Chapter I

INTRODUCTION & LITERATURE REVIEW
INTRODUCTION

Around the world allergic diseases are recognized as an important cause of morbidity which affect the quality of life. The costs of allergic diseases to the health services are extremely high. According to one report from the USA during early eighties, the total amount spent on such diseases in one particular year was above one billion dollars which does not take into account the economic impact on the quality of life that allergies have for the sufferer or for his family [6]. Recently, Lichtenstein (1993) reported that asthma alone accounted for an estimated direct medical expenditure to the tune of 3.6 billion dollars in 1990, equivalent to nearly 1% of all health care costs in USA [136]. The prevalence of allergic diseases is on increase, throughout the world, since last few decades [38,41,78,193]. It has recently been estimated that upto 20 % of the human population in both developed and developing countries, suffers from such diseases [233].

Allergy is one of the ancient health problems associated with human beings. Historically, the first ever recorded incidence of allergy dates back to an era, 4500 years old [136]. The term ‘Allergy’ was coined by Von Pirquet in 1906 and was defined as “an altered and accelerated reaction of a person to a second or subsequent exposure of a substance to which he/she been sensitized during first exposure” [241]. The very first skin test was performed by Blackley in 1873 on himself [28]. Dale and Laidlaw in 1911, demonstrated that asthma, hay fever and anaphylaxis were the manifestations caused by the liberation of histamine like substances [56]. The presence of ‘reagenic serum factor’ in allergic patient’s serum and its possible involvement were described by Prausnitz and Kustner in 1921 [187]. It took almost 47 years, however, for the ‘reagenic serum factor’ to receive a proper identification in the form of Immunoglobulin E. Ishizaka and co-workers raised an antiserum that precipitated the reagenic activity of the serum from allergic patients [106-107]. At the same time, Johansson and Bennich discovered a myeloma protein and called it IgND, which was present in higher amount in allergic patients [111] The WHO in 1968 decided a new name Immunoglobulin E (IgE) for the ‘reagenic serum factor’ [25].
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Introduction and literature review

TYPES OF ALLERGY

Gell and Coombs (1963), described four types of allergic reactions which, however, do not necessarily occur in isolation from each other [86]. Type I hypersensitivity or Immediate allergy occurs when an IgE response is directed against innocuous environmental antigens. The resulting release of pharmacological mediators by IgE sensitized mast cells produce an acute inflammatory reaction with symptoms such as allergic rhinitis, extrinsic bronchial asthma, allergic dermatitis, urticaria and rhinoconjunctivitis. Type II or Antibody dependent cytotoxic allergy occurs when antibody binds to either self antigens or foreign antigens on cells, and leads to phagocytosis, killer cell activity, or compliment mediated lysis. Type III allergy occurs when immune complexes are formed in large quantities, or can not be cleared adequately by the reticulo-endothelial system, leading to serum sickness type reactions. Type VI or Delayed type hypersensitivity (DTH), is seriously manifested when antigens are trapped in a macrophage and can not be cleared. T cells are then stimulated to produce lymphokines which mediate a range of inflammatory responses. Three different types (viz. contact, tuberculin and granulomatous) of delayed hypersensitivities have been recognized. Of these four types of allergies, type I is more common and is mediated by IgE antibodies and recognized by the term Allergy.

INHERITANCE OF ALLERGIC DISEASES

Tendency to develop type I allergies is familial. It was first noticed by Coka and Cooke in 1923, who coined the term 'Atopy' to describe this familial occurrence of immediate allergy caused by natural sensitization to common antigens entering via the respiratory and gastrointestinal tract mucosae [51]. Manifestations of atopic diseases vary in severity from mild to chronic and life threatening anaphylactic reactions and have relation with the level of serum IgE antibodies. These diseases affect people of all age groups. Genetic factors [144,148] along with environmental factors are known to be associated with serum IgE levels [154]. Low level of serum IgE in human population has been identified as dominant trait [144,230]. Studies have shown the existance of an association between certain HLA phenotypes and the ability to acquire atopic diseases. For instance, the response to certain ragweed allergens is strongly associated with the DR2 haplotype (ragweed allergens Amb a 5,
molecular weight 5 kDa; and Amb t 5, molecular weight 4.4 kDa), as well as with the DR5 haplotype in the case of Amb a 6 (molecular weight 11.5 kDa). The IgE response of the specific allergen from the rye grass (Lolium perenne) is strongly associated with DR 3 (allergens Lol p 1, molecular weight 27 kDa. Lol p 2 and 3, molecular weight for both is 11 kDa). Except for Lol p 1, all these allergens have a relatively low molecular weight. These associations have been reported as highly significant [7,11,30,82,88,101,145-146,149-150,152,230]. The exact mode of inheritance of atopic diseases is unknown and so far, no single dominant or recessive gene predisposing to allergy has conclusively been identified. Study by Cookson et al. (1989), indicated autosomal dominance as the mode of inheritance. Later, molecular linkage studies showed that the transmission of atopy is a dominant trait, and allowed a putative atopy gene to be mapped to chromosome 11q13 [52-53,97,161] however, these reports were not confirmed by other workers [4,36,95,139-140,190]. Atopic diseases are believed to be multifactorial in origin affected by polygenic inheritance [181]. A newborn’s risk of developing atopic diseases based on family history is shown in Table 1. The chances are highest when both parents have same type of manifestations.

**TABLE 1:**

Newborn’s risk of developing atopic diseases based on family history [48]

<table>
<thead>
<tr>
<th>FAMILY HISTORY</th>
<th>RISK PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both parents are atopic with same manifestations.</td>
<td>50-80</td>
</tr>
<tr>
<td>Both parents atopic</td>
<td>40-60</td>
</tr>
<tr>
<td>One parent atopic</td>
<td>20-40</td>
</tr>
<tr>
<td>One sibling atopic</td>
<td>25-35</td>
</tr>
<tr>
<td>Neither parent atopic</td>
<td>5-15</td>
</tr>
</tbody>
</table>
IMMUNOGLOBULIN E (IgE)

Immunoglobulin E is present in very low quantities (50-300 ng/ml in comparison to 10 mg/ml of IgG) in normal individuals, however, its concentration shoots up to 700 ng/ml in case of allergic diseases [69]. It has a ‘Y’ shaped structure common to all classes of immunoglobulins. It is composed of two light and two heavy chains that are covalently linked by disulfide bonds. Light chains may be κ or λ. Heavy chains (H chains), unique to IgE, contain five complete domains (VH, CH1, CH2, CH3, and CH4) each with an intradomain disulfide bond [226]. Analysis of amino acid sequence provided evidence that a common ancestral gene duplicated to form an ancestral μ and α chain, and a separate ancestral gene for ε and γ chain. Because ε and γ chain share a common aneister, they lack a c-terminal octadecapeptide capable of binding J chain; thus IgE and IgG are always monomeric immunoglobulins. IgE has five domains with two interesting antigenic determinants, D1, which is heat stable, and D2, located closer to the carboxyl terminal which is degraded by heating at 56°C for two hours. IgE contains 12% carbohydrate; this accounts for its molecular weight of 188-196 kDa. IgE is constrained by two widely spaced intraheavy chain bonds and two interdomain bonds with its Cε1 domains; this is probably why IgG is more easily ‘salted out’ of solution than IgE [105,130]. High affinity is a biologic compensation for the low concentration in the serum and tissue fluids. This affinity has been quantified for IgE and basophils as 10⁹/M compared with 10⁶/M for IgG and monocytes, there is a 10000 folds difference [91]. Since the half life of IgE in circulation is only 2-3 days (Table 2), IgE’s high affinity also compensates for its rapid catabolism.

TABLE 2

Biological properties of various immunoglobulins [91].

<table>
<thead>
<tr>
<th>Properties</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA1</th>
<th>IgA2</th>
<th>IgM</th>
<th>IgD</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen binding sites</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5-10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Serum concentration (mg/ml)</td>
<td>5-12</td>
<td>2-6</td>
<td>0.5-1</td>
<td>0.2-1</td>
<td>0.5-2</td>
<td>0-0.2</td>
<td>0.5-2</td>
<td>0-0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Half life in days</td>
<td>23</td>
<td>23</td>
<td>16</td>
<td>23</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>2-3</td>
</tr>
</tbody>
</table>
The IgE concentration in the serum of healthy individual is extremely low, a geometric mean in adults of 13 kU/L (1 unit of IgE corresponds to approximately 24 ng) has been reported [248]. IgE levels in healthy human subjects is known to vary from 1-1000 IU/ml [194]. From India, Saha et al. (1976) reported IgE levels as high as 820 IU/ml in normal Indian subjects [198]. Patients with atopic diseases, including allergic asthma, generally have moderately elevated IgE levels. Serum IgE levels are markedly elevated in approximately 90% patients of atopic dermatitis [112]. In some instances, most notably in seasonal allergic rhinitis, a significant positive correlation between the quantity of specific IgE antibodies and the intensity of the patients symptoms has been demonstrated [154]. Such correlations provide indirect evidence that the IgE antibodies play a role in the pathogenesis of atopic respiratory diseases and suggest the quantitation of IgE antibodies may be useful in the assessment of IgE mediated respiratory symptoms.

IgE RECEPTORS

One of the critical steps in the allergic reactions is the binding of the IgE to its high affinity receptors. Two different receptors for IgE on cells are known. Fc,RI, the classical high affinity receptor, is found mainly on mast cells and basophils and is a member of immunoglobulin supergene family. Fc,RI is a tetrameric complex composed of an α chain, a β chain and a dimeric γ chain. Although, it is the α chain that binds IgE, the β and γ chains are required for the insertion of the α chain into the membrane and for signal transduction. The extracellular portion of the α chain (α1-1) is sufficient for the binding of IgE molecule. The interaction between IgE and Fc, RI is depicted in figure 1. The IgE molecule bind with its convex surface facing the membrane. The receptor binding site, located in the N-terminal segment of C, 3 that adjoins C, 2 is accessible only on the convex surface. The second site is generally occluded on the concave surface. This is in agreement with the presence of a single α chain in the receptor, and its affinity for single ω chain. The Fc portion of IgE contains two copies of the Fc,RI binding sites. The Fc,RI is quite distinct from the low affinity trimeric Fc receptors for IgE (Fc,RII a & b) found on normal B cells, monocytes, eosinophils, macrophages, NK cells, Langerhans' cells and follicular dendritic cells. These low affinity receptors have not evolved from the
immunoglobulin supergene family but have substantial homology with several animal C-type lectins and have a role in IgE regulation [200,226].

ALLERGEN

An allergen is defined as an agent that triggers allergic reactions in an individual upon exposure through inhalation, contact and ingestion. It represents a special type of antigen that is capable, at extremely low doses, of inducing the synthesis of IgE antibodies in genetically predisposed individuals [154]. The major classes of allergenic sources are house dust mites, pollen, fungi, food and animal danders. An allergen is usually encountered by mucosal surfaces of the eyes, respiratory and gastrointestinal tracts. The allergic response is a local event occurring at the site of allergen’s entry. IgE first sensitizes local mast cells by binding to their receptors, and the excess enters the circulation and binds to the circulating basophils and fixed tissue mast cells throughout the body. Very small quantity (picograms) of the allergens, is adequate for stimulating IgE synthesis. Such exposures often go unnoticed and occurs from diverse and unsuspected sources. On re-exposure to the same allergen, the divalent allergen molecule binds to IgE coated mast cells and basophils which degranulate and release various vasoactive chemical mediators (Table 3) of inflammation and produce symptoms, like vasodilation, contraction of the smooth muscles of bronchi, edema of the mucous membranes, hypersecretion of watery nasal fluid and contraction of the bronchial passage, hay fever, asthma and other atopic diseases as the case may be.

TABLE 3

Mediators of allergic inflammation [69,136].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Mediators</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arylsulfatase*</td>
<td>Inactivates slow release of substance of anaphylaxis (SRS-A)</td>
</tr>
<tr>
<td>2</td>
<td>Chymase*</td>
<td>Chymotrypsin.</td>
</tr>
<tr>
<td>3</td>
<td>ECF -A*</td>
<td>Chemotactic for eosinophils.</td>
</tr>
<tr>
<td>4</td>
<td>Heparin*</td>
<td>Anticoagulant.</td>
</tr>
<tr>
<td>5</td>
<td>Histamine*</td>
<td>Contracts smooth muscles and increases vascular permeability, stimulates nerve endings, stimulates secretion of mucus in the airways</td>
</tr>
</tbody>
</table>
Before Binding

After Binding

IgE

FcεRI

α β γ

FIGURE 1:
Diagrammatic representation of the binding of IgE antibody to its high affinity receptor (Fcε RI).

All known allergens, with few possible exceptions, have at least three unique features in common:

1. They are always proteins or bound to proteins as haptens because only proteins induce CD4+ Th2 type cellular response which finally manifest itself in higher production of IgE molecules.
2. They have presence and spacing of two or more allergenic determinants kind of arrangement which allows the cross-linking of adjacent IgE molecules on mast cell or basophil.
3. They are required in very low dose to initiate an allergic reaction [69].

CELLULAR AND MOLECULAR REGULATION OF IgE SYNTHESIS

It is well established that T cells play an important role in the regulation of IgE synthesis. Although, initially a balance between Th and Ts cell activities was considered as a regulatory parameter of IgE synthesis, more recent studies showed that there exist a fine balance between Th1 and Th2 subsets of Th cells and these subsets regulate IgE production by a cascade of cytokines (molecules produced by cells of the immune system playing direct role in the immunologic communication network). Early stimulation by IL-12 generally promotes the formation of Th1 subset from naive Th0 cells, whereas initial environment of IL-4 results in the differentiation of Th0 cell into Th2 subsets [201]. IgE synthesis results from the collaboration between Th2 subsets of CD4+ cells and B cells. Initiation of IgE synthesis by human B cells however, requires at least 2 signals. The first signal is delivered by IL-4, which
induces IgE heavy chain gene switching to the ε locus [62,183]. The second signal is triggered by B cell activities such as the actual physical contact between T and B cells, delivered through cognate and non-cognate interactions as shown in figure 2 [182,238]. So far, a number of cytokines like IL-4, IL-5, IL-6, IL-13 and TNF-α have been identified as upregulatory factors of IgE synthesis. On the other hand, cytokines such as INF-γ, TNF-β, TGF-β and IL-8 have been described as inhibitors or suppressors of IgE synthesis [24].

**BENEFICIAL ROLE OF IgE**

IgE antibodies although a minor component in serum, play a major role in allergy; a pathological condition, yet one may wonder why this particular class of antibody present in minute amounts in normal conditions in human, has evolved and persisted through evolutionary pressure just to be the source of strong immunopathological disorders, all the more so since many studies [85,226] have clearly established and redefined complexity of the regulatory network controlling its production. One of the most beneficial role that has been assigned to IgE by reports on circumstantial [102,178] and direct [42,63,84,124] evidences, is the protection against metazoan (helminthic) parasites.

**THERAPIES OF TYPE I ALLERGIC DISEASES**

Immunotherapy/hyposensitization is being used throughout the world for the treatment of type I allergic disorders. It involves repeated injections of increasing doses of crude extract of the offending source of allergens. Introduced more than eight decades ago by Noon, it is one of the oldest ways of allergic disease management which are still in use [81,172]. Although beneficial clinically, the exact underlying mechanism is yet unkown. Effects like rise in blocking type of IgG antibodies [135]; suppression of seasonal rise in IgE antibodies [175]; reduced basophil reactivity and sensitivity to allergens [188]; and induction of B cell tolerance and induction of suppressor T cells, in patients undergoing hyposensitization were reported by Norman in 1980 [175]. These changes were held responsible for the observed relief from the allergic symptoms. However, this kind of therapy is not always risk free as several fatal systemic reactions have also been reported [138]. New and improved immunotherapy approaches in safe and effective treatments of allergic diseases include use of alum precipitated allergen extracts [174]; use of
FIGURE 2:
Role of T cells and cytokines in the regulation of IgE synthesis
purified allergens [125], application of allergoids and polymerized allergens [117,173], and liposome mediated delivery of allergens [12-15] Other pharmacological therapies for type I allergies include drugs like antihistamines, inhaled steroids etc. However, these antihistamines do not suppress IgE production but provide only symptomatic relief and are not free from side effects [141,169,224]

POLLEN AS A SOURCE OF ALLERGENS

Pollen grains are amongst the earliest known allergen sources and at present the major cause of morbidity among atopic patients [127] A single anemophilous (wind pollinated) plant may liberate millions of pollen grains, the tonnage of wind borne pollen produced globally in a year is really most astonishing Among numerous allergenic pollen producing members of the plant kingdom, the grass family Gramineae (Poaceae) occupies an important place. It represents a uniquely cosmopolitan array of over 400 genera with nearly 4500 species primarily of wind pollinated nature Members of this family covers roughly 20% of the world’s surface [59] Several hundreds of them are cultivated as crops and cereals throughout the world.

INCIDENCE OF POLLEN INDUCED TYPE I ALLERGIC DISEASES

The term “hay fever”, recalls the clinical symptoms on exposure to flowering hay fields. John Bostock (1819) suspected pollen as the cause of “hay fever” [33]. Later, several workers like Ordman (1945), Feinberg (1946), Saad (1958a,1958b), Ogden and Lewis (1960) established that pollens are responsible for different symptoms of allergic disorders [76,177,180,196-197]. Seasonal occurrence is the hallmark of the pollen sensitivities, usually at the time when pollen grains are most frequent in the atmosphere. Clinically, pollen induced allergies include manifestations such as allergic rhinitis, bronchial asthma, contact dermatitis etc. Six to twelve percent cases of pollen induced allergic rhinitis have been reported from the USA, Canada, Australia and New Zealand by Smith and Slavin (1988) [213]. Up to 6% pollen related asthma cases were reported by Smith (1983) from different parts of the world such as Britain, USA, Canada, France and Australia [212]. According to one report nearly 19% of the American school population suffers from “hay fever” [80]. Fourteen and twenty percent cases of “hay fever” are reported from New Zealand and Republic of Maldives, respectively [242,245]. Cases of increase in seasonal
rhinitis have also been observed all over Europe by Blenkinsopp and Blenkinsopp (1989) [29]. Studies by Wig and Guleria (1961), and Shivpuri (1968), showed 18-32% of respiratory allergy and allergic asthma cases from North Indian chest clinics [204,244]. Later, it was estimated that, in India, nearly 10% population suffers from allergic diseases [239]. Also, one percent population is known to have bronchial asthma [5]. Vishwanathan et al. (1965), reported that 1.6% of urban and 2.7% rural population in the state of Bihar, had bronchial asthma [240]. Recent epidemiological surveys in India indicate that 11-16% of the population suffer from respiratory disorders [206].

PATHOPHYSIOLOGY OF POLLEN INDUCED ALLERGIC SYMPTOMS

Type I respiratory allergic diseases are induced by pollen when it makes contact with the upper respiratory tract, oral cavity and the eye. The atopic individual in such cases may suffer from considerable irritations of the eye immediately on contact with the pollen grain, whereas in the case of pollen induced bronchial asthma, after inhalation, the pollen gets deposited in the upper ciliated part of the respiratory tract and then symptoms develop in the ciliated part of the lungs which show accumulation of fluid and secretion in the terminal bronchioles. Pollen, after inhalation, remains in the upper respiratory tract for about 30 minutes [143]. During this period allergenic components must be released from the pollen. The moist atmosphere of mucous membranes facilitate the release and solubilization of allergens from pollen grains which then penetrates the mucous tissues. Alternatively, during rainy season pollen falling on the ground may release the allergens in the rain water which during dry period get airborne along with the dust particles and are inhaled and make contact with the mucous membranes. Since many of the pollen allergens/proteins have proteolytic activity, they may destroy the epithelial surface layer thereby increasing their contact with the immune system. The contact of the allergens with T and B cell of the immune system brings about sensitization of the subject through IgE mediated reactions as shown in figure 3. After second exposure, pollen allergens bind to the adjacent specific IgE molecules linked to mast cells/basophils surface leading to their degranulation which causes the release of mediators from these cell.
FIGURE 3:
CLIMATIC CONDITIONS AND POLLEN ALLERGY

Climatic factors such as temperature, relative humidity, rainfall, wind velocity and direction are also associated with wind borne pollen allergies. From Bangalore, Agashe and Alfadil (1989) have reported high temperature and low relative humidity as key factors which lead to the enhanced liberation and distribution of pollen grains in the atmosphere [2]. Similarly, gradual warming of the atmosphere over Austria has been implicated in increase in birch pollen sensitivity among local population. It is suggested that rise in temperature causes stress on birch which in turn, causes increased synthesis of Bet v 1 and 2 (prolin) allergens in its pollen grains [110]. Increased pollen counts were observed in London after heavy rainfalls. Such observations indicate the role of rainfall in pollen prevalence in the atmosphere [176].

Role of wind velocity and direction in the transport of pollen grains was addressed by Hjellmrous (1992), who reported the transport of allergenic betula pollens over a long distance of central Europe to Stockholm regions in Scandinavia [96]. Wind drift is known to play an important role in transport of Abies, Alnus, Betula, Pinus and Quercus pollen from hilly Himalayan regions to the Gangetic planes in India (141). It is observed that stable, warm sunny, windless climatic conditions favours high prevalence of airborne polien grains in the atmosphere [92].

AIR POLLUTION AND POLLEN ALLERGY

With increase in industrialization, environmental pollution has become an additional major health hazard. Role of air pollution in allergic diseases is well documented [185,191,195]. Some association between different organic and inorganic air pollutants and airborne pollen grains is quite obvious in heavily polluted regions. This association was studied in-situ by Behrendt et al (1991, 1992). A morphological evidence for pre-activation of pollen by organic extract of airborne particulate matter was provided by electron microscopy and immunoblot analysis. It was shown that the generation and release of allergenic aerosols from pollen in humidified air is initiated and mediated by substances adsorbed in airborne particulate matters. It was therefore, suggested that air pollution enhances the risk of allergy and may also be responsible for increasing rate of pollen allergies in heavily polluted localities throughout the world [22,23,119]. Interference of air pollutants at the level of sensitization, elicitation of symptoms and chronication of diseases has
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been emphasized by Behrendt et al. (1991, 1992) [22-23]. Hitzfeld et al (1993), opined that air pollution affects the mucosa of the respiratory tract, leading to an increase in sensitization [94]. Pollutant gases like SO₂, NO₂ and O₃ along with airborne particulate matter (with average diameter of 5 μm or less) especially diesel exhaust particulate have both adjuvant activity for specific IgE production against common allergens in experimental animals and enhancing effects on allergic symptoms with in sensitized individuals [27].

THE POLLEN

Most of the wind borne pollen grains are yellowish bodies, varying from 10-200 μm in diameter and often bearing globules of surface lipids. The outer envelop or “exine” is often sculptured and bear aperture in the form of circular pores or elongated furrows. The pollen of grasses are characterized by single pore with annular thickening, generally varying from 20-50 μm for wild grass and larger than 50 μm for cereals. Sporopollin, a highly resistant biogenic polymer forms the “exine”. Sporopollin is remarkable for its resistance in both physical and enzymatic degradation and is known to be the product of polymerization of carotenoid pigments and esters. The exine layer is extremely reduced or even absent from the germinal pore region. Pectocellulose or cellulose rich middle layer or “intine” has further been shown to consist of two layers, outer pectic polysaccharides layer called “exintine” and inner cellulotic layer called “endintine”. In general, pollen grains contain 13-37% carbohydrates, 11-20% proteins, 1-4% lipids and 1-2% inorganic compounds [246]. Besides other proteins, pollen grains are known to carry a number of enzymes such as amylase, catalase, diastase, succinic anhydrase, cytochrome system, upto 5 types of isomerases, 11 types of lyases, 23 types of different transferases, 24 types of different oxidoreductases, 33 different types of hydrolases [104].

ALLERGENS LOCALIZATION

The quick manifestations of allergic symptoms indicate that allergens are localized either on the pollen surface or they diffuse out from pollen immediately upon contact with the moist surface of the eyes and upper respiratory tract [100,129]. Tsinger and Petrovikaya (1961), reported for the first time that various proteins are present in the pollen wall [229]. This particular location suggests that these proteins are strategically situated for immediate contact with stigma surface where possibly
they are involved in recognition events. A majority of proteins from pollen grains are of enzymatic nature, particularly the hydrolytic type. The exine region mainly accommodates esterases whereas intine holds acid phosphatases [44]. The intine proteins are concentrated at the germinal pore as radially or tangentially oriented leaflets.

Generally soluble proteins are responsible for the allergic manifestations Amb a 1, a ragweed pollen is held in the inner cellulosic intine of the pollen grain wall [129]. Howlett et al. (1973), studied the kinetics of proteins (including Amb a 1) release from whole pollen of ragweed. It was estimated that preparation time of mere 30 seconds was sufficient for the antigen to be lost from the pollen [100]. Allergens from the intine region were released through the germinal aperture during germination. Lol p 1, a pollen allergen from rye grass has been located in the cytosol using immunogold labelling technique and anti Lol p 1 monoclonal antibodies [208]. In contrast, by using a specific monoclonal antibody, Lol p 5 (previously designated as Lol p 9 [126]) allergen was found to be associated with starch granules [225]. A single grass pollen grain is known to carry approximately 700 such granules. These granules may vary from 0.6-2.5 µm in size [225]. Amyloplast, a kind of chloroplast has been identified as the site of production of such starch granules. Their localization suggests that Lol p 5 must be transported from the cytosol to the lumen of the amyloplast during development. Lol p 5 in association with starch granules of rye grass pollen represent one of the few respirable major allergens [77]. Pollen grains on the other hand, are too large (>12 µm) to be respirable. However, pollen allergens are known to occur naturally in the environment associated with particles as small as 0.1 µm or even less [218]. Identification of allergen containing micronic particles was reported for grass pollen by Stewart and Holt (1985) [221]. Several possible explanations like pollen fragments produced by physical degradation in the environment, the outer lining in the grass pollen is coated with orbicules which could be released in to the environment and allergen containing aerosols that bind to physical particulate matter in the environment of various submicronic sizes have been suggested for such associations [216]. Presence of Lol p 5 in starch granules suggest a hypothesis for the origin of submicronic particles. Lol p 5 attached to starch granules represent an excellent tool to unravel the mechanism of bronchial asthma.
It has been suggested that starch granules are capable of eliciting IgE mediated responses and can also trigger an attack of extrinsic bronchial asthma [225].

IMPORTANT ALLERGENIC GRASS POLLENS: WORLD-WIDE DISTRIBUTION

In tropical and sub-tropical areas like India, airborne grass pollen are encountered throughout the year but in temperate regions of the Northern hemisphere, a peak of anthesis from mid-May to mid-July is typical. Most of the wind borne grass pollen throughout Canada and Northeastern two third of the USA is derived from the Kentucky blue grass (Poa pratense), Orchard (Dactylis glomerata), Timothy (Phleum pratensis) and Red top grass (Agrostis alba). Bermuda grass (Cynodon dactylon) is also a major contributor of wind borne pollen throughout Southern half of the USA and the Pacific coast. In the Southern USA, Bahia (Paspalum notatum) has been demonstrated as a major contributor of pollinosis. Other important species include Rye grass (Lolium perenne), Velvet grass (Holcus lanatum), especially in the Pacific Northwest of the USA and adjacent Canada, and the Sweet vernal grass (Anthoxanthum odoratum) which flowers in spring throughout many states of the USA [215]. Grasses as a source of major allergenic wind borne pollen have also been reported from Australia. Important species include Lolium perenne and Phalians aquatica (the Canary grass) [210-211]. Role of grass pollen in initiating allergic reactions and their abundance in the atmosphere have been documented from New Zealand by Hillas and Wilson (1979) [93]. Wind borne pollens of the Kikuyu grass (Pennisetum clandestinum) have recently been reported as a causative factor of allergic disorders from South Africa [186]. Pennisetum sp pollen along with other types have also been reported as major aeroallergen from Japan [108,203]. From China, reports are available which demonstrate Gramineae pollens as dominant aeroallergens [49]. Grass pollen contribute up to 23% of the total airborne pollen spectrum of Taipei basin region in Taiwan [50]. In the European regions of the world, Emberlin (1994) analyzed the annual variation in grass pollen in London during 1961-1990 [70]. Studies showed grasses as major contributor of wind borne pollen in different localities of Europe [43,58,70,133,160,168,189,217,223,247] In Europe, the grass pollen season is restricted mainly to warmer months and it is a major cause of pollinosis during May-July [55]. Based on clinical studies Lewis and Imber (1975) reported 43.7% patients allergic to Vernal and Orchard grass
pollen, 36.6% to Red tcp grass pollen and 35.4% to June grass pollen [134]. Similarly, Erikson et al. (1987) reported 23.11% cases of pollen allergies from Mediterranean regions [71]. Again from the same region, Bousquet et al. (1984) reported 85% cases of pollen sensitivities [35].

**IMPORTANT ALLERGENIC GRASS POLLEN: DISTRIBUTION IN INDIAN SUB-CONTINENT**

Grass pollen allergy is also very common amongst Indian population [220]. This problem is much more complex and trickier than Western and Scandinavian countries. India hosts approximately 1180 species of grasses from nearly 239 different genera representing the family Gramineae (Poaceae) [17]. Nearly 70% of the total cultivated field area is covered by the members of the grass family. Majority of the species are wild growing in nature. They flower all the year round and this can largely be attributed for the vast geographical area ranging over many latitudes, the marked elevations and varied climate that include from arid to humid and tropical to arctic conditions. Cunningham (1873) was the first aerobiologist to initiate systematic aerobiological survey in India [54]. After a gap of almost 75 years Kasliwal et al. (1958, 1959) carried out such surveys in Jaipur [122-123]. Shivpuri et al. (1960) initiated such studies in Delhi metropolitan area [205]. Since then various aerobiological reports have been published from different regions of India. Grasses were found to be most dominant accounting up to 25% of the total aeroallergens in India [120]. Due to great diversity in climatic conditions, it is difficult to generalize the Indian aerobiological data on grass pollens. For the sake of simplicity, various ecozones of Indian sub-continent can be divided into 4 major regions viz 1. Himalayan region; 2. Gangetic region; 3. Central region; and 4. Peninsular region. Pollen species causing allergy are quite variable in these different ecozones which makes it very important to identify pollenosis related grass species from every region. In a landmark study in the form of ‘All India Coordinated Project on Aerobiology (AICP)’, main grass pollen season has been observed during the months of September through November, although flowering occur throughout the year [170]. Twenty seven different localities were included in this study representing all the 4 major regions of Indian subcontinent. The data from AICP and other similar studies has been summarized in the form of table 4.
## TABLE 4:

Important airborne grass pollen of India based upon various aerobiological surveys [131].

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>PREVALENCE PERCENTAGE</th>
<th>IMPORTANT SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIMALAYAN REGION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almora</td>
<td>less than 10</td>
<td></td>
</tr>
<tr>
<td>Darjeeling</td>
<td>51.69</td>
<td>Cynodon, Zea mays, Triticum</td>
</tr>
<tr>
<td>Guhawti</td>
<td>22.88</td>
<td></td>
</tr>
<tr>
<td>Imphal</td>
<td>38.44</td>
<td>Cynodon</td>
</tr>
<tr>
<td>Kashmir</td>
<td>-</td>
<td>Imperata, Sorghum, Pennisetum, Cynodon</td>
</tr>
<tr>
<td>Mirik</td>
<td>51.69</td>
<td></td>
</tr>
<tr>
<td>Shillong</td>
<td>6.22</td>
<td></td>
</tr>
<tr>
<td>Tripura</td>
<td>-</td>
<td>Cenchrus, Cynodon, Imperata, Pennisetum, Sorghum, Zea mays</td>
</tr>
<tr>
<td><strong>GANGETIC REGION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amritsar</td>
<td>50.00</td>
<td></td>
</tr>
<tr>
<td>Bareilly</td>
<td>34.90</td>
<td>Oryza, Pennisetum, Sorghum, Zea mays</td>
</tr>
<tr>
<td>Calcutta</td>
<td>30.00-39.00</td>
<td>Cynodon, Panicum, Setana, Zea mays</td>
</tr>
<tr>
<td>Delhi</td>
<td>-</td>
<td>Avena, Cenchrus, Cynodon, Imperata, Poa, Pennisetum, Sorghum, Vetvana Zea mays</td>
</tr>
<tr>
<td>Falta</td>
<td>32.50</td>
<td>Cynodon, Sorghum</td>
</tr>
<tr>
<td>Gaya</td>
<td>-</td>
<td>Cynodon, Panicum, Pennisetum, Oryza</td>
</tr>
<tr>
<td>Gorakhpur</td>
<td>-</td>
<td>Eragrostis, Sorghum, Zea mays</td>
</tr>
<tr>
<td>Jaipur</td>
<td>22.87-70.00</td>
<td>Cynodon</td>
</tr>
</tbody>
</table>
### CENTRAL REGION

<table>
<thead>
<tr>
<th>City</th>
<th>Value</th>
<th>Plant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhopal</td>
<td></td>
<td>Cenchrus, Cynodon, Imperata</td>
</tr>
<tr>
<td>Gwalior</td>
<td>20 41</td>
<td>Cenchrus, Cynodon, Imperata, Pennisetum</td>
</tr>
<tr>
<td>Nagpur</td>
<td>33 30</td>
<td></td>
</tr>
<tr>
<td>Raipur</td>
<td>68 66</td>
<td>Cynodon, Ischaelema</td>
</tr>
<tr>
<td>Rewa</td>
<td>28 44</td>
<td></td>
</tr>
<tr>
<td>Sagar</td>
<td>58 93</td>
<td>Eragrostis, Cymbopogon</td>
</tr>
</tbody>
</table>

### PENINSULAR REGION

<table>
<thead>
<tr>
<th>City</th>
<th>Value</th>
<th>Plant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aurangabad</td>
<td>80 64</td>
<td></td>
</tr>
<tr>
<td>Bangalore</td>
<td>13 00</td>
<td></td>
</tr>
<tr>
<td>Bhavnagar</td>
<td>70 26</td>
<td></td>
</tr>
<tr>
<td>Bombay</td>
<td>42 00</td>
<td>Dicanthium</td>
</tr>
<tr>
<td>Calicut</td>
<td></td>
<td>Brachiara, Chloris, Digitaria, Panicum, Pennisetum, Setana</td>
</tr>
<tr>
<td>Mysore</td>
<td>30 85-55 20</td>
<td>Chloris, Cymbopogon, Cynodon</td>
</tr>
<tr>
<td>Rajamundry</td>
<td>38 00-41 00</td>
<td></td>
</tr>
<tr>
<td>Tirupati</td>
<td>38 62</td>
<td>Oryza, Pennisetum, Sorghum, Zea mays,</td>
</tr>
<tr>
<td>Vellore</td>
<td>58 95</td>
<td></td>
</tr>
<tr>
<td>Vishakhapatnam</td>
<td>22 66</td>
<td>Cynodon</td>
</tr>
</tbody>
</table>
Grass pollen showed a very low percentage in the air of the Himalayan region although, in the foothill towns their percentage went up to 12-40%. Possibly this could be due to the fact that thick canopy of tall trees in the hilly areas restrict the movement and the easy flow of pollen of these herbaceous plants in the air as suggested by Chaturvedi et al. (1992) [47]. However, exceptionally high percentage of grass pollens in the air was observed at Mirik in this particular hilly region. On the other hand, the open land area of the plains may be the reason for the observed high (above 55%) percentage of occurrence of air borne grass pollen in the Central and Peninsular regions. Aurangabad, in the Peninsular region, showed a very high percentage (about 80.64%). Up to 40% of grass pollen prevalence was reported from the atmosphere of Gangetic region [170]. Table 5 summarizes the clinical results of studies carried out with grass pollen extracts at various places [131]. It can be concluded that allergic grasses of Indian sub-continent are quite different from other parts of the world with an exception of Bermuda grass (Cynodon dactylon) which is also a major source of allergenic pollens in India. Among wild growing species, pollens of Cenchrus ciliaris, Cynodon dactylon, Imperata cylindrica, Panicum sp., Chloris sp. and Vetiveria sp. have been shown to be the important aeroallergens from all 4 major regions. Similarly from the section of cultivated species of family Gramineae, Pennisetum typhoides, Sorghum vulgare, Zea mays and Oryza sp. have been identified as clinically important sources of aeroallergens [170].

**TABLE 5:**

Percent skin test positivity to Important allergenic grass pollens of India based upon various clinical studies.

<table>
<thead>
<tr>
<th>Pollen species</th>
<th>Aligarh</th>
<th>Bangalore</th>
<th>Bhopal</th>
<th>Delhi</th>
<th>Guntur/</th>
<th>Kanpur</th>
<th>Jaipur</th>
<th>Lucknow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cenchrus</td>
<td>3.0</td>
<td>10.0</td>
<td>41.2</td>
<td>8.8</td>
<td>3.4</td>
<td>-</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Cynodon</td>
<td>16.0</td>
<td>4.7</td>
<td>4.9</td>
<td>47.5</td>
<td>9.1</td>
<td>13.0</td>
<td>10.0</td>
<td>60.3</td>
</tr>
<tr>
<td>Dicanthium</td>
<td>-</td>
<td>-</td>
<td>5.8</td>
<td>6.3</td>
<td>6.5</td>
<td>-</td>
<td>48.1</td>
<td></td>
</tr>
<tr>
<td>Imperata</td>
<td>20.0</td>
<td>1.6</td>
<td>16.0</td>
<td>8.3</td>
<td>9.4</td>
<td>6.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Panicum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paspalum</td>
<td>-</td>
<td>-</td>
<td>16.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pennisetum</td>
<td>12.0</td>
<td>4.0</td>
<td>-</td>
<td>31.6</td>
<td>-</td>
<td>11.7</td>
<td>10.0</td>
<td>48.4</td>
</tr>
</tbody>
</table>
BIOCHEMISTRY OF GRASS POLLEN ALLERGENS

Although grass pollen allergy is known for almost 100 years, serious research on biochemical nature of grass pollen allergens started only in the later half of this century, which led to accumulation of sizable database on their immunobiochemical features. More than 160 proteins (in the molecular weight range of 3 to 80 kDa) of 73 different species of plant and animal origin, causing type I allergic reactions have been identified [137]. It has recently been suggested that a purified allergen must be named strictly according to the guidelines laid down by 'IUUIS Allergen Nomenclature Sub-Committee of WHO [243]. An allergen is named according to the accepted taxonomic name of their source as follows: the first three letters of the genus, space, the first letter of the species, space, and an Arabic number which should be in the order of their identification. For example, Lol p 1 refers to the first pollen allergen identified from Lolium perenne. To avoid possible ambiguities in cases where genus and species both give rise to same name, additional letter should be added, like Ves v 5 and Ves vi 5 to differentiate allergens from Vespula vulgaris and Vespula vidua, respectively (because allergen from Vespula vulgaris was characterized before that Vespula vidua). These guidelines were later discussed by King et al (1995) [126]. However, there are still some confusing names prevalent in the literature, like Lol p 11 and Phl p 11 which should not have the same number because they are not homologous; Lol p 11 is a trypsin inhibitor whereas Phl p 11 is a member of profilin family.

Various methodologies were employed to extract aqueous and non-aqueous contents of the pollen for the study of their allergenicities, however, ether soluble fatty components of grass pollens were largely found to be allergenically inactive in most of the allergic subjects [16,37]. On the other hand, most of the allergenicities resides in the aqueous extract of the pollens [154]. Kammenn (1912), initiated biochemical studies on rye grass pollen and extracted 'pollenotoxin'; a soluble and stable albuminous protein fraction with skin reactivity [121]. Newell (1941), Bernton (1949), and Sherman (1959), reviewed the immunoochemical properties of grass pollen
allergens [26,171,202]. Later, Marsh et al. (1965) isolated various allergenic fractions from rye grass pollen and categorized them into different groups (I, II, III) according to their immunochemical and physiochemical characteristics. These were shown to be antigenically as well as allergenically distinct from each other. Their distinct allergenic nature was determined by direct skin testing on sensitive patients. The molecular weights of group I, II, III and IV were found to be 27 kDa, 11 kDa, 11 kDa and 50 kDa, respectively [114,147,151]. Eighty five to ninety percent allergic patients showed sensitivity to Lol p 1 [83]. Lol p 2 was reported as an acidic protein with 97 amino acid long monomeric protein with no cysteine, glutamine and glycosylation [10]. Lol p 3, on the other hand, is a basic non-glycosylated protein with two major isoforms. Lol p 3b has two isoelectric forms. Lol p 2a and Lol p 3b both have same molecular weight with 97 amino acids but share only 57% sequence identity [9]. Over the years, allergens from various different groups have been reported of which, group I and V are suggested as the most important groups of allergens [89,157]. Group I allergens from rye grass (Lol p 1) and group V allergen from timothy grass (Phl p 5) pollen constitutes about five and six percent of the total protein extracts, respectively [157,209]. Similarly, group I allergen of Bermuda grass (Cyn d 1) constitute the major protein component of the pollen extract as its amount in the source was estimated to be 15% (w/w) [157]. Lol p 1 has about 5% carbohydrate which consists mainly of mannose and galactose [99,115]. Complete digestion of Lol p 1b with cellulase or β-galactosidase does not reduce the allergenic activity of the allergen which together with other evidences discussed by Marsh et al. (1966), leads to ruling out of the possibility that carbohydrate moieties constitute an important allergenic determinant, however its requirement for binding with lymphocytes was suggested [153]. Augustein (1959) demonstrated that several carbohydrates do not affect the allergenic activities of Timothy and Cocksfoot grass pollen extracts [16]. In contrast, some studies have implicated the role of glycan components in IgE binding [98]. Complete proteolytic digestion of Lol p 1b and Lol p 1c completely destroys the allergenicity and hapten like inhibitory activity. This shows that protein structures are involved in allergenic determinants and that the portion of the three dimensional structure probably constitute the principle determinant [153]. However, this claim was not found true in various other grass pollen allergens that retained their IgE binding capacities even after denaturation and digestion when subjected to SDS-PAGE and
immunoblotting like techniques [79] Most important allergens are known to be acidic in nature [158,209], however, a few basic allergens are also reported from grass pollens [90,209,237]

The term 'isoallergen' is used to denote a member of a group of allergens isolated from a specific source and which possess experimentally indistinguishable immunologic properties and a closely related physicochemical structure to other isoallergens, usually differ slightly from one another on IEF (pl) due to minor structural differences such as the nature of their carbohydrate moieties or differences in the degree of protein amidation or genetic variations [113,154,209] Group I allergens of rye grass consists of four isoelectric variants, i.e. 1a-1d, in the order of decreasing pl's. Also group II and III allergens exists in at least two isoallergenic forms. Isoallergen Lol p 2a, an isoallergen of Lol p 2, can be separated by electrophoresis or ion exchange chromatography from Lol p 2b which in turn contain more isoelectric forms [154,209]. It is becoming clear that many isoallergenic forms of allergens (such as Cyn d 1, Phl p 1, 4, 5a, 5b, and some minor allergens like group XI) exist in nature [20,45,214,222]. These isoallergens differ from one another in one to several amino acids. Some isoallergens have a very high degree of identity with homologous allergens in related species. The existence of many isoallergens have several implications for possible use of peptides in immunotherapy. T cell epitopes identified by using T cells that react with recombinant allergens and their peptides might represent only a proportion of the repertoire of allergen specific T cell present in the human body. Various studies have shown that multiple isoallergens are involved in the sensitization to Bet v 1, the major birch pollen allergen. Some of the isoforms studied have complete T cell activation capacity, but fail to bind IgE in vitro, and induce very weak skin prick test response. Such isoforms have been suggested for immunotherapy, since they might be safer than natural extracts, owing to decreased IgE binding and the decreased risk for induction of anaphylactic reactions [65]. On the basis of four hydrophilic regions and one amphipathic region, 4 B cell sites (7-15, 31-40, 44-50, 75-85) and one la/T cell site (73-83) were predicted by Ansari et al. (1989a, 1989b) for rye grass allergen [9-10].

Bousquet et al. (1991) observed that the patients allergic only to grass pollen are immunologically different from patients allergic to multiple pollen species. They
suggested that each allergic patient may be treated with only the constituents to which the patient is allergic rather than injecting crude extract having constituents to which the person may not be allergic and to which he or she may become sensitized in the course of treatment [34]. Such observations together with report that ruled out the possibility of using ‘broad range extract’, inspired the early researchers to purify the allergenic proteins from crude extracts [60]. Johnson and Marsh (1965) used a combination of ion exchange and gel filtration column chromatography to purify rye grass pollen allergen which were subsequently classified as group I-III [114]. Cyn d 1, a major allergen from Bermuda grass, was isolated by Mathiessen et al. (1991), by using Con-A Sepharose affinity chromatography and Carboxymethyl Sepharose chromatography [158]. Mecheri et al. (1985) isolated and characterized Ag Dg 1, a major allergen with molecular weight of 33 kDa and pl 5.9, from Orchard or Cocksfoot grass pollen [159]. Combination of gel filtration and isoelectric focussing helped Ekrammoddoullah et al. (1983) to isolate a high molecular weight basic allergen (HMBA) from the pollen extract of rye grass. It was found out to be a glycoprotein of 56.8 kDa and later was named as Lol p IV [68]. Kundu et al. (1988) fractionated Cynodon dactylon extract over DEAE cellulose and sephadex G 75 columns, to obtain two allergenic proteins with molecular weight of 11 kD (III A) and 20.2 kD (III B). These proteins reacted with 48 % and 79 % of grass pollen allergic patients’ skin respectively. Allergen with molecular weight of 20.2 kD (III B) was further analyzed for amino acid composition which revealed the high content of glycine in this protein [132]. Later, the monospecificity of monoclonal antibodies was exploited in the isolation of major allergen of Kentucky blue grass called Ag 27. A reverse immunosorbtent, prepared by coupling monoclonal antibodies to CnBr activated sepharose 4B, was used to absorb KBG-R. Bound material was recovered by acid elution and was named Ag 27, with molecular weight of 47 kDa in native form [66]. To date, upto 50 allergens from various sources have been purified but the number of purified grass pollen allergen is far from being satisfactory [46].

Jaggi et al. (1989) characterized Lol p 4 by RIA using monoclonal antibodies to establish the presence of two distinct antigenic epitopes which together constitute the allergenic region of Lol p 4 [109]. Earlier studies with polyclonal rabbit antisera suffered from the problems of identification of allergenic and non allergic antigens and of specificities. The availability of monoclonal antibodies produced by hybridoma
technology opened new avenues for a) the isolation of pure individual components in a single step by the use of reverse immunosorbents; b) standardization of allergenic preparations used for the immunotherapy; c) the antigenic analysis of the grass pollen allergens. Various authors have reported monoclonal antibodies against allergen extracts of various pollen grains [32,66,72,74,118,167,184]. These monoclonal antibodies can be classified into three different categories: (1) specific to the allergen against which they were raised; (2) which are specific to group I allergens from a limited number of grass pollens; (3) which are specific to group I allergens from a large number of grass pollens. These monoclonal antibodies helped in the identification of common as well as unique epitopes on pollen allergens. Taxonomically ordered antigenic/allergenic variations were found between pollens of 22 different grass species representing many groups [207]. Although extensive aerobiological and clinical investigations have established the role of grass pollens in causing allergic diseases in India, not much information is available about the immunobiochemical nature of grass pollens of Indian sub-continent [132,220].

Purification of allergens from pollen extract is an extremely cumbersome, less efficient and time consuming process. In addition, the presence of isoforms of allergens further complicate the matter [235]. Recombinant DNA technology offers the possibility to isolate allergen encoding cDNA from expression libraries constructed from mRNA of pollen origin. Screening such libraries with grass pollen allergic patient’s IgE allows the isolation of these allergenic cDNA followed by determination of the DNA sequences and protein synthesis in suitable expression system. To date, cDNA cloning has been performed for at least 10 major allergens from grass pollens [137,165,235]. A detailed biochemical database on grass pollen allergens has been summarized in Table 6. cDNA encoded recombinant allergens have been found out to be equally potent in their allergenic properties when compared with their native counterparts [235]. cDNA cloning offers the advantage of getting allergens in the purest form; in unlimited amount; even when the pollen season is over; identification of the allergenic/antigenic epitope structures, and development of new therapeutics based on their epitope analysis.
# Table 6:
Immunobiochemical characteristics of well known grass pollen allergens

[21,137,166,222]

<table>
<thead>
<tr>
<th>Pollen source</th>
<th>Scientific name</th>
<th>Antigen designation</th>
<th>Mr (kD)</th>
<th>MW (Da)</th>
<th>Molec. weight</th>
<th>Amino acid sequence</th>
<th>Allergy association with HLA class</th>
<th>B cell epitope</th>
<th>T cell epitope</th>
<th>Biological function</th>
<th>Reactions with allergen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermuda</td>
<td>Cynodon dactylon</td>
<td>Cyn d 1</td>
<td>29.32</td>
<td>6473</td>
<td>(6.7) P</td>
<td>glycopeptide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes grass group 5 allergens</td>
</tr>
<tr>
<td>Canary</td>
<td>Phleum pratense</td>
<td>Poe p 1</td>
<td>33.56</td>
<td>5486</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes grass group 1 allergens</td>
</tr>
<tr>
<td>Kentucky blue</td>
<td>Phleum pratense</td>
<td>Poe p 2</td>
<td>26.30</td>
<td>3035</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes N terminal to Poe p 3</td>
</tr>
<tr>
<td>Kentucky blue</td>
<td>Phleum pratense</td>
<td>Poe p 10</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes N terminal to Poe p 3</td>
</tr>
<tr>
<td>Meadow fescue grass</td>
<td>Festuca arundinacea</td>
<td>Fes a 1</td>
<td>21.4</td>
<td>37</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>Lolium perenne</td>
<td>Lol p 2</td>
<td>26.63</td>
<td>5160</td>
<td>C</td>
<td>glycopeptide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Redtop</td>
<td>Agrostis palustris</td>
<td>Agp a 1</td>
<td>10.94</td>
<td>5002</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>Lolium perenne</td>
<td>Lol p 3</td>
<td>9.04</td>
<td>583</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Timothy</td>
<td>Phleum pratense</td>
<td>Poe p 4</td>
<td>12.2</td>
<td>38</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>YesPhase 1, 2</td>
</tr>
<tr>
<td>Timothy</td>
<td>Phleum pratense</td>
<td>Poe p 2</td>
<td>10.12</td>
<td>40</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Timothy</td>
<td>Phleum pratense</td>
<td>Poe p 10</td>
<td>12.2</td>
<td>39</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Timothy</td>
<td>Phleum pratense</td>
<td>Poe p 11</td>
<td>29.31</td>
<td>4.5</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes Phase 1, 2</td>
</tr>
<tr>
<td>Timothy</td>
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<td>Poe p 12</td>
<td>11</td>
<td>3.244</td>
<td>C</td>
<td>-</td>
<td>-</td>
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</tr>
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<td>Phleum pratense</td>
<td>Poe p 5</td>
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<td>3.9</td>
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<td>-</td>
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</tr>
</tbody>
</table>

Structural data with respect to glycopeptide: protein only.

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CROSS-REACTIVITY AMONG GRASS POLLEN ALLERGENS

Cross-reactivity among grass pollen extracts has been observed from various parts of the world. Various in-vitro and in-vivo systems notably RAST inhibition, ELISA inhibition, CRIE, and SPT have provided sufficient evidences for the presence of cross-reactivity among different species of grasses. Martin et al. (1985) observed a high degree of shared allergenicity between brome, Western wheat and quack of the western grasses and six Northern grasses of the USA [155]. Cross-reactive group I allergens have been demonstrated in a number of different grass pollens [207]. Similarly, group V allergens have been observed in 8 grasses [156]. Shared antigenic and allergenic analysis of pollen extracts of five members (Cenchrus ciliens, Cynodon dactylon, Imperata cylindrica, Pennisetum typhoides and Sorghum vulgare) of family Poaceae, by means of Intradermal test, rocket immunoelectrophoresis, ELISA, and ELISA-inhibition, was carried out by Sridhara et al. (1995) [220]. Shared allergenicity among different grass pollen extracts have shown that common epitopes exist between them. Cross-reactivities among grass pollen allergens have been well documented even at T-cell epitope levels [19,64,162-164,199]. Major grass pollen allergens belonging to group I, II, III and IX were shown to share cross-reactive T-cell epitopes [19]. This kind of observations have direct implications in designing peptide based therapeutic modalities with an aim to better clinical management of grass pollen allergies. Another classical example of allergen cross-reactivity is the presence of profilin, a 14-18 kD ubiquitous protein which exist in various grass pollens and plants of widely separated families. Although profilin has never been reported as major allergen from any single grass pollen source, it is present in almost all and binds to patient’s IgE. This protein offers itself as a good candidate for the molecular analysis of phenomenon of cross-reactivity amongst allergens from different sources [236]. Broadly, three different types of cross-reactivities have been described:

A. Based on taxonomic relationships

1) Intraspecific:

Extensive sequence homologies results in this kind of cross-reactivity [19]

2) Interspecific:

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Monoclonal antibodies specific to different grass pollen allergens like group I allergens of rye and group V allergens of timothy pollen were shown to cross-react with corresponding allergens of a number of other grasses [72-74, 207, 209]. N-terminal sequence analysis has also confirmed the existence of this kind of cross-reactivity [179, 192].

3) Intergeneric:

Group I and IX allergen specific antibodies have been shown to identify allergens from unrelated cultivated cereals, brassicas and some other dicotyledonous plants. Presence of profilins in almost all plant species is another example of intergeneric cross-reactivity [1, 128, 162, 227].

B. Evolutionary conserved protein structures.

A classical example of this kind of category is again profilin which has been found out to be a conserved protein in eukaryotic cells and is responsible for most of the cross-reactivity between birch pollen allergen and extracts of vegetables such as carrot and potatoes [1].

C. Presence of carbohydrate chain of less phylogenetically related glycoproteins.

Presence of cross-reactive carbohydrate determinants among allergens of phylogenetically unrelated sources have been reported. This kind of cross-reactivity is much more pronounced in vegetable and invertebrate sources [1].

Sequence homologies among different proteins have suggested the presence of motif structures (patterns of conserved amino acid residues). Seven motifs have been identified from comparison of 4 major grass pollen allergens (group I, II, III and IX). Similar analysis of a total of 9 polypeptides led to the identification of a single motif of 5 amino acid residues, of which 3 were contiguous. Moreover, this motif comprised a pentapeptide (AG-LE) which constitute the backbone of a peptide which has been shown to be a T and B cell epitope of Poa p 5 allergen. This motif has been found on the C-terminal (which is the most allergenic/antigenic part of these allergens) of all the proteins examined. It has been speculated that these residues may be involved in induction of and binding to IgE by these proteins [162]. However, the exact cellular and molecular basis of cross-reactivity is still unknown.
BIOLOGICAL FUNCTIONS OF GRASS POLLEN ALLERGENS

Grass pollen allergens have not been explored in detail with respect to their natural biological functions. However, significant demonstratable sequence homologies with proteins of known functions and some direct evidences suggested enzymes, enzyme inhibitors, transport, regulation and structural roles for these allergenic proteins [222]. Group V allergens have mainly been identified as ribonuclease, a kind of hydrolytic enzymes. It is speculated that these ribonucleases may play an important role in ensuring ‘self incompatibility’, during pollination events [18,39]. Group XI (Lol p 5, an 18 kDa protein) allergens have been described as trypsin inhibitors (serine protease inhibitor) from the pollen extract of rye grass [90]. It is suggested that trypsin inhibitors, by the virtue of their antiprotease nature, may initiate or modulate the allergic reactions i.e. by blocking mast cell trypsin [3]. Similarly, group X allergens have been recognized as cytochrome C (proteins involved in electron transport) [8,67]. The classical example of allergen with structural/regulatory role is that of ‘profilin’. It is a 14-18 kDa ubiquitous protein, present in almost all living cells. These proteins are thought to act in the process of actin (a structural protein) polymerization; defining cell shapes and movements [234]. Anti-profilin IgE binds both plant as well as animal profilin [232-233]. This finding indicates the possible role of auto-immune factors in the triggering of IgE mediated allergies. However, it may be assumed that these functions may not be directly linked to inducing IgE antibodies. Nevertheless, it is deemed too early to dismiss the possible relationship between structure and intrinsic biological activities of allergens, i.e., to overlook the question as to what are the structure-function characteristics of a molecule that determine it to be an allergen.

B - AND T - CELL EPITOPES OF GRASS POLLEN ALLERGENS

The availability of B and T cell epitopes may be useful not only for the diagnosis of allergies but also for the development of therapies which may result in the elimination/suppression of Bc cells responsible for the production of IgE antibodies, or blocking of the antigen binding sites of the antibodies, thus preventing their being cross-linked by the multivalent antigens or its degradation products resulting from its processing by the patients’s appropriate cells [165]. Similarly, the availability of T cell epitopes may lead to the development of new immunotherapies.
based on the concept that appropriately modified epitopes may render T cells, involved in the upregulation of Bc cells, unresponsive, or anergic to specific peptides and that one may thus switch off the production of IgE antibodies [165]. In atopic patients, symptoms are induced by a spectrum of type 2 cytokines produced by allergen specific CD4+ T cells [57]. Therefore allergen specific T cells are obvious targets for intervention in atopic diseases and a detailed characterization of epitopes present on major allergen might lead to improved form of therapy, using either synthetic peptides or recombinant allergens [165]. A large proportion of allergen specific T cells are specific for major allergens. Upto 50 major allergens from different sources have been cloned [166]. Availability of some of these recombinant allergens (cDNA) has allowed the identification of T cell epitopes, as well as an estimation of their relative importance in inducing T cell activation. Epitope mapping studies have shown that allergens contain multiple T cell inducing T cell epitopes dispersed throughout the molecule [116]. Presence of isoallergenic forms explain the cross-reactive T cell epitopes on allergens. As a result of structural homologies, major allergen specific T cells might cross-react with allergens from different species within the same genus; a phenomenon observed with group I allergen specific T cells that cross-react group I allergens from different genera of grasses [40,219]. Allergen derived T cell synthetic peptides can be used in anergizing Th2 clone, this feature indicate their future therapeutic role [75].

Among hundreds of grass species, many have been domesticated by man for agricultural and horticultural purposes. Those cultivated for food, are classified into various groups such as cereals, millets, etc. After cereals, millets occupies a place of prime importance with \textit{Pennisetum typhoides} being the most important species. Due to its tremendous production potentials, particularly in less fertile, intense heat, and moisture deficit areas, \textit{Pennisetum typhoides} alone accounts for upto 40% of world’s total millet production. It is commonly known as Pearl millet, Spiked or Bulrush millet in English and Bajra in Hindi [87,228].

Due to its ability to grow on a variety of soils and under stress conditions, the \textit{Pennisetum typhoides} is cultivated for grain, fodder, forage, green chop and silage, in the arid regions of Africa, Asia, Europe, North and Latin America. During 1985, it was grown over an approximate area of 21.41 million hectares in the world, which fall
roughly below 15°W-90°E longitude and 5°S-40°N latitude. In the USA, Canada, South Africa, Italy, Japan, Australia, Southern parts of the erstwhile USSR, and some parts of India and Pakistan, it is also grown for forage and fodder purposes. In India, it is grown all over the country with a few states such as Himachal Pradesh, Jammu and Kashmir, Assam being the exception. In general, millets are grown in about 20 million hectares in India, of which, area upto 12.50 million is covered by *Pennisetum typhoides* alone.

States like Rajasthan, Haryana, Punjab, U.P., Gujrat and Maharastra accounts for 5/6th of the total area under *Pennisetum typhoides*. Area under this crop represent 30% of the total acreage of the world and 11% of the total cereal production in India. These figures thus indicate the importance of *Pennisetum typhoides* in Indian agriculture. It is grown mostly during June to October and as a winter crop from November to February or as a summer crop in March to June [87, 228].

*Pennisetum* is one of the largest genera in the tribe Paniceae with around 140 different species, distributed throughout the world. A native to Africa, from where it was introduced to the other parts of the world. The earliest mention of pearl millet is by Al Idrisi (1154 AD), an Arab scholar, in his account of his travel in north Africa and Spain. However, the first mention of the pearl millet in western literature is attributed to Leo Africanus, a 16th century Moorish slave in the service of pope for whom he wrote an account of Africa. It is believed that it was domesticated along the southern margins to the central highland at the beginning of present day phases between 3000-2000 BC. Archeological remains in the form of carbonized grains indicate that pearl millet reached the north west coast of India at Gujrat by 1000 BC [87].

*Pennisetum typhoides* is an annual plant which can be grown round the year under tropical conditions. It bears a long inflorescence (upto 60 cm) with terminal spike consisting of a central rachis on which fascicles are closely packed, each of which consists of one or more spikelet and lot of bristles. Each spikelet in turn has two flowers, where the lateral one is invariably male and other bisexual. Anthesis in pearl millet is accomplished in two phases. The first phase involves the bisexual floret while the second involves staminate floret. It has been generally observed that anthers in the sessile male floret emerge 2-3 days after the anthers have emerged in
the bisexual florets, thus most of the head continue to shed pollen for a period of 4-6 days. It has been noted that the largest number of anthers emerged between 8 PM and 2 AM, the maximum being at 10 PM [87].

Extensive aerobiological and clinical studies have demonstrated that allergenic grass pollen prevalent in Indian sub-continent are different from those found in the Western countries [170,204-205]. Pennisetum typhoides, has been identified as a major source of allergenic pollens in India [219]. However, no information is available on the immunobiochemical nature of pollen proteins from Pennisetum typhoides. It is thus important to study the immunobiochemistry of its pollen proteins.

AIMS AND OBJECTIVES

1. To characterize the pollen extract of Pennisetum typhoides with respect to its allergenic and antigenic components for the diagnosis and immunotherapy of pollen allergy.
2. To identify the major allergenic component(s) of the pollen extract of Pennisetum typhoides.
3. To isolate, purify and characterize the major allergenic protein from the pollen extract of Pennisetum typhoides.
4. To study the cross reactivity of Pennisetum typhoides pollen extract with those of other grass pollens.
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