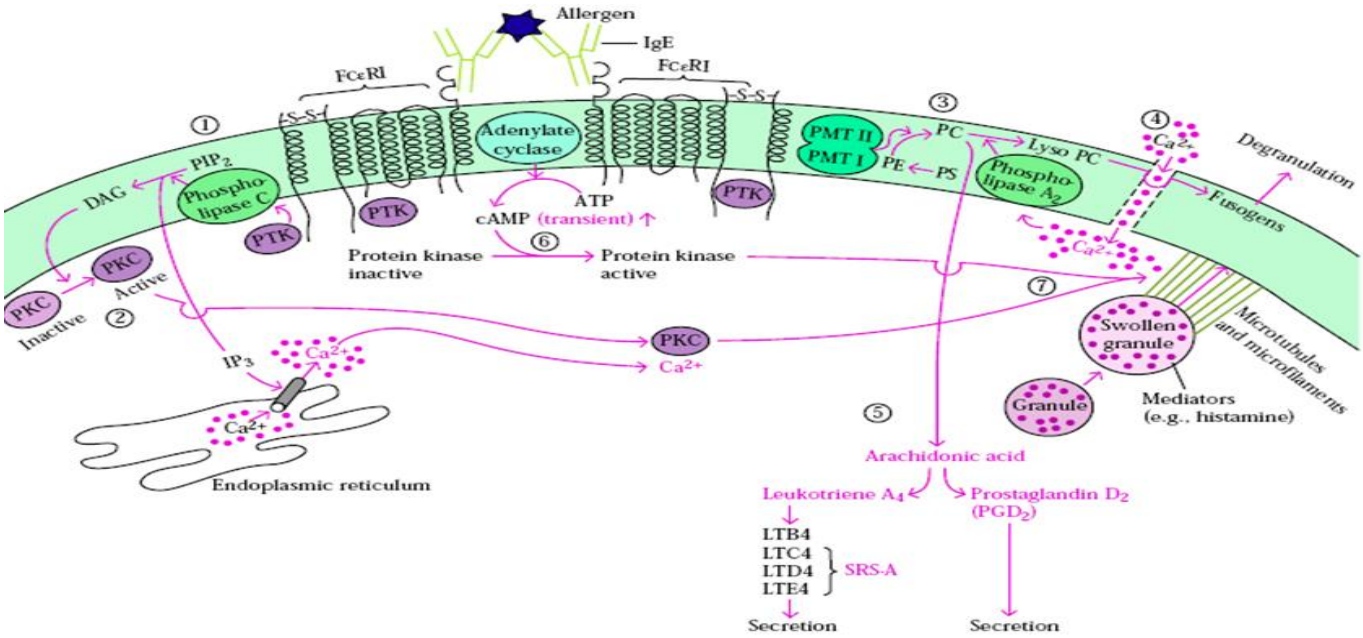


Figure 10: Diagrammatic depiction of biochemical events in mast cell activation and degranulation
Source: Author[Modified from Kuby's Immunology 4th ed. by R. A. Goldsby, T. J. Kindt and B. A. Osborne]

9. Mediators of Type-I Hypersensitivity

Mediator molecules are pharmacologically active agents that are released from the mast cells and basophils in the process of degranulation. It is these mediators which are responsible for biological effects or clinical manifestation of type I hypersensitivity. They act as amplifying terminal effector mechanisms which act upon local tissues and secondary effector cells e.g. neutrophils, eosinophils lymphocytes and platelets etc. These mediators from mast cells and basophils have a protective, defensive role against parasitic infections where they cause vascular permeability and vasodilation to bring an influx of plasma and inflammatory cells to attack the pathogen. But the same phenomenon in case of an inappropriate, non-pathogenic, allergenic induction cause deleterious effects such as in case of type-I hypersensitive reactions.

Based on the synthesis and storage, these mediators are classified as primary or secondary mediators, shown here with their biological activity:

- **Primary Mediators:** They are pre-manufactured mediators which remain stored in granules of mast cells and basophils. Histamine, serotonin, heparin, proteases are some of the common primary mediators which are listed below along with their biological roles (Table 4).

Primary Mediator	Biological Effect
Heparin, histamine	Smooth muscle contraction and increase in vascular permeability
Serotonin	Smooth muscle contraction and increase in vascular permeability
Neutrophil chemotactic factor (NCF-A)	Chemotaxis of neutrophils
Eosinophil chemotactic factor (ECF-A)	Chemotaxis of eosinophils
Proteases (tryptase, chymase)	Bronchial mucus secretion, generation of complement split products, degradation of blood vessel basement membrane

Table 4: Some of the common primary mediator molecules with their biological roles

- **Secondary Mediators:** They are not pre-formed like primary mediators but are either synthesized upon target cell activation or released upon breakdown of membrane phospholipids during the process of degranulation. Some of the common secondary mediators are leukotrienes, prostaglandins, cytokines (IL-1, IL-4, IL-5, IL-6, TNF-α etc.) and platelet activating factor. Their biological functions are listed below (Table 5).

Secondary Mediator	Biological Effect
Leukotrienes	Contraction of pulmonary smooth muscles and increased vascular permeability. Also called as slow reactive substance of anaphylaxis (SRS-A)
Prostaglandins	Contraction of pulmonary smooth muscles, platelet aggregation, vasodilation
Cytokines	IL-1 and TNF-α: Systemic anaphylaxis IL-4 and IL-13: IgE production increase IL-5: Eosinophil activation and generation
Bradykinin	Contraction of smooth muscles and increased vascular permeability
Platelet activating factor	Contraction of pulmonary smooth muscles, platelet aggregation

Table 5: Some of the common secondary mediator molecules with their biological roles

The difference observed in clinical manifestations of type-I hypersensitive reactions in different tissues or different species are may be because of variation in primary and secondary mediators.

10. Clinical Manifestations of Type-I Hypersensitivity

The clinical manifestations of type-I hypersensitive reactions are classified as localized anaphylaxis (e.g. eczema, asthma, food allergy and rhinitis or hay fever) or systemic anaphylaxis depending on the extent of tissue/ organ damage whether its localized to target site or systemic. Various type-I hypersensitivity clinical manifestations are:

➢ **Systemic Anaphylaxis:**

- It's a shock like fatal condition which involves throughout body symptoms.
- Its symptoms are observed within minutes of type-I hypersensitive reaction
- Common allergens which can cause systemic anaphylaxis in susceptible individuals are venom of bees, wasps and ants; drugs such as antitoxin, penicillin and insulin; food such as nuts and seafood etc.
- The widely used drug for treatment of systemic anaphylaxis is epinephrine. Epinephrine reverts back the effects caused by mediators. It reduces the vascular permeability and relaxes the smooth muscles, blocks mast cell degranulation by increasing cAMP level and prevents vascular collapse by improving cardiac output.
- Guinea pig is the preferred animal model for studying systemic anaphylaxis.

➢ **Localized Anaphylaxis:**

- In this response, reaction is restricted to specific target tissue or organ only usually epithelial tissue at the site of allergen entry.
- It is also called as 'atopy' i.e. the inherited tendency to manifest localized anaphylaxis.
- Common examples of localized anaphylaxis are: Allergic rhinitis or hay fever, atopic dermatitis or allergic eczema, food allergies and asthma.

Allergic rhinitis (hay fever): It is caused by reaction of airborne allergens with sensitized mast cells in nasal mucosa and conjunctiva. Vasodilation and increased vascular permeability result due to release of mediators which cause sneezing and coughing, watery conjunctivae and nasal mucosa.

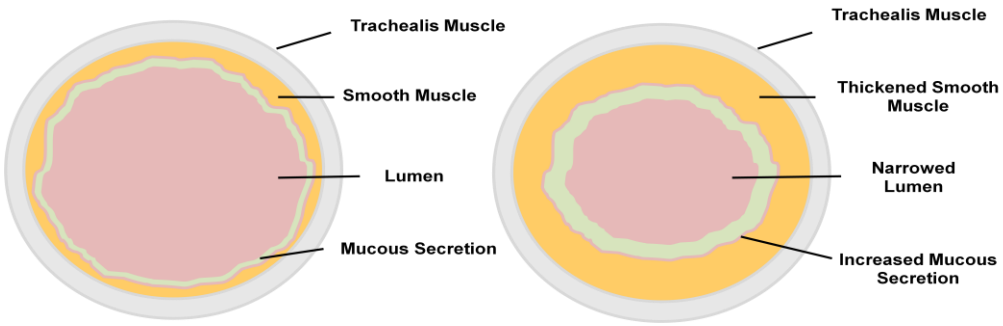


Figure 11: Some of the common differences in cross-section of trachea of normal individual and hay-fever affected one Source: Author

Hay Fever v/s Normal Colds		
Characteristic	**Hay Fever**	**Colds**
Symptom	Runny nose with thin watery discharge	Runny nose with watery to thick yellow discharge
Fever	No	Yes
Sneezing	More common than cold	Less than hay fever
Onset	Immediate on exposure to allergen	1-3 days after exposure to cold virus
Duration	Lasts till the exposure to allergen	5-7 days

Table 6: Difference between hay fever (allergic rhinitis and colds)

Atopic dermatitis (Allergic eczema): Erythematous and pus-filled skin eruptions generally seen in atopic infants (Fig. 12) are another example of localized anaphylaxis.

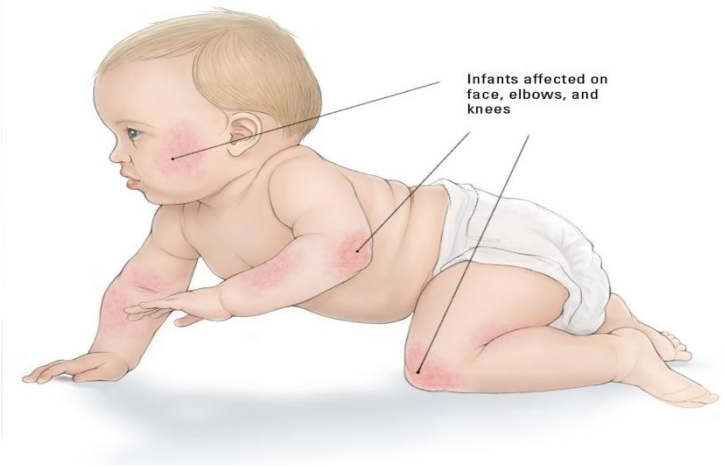


Figure 12: Common sites of allergic eczema (atopic dermatitis) in infants

Source:http://dyersburgskinandallergyclinic.com/?page_id=387

Food allergies: Susceptible individuals show localized anaphylaxis against a number of food items. Depending upon the site of allergen entry, symptoms may include diarrhoea and

vomiting (in gastro-intestinal tract), asthmatic attacks (in respiratory tract) or atopic urticaria or hives i.e. erythematous and edematous eruptions in skin (Fig. 13).

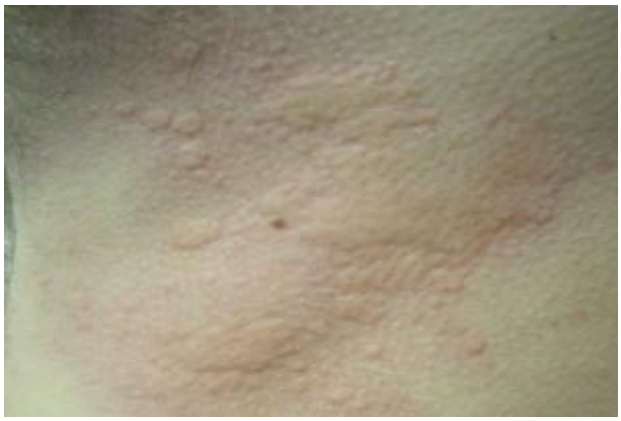


Figure 13: Hives or Urticaria developed on the skin as an outcome of allergy to food allergen

Source: https://www.firstderm.com/skin-guide/urticaria-hives/

Asthma: Asthma can be intrinsic (i.e. allergen independent, generally cold or exercise induced) or allergic (i.e. allergen dependent, induced by dust, fumes, pollens, insects etc.). In asthma the target site is lower respiratory tract (Fig. 14). Broncho-constriction and airway obstruction are common features of asthmatic patients. The asthmatic response is divided into early and late phase response. Early response involves leukotrienes, prostaglandins and histamine which cause vasodilation, bronchoconstriction and mucous secretion. Late phase involves mediators such as IL-4, IL-5, TNF-α, platelet activating factor etc. which recruit eosinophils and neutrophils. These inflammatory cells cause tissue damage by releasing toxic enzymes, cytokines and free radicals etc. These effects cause accumulation of thick mucous and cell debris which blocks the airway. Hypertrophy of bronchial smooth muscles, thickening of basement membrane and formation of mucous plug are other effects observed in asthmatic patients. Curschmann's spirals are the mucous plugs containing clusters of detached epithelial cells and inflammatory cells and spirals of bronchial tissues. They are named so after their discoverer, German Physician Heinrich Curschmann.

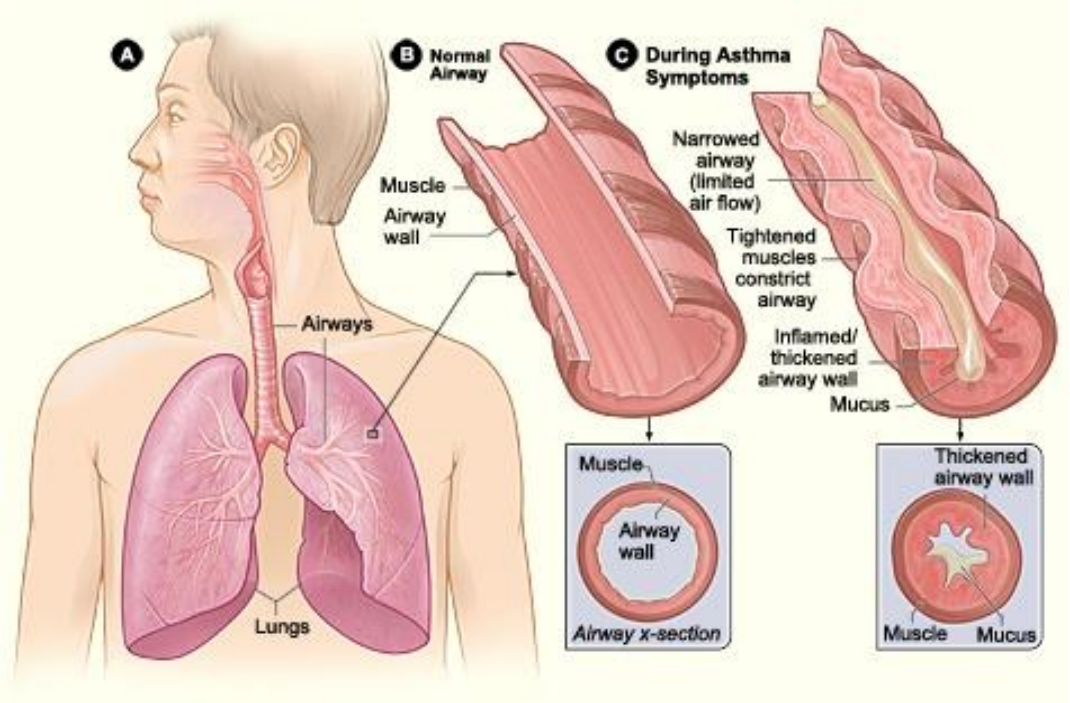


Figure 14: A) Overview of affected sites in asthma, B & C)Cross section of normal and asthmatic individual's lung showing characteristic differences
Source: http://www.nhlbi.nih.gov/health/health-topics/topics/asthma

11. Regulation of Type-I Hypersensitivity

The allergenicity of an allergen or in turn, the level of IgE response by an allergen depends upon various factors such as:

- **Allergen dose:** Dos of the allergen is a critical factor for defining the allergenicity. For example, repeated low dose of allergen has been shown to induce a persistent IgE response in BDF1 mice but higher dose resulted in transient IgE production and shift towards IgG.

- **Genetic constitution of the individual**: Some strains of mice such as SJL strain do not mount an IgE response to allergen whereas some other strains such as BDF1 has high propensity for IgE production. In humans, genetic component has been shown to

influence susceptibility to type-I hypersensitivity. If both the parents are allergic, 50% chances are there that their child will also be allergic. And, if one of the parent is allergic, chances are 30% that the child will manifest the symptoms of type-I hypersensitivity. These examples clearly indicate the role, genetic constitution of an individual plays in regulating the allergic response of an individual for an allergen.

- **Mode of allergen presentation**: Immunization of Lewis-strain rats with keyhole limpet hemocyanin (KLH) with aluminium hydroxide gel or *Bordetella pertussis* as an adjuvant induced very strong IgE response whereas KLH with complete Freund's adjuvant produced largely an IgG response. It shows that the mode of allergen presentation plays an important role in defining the response of an individual to a particular antigen.

- **Relative level of T_H1 and T_H2:**The cytokines released from T_H2 cells i.e. IL-3, IL-4, IL-5 and IL-10 stimulate the type-I hypersensitivity whereas the cytokines released from T_H1 cells e.g. IFN-γ inhibit the type-I hypersensitivity. IL-4 and IFN-γ particularly play pivotal role in stimulating or inhibiting the type-I hypersensitive response respectively. And, since IL-4 and IFN-γ are secreted from T_H1 and T_H2 respectively, the relative activity of these two cells subsets regulates an individual's response to allergen.

12. Detection of Type-I Hypersensitivity

The identification and assessment of type-I hypersensitivity can be done by a number of medical tests such as:

- **Wheel and flare test:** The simplest, inexpensive and easy testing mechanism for type-I reaction is skin testing where an intradermal injection or superficial scratching of potent allergen is applied on specific skin sites preferably arm or back of the individual. If the individual is allergic to that particular antigen, localized wheel and flare reaction is observed within 30 minutes due to mediators released at that site (Fig. 15).

  Advantage: Simple, Inexpensive, fast and allows screening of large number of allergens at one time.

Disadvantage: It may cause systemic anaphylaxis, tissue damaging late phase reaction and sensitization of allergic individual to new allergens.

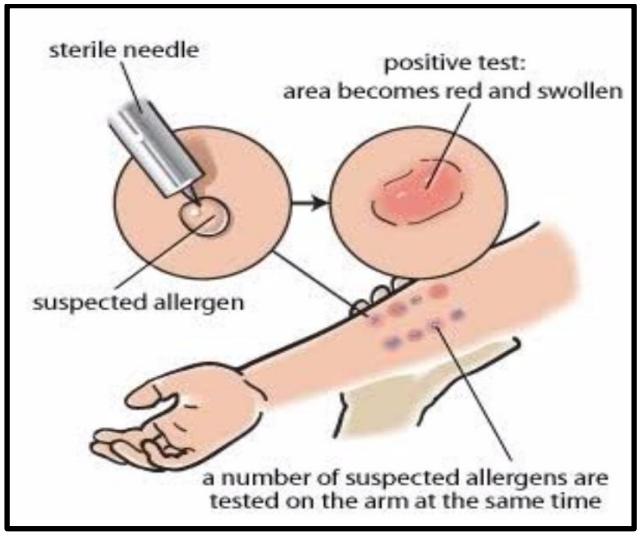


Figure15: Wheel and flare test for assessing type-I hypersensitivity against multiple allergens

Source: http://www.medbroadcast.com/procedure/getprocedure/Allergy-Skin-Test

- **Radio-immunosorbent test (RIST):** It is a radioimmunoassay based highly sensitive technique which determines the total IgE in patient's serum. In this method, patient's serum is reacted with rabbit anti-IgE, coated on paper disk or agarose beads. After washing, radio-labelled rabbit anti-IgE (^{125}I labelled) is added. The radioactivity of beads or disks is measured by gamma counter, which corresponds to amount of IgE in the patient. A simplified pictorial depiction of the same if shown below (Fig. 16):

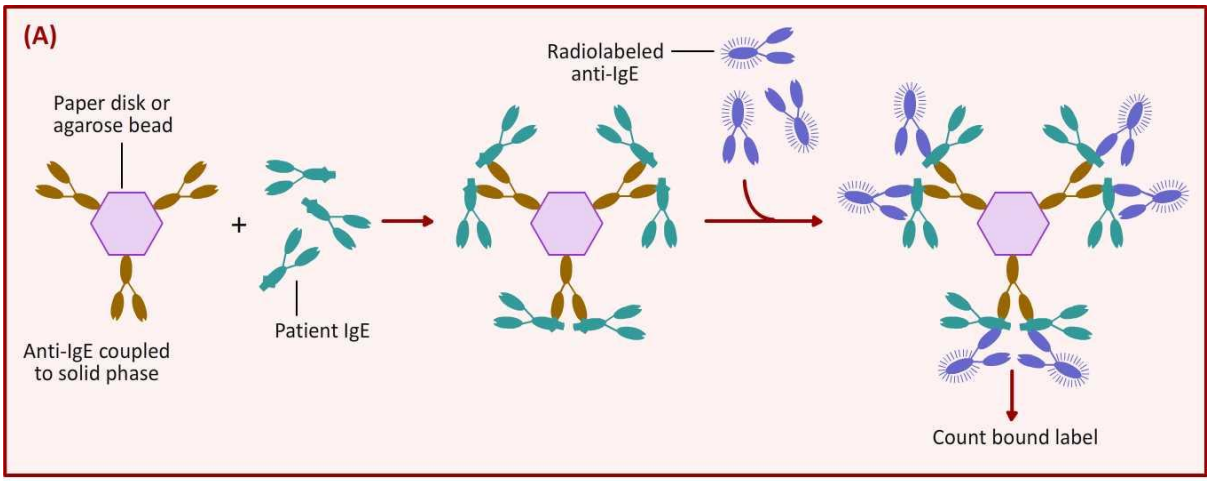


Figure 16: Principle of radio immuno-sorbent test (RIST)

Source: Author

- **Radio-allergosorbent test (RAST):** Unlike RIST, where total IgE in patient's serum is detected, RAST is used to detect serum IgE specific for a given allergen. In RAST, the allergen is coupled to a solid phase and then reacted with patient's serum. Unbound antibodies are washed away. The remaining allergen bound IgE is reacted with radio-labelled rabbit anti-IgE (^{125}I labelled) and then radioactivity is measured (Fig. 17). Fluorescent labelling can be used instead of radioactivity in both RIST and RAST.

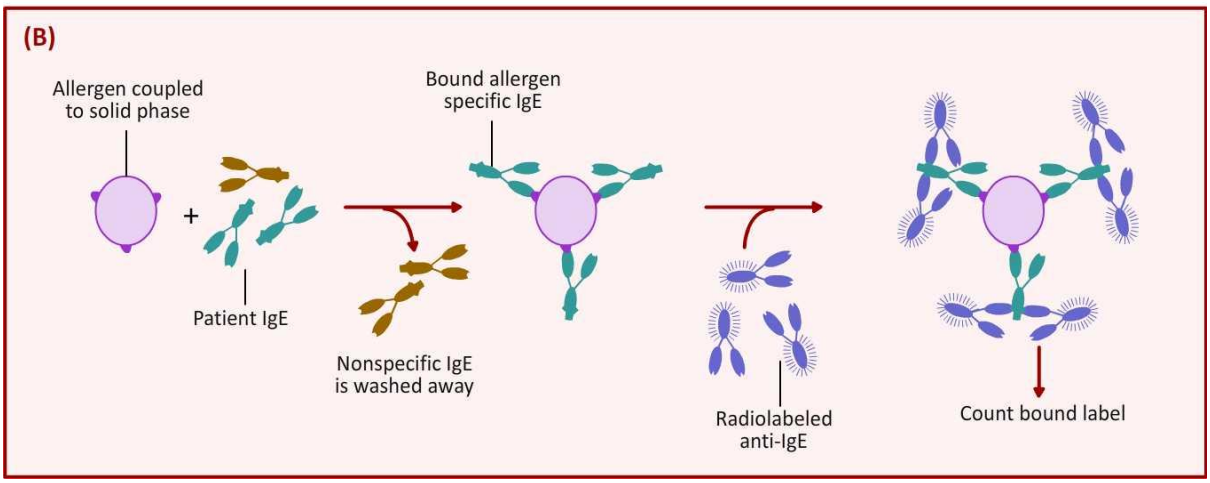


Figure 17: Principle of radio allergo-sorbent test (RAST)

Source: Author

13. Therapy/ Precaution for Type-I Hypersensitivity

Starting from the exposure of allergen to production of atopic disease, there are several points which can be targeted for therapy. These points can be:

- **Avoidance of allergen:** The first step in controlling type-I hypersensitivity is to identify and avoid the allergen itself. Though most of the time, its impractical to avoid keep oneself away from all the allergens but still measures can be taken to avoid the known allergens e.g. dust, pets, pollens etc.
- **Modulation of immunological response:** Immunotherapy with repeated injection of low dose of allergen has been a long time practice for minimizing the severity or even elimination of type-I reaction **(Hyposensitization).** Such repeated injections cause a shift towards IgG production or T-cell mediated suppression that turns off IgE response. IgG here is called as *blocking antibody* because it competes with IgE for allergen and binds with allergens so that it's not available to cross-link bound IgE on mast cells and thus cease the allergic reaction.
- **Stabilization of the triggering cells:** Drugs such as inhalant isoprenaline and sodium cromoglycate render mast cells resistant to triggering. Sodium cromoglycate blocks calcium ion influx into mast cells and maintains them in normal resting physiological states. In this way mast cells are inhibited from degranulation, chemotaxis and mediator release.
- **Antagonism of mediators:** Various antagonists of type-I hypersensitivity mediators have been employed to cure allergy. Histamine H_1-receptor antagonists, β_2 antagonists (e.g. salmeterol, formoterol), leukotriene antagonists (e.g. pranlukast), phosphodiesterase inhibitor (e.g. theophylline) are some of such examples of mediator antagonists.
- **Attacking chronic inflammation:** Some drugs have impeding effects on atopic disease at many stages. For example, cetrizine has its effect on histamine receptor and eosinophil recruitment both. Similarly corticosteroids stabilize macrophages and inhibit activation and proliferation of Th2 cells. Other examples include budesonide, mometasone furoate etc.

Drug	Mechanism of action
Cromolyn sodium	Blocks Ca^{2+} influx in mast cells
Antihistamine	Block histamine receptor on target cells
Inhalent isoprenaline	Stabilizes the mast cell triggering
β_2 antagonists (e.g. salmeterol, formoterol)	Act as bronchodilators and protect against bronchoconstriction
leukotriene antagonists (e.g. pranlukast)	Protect against bronchoconstriction
phosphodiesterase inhibitor (e.g. theophylline)	Prolongs high cAMP level in mast cells
Adrenaline/ epinephrine	Stimulate cAMP production
Cortisone	Block histidine to histamine conversion, stimulate cAMP production

Table 7: Some of the common drugs used in type-I hypersensitivity

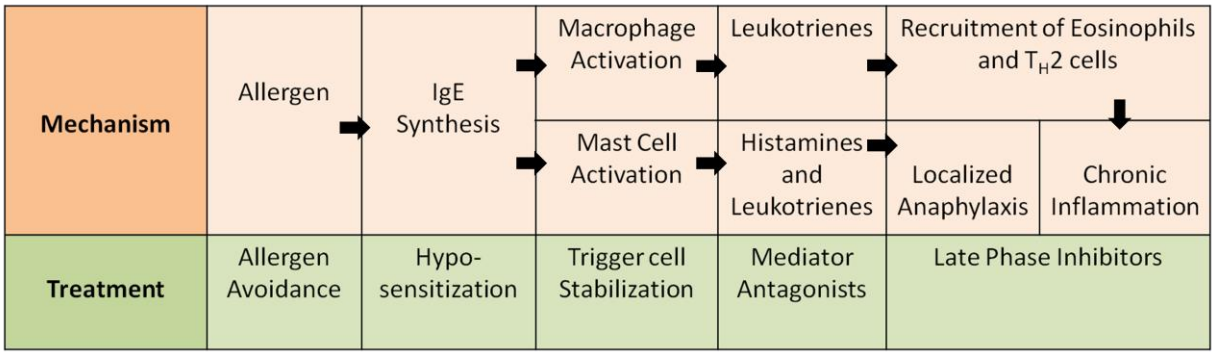


Figure 18: Events and possible treatment approach during atopic allergies
Source: Author [Modified from Roitt's Immunology 10th edition (2001) by IvanM. Roitt and Peter J. Delves]

14. Summary

- The concept of allergy as given by Von Pirquet in 1906 refers to overall response of an individual to an antigen. If response if protective in nature, it is called as immunity. Whereas the harmful deleterious effect due to inappropriate immune response is termed as hypersensitivity.
- Another term for similar response was proposed earlier by Richet and Portier to denote 'over-reactive' immune response.
- Cooke and Coca proposed a generalized term 'hypersensitivity' which was classified by Gell and Coombs in four different types based on branch of immune response involved, time taken to show the symptoms, effector mechanism etc.
- Type-I hypersensitivity is IgE based allergic reaction raised against allergens. It is a humoral immunity based immediate type hypersensitive reaction. Ex. Asthma. Type-II hypersensitivity is IgG mediated cytotoxic hypersensitivity which is also humoral immunity based immediate type hypersensitive reaction. Ex. *Erythroblastosis fetalis*. Type-III hypersensitivity is immune complex mediated, humoral, immediate type hypersensitivity. Ex. Rheumatoid arthritis. Type-IV hypersensitivity is cell-mediated hypersensitivity. It is cell mediated immunity based, delayed type hypersensitive reaction. Ex. Contact dermatitis.
- Type-I hypersensitivity is an immediate type hypersensitivity within the humoral branch of immunity which is induced by antigens called as 'allergens'. It is similar to normal humoral immune response except the fact that plasma cells secrete IgE antibody in response to activation of allergen-specific T_H-2 cells.
- The components involved in type-I hypersensitivity are allergens air-borne, food-borne or drugs), immune cell (T_H2 cells, mast cells, basophils, B cells), various cytokines, IgE, Fc receptors for IgE (type I and II) and various mediator molecules (Primary and secondary). The degranulation of basophils and mast cells is the key point of type-I hypersensitivity.
- The generalized mechanism of type-I hypersensitive reaction involves sensitization and degranulation phase. Sensitization phase involves exposure to allergen, activation of T_H2 cells, release of IgE from plasma cells and binding of these IgE with Fc receptor of mast cells. Degranulation phase involves release of mediators on re-exposure of same allergen and downstream effect or functions.
- Clinical manifestation of type-I hypersensitivity includes systemic anaphylaxis and localized anaphylaxis i.e. allergic dermatitis, hay fever, asthma etc.

- Various factors regulate type-I hypersensitive response such as: dose and type of allergen, processing of allergen, genetic constitution of the individual and the relative ratio of T_H1 and T_H2 cells.
- Wheel and flare test, Radioimmunosorbent assay (RIST) and Radioallergosorbent assay (RAST) are common medical tests to detect the type-I hypersensitive individuals for a particular allergen.
- The treatment/ precaution involves avoidance of allergen, modulation of immunological response, stabilization of the triggering cells, antagonism of mediators and attacking chronic inflammation. Various drugs are available to cure type-I hypersensitive reactions now.