CHAPTER 1

INTRODUCTION

The World Allergy Organization report, based on a survey conducted in 2004 among 1.39 billion people from 33 countries, estimated the prevalence of allergies worldwide at 22%. The numbers of incidences have been reported to be increasing over the past 15 years and continue to be on the rise (Birch et al 2010, Warner et al 2006). In the US alone, more than 50 million people have been estimated to suffer from some form of allergy, and 20 million from asthma alone (Hoffman et al 2005). While majority of the incidences of allergies have been reported from Ukraine, Japan, Bulgaria, Sweden and the UK, Colombia, Italy and the Phillipines recorded the lowest spread (Warner et al 2006). In India, allergies and asthma have an estimated prevalence of 18.38%, with dust mites being the most common cause of the incidences reported from the region, with estimates for asthma standing at 29.5%. Allergies from pollution have been estimated to have risen from 20 to 27.5% (Paramesh 2002). A survey conducted among the farm lands in W. Bengal, India, indicated that the pollen from Saccharum officinarum (54.86%) followed by Azadirachta indica (52.93%) and Phoenix sylvestris (44.09%) contributed the most towards pollen allergies and allergic rhinitis in India (Chakraborty et al 1998). The morbidity associated with the disorder is accentuated by the lack of proper diagnostic strategies or health care provisions. Allergy has been touted as a disorder most common to the developed world (Paramesh 2002). This may however be an observational discrepancy, arising out of lack of awareness, lower levels of reporting and misdiagnosis observed in the developing nations (Clark et al 2009, Khaled
et al 2009). This statement is substantiated by statistics from the country-wise allergist: patient ratio (LSAI survey), where even the existing statistics are from Europe, the Americas and West Asia; statistics from Africa and S. Asia are less well documented. While Germany has the highest allergist: patient ratio (1: 16,000 allergist per head of the population), Bangladesh has reported the existence of 0 certified specialists in allergy treatment and diagnosis. The condition in Bangladesh, is an indication of the degree to which statistical databases with respect to allergy are maintained in the developing nations, as well as, the significance attached to them, thereof (Warner et al 2006).

An allergen maybe defined as any antigen or stimulus capable of inducing an IgE mediated immune response. A homology study has determined allergens to be proteins with a conserved, approximately 30 amino acid long, inter-domain groove sandwiched between an α- helix and an anti-parallell β- sheet (Furmonaviciene et al 2001). Physical and environmental stimuli ranging from temperature, to electric and magnetic fields have been reported to induce allergic reactions in atopic individuals, by means of activating cell surface receptors of temperature, pressure etc (Hillert et al 2002). Depending on the nature of allergens, their dosage and routes of entry, different types of allergies have been reported (Constantin et al 2009).

1.1 TYPES OF ALLERGIES

1.1.1 Food Allergies

Allergic reactions have been reported to occur in response to protein allergens present in many foods, with citrus fruits, eggs, tomatoes, strawberries, fish, peanuts and tree nuts being the most commonly implicated (Clark et al 2009, Constantin et al 2009). A NIAID survey estimated that approximately 8% of children up to 6 years of age are susceptible to food allergies, while the condition is prevalent in 3-4 % of adults in the Western
countries and 1-2% adults in the US alone (Sicherer and Sampson 2009). Food allergies, when they are IgE dependant and hence relevant to this study, manifest themselves in the form of gastro-intestinal anaphylaxis, oral allergy syndrome, allergic eosinophilic gastroenteritis, atopic dermatitis, urticaria, and even respiratory tract manifestations such as asthma and allergic rhinitis (NIH Expert Panel 2006, Wood 2003).

1.1.2 Drug Allergies

Drug allergies contribute to approximately one-third of all adverse drug reactions. Drug allergies occur in response to antibiotics, sulfonamides, NSAIDS, with the world-wide prevalence rates placed at 7.3% in normal child population, and 1.8-2.8% of all cases of adverse drug reactions. The projected Figure stands at 10-15% as proposed by a meta-analysis study. The allergic manifestations exhibited by many common anti-biotics in the general population stands at 12.3% for cefaclor, 8.5% for sulfonamides, 7.4% for penicillins and 2.6% for cephalosporins. Drug allergies to specific drugs such as the penicillins have been mapped to polymorphisms in specific genetic loci in the HLA genes, while multiple drug reactions occur on account of structural similarity and consequent cross-reactivity (Demoly and Bousquet 2001). Drug allergies are most commonly manifested in the forms of cutaneous eruptions (urticaria, erythema and eczema), gut anaphylaxis and systemic anaphylaxis characterized by respiratory distress and shock (Vervloet and Durham 1998, Rozieres et al 2009).

1.1.3 Insect Venom Allergies

Venom allergies occur in response to particularly two sub-groups of class Insecta namely, Vespidae comprising the common Wasps and Apidae, the Bees. Cross-reactivity has been observed in between the venom mixture from these two sub-groups. Insect venom allergies most commonly manifest
themselves as systemic anaphylaxis and shock, with 3.3% of all anaphylaxis deaths falling under this class. Immunotherapy and vaccination, especially via the mucosal route has been found to be the most efficacious in the treatment of this form of allergy, on account of its severe nature and the short duration available for treatment (with epinephrine), after onset (Bonifazi et al 2005). However, as in the case of treatment with whole Wasp venom preparations, sensitization with 30µg has proven cytolytic in vitro and toxic and fatal, in vivo, in the mouse insect venom allergy models. Recombinant allergens, such as the rVesv5, are now being examined as vaccine alternatives, on account of their low toxicity and ability to induce both B and T cell responses (Winkler et al 2003). The cost-effectiveness of such treatment strategies, still remain to be evaluated (Muller et al 2009).

1.1.4 Chemical Allergies

A wide variety of entities ranging from wheat pollen, dust mites and grass pollen to pesticides, perfumes and automobile exhaust classify as chemical allergens. Most of the chemical allergens are aero-allergens and consequently, allergic rhinitis, followed by conjunctivitis is the most common manifestations. Asthma occurs in approximately 3% of the cases of chemical allergies. Neither the statistics, nor the mechanisms of chemical allergic sensitization are very clear. However, three separate studies from North Carolina, USA, Nottingham, UK and France have placed the incidence of chemical allergies as opposed from chemical sensitivities at 30% of the tested population, reporting allergies less than once a month. Latex allergy is another common feature prevalent among the clinicians (Meggs et al 1996).

1.1.5 Allergic Reactions

A percentage of the population reacts with allergic manifestations to a range of physical and environmental stimuli such as temperature, electric
and magnetic fields, as well as many chemicals. Currently no diagnostic procedures are followed to immunologically classify these reactions as allergies. It is believed that many of these conditions are either neurogenic in origin or arise from somatic or limbic sensitization. While a 1995 study based on physician’s diagnosis in the USA, placed the occurrence of Multiple Chemical Sensitivities (MCS) at 6.3%, a more recent (2002) study from Sweden estimated the overall prevalence of sensitivities, to electric and magnetic fields in the cohort, at 1.5%. Allergic reactions have been reported to co-exist with asthma (20.1%), hay fever (27.4%), allergic conjunctivitis (31.8%) and manifest themselves as, eye irritation, dry throat and cutaneous manifestations among other symptoms that are not typical of immune mediated hypersensitivities. These include symptoms such as headache, dizziness and loss of concentration, giving credence to the hypothesis of neurogenic origin of the disorder (Hillert et al 2002).

1.2 MANIFESTATIONS OF ALLERGIES

1.2.1 Asthma and Bronchitis

Asthma is the pathology of upper and lower respiratory tracts resulting from airway obstruction and manifests itself as wheeze, breathlessness, positive or increased alveolar pressure resulting from hyperinflation; under extreme exacerbation the condition results in asphyxiation and death (Rainbow and Browne 2002). Clinically, the condition presents itself as reversible airway obstruction, eosinophilic airway inflammation, airway hyperresponsiveness, airway remodeling, mucus hypersecretion and increased sensitivity to bronchogenic stimuli. Bronchitis represents a milder version of the disorder, with the pathology being limited to the bronchia. Asthma is one of the leading causes of morbidity in the world. Over 300 million people are affected world wide, of which over 90% of the cases in children are from allergic asthma, while 50-60% of the cases in
adults are documented to be atopic (Heijink and Van Oosterhout 2006). However, in recent years, a decline has been observed in the reported morbidity, on account of better management of the disorder, relative to other allergic manifestations. The disorder occurs in response to various triggers such as aero-allergens, airway infection, exercise and stress. Allergens triggered asthma implies confirmation of underlying atopy. The disorder has been observed to have a higher incidence in boys, among children under 6 years of age, the prevalence of the disorder becomes common to women, later on in life. While the underlying reason behind the gender difference is partly hormonal, an increase in IgE serum levels has been observed in boys between 2-4 years of age, compared to girl children of the same age group. In addition, all conditions predisposing towards asthma have been proven to have higher levels of serum IgE such as smoking, socio-economic class and heredity (Boschetto et al 2003). Systemic corticosteroids in combination with β-adrenergics represent the standard treatment protocol for asthma. However, oral corticosteroids and to a lesser degree, higher doses of inhaled corticosteroids have also been shown to provide similar results (Holgate and Polosa 2008). While epinephrine has been shown to possess cardio-vascular side-effects, most of the other standard treatment procedures (heliox, α-cholinergics and Ipratropium Bromide- bronchodilators, leukotriene modifiers) have been shown to be ineffective as mono-therapies. Mucolytic agents have adverse effects (Rodrigo et al 2004).

1.2.2 Anaphylaxis

Anaphylaxis has been defined by Choy et al as a condition of urticaria associated with one or more of the following conditions: shock, asphyxiation or collapse, bronchospasm, upper airway symptoms, gastrointestinal symptoms such as acute vomiting and diarrhea or pain that is potentially life-threatening. The cases of anaphylaxis around the world are
under-reported due to misdiagnosis. The existing statistic of 3-30 reports per 100,000 of the population is a reflection of the scenario. While mild anaphylactic reactions are characterized by runny nose, cough or vomiting with urticarial reactions, severe anaphylactic reactions result in acute gastrointestinal, respiratory or cardio-vascular symptoms. Elevated mast cell tryptase levels are characteristic of the condition; however, its diagnostic application is limited by the lowered levels of the serum enzyme, 6 h after onset of anaphylaxis (Tejedor Alonso et al 2004).

1.2.3 Allergic Rhinitis

Allergic rhinitis alone affects 155 million people world wide and over 80 million people in the Europe (Heijink and Van Oosterhout 2006). Pollinosis is allergic rhinitis occurring specifically in response to pollens as allergens. The condition is characterized by runny nose, sneezing, itchy and watery eyes and burning sensations in the throat. Japanese cedar pollen, orchard grass and ragweed pollen represent the three major pollen allergens in the world, both in terms of the number of reported incidences, as well as, in terms of disease severity. In one study, perennial allergic rhinitis was documented at 20% on an average, in the Japanese population. The prevalence of seasonal allergic rhinitis was estimated at 15% in the cohort of children, less than 7 years of age, studied by Kulig et al (1998). The predisposing factors to allergic rhinitis are both environmental, as well as genetic. Afforestation, the nature of the pollen, duration of pollination all contribute towards the increase in the reported incidences of allergic rhinitis, while, linkage associations have been observed between specific chromosomal loci and the condition. One major linkage loci is the 4q24-q27. Although, the numbers of alleles specifically linked to allergic rhinitis are few, the linkages are common to asthma and allergic rhinitis. In addition specific candidate genes such as Il-4, IL-4R, sSTAT-6, FceRIβ (Gly 237 var),
HLA-D, IFN-γ have all been implicated in the pre-disposition towards allergic rhinitis (Arakawa and Morikawa 2004).

1.2.4 Contact Hypersensitivity (Atopic Dermatitis and Conjunctivitis)

Atopic dermatitis (AD) or “constitutional dermatitis”, as it is classified, according to the revised nomenclature of allergic diseases, is a genetically determined, IgE mediated, delayed type hypersensitivity reaction of the skin, characterized by eczema and erythema, cutaneous eruptions, dry skin and intense pruritis. AD may be classified as intrinsic (non-atopic) and extrinsic (atopic) similar to classifications of the asthma and allergic rhinitis. Extrinsic AD (EAD) is characterized by high serum IgE levels (>150kU/l), both allergen-specific and non-specific, positive skin prick test and intracutaneous test reactions of the immediate type to the common environmental allergens, elevated IL-4 and IL-13 levels, CD23+ B cells, and ratio of over 1.5 of FcεRI : FcεRII. The condition has an early onset, with a slight male predominance. The causal allergens behind AD include both food and aero-allergens. Following initial sensitization, AD often progresses to bronchial asthma, asthma or anaphylaxis. Allergens have also been shown to cross-react with auto-antigens from the skin, resulting in the production of auto IgE antibodies and a consequent auto-immune disease (Saloga and Knop, 2000). AD is characterized by a pre-dominance of cell-mediated immunity (T-cells, eosinophils, and to a lesser extent mast cells and basophils) in the skin lesions, especially in the chronic phase of the disease. Cetrizine, a H1-histamine antagonist is commonly used in the treatment of the disorder (Schmid-Grendelmeier et al 2001).

1.2.5 Gastro-intestinal Allergic Disorders

Gastro-intestinal allergic disorders comprise a spectrum of disorders ranging from gastro-intestinal anaphylaxis to oral allergy syndrome
and eosinophil mediated gut allergy. The condition is characterized by acute diarrhea, pain, vomiting and nausea, with inflammation observed in the large and small intestines in different murine allergy models. 2-6% of the populations worldwide are currently reported to be suffering from food anaphylaxis, which also may involve systemic anaphylaxis, under certain conditions. Elevated levels of gut mast cells and eosinophils, characterize the condition. Unlike systemic anaphylaxis, gut anaphylaxis and gut allergy follows a classic IgE mediated hypersensitivity and is dependant on FcεRI, serotonin and PAF. The reduced numbers of granulated mediators in the mucosal mast cells are thought to be responsible for the reduced disease morbidity associated with the gut anaphylaxis, relative to systemic anaphylaxis. Mast cell depleting or neutralizing molecules, anti-serotonin in combination with PAF receptor antagonists, anti-IgE molecules such as the commercially available Omalizumab and c-kit inhibitors are recommended for the treatment of the condition (Brandt et al 2003).

Allergy is thus a multi-factorial disorder at the interface of genes and environment (Huang et al 2001). The paradox lies in the commonality of the underlying molecular pathology (Hunskaar and Fosse 1990). At the molecular level, the redundancies in the cellular signaling mediators contribute to the complexity of the disorder and the ineffectiveness of the existing treatment strategies. The human system, in general responds to common environmental allergens with a low grade IgG and Th1 mediated immune response. The pathology sets in, in those individuals, in whom, the system loses the Th2/Th1 immunological balance and shifts to a Th2, IgE mediated immune response. It is this dysfunction at the molecular level that is clinically termed allergy (Kay 2000).
1.3 ALLERGIC SIGNALING: MOLECULAR MECHANISM DRIVING ALLERGIC PATHOLOGY

Any allergenic protein, as was previously defined, is processed by the Antigen Presenting Cells (APCs), the dendritic cells (DCs) and the macrophages; induces a Th2 type of immune response predominated by the synthesis of IL-4, IL-5 and IL-13, all of which cytokines, push the B-cells to undergo IgE class switching. The Th2 cytokines, along with IgE stimulates the high-affinity IgE receptor, FcεRI, density on tissue mast cells and blood basophils. To these cell-surface receptors, the allergen specific IgE binds, and the individual is then sensitized to the specific allergen and its cross-reactors; depending on the dosage and the route of entry, the degree of sensitization and hence the nature of the subsequent allergic pathology varies (Pandya et al 2002).

Figure 1.1 Mechanism of allergic sensitization: Immunological Pathways in Asthma

1.4 CELLULAR MEDIATORS OF ALLERGY

1.4.1 T Lymphocytes

T cells are the central orchestrators of the adaptive immune response. The antigens processed by the APCs are presented to the T cells at the lymph nodes, where they differentiate into either the Helper T cells (Th1, Th2), Cytotoxic T cells (Tc) or the regulatory T cells (Treg) subtype, based on the nature of the immunogen, as well as the cellular chemokine and cytokine milieu (Heijink and Van Oosterhout 2006). This T cell activation and differentiation is dependant upon both the stimulatory as well as the co-stimulatory signals presented to the naïve T cells. It has been shown that, the chemokine receptor CCR7, is involved in the homing of the T cells to the LNs, just as is the case with dendritic cells. Further, CCR7 is also essential for the primed T cells to leave the site of inflammation, such as the skin or the lungs. The CXCR4-CXCL12 axis has also been shown to be responsible for eosinophilia and airway hyper-responsiveness observed in asthma patients; while CCR4 and 8 are specifically found only on the TH2 cell subtypes. They are thought to play a role in T cell migration (Pease and Williams, 2006).

CTLA 4, the Cytotoxic T Lymphocyte Antigen 4 or the CD 28 is another major cell surface antigen, responsible for the co-stimulatory signaling pathway involved in T cell proliferation and Th2 cytokine production. Targeting CTLA4 has been shown to produce lowering of serum IgE levels, airway eosinophilia and IL5 production in mild asthmatics, as opposed to moderately severe asthmatics. This is an indication of the potential therapeutic use of CTLA4 as a target for anti-asthmatic medication, at least in patients with mild asthma (Heijink and Van Oosterhout 2006). Further, another subset of T cells, the recently classified, IL10 producing regulatory T cells (Treg) are known to suppress both the TH1 and TH2 type of cells and thus are important therapeutic targets in the treatment of allergic diseases (Pease and Williams 2006).
1.4.2 B Lymphocytes

B cells are the antibody producing lymphocytes; their activation, differentiation and class-switching take place in the bone-marrow. The antibody producing plasma cells, generally in response to an antigen undergo clonal selection, followed by IgM, IgA and IgG production, in that order. However, when the immunogen is an allergen, and when the presentation of the allergenic peptide is done in a milieu of Th2 cytokines and chemokines, especially, the interleukins, IL 4, 5 and 13, then the plasma cells undergo a class-switching to produce IgE antibodies. It is these, allergen-specific IgE antibodies, that mediate the classic IgE based immediate type allergic reactions. Thus B lymphocytes are central effector cells in the allergic reactions (Lebman and Coffman 1988).

1.4.3 Dendritic Cells

Dendritic cells (DCs) are the primary antigen presenting Cells (APCs), present at the interface of the animal system and environment. Dendritic cells normally mediate the adaptive immune response. This involves recognition and processing of the foreign antigens and homing to the nearest draining lymph nodes (LNs) rich in naïve T cells (Banchereau and Steinman 1998). The DC mediated presentation of the antigen to the CCR4 bearing naïve T cells, primes and clonally selects the T cells to mount either a Th1 or Th2 type immune response, depending on the chemokines expressed and the nature of the immunogen. Different subsets of DCs exist, such as the myeloid, plasmacetois, lung-derived and the monocyte derived cells. The homing of the lung-derived DCs to the LNs require the presence of the chemokine receptor CCR7, and in its absence, clinical features and symptoms of asthma are known to develop. The transcription of this receptor gene is under the control of the transcription factor runt-related transcription factor 3; the gene for which has been shown to be present at the chromosomal loci
1p36, which has been linkage mapped to increased serum IgE levels and asthma (Pease and Williams 2006).

### 1.4.4 Eosinophils

The presence of eosinophils marks the late phase of allergic responses and together with the mast cells are involved in chronic allergy. These cells are derived from the bone-marrow stem cells and recruited from the peripheral blood to sites of allergic inflammation or parasitic infection. The cells are granulated; the granules contain Eosinophil peroxidase, major basic protein, Eosinophil derived neurotoxin, as well as other pre-formed and de novo synthesized mediators. The trafficking of eosinophils occur under the influence of specific transcription factors GATA1/2, c/EBP, chemokine eotaxin, as well as Th2 cytokines, IL-3, 5 and 13. In addition these cells also bear surface receptors for a variety of pro-inflammatory mediators, including the IgE receptor, FcεR (Pease and Williams 2006).

### 1.4.5 Basophils

Basophils are the counter parts of the tissue mast cells, in the blood. It was assumed that these cells are derived from the bone-marrow stem cells. However, recent schools of thought tend towards a common lineage of origin for both basophils and mast cells. Basophils play a role in systemic anaphylaxis in cases of allergic inflammation, and are also recruited to sites of allergic inflammation or in response to parasitic infection or any type of Th2 mediated inflammatory response. Their role in inflammation is less well defined; but these cells are known to produce the cytokines IL4, IL13 and TNF-α. The recruitment of blood basophils to the sites of inflammation is dependant on 2 chemokines, namely, CCL5 and CCL 11, with CCL 11 being the most potent in vitro. These chemokines react with the CCR 1, 3, 5 receptors. While CCL 11 is involved in the dermal recruitment of basophils,
CCL 5 has been shown to be more involved in the recruitment of basophils to the airway mucosa, as demonstrated in allergic patients. Downstream, CCL 5 has been shown to drive the transcription of the Histidine Decarboxylase mRNA, responsible for the histamine production in basophils. Activated basophils also produce CXCR4, but not CCR 4. Degranulation of the basophils is driven by the chemokines CCL2, 3, 7 and 13, but not CCL 11. CCL 11 is thought to be responsible for the allergic inflammation, consequent of mast cell degranulation (Pease and Williams 2006).

1.4.6 Mast Cells

Mast cells are the central players mediating the classic IgE mediated allergic reaction. Mast cells are tissue-specific and exist as different sub-groups, depending on the resident tissue type. Accordingly, Langerhans cells occur in the skin and kupffer cells in the liver. Mast cells possess the surface high affinity, IgE receptor FcɛRI in high density. They are highly granulated, arising out of myeloid progenitors. The granules contain pre-formed mediators such as histamine, serotonin, tryptase, chymase and other proteoglycans all of which lead to smooth muscle contraction and tissue injury in cases of allergy, while conferring protective effects during parasitic infections. In addition, certain mediators such as β-hexoseaminidase, are present whose function is unknown, but serve as markers of the degranulation response. It is this enzyme β-hexoseaminidase, which presence has been exploited as a measure of degranulation in the in vitro experimental allergy model used in the study. In addition, de novo synthesized secondary mediators of allergy, namely, the lipid mediators, prostaglandins and lekotrienes are also packed within granules to ensure their release on to the cell exterior. Finally, mast cells fix the onset of late phase allergic reactions with the release of cytokines, chemokines and growth factors (Brown et al 2008). However, these cells are characteristic of the early phase of allergy, as
indicated by its significance in the IgE mediated allergic reactions (Pease and Williams 2006).

1.5 **RBL-2H3: ITS SUITABILITY AS A MODEL FOR MAST CELL AND ALLERGY**

RBL-2H3, a variant of the rat basophilic leukemia cell line has been used extensively for the study of FcεRI, as well as mast cell secretion effectors, including calcium influx, phospholipases and protein kinases (Collado-Escobar et al 1990). RBL-2H3, a variant of the rat basophilic leukemia cell line is derived from RBL-HR⁺, a histamine releasing cell line, which in its turn was derived from the RBL-1 cell line. RBL -1 cell line is a tumor cell line developed from a clinical case of human basophilic leukemia. The leukemic basophils were transplanted into rats sub-cutaneously and later tumors developed intra-peritonially. The tumor cells were then transferred onto in vitro culture conditions representing the RBL-1 cells.

The derived RBL-2H3 variant is characterized by the presence of IgE binding FcεRI, the expression of rat mast cell protease II and the c-kit receptor tyrosine kinase also expressed in the human mast cell line, HMC-1. Thus the RBL-2H3 cells share characteristics of mucosal mast cells, although they are derived from the basophilic leukemia cells. However, RBL-2H3 varies from the MMCs in terms of ultra-structural and phenotypic differences. However, it has been proven that basophils and mast cells are functionally similar; the RBL-2H3 cell line sharing characteristics of both basophils and mucosal mast cells, may thus be perceived as an advantage in the study of FcεRI-IgE interactions, calcium flux, IgE and A23187 mediated degranulation, and signal mediator interactions (Passante and Frankish 2009).
Figure 1.2 Mast cell exocytosis

Adapted from J.M Brown et al “The mast cell and allergic diseases: role in pathogenesis and implications for therapy,” Clinical and Experimental Allergy, 38, 4-18.

RBL-2H3 was thus the in vitro model of choice for this study which aimed to delineate the various molecular targets of pharmacotherapy in allergic signaling, to identify potential anti-allergic molecules and to map the molecular targets of their anti-allergic potential.
1.6 MOLECULAR MEDIATORS OF ALLERGY

1.6.1 Construction of the FcεR

The high-affinity IgE receptor, FcεR is a heterotetramer and comprises of one α, one β and two γ chains. The α chain lies on the cell surface and forms the IgE binding site, the β chain forms the inter-membrane domain, while the γ chains form the cytosolic domain. The γ chains possess Tyrosine phosphorylation sites in specific motifs, called the Immuno Tyrosine-based Activation Motifs (the ITAMs). Trans-phosphorylation of the tyrosine residues within these motifs leads to receptor activation, cross-linking and subsequent membrane toggle that marks the activation of the allergic signaling cascade (Zhu et al 2002).

1.7 PRIMARY MEDIATORS OF ALLERGY

1.7.1 The Tyrosine Kinases and Phosphatases

The activated ITAMs then phosphorylate/activate the receptor attached Lyn kinase which then phosphorylates and recruits the Syk kinase. The Syk kinase then activates the kinases, the PI3K and Btk and the SH2 domain containing signaling adaptors, the Shc, Grb2, Sos and Vav. At each stage, the number of molecules of effectors activated by each mediator increases and the magnitude of the allergic signal amplifies (Daëron 1997, Kopec et al 2006, Rivera and Olivera 2008). The phosphorylation/activation of the Lyn and Syk kinases are held in check by the phosphatase, SHIP (SH2 domain containing Inositol 5-Phosphatase), the negative regulator of the signal. Hydrolysis of the tyrosine phosphorylation by SHIP, prevents the kinases from being recruited into the lipid rafts, where the receptor cross-linking and membrane toggle actually takes place (Kepley et al 2004). Thus studying the phosphorylation states of the various cellular kinases would provide an insight into the effects of the candidate drug isolates on the cellular
activation status. This was studied with the help of anti-phospho Tyrosine antibody, directed against the ITAM motifs, but which picks up the phosphorylation states of all the cellular kinases and hence the cellular activation status.

1.7.2 Lipid Rafts and Cholesterol Sequestration

Lipid rafts represent dynamic regions within the plasma membrane that do not exist in fluid equilibrium with the rest of the fluid-mosaic of the membrane. These regions are rich in cholesterol, sphingolipids and Glycosyl Phosphatidyl Inositol (GPI)-anchored proteins. It has been shown that, sequestration of cholesterol from these micro-domains interfere with their integrity, and thus disrupt receptor aggregation and cross-linking, thereby interfering with the allergic signaling. However, excessive depletion of cholesterol from the plasma membrane interferes with the integrity of the membranes themselves and thus the integrity of the cell (Fielding and Fielding, 2004). The FcεRs aggregate within these micro-domains upon receptor occupation and trans-phosphorylation, undergo allergenic cross-linking with other IgE bound receptors and the membrane toggle and signal transduction ensues. To study the effect of the candidate drug isolates on the membrane and cytosolic cholesterol levels, a cholesterol loaded cell model was adopted (Hoessli et al 2000, Simons and Toomre 2000).

1.7.3 Membrane Toggle: Role of Actin in Allergic Signaling

The sub-membranous meshwork of actin cytoskeleton plays a crucial role in a variety of prokaryotic and eukaryotic cellular processes including filopodium formation, phagocytosis, endocytosis, granule exocytosis, chemotaxis, cell division, cell polarization at immunological and neuronal synapses. In addition, the membrane actin functions as both an effector as well as a signal transducer of membrane receptor activation
signals, in a variety of cell types (Castellano et al 2001, Lesourne et al 2005). In neutrophils, basophils and mast cells, the central cellular effectors of allergic pathology, actin modulates the exocytosis of pre-formed granules containing pharmacological effectors of inflammation and tissue injury. The regulation takes place both at the level of membrane cytoskeleton, as well as the free cytosolic F-actin, that polymerizes to form the microtubular structures propelling the granules towards cell membrane and consequent exocytosis (Jog et al 2007). While Frigeri et al (1999) demonstrated that mast cell degranulation is directly proportional to inhibition of F-actin polymerization in a dose dependant manner, recent studies establish that actin polymerization and reorganization might in fact be a constitutive negative regulator of degranulation (Lesourne et al 2005, Vilarino and McGlashan 2001).

It has now been established that the actin dynamics observed upon FcεRI activation, temporally regulates mast cell degranulation in a bi-phasic manner (Nishida et al 2005). In response to the early receptor activation signals, the cytoskeletal actin platform undergoes extensive reorganisation and in the process amplifies the transduced signal via a family of 60 actin binding proteins (Eitzen 2003). The ITAM phosphorylated receptor activates tyrosine kinases Lyn, Syk and Btk which in turn activate the Phospholipases A, C and D. Phospholipase A is responsible for the de novo synthesis of the secondary messengers, the Prostaglandins and Leukotrienes. Phospholipase C activates PKC. Diacyl glycerol (DAG), the product of Phospholipase D action on membrane phospholipids, is responsible for the reinforcement of PKC signals (Davey et al 2008). The active PKC translocates to the site of actin polymerization, binds to WASP or N-WASP via its Plecstrein Homology (PH) domain, recruiting other F-actin binding proteins, such as profilin and coifflin, thus aiding in further actin polymerization or depolymerization (Yao et al 1999).
Activation of the high-affinity IgE receptor FcεRI, by the multivalent antigens (allergens) is a highly transient phenomena. It is dependant on the localization of the FcεRI-IgE complexes and the membrane associated kinase Lyn, within the specialized cholesterol rich plasma membrane domains, the lipid rafts. Holowka et al (2000) and Lesourne et al (2005) have shown that these receptor raft associations are F-actin dependant and mediated via the FcγRIIB co-aggregation. Further, the co-aggregation of SHIP 1, a negative regulator of ITAM phosphorylation and FcεRI activation has also been shown to be F-actin dependant (Lesourne et al 2005). F-actin has thus been shown to not only temporally, but also spatially regulate the degranulation signals. Modulation of F-actin and actin dynamics is thus a logical pharmacological target. To this end, the effect of the Methanolic extract of Solanum Xanthocarpum and the compound isolated from it and Solasodine on membrane actin dynamics were studied using Western blotting and Immuno-fluorescence techniques. For further investigation of the isolates from Solanum Xanthocarpum on membrane and total F-actin content, three different experimental models were adopted, a SDS-PAGE membrane actin content analysis, confirmation of the results with immunoblotting and a Alexa_Fluor 488 based immunofluorescence study. A time course analysis on FcεRI activated cells was performed to determine the membrane actin dynamics in the in Vitro allergy model.

1.7.4 Wiskott Aldrich Syndrome Protein (WASP): Regulator of Actin Polymerization

WASP is a multi-domain protein with binding sites for a host of cellular mediators. The binding domains in WASP include the IQ domain for calcium dependant calmodulin binding, the GBD for binding the Rho GTPase Cdc42, the PRD for binding proteins with poly proline sequences such as WIP, the N-terminal EVH1 domain for VASP binding, the PH domain for
binding PKC and other protein kinases, a host of actin binding proteins such as coifflin, filamin and profilin binding sites, as well as the C-terminal VCA domain for the actin polymerizing Arp 2/3 complex interaction (Badour et al 2003, Benesch et al 2005, Castellano et al 2001, Miki et al 1998, Miki and Takenawa 1998). Thus WASP is structurally suited to integrate the plethora of cellular signals and is centrally placed to control cytoskeletal actin dynamics and F-actin polymerization. Cory et al (2003) have shown that the constitutive phosphorylation exhibited by WASP Ser 483/484 residues is rate-limiting for Arp2/3 complex binding, increasing the binding 7 fold. This additional regulatory step is thought to increase the Arp 2/3 complex turnover and indirectly, actin polymerization. The study however, does not rule out stimulus-induced changes in the Ser 483/484 phosphorylation status under specific cellular environments. The phosphorylation state of the Serine 483/484 residues was studied upon treatment in comparison of the Methanolic extract and the potential candidate drug isolated.

1.7.5 Calcium Signaling

The process wherein calcium is released from the intracellular pools such as the Endoplasmic Reticulum (ER), causing a subsequent increase in the intracellular calcium concentration \([Ca]_i\) is termed the Store Operated Calcium Entry (SOCE). The resultant rise in \([Ca]_i\) is due to its influx from the extracellular pools. For the purposes of distinguishing the cause and effect, these two processes have been referred to as Store Operated Calcium Entry (SOCE) and Capacitative Calcium Entry (CCE) respectively, in this study, although the terms have been used inter-changeably by many researchers (Oka et al 2005, Sandoval et al 2007). SOCE occurs in response to receptor activation in mast cells in particular, and in response to a host of other stimuli such as insulin, receptor tyrosine kinase activation and activation of tyrosine kinases in different cell types and cellular milieu. In basophils and mucosal
mast cells, including the RBL-2H3 mast cell line, FcεRI is the receptor transmitting the signal for calcium release. Upon receptor activation, the transient depolymerization of the actin cyto-skeletal network induces resultant small rises in the levels of peri-plasmic calcium concentration (Oka et al 2002). This small rise in calcium is thought to provide the impetus for the activation of immediate downstream signals including the actin polymerizing proteins and Phospholipases (Benhamou et al 1992). It is interesting to note that, WASP or N-WASP function has been shown to be calcium dependant, via their calmodulin binding IQ domain (Piviniouk et al 2003).

PLC releases IP3, a product of membrane phospholipid hydrolysis; IP3 binds to its receptors (IP3R) in the ER, resulting in SOCE (Piviniouk et al 2003). SOCE then causes CCE, a concurrent opening of the calcium channels in the plasma membrane, the SOCs (Store Operated Calcium Channels), such as the non-voltage gated I_{CRAC} calcium channels as well as the Na/H ion exchanger (NHE). The exact mechanism by which SOCE opens the calcium channels in the plasma membrane is not well understood; but studies postulate two possible mechanisms, the conformational coupling and the calcium inducible factor (CIF) induced influx. In the conformational coupling mechanism, microtubules are thought to directly relay the calcium signals from the ER to the plasma membrane calcium channels (Parekh and penner, 1995). While in the latter case, a soluble CIF is thought to be activated upon IP3R activation or by the store-released calcium signals themselves; the CIF then travels to the plasma membrane opening the calcium channels (Oka et al 2005).

This rise in the pool of intracellular calcium concentration [Ca]_i results in transient cellular acidification, followed by sustained alkalinization. These changes in the intracellular pH (pH_i) are thought to be essential for many cellular processes including granule exocytosis, as well as in the
regulation of MAP kinases and the protein kinases PKB/Akt and PKC phosphorylation and activation (Hidalgo et al 2004, Sandoval et al 2007). Calcium mediated activation of N-WASP and PKC play an important role in actin microspike polymerization and granules movement. PKC is also known to mediate membrane fusion of the transmitted granules and consequent degranulation, via its effect on the cytoskeletal network (Taunton et al 2000). On the other hand, MAP Kinases and PKB activation are known to mediate the secondary and late phases of the allergic reaction, namely, prostaglandins and cytokines synthesis, activation and release (Sandoval et al 2007). Thus assessing the effects of potential anti-allergic molecules for their effects on SOCE and CCE becomes of paramount importance. Although dependant on SOCE, CCE has a more significant and direct effect on degranulation, on account of the high concentrations of intra-cellular calcium waves generated (Beaven et al 1984). The intracellular calcium concentrations, upon CCE and SOCE were measured upon loading the cells with the fluorophore, FURA 2A/M and the calcium measurements expressed as Relative Fluorescence Units (RFUs).

1.8 SECONDARY MEDIATORS OF ALLERGY

1.8.1 The Prostaglandin Bio-synthetic Enzymes: PLA2 and COX

Prostaglandins, Leukotrienes and Thromboxanes are the pain effectors in allergic reactions. These are the secondary mediators in allergic reactions; present in pre-formed granules in the cytoplasm, the granules are released upon receptor activation, resulting in pain, edema, inflammation and tissue injury (Kunikata et al 2005). De novo synthesis of these lipid secondary mediators requires the activation of PLAs, of which two functional isoforms exist, the secretory PLA2 (sPLA2; groups IIA, IID, V, X) and the cytosolic PLA2 (the cPLA2; group IVA), (Murakami et al 2002). The PLA2 isoforms exhibit a functional segregation in terms of temporal and cell-specific
coupling and regulation of the downstream enzymes in the lipid mediator bio-
synthesis. Upon receptor induction, the PLAs, in particular, the cytosolic
PLA2α (cPLA2α; group IV A) cleaves the membrane glycerophospholipids to
release the C-20 fatty acid, the arachidonic acid (Ueno et al 2001).
Arachidonic acid is the substrate of two lipid mediator bio-synthetic enzymes,
the COX (Cyclo-oxygenases) and LOX (lipooxygenases). While COX
isoforms 1 and 2 are responsible for the bio-synthesis of Prostaglandins and
Thromboxanes, LOX isoforms mediate the synthesis of Leukotrienes. The
COX enzymes are protein dimmers, with a PG synthase domain coupled to it;
the terminal PG synthase is functionally a hydroperoxidase and converts the
PGE2 and PGH2 produced by the COX1 and COX 2 respectively to the PGs
[thromboxane (TXA2), PGD2, PGE2, PGF2 α and PGI2]. While the
constitutive COX 1 is of physiological significance, produced immediately
after the activation stimulus, the inducible COX2 mediates inflammation and
tissue injury via the production of pro-inflammatory cytokines and PGs.
COX2 activity occurs in the delayed phase of inflammation and is sustained
until the inflammation is resolved by the induction of a variant protein of
COX2 gene expression, named COX4. COX3 is a variant of COX1 gene
expression, expressed in the anterior hypothalamus, inhibition of which
results in hypothermia (Ueno et al 2001). The Cox activity analysis was
performed using a colorimetric kit based assay, while the PLA2 activity was
measured as a function of release of the C14-labelled arachidonic acid.

1.9 LATE PHASE MEDIATORS OF ALLERGY

1.9.1 NFκB Activation: p65 Nuclear Translocation and IκB Degradation

NFκB comprise a family of 6 Rel proteins, including the NFκB1
(p50), NFκB2 (p52), RelA (p65), RelB, c-Rel and v-Rel proteins. Upon
activation, the protein subunits form homo- or hetero-dimers and translocate
to the nucleus where they regulate the transcription of target genes via their
DNA binding activity. The activation of NFκB occurs in response to various stimuli, both physiological and receptor mediated (Karin and Neriah 2000). Among the receptor mediated activation, canonical and non-canonical regulation of NFκB exists. While the canonical NFκB activation occurs in response to inflammatory stimuli such as LPS, TNF-α, LTβ-4, allergens and bacterial antigens, the alternative pathway regulates growth factor mediated signals involved in lymph node architecture, thymocyte differentiation and immunological memory. While the canonical pathway is regulated by Iκ Kinase (IKK2), the alternative pathway is dependant on (NFκB Inducing Kinase) NIK-IKK1 mediated activation of Rel dimers. Regulation of the NFκB activation occurs either via the uncoupling of inhibitory proteins, the Inhibitors of κB (IκB) or by a precursor processing mechanism. The former is the classical NFκB activation pathway, where upon activation, the inhibitory IκB proteins (α, β, ε) undergo IKK2 mediated ubiquitination and degradation, releasing the Rel (most commonly, the RelA: p50) dimers for nuclear translocation. In the latter, the whole length nfkβ1 and 2 gene products, the p105 and p100, synthesized in response to stimuli, undergo post-translational processing to yield the functional p52 and p50 subunits. The nfkβ 2 (p100) prior to processing and in response to LTβ-4 signaling, has been shown to act as an inhibitor of the RelA: p50 NFκB heterodimer; p100 is now classified as IκB-δ and is thus thought to link the canonical immunological stimuli to the non-canonical developmental stimuli. This gains significance in immunological memory as well as in signal amplification (Basak et al 2007).

In mast cells, upon FcεRI activation, the calcium dependant PKC-β has been shown to activate Rel via the NFκB specific, Bcl110-Malt1 mediated IKK induction and IκB degradation (Klemm et al 2006). This pathway occurs independently of the Akt-Cot mediated activation of NFκB, another NFκB specific pathway demonstrated in response to Platelet Activating Factor
(PAF), but not TNF signal. The latter pathway has been shown to synergize with the former in amplifying the activation signal (Kane et al. 2002). Although in rats, NF-AT has been shown to be the primary transcription factor, NFκB is also known to play an important role in cytokine synthesis and late phase allergic reactions (Prieschl et al. 1995). This makes it a suitable pharmacological target for the inhibition of TNF induced neutrophil and lymphocyte recruitment, tissue damage and inflammation. The p65 nuclear translocation levels and the IκB degradation levels in the nuclear and cytosolic cellular fractions were studied using anti-p65 and anti IκB antibodies respectively.

1.9.3 Protein Kinase B/AKT

Akt is a pleiotropic serine/threonine protein kinase (PKB), involved in the signal transduction regulating cell survival, metabolism, transcription and translation. It is the calcium independent effector kinase of PI3K, with an N-terminal Plecstrein Homology (PH) domain and a C-terminal catalytic domain (Hemmings 1997, Kitaura et al. 2000, Mitsiades et al. 2004). The products of PI3K phosphorylation of phosphatidyl inositols, namely, the phosphatidyl inositol 3, 4, 5-triphosphate (PIP3) and the phosphatidyl inositol 3, 4- bisphosphate (PIP2), recruits Akt via its PH domain. Consequent to membrane anchoring, the threonine 308 the serine 473 residues in its catalytic domain undergo phosphorylation/activation via the PIP3 recruited effector kinase PDK-1 (Kitaura et al. 2000). The PDK-1 mediated phosphorylation of Akt occurs in conjunction with an as yet unspecified kinase enzyme, possibly, the PKBK (Kitaura et al. 2000). Thus the activation of Akt occurs simultaneous to Raf (MAP3K) activation, downstream to FcεRI activation. Alternatively, Akt is also activated by ERK1/2, participating in the negative feedback control of MAPK signaling. Both these signaling mechanisms are activated upon FcεRI induction and thus the activation status of Akt in
allergic signal transduction gains significance (Djouder et al 2001). The phosphorylation status of the cellular Akt levels at two specific time points were studied using phosphor-specific anti-Akt antibody. The time points for receptor activation was determined based on a time-course analysis in FceRI activated allergy model.

1.10 Treatment Strategies for Allergy

One of the most striking characteristic features of human allergy is the diversity of its manifestations with the differences observed among the different organs. In a single organ, allergic injury may manifest itself in many different ways. For instance, contact dermatitis of the skin may variously be characterized by eczema, wheal and flare, purpura, vesiculation or scaling. (Talmage 1957). Also, depending upon the dosage of allergen challenge and hereditary predispositions, the intensity, manifestation and disease progression varies among the different subsets of atopic individuals. The acute immediate hypersensitivity reactions may develop into the more sustained late-phase allergy that physiologically resembles a generalized, pathological inflammatory response. However, at the molecular level, the pathology is found to be Th2 mediated (Kay 2001). Treatment strategies depend on modulation of the immune response so as to interfere with the function of IgE antibodies, interruption of the release of antigen-induced autacoids (histamine and eicosanoids) from IgE-sensitized cells, inhibition of the autacoid effect at receptor sites, and the resolution of allergic inflammation (Kay 2001).

Anti-histamines were introduced more than 50 years ago. The first generation of oral anti-histamines suffered from sedative and performance depressing effects, leading to the development of second generation anti-histamines, such as the histamine receptor antagonists, which have less sedative effects and are more pharmacologically selective. However, the
efficacy and side effects of such regimens have not been rigorously evaluated, and next-day sedation has been observed with such a regimen (Plaut et al 2005). Anti-leukotrienes, leukotriene and PAF receptor antagonists (Izquierdo et al 2003), mucolytic agents (Poole and Black 2010), \( \alpha \)-adrenergic agonists such as ephedrine and pseudo-ephedrine are also available (Sheikh et al 2009), but these treatment strategies are relatively ineffective as a monotherapy, in addition to having undesirable side effects. In addition, mast cell stabilizers and anti-cholinergics are used for symptomatic treatment and are also useful in alleviating the immediate degranulation process. However, these agents fail to inhibit the FceR mediated cell activation. In recent years, corticosteroids have been in use, as the first line of therapy against almost all classes of allergic manifestations, due to their efficiency of action (Rodrigo et al 2004, Taube et al 2004).

1.11 CORTICOSTEROIDS AND ALLERGY

Corticosteroids are effective in the treatment of allergic inflammation, in particular. Systemic corticosteroids represent the standard therapy in the treatment of acute asthmatic patients. Oral and inhaled corticosteroids are also being used in the treatment of mild to moderate cases of asthma (Rodrigo et al 2004). Corticosteroids primarily act by suppressing the synthesis of Th2 cytokines, the IL 4, 5 and 13 via the down regulation of their gene transcription. The promoters for these genes have (GRE), the Glucocorticoid receptor Response Element, to which the corticosteroid-glucocorticoid receptor (GR) complex binds and regulates transcription. Further, the GR also binds to co-stimulatory signals, that recruits and activates histone deacetylase. This enzyme, deacetylates core histones bound to the glucocorticoid responsive genes, and up regulates transcription. Thus corticosteroids act by their direct interaction with the transcription factors
such as NFkB and AP-1, as well as by their interaction with the accessory signals (Franchimont 2004).

However, corticosteroids are known to up regulate serum IgE levels, which is typical in clinical cases of patients with corticosteroid dependant asthma. This is via their up regulation of the CD40 Ligand found on T cells. This contradictory effect of the corticosteroids in the treatment of allergy, can be overcome by the use of supportive anti-IgE therapy. For this, monoclonal anti-IgE antibodies currently in the market, is of significance. Alternatively, pharmacopeia targeting CD40 ligand or serum IgE production would prove beneficial. Further, the continued use of corticosteroids is thought to be deleterious on account of systemic corticosteroid effects (Franchimont 2004). Consequently, the full spectra of allergic manifestations remain unaddressed (Grzela et al 2004). The dosage of corticosteroids used in allergy treatment, gains significance in this context. At low doses, corticosteroids exhibit the documented Th2 inhibitory effects, while the rise in serum IgE; levels is observed at higher doses. Also, at reduced doses, the deleterious side-effects maybe minimized. Thus the identification of corticosteroids with lower IC50 concentrations would prove much more beneficial and more cost-effective in the treatment of mild to severe cases of allergies and asthma (Self et al 2009).

1.12 IDENTIFYING THE MOLECULAR TARGETS FOR ALLERGY TREATMENT

The shortcomings of these standard treatment strategies lie in their narrow and non-selective pharmacological targets as well as in failing to eliminate the molecular cause of allergy (Grzela et al 2004). Although, the physiological manifestations of allergy are varied and numerous, at the molecular level, they fundamentally remain an effect of genetically determined IgE class switching and consequent FcεRI receptor activation
Upon FcεR activation, alternative and often redundant signaling cascades are activated, with consequent mast cell degranulation, release of pharmacological mediators, tissue damage and late phase inflammation. One striking example is the redundancy in the PKC signal cascade. The calcium dependant PKC-β, is the isoform that contributes to 70% of mast cell degranulation. However, in its absence, in physiological concentrations, the normally inhibitory PKC-δ mediates the exocytotic process (Ozawa et al 1993). Identifying the various cascades involved in allergic signaling would thus be a major goal of causal allergy treatment strategy.

Dexamethasone is a corticosteroid, exhibiting anti-inflammatory properties that spread across both Th1 and Th2 types of inflammation (Franchimont 2004). In RBL-2H3, it has been shown that, 0.1μM dexamethasone resulted in the suppression of various stimulatory events in response to antigen (Collado-Escobar et al 1990). Dexamethasone has been shown to exert its anti-allergic effects via blockage of the IgE mediated Ca\(^{2+}\) influx and the inhibition of [14C]-arachidonic acid release. The effects have been attributed to the inhibition of both phospholipase A2 and phospholipase C pathways with the consequent inhibition of the IgE receptor-mediated phosphoinositide breakdown (Berenstein et al 1987).

Wortmannin, a furanosteroid metabolite of the fungi Penicillium funicolosum Talaromyces (Penicillium) wortmannii, is a specific, covalent inhibitor of phosphoinositide 3-kinases (PI3Ks). It has an in vitro inhibitory concentration (IC50) of around 5 nM, making it a more potent inhibitor than LY294002, another commonly used PI3K inhibitor. It displays a similar potency in vitro for the class I, II, and III PI3K members, although, it can also inhibit other PI3K-related enzymes such as mTOR, DNA-PK, some phosphatidylinositol 4-kinases, myosin light chain kinase (MLCK) and mitogen-activated protein kinase (MAPK) at high concentrations.
(Vanhaesebroeck et al 2001, Ferby et al 1996). In addition, Wortmannin strongly inhibits the antigen-stimulated phosphorylation of both serine and tyrosine residues on PLC\(\gamma_1\) with little inhibition of PLC\(\gamma_2\) phosphorylation. Wortmannin also blocks the antigen-stimulated translocation of PLC\(\gamma_1\) to the plasma membrane (Barker et al 1997, Barker et al 1998).

Cyclosporin A is a fungal polysaccharide that has been shown to inhibit Fc\(\varepsilon\)RI-mediated exocytosis in rat basophilic leukemia (RBL) cells and human peripheral blood basophils in a dose-dependent manner (Charles, 1996). It has been shown that Cyclosporin A exerts its anti-allergic effects via targeting of the Akt/PKB signaling cascade (Ohkawa et al 2003). It has further been documented that, of the three test molecules discussed above, CSA is the least effective as an anti-allergic agent (Matsubara et al 1998). It is thus established that each of the three molecules, exert their anti-allergic effects, via the inhibition of alternative allergic signals.

This study has looked to comparatively assess their anti-allergic potential, at the level of primary, secondary and late-phase molecular mediators of allergy. In view of what has already been established as their molecular mechanisms of action, the study throws light on the key signaling cascade mediating allergic hypersensitivity. Further, based on the study, additional molecular targets have been identified, the negligence of which could be responsible for the shortcomings of the test molecules as well as in the currently available drugs. The study highlights multiple molecular targeting or combinatorial approach as an effective treatment strategy for allergy.

1.13 PLANTS AS A SOURCE OF ANTI-ALLERGIC MOLECULES

There is a growing demand for cost-effective and safer alternatives to the existing therapies for allergy. In recent years, this lacuna is increasingly
filled by the growing list of natural products, especially those sourced by indigenous medicinal herbs (Susanne and Thiericke 1999). It has been confirmed by the World health Organization (WHO) that approximately 80-85% of the world’s health care needs are met by natural products, including antibiotics. Of this, about 25% come from plants, another 25% from fungi and the rest by varied microbiota and animal products (Kong et al 2003). Plants provide therapeutic, bio-active molecules against a host of illnesses ranging from diabetes, cardio-pulmonary, neurogenic disorders and cancer to alcohol abuse, emetics and receptor-specific inhibitors, with specific molecular targets. In particular, the secondary metabolites from plants and its defense molecules provide the therapeutic phyto-chemicals (Duke 1990).

Of the documented and classified 250,000 to 350,000 plant species existing worldwide, about 35,000 species are currently under study. Among the currently available drugs in the market, around 120 distinct chemical substances are either directly sourced by plants, or are derived from phytochemicals, in addition to semi-synthetic derivatives synthesized using natural products as templates. To date 7 anti-cancer molecules have been approved by the US FDA, including the taxol/ paclitaxel, vincristine, vinblastine, topotecan, irinotecan, etoposide and teniposide. A few more molecules such as Calanolide, Conocurovone, Michellamine and Prostratin are under research for both their anti-cancer and anti-HIV activities. Aspirin, morphine, quinine, emetine and many such common drugs are all plant derived, and represent the fore-runners in the field of Natural product research and phyto-medicines. With regards to allergy, several synthetically manufactured drugs based on natural product templates exist. Examples are the bronchodilator Salbutamol (adrenoreceptor stimulant) and atenolol, a β-blocker, both of which are used in the treatment of asthma. Among the plant families studied, the Solanaceae family has contributed the most towards the discovery of anti-allergic compounds, especially in recent years. Examples are Atropine and Hyoscine derived from Belladona (Kong et al 2003).
Solanum xanthocarpum, commonly called the yellow-berried night shade, is a member of the family Solanaceae. The plant is rich in steroidal glycoalkaloids and saponins (Paul et al 2008). Various compounds such as coumarins, scopolin, scopoletin, esculin, esculetin, solasodine, solasonine, solamargine have been isolated from it (Tupkari et al 1972). Studies have established S. xanthocarpum as a larvicide, molluscicide, anti-helmintic, anti-pyretic, laxative and aphrodisiac (Paul et al 2008). In traditional Indian medicine, this herb has been classified as a mast cell stabilizer, with documented uses in the treatment of asthma and bronchitis in vivo (Gupta 1994). Studies to date have focused on the clinical efficacy of the herb in the treatment of respiratory disorders (Govindan et al 2004). However, the entire spectrum of anti-allergic potential of S. xanthocarpum, as well as its molecular mechanism of action remains to be deciphered. Also, the bio-active principle(s) responsible for its anti-allergic efficacy remains to be identified. The effect of the drug molecule interactions need to be studied to determine their possible antagonistic, synergistic or additive effects and the testing of the “whole plant” concept. Further, their toxicity to normal human cells remains to be evaluated. This study aimed to fill these gaps in the existing knowledge base.

1.14 RATIONALE OF THE STUDY

1. To contribute to the molecular level understanding of the disorder in order to make the pharmacological targets more well-defined.

2. To identify cost-effective, small molecule inhibitors, those are comprehensive and targeted.

Based on these criteria, the following objectives were defined for the study.
1.15 OBJECTIVES OF THE STUDY

Objective 1

To comparatively analyze the potential and short comings of existing anti-allergic molecules that act via differing molecular mechanisms – Dexamethasone, Cyclosporin A, Wortmannin and thereby identify the principal and ancillary pharmacological targets.

Objective 2

To isolate and structure elucidate anti-allergic small molecule(s) from an edible/ medicinal plant, in terms of degranulation inhibition.

Objective 3

To biologically characterize the isolated molecules in an in vitro allergy model and to assess their effects on the identified, multiple pharmacological targets

- membrane stabilization
- inhibition of allergic signaling
- cholesterol sequestration
- inhibition of secondary (prostaglandins) and late phase (cytokine and transcription factors) mediators of allergy

Objective 4

To investigate synergistic, additive or antagonistic effect of the isolated molecules in their various combinations as well as to design a combination therapy, that would comprehensively target the multiple molecular mediators of allergy.