

LECTURE: 08

Title: IMMUNOLOGICAL MECHANISM IN TISSUE DAMAGE
TYPE-I- HYPERSENSITIVITY "IMMEDIATE HYPERSENSITIVITY"

LEARNING OBJECTIVES:

The student should be able to:

- Define the Terms "hypersensitivity reactions, allergen, anaphylactic".
- Explain the Coombs & Gell classification for type I reactions.
- Describe type I hypersensitivity.
- Define the term "Atopic allergies", and give examples.
- Specify the location of the type I occurrence.
- List the seriousness of the type of symptoms results from Type I.
- List some common allergens that cause type I activation.
- Explain the initiation "triggering" process of Type I reactions, including the involved immunological elements either cellular or humoral, and distinctive cellular receptors, and the different stages of the reaction.
- Determine the predominant location of the involved immune cell (s).
- Explain the role of the process of cellular cross-linking in type I hypersensitivity.
- Enumerate the factors that control the strength of type I reaction.
- Enumerate some primary and secondary pharmacological mediators that cause the allergic reactions such as:
 - Primary mediators are made and stored in cytoplasmic granules these are; histamine, proteases, and chemotactic factors.
 - Secondary mediators are formed after activation and include; platelet activating factor, leukotrienes, prostaglandins, bradykinin and cytokines.
- List some examples of type I hypersensitivity reactions such as:

- Anaphylaxis - Atopic asthma - Atopic eczema (familial predisposed) – Drug allergy – Hay fever, allergic rhinitis.
- Describe the clinical and pathological manifestation of type I hypersensitivity.
- Enumerate the factors that control the strength of type I reaction.
- Enumerate the diagnostic and investigating tests for type I reactions.
- Explain the reason why some people are allergic for a particular substance and the others not?.

LECTURE REFERENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE. IMMUNOLOGY. 6th edition. Chapter 21. pp. 324-341.

2. HANDOUT.

Hypersensitivity – Type I

- Production of IgE in genetically predisposed, i.e., atopic, individuals occurs in response to repeated low-dose exposure to inhaled allergens such as dust mite, cat dander or grass pollen.
- IgE antibodies bind to specific receptor, FcεRI, on mast cells and basophils. When bound IgE is cross-linked by specific allergen, mediators including histamine, leukotrienes and cytokines are released.
- Allergic diseases include anaphylaxis, seasonal hayfever, atopic dermatitis and allergic asthma. Therapy includes antihistamines, adrenaline, bronchodilators, corticosteroids, reducing exposure to allergens and specific allergen immunotherapy.
- The severity of symptoms depends on IgE antibodies, the quantity of allergen, and also a variety of factors that can enhance the response including viral infections and environmental pollutions.

- Multiple genetic loci influence the production of IgE, the inflammatory response to allergen exposure and the response to treatment. Polymorphisms have been identified in the genes, in promoter regions and in the receptors of IgE, cytokines, leukotrienes and the β_2 -receptors.
- The biological role of immediate hypersensitivity is to control helminth infections such as schistosomiasis, hookworm of *Ascaris*. However, it is likely to be a combination of effector TH2 cells, basophils and eosinophils, as well as IgE antibodies on mast cells that control these worms.

The adaptive immune response provides specific protection against infection with bacteria, viruses, parasites and fungi. In particular, it is able to provide rapid protection against a repeated challenge with the same or similar foreign organism or toxin. By contrast, some immune responses can give rise to an excessive or inappropriate reaction; this is usually referred to as hypersensitivity. Hypersensitivity may occur as an exaggerated form of an appropriate response, for example to a virus, or from a response to an antigen that has no toxic potential, for example asthma with inhaled cat dander or eczematous response of the skin to jewellery containing nickel. Typical examples of hypersensitivity include contact sensitivity, antibody mediated responses against self antigens, and immune complex deposition in the kidneys, joints or skin. However, the most common forms of hypersensitivity are allergic responses characterized by wheal and flare skin responses to the relevant antigen, which are mediated by IgE antibodies binding to mast cells. Coombs and Gell classified hypersensitivity reactions into four forms, Type I-IV. The classification is a useful guide to understanding the different forms of response. However, some conditions do not fit easily into the classification and only the terms Type I and Type IV are used routinely (**Figure-1**).

Type I or immediate hypersensitivity is characterized by the production of IgE antibodies against foreign proteins that commonly present in the environment, for example pollens, animal dander or dust mites. These antibodies bind specifically to a high-affinity receptor on mast cells and basophils, which are the only human cells that contain histamine. Subsequently exposure to the same antigen will lead to rapid release of histamine, and more gradual release of other mediators including leukotrienes and cytokines. The conditions that are associated with Type I hypersensitivity include hayfever, asthma, atopic dermatitis and anaphylaxis. Type II or antibody-mediated food allergy reactions occur when antibodies, either of the IgG or IgM isotypes are produced against surface antigens present on cells of the body. These antibodies can trigger cytotoxic reactions either by activating complement (c) (e.g. autoimmune haemolytic anaemia) or by facilitating the binding of natural killer cells (NK). Type III or immune complex disease occurs when excess complexes are formed in the circulation that cannot be cleared by macrophages or other cells in the reticuloendothelial system. The formation of immune complexes requires significant quantities of antibodies and antigen (typically μm quantities of each). The local accumulation of complexes can trigger either a complement or a cell-mediated local reaction. The classical diseases in which immune complexes are thought to be involved are systemic lupus erythematosus (SLE) and serum sickness. Finally, Type IV or cell-mediated reactions are those in which specific T cells are the primary effector cells. The simplest examples of T cells causing unwanted responses are

contact sensitivity (e.g. to nickel or poison ivy) and graft rejection. However, specifically sensitized T cells also play a role in the chronic hypersensitivity skin responses of leprosy or tuberculosis, and are an important part of the exaggerated response to viral infections such as measles.

IMMEDIATE HYPERSENSITIVITY

Historical introduction

The classical allergic disease is seasonal hayfever caused by pollen grains entering the nose (rhinitis) and eyes (conjunctivitis). In severe cases patients may also get seasonal asthma and seasonal dermatitis. Charles Blackley in 1873 demonstrated that pollen grains placed into the nose could induce rhinitis. He also demonstrated that pollen extract could produce a wheal and flare skin response in patients with hayfever. The wheal and flare skin response is an extremely sensitive method of detecting specific IgE antibodies. The timing and form of the skin response is indistinguishable from the local reaction to injected histamine. Furthermore, the immediate skin response can be effectively blocked with antihistamines. In 1903 Portier and Richet discovered that immunization of guinea-pigs with a toxin from the jellyfish *Physalia* could sensitize them so that a subsequent injection of the same protein would cause rapid onset of breathing difficulty, influx of fluid into the lungs, and death. They coined the term anaphylaxis (from the Greek *ana*=non, and *phylaxis*=protection) and speculated about the relationship to other hypersensitivity diseases. They noted that human anaphylaxis had no familial characteristics (unlike most of the other allergic diseases) and that natural exposure to inhaled allergens did not cause anaphylaxis or urticaria. Subsequently, it became clear that injection of any protein into an individual with immediate hypersensitivity to that protein can induce anaphylaxis. Thus anaphylaxis occurs when a patient with immediate hypersensitivity is exposed to a relevant allergen in such a way that antigen enters the circulation rapidly; this can occur after a bee sting, an injection of penicillin eating an allergen such as peanut or shellfish, or following a therapeutic allergen injection for hyposensitization (**Figure-2**). The term allergen was first used by von Pirquet to cover all foreign substances that could produce an immune response. He included those substances that could induce 'supersensitivity' the word they used for allergy. Subsequently the word 'allergen' came to be used selectively for the proteins that cause 'supersensitivity'. Thus, an allergen is an antigen that gives rise to immediate hypersensitivity.

Characteristics of allergens

Substances that can give rise to wheal and flare responses in the skin and to the symptoms of allergic disease are derived from many different sources. When purified they are almost all found to be proteins and their size ranges in molecular weight from 10000 to 40000 Daltons. These proteins are all freely soluble in aqueous solution but have many different biological functions including digestive enzyme, carrier proteins, calycons and pollen recognition proteins. Any allergen can be described or classified by its source,

route of exposure and the nature of the specific protein (**Figure-3**). Extracts used for skin testing or in vitro measurement of IgE antibodies are made from the whole material which contains multiple different proteins, any of which can be an allergen. Indeed, it is clear that individual patients can react selectively to one or more different proteins within an extract. Estimates of exposure can be made either by visual identification of particles (e.g. pollen grains or fungal spores) or by immunoassay of the major allergens (e.g. Fel d 1 or Der p 1).

IMMUNOGLOBULIN E

In 1921 Küstner, who was allergic to fish, injected his own serum into the skin of Prausnitz, who was allergic to grass pollen but not fish, and demonstrated that it was possible to passively transfer immediate hypersensitivity (the Prausnitz Listner or P-K test). Over the next 30 years it was established that P-K activity was a general property of immediate hypersensitivity, and that it was allergen specific, i.e. behaved like an antibody. In 1967 Ishizaka and his colleagues purified the P-K activity from a patient with ragweed hayfever and proved that this was a novel isotype of immunoglobulin: IgE. However, it was obvious that the concentration of this immunoglobulin isotype in serum was very low. The initial antisera to IgE made it possible to identify a patient with multiple myeloma whose serum contained a very high concentration of IgE (~10 mg/ml). Purification of this myeloma protein led to the full structure of IgE and also to the production of protein antisera. Antisera to IgE are used in the radioallergosorbent test (RAST) to measure IgE antibodies in serum, as well as for measuring total serum IgE is distinct from the other dimeric immunoglobulin because it has an extra constant region domain, a different structure to the hinge region, and binding sites for both high and low-affinity IgE receptors, FcεRI and FcεRII, respectively (**Figure-4**). The primary cells that bear FcεRI are mast cells and basophils which are only cells in the human that contain significant amounts of histamine.

The properties of IgE can be separated into three areas: the characteristics of the molecules including its half-life and binding to IgE receptors; the control of IgE and (IgG4) antibody production by T cells; and the consequences of allergen cross-linking IgE on the surface of mast cells or basophils.

Half-life of IgE

The concentration of IgE in the serum of normal individuals is very low compared to all the other immunoglobulin isotypes. Values range from <10 to 10000 IU/ml, and the international unit IU is equivalent to 2.4 ng. Most sera contain less than 1 µg IgE/ml. The reasons why serum IgE is so low include: (i) serum IgE has a much shorter half-life than other isotypes, ~2 days compared with 21-23 days for IgG; (ii) IgE is produced in small quantities and is only produced in response to a select group of antigens (allergens and parasites); and (iii) IgE antibodies are sequestered on the high affinity receptor on mast cells and basophils. The half-life of IgE in the serum has been measured both by injecting

radiolabeled IgE and by infusing plasma from allergic patients into normal and immune-deficient patients. The half-life of IgE in serum is less than 2 days; by contrast, IgE bound to mast cells in the skin has a half-life of ~10 days. However, the low quantities of IgE in the serum must reflect a more rapid breakdown of IgE, as well as removal from the circulation by binding onto mast cells. The most important site of breakdown of IgE is being thought to be within endosomes where the low pH facilitates breakdown of free immunoglobulin by cathepsin. Serum is constantly being taken up by endocytosis. Most macromolecules including IgE degrade in the endosome. One major exception is IgG, which is protected by binding to the neonatal Fc gamma receptor, FcγRn (**Figure-5**).

Placental transfer of antibodies

In cord blood the concentration of IgE is very low indeed, generally less than 1 IU/ml (i.e. <2 ng/ml). Thus there appears to be almost no transfer across the placenta. By contrast, IgG including IgG antibodies to allergens such as those from dust mite or cat are very efficiently transferred across the placenta. This process also involves endocytosis and receptor-mediated transport. Passive transfer of IgE to the fetus may be blocked because IgE is broken down in the endosomes or because an Fc receptor is essential for transport, and there is no receptor for IgE on the cell that comprises the placenta tissues.

ROLE OF T-CELLS IN THE IMMUNE RESPONSE TO INHALANT ALLERGENS

Experiments in animals have established that the production of IgE is completely dependent on T cells. It is also clear that T cells can suppress IgE production. The T cells which can suppress IgE production act predominantly by producing interferon- γ (IFN γ), and are produced when the animal, for example mouse, rat or rabbit, is primed in the presence of Freund's complete adjuvant. This adjuvant, which includes bacterial cell walls and probably bacterial DNA, is a very potent activator of macrophages. With the discovery of TH1 and TH2 cells, it became clear that IgE production is dependent on TH2 cells and that any priming that generates a TH1 response will inhibit IgE production. The main cytokines that are specifically relevant to a TH1 response include interleukin-12 (IL-12) produced by macrophages and IFN γ produced by T cells. By contrast, the primary cytokines relevant to a TH2 response are IL-4 (IL-13), IL-5 and IL-10 (**Figure-6**). It is clear that from experiments in mice and humans that the expression of gene for IgE is dependent on IL-4. Thus if immune human B cells are cultured with anti-CD40 and IL-4, they will produce IgE antibodies.

Cytokine regulation of IgE production

In human IgE antibodies are the dominant feature of the response to a select group of antigens and most other immune responses do not include IgE. The classical allergens are inhaled in very small quantities (5-20ng/day) either perennially indoors or over a period of weeks or months outdoors. Immunization of mice with repeated low-dose antigen is a very effective method of inducing IgE responses. By contrast, the routine immunization

of children with diphtheria and tetanus toxoid does not induce persistent production of IgE antibodies. This is clear because we do not routinely take precautions against anaphylaxis when administering a booster injection of tetanus. The main factors that influence the development of T cells into the TH1 or TH2 pathways are the cytokines produced at the time of priming, in particular IL-12 and IL-4. IL-12 can be produced by macrophages or dendritic cells and is directly involved in the enhancement of IFN γ production and the associated differentiation towards the TH1 phenotype. As T cells differentiate, TH1 cells express the functional IL-12 receptor with IL-12 β 2chain; by contrast, TH2 cells express only part of the IL-12 receptor and this part is non-functional. IL-4 is important in the differentiation of TH2 cells and is also a growth factor for these cells. Since IL-4 is produced by TH2 cells, it is at least in part acting in autocrine fashion. The interaction of IL-4 with T cells can be blocked either with an antibody to IL-4 or with a soluble form of the IL-4 receptor. The release of soluble IL-4R from T cells may be a natural mechanism for controlling T cell differentiation. It follows that inhaling recombinant soluble IL-4R is a potential therapeutic strategy to control allergic responses in the lung.

The relationship between IgE and IgG4

The genes for immunoglobulin heavy chains are in sequence on chromosome 14. The gene for epsilon occurs directly following the gene for gamma-4. Both of these isotypes are dependent on IL-4 and they may be expressed sequentially (**Figure-7**). The mechanisms by which IgG4 is controlled separately from IgE are not well understood but this may include a role for IL-10. Thus, immunotherapy for patients with anaphylactic sensitivity to honey bee venom will induce IL-10 production by T cells, decreased IgE and increased IgG4 antibodies to venom antigens. Recently, it has been shown that children raised in a house with a cat can produce an IgG including an IgG4 antibody response without becoming allergic. Thus a modified TH2 response (increased IgG4 and decreased IgE) represents an important mechanism of tolerance to allergens (**Figure-8**).

ALLERGENS: THE ANTIGENS THAT GIVE RISE TO IMMEDIATE HYPERSENSITIVITY

Properties of the proteins

In mice a wide range of proteins can be used to induce an IgE antibody response. The primary factors that influence the response are the strain of mouse, the dose and adjuvant used. Thus repeated low-dose immunization with alum or pertussis (but not complete Freud's adjuvant) will produce IgE responses. However, the dose necessary to induce a response varies greatly from one strain to another.

The allergens that have been defined have similar physical properties (i.e. freely soluble in aqueous solution with a molecular weight between 10000 and 40000 Da), but are very diverse biologically. Cloning has revealed sequence homology between allergens and

diverse proteins including calycins, pheromone binding proteins, enzymes and pollens recognition proteins. Although many of the allergens have homology with known enzymes, this is not surprising since enzyme activity is an important property of proteins in general. However, some important allergens, for example Der P 2 from mites, Fel d 1 from cats and Amb a 5 from ragweed pollen, have neither enzymic activity nor homology with known enzymes. Thus enzymic activity is not essential for immunogenicity. Nevertheless, the Group I allergens of dust mites are cysteine proteases and in several model situations it has been shown that this enzymic activity influences the immunogenicity of the protein. Thus cleavage of CD23 or CD25 on lymphocytes by Der p I can enhance immune responses. Alternatively, it has been shown that Der p I can disrupt epithelial junctions and alter the entry of proteins through the epithelial layer. The interest in this property is increased because many different mite allergens are inhaled together in the faecal particle so that the enzymic activity of one protein (i.e. Der p 1) could facilitate either the physical entry or the response to other mite proteins. However, the lungs contain many different naturally occurring proteases (as well as anti-proteases) which are just as potent as these allergens.

The primary characterization of allergens relates to their route of exposure. This includes inhaled allergens, foods, drugs, antigens from fungi growing on the body (e.g. *Aspergillus*) and venoms. The routes are important because they define the ways in which the antigens are presented to the immune system. Since antigen presentation may well be the site at which genetic influences play the biggest role, the properties of the different groups need to be considered separately.

Inhalant allergens

The inhalant allergens are the primary causal agents in hayfever, chronic rhinitis and asthma among school age children or young adults; they also play an important role in atopic dermatitis. Almost all the evidence about the genetics of allergic disease relates to inhalant allergens. Allergens can only become airborne in sufficient quantity to cause an immune response or symptoms when they are carried on particles. Pollen grains, mite faecal particles, particles of fungal hyphae or spores and animal dander are the best defined forms in which allergens are inhaled (**Figure-19**). In each case it is possible to define the approximate particle size and the quantity of protein on the particle as well as the speed with which the proteins in the particle dissolve in aqueous solution (**Figure-3**). Thus for gas pollen, mite faecal pellets and cat dander, the relevant allergens are present in high concentrations (up to 10 mg/cm³), the particles are 'large' (i.e. 3-30 µm diameter) and the allergens elute rapidly in aqueous solution. The allergens within these particles will be delivered to the nasal epithelium because a large proportion of particles of this size will impact on the mucous membrane during passage of inhaled air through the nose.

Quantities inhaled

Estimates of the quantity of mites or pollen-derived proteins inhaled vary from 5 to 50 ng/day. Thus exposure to some allergens may be as little as 1 µg/year. This is very important because it probably explains why the immune response is consistently of this

one kind, i.e. immediate hypersensitivity, and also why no respiratory diseases, other than asthma, have been associated with these allergens. The quantities inhaled also seriously restrict the models about how allergens contribute to asthma. Inhaling between 10 and 100 particles per day will produce localized areas of inflammation in the lungs but would not be expected to induce acute bronchospasm. Equally, the quantities inhaled severely restrict the quantity that enters the blood stream and make it extremely unlikely that the fetus is sensitized or primed to inhaled allergens *in utero*.

Food allergens

While a very large number of food proteins can occasionally give rise to IgE responses, only a small number are common causes of allergic responses. These include egg, milk, peanut, soy, chicken and shellfish. In contrast to inhaled allergens, these proteins are often eaten in very large quantities (i.e. ~10-100 g/day). In general only a small fraction of the food proteins are absorbed. However, small peptides can be freely absorbed and may be recognized by T cells and even by IgE antibodies in a minority of individuals. Nevertheless, the bulk of the allergic and anaphylactic responses to foods are thought to be related to food proteins that have not been digested, either triggering mast cells in the intestine or entering the circulation.

Tertiary structure of modified allergens and peptides

Many different allergens have been cloned, and for a few the tertiary structure is now known either from X-ray crystallography (i.e. Bet v 1), by nuclear magnetic resonance (NMR) (e.g. Der p 2) or by modeling relationships to known homologues (**Figure-10**). Knowledge of the tertiary structure makes it possible to predict surface residues and to define IgE binding sites using sites-directed mutagenesis. This approach has the potential to design molecules which have decreased IgE binding properties but with preserved T cell epitopes. Given the importance of T cells to the control of IgE antibody production and their potential role in the recruitment of inflammatory cells, it is logical to try to use molecules that will directly 'desensitize' T cells. The approach used has been to produce peptides of different lengths. Therapeutic trials have been carried out with peptides from ragweed pollen antigens and the cat allergen Fel d 1. The results show that peptide recognition is restricted by the HLA-DR type of the patient, which means that a wide range of peptides are necessary for treatment. In addition, there is clear evidence that peptides can produce a significant response in the lungs (**Figure-11**). This is the clearest evidence yet that T cells in the lung can contribute to an asthmatic response.

MAST CELLS AND BASOPHILS

The only human cell types that contain histamine are mast cells and basophils. In addition, there are the only cells that express the high-affinity receptor for IgE FcεRI under resting conditions. Under most circumstances the primary and most rapid consequence of allergen exposure in an allergic individual is cross-linking of IgE receptors on these two cell types. Basophils are circulating polymorphs which are not

present in normal tissue but can be recruited to a local site by cytokines released from either T cells or mast cells. By contrast, mast cells cannot be identified in the circulation but are present in connective tissue and mucosal surfaces throughout the body (**Figure-12**). Mast cells in different tissues are morphologically and cytogenetically distinct. Both the cells contain histamine and the biology of these cells may be very different in other species. For example, in the rabbit the histamine content of the peripheral blood is almost all in platelets, in the mouse there are few if any circulating basophils and in rats the degranulation of mast cells appears to be one granule at a time (**Figure-12**). By contrast, human granules tend to fuse and release their contents together (**Figure-13**).

Connective tissues and mucosal mast cells

Mast cells were originally identified by Ehrlich who named them (Mast=well fed, or fattening, in German) because of the distinctive, tightly packed granules. Mast cells in different tissues can be distinguished by staining for proteases, and the content of these enzymes may be relevant to their role in allergic diseases. The granule proteases of mast cell have been cloned and sequenced and are distinct for two types of mast cells (**Figure-14**). Mucosal mast cells are characterized by the presence of tryptase without chymase. By contrast, connective tissue mast cells contain both chymase and tryptase. These enzymes may play a direct role in the lung inflammation of asthma, either by breaking down mediators or in the case of tryptase by acting as a fibroblast growth factor. Basophils contain very little of either of these proteases. Staining of basophils in tissue sections requires special fixation and staining. Without this staining the granules in basophils cannot be identified and the cells appear as neutrophils (i.e. polymorphs without special granules).

Local accumulation of mast cells and basophils

Although mast cells are present in normal non-inflamed tissue, their numbers are increased in response to inflammation. It is assumed that this accumulation is T cell dependent, since in rats infected with *Nippostrongylus brasiliensis* accumulation of mast cells in the gut is dependent on T cells and can be suppressed by corticosteroids. In guinea-pigs the immune response to tick bites includes a large local accumulation of basophils. Indeed, the tick is thought to be killed by basophils that it ingests. In allergic individuals mast cell recruitment has been demonstrated both in the skin in response to repeated allergen exposure and in the nose during the pollen season. In both situations basophils are also recruited. In the nose the recruitment of cells represents a shift so that mast cells move from the subepithelium into the epithelium while basophils appear in the nasal mucus. This process, which brings histamine-containing cells closer to the site of entry of allergen, is one of the ways in which allergic individuals become more sensitive. It is likely but less well established that equivalent processes occur in the human lung and gut.

Degranulation of mast cells and basophils

The process of degranulation in human mast cells and basophils involves fusing of the membrane of the granules containing histamine with the exterior cell membrane (**Figure-13**). The granule membrane becomes part of the cell membrane; the granule contents rapidly dissolve and are secreted, leaving behind a viable degranulated or partially degranulated cell. This process is initiated in most cases by cross-linking of two specific IgE molecules by their relevant allergen. When two IgE receptors (FcεR1) are cross-linked, signal transduction occurring through the gamma chains of the receptor leads to influx of calcium, which initiates both degranulation and the synthesis of newly formed mediators (**Figure-15**).

Cross-linking of IgE antibodies on FcεR1 by allergens is the primary methods by which mediators are released from basophils and mast cells; however, other mechanisms can be involved. Experimentally, degranulation can be triggered through FcεR1 by using lectins such as phytohaemagglutinin (PHA) or concanavalin A (Con A), or with antibodies to the α chain of the receptor. Histamine release can also be triggered by agents that act on other receptors on the cell surface. Typical examples include the complement components C5a and C3a; drugs such as codeine or morphine; the antibiotic vancomycin; and contrast media used for imaging the kidneys. Acute reactions to these agents which are not thought to involve IgE antibodies are referred to as anaphylactoid.

GENETICS OF ALLERGIC DISEASE

Hayfever and asthma are strongly hereditary

Children with one allergic parent have a 30% chance of developing allergic disease while those who have two allergic parents have as high as a 50% chance. Systematic studies of allergic diseases are difficult because the phenotypes for diseases, such as hayfever and asthma, are not well defined and depend on the approach used to make the diagnosis (**Figure-16**). Thus asthma defined by a parent questionnaire is less specific than asthma defined by testing of specific or non-specific bronchial hyper-reactivity. Furthermore, studies on asthma are complicated because both IgE antibody responses and bronchial reactivity are genetically controlled. Indeed, it is important not to confuse simple genetic diseases like cystic fibrosis or haemophilia with complex traits such as asthma or Type II diabetes. Thus, it is not at all surprising that multiple genes have been associated with asthma in different populations. A further major problem in genetic analyses of allergic disease comes from the progressive increase in asthma between 1960 and 2000. Clearly this increase cannot be attributed to genetic change and implies that some of the genes identified would only influence asthma in the presence of other changes influencing the environment or lifestyle. This is referred to as a gene: environment interaction.

Multiple genes or genetic regions are associated with asthma. Analyses of the genetics of immediate hypersensitivity have identified both allergen specific and non-specific influences. Thus there are HLA associations with atopy in general and also with

sensitization to specific allergens. However, these genetic studies have given the clearest associations when fully purified allergens are used to test sensitization (**Figure-17**). The genetics of asthma has been studied both by genomic screening and by using candidate genes. Genomic screen identifies regions of the genome which link to asthma so that this region can be examined to identify specific genes. If a candidate gene is identified, it is possible to examine the gene for polymorphisms that link to asthma. However, a brief consideration of the possible targets makes it clear how complex the analysis of asthma is likely to be, and indeed is proving to be (**Figure-18**). Typical examples include polymorphisms of the promoter region for IL-4 and polymorphisms of the gene for IL-5, either of which could directly influence the inflammatory response to allergens. Alternatively, a series of polymorphisms have been identified that influence the response of asthma to treatment. These include variants of the β_2 -adrenergic receptor α chain, and genetic differences that influence the efficacy of leukotriene antagonists. In the last 10 years multiple 'genes' or genetic regions associated with asthma have been identified. At present, it appears that the overall effects are too complex to be of any practical significance. Certainly it is most unlikely that gene transfer will ever be of significance. However, as genetic screening becomes easier, pharmacogenetics may well become an important method for identifying the best drugs for individual patients in the management of chronic diseases such as asthma.

SKIN TESTS FOR DIAGNOSIS AND INVESTIGATION

The primary method for diagnosing immediate hypersensitivity is skin testing. The characteristic response is a wheal and flare (**Figure-19**). The wheal is caused by extravasation of serum from capillaries in the skin which results from a direct effect of histamine. This is accompanied by pruritus (also a direct effect of histamine) and a large erythematous flare which is mediated by an axon reflex. This skin response takes 5-15 minutes to develop and may persist for 30 minutes or more. Techniques for skin testing include a prick test, in which a 25 gauge needle or a lancette is used to introduce 0.1 μ l of extract into the dermis. Alternatively, an intradermal injection of 0.02-0.03 ml is used. All antigen injections have the potential to cause anaphylaxis but the intradermal test which introduces ~200 times more extract should always be preceded by a prick test.

Skin tests are evaluated by the size of the wheal compared to a positive (histamine) and negative (saline) control; in general, a 3x3 mm wheal in children and a 4x4 mm wheal in adults can be considered a positive response to a prick test. A positive skin test indicated that the patient has specific IgE antibodies on the mast cells in their skin. In turn this implies that bronchial or nasal challenge would also be positive if sufficient antigen was administered. In most cases (i.e. ~80%) where the skin test is positive, IgE antibody will be detectable in the serum. However, blood tests for IgE antibody are generally less sensitive than skin tests.

Relevance of a positive skin test (or IgE antibody assay)

Epidemiologically, sensitization to a relevant inhalant allergen is a 'risk factor' for allergic disease. Thus, an individual with a positive skin test to grass pollen is up to ten times more likely to have hayfever during the grass pollen season (odds ratio ≥ 10) than a skin test negative individual. Equally an individual with a positive skin test to dust mite or cat allergen is more likely to have asthma (odds ratios 2-6). It is assumed that allergen exposure contributes to the risk, but that relationship is not simple. However, positive skin tests are common, and in individual cases they may not be relevant. This may be because the patient is not exposed to the allergen, i.e. rhinitis symptoms occurring at a time of year when pollen is not present. Alternatively, up to one third of skin test positive patients do not experience symptoms even when they are exposed.

Late and delayed skin responses

Late reactions can occur following an immediate response to allergen either in the skin or the lungs. A late skin response is only common following a large immediate response, i.e. wheal size 10x10 mm. This response, which is diffuse, erythematous and indurated, generally starts 2-3 hours after the wheal and may last up to 24 hours. The late reaction is considered to be a model of the events that lead to persistent inflammation in the nose, lungs or skin.

Late reactions probably include several different events: (i) the direct effects of prostaglandins, leukotrienes and cytokines released by mast cells following the initial release of histamine; (ii) infiltration of lymphocytes, eosinophils, basophils and neutrophils into the local site mediated by chemokines and other cytokines released from mast cells; (iii) release of products from the infiltrating cells. In general, these different events are occurring in parallel over a period of hours.

Eczematous patch test (atopy patch test)

The infiltration of cells into the skin that occurs in the 24 hours after an allergen is applied can be studied in several ways: by local intradermal injections; by applying a patch of allergen on gauze that stays on the skin for 2 days; or by fixing a chamber containing allergen over a denuded area of skin. The skin chamber allows repeated sampling while the other two techniques require biopsy of the skin. In the patch test 10 μg allergen is applied on a gauze pad 2.5 cm^2 , and the biopsy is carried out at 24 or 48 hours. A positive patch response induces macroscopic eczema, spongiosis of the epidermis (a hallmark of eczema) and an infiltrate of cells into the dermis (**Figure-20**). The cellular infiltrate includes eosinophils, basophils and lymphocytes. With persistent allergen at a site, i.e. 6 days, the eosinophils degranulate locally. This is in keeping with the evidence that the skin of patients with eczema has large quantities of the eosinophil granule major basic protein (MBP), even though very few whole eosinophils are visible (**Figure-21**).

Biopsy of patch tests also yields T cells that are specific for the allergen for example dust mite, thus establishing that antigen challenge. Some groups have succeeded in cloning

allergen specific T cells from the skin of eczema patients without a preceding patch. This is never possible from normal individuals or allergic patients without eczema.

Answering whether allergen specific T cells are present at local sites is important because T cells could play a role both as effector cells and in the recruitment of other cells. Establishing whether T cells play an effector role is relevant to the nose in rhinitis, the lungs in asthma, the conjunctiva in hayfever, as well as to the skin in atopic dermatitis.

Biopsy of patch test sites has also established that the Langerhans' cell in the skin of patients with eczema express FcεR1. Thus, these antigen presenting cells use IgE antibodies to help capture allergens and to increase the efficiency of antigen presentation.

Passive transfer of patch test response – Following injection of serum from a patient with atopic dermatitis into the skin of a non-allergen individual, a patch test can be carried out. Biopsy of this passively transferred response reveals large number of eosinophils. Thus, at least part of the eczematous response can be passively transferred. The mechanisms for this response are thought to be: (i) passive sensitization of IgE antibodies onto mast cells in the dermis; (ii) triggering of the local mast cells with allergen to release histamine, leukotrienes and cytokines; (iii) recruitment of eosinophils by IL-5 as well as by chemokines such as RANTES or eotaxin.

FACTORS THAT INFLUENCE THE SYMPTOMS OF ALLERGEN DISEASE

The diagnosis of allergy is made by skin tests or by serum assays of IgE antibodies. These antibodies form part of an immune response which also includes antibodies of other isotypes (IgG1, IgG4 and IgA), as well as T cells that are characteristically TH2. The release of histamine within 15 minutes after allergen exposure can only explain a small proportion of allergic disease. In particular, the chronic inflammation in the lungs of patients with asthma and in the skin of patients with atopic dermatitis has many features that cannot be explained by histamine. First, the time course is too long; secondly, there is a cellular infiltrate in these tissues; and third, there are major differences in disease between patients who have apparently similar IgE antibodies in their serum and skin (**Figure-22**).

Several different pathways contribute to chronic symptoms and can alter the severity of allergic disease:

1. Local recruitment of mast cells and basophils, combined with increased 'releasability' of these cells, allows increased response to the same allergen challenge. This mechanism plays a major role in the increased symptoms in the nose during the pollen season.
2. Release of leukotrienes, chemokines and cytokines from mast cells or basophils. The mediators can have direct effects on blood vessels and smooth

muscles. IL-5, tumour necrosis factor (TNF) and chemokines are each thought to contribute to the recruitment of inflammatory cells.

3. The action of T effector cells: T cells release a wide range of cytokines which can have direct inflammatory effects. Separating out the different factors influencing chronic allergic symptoms is difficult. Information may be obtained from: (i) the effects of different drugs, particularly disodium cromoglycate, leukotriene antagonists and anti-IgE; and (ii) passive transfer experiments. The fact that cromoglycate can inhibit both the immediate and the late response of the lung to allergen challenge has been taken as evidence that the late reaction is also dependent on mast cell triggering. However, it is clear that cromoglycate can only control part of the chronic inflammation. By contrast, steroids, which are an effective treatment for most of the inflammation in asthma, act selectively on the late response. However, the response to steroid treatment cannot be used to distinguish the delayed effects of the mast cell triggering from the direct effects of T cells, since steroids can inhibit both of these mechanisms (**Figure-23**).

Local or systemic injection of IgE antibodies can passively transfer the wheal and flare skin response. However, passive transfer of serum from an allergic patient (i.e. containing IgE antibodies) into the skin of a non-allergic individual, can also transfer some aspects of the delayed or cellular response. Both late reactions following an intradermal injection of allergen and the patch test response at 48 hours can be passively transferred. This experiment demonstrates that cross-linking of IgE antibody on mast cells in the tissue of a non-allergic individual can lead to local recruitment of eosinophils in the absence of antigen-specific T cells. Thus, in any analysis of the factors influencing the severity of allergic disease, for example response to pharmacological treatment or response to immunotherapy, it is necessary to consider the relevance of both mast cells *and* effector T cells.

ASTHMA AND BRONCHIAL REACTIONS TO INHALED ANTIGENS

Evidence that allergens contribute to asthma

The causal role of bee venom in anaphylaxis or grass pollen in seasonal hayfever is obvious because these diseases occur in individuals who have positive skin tests and the symptoms are directly related to increased exposure. By contrast, the role of inhaled allergens in chronic asthma is less obvious because exposure is perennial, the patients are often not aware of the relationship and only a proportion of skin test positive individuals develop asthma. The evidence that allergens derived from dust mites, cats, dogs, the German cockroach or the fungus *Alternaria* contribute to asthma comes from several different lines of evidence:

1. The epidemiological evidence that positive skin tests or serum IgE antibodies are a major risk factor for asthma.
2. Bronchial challenge with nebulized extracts can produce both rapid bronchospasm, within 20 minutes, and a late reaction, in 4-8 hours, which is characterized by renewed mediator production and a cellular infiltrate.
3. Reduced exposure to allergens can lead to decreased symptoms and decreased non-specific bronchial reactivity. This avoidance can be achieved either by moving patients to an allergen-free unit or by controlling exposure in the home.

The bronchial walls of patients with asthma are characterized by increased mast cells, lymphocytes of the TH2 type, eosinophils and products of eosinophils. In addition, there is increased mucous production secondary to goblet cell hyperplasia, epithelial desquamation and collagen deposition below the basement membrane. These changes are a reflection of chronic inflammation, and it is generally considered that eosinophils play a major role in these events (**Figure-22**). However, recent evidence that anti IL-5 treatment has limited effects on asthma, although it decreases circulating eosinophils, suggests that other cells may play in an important role. Thus, basophils, mast cells, effector T cells and macrophages may all contribute to the non-specific bronchial reactivity.

Analysis of bronchoalveolar lavage (BAL)

Analysis of bronchoalveolar lavage (BAL) after an allergen challenge demonstrates the presence of products derived from mast cells (histamine, prostaglandins and leukotrienes) and eosinophils (major basic protein and eosinophils cationic protein). Furthermore, MBP is present in biopsies of the lungs and can produce epithelial change typical of asthma in vitro (**Figure-24**). The subepithelial collagen deposition present in many patients with asthma is probably a reflection of fibroblast responses to local inflammation. Although it has been suggested that these changes, which are referred to as 'remodeling', can lead to progressive decreases in lung function, the evidence of this view is not clear. In particular, progressive loss of lung function is unusual in asthma and there are no studies showing a correlation between the extent of collagen deposition and changes in lung function. None the less, inhaled steroids which can block many different aspects of inflammation are an effective treatment for asthma.

Bronchial hyper-reactivity

Non-specific bronchial hyper-reactivity (BHR) is present in patients with asthma and is major feature of the disease. Thus, airway obstruction, induced by cold air or exercise, and nocturnal asthma all correlate with non-specific bronchial reactivity. BHR can be demonstrated by challenging the lungs with histamine, methacholine or cold air cold air. The mechanism by which exercise or cold air induces a bronchial response is thought to be evaporation of water with associated cooling of the epithelium. However, it is unclear whether this process triggers nerve endings directly or by causing local mediator release.

Markers of inflammation in patients with asthma

Bronchoscopy is not possible in patients with asthma except as a research procedure. Therefore the only evidence for inflammation of the lungs that can be obtained routinely is indirect. Peripheral blood or nasal smear eosinophils are increased in most patients presenting with an acute episode of asthma (**Figure-25**). In addition, nasal secretions may have increased eosinophil cationic protein (ECP) and IL-8. Additional evidence about inflammation in the lungs can be obtained either from exhaled air or condensates for exhaled air. Nitric oxides gas is increased, i.e. ≥ 10 ppb, in patients with asthma, and this decreases following systemic or local steroid treatment. In addition, the pH of the condensate decreases during acute episodes. The increased exhaled nitric oxide may reflect upregulation of the enzyme nitric oxide synthase. Alternatively, the nitric oxide gas could also increase acutely as a consequence of the fall in pH which is due to decreased ammonia production in the lung epithelium.

In adults further information about the inflammation in the respiratory tract can be obtained from computerized tomography (CT) of the nasal sinuses. Extensive pacification of the sinuses is present in approximately one-third of patients presenting with acute asthma. This reflects both chronic sinusitis, which is a major feature of late-onset asthma, and also sinus inflammation secondary to acute rhinovirus infection. Whether the changes in the sinuses are a reflection of similar effects occurring in the lungs or a source of mediators or T cells that contribute to lung inflammation is not clear.

IMMUNOTHERAPY WITH ALLERGEN EXTRACTS

Immunotherapy (or hyposensitization) with allergen extracts was introduced in 1911 by Noon and Freeman. At that time they were trying to establish immunity against pollen toxin. The treatment requires regular injections of allergen over a period of months. It is an established treatment of seasonal hayfever and for anaphylactic sensitivity to bees, wasps and hornets. In addition, immunotherapy is an effective treatment for selected cases of other allergic diseases including asthma. The dose is increased progressively, starting with between 1 and 10 ng and increasing up to ~ 10 μ g allergen per dose. The response to treatment includes an increase in serum IgG antibodies, a striking decrease in the response of peripheral blood T cells to antigen in vitro, and a marked decrease in late reactions in the skin. Over a longer period of time there is a progressive decrease in IgE antibodies in the serum (**Figure-26**). The change in antibodies, lymphocyte responses and symptoms could all be secondary to changes in T cells. Given the known mechanisms of allergic inflammation a response of T cells to allergen injections could influence symptoms in several ways:

- Decreased local recruitment of mast cells and basophils.
- Decreased recruitment of eosinophils to the nose or lungs.

- Increased IgG including IgG4 antibodies with progressive decreases in IgE. The IgG antibodies may act as blocking antibodies by binding allergen before it cross-links IgE on mast cells.

Some studies of cytokine RNA have suggested that immunotherapy produces a shift in T cell from a TH2 profile (i.e. IL-4 and IL-5) towards a profile that is more typical TH1 (i.e. IFN γ). Although this could explain decreased help for IgE, and decreased eosinophil recruitment, this would not explain the production of IgG4. The expression of the gene for IgG4 is dependent on IL-4, and may also require the cytokine IL-10. This, the response to immunotherapy is better seen as a modification of the TH2 response.

NEW TREATMENTS FOR ALLERGIC DISEASE

New approaches to allergen specific immunotherapy

Peptides from the primary sequence of an allergen can stimulate T cells in vitro

These peptides usually ~20 amino acids in length, stimulate T cells *in vitro*. In theory, peptides provide a mechanism for stimulating or desensitizing T cells without the risk of anaphylaxis that is always present with traditional allergens. Whether incomplete stimulation of T cells by peptides can lead to 'tolerance' or a change in the cytokine profile is not clear. Problems include surprisingly severe reactions in the lung and the fact that multiple peptides are necessary to allow presentation of antigen to patients with different HLA types.

Modified recombinant allergens have decreased binding to IgE

Genetically modified recombinant allergens that have decreased binding to IgE antibodies can be produced. The advantage of these is that the primary sequence with the T cell epitopes is preserved. Even if the molecule is extensively modified, any full-length protein has the potential to induce anaphylaxis in allergic individuals. Thus, the use of genetically modified molecules would always require precautions similar to those for traditional immunotherapy. A potential but unlikely problem is that patients would develop IgE antibodies against new epitopes.

Adjuvants can shift the immune response to TH1

Adjuvants 'attached' to allergen molecules have been designed to shift the immune response from TH2 towards TH1. Possible co-molecules that act like an adjuvant include the cytokine IL-12 or immunostimulatory sequences (ISS). ISS are DNA sequences such as cytosine phosphoguanidine (CpG), that are common in bacterial DNA, and which have profound effect on the mammalian immune system. In mice combining an antigen with two or three molecules of CpG can induce a TH1 response or down-regulate IgE responses. Combining CpG with allergen not only influences the response but also

reduces the reactivity of the allergen with IgE. Thus immunization with allergen and CpG may produce a greater immune response with less potential for an acute allergic reaction.

DNA vaccines are designed to change the immune response

The concept of immunizing with the gene for an antigen is well established, i.e. DNA vaccines. This approach has potential for the treatment of allergy because the DNA vector can be designed to change the immune response. Prokaryotic DNA includes CpG motifs so that as the antigen is expressed, it will induce a TH1 response. Experiments with DNA vaccines have been very successful in mice, both in inducing a TH1 response initially and in controlling an existing IgE antibody response. However, the consequences of expressing an allergen within the tissue of an allergic individual are not known. Equally, it is not clear whether inducing a TH1 response to a ubiquitous allergen such as cat would give rise to other forms of inflammatory disease.

New approaches to non-specific therapy

Humanized monoclonal anti-IgE treatment may reduce sensitivity –

Antibodies directed against the binding site for FcεRI on IgE can bind to IgE in the circulation but not when it is attached to mast cells or basophils. Thus an antibody of this kind can remove IgE from the circulation but will not induce anaphylaxis. Starting with a mouse monoclonal antibody to IgE, the molecule has been chimerized with human IgG1 and then progressively humanized so that less than 10% of the molecule is derived from the original mouse sequence. This molecule can be safely injected into patients and will bind IgE with very high affinity. In clinical trials treatment with anti-IgE antibodies has reduced the symptoms of both asthma and hayfever. In addition, continued treatment which controls free IgE below 10 ng/ml leads to a progressive decrease in the number of IgE receptors on mast cells. Thus, the treatment may achieve a secondary effect further decreasing the sensitivity of histamine-containing cells to allergen. The role of anti-IgE in treating food allergy, atopic dermatitis and drug allergy remains to be established.

Recombinant soluble IL-4 receptor can block biological activity of IL-4 – Given the central role of IL-4 in the TH2 response, it is not surprising that several efforts have been made to block its action. These include a mutated IL-4, (Y124D) antibodies to IL-4 and recombinant soluble IL-4 receptor. The soluble IL-4 receptor (sIL-4R) has proved effective in clinical trials of allergic asthma. The mechanism is that sIL-4R binds to IL-4 before it can react with the receptor on T cells or B cells, and thus blocks its biological activity. However, it is less clear which of the many actions of IL-4 is relevant to the clinical effects. Blocking the action of IL-4 on B cells may reduce IgE production but would probably require several weeks to produce a clinical effect. However, the autocrine effect of IL-4 on TH2 cells may be an essential growth factor. The efficacy of sIL-4R provides further evidence for the role of T cells in allergic disease.

Humanized monoclonal anti-IL-5 decrease circulating eosinophils –

Anti-IL-5 (like anti-IgE) is a humanized mouse monoclonal antibody. Following successful studies in baboons, anti-IL-5 has been shown to decrease circulating eosinophils in patients. Thus it is assumed that binding IL-5 produced by T cells (or mast cells) can decrease the production of eosinophils in the bone marrow. However, the results do not answer whether the treatment acts on IL-5 in the circulation or on IL-5 produced by T cells (or mast cells) locally in the bone marrow and/or the respiratory tract.

THE BIOLOGICAL ROLE OF IgE

The biological role of IgE has been studied both in relation to human disease and using animal models. In tropical countries total serum IgE is usually much higher than in the West. Typical mean serum IgE levels among rural populations in tropical countries are 1000-2000 IU/ml compared to ~100-200 IU/ml. The best established cause of increased IgE is infection with helminthes, for example ascaris, hookworms or schistosomiasis. Elevated serum IgE is not a feature of protozoal infections such as malaria and trypanosomiasis, or of bacterial infections such as tuberculosis or leprosy. The hypothesis is that IgE antibodies play a critical role in the defence against helminthes, primarily by acting as a great keeper. A good example is to consider the possible role of immediate skin sensitivity in the protection against schistosomiasis. As the schistosomules enter through the skin of a sensitized individual, they will trigger mast cell degranulation with release of histamine, leukotrienes and cytokines.

These mediators lead to the local accumulation of serum (which contains IgG antibodies) and eosinophils. IgG antibodies bound to schistosomules interact with Fc γ R on eosinophils leading to degranulation on the surface of the worm which kills it **Figure-27**). Another important protective mechanism against helminthes is the expulsion of worms from the gut. This involves increased mucus production, activated peristalsis, as well as a role for eosinophils and mast cells. Whether IgE and IgE antibodies are essential for this expulsion is not clear. However, the best analysis is that protection against worm infection is a primary role of the TH2 system. This includes roles for effector T cells, mast cells, basophils and IgE antibodies. Furthermore, the primary mechanisms are focused on preventing entry of new worms. Given that one third of the world's population is infested with helminthes, it is clear that a protective system could have sufficient survival advantage to explain the presence of an effective TH2 system. Perhaps, as a consequence of living in the West without helminth infection, a large proportion of the population generates a TH2 based allergic response against irrelevant antigens such as those on pollen grains, cat dander or mite faecal particles.

Four types of hypersensitivity reaction

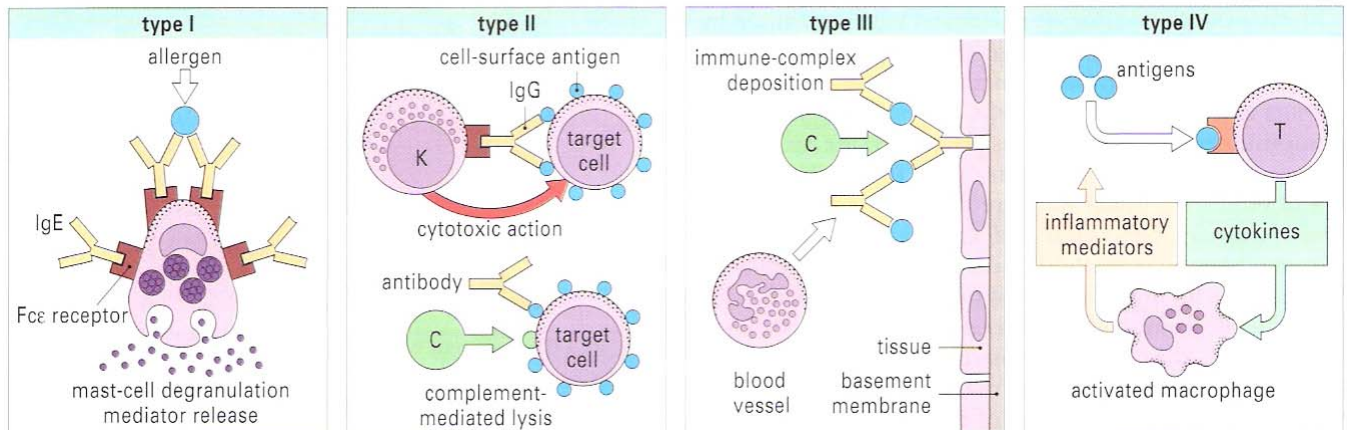


Figure-1 There are four types of hypersensitivity reaction. Type I mast cells bind IgE via their fc receptors. On encountering allergen the IgE becomes cross-linked, inducing degranulation and release of mediators that produce allergic reactions. Type II antibody is directed against antigen on an individual's own cells (target cell) or foreign antigen, such as transfused red blood cells. This may lead to cytotoxic action by K cells, or complement-mediated lysis. Type III immune complexes are deposited in the tissue. Complement is activated and polymorphs are attracted to the site of deposition, causing local tissue damage and inflammation. Type IV antigen-sensitized T cells release lymphokines following a secondary contact with the same antigen. Cytokines induce inflammatory reactions and activate and attract macrophages, which release inflammatory mediators.



Figure-2 Anaphylaxis and urticaria. (1) The anaphylactic response to bee venom is a patients who has IgE antibodies to the venom protein, Phospholipase A. The immediate reaction occurs within 20 minutes and is mediated by the release of histamine and other mediators from mast cells. The patient shown had been stung on the face, but the reaction can become generalized, leading to a fall in blood pressure, generalized urticaria and/or bronchospasm (i.e. anaphylaxis). (2) Diffuse urticaria on a patient with severe chronic urticaria. The lesions have a raised edge and come up within minutes or hours. The lesions almost always resolve within 12 hours leaving no trace on the skin.

Properties of allergens

source	airborne particles	Dimension of airborne particle (μm)	allergen		
			name	MW (kDa)	function/homologies
dust mite <i>D. pteronyssinus</i>	faeces	10–40	Der p 1 Der p 2	25 13	cysteine protease (epididymal protein)
cats <i>Felis domesticus</i>	dander particles	2–15	Fel d 1	36	uteroglobin
German cockroach <i>Blattella germanica</i>	frass saliva	≥ 5	Bla g 2 Bla g 4 Bla g 5	36 21 23	aspartic protease calycin glutathione-S-transferase
rat <i>Rattus norvegicus</i>	urine on bedding?	2–20	Rat n 1	19	pheromone binding protein
grass	pollen	30	Lol p 1	29	not known
fungi: <i>Alternaria alternata</i> <i>Aspergillus fumigatus</i>	spores spores	14×10 2	Alt a 1 Asp f 1		not known mitogillin

Figure-3 When a patient becomes 'allergic' to one of the well recognized sources of allergens, they have actually produced an IgE antibody response to one or more of the proteins that are produced by mites, trees, grass, cats or fungi. The proteins are predominantly water soluble with a molecular weight (MW) ranging from 1000 to 40000 kDa. In many cases the function of the proteins is known, but it is not clear whether function such as enzymic activity alters the ability of these proteins to induce an allergic response. The properties of the particles carrying these allergen are very important because they influence both how much becomes airborne, and also where the allergen is deposited in the respiratory tract. The dimensions of the particles airborne vary from $\geq 2\mu\text{m}$ for mite faecal pellets and some pollen grains (sizes are given as diameter in micrometers).

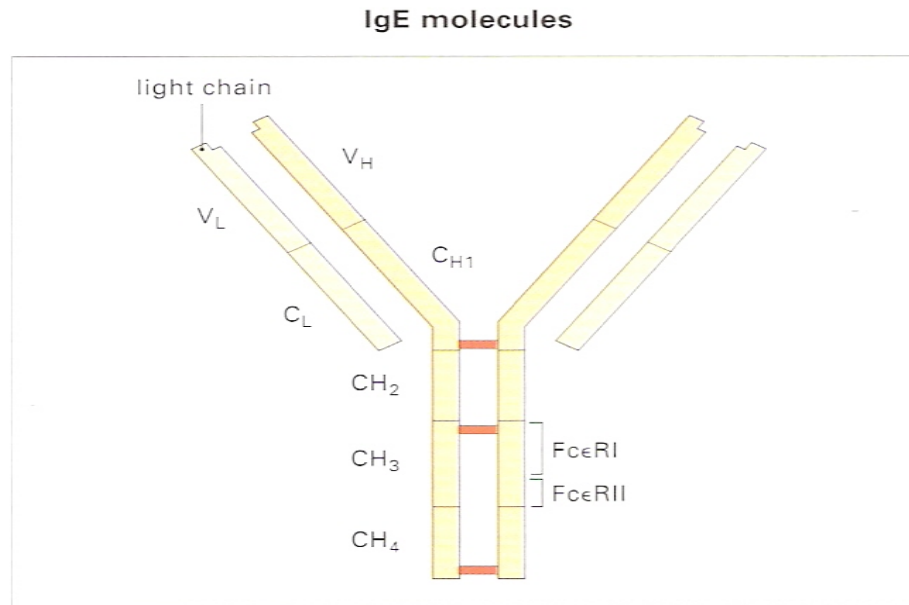


Figure-4 The IgE molecule has four heavy chain constant regions. The binding sites for the high-affinity IgE receptor $Fc\epsilon RI$, and for the low-affinity receptor $Fc\epsilon RII$, or CD-23 are shown. Monoclonal antibodies to the binding site for $Fc\epsilon RI$ also block $Fc\epsilon RII$.

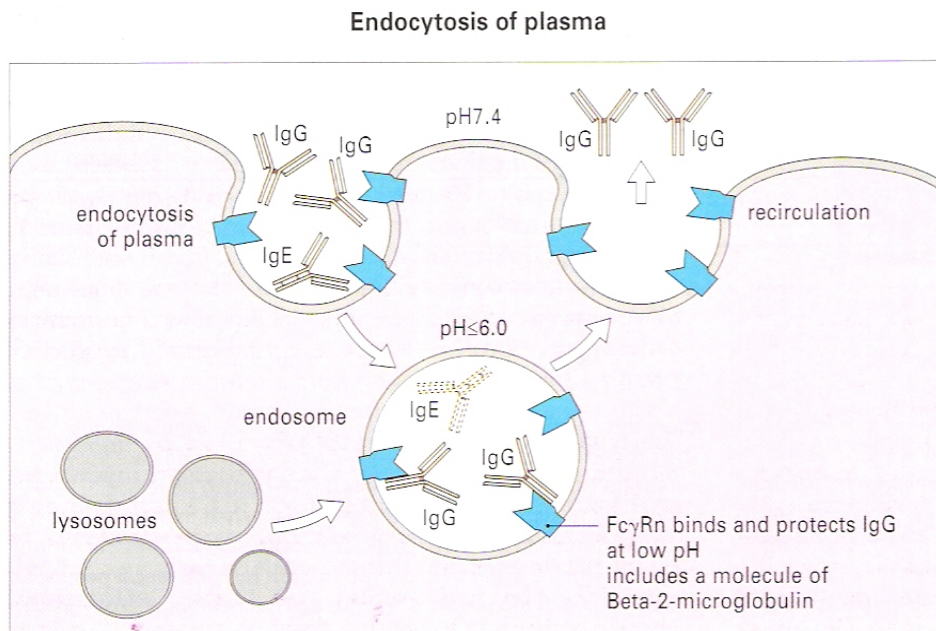


Figure-5 Endocytosis of plasma contributes to the short half-life of IgE, as plasma proteins are taken up and the pH falls because of lysosomes combining with the endosome. At low pH IgG including IgG4 molecules bind to the neonatal Fc gamma receptor ($Fc\gamma Rn$). By contrast, IgE molecules do not bind to $Fc\gamma Rn$ so they are not protected and are digested by cathepsin. As the endosomes recirculate, the pH rises to 7.4 and the undamaged IgG molecules are released into the circulation. The $Fc\gamma Rn$ includes a

molecule of β_2 -microglobulin. In keeping with this model the half-life of IgG is shorter than normal in mice that have had the gene for β_2 -microglobulin removed for knockout.

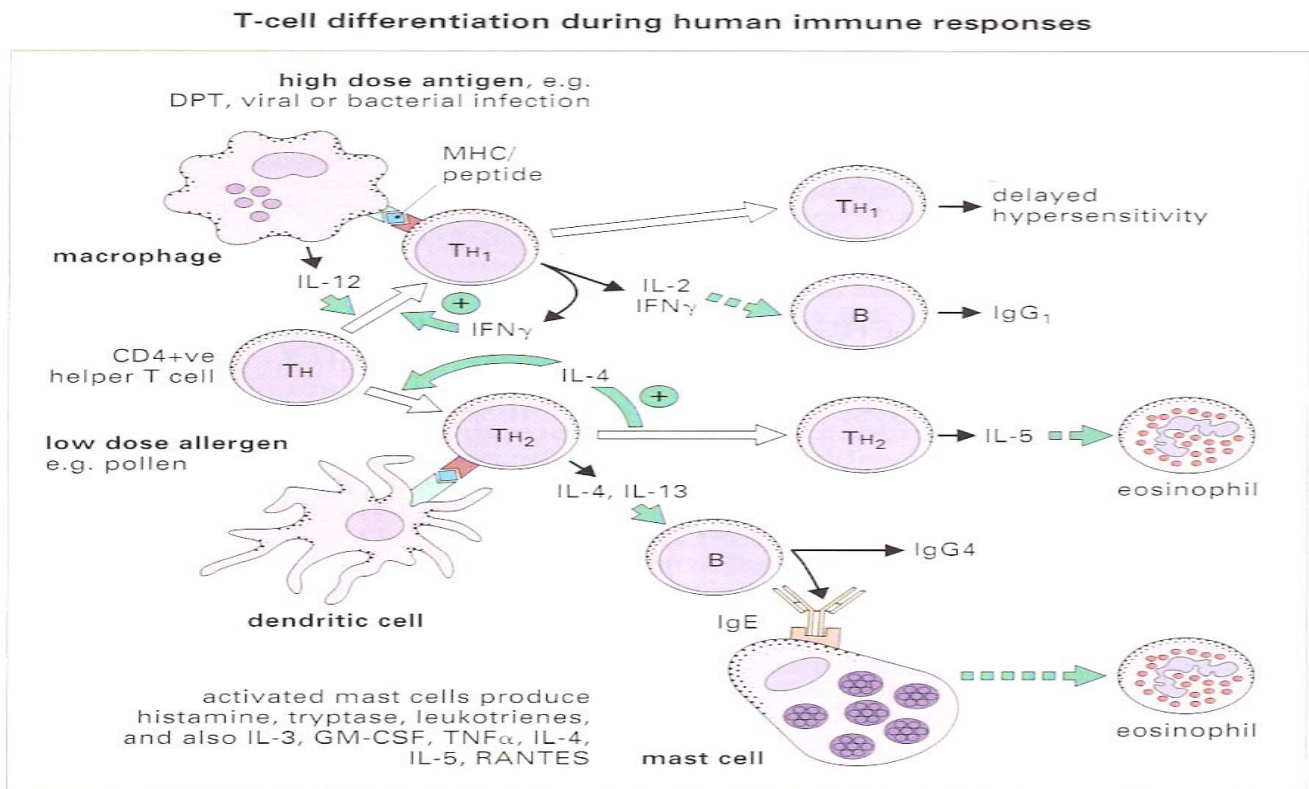


Figure-6 The differentiation of TH cells depends on the antigen source, the quantity of allergen and the cytokines produced. Bacterial antigens or a high doses of antigen will induce IL-12 from macrophages. In