INTRODUCTION

Allergy is the change of the reactivity of the host to an agent (allergen) on second or subsequent occasion . This include both useful and harmful effects . Recently the term allergy has become restricted to the latter and is now synonymous with type I hypersensitivity ***(Brostoff and Scadding , 1992)*** .

Antigens that are innocuous to most people may cause allergic disease in an atopic individual . The term allergen is applied specifically to those antigens that can cause allergic symptoms when exposed to them by inhalation , ingestion , contact or injection ***(Philip et al., 1987)*** .

So , extrinsic bronchial asthma , rhinitis , conjunctivitis , atopic eczema , urticaria , angiodema , food / drug allergy and even anaphylaxis are conditions in which allergy is a major underlying factor ***(Stevenes , 1996)*** .

Bronchial asthma is a disease of the airways that is characterized by increased responsiveness of the tracheobronchial tree to a multiplicity of stimuli . Functionally the hallmark of this illness is widespread narrowing of the airways that change in severity either spontaneously or as a result of therapy . Clinically , the disorder is manifested by paroxysm of cough , dyspnea and wheezing which generally occur together . However , patients may present with only cough or dyspnea (***McFadden , 1988)*** .

Diagnosis of allergy can be done by in vivo tests such as scratch , prick or intradermal skin tests , but these tests carry the hazard of being traumatic with high percentage of false positive and negative results ***(Stite , 1987)*** . A more sensitive methods are in vitro quantitative measurement of total and specific IgE which can be measured by various

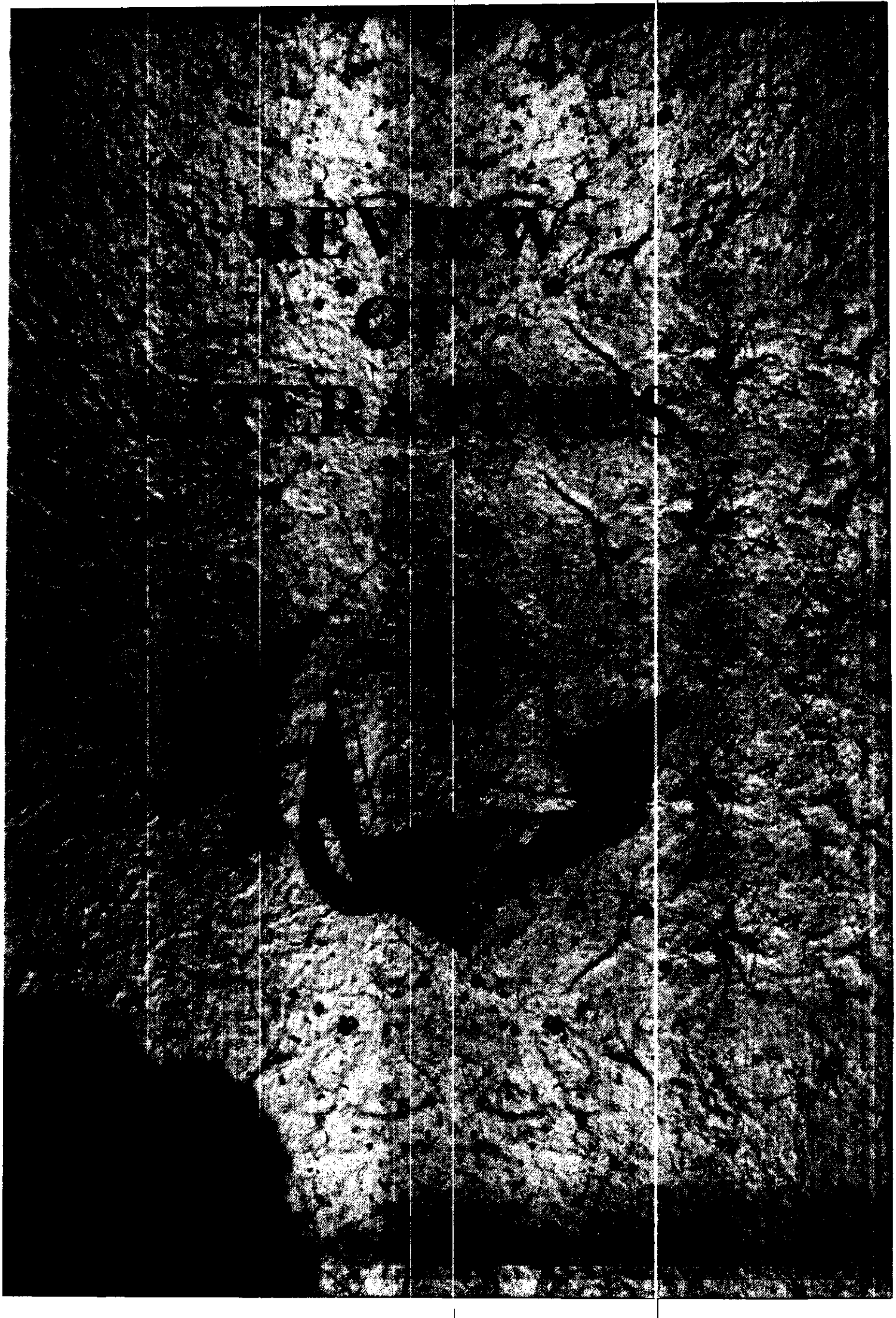
methods such as ELISA and RAST , but these tests are expensive (***Mangi, 1985)*** . Other non specific parameters includes detection of adhesion molecules , cytokines such as ILs , TNF , INF , special complement component such as C3a and C5a , and proinflamatory mediators such as leucotriens and PGD2 ***(Cavaillon , 1993)*** .

Treatment of bronchial asthma is based on three parameters which are avoidance of allergen , various drugs , and immunotherapy which is preserved as a last step if the other measures failed ***(Lerch and Muller, 1998)*** .

AIM OF WORK

The aim of this work is to :

- determine the percentage of cases of bronchial asthma due to different allergens in Qalubia governorate locality which are house dust , hay dust , cotton dust , fungal allergens , wool , feathers and hair of different origin).
- detect the most common allergen in the Qalubia governorate locality responsible for cases f bronchial asthma .
- find any correlation between the efficacy of intradermal skin test and detection of specific IgE for the most common allergens in cases of bronchial asthma in Qalubia governorate locality .



IMMUNE RESPONSE

The term immune response refer to all the mechanisms used by the body as protection against environmental agent that are foreign to the body. These agents may be microorgonisms or their products , foods , chemicals , drugs , pollen or animal products *(Duijvenstin and Hamann , 1989)* .

The immune response is triggered by materials called antigens . An antigen is a macromolecule capable of eliciting the formation of immunoglobulins (antibodies) or sensitized cells in an immunocompetent - host . The antigen will then specifically react with its antibody that have been included . Immunogen is a term that has come into use to emphasize the occurrence of a host response . An immunogen is any substance that is capable of inducing an immune response , while an antigen is a substance that can react with antibody , but is not necessarily able to evoke immune response , but the converse is not true . However many times the terms used synonymously *(Stevenes , 1996)* .

Once the host had its first contact with the antigen , initiating a primary response , lymphocytes producing antibodies or antigen binding sites multiply . Some of the cells survive as memory cells ; when the host encounters the same antigen a second or subsequent time , the response is accelerated and will be more intense than in the first time because there are more lymphocytes primed to recognize the antigen and the expanded number of memory lymphocytes are ready to react quickly . Memory cells are responsible for the phenomenon known as the secondary or anamnestic response. *(Eugene , 1995)* .

Immune response may be innate or acquired . Innate immune response conferred by all those elements with which an individual is born and which are always present and available. These elements include physical barriers such as skin , mucous membranes , and cough reflex . Chemical barriers as pH of saliva and stomach , and secreted fatty acids , interferon and other substances released by leucocytes as well as a variety of proteins , and the enzyme lysosome . Other internal elements include phagocytic cells such as granulocytes , marcrophages and microglial cells of the central nervous system . Acquired immune response is more specific than innate immune response . It's acquired by contact with foreign invaders . Contact with the invader trigger a chain of event that lead to the activation of certain cells (lymphocytes) for synthesis of proteins . There are two arms of acquired immunity ; one is mediated by B cells and circulating antibodies hence it is termed the humoral immune response. The other is mediated by T cell that not synthesize antibodies but instead synthesis and release various cytokines that affect the cells hence the name cell mediated immune response (CMI) ***(Gallatin et al., 1986)*** .

Both B and T cells have recognition sites (receptors) on their membranes . These recognition sites are complementary in configuration to antigenic determinant sites on antigens . If the recognition site and the antigenic determinant fit together , an immune response is triggered and the lymphocyte is stimulated to divide . The new daughter cells will each have the same lymphocyte recognition site as the parent cell .This type of lymphocyte division is known as clonal expansion , and continues as long as there is antigenic stimulation ***(Evans , 1995)*** .

Regulation of the Immune Response :-

When an immune response is mounted to a foreign antigen , lymphocytes and other cells involved in it proliferate at a rapid rate . Once the threat of the foreign invasion is removed, the response must necessarily be turned off . Disastrous results could ensue if the response went unchecked , as occurs in certain disease such as cancers of the lymphocytic system . Thus , the immune response has mechanisms that regulate the response *(Eugene , 1995)* .

Since antibodies are usually formed only after specific antigenic stimulation of lymphoid cells , the persistence of the antigen is required for continued proliferation of antibody - forming cells and high levels of antibody in serum . When the antigen is no longer available , the corresponding antibodies are not formed and the remaining gradually decrease in quantity as they are degraded . Plasma cells have a limited life span (a few days) , so if they are no longer stimulated by antigen they also disappear *(Atassia , 1977)* .

Antibody molecules also have a definite life span . They either leave the body in secretions or excretions or are eventually degraded by proteolytic enzymes . The half - life of antibody molecules varies considerably , depending on their class and location (that is , whether they are free , cell fixed , or transported into secretions) . The average half - life of most antibody molecules of the IgG class in human circulation is about 19 to 25 days . A unique property of IgG is that it exerts a regulatory influence on the rate of its own catabolism ; the higher the serum concentration of IgG , the more rapidly the molecules of IgG are degraded *(Berzofsky and Berkower , 1993).*

Antibodies bind to antigenic determinants , making them unavailable to bind to lymphocytes . Thus , once there is large amount of a particular antibody bound to the antigenic determinants , plasma cells and T cells will not be stimulated by the antigen to divide . There are also suppressor T (Ts) cells that inhibit B cells maturing to plasma cells and prevent T cells from dividing . As a result antibodies are not formed and T cell receptors are not available . Thus , the immune response is shut down ***(Hoop and Woods , 1981)*** .

Aberration of the Immune Response :

Sometimes there is aberration in the immune response in the form of hyperfunction or underfunction . Hyperfunction is manifested in hypersensitivity and autoimmune diseases . In hypersensitivity there is altered response to materials introduced into the body , while in autoimmune disease there is abnormal response to the body's own component . Underfunction is manifested in immune deficiency diseases and in tumors . In immune deficiency diseases there is deficiency in any component of the immune response (e.g, B cells , T cells) . In tumors there is failure of the body to detect tumor cells and kill them ***(Kuby , 1992)*** .

HYPERSENSITIVITY

Hypersensitivity is an abnormal immune response which produces damage , either histopathological or physiological in the host . Early bacteriologists were well acquainted with some forms of bacterially induced hyper - sensitivity which they termed anaphylaxis or anaphylactic shock and which they distinguished from toxic phenomena by the specificity of the response and its reproducibility in the same host after months or years *(Portier and Richet 1902 ; Richet 1913)* .

The hypersensitivity could be transferred between animals by serum ; the resulted anaphylaxis could be local with an inflammatory reaction at the injection site or generalized with shock and respiratory distress due to bronchospasm and urticaria . Analogous reactions were observed in man often with antigens of non - bacterial origin such as therapeutic horse serum *(Von pirquent and Schick 1905 ; Otto, 1907)*.

Hypersensitivities are of different types with varying mechanisms and have been classified by *Gell and Coombs (1963)* into anaphylactic , cytotoxic , immune complex and delayed types . Although this classification has deficiencies, it does define the four major mechanisms and describes the main clinical events which occur with each type of reaction . The problems with the classification are that patients do not always present in classification and that several types of hypersensitivity may be seen in the same patient at a given time .

Gell and Coombs (1963) used the term allergy instead of hypersensitivity . Most immunologists have however continued to use term hypersensitivity and have reserved allergy for the anaphylactic responses of IgE - mediated type I reactions *(Parratt , 1990)* .

TYPE I HYPERSENSITIVITY :-

The distinguishing feature of type I hypersensitivity is the short lag time , usually seconds to minutes between the exposure to antigen and the onset of clinical symptoms . The key reactant in type I or immediate hypersensitivity reactions is IgE . Antigens that trigger the formation of IgE are called atopic antigens or allergens . Atopy refers to an inherited tendency to respond to naturally occurring inhaled and ingested allergens with continual production of IgE . Typically patients who exhibit allergic or immediate hypersensitivity reactions usually produce a large amount of IgE in response to small concentration of antigen . IgE levels appear to depend on the interaction of both genetic and environmental factors ***(Atkinson and Mills , 1988)*** .

Prausnitz and Kustner (1921) were the first researchers who showed that a serum factor was responsible for type I reactions . They found that when serum is transferred from an allergic individual to a nonallergic one , and when the second is challenged intradermaly with specific antigen , a type of reaction appears is known as passive cutaneous anaphylaxis . While this experiment was conducted in 1921 , it was not explained until 1967 where the serum factor responsible namely IgE was identified ***(Goust , 1993)*** .

Triggering of type I reactions by IgE :-

Immunoglobulin E (IgE) is a glycoprotein of molecular weight of about 190,000 ***(Geha , 1992)*** .

The normal newborn has virtually no IgE in its cord serum (± 1-2 IU ml) . By the age of 1.5- 4.5 months , the mean is 9 IU / ml in healthy infant . This increase to 32 IU / ml between 9 months to 3 years of age .

The adult level in about 90 IU / ml with a range of 29 - 800 IU / ml *(Frick, 1983)*.

Total IgE can be measured by several methods , as single radial immunodiffusion , electroimmunoassay or radioimmunoassay . Specific IgE can be measured by radioallergosorbent test (RAST) method *(Augustin , 1978)*.

Sensitization :

The IgE response is a local event occurring at the site of allergen entry at mucosal surfaces and at local lymph nodes . This IgE will first sensitize local mast cells and spill - over IgE that enters the circulation and binds to receptors on circulating basophils and tissue mast cells throughout the body *(Brostoff and Scadding , 1992)*.

Structure of IgE :

IgE comprises two heavy and two light chains , the IgE heavy chain having five - domains . The major characteristics of IgE are its heat liability and tight binding to the Fc receptors on mast cells and basophils which are termed the high affinity receptors or FcεRI . There are also low affinity IgE receptors on other cell types - FcεRII . The heat liability reflects alterations in the Fc portion of the molecule following which it no longer sensitizes skin mast cell . The antigen binding capacity which resides in the Fab portion is preserved *(Chen , 1991)*.

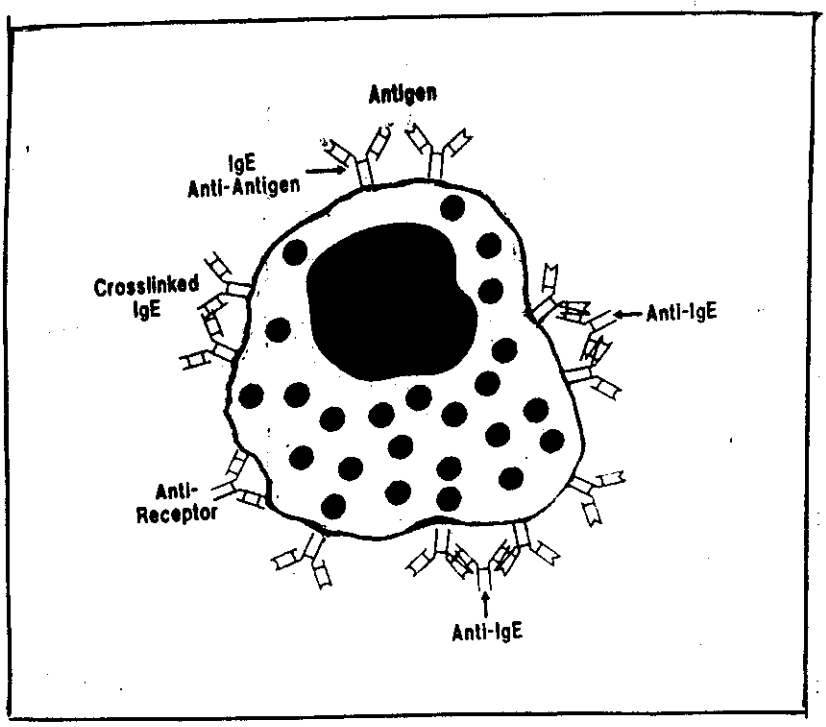
Although the serum half life of IgE is only 2-5 days , mast cells in human skin may remain effectively sensitized for up to 12 weeks following passive sensitization with atopic serum containing IgE *(Hamilton and Adkinson , 1992)*.

Structure and Function of Fc receptors for IgE :

a- Receptor Fc∈RI :

It's the high affinity receptor of IgE and is a tetramer of polypeptides. In IgE - FcRI interaction , the receptor interacts with regions of the CH_2 and CH_3 portion of the IgE heavy chain with a high binding constant (approximtely 10^{10}) . The interaction of monovalent IgE with the receptor complex does not activate mast cells or basophils since no histamine release occurs . It is the cross linking of surface bound IgE with antigen and other molecules which stimulates degranulation ***(Terr , 1994 a)*** .

Fig (1) : Represent various modes of cross linking receptors leading to mast cell activation and degranulation :



b- Receptor Fc∈RII :

It's the low affinity receptor of IgE . The exact molecular structure of it has not yet been described but a hypothetical mode has been made . Unlike other Fc receptors , Fc∈RII which is identical to CD23 is not a member of the immunogobulin superfamily but belongs to a primitive superfamily of animal lectins . A common feature of these molecules is that they lie upside down in the cell membrane with an intracytoplasmic NH2 end and an extracellular COOH end . Cleaving the Fc∈RII produce a soluble CD23 molecule that can bind IgE - IgE binding factors (IgE - BF) ***(Tharp , 1990) .***

The interaction of IgE with Fc∈RII is independent of any lectin - like activity that the receptor may have . Binding has been mapped to the CH_3 region slightly downstream to the area which binds to the high affinity receptor . Interestingly (IgE - BF) (soluble Fc∈RII / CD23) can inhibit IgE binding to Fc∈RII and possible to Fc∈RI by steric hindrance .Thus Fc∈RI / CD 23 can regulates allergic reactions in two ways by soluble IgE-BF inhibiting IgE binding and by regulation of IgE biosynthesis through feedback on B cells ***(Widmann , 1989) .***

Other Fc∈R - bearing cells :

The low affinity receptor (Fc∈RII) is carried by about a quarter of B cells and by monocytes which show increased expression during the pollen season , There is a much higher level of receptor on monocytes from patients with atopic eczema which is suppressed by treatment with corticosteroids . Alveolar macrophages with Fc∈RII can be sensitized with allergen specific IgE to release enzymes after exposure to the relevant allergen and this could play an important role in allergic lung disease

Eosinophils and platelets can be also activated through IgE dependent mechanisms leading to substantial damage to parasites such as schistosomes *(Terr , 1994 b)*.

IgE levels in disease :

Serum levels of IgE are minute compared to those of IgG even in highly allergic subjects . Levels of IgE are raised in atopic individuals and even more so in those with parasitic infections . When considering the possibility of atopic disease , a raised level of IgE aids the diagnosis but a normal level does not exclude it *(Brostoff and Scadding 1991)*.

A recent survey by *Sampson and Metcalf , (1992)* has shown that up to 30% of random group of 5000 subjects has a positive wheal and flare reaction on skin testing to one or more common allergens , but only 10-15% of the population is clinically allergic . Thus these subjects can produce specific IgE but lack some factor which precipitates the actual symptoms of atopy . They concluded that the higher IgE level , however, the more likely the presence of atopy .

Control of IgE Production :

The regulation of IgE production appears to be a function of T cells. Interleukin - 4 is essential for IgE synthesis as it is responsible for the final differentiation that occurs in B cells committing particular B cell to IgE production . Interleukin-2 , 5 and 6 also play a role probably as sequential growth and differentiation factors that select for IgE synthesis *(Tharp , 1990)*.

While actual antibody synthesis is regulated by the action of cytokines , the tendency to respond to specific allergens appears to be

linked to inheritance of certain major histocompatability complex (MHC) genes . Various human lymphocytic antigen (HLA) class II antigens seem to be associated with a high response to individual allergens . As an example individuals who possess the HLA antigens B7 and DR2 are more likely to respond to a specific ragweed antigen .The nature of this association is unclear at this time *(Goust , 1993)* .

As soon IgE is synthesized it become bound to high affinity receptors of the Fc portion of the heavy chains called FcεRI receptors these receptors are found on basophil and mast cells . Other cells such as lymphocytes , platelets and eosinophils also have receptors for IgE but the binding is not as strong , and hence these cells play a minor role in allergic reactions . Binding of IgE to cell membrane increases the half- life of IgE from 2 to 3 days up to 8 to 14 days . Once bound , IgE serves as an antigen receptor on mast cells and basophils , cross linking of antibody molecules triggers release of mediators from these cells *(Stevens , 1996)*.

Role of Mast Cells And Basophils :-

Basophils represent approximately 1% of the white blood cell in peripheral blood . They have a half life of about 3 days . They respond to chemotactic stimulation and tend to accumulate in inflammatory reactions. Basophils have twice as many receptors for IgE as do mast cells *(Miller , 1991)* .

Mast cells are derived from precursors in the bone marrow that migrate to specific tissue sites to mature . While they are found throughout the body they are most prominent in the skin , the upper and lower respiratory tract , and the gastrointestinal tract *(Tharp , 1990)* .

In the respiratory tract they are mostly located in the walls of the airways (60%) and alveoli (40%). Within the airways they are located predominantly between the basement membrane and the ciliated pseudo - stratified epithelium , although occasional cells are intraepithilial and onto bronchial lumen . These latter cells would come into immediate contact with the inhaled antigens and might be expected to be of major importance in modulating the initial phase of the allergic response ***(Barnes et al., 1992)***.

In the bronchi mast cells are also found in the connective tissue stroma beneath the basement membrane in blood vessel walls and occasionally between smooth muscle fibers however it is those mucosal - Type mast cells of the airways that have attracted most attention in relation to the pathogenesis of asthma ***(Auguis and Keller , 1986)*** . It is suggested that the inhaled allergens activate epithelial mast cells for the secretion of chemical mediators and that in turn provides the irritating stimulus for the activation of mast cells located deeper in the airways ***(Lia and Holgate 1988)***.

In normal subjects mast cells recovered by bronchoalveolar lavage (BAL) constitute 0.32 ±0.05 of the total nucleated cells and are likely to originate from both the alveoli and bronchi ***(Auguis and Keller , 1986)***. In extrinsic asthmatic this figure significantly increases to 1.41 ± 0.20 than the normal control and correlated significantly with the severity of the disease as indicated by measured indices of both airway obstruction and of hyperreactivity ***(Barnes et al., 1992)***.

Wardlow et al (1985) reported an up to tenfold increase in the relative number of mast cells in patients with allergic and non allergic asthma and the cells exhibited increased spontaneous release of histamine

and were hyperresponsive to both IgE - dependent stimulation and inhibition of mediator secretion by sodium cromoglycate .

It was found that mast cell hyperplasia occurring in the bronchial mucosa in asthma was induced in response to the release of growth factors e.g interleukin - 3 from allergen stimulated T - lymphocytes . In support of the number of mucosal type mast cells in the nasal epithelium increases up to twenty fold that this increase is inhibited by prior exposure to steroids which are known to block and reverse the T lymphocyte dependent hyperplasia ***(Vigas et al ., 1987)*** .

Ultrastructure of Mast Cells :

Human mast cells have a diameter ranging from 5-15 μm . The characteristic feature is the presence within the cytoplasm of large numbers of secretory granules which can exhibit a variety of different ultrastuctural patterns . The most common granule type contains cylindrical scrolls which may be characteristic of the MC_T (mast cell containing tryptase only) phenotype while other mast cells contain granules whose matrices appear as highly ordered crystals or electron - dense particles . In addition the cells also contain cytoplasmic lipid bodies ***(Pearce , 1992)*** .

Human mast cell contains between 80-300 membrane bound granules ***(Lai and Holgate 1988)*** . On activation the granules become swollen and amorphous and their membrane fuse to produce chains that enlarge to form tortuous cytoplasmic channels . The later eventually open to the exterior through multiple points on cell surface thereby permitting the release of histamine ***(Barnes et al ., 1984)*** . These pores progressively widen ultimately allowing the entry of extracellular markers.

The opening of the degranulation channels is accompanied both by increasingly prominent filament in the intervening cytoplasm and by the convolution of the plasma membrane into folds and projections *(Barnes et al., 1992)*.

The bronchoalveolar lavage (BAL) cell broadly resembles that from the lung parenchyma . However , there are generally fewer granules some of which are characteristically basket shaped or partially disrupted and numerous lipid bodies and cytoplasmic folds and projections . Overall, the cell appears to be in a partially activated state *(Pearce , 1992)*.

Mast cell Heterogenecity :

Mast cells are ideally situated to take part in immediate hypersensitivity reaction as they are found in greater numbers in those areas of human body that come into contact with the external environment, namely in the skin , the respiratory tract from the nose to the lung , the conjunctiva , and the gastrointestinal tract . Mast cells from different sites vary in their functional properties and in particular may differ in their responses to secretory stimuli and to various anti-allergic drugs . There are two mast cell types, in human skin , lung , and small bowel based on their distinct protease content . All mast cells examined showed immunoreactivity with human tryptase , a four chained neutral protease which constitutes nearly 23% of the total cell protein and is found in close ionic association with heparin proteoglycan . However , there were marked variations in the immunoreactivity to human chymases antibody . In the skin and intestinal submucosa more than 85% of the cells were shown to contain the cyhmase and were called MC_{Tc} i.e mast cell containing tryptase and chymase . In contrast the majority of the mast cells

of the intestinal mucosa and lung did not contain demonstrable amounts of this enzyme and were termed MC_T i.e mast cell containing tryptase only ***(Irani et al 1986)***.

Agiuus et al ., (1989) showed that sequential staining with Alcian Blue and safranin O clearly differentiates between lung and skin mast cells with the latter taking up the metachromatic Safranin O stain but not the former.

Although both population of cells respond to anti-human IgE , only skin mast cells secrete , in response to compound CPD 48/80 polysine and substance P which are non IgE - dependent stimuli for mast cell activation. In cases of non - immunological stimuli histamine release reaches a maximum 10-30 seconds after challenge , is dependent upon intact oxidative phosphorylation but only partially dependent on the presence of extracellular calcium . These secretory responses may be clearly differentiated from IgE - stimulated histamine release which reaches a maximum 5-10 minutes after challenge and is totally dependent on the availability of extracellular calcium . Mast cells from human lung are also sensitive to the inhibitory effect of sodium cromoglycate whereas those of human skin are not ***(Church 1986 ; Benyon et al ., 1987)*** . Mast cells from human skin , mastocytosis spleen , and lung generate approximately equivalent amounts of prostaglandin D2 (50-70 ng / 10^6 mast cells) , but have less capacity to synthesize leukotrien C4 (LTC4) ***(Lia and Holgate 1988)*** . Table (1) summarize the major difference between different types of mast cells from human sources .

Surface mast cells obtained by bronchoalveolar lavage (BAL) in both normal and asthmatic subjects respond to IgE - dependent stimulation by the secretion of histamine with a similar time coarse , calcium and

energy requirement to that described for mast cells mechanically or enzymatically dispersed from resected lung tissue *(Leung et al 1985).* Both spontaneous and stimulated mediator release is higher from mast cells recovered from lungs of asthmatic subject compared with lungs of normal *(Flint et al ., 1985)*

Table (1) : Difference between human lung and skin mast cells *(Lai and 1988) .*

	Human lung MC	**Human skin MC**
Morphology	No distinguishable feature	
Specific protease	Tryptase only	Tryptase and chymase
Biogenic amines	Equivalent amounts of histamine	
Proteoglycan	Heparin (? less sulfated)	Heparin
Staining	Alcian blue positive only	Alcian blue and safranin O+ve (pH 2.0)
Secretagogues	Anti - IgE and A 23 187 only .	Anti - IgE A 23 187 , CPD 48/80 basic ployamines .opiate, and substance P .
Cromoglycan effect	Inhibitory	No effect
Arachidonic Metabolites	Equivalent amounts PGD2	Little LTC4
Growth factor	T- Lymphocyte - dependent	Fibroblast - dependent

MC = mast cells

PGD 2 = prostaglandin D2

LTC4 = Leukotrien C4

Thus there is evidence for the existence of heterogeneity between mast cells from different human tissues and that heterogenecity does occur to some degree between mast cells within the lung and between normal subjects and subjects with asthma *(Lai and Holgate 1988)* . **Table (1)**

Mechanism of Ig-E Dependent Mast Cell Activation :

Mast cells have more than 130.000 Fc receptors which bind with high affinity to the C4 domain of IgE . Cross - linkages of cell bound IgE with bridging of IgE - Fc receptors is the stimulus that initiates the mast - cell secretory response . The early cellular events which occur during the first 30 seconds of IgE-receptor bridging include the disorganization of membrane phospholipid and an early rise in the cellular level of cyclic 3,5 adenosine monophosphate . It has been proposed that in rat serosal mast cell and human lung mast cell , bridging of IgE receptors stimulates the progressive methylation of membrane phosphatidylethanolamine to phosphatidylcholine . It has been further suggested that this event is involved in the opening of membrane calcium channels and makes available phopholipids for cleavage by phospholipase A2 which release arachidonic acid for subsequent oxidative metabolism to newly formed mediators . However , recent experiments have failed to confirm an obligatory requirement for phospholipid methylation for immuunological mast cell activation *(Ishizaka et al ., 1983)* .

More recent work by ***Lai and Holgate (1988)*** , suggests an important role for phosphatidylinositol whose metabolism involving the enzymes 2,3 phosphodiestrase (phospholipase C) which generates polyphosphoinositides and diacylglycerol . The former has been closely linked to the release of Ca^{+2} from intracellular sarcoplasmic reticulum

while the latter activates the membrane enzyme protein kinase C in the presence of Ca^{+2} and ATP protein kinase C is able to phosphorylate selected proteins e.g myosin light chain that form part of the cascade leading to the degranulation .

Whatever the initial biochemical events for mast cell activation these eventually result in the influx of water and anions across the perigranular membrane which partially solubilize the preformed mediators contained within by neutralizing the acid radicals of glycosaminoglycan (GAG) side chains of heparin ***(Caulfield et al ., 1980)*** .

With acquisition of cytoplasmic membrane vesicles the granules swell , assumulate intermediate filaments around their membrane and then move towards each other and the plasma membrane , where juxtaposition of the two membranes results in release of the chemiosmosis . In mucosal mast cell a further form of secretion involving the "budding - off" small packages of membrane bound mediators also occur (piecemeal secretion), and probably account for the increased basal secretion observed in asthma. The rate at which the preformed mediators are released from the granule matrix once it is externalized depends upon their ionic association with heparin . Histamine and exoglycosidases exchanges rapidly , while tryptase , chymase and carboxypeptidase B remain complexed with heparin for sometime after degranulation ***(Schwartz and Austen , 1988)*** .

Gall (1994) reported that stem cell factor (SCF) is a major if not the major regulator of mast cell and also can influence the response of mast cell to activation through the FcRI , the receptor that confers immunological specificity to mast cell activation and that regulate mast cell effector function in IgE- dependent immunological and allergic response .

The Role of Mast cells in the early Asthmatic Reaction :

Current evidence suggests that the immediate bronchoconstrictor response to inhaled allergens is largely mediated by mast cell products . Allergen challenge of extrinsic asthmatics lead to a release of histamine into the systemic circulation and the secretion of amine , together with the other mast cell associated mediators as tryptase and PGD2 into bronchalveolar lavage (BAL) fluid *(Wanzel et al ., 1988)* .

Pulmonary mast cells are clearly activated in the course of the asthmatic response . About one half of this response is due to liberated histamine and the remainder to leukotriens , thromboxan and prostaglandins *(Barnes et al ., 1992)* . **Fig (2)**

The role of Mast cells in the Late Asthmatic Reaction :

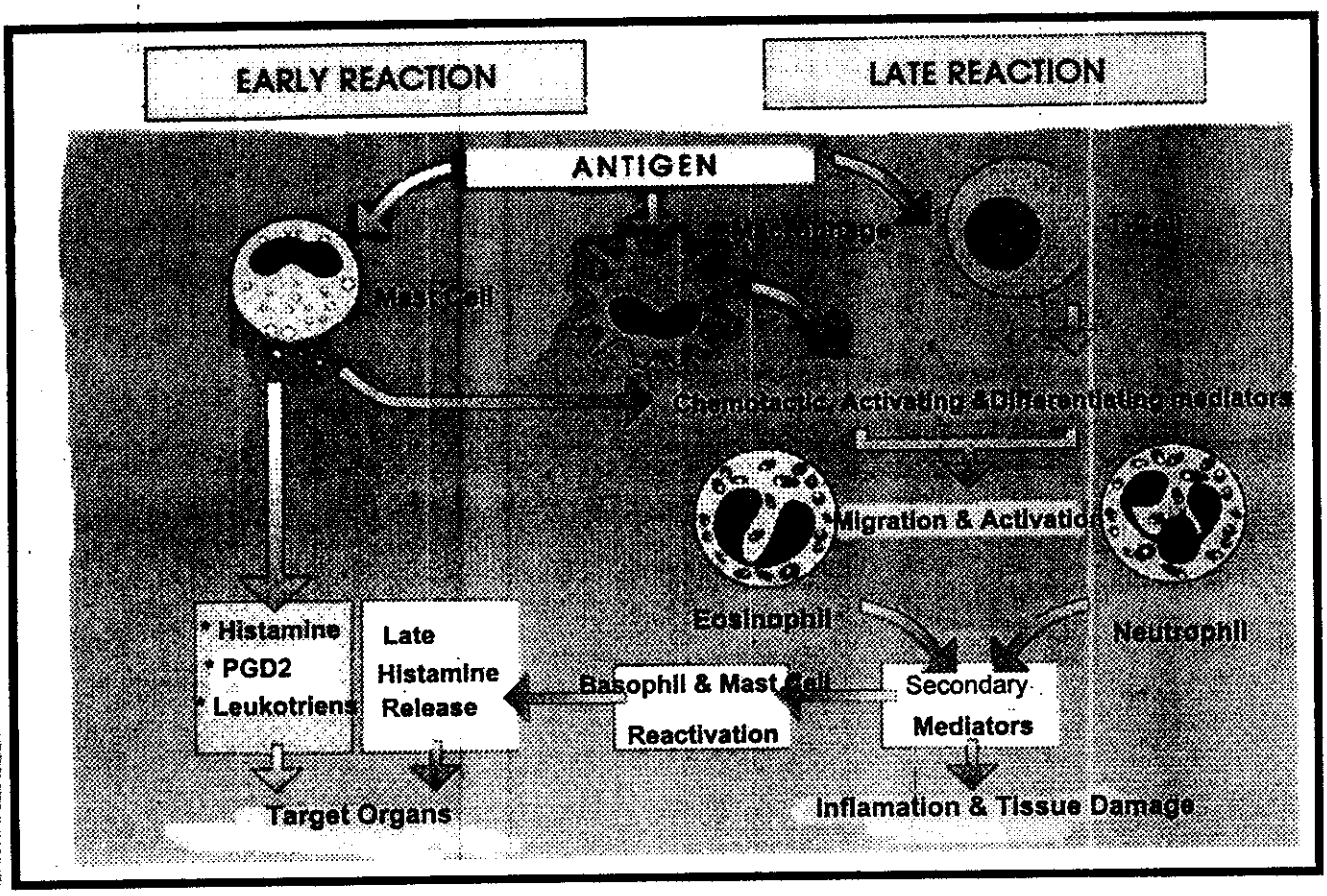
The role of mast cells in such reaction has been the subject of considerable debate . Release of histamine into the systemic circulation in the late phase response is controversial but has reported by ***Kay (1986)*** .

Church et al ., (1987) reported that the late asthmatic reaction is dependent upon the release of mediators from cells other than mast cell namely eosinophils and neutrophils with mediators from activated macrophages being responsible for this recruitment .

Recent studies demonstrated the production of various cytokines by mast cell . These studies have shown that immunological activation of tissue culture derived murine mast cells lead to increased levels of mRNA and / or secretion of a large range of cytokines including tumor necrosis factors - 2 (TNF-2) , granulocyte / macrophage colony stimulating factor (GM- CSF) , interferon - γ (INF - γ) , interleukins (IL) - 1,3,4,5 and 6 and four members of the macrophage inflammatory protein (MIP) gene

family namely T-cell activator -3 (TCA-3) MIP-1α and MIP-1β. Identification of these molecules raises the possibility of wide range of a potential roles for the mast cell in pathological responses . The release of cytokines could recruit , prime and active neutrophils , macrophages basophils , and eosinophils , increase immunoglobulin secretion and regulate the proliferation and phenotype of other mast cells *(Barnes et al , 1992)* . **Fig (2)**

Fig (2) : represent early and late asthmatic reactions .



Mast cells in Non - Allergic Asthma :

The pathological changes occurring in the airways in non allergic asthma are almost identical to those described in the form associated with atopy . Thus , it is tempting to speculate that mast cells may be involved in the pathogenesis of this variant form of the disease ***(Lai and Holgate, 1988).***

Studies have shown that human mast cells may be activated to release mediators by number of mechanisms other than through IgE . It is uncertain if IgG subclasses and particular IgG_4 have the capacity to sensitize mast cells and initiate mediator secretion . There is little doubt that immunoglobulins and complement may be deposited along the basement membrane of the airways in severe chronic form of asthma and that these components may indeed play a role in maintaining mast cell mediator secretion . The complement derived peptide C_{5a} , through interaction of specific receptors on the surface of mast cells induces a slow release of chemical mediators . The carbohydrate component of bacterial cell wall may degranulate mast cells by a non - immunological mechanism ***(Norn et al., 1989).***

Major basic protein , an eosinophilic product has been demonstrated to be capable of stimulating histamine release from human basophils and isolated rat peritoneal mast cells but whether a similar mechanism exists for human mast cell has yet to be determined ***(Hugli 1989)*** .

MEDIATORS RELEASED FROM GRANULES :-

Preformed Mediators :

Cross - linking of surface - bound IgE by a specific allergen causes changes in the cell membrane that result in the release of mediators from

the cytoplasmic granules . These preformed mediators include histamine , eosinophil chemotactic factor of anaphylaxis (ECF-A) neutrophil chemotactic factor , proteolytic enzymes , and heparin . Release of these substances is responsible for the early phase symptoms as in allergic reactions which occur within 30 to 60 minutes after exposure to the allergen . The effect of each of these mediators is summarized by ***Irani et al., (1986)*** and is presented in table (2) .

Histamine is a vasoactive amine with a molecular weight of 111KD. Its effects which appear within 30 to 60 seconds after release are dependent on activation of specific receptors found on cells in various types of tissue . Activation of histamine 1 (H1) receptors results in contraction of smooth muscle in bronchioles , blood vessels and the intestines . In addition there is increased capillary permeability , altered cardiac contractility and increased mucous gland secretion in the upper respiratory tract . Binding to histamine 2 (H2) receptors increases gastric acid secretion , airway mucus production and permeability of capillaries and venules . Histamine 3 (H3) receptors are found only in neural tissue and the effects are more limited ***(Kaliner and Lemanske , 1992)***.

In the skin , histamine is responsible for local erythema or redness and wheal and flare formation . Contraction of the smooth muscle in the bronchioles may results in airflow obstruction . Increased vascular permeability may cause hypotension or shock depending on the route by which an individual is exposed to the triggering allergen one or more of these effects may be seen ***(Tharp , 1990)***.

Esoinophil Chemotactic factor of anaphylaxis (ECF-A) is another preformed factors released from granules . This attracts eosinophils to the

area as well as inducing expression of eosinophil receptors for C3b . ***(Capron et al ., 1988)*** .

A second chemotactic factor , neutrophil chemotactic factor (NCF) is less well characterized than ECF-A . It appears to be a heat - stable molecule with a molecular weight of approximately 600,000 that acts as an attractant for neutrophils ***(Joseph et al .., 1989)*** .

Proteolytic enzymes that are released include tryptase and chymase. Tryptase cleaves kininogen to generate bradykinin which induces prolonged smooth muscle contraction , increases vascular permeability and increase secretory activity . In addition , complement component C3 is converted to C3a and C3b . Chymase converts angiotensin I to angiotensin II , which increase secretion of aldosterone . Aldosterone cause sodium retention with a corresponding increase in blood pressure ***(Schwartz and Austen , 1988)***

Heparin , a proteoglycan molecule is found associated with histamine within mast cell granules .When degranulation occurs the two dissociate and each has specific effects upon release from the cell . Heparin acts as an anticoagulant by binding to and enhancing the activity of antithrombin III . In addition heparin inhibits thrombin as well as other serine protease coagulation factors . It also inhibits complement activation at several steps in the cascade ***(Schwartz et al ., 1985)*** .

Table (2) Mediators of immediate hypersensitivity *(Schwartz et al ., 1985)* :

	Mediator	Structure	Actions
Preformed	Histamine	MW 111	Smooth muscle contraction . vasodilatation increased vascular permeability ,
	*ECF - A	MW 380-2000	Chemotactic for eosinophils .
	**NCF	MW 750.000	Chemotactic for neutrophils.
	Heparin	Proteoglycan MW 60.000	Anticoagulant .
	Tryptase	Tetramer MW 130.000	Cleaves kininogen inactivates fibrinogen converted C3 to C3b.
	Chymase	Neutral protease	Converts angiotensin I to angiotensin II .
Newly Sensitized	#PGD2	Cyclooxygenas products of arachidonic acid	Vasodiltation increased vascular permeability .
	PGE 2 , PGF2 , PGI 2	Cyclooxygenas products of arachidonic acid	Vasodiltation , chemotactic for eosinophils and neutrophils .
	^LTB4	5-Lipoxygenase products of arachidonic acid .	Increased vascular permeability .
	LTC4 , LTD4, LTE4	Arachidonic acid products.	Bronchoconstriction mucous secretion .
	Platelet activating factor	Phospholipid MW 300 - 500	Platelet aggregation , chemotactic for neutrophils and eosinophils

*ECF-A : Eosinophil Chemotactic Factor of Anaphylaxis .

**NCF : Neutrophil Chemotactic Factor .

PG : Prostaglandin .

^ LT : Leucotriens .

Newly Synthesized Mediators :

In addition to immediate release of preformed mediators , mast cells and basophils are triggered to synthesize certain other reactants that are responsible for a late phase allergic reaction seen within 6 to 8 hours after exposure to antigen . These newly formed mediators include platelet activating factor , prostaglandins especially D2 and leukotrienes B_4 , C_4 , D_4 and E_4 (LTB_4 , LTC_4 , LTD_4 LTE_4) . Prostagtandins as well as leukotrienes are derived from arachidonic acid , a membrane lipid , by two separate metabolic pathways . In one pathway the enzyme 5 - lipoxygenase cleaves arachidonic acid to generate leukotrienes . The other pathway uses cyclooxygenase which results in prostaglandin production ***(Mac Glashan et al., 1989).***

Prostaglandin D2 (PGD 2) is the major product of the cyclooxygenase pathway . When released by mast cell it mimics the effects of histamine causing vasodilatation , increased vascular permeability and bronchial constriction . In skin reaction PGD2 triggers wheal and flare formation . Prostaglandins E_2 F_2 and I_2 are all potent vasodilators . Thus the overall effect of the prostaglandin is to enhance and potentiate the action of histamine ***(Hardy et al., 1988)*** .

Leukotriens resulting from the 5-lipoxygenase pathway of arachidonic acid metabolism are also responsible for the late - phase symptoms of immediate sensitivity . Leukotrienes C_4 , D_4 and E_4 were originally collectively named the slow - reacting substances of anaphylaxis (SRS-A) . LTC_4 and LTD_4 are 100 times more potent than histamine is causing increased vascular permeability , bronchoconstriction and increased mucus secretion in small airways . In the intestines leukotrienes induce smooth muscle contraction . Systemically they may produce

hypotension as a result of diminished cardiac muscle contractility and decrease blood flow *(Beasley et al ,. 1988)* .

Leukotriene B_4 , is a potent chemotactic factor for neutrophils and eosinophils . The appearance of eosinophils is especially important as a negative feedback control mechanism . Eosinopils release histaminase which degrades histamine and phospholipase D which degrades platelet activating factor . Additionally superoxides created in both eosinophils and neutrophils cause the breakdown of leukotrienes *(Steel and Kaliner , 1989).*

Platelet activating factor (PAF) is a phospholipid released by monocytes , macrophages , and neurtophils as well as by mast cells and basophils . The effects of PAF include platelet aggregation , chemotaxis of eosinophils and neutrophils , increased vascular permeability and contraction of smooth muscle in the lung and intestines *(Peter et al ., 1990)* .

Clinical Manifestations of Immediate Hypersensitivity :-

The clinical manifestations due to the release of both preformed and newly synthesized mediators from mast cells and basophils vary from a localized skin reaction to a systemic response known as anaphylaxis . Symptoms depends on such variables as route of exposure , dosage and frequency of exposure . If an allergen is inhaled it is most likely to cause respiratory symptoms such as asthma or rhinitis . Ingestion of an allergen may result in gastrointestinal symptoms and injection into the bloodstream can trigger a systemic response *(Brostoff and Scadding 1992)* .

Anaphylaxis is the most severe type of allergic response as it involves multiple organs and may be fatal . The term anaphylaxis was

coined by biologists ***Portier and Richet (1902)*** and it literally means "without protection" . Anaphylactic reactions are typically triggered by glycoproteins or large polypeptides . Smaller molecules such as penicillin are haptens that may become immunogenic by combining with host cells or proteins . Typical agents that induce anaphylaxis include venom from insects in the Hymenoptera family , drugs such as penicillin , and foods such as seafood or egg albumin ***(Roitt , 1991) .***

Clinical signs begin within minutes after antigenic challenge and may include bronchospasm , laryngeal edema , skin manifestation such as urticaria (hives)and angioedma , diarrhea and / or vomiting and intractable shock due to the effect on blood vessels and smooth muscle of the circulatory system . The severity of the reaction depends on the buildup of IgE on mast cells and basophils . Massive release of reactants especially histamine from the granules is responsible for the ensuing symptoms . Death may results from asphyxiation due to upper airway edema and congestion irreversible shock or a combination of these ***(Roitt et al ., 1989) .***

Rhinitis is the most common form of atopy or allergy . It affects between 5 and 22 % of the population and is characterized by sneezing, rhinorrhea or runny nose , nasal congestion and itching of the nose and eyes . Pollen , mold spore , animal dander , and particulate matter from house dust mites are examples of airborne foreign particles that act directly on the respiratory mucous membranes to trigger rhinitis ***(Naclerio , 1992).***

Particles no larger than 2 to 4 μ M in diameter may reach the lower respiratory tract to cause asthma . Asthma is derived from the Greek word for panting or breathlessness . The airflow obstruction is due to bronchial

smooth muscle contraction , mucosal edema and heavy mucus secretion . All of these changes lead to an increase in airway resistance making it difficult for inspired air to leave the lungs . This trapping of air creates the sense of breathlessness *(Kaliner and Lemanske , 1992)* .

Food allergies are another example of type I immediate hypersensitivity reactions . Two of the most common food allergies involve cow's milk and eggs . Symptoms limited to the gastrointestinal tract include cramping , vomiting and diarrhea . While spread of antigen through the bloodstream may cause hives and angioedema on the skin as well as asthma or rhinitis *(Sampson and Metcalf , 1992)* .

GENETIC MECHANISMS OF HYPERSENSITIVITY :-

Coleman et al., (1997) found that atopy and the atopic disorders are likely to result from multifactorial inheritance . With interaction between genetic and environmental factors . It has been proposed that at least two major mechanisms ; non-antigen specific (total IgE levels) and antigen specific (specific IgE antibodies and skin tests) regulate the immune response to allergens in humans : firstly a gene / genes independent of the human leucocyte antigen system which is involved in the regulation of total IgE levels , and secondly a specific immune response gene / genes associated with major histocompatibility complex class II genes which are involved in antigen - specific mechanisms .

Genetic analysis by *Marsh et al., (1995)* revealed evidence for linkage of five markers in chromosome 5q 31.1 especially IL4 with a gene controlling total serum IgE levels . No linkage was found between these markers and specific IgE antibody levels .. This provide evidence for a possible link between asthma and the IL4 gene .

When ultrapure (> 99.5% pure) allergens are used there is a good association between HLA and specific IgE response .This is more impressive for very low dose exposure and low molecular weight minor determinants such as the ragweed allergen , than for abundant high molecular weight allergens . With the former over 90% of IgE responders are HLA-DW2 , whereas with the latter there is as yet no HLA association ***(Rihs and Baur , 1994)***.

Among patients attending allergy clinic there was significantly higher proportion with HLA - B8 and HLA - Dw3 in the skin test positive group than in the antibodies to ragweed those with HLA - B8 have higher titers of antibody and also higher levels of LgE . This HLA profile is also strongly associated with autoimmune disease raising the possibility of defective T suppressor activity in the development of both autoimmune and IgE responses ***(Terr , 1994a)***.

Recently , ***Stevens (1996)*** have re-examined the inheritance of atopy in more than 500 individuals from a number of extended families and have shown that 90% of young atopic asthmatics had at least one parent with demonstrable atopic IgE responsiveness (IgER) . suggesting a decisive genetic mechanism .

Another extended family studies showed clear vertical transmission of IgER suggesting autosomal dominant inheritance . Supporting this 61% of children from one responder and one non - responder parent had IgER This value is higher than that in previous studies probably because extensive testing and questioning employed . This suggests that there could be one gene locus largely responsible for IgER , but that this can be modified by other genetic and environmental factors ***(Brostoff and Scadding , 1992)*** .

ALLERGNS AND AEROALLERGENS

Antigens that are innocuous to most of the population may cause allergic disease in an atopic individual exposed to them by inhalation, injection or ingestion . The term allergen is applied specifically to those allergens (antigens) that can cause allergic symptoms . Atopic individuals however are not sensitive to some antigens even when they are exposed heavily to them although the same antigens may cause severe troubles in other atopic persons . The factors that cause atopic individuals to develop sensitivity on exposure to some antigens and not to others have long been suspected to be genetically controlled *(Cockroft , 1992)* .

Allergens from a number of sources , such as pollens , animal danders , milk , egg , and insect venom , have been isolated and characterized . The known allergens are usually proteins and range in molecular weight from 2800 to 65000 daltons . Chemical studies of protein allergens provide , no evidence for structural peculárities , that could explain their ability to cause disease in atopic individuals . Polysaccharides, such as dextran and pneumococcal polysaccharide , can be allergens but are so less commonly . Simple , chemical compounds that are active with tissue proteins can also cause allergic problems *(Philip et al., 1987)* .

INHALED ALLERGENS :

Certain wind - borne plant pollens , mold spores , and insect parts , are spreading in sufficient quantities over large areas to cause a portion of the susceptible population to develop hay fever or asthma . In addition there may be sufficient local contamination by animal danders , house dust, plant dusts , or other inhalant materials at home or at work to cause

respiratory symptoms in exposed allergic individuals (***Elsayed et al., 1980***).

Plant Pollens :

Of inhaled allergens plant pollens are the principal offenders and some plants release enough pollen to produce nationwide seasonal epidemics of respiratory disease affecting as such as 5 % of the population. Most plants depend on insect pollination and produce pollen suitable for spread by temporary attachment to insect bodies . The more perfectly adapted a plant is to insect pollination , the less pollen it produces that is spread randomly by wind . Contrarily a small number of species (referred to anemophilous) depend on wind for easily borne long distances by air currents . The essential features for establishment of an etiologic relationship between a pollen and a respiratory allergens are referred to as Thommen's postulates which consist of the following :

1- The pollen must be wind - bone .

2- The pollen must occur in large quantities .

3- The plant must be widespread .

4- The pollen must contain an excitant of hay fever (***Pollart et al., 1988***).

The small number of species of air - borne plant pollens that are significant in causing hay fever are produced in definite seasons characteristic for each plant . The dates of pollination of a species may vary by only a week or two from years in any one locale but may be quite different in another climate . Only direct sampling of the air in locality will define the time of occurrence of pollens significant in human allergy ***(Marsh and Norman , 1988)***.

Ogden and Raynor (1967) found that air - born pollens vary in diameter from 10 to 100 μ ; particles that are smaller or larger are not suitable for dispersion from plants by air currents . Particles of 10 to 100 μ in diameter are removed from inhaled air by impingement on the moist surfaces of the nasal membranes . In mouth - breathers such particles land in the pharynx and trachea but are too large to be inhaled deeply into the lungs . However , The aerobiological samples of pollen articulates are small enough to be inhaled into the small bronchi .

Animal Danders :

Some very potent allergens pollute the air in a circumscribed locals such as a house , barn , or factory . The variety in this group is large . Animal danders cause allergic rhinitis and asthma since domesticated animals shed desquamated skin epithelium which is a potent allergen ***(Shamic et al ., 1990)*** .

Persons who are sensitive to animal danders are often sensitive to animals serum proteins , which are found in extracts of horse danders , cat dander , and dog dander . However , albumin is always less reactive than dander allergens ***(De Groot et al ., 1991)*** .

House Dust :

House dust extracts commonly cause wheal and erythema reactions in atopic individuals with perennial rhinitis or asthma , or episodic symptoms on exposure to unusual concentrations of dust . House dust is a mixture of epidermal products of man and animals , bacteria , molds, degeneration products of fibrous materials , and remains of food , plants and insects . As people do give positive skin tests to extracts of these

different sources of house dust allergen i.e mites , dandruff etc . All or most of these materials are found in different degrees of denaturation and may provide the nutritive sources of some microorganisms inside which further formation of new structure of antigens (Ags) can occur *(Warner, 1976)* . The question arises as to which one of these many dust components represents the chief or sole source of dust allergens . The likely answer is that different people are sensitive in varying degrees to the different dust allergens *(Aas , 1976)* .

Mites :-

House dust mites are the most common sensitizing allergens . There are 47 species of mites , the most common species are Dermatophagiod farinae and Dermatophagoid pternoyssus *(Duff and Platts , 1992)* .

Mites inhabit areas in the home that hold moisture such as mattresses carpets and stuffed furniture . A single mattress can contain more than 100.000 mites whereas dust samples may contain more than 100,000 mite fecal particles / gm of dust *(Duff and Platts , 1992)* .

There are two major groups of mites allergens . group 1 protein (Der pI and Der FI) which have molecular weight 24.000 and group II (Der P II and Der F II) which have molecular weight of 15.000 . Both have been isolated from Dermatophagoid pteronyssus and Dermatophagoid farinae . The group I mite allergens are present in fecal material and have been shown to be a digestive enzyme of the mite *(Platts and Chapman , 1987)* .

The two groups are completely unrelated and show no cross reactivity . Commercially available dust mite extracts are either made from whole culture which is rich in fecal particles and group I allergens , or

from extracts of isolated mites in which there may be nearly equal quantities of both groups ***(Ford et al., 1985)*** .

Several of evidences uphold the concept that Dermatophagoides is the major source of house dust allergens in many parts of the world :-

- Mattress dust long - considered the most potently allergenic dust , is more heavily infested with these mites than dust from other sources ***(Freedman , 1976)***
- There is an annual peak of symptoms in dust - sensitive patients during the months of highest temperature and humidity (usually August to October in the northern hemisphere) . At the same time the mite population in dust rises to a maximum and then declines again ***(Ford et al., 1985)*** .
- Patients with clinical evidence of house dust sensitivity and positive skin reactions to house dust almost invariably react to extremely dilute solutions (0.000001 percent) of extracts of Dermatophagoides prepared from mite population grown in the laboratory ***(Aas , 1976)*** .

Clapman and his colleagues (1987) , reported that it is possible to measure the major mite allergens in house dust using simple immunoassays based on monoclonal antibodies . Levels of Der PI in dust vary from <0.1 to > 100 μ gm / gm dust . The variation related to local and seasonal differences in humidity as well as local difference in temperature and cleaning .

Platts and his Colleagues (1987) reported that an association can be inferred between a given level of mite allergens and asthma :

- 2 μ gm Der PI / gm of dust (or 10 mites / gm of dust) or more should be regarded as risk factor developing mite hypersensitivity and symptomatic asthma .

- 10μ gm Der PI / gm of dust (or 500 mites / gm of dust) more should be considered as a higher level of risk for acute attacks of asthma in mite allergic individuals .

The immune response to mite allergens include T cells as well as IgE antibodies . These T cells have shown to produce interleukins when stimulated ***(Platts and Chapman 1987)*** .

Witt et al ., (1986) , found that dust mites allergens are largely produced in the form of fecal particles and become airborne . Reported insults at the site of fecal particle deposition could produce a cumulative "inflammatory " effect in the lung in which mediators release , eosinophil infiltration and epithelial damage are involved . These inflammatory reaction include a role for T cell as well as IgE antibodies .

In large proportion of allergic asthmatic patients these inflammatory effects on the lung and the associated bronchial hyperreactivity will at least partially heal if the patient is effectively excluded from continued allergen exposure ***(Platts et al ., 1988).***

Molds :

Molds (fungi) are found throughout the indoor and outdoor environments . Molds can be a source of indoor perennial exposure often growing on shower curtains , damp basement , and on indoor plants . The most commonly identified indoor molds are Alternaria , Cladosporium , Aspergillus , Penicillium , Mucor , Chatomium , Candida , Rhizopus and

Smuts species . Extracts of these fungi are commonly used in diagnosis and treatment of respiratory allergy *(Jelks , 1985)*.

The role of molds as allergens has been less completely evaluated than that of other inhaled allergens . Nevertheless , the spores of a number of molds and fungi seem to fulfill the requirements of Thommen's postulates . These molds are widespread in nature , occur in soil or decaying organic matter , and propagate by microscopic spores blown about by wind *(Soloman , 1985)*. Mold spores may be identified by their morphological appearance on microscopic examination on glass slide, or by the colonies appearing on plates of sabouroud's culture medium that have been exposed for a time and then incubated *(Jelks , 1985)* . Some mold spores have definite season as for particularly Alternaria and Cladosporium (Hormodendrum) , which are found from May to December in the Eastern United states . Alternaria has a definite peak in late September or October whereas Cladosporium incidence is highest in July *(Philip , 1987)*. These spores seem to account for the symptoms of hay fever , that some people have in July or early August in the gap between the grass and ragweed pollen seasons *(Soloman , 1985)*.

El-Hefny et al., (1986), found that Cladosporium and Alternaria are the most common indoor fungi in Egypt followed by Aspergillus and Penicillium . In general , fungi are more common in low socioeconomic class houses than higher classes .

Asthmatic patients with hypersensitivity to Aspergillus may present either by asthma alone or asthma and pulmonary infiltrates (Allergic Broncho Pulmonary Aspergillosis - ABPA) *(Hinson et al., 1969)*.

Mc Carthy and Pepys (1971) , described diagnostic criteria of ABPA by the occurrence of transient pulmonary infiltrates together with peripheral esoinophilia and evidence of type I allergy to Aspergillus .

There may be also elevated serum level of total IgE *(Malo et al ., 1977 a)* . However most people with extrinsic bronchial asthma and type I allergy to Aspergillus species do not have pulmonary infiltrates *(Malo et al ., 1977 b)* .

Industrial Dusts :

Industrial exposure to dusts arising from a variety of plant materials can cause rhinitis asthma or urticaria . Refining of oil from cotton seeds leaves a highly allergenic meal of cotton seed which may cause asthma or rhinitis among workers in plants handling cotton seeds or among farm workers who use the meal as a fertilizer *(King and Norman , 1978)* . Two other common examples of industrial exposure are green coffee beans and castor beans , both of which occasionally cause severe reaction in those who handle them in an unrefined state . Castor bean allergen is extremely potent and may be isolated from castor bean meal in a partially purified state by the same methods used for isolating cotton allergen . This allergen is to be distinguished from the potent toxin of castor beans , ricin , which is not implicated in respiratory allergy . Castor oil for medicinal purpose is free of the toxin and the allergen *(Philip et al ., 1987)* .

INDUSTRIAL CHEMICALS :

Although inhalant allergy appears to occur mainly in connection with exposure to moderately complex protein antigens , similar reactions to simple chemicals have been observed frequently . Examples of

compounds reported to cause such reactions are toluene diisocyanate (TDI) , trimellitic anhydride (TMA) , phthalic anhydride , chloramine T , tannic acid , phenylmercuric acetate , and platinum salts . In each case wheal and erythema skin reactions and passive transfer of skin - antibodies have been demonstrated . The most common sensitivity is TDI which is extensively used as plasticizing agent , but the mechanism of reaction has been most thoroughly worked out in TMA sensitivity *(Zeiss et al., 1977)*. TMA is a haptenizing chemical which reacts readily with body proteins to form trimellity - new antigenic determinants . Trimellityl human serum albumin prepared in vitro can be used as an antigen to detect IgE and IgG antibodies in sensitive patients *(Philip et al., 1987)* .

FOOD ALLERGENS :

Many patients have symptoms attributed to allergy to food in the absence of demonstrable IgE - mediated reactivity to antigens in the food . Gastrointestinal allergy is a diagnosis frequently entertained , occasionally evaluated and rarely established *(Ingelfinger et al., 1949).*

The suggestion that the antigen is absorbed from the intestine may have been partially digested and therefore altered to a new antigenic specificity , even though proteins can be absorbed from the intestine in the native state. It is not essential , that all of protein be digested before intestinal absorption . There are of course a number of individuals who have IgE-mediated reaction to undigested food antigens *(Marsh and Goodfriend , 1979)* .

Allergy to cow's milk is the most frequently suspected food allergy in children , while egg sensitive individuals are usually reactive only to the

egg white which contains several well characterized proteins ***(Philip et al., 1987)***.

INJECTED ANTIGENS :

Allergy to stings of insects of the Hymenoptera order , such as bees, yellow jackets , wasps , hornets and stinging ants , affects 0.4 to 4 % of the population of the United States . Also the injection of various drugs can cause allergic reactions e.g penicillin ***(Gluck and Pacin , 1986)***.

STANDARDIZATION OF ALLERGEN EXTRACT POTENCY

Allergens extracts have been used for the diagnosis and treatment of allergic diseases for the last 70 years *(Yman et al., 1980)*.

The simple and straightforward way to produce an allergen extract is usually to add the allergenic insoluble material to a buffered aquous solution and after a while , to remove insoluble material . Production has until recently been guided and standardized by a fixed relationship between the weight of the allergenic raw material and the volume of the extraction fluid . Certain special procedures have been carried out with the aim of improving quality *(Lockey , 1988)*.

Most allergen extracts have been produced in a proportion of 1/10 . 1/50 pr 1/100 these extracts are the stock solutions and the manufacturing weight by volume ratio (w/v) data are the only information about the strength of the extracts . Diagnostic and therapeutic dilution have been graded accordingly . It is evident that the raw material from such varying source as pollen on the one hand and house dust on the other are likely to contain quite different proportions of active allergens and the proportion between allergenic and non - allergenic extractable material will vary accordingly *(Schroder , 1980)* .

Different Methods of Standardization :

1- Weight by Volume

The first attempt to express the strength of an allergenic extract was the use of weight by volume (w/v) ratio a method that is still in common use today *(Marsh and Goodfriend , 1979)* . Weight by volume is merely the expression of a ratio of the weight of any allergen extracted in a given

volume of solution for example 1g of pollen extracted in 20 ml of solution results in a 1: 20 w/v concentrations as does 10 g of pollen extracted in 200 ml of solution *(King and Norman 1962)*.

2- Pollen or Allergen Unit:

In an attempt to express weight by volume strength in whole numbers rather than dilutions or fractions . ***Noon (1911)***, proposed the pollen unit (also known as the Noon unit) . While he defined as amount of material extracted from 0.001 mg of pollen (1:1 w/v - 1 million pollen unit) . ***Fadal and Nalebuff (1980)*** have extended Noon's dilution to include the amount of material extract from 0.001 mg of any allergen (rather than pollen only) and have designated this as an " allergen unit " .

3- Protein Nitrogen Units

In ***1933 Cooks and Stull*** proposed the protein nitrogen content of an extract be used as standard of potency . The protein content of an extract is expressed in protein nitrogen units (PNUs) and is determined by the micro-kjeldhl method . Many investigators have outlined the shortcomings of expressing extract strength in PNU . ***Baer et al., (1970)*** evaluated commercial extract of short ragweed pollen for PNU and antigen E (Amb a 1) content and reactivity in ragweed sensitive individuals by skin test and in vitro histamine release , they found that the PNU content as labeled or as assayed in their laboratory did not correlate with either antigen E (Amb a 1) content or the degrees of biologic reactivity . They further demonstrated 100 fold and greater differences between extracts labeled to have the same PNU content . PNU is not a measure of allergenically active proteins , because most of the proteins in allergens

extracts are not allergenic . PNU is principally a measure of the amount of allergenically inactive proteins . Manipulations in manufacturing processes such as freezing and thawing and pollen grain disruption have been used to produce extracts with elevated PNU but not of increased the biologic activity . ***Gleich et al ., (1976)*** found that PNUs were not a reliable measure of potency and demonstrated a lack of correlation between the skin test activity of an extract and its protein nitrogen content .

4- Qualitative Methods

Qualitative methods for analysis of allergenic extracts consist of separation of the various proteins but may also include differentiation of the allergenic (IgE - binding proteins) from the proteins that may be extraneous ***(William et al ., 1992)*** .

a- ***Polyacrylamide Gel Electrophoresis (SDS-PAGE)***

Polyacrylamide Gel electrophoresis (PAGE) is a method of protein separation whereby the proteins are induced to migrate in gel containing sodium dodecyl sulfate (SDS) at rates that vary in relation to the individuals molecular weights of each protein producing a banding pattern when stained . By including reference marker proteins of known molecular weight on the same gel , the molecular weight of the various extract proteins can be calculated ***(Yman et al ., 1980)*** .

b- ***Crossed Immunoelectrophoresis (CIE)***

Another electrophoretic protein separation method involves thin - layer get electrophoresis of the proteins in an allergic extract in one direction followed by a second electrophoresis at 90° to the first into a

different gel containing precipitating animal antiserum specific for the allergen being analyzed once stained the banding patterns can be compared with patterns produced by other extracts of the same allergen or data from other assays . CIE requires the production of animal serum against the specific extract . If the immunized animal fails to respond to one or more components of the allergen they are not visualized and therefor appear not to exist *(Weeke and Lowenstein , 1973)* .

c- ***Crossed Radioimmunoelectrophoresis (CRIE)***

CRIE consists of the performance of CIE modified by exposing the gel containing the unstaining precipitin arcs to human IgE antibodies that bind only to the allergenic proteins ; after the unbound IgE antibodies are washed away radiolabeled anti-human IgE is applied that binds to the bound IgE antibodies , the excess the bands visible by autoradiography represent those proteins in the extract that have reacted with the antibodies contained in the human serum pool *(Weeke and Lowenstein 1973)* .

d- ***Isoelectric Focusing (IEF)***

IEF is yet another electrophoretic technique preformed in thin layer gel separating extracts on the basis of the isoelectric point of the individual protein . Like SDS- PAGE , IEF makes all the protein in an extract visible including both allergenic and nonallergenic but unique protein banding pattern for each pollen extract can be produced by IEF . These patterns are very helpful in establishing or verifying the identity of the pollen used to produce an allergenic - extract *(Landay and Folds . 1992)* .

e- ***Immunoblotting***

Immunoblots can be prepared from IFE or SDS - PAGE gels by either passive or electrophoretic transfer to nitrocellulose paper, application of human IgE antibodies and radiolabeled anti - human IgE producing an autoradiographic pattern (as was the result in CRIE) ***(Proffitt , 1990)***.

5) Quantitative Methods

a- Enzyme Analysis

Many Hymenoptera venoms contain enzymes that are allergenic eg , phospholipase -A and hyaluronidase in both honeycombe and vespid venoms , and acid phosphatatse in honeybee venom . In such instances the measurement of these enzymatic activities serves to both characterization and standardization of hymenoptera venom allergenic extracts. Use of enzyme content as standard of potency for allergenic extracts other than hymenoptera appears to have severe limitations ***(Lee, 1991)*** .

b - Radial Immunodiffusion (RID)

Assays for a single major allergen in an extract can provide an accurate standard of potency . The measurement of antigen requires the production of specific antisera to that single allergen which can be incorporated into gel for use in a simple RID assay . After wells are cut in the gel that has been impregnated with antisera , extract is applied in the well . Upon diffusion a precipitin ring will form and become visible . The diameter of this precipitin ring will be directly proportional to the antigen content of the allergenic extract . Single major allergen analysis can be

performed by alternative tests using monoclonal antibodies and may have advantages over RID assays in the future ***(Yolken , 1990)*** .

c- Protein content

The total protein content of an extract is important in evaluating the source materials and the consistency of the extraction procedure . It also permits comparisons of the various gel procedures as the quality of the pattern is dependent on the total protein load . A variety of protein assays are available , although most have limitations for use with allergic extracts due to the complexity of the allergen mixture and the presence of stabilizers and preservatives . It has been shown that protein nitrogen unit (PNU) assays may give significantly varying results ***(Aas et al ., 1978)*** .

d- Leukocyte Histamine Release

Histamine release from peripheral leukocytes in the blood of allergic people is an in vitro assay that correlates with skin testing results . But it is not in common use of assay of allergenic extracts because it requires a continual supply of fresh blood cells ***(Aas et al ., 1978)*** .

6) RELATIVE POTENCY ASSAYS

The potency of drugs and most vaccines is specified by the results of assays for identity and quantity of each component . It is not currently feasible to identify and quantify each allergenic component of allergenic extract , but it is possible to perform comparison assays using in vivo and in vitro methods . Determination at relative potency by either methods requires simultaneous parallel multiple comparisons of two or more extract at multiple doses for a comparative analysis , one of the extracts must be

the standard against which the other extract of known potency . In addition to the in vitro methods described , extensive skin testing by intrademal titration would also be performed by most in vivo and in vitro methods relative potency is a determination of functional activity of the test extract . *(William et al ., 1992)* .

7) Radioallergo Sorbent Test (RAST) Inhibition

RAST has been widely employed as an in vitro diagnostic test to quantitative allergen - specific IgE antibodies in sera of allergic patients . Shortly after its introduction , the test was modified for use in measuring the allergenic activity of extracts . This system now allows for direct comparison of the allergenic activity of two or more extracts in parallel and can produce accurate quantitative results *(Turgeon , 1990)* .

8) In Vivo Methods

Skin testing is the most widely used in vivo methods for standardizing allergenic extract . The skin test methods used for standardization differ greatly from most skin test procedures used in clinical practice for the diagnosis of allergies . Accurate and precise skin testing allows the qualitative and quantitative in vivo tests for standardization and provides methods for standardization when no in vitro test is feasible for a given extract *(William et al ., 1992)* .

Histamine Equivalent Prick Testing (HEP)

Skin testing by the HEP method is performed by prick testing of 20 or more patients who have a clinical history of sensitivity and positive skin tests to allergen to be standardized . The potency is calculated from the

whealing response ; those factors appears to limit the accuracy of the methods for standardizing allergenic extract *(William et al., 1992)*.

Interadermal Dilution for 50 mm Sum of Erythema Determines the Allergy Unit (ID_{50} EAU) :

The extracts standardized by this methods are labeled in allergy units milllions (AU) . Patients are selected who are clinically allergic to the allergen to be tested and are skin test positive by puncture methods . At least 15 patients are subjected to titration skin test in which 0.05 ml of threefold (Log_3) serial dilutions of both the test and reference standard are tested simultaneously on each patient by intradermal injection .It has become apparent that some allergens are more potent than others indicating that the highest concentration of the most potent allergens (eg rye grass pollen) should be assigned standardized units significantly greater than the units used for the highest concentration of less present allergens (eg mites) *(Peter , 1989)* .

BRONCHIAL ASTHMA

DEFINITION :

Asthma has been known and described for more than 2000 years. The word asthma derived from the greek word means panting (Breathing quickly) or gasping for breath of severe nature , or breath with an open mouth *(Gregg , 1983)*.

Sir John Floyer in his study on asthma (1698) used the term in its general sense but confined himself largely to discussing the episodic type from which he himself suffered . Henry Hyde Salter's (1860) used it specifically to describe this type of breathlessness *(Sakula , 1984 ; Sakula, 1985)*.

Ciba foundation Guest Symposuim (1959) , suggested the following definition " Asthma refers to the condition of subject with wide - spread narrowing of the bronchial airways , which changes its severity over short periods of time either spontaneously or under treatment and not due to cardiovascular disease " . The clinical characteristics are abnormal breathlessness which may be paroxysmal , or persistent wheezing and in most cases relieved by bronchodilator drugs and cotricosteriods .

The American Thoracic Society (1962) , defined asthma as " A disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by wide - spread narrowing of the airways that changes its severity either spontaneously or as a result of treatment ".

Herxheimer (1975) defined asthma " Attacks of dyspnea caused by bronchial obstruction occurring at any time of the day or night and early morning" .

Farr (1985) reported that asthmatic subjects could tolerate an average of only 3 breaths of 10mg /ml of methacholine before the induction of bronchospasm as measured by timed vital capacity compared to normal , who could breath 150 or more breaths of the same solution . Far defined asthma as a reversible obstructive airways disease of unknown etiology until proved otherwise .

Asthma results from the interaction of genetic and many environmental influences on the tone or reactivity of the airways ; the response varies from one individual to another and from time to time . No single definition will ever cover all these variables in a way that is likely to be useful . Currently it is popular to define asthma in terms of bronchial reactivity , although this leads to the uncomfortable recognition that some quite typical asthmatic subjects have normally reactive airways between attacks ***(Seaton and Doglas , 1989)***

Asthma was recently defined as " a chronic desquamative eosinophilic bronchitis " ***(Bousquent et al ., 1990)*** .

Another recent definition by the ***National Heart , Lung And Blood Institute (1991)*** was that asthma is a lung disease with the following characteristics , airway obstruction that is reversible (but not completely) either spontaneously or with treatment , airway inflammation and increased airway responsiveness to a variety of stimuli .

The International Consensus Report (1992) agreed on an operational definition of asthma as " a chronic inflammatory disorder of the airways in which many cells play a role , including mast cells and eosinophils .

CLASSIFICATION OF ASTHMA

Asthma is classified as :

1- Extrinsic Asthma :-

The development of asthma following exposure to specific allergen(s) is now recognized as IgE - mediated asthma and is classified as extrinsic . It may also have a nonallergic bronchospastic component and are then classified as having mixed asthma. Extrinsic asthma is seen more often in younger patients especially children ***(Aas , 1969)*** .

2- Intrinsic Asthma :-

Asthmatic subjects (usually adults)with no history of atopic disease who have negative allergy skin test reactions and normal levels of serum IgE are classified as intrinsic asthmatic . Asthma is other triggered in each patients by infections especially viral respiratory tract infections or sinusitis . Emotional stress , air pollution , tobacco smoke , and extremes of heat or cold and humidity can also trigger intrinsic asthma . It tends to be more severe and more difficult to manage than the extrinsic type ***(Amerian Thoracic Society , 1962)*** .

3-Exercise - Induced Asthma :-

Evidence has suggested that the bronchospasm associated with exercise may result from heat and water loss in the bronchial airways . Although asthma of this type is common in all asthma groups, a small population develops symptoms only during exercise , especially when hyperventilating . Exercise asthma usually occurs within 5 to 10 minutes after the exercise has been completed . The evidence suggests that both

mast cells and reflex mechanisms are involved in the etiology of exercise - induced asthma *(Barnes , et al ., 1992)* .

3- Occupational Asthma :-

Asthma that occurs in the work place after exposure to particular dust or gases is termed occupational asthma . Examples of occupational asthma include baker's asthma , woodworker's asthma , and wrapper's asthma . Some of these individuals are also atopic suggesting that preexisting bronchial reactivity may be necessary to develop clinically apparent asthma in response to occupational exposure to a specific agent . *(Philip et al ., 1987)* .

Asthma symptoms associated with exposure to specific etiological agents may also result from direct irritant effects causing vagal - mediated bronchoconstriction . In some instances , particularly after exposure to biologic products , the agent may causes an IgE - mediated response *(Zeiss , et al , 1977)* .

5-Aspirin Induced Asthma :-

Ingestion of aspirin or other nonsteroidal anti - inflammatory agents such as indomethacin will elicit symptoms and may even produce asthma . The reaction usually occur within few minutes to 2 hours after ingestion . The causes maybe related to imbalance of cyclo-oxygenase pathways resulting in deficiency of bronchodilating prostaglandin E (PGE) and over production of bronchoconstricting prostaglandin such as PGF , PGD2 . Alternately , it has been speculated that those agents block cyclo-oxygenase pathway of arachidonic acid metabolism , causing increase in Lipoxygenase products such as the potent bronchoconstrictor leucotrienes

LTC4 , LTD4 , LTE 4 collectively recognized as slow reacting substance of anaphylaxis (SRS-A) *(Barnes , et al ., 1992)* .

EPIDEMIOLOGY OF ASTHMA :

Studies on the epidemiology of asthma are difficult to interpret because of variations in definitions of asthma , study populations , survey and sampling methods . ***Barnes et al ., (1992)*** found that 11 percent of 2700 children surveyed had asthma . They revealed a male - to female ratio of about 2/1 in childhood with this difference disappearing in adolescence and , after adolescence becoming higher in females . Asthma tends to be diagnosed that at least a third of all cases of asthma begin before the age of 10 .The vast majority of childhood asthma develops before 8 years of age and 50 percent before 3 years *(American Thoracic Society , 1962)* .

In a study by ***Botros , (1983)*** of the natural history of asthma has discovered significant spontaneous improvement by age 10 to 14 . Children have had fewer episodes of asthma beginning after age 3 tend to " outgrow " their asthma by age 10 ; early onset asthma before 3 years of age and severe asthma are more likely to persist . Many individual who "outgrow " their asthma as children frequently have a recurrence in adult life . Long - term studies indicate that children with only occasional wheezing before the age of 7 years are likely to be wheeze - free during adolescence . However , as adults 45% again have episode of wheezing . Children with frequent wheezing , however , are less likely to remit during adolescence and as adults 80% again have evidence of wheezing *(Elliot et al ., 1987)* .

PATHOGENESIS of BRONCHIAL ASTHMA

Bronchial response to allergen can be divided into immediate and late response . The immediate response is an episode of airflow obstruction which is maximal 10-20 minutes after allergen inhalation and resolves spontaneously in 1-2 hr . This response is predominantly bronchospastic in nature *(Pepys , 1973)*. It is caused by the combined release of histamine , prostaglandins and leukotreins (*Sechellenberg et al., 1985)* .

The late sequel include the late asthmatic response , allergen induced increase in airway responsiveness and recurrent nocturnal asthma. All of which are due to , in whole or in part , to airway inflammation *(Cartier et al ,. 1982)*.

The late asthmatic response is an episode of airflow obstruction that develops after spontaneous resolution of the early response between 3 and 5 hours after exposure . Resolution usually begins by 6-8 hours but may require in excess of 12 hours . It was felt that it might represent a type III precipitin - mediated immune complex reaction *(Pepys , 1973)*. However *Kirby et al ., (1986)* reported that the late responses is a part of the late (inflammatory) sequel of the IgE - mediated allergic reaction .

ROLE OF IMMUNOGLOBULIN - E (IgE)

Mohamed (1982) found a mean value of serum IgE of 20.21 ± 2.9 IU / ml in normal Egyptian children using phadezym PRIST method .

Hammad (1975) found an obvious correlation between the amount of total IgE and the duration of clinical manifestation of respiratory allergy.

El- Helaly et al ., (1977) , found a high level of serum IgE in cases of asthma with atopic dermatitis than in cases with asthma alone or cases

with local dermatitis . This signifies that IgE level correlates well with both the severity and extent of atopic reaction . The increased frequency of raised IgE values in allergic asthma has suggested a relationship between prolonged exposure and IgE production .

The induction of IgE synthesis requires two signals . One signal is delivered by a cytokine , interleukin-4 (IL-4) , and is IgE isotype specific. The other signal is a B - cell activating signal *(Geha , 1992)* .

Burrows et al ., (1991) reported that airway hyperreactivity is closely linked to serum IgE level even in children with no clinical expression of allergy. This finding suggested that allergic inflammation of the airways can be present in children who are asymptomatic with no clinical features of atopy , and that this inflammation causes either temporary or persistent airways hyperresponsiveness .

Sharaf El - Din , (1982) , found a significantly higher level of serum IgE in asthmatic than normal controls , with no significant difference between atopic and non - atopic asthma . However , ***Botros (1983)*** , found higher levels of IgE in sera of patients with extrinsic than in those with intrinsic asthma . Higher levels were found in atopic asthma with positive skin tests to different allergens . The levels were much higher during exacerbations of asthmatic attacks .

Corticosteriod treatment has a negligible effect on IgE levels ***(Freedman , 1976 ; Botros , 1983)*** , whereas in some patients , IgE is increased by treatment with cromoglycan sodium perhaps due to suppression of symptoms thereby allowing the patient greater allergen exposure ***(Freedman , 1976)*** .

Wraith et al ., (1989) reported that 100% of patients with history of immediate symptoms to food allergens had specific IgE to the same food allergens.

Stingl and Maure (1997) , found that in atopic individual , cutaneous antigen - presenting cells (APC) , i.e . Langerhans cells (LC) and dermal dendritic cells (DDC) ,frequently display anti - IgE reactivity. While earlier observations suggested that this phenomenon results from the binding of (complexed) IgE to low affinity IgE receptor (FcεRII / CD23). They mentioned also that LC and DDC , as well as peripheral blood dendritic cells and monocytes from atopic individuals , can bind monomeric IgE via the high - affinity receptor for IgE (FcεRI) . In vivo , FcεRI - IgE dependent allergen presentation may critically lower atopic individuals threshold to mount allergen - specific IgE production (Type I reactions) and perhaps even in the occurrence of T - cell - mediated delayed - type hypersensitivity reactions in allergen - exposed tissues .

Biber , (1997) found that LCs from normal and atopic individual use FcεRI to maximize antigen uptake via specific IgE and subsequent presentation to T cells and may be responsible for driving the T cell responses in either TH0 , TH1 , TH2 type .

ROLE OF T - LYMPHOCYTES :-

T - lymphocytes play a considerable role in the regulation and expression of the inflammation associated with allergy and asthma . The T - cell derived lymphokines , interleukin -4 (IL - 4) , interleukin - 5 (IL-5) and interferon gamma (IFN - γ) , are involved in the regulation of IgE production , some lymphokines as IL-5 , IL-3 , and granulocyte monocyte-colony stimulating factor (GM - CSF) are active in the control of

eosinophil production by the bone marrow and in the regulation of mast cell differentiation . Others (e.g. eosinophil chemotactic factor of anaphylaxis [ECF-A] and neutrophil chemotactic factor [NCF]) have chemotactic activity for neutrophils , eosinopils and basophil granulocytes as well as moncocytes and can activate or degranulate these effector cells . T - lymphocytes also play a general role in the regulation of specific immune response and are possible target cells for desensitization immunotherapy ***(Kay , 1992)*** .

The frequency of allergen - specific T - lymphocytes in peripheral blood is increased in atopic asthma and correlates with the severity of bronchial response to allergen inhalation ***(Burastero et al ., 1993) .***

Major subsets of T cells consist of helper and suppressor types . Helper cells (CD4) consist of at least 2 phenotypically and functionally distinct subtypes : inducer cells (CD4+ , Leu8+ , or TQ +) which influence the induction of mature helper cells and suppressor cells ; and helper cells (CD4- , Leu8- , TQI-) , which influence antibody production of B cells . These 2 subtypes of the CD4 class are recognized phenotypically by the simultaneous expression of CD4 molecules and either Leu8 or TQI (which have not yet received CD designation) . So far, no single reagent has been developed that can identify these population . Suppressor cells (CD8) can similarly be subdivided into so - called true suppressor cells (CD8+ , CD11 +) , which influence B cell antibody function , and cytotoxic T cell (CD8- , CD11-) . Combination of monoclonal antibodies are thus used to detect these two important T cell subsets ***(Stites , 1987) .***

Wierenga et al ., (1990) , reported high frequency of CD4 + T lymphocytes of atopic donors .

Gonzalez and his Colleagues (1987), found a difference in T - cell subsets in bronchoalveolar lavage (BAL) of asthmatic who respond with an early reactions alone (relative increase in CD4 and CD8) compared with the dual asthmatic responders (increase in CD4 only), suggesting that those who go on to develop late reaction to allergen challenge may have a relative inability to recruit CD8+ T lymphocytes to the lung .

Corrigan and his colleagues (1988), reported that activated T cells express 3 activation markers , the class II histocompatibility antigen (HLA - DR) , the receptor for interleukin - 2 (IL - 2 R) and the very late activation antigen (VLA - 1) . These markers could be identified in the peripheral blood of patients with acute severe asthma and that the percentage of activated cells decreased after therapy and clinical improvement .

The interleukin - 2R T lymphocytes were exclusively of the CD4+ phenotype which provided evidence that CD4+ T lymphocytes activation may be a feature of the asthma pathogenesis ***(Kay , 1991)***.

Helper (TH2) lymphocytes and other resident airway cells (Mast cells , epithelial cells and possible macrophages) might participate in the induction of the local inflammatory reaction observed in bronchial asthma . During the late asthmatic reactions , cytokines are able , through an enhanced adhesion molecule expression on endothelial cells , to facilitate the bronchial cellular influx . These cells may secrete proinflammatory mediators such as tumor necrosis factor , interleukin - 1 , interleukin - 6 , interleukin -8 and platelet- activating factor . The epithelium is itself capable of mediator production , and it may function as an effector cell not simply as a passive target cell . After the initial stimulus the cytokines and

chemoattractants active bronchial microvascular endothelial cell and / or circulating leukocyctes and so , initiating , a leukocyte - endothelial adhesion cascade . Once arrested in the microcirculation leukocytes then diapedese between endothelial cells and migrate through extracellular matrix along a chemoattractant gradient . Finally leukocytes traverse the basement membrane and pseudostratified columnar epithelium to gain access to airway lumen . This step of epithelial transmigration is thought to include leukocyte - epithelial cell adhesion and deadheion , or in the case of epithelial damage , adhesion and cytotoxicity . Completion of these steps allows for normal host defense or result in epithelial desquamation of the asthmatic airway ***(Pilewski and Albelda , 1993)*** .

Mudd et al ., (1995) reported that IgE may also be involved in the uptake and processing of allergens . Such IgE - mediated antigen presentation may lead to a continuous (over) activation of the immune system . In addition , it may be a cause for the advance of disease from a single allergy to " multi - allergy " syndrome .

Coleman and his Colleagues (1994) , postulated that in vivo T cell response might influence , via the production of cytokines the behavior of mast cells in situ . In support of the hypothesis , they found that mast cells isolated from chemical allergen - sensitized mice show enhanced responsiveness to IgE - dependent activation in vitro .

Maliszewski and his colleagues (1994) , investigated the role of soluble interleukin - 4 receptor (sIL - 4R) as a regulator of IL - 4 mediated activities in vivo . They found that recombinant sIL - 4R can not only antagonize function mediated by endogenous IL- 4 , but also potentiate the biological activity of exogenously administered IL - 4 .

Jansen and Kapsenberg (1994) declared that IL- 4 play a crucial role in the regulation of the production of specific IgE by B cells . IL - 4 appears to be the immunoregulatory cytokine , and its effects are antagonized by IFN - gamma , and vice versa . ***O'Brien et al ., (1996)***, found that proliferation and differentiation of Th2 subsets producing predominantly IL -4 and IL- 5 and not IFN - gamma provide an essential signal for isotype switching to IgE in B - cells , on the one hand , and direct the activation and influx of inflammatory effector cells such as eosinophils , on the other hand .

Amongst the numerous pairs of surface adhesion molecules, the CD23 - CD21 pair seems to play a key role in the generation of IgE . The CD23 molecules is positively and negatively , regulated by factors which increase or decrease IgE production , respectively , Antibodies to CD23 have been shown to inhibit IL - 4 induced human IgE production in vitro and to inhibit antigen - specific IgE responses ***(Dessaint and labalette , 1994)***.

Bonnefoy et al (1996) , found that at least two cell - derived signals have been shown to be necessary for the induction of immunoglobulin isotype switching in B - cells . The first signals is given by either of the soluble lymphokines , interleukin (IL - 4 or IL-13) , but this alone is insufficient to trigger secretion of immunoglobulin E (IgE) . The second signal is provided by a physical interaction between B - cells and activated T - cells , basophils and mast cells , and it has been shown that the CD40 (on B cells) / CD40 ligand (CD40L)(on active T cells) pairing is crucial for mediating IgE synthesis , and that activation of T cells needs interaction between B7 on antigen presenting cells and CD28 on T cells .

Role of Adhesion molecules :

Asthma , whether extrinsic or intrinsic , is characterized by marked inflammation of the bronchial mucosa and submucosa . This inflammation which is responsible for the bronchial hyperreactivity observed in asthmatic patients is partly due to the massive recruitment of inflammatory cells , and of T cell monocytes and eosinophils in particular . This cell migration is regulated by the adhesion molecules . These adhesion molecules , which are veritable markers of inflammation are expressed by the vascular endothelium which are , intercellular adhesion molecule 1 , endothelial luminary adhesion molecule 1 , and vascular cellular adhesion molecule 1 (ICAM - 1 , ELAM -1 , VCAM -1 respectively) and by the bronchial epithelium (ICAM -1) . The cooperation observed between the main agents of the allergic inflammatory reaction (macrophage , T cell and mast cell) and the vascular endothelial cell , demonstrates that the mediators released by the allergic response are able , through the expression of adhesion molecule to facilitate adhesion of blood cells , essentially the leucocytes , lymphocytes and eosinophils to adhere to the vascular endothelium and to the extracellular matrix , and promote the migration of leucocytes from the vascular compartment towards the submucosal tissue which is the site of the inflammation reaction . They help in the process of specific antigen recognition by the T - cell , they act as T - cell costimulatory cell proliferation and the regulation of cell growth ***(Calderon and Lockey , 1992)***.

Four main families of adhesion molecules have been described the selectins (on neutrophils , eosinophils and monocytes as ELAM-1) , the integrins (on neutrophils , dendritic cells and monocytes as very late

antigen [VLA] and leucocyte function associated antigen [LFA]) , the immunoglobulin superfamily (on T cells and antigen presenting cells as ICAM and VCAM) and the cadherins . The adhesion molecules intervene at several levels during the course of asthma disease , for example , the expression of ICAM -1 , which is the LFA -1 ligand , is increased on the cells of the vascular endothelium or those of the bronchial epithelium especially after activation by histamine , likewise the ICAM - 1 molecule is known to be the rhinovirus receptor on the cells of the respiratory epithelium . ***Holagte (1993)*** , suggested that a high percentage of asthma attacks are due to the presence of rhinovirus . Increased expression of ICAM - 1 on bronchial epithelium in the asthmatic patient would increased the ability of this epithelium to bind rhinovirus , thus forming a kind of viscous circle .

Wegner (1990) , found that ICAM-1 is increased in vascular endothelial cells and bronchial epithelial cells after allergen exposure . An eosinophil - rich inflammatory infiltrate and non - specific bronchial hyperreactivity were also found . He also mentioned that administration of anti - ICAM-1 antibodies caused simultaneous reduction of the eosinophil infiltrate and the bronchial hyperreactivity .

The involvement of adhesion molecules in inflammatory infiltration has been investigated in 3 different cells models ; alveolar macrophages , T cells , and endothelial cells both in vitro and vivo. These three models demonstrate that there is close cooperation between the endothelial cell and the macrophages , T cells and mast cells , and support the idea that the adhesion molecules constitute a prime target for the treatment of the allergic inflammatory reaction ***(Lassalle et al ., 1993)*** .

Clinical investigation of bronchial biopsies in asthmatic patients demonstrated that there was increased expression of ICAM-1 on the bronchial epithelium , and of both ICAM-1 and E-selection in the vascular lumen . These findings , reflect the involvement of adhesion molecules in asthmatic ' disease at the epithelial level as well as at the endothelial level , ***(Vignola and Bousquet , 1993) .***

DIAGNOSIS OF ATOPIC BRONCHIAL ASTHMA

The basis of an allergy diagnosis is the patient's case history . In patients with inhalant allergy , an accurate diagnosis is often received when the case history is supported by the result of skin prick test (SPT) , or in vitro test for allergen specific IgE in the serum which could be used when there is suspicion of allergy against only one or a few allergens , whereas SPT should be used when testing with many allergens is necessary *(Eriksson , 1994)* .

[I] IN VIVO SKIN TESTS :

Skin testing is the method generally used to confirm sensitivity in patients with atopic disease or anaphylaxis . The skin is a convenient organ to test , since it is equipped with all the elements necessary for eliciting a localized controlled allergic reaction even though the disease is targeted to another organ *(Stites , 1987)* . It is one of the preferred in vivo method for assessing the presence of specific IgE antibodies against an allergen *(Arreguin et al ., 1995)* .

Skin testing occupies an intermediate position between in vitro tests which demonstrate specific immune response only (e.g tests for IgE antibodies , tests for immune complexes , tests for lymphocyte stimulation) and in vivo provocation tests which demonstrate the ability of the disease target organ to respond immunologically to the allergen . In positive cases of skin tests , within minutes after introduction of the allergen , histamine released from skin mast cells cause vasodilatation (erythema) , localized edema from increased vascular permeability (wheal) , and pruritis . The

skin reacts to allergen in almost all patients with type I allergy *(Kustner , 1987).*

Sarpong and Karrison (1998) reported that asthmatic children with sensitivities to many allergen were at increased risk of having more severe asthma .

Antihistaminic drugs inhibit or diminish skin test responses and have to be stopped 24 hours or more before testing *(Abba , 1987)* .

The common allergens used for diagnostic skin testing are listed in table (3) .

TYPES OF SKIN TESTS :

(A) ***EPICUTANEOUS TESTS :***

Epicutaneous tests (prick , scratch , and patch tests) are applied to the volar surface of the forearm or even the back when large number of tests are required . It is the procedure less likely to cause systemic anaphylaxis because of less amount of allergen introduced into the skin *(Norman , 1980)* .

In **prick testing** a single drop of concentrated aqueous allergen extract in buffered saline diluent at pH 6.0 is placed on the skin , which is then pricked lightly with a sterile needle point at the center of the drop . The needle is pushed slightly into the skin on 45 degree angle , and is then snapped upward to break , through the horny layer without drawing blood. After 20 minutes , the drop is wiped off and the reaction is recorded . The test is considered positive if there is erythema more than 20 mm with or without a wheal (Table 4) . A negative diluent control must be included but a positive control of histamine or codeine (non - specific mast cell mediator releasing agent) is optional in routine use *(Abba , 1987)* .

A late phase reaction is not usually elicited by prick testing and a negative prick test should be repeated intracutaneously ***(Roesel , 1978)*** .

Chase (1981) , reported that concentrations of allergens 10 to 100 times greater than the highest concentration used for intradermal injection are required .

Table (3) : Common allergens used for diagnostic skin testing ***(Paul and Van Arsdel , 1983)*** .

Pollens	Other Inhalants
Trees	**House dust mites**
■ Alder	**Epidermal allergen**
■ Ash	■ Cat
■ Birch	■ Cattle
■ Cotton wood	■ Dog
■ Elm	■ Horse
■ Hazel	■ Bird
■ Hickory	■ Others
■ Junipar	**Molds**
■ Maple	Aspergillus
■ Mountain cedar	Alternaria
■ Oak	Cladosporium
■ Pecan	Fursorium
■ Poplar	Helminthosporium
■ Sycamore	Monilia
Grasses	Pericillium Rhizopus
■ Bermuda	**Algae**
■ Blue	**Insects**
■ Fescue	- Caddis fly (limited distribution)
■ Orchard	Mayfly
■ Rye	**Weeds**
■ Timothy	■ Dock
■ Vernal	■ English Plantain
	■ Lamb's cuarters
	■ Marsh elder
	■ Ragweed
	■ Russian thistte
	■ Sageehrush

Scratch tests : are done by making a short liner scratch in the skin , 2-3 mm length without drawing blood using the needle tip , to which the allergen is then applied . It is not recommended because it frequently causes non - specific irritation , it is painful , and occasionally causes scarring ***(Norman , 1980)*** .

Patch tests : the allergen are dissolved in water or in soft paraffin ointment and are applied , in sequence to patch test dressings and fixed to the skin with adhesive tape (preferably applied on the back) . After 48-72 hours the test dressings are discarded and test sites are inspected for inflammation ***(Rook et al ., 1979)*** .

(B) INTRACUTANEOUS (INTRADERMAL) TESTS :-

In the intradermal test , a measured quantity of allergen is introduced into the skin for detection of IgE - mediated (atopic or anaphylactic) , IgG - mediated (immune complex) , or effector T lymphocyte - mediated (cellular or delayed hypersensitivity) responses ***(Woir , 1979)*** .

Intradermal tests should be applied to the arm or forearm only (back is avoided) , so that a tourniquent can be used in the event of an unexpected systemic reaction . Epinephrine 1/1000 aqueous solution and an antihistaminic for injection should be readily available all the time ***(Norman , 1980)*** . A tuberculin syringes with 27 - gauge needle is used , and the volume to be injected depends upon the immunological effector mechanism . For IgE - mediated sensitivities , the recommended volume ranges from 0.005 to 0.02 ml . This is to avoid injury and release of histamine by mast cells and basophils present in the cutis below the horny layer which will give confusing results ***(Abba , 1987)*** .

In most cases , Intradermal testing in suspected IgE - mediated disease is performed only for allergens giving 0 to 1+ responses to prior prick testing . The intradermal test is approximately 100 times more sensitive and results in a larger wheal and flare reaction than occurs with the prick test method . The reaction is read after 20 min (table 4) ***(Kustner, 1987)***.

Table (4) Wheal and erythema in skin tests : ***(Kustner ,1987)***

Reaction	Prick test	Intradermal test
Neg.	■ No wheal or erythema .	■ Same as control .
1+	■ No wheal , eryth . < 20 mm in diameter .	■ Wheal twice as large as control, erythema < 20 mm
2+	■ No wheal , eryth > 20 mm.	■ Wheal twice as large as control , eryth > 20 mm.
3+	■ Wheal and erythema.	■ Wheal 3 times as large as control erythema .
4+	■ Wheal with pseudopods, erythema.	■ Wheal with pseudopods , erythema .

A negative diluent control is necessary . A positive histamine or histamine - release control , or both is optional .

After the immediate wheal and erythema subside , a late phase 6-12 hours reaction appears in some cases . The diagnostic significance of the late phase is uncertain ***(Kustner , 1987)***.

Serial dilution titration is a semiquantitative form of intradermal testing in which 5 or 10 fold increasing concentrations of allergen extract are tested for each allergens until a positive result occurs . The main

purpose of serial titration is to determine a starting dose for immunotherapy that will avoid the risk of systemic reaction *(Stite , 1987)*.

Advantages of Skin Testing : -

Skin testing is simple , convenient inexpensive , safe if done properly , the results are available with no delay beyond the time required for the allergic response , there is no possibility of sample (i.e patient) error , many allergens can be tested simultaneously and discomfort is minimal *(Norman , 1980)* .

Disadvantages of Skin Testing :-

First , it is important to discontinue certain inhibitory drugs. Occasionally , skin testing is prohibited for lack of available skin because of generalized dermatitis . The procedure may be unacceptable to small children and some adults . A marked local reaction in sensitive patient may occur e.g local necrosis . There is always a potential for a systemic reaction e.g fever , anaphylaxis or flare of the disease . It is more with the intradermal than the prick method . Also false negative and false positive can exists *(Stite , 1987)*

Limitation of Skin Test :

False Negative Results :-

This may result from an improper antigen concentration , bacterial contamination ; exposure to heat or light ; adsorption of antigen on container walls ; faulty injection (too deep , leaking) ; improper reading of reaction ; washout of the antigen by bleeding . If the individual is allergic to the allergen metabolites , the allergens acts as a hapten that