INTRODUCTION
Aerobiology is the study of airborne bioparticulates in terms of their source, release, dispersal and impact on living organisms after deposition. With the human immune system, the respiratory organ is the direct target of inhaled airborne particles, resulting into a variety of adverse effects including infection and hypersensitivity. A normal adult inhales about 14-15 m³ of air daily. Pollen grains and fungal spores are among the most numerous airborne bioparticles. Occurrence of allergic diseases, e.g., bronchial asthma, seasonal rhinitis, conjunctivitis, dermatitis etc. have been known from early historic time. Pollen grains are the earliest known allergens (Blackley, 1873) and are at present the major sources of morbidity among atopic subjects (Kjellman, 1993). The term “hay fever” (seasonal allergic rhinitis) results from the clinical observation of nasal symptoms on exposure to grass pollen from the flowering hay fields. Subsequent extensive and systematic studies have shown the allergenic significance of different airborne pollen grains in such episodes. Now, the correlation between the onset of different airborne pollen seasons and the occurrence of a patient’s symptom is well-established.

The occurrence of pollen grains in air has implications for medical, especially clinical practice. The load of airborne pollen, in a particular place changes from season to season and from year to year, depending on changes in ecological and climatic conditions. Quantification is therefore essential to allow the construction of pollen calendars for different areas to aid the patients and clinicians in the identification of causal factors (Cosentino et al., 1995).

The formulation of a pollen calendar requires a detailed study of the morphology of pollen grains from vegetation of the study area, based on ecofloristic survey, to allow correct identification. Dispersal of pollen into the air is highly dependent on meteorological parameters and this relationship has to be defined before the occurrence of allergenic pollen can be forecast (Antepara et al., 1995).

The diagnosis and treatment of allergic disorders caused by pollen generally require preparation of extracts comprising a complex mixture of proteins, lipids, carbohydrates, nucleic acids and lectins. Unless the methods of standardization are available, there could be batch to batch variation in the content of extracts, which can result in unreliable diagnosis and therapy of patients. Reliable standardization requires the identification of the specific allergenic components in the extracts, which initiate the allergy, and further their isolation and purification from the crude extracts.
The presence of cross reactive allergenic or antigenic components within the pollen types belonging to same family is now well-established. For example, grass pollen extracts have been classified into six major groups, of which, I and V are allergenically significant and among ten grass species, group V allergens are common in the eight (Matthiesen & Lowenstein, 1991a; 1991b). Shared allergenicity among different grass pollen extracts have shown the presence of common epitopes (Westphal et al., 1988; Klysner et al. 1992). These shared allergenic components could be very effective in the immunotherapy of patients suffering from allergy against an array of grass pollen types.

Clinically, the essential information for a pollinosis patient is not actually the numerical concentration of airborne pollen present in the air, but mean concentration of the relevant allergen or antigen present in air. Studies of airborne antigens and allergens, started in 1980s (Agarwal et al., 1984; Schumacher et al., 1988; Takahashi et al., 1993; Ekebom et al., 1996) using a range of immunochemical methods. Such methods could help the decrease of the need for routine aerobiological surveys when quantifying a specific type of allergen, but would not enable to detect a number of allergens types at a time.

Allergy in the developing countries, such as India, was for a long time neglected and allergic diseases were considered to be uncommon in these countries (Turner, 1989). Over the last three decades, studies have shown that allergic disorders are quite common in India (Chanda & Sarkar, 1972; Shivpuri & Agarwal, 1982; Agarwal & Jhamb, 1995) and the incidence of different types of aeroallergen, e.g., pollen (Malik et al., 1991), fungal spores (Al-Doory & Domson, 1984), animal danders (Gupta et al. 1996), house dust (Mitra & Chatterjee, 1990) have been studied. However, the concern in the population, the need has been felt for systematic studies of allergenic pollen grains in different areas of India, characterized by a rich vegetation.

Aeropalynological Researches : A Global Scenario

Pollen grains are the carriers of male genetic materials in higher plants. For the purpose of reproduction, they have to be transmitted from flower to flower, for which they utilize a range of different methods, of which air dispersal is an important pathway. For this reason, pollen grains are important airborne bioparticulates. John Bostock (1819) was the first to suspect that pollen grains caused hay fever, but it was Blackley (1873) who provided the experimental proof that grass pollen grains caused these
symptoms and trapped them from the air. More than 40 years later, Scheppegrell (1916) felt the need for further field exploration and surveys to record airborne allergens. F. C. Meier (1935) coined the term “aerobiology” as the scientific discipline focussed on the airborne biomass, i.e., pollen grains, spores, insect debris and other biological material, also referred to as the bioaerosol. Therefore, aerobiology is a multidisciplinary subject and study of inhalant allergens require input from botanists, plant pathologists, meteorologists, physicists, biochemists, physicians, environmentalists and mathematical modellers.

To make an aerobiological pollen survey in a particular area, it is first necessary to make an eco-floristic survey of the area and to study the detailed pollen morphology of the species found to enable proper identification of airborne pollen grains. Such studies on pollen morphology were started by Wodehouse (1935), followed by great contribution by Prof. G. Erdtman summarised in “Pollen Morphology and Plant Taxonomy” (Erdtman, 1952) and in “Handbook of Palynology” (Erdtman, 1969).

Gravity sampling of airspora and their visual demonstration was performed even in the second half of 19th century by Pasteur (1861), Cunningham (1873) and Blackley (1873). Initiation of aerobiological survey was made by Durham in 1925 and he devised a shelter for gravity slides (1946), which was adopted by American Academy of Allergy. This sampler was based on the principle of simple gravity deposition onto gelatin coated slides which was followed by other workers (Hyde & Williams, 1945; Hyre, 1950). Subsequently, volumetric samplers based on aerodynamic principles were designed (Ogden et al., 1974) and among them the Hirst automatic volumetric trap (Hirst, 1952), rotorod sampler (Perkins, 1957; Harrington et al., 1959), Andersen sampler (Andersen, 1958) and Burkard volumetric sampler (Burkard Manufacturing Co. Ltd., England) are the more versatile collectors of bioaerosols including pollen grains.

The dominant airborne pollen types in different parts of the world as recorded by several workers in their survey are represented in the following table:

<table>
<thead>
<tr>
<th>Country</th>
<th>Workers</th>
<th>Common Airborne Pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Meier, 1941, Moss, 1965, Ong et al., 1995</td>
<td>Casuarina, Myrtaceae, Pinus, Poaceae, Quercus</td>
</tr>
<tr>
<td>China</td>
<td>Chen &amp; Zhang, 1985, Chen et al., 1988</td>
<td>Artemisia, Euphorbia, Morus, Pinaceae, Poaceae</td>
</tr>
<tr>
<td>Country</td>
<td>Workers</td>
<td>Common Airborne Pollen</td>
</tr>
<tr>
<td>------------------</td>
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<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Denmark</td>
<td>Goldberg <em>et al.</em>, 1988</td>
<td><em>Alnus, Artemisia, Betula, Poaceae</em></td>
</tr>
<tr>
<td>Egypt/Turkey/Iran</td>
<td>Saad, 1959</td>
<td><em>Acer, Cheno-Amaranthaceae, Cupressus, Cyperaceae, Morus, Plantaginaceae, Pinus, Poaceae, Populus</em></td>
</tr>
<tr>
<td></td>
<td>Ritchie, 1986 - Egypt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ghazaly &amp; Fawzy, 1988</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ozkargoz, 1967 - Turkey</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shaffiee, 1976 - Iran</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Charpin <em>et al.</em>, 1966</td>
<td><em>Alnus, Asteraceae, Betula, Chenopodiaceae, Cupressus, Pinus, Poaceae, Quercus</em></td>
</tr>
<tr>
<td></td>
<td>Michel <em>et al.</em>, 1976</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Stix, 1977</td>
<td><em>Betula, nettle, Poaceae, Pinus, Quercus</em></td>
</tr>
<tr>
<td>Italy</td>
<td>Caramielo <em>et al.</em>, 1990</td>
<td><em>Chenopodiaceae, Cupressaceae, Fraxinus, Oleaceae, Parietaria, Pinus, Poaceae, Populus, Quercus</em></td>
</tr>
<tr>
<td></td>
<td>Famularo <em>et al.</em>, 1992</td>
<td></td>
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<tr>
<td></td>
<td>Fornaciari <em>et al.</em>, 1996</td>
<td></td>
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<tr>
<td>Japan</td>
<td>Higuchi <em>et al.</em>, 1977</td>
<td><em>Cryptomeria, Pinus</em></td>
</tr>
<tr>
<td></td>
<td>Ishizaki <em>et al.</em>, 1987</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>Andrupo, 1945</td>
<td><em>Betula, castanea, Corylus, Oxyria, Poaceae, Salix</em></td>
</tr>
<tr>
<td></td>
<td>Johansen, 1991</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>Zawisza <em>et al.</em>, 1993</td>
<td><em>Artemisia, Betula, Corylus, Poaceae, Populus, Quercus</em></td>
</tr>
<tr>
<td>Spain</td>
<td>Gálán <em>et al.</em>, 1989</td>
<td><em>Alnus, Chenopodiaceae, Corylus, Cupressus, Morus, Olea, Pinus, Poaceae, Quercus, Taxus</em></td>
</tr>
<tr>
<td></td>
<td>Belmont &amp; Roure, 1991</td>
<td></td>
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<tr>
<td>South Africa</td>
<td>Ordman, 1970</td>
<td><em>Acacia, Cannabis, Fabaceae, Morus, Pinus, Poaceae, Prosopis</em></td>
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<td></td>
<td>Hawke &amp; Medows, 1989</td>
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<tr>
<td></td>
<td>Cadman, 1990</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cadman &amp; Dames, 1993</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>Kotzamanidau &amp; Nilsson, 1977</td>
<td><em>Betula, Pinus, Poaceae, Quercus, Ulmus</em></td>
</tr>
<tr>
<td></td>
<td>El-Ghazaly <em>et al.</em>, 1993</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>Leuschner, 1974</td>
<td><em>Aesculus, Artemisia, Salix</em></td>
</tr>
<tr>
<td>Thailand</td>
<td>Dhorranintra <em>et al.</em>, 1991</td>
<td><em>Casuarina, Cyperaceae, Mimosa, Poaceae, Urtica</em></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Spieksma, 1986</td>
<td><em>Artemisia, Chenopodiaceae, Plantago, Rumex, Urtica</em></td>
</tr>
</tbody>
</table>
The longest continuous pollen record was reported from Cardiff, U.K., where sampling was carried out from 1943 (Hyde & Williams, 1943) until the present (Mullins & Emberlin, 1997). Smart & Knox (1979) studied the seasonal and diurnal changes of grass pollen in the air of Melbourne. Jäger et al. (1996) studied the trend of five airborne tree pollen types (Alnus, Betula, Corylus, Pinus & Ulmus) of Vienna (Austria), Stockholm (Sweden), Turku (Finland) and Throndeim (Norway) in terms of start, peak, end and duration of season. The overall view showed that Poaceae is the most dominant airborne pollen throughout the world. In most of the European countries, Australia and USA, Betula, Pinus and Quercus are the important airborne pollen types.

Chen & Huang (1980) recorded 56% contribution of tree species to the total aeropol len load of Taiwan. Seasonal variation of pollen types causing sugi-pollinosis in Japan was recorded where it was found that the number of the patients had doubled in 1988 compared to 1987 (Sado & Takeshita, 1991).

Besides volumetric surveys of airborne pollen grains, other studies have indicated direct or indirect visualization of pollen allergens. An indirect immunofluorescent technique for counting airborne pollen allergen on Burkard sampling tapes was introduced by Schumacher et al. (1988) and immunoblotting and chemiluminescence by Takahashi et al. (1993, 1995) and Ekebom et al. (1996). Such direct visualization and detection of allergens from pollen grains are very useful for all kinds of allergy research.

Aeropalynological Researches in India

Cunningham (1873) pioneered the aerobiological researches in Calcutta, India. After a long gap, Sangvi et al. (1957), Kasiwal et al. (1959) conducted such studies in Rajasthan and Shivpuri et al. (1960) in Delhi. Since then, some studies of different
aspects of the aeropalynoflora have been made in different parts of India.

Eastern India:


Western India:


Northern India:

Lakhanpal & Nair (1958) reported the presence of 30 pollen types in Lucknow and Almora. Dua & Shivpuri (1962) studied the airborne pollen of Delhi. Later, aeropalynoflora of Delhi was studied in detail to prepare a pollen calendar (Singh & Shivpuri, 1971). Singh & Babu (1982), Singh (1984) studied the aeroallergen variation along with seasonal variation. Malik et al. (1991) reported dominant pollen types of Delhi, e.g., Poaceae, Cheno-Amaranthaceae, *Ailanthus, Ricinus, Holoptelea*, etc. Munshi (1997) reported the pollen of *Platanus orientalis, Narcissus, Salix*, etc. to be the major airborne types in Srinagar.

Southern India:

Visakhapatnam, Bangalore and Kodaikanal are some of the places in south India, from where major airborne pollen types, e.g., Poaceae, *Casuarina, Parthenium, Cocos, Cyperaceae, Pinus, Eucalyptus* etc. are reported (Agashe et al., 1983; Reddi & Ramanujam, 1989; Maribhat & Rajasab, 1992; Sathees et al., 1992; Avasthi & Agashe, 1997).
Based on aerobiological data obtained, pollen /flowering calendars were prepared for Calcutta, Sambalpur, Gulbarga, Imphal and Kodalkanal by Chanda (1973), Panda et al. (1992), Maribhat & Rajasab (1992), Singh & Devika (1992) and Sathees et al. (1992) respectively. Council for Scientific and Industrial Research published a book containing pollen calendars for 12 different states (Singh et al., 1992).

**Respiratory Allergic Disorders: A Brief Review**

The immune system has developed to protect the human body against the harmful effects of environmental bioparticulates through the action of effector molecules which induces the elimination of the foreign substances. Under certain circumstances the response may be diminished leading to immunodeficiency, or heightened, to cause the hypersensitivity.

When an adaptive immune response occurs in an exaggerated form, it may cause tissue damage and the term hypersensitivity is applied. An altered immune response generally occurs to a second or subsequent exposure against a foreign substance to which the body has already been sensitized (Henson, 1985). Hypersensitivity is the characteristic of an individual concerned. Coombs and Gel 1 (1963) described four types of hypersensitive reaction. These are:

**Type I (Anaphylactic Hypersensitivity)**

A type I response is initiated by the antigen reacting with tissue mast cells passively sensitized by antibodies elsewhere, leading to pharmacologically active mediator release. The reaction is manifested within seconds or minutes after exposure and referred as immediate hypersensitivity. It includes general anaphylaxis and local manifestation of symptoms in various organs or systems. The examples include bronchial asthma, rhinitis, urticaria, vomiting, diarrhoea etc.

**Type II (Antibody Dependent Cytotoxic/Cytolytic Hypersensitivity)**

In a type II reaction, the antibody is directed against the antigen on an individual’s own cells (target cells) or foreign antigen, e.g., transfused red blood cells. This may lead to cytotoxic action by killer cells or by cytostimulating complement mediated lysis. The examples of this reaction are mismatched blood transfusion reaction and organ transplant rejection. Janeway and Traverse (1995) proposed further subdivision of the classic Coombs & Gell type II reaction into type IIa (cytotoxic) and type IIb (cytostimulating) response.
Type III (Arthus Reaction/Toxic Complex Mediated Hypersensitivity)

In a type III reaction, the immune complexes are deposited in the tissue, the complement cascade is activated and polymorphs are attracted to the site of deposition causing local damage. Examples include the Arthus reaction, serum sickness etc.

Type IV (Delayed or Tuberculin Type Hypersensitivity)

Type IV reactions are initiated by the action of antigen sensitized T-lymphocytes releasing lymphokines following a secondary contact with the same antigen. Lymphokines induce inflammatory reaction and activate macrophages which release mediators. The reaction takes more than 12 hours to develop. Examples include tuberculin hypersensitivity, graft rejection, contact dermatitis, etc.

The effector cells in classical type IV reaction is the CD4+ type 1, whereas in the tissue damage mediated by cytotoxic T cells, it is the CD8+ type. So, Janeway & Traverse (1995) suggested two forms of type IV reaction - type IVa and type IVb since the initiating event involves the T cells with distinct characteristics.

Type I hypersensitivity is characterized by an allergic reaction, that occurs immediately following contact with antigen, which is referred to as the allergen. The term “allergy” (allos: altered, ergos: action) was coined by von Pirquet (1906) and defined as an acquired, specific, altered capacity of the immune system to react against a second/subsequent exposure to an allergen to which the body has already become sensitized (Lowenstein et al., 1987). It is only in recent years that “allergy” has become synonymous in popular terms with type I hypersensitivity (Roitt et al., 1993). Such restricted meaning was not originally intended by von Pirquet.

Allergic reactions are dependent on the specific triggering of the unique antibody, i.e., immunoglobulin E (IgE) sensitized mast cell, which release mediators to produce inflammatory reactions.

The common allergens have been classified according to the route of exposure into the following types:

i) Inhalants (e.g., bioaerosols including pollen and spores)
ii) Ingestants (e.g., food substances)
iii) Injectants (e.g., insect venom, injected medicines)
iv) Contactants (e.g., cosmetics)
Among these, inhalants are most important as the causative agents of respiratory allergic disorders from systemic vasodilation and smooth muscle contraction in the lungs.

The manifestation of type I hypersensitivity in the respiratory system is relevant to the present study and is, therefore, described a bit more in details:

* **Allergic Rhinitis**

Allergic rhinitis, commonly referred to as hay fever, is an IgE mediated reaction affecting the upper respiratory tract. It results from an interaction between an aeroallergen with sensitized mast cells in the conjunctiva and nasal mucosa to induce mediator release and is manifested by nasal congestion, sinus headache, running nose, watery eyes, itching and sneezing (Coleman et al., 1992). The overall estimate of the incidence of allergic rhinitis ranges from 2-10% (Evans, 1993). An increase of seasonal rhinitis has been found to occur in almost all European countries (Blenkinshopp & Blenkinshopp, 1989). In the United Kingdom, a four-fold increase in the number of general practitioner consultation for rhinitis has been observed over past 20 years (Burr et al., 1989; Ninan and Russell, 1992).

** Bronchial Asthma**

Bronchial asthma is the usual manifestation of IgE-mediated anaphylaxis in the lower respiratory tract. The resulting constriction of bronchioles and airways obstruction cause difficulty in breathing, often associated with wheezing. Briefly, asthma is characterized by large differences in resistance to flow in the airways of lungs over short period of time (Weiss & Segal, 1985). It is a lung disease with following characteristics:

i) Airways obstruction which is sometimes irreversible

ii) Airway inflammation

iii) Increased airway responsiveness to a range of stimuli.

The incidence of asthma differs between countries and shows a trend of increase (Ayres, 1986; Massicot & Cohen, 1986; Flemming & Crombie, 1987). In India, it has been reported that almost 10% of population suffer from allergic disorders (Viswanathan, 1964; Singh & Singh, 1994).

**Historical Background**

The first report of anaphylactic shock was mentioned by Menes in 2641 BC reporting the death of an Egyptian pharaoh from a wasp (kehb) sting which has some
controversy (Avenberg & Harper, 1980). Asthma (noisy breathing) was first reported in the Nei Ching by Huang Ti in 2698 BC, which is the oldest treatise of internal medicine (de Weck, 1997). In 460-375 BC, the occurrence of asthma and allergy was reported by Hippocrates (Marketos & Ballos, 1982). Later, Galen described allergic reactions in 131-201 AD (Daremberg, 1854). During the period of 1135-1204, Moses Malmonides, the physician of Sultan Saladin wrote the famous “Treatise of asthma” (Cohen & Samter, 1992). Several centuries later, Richet (1902) first described the anaphylaxis in animals and suggested that this was similar to hay fever or asthma in human. Prausnitz & Küstner (1921) demonstrated passive transfer of immediate skin reactivity caused by fish allergen by injection of serum from an allergic patient into a non-allergic subject. The serum component was referred to as reagin - the active substance. Coca coined the term “atopy” to describe a genetic predisposition to respond to environmental allergens with the production of specific IgE antibodies (Coca & Cooke, 1923).

In 1967, Ishizaka and coworkers described an immunoglobulin, gamma E as the carrier of skin-sensitizing activity. Simultaneously, Johansson & Bennich (1967) identified a unique myeloma protein called IgND having similar properties to reagin. Collaborative studies of these two groups revealed that IgND and gamma globulin E were identical (Bennich et al., 1969). This molecule was designated as IgE by World Health Organization (WHO) Committee on Nomenclature for Human Immunoglobulins (Bennich et al., 1968). The role of IgE in mediating allergy is now well established.

Role of Different Effector Molecules and Inflammatory Cells in the Immunity of Type I Respiratory Allergy

I. Immunoglobulins

* IgE

Immunoglobulin E comprises a class of antibody significantly related to allergic disorders. IgE response is a local event in the body at the point of encounter with the allergen. Kleinjan et al. (1997) demonstrated the presence of grass pollen/dust mite specific IgE on airway mucosal cells by histochemical studies. Local production of IgE by B cells depends on antigen presenting cells (APC) and co-operation between B and T-helper (Tₜ) cells. After local sensitization of mast cells, the spill-over IgE enters into the circulation and binds to the receptors on circulating basophils and tissue fixed mast cells throughout the body. IgE is a trace serum protein, comprising only 0.001% of total serum immunoglobulin. Elevation of specific IgE occurs in allergic disorders but
it is grossly elevated by parasites. In most atopic subjects (Roitt et al., 1993) the concentration of specific IgE is greater than 450 IU/ml (1 IU = 2.4 ng/ml). IgE comprises two heavy and two light chains like other immunoglobulins, but it has five domains in the heavy chain by contrast with four in the others. A part of the Fc region (cell binding site) of IgE is involved in binding to Fce receptors (FceR) on mast cells and basophils (Ishizaka et al., 1967). This Fc region is thermolabile whose activity is destroyed by heating at 56°C for 40 minutes (Dorrington & Bennich, 1973). IgE binds with greater affinity to mast cells and basophils than to lymphocyte, macrophage, eosinophil, complements etc. (Ishizaka & New Comb, 1970; Capron, 1986).

There are two groups of receptors for the IgE molecule. One is a high affinity (FceRI) receptor on mast cells and basophils (Kulczycki & Metzger, 1974), and the other is a low affinity receptor (FceRII) on B and T lymphocytes, eosinophils, platelets, and macrophages (Kikutani et al., 1986; Capron, 1986; Nutman et al., 1987). FceRII has two subgroups, i.e., FceRIIa and FceRIIb. The first type is involved with the expression of B-cells while the other is associated with the stimulation of interleukin IL-4 on peripheral B and T cells, monocytes and eosinophils (Yokota et al., 1988). IgE receptors are analogous to soluble binding factors.

IgE synthesis is induced generally by cognate/non-cognate B-T cells interactions (Vercelli & Geha, 1993), production of IL-4 (Snapper & Paul, 1987), IL-13 and IFN-Y (gamma interferon) for suppression (Pene, 1993).

The IgE response is regulated through two IgE binding factors, IgE potentiality and suppressives (Ishizaka, 1989). These factors are regulated by T-Cell glycosylation enhancing (GEF) and inhibiting (GIF) factors.

IgE production is controlled genetically and a linkage has been suggested between IgE regulating genes and the HLA gene complex (Marsh et al., 1981).

The IgE response to allergens present several disadvantages in the host raising the question why IgE has evolved. This unique antibody plays an important role in parasitic worm infections. Since one-third of the world's population suffers from parasitic worm infections which may have presented the evolutionary pressure resulting into the development of IgE. Allergy can thus be the result of an unfortunate by-product of this evolutionary step (Roitt et al., 1993).
**Other Immunoglobulins**

Apart from IgE, IgA and IgG also appear to be important in type I respiratory allergy, but their roles have not yet been completely elucidated. Eosinophils, cell which play an important role in allergic disorders, carry receptors for IgA, which appears to induce their activation and release of mediators (Capron et al., 1989).

The function of IgG as mast cell activators was first reported by Parish (1970). In hay fever patients, the IgG antibody against grass pollen mainly belongs to IgG subclass (Devey et al., 1976), which contributes approximately 4% of total IgG. This group of antibody has been reported as a good inducer of IgE for pollen or grass allergen, honey bee venom, house dust etc., and is suggested to be a prominent antibody in chronic asthma patients (Aalberse et al., 1983). Lately, IgG₄ is being studied with IgE to determine its role in the allergic response (Alenius et al., 1992; Panzani et al., 1993). During immunotherapy, inverse correlation between IgG₄ and IgE in some subjects was detected (Ito et al., 1993), while in the other, there was no such relationship, making the role of Ig₄ a matter of controversy and research (Halpern, 1993).

II. Mast Cells & Basophils

Mast cells and basophils were first described by Ehrlich (1879). Basophils constitute fewer than 0.2% of leucocytes in circulation. Mast cells reside in the body tissue and most (80%) are present in the airways with only 0.01-1% in the lungs (Kaliner, 1980). Basophils are short lived (<two weeks) while mast cells are long lived and appear to proliferate (Siragnian, 1993). Degranulation of mast cells in response to the binding of allergen to receptors leads to the release of mediators (e.g., histamine, heparin, chondroitin sulphate A & E, NCF-A, ECF-A, proteases, cytokines (IL-1, TNF-) and newly generated lipid mediators (LTB₄, LTC₄, PGD₂, LTE₄) (Schwartz & Huff, 1993).

Jarjour et al. (1991) reported that spontaneous release of histamine in bronchial mast cells was greater in asthmatics than in a control population with concentration of tryptase, a mast cell marker also elevated (Schwartz and Huff, 1993). Basophils are also important in allergic disorders (Marona, 1989) but more in the pathogenesis of chronic asthma than in the acute phase (Schroeder et al., 1995). Though not present in the airways, basophils are important in hay fever and rhinitis (Barnes, 1993).

III. Eosinophils

Eosinophils are enhancers of tissue inflammation by generating cytokines, such as
IL-1, IL-3. Eosinophil infiltration and concentration elevation is a characteristic feature of asthma and rhinitis (Adelroth et al., 1990). Besides enhancing inflammation, these cells decrease the inflammation by releasing histaminase and aryl sulphotase to inactivate histamine and SRS-A (Brostoff & Hall, 1993).

IV. Monocytes & Macrophages

Monocytes and macrophages are involved in allergic reaction through the presence of FcγRII receptors (Joseph et al., 1983; Lane et al., 1994). They release mediators in the form of chemotoxins (PAF, TNF etc.) and activators (IL-1, IL-6 and IL-8) for eosinophils, neutrophils, fibroblast etc. (Mensing & Czarnetzki, 1984; Kelley, 1990). The mediators of macrophage cause bronchoconstriction by smooth muscle contraction (Marom et al., 1983).

V. Neutrophils

There is no direct evidence of the involvement of neutrophils in allergic inflammation except for their marked increase in the number (Laitinen, 1989) in severe asthmatics having a good positive correlation with occurrence of symptoms (Kelley et al., 1988).

VI. Platelets

Besides their involvement in blood clotting, through mediators like histamine, thromboglobulin, PAF and others, platelets are associated with inflammation (Page, 1988; Degaetano et al., 1989).

VII. Complement

The role of complement in allergic reaction is a subject of dispute. The presence of these components in bronchial mucosa of asthmatics has been reported (Molina et al., 1977), while others found no difference between asthmatics and the normal population (Srivastava et al., 1983).

Predisposing and Contributing Factors of Allergic Diseases

The primary determinants of susceptibility to allergy are mainly genetic and environmental. Genetic factors determine the overall risk of allergy, while environmental factors effect specific sensitivity to a particular allergen (Ownby, 1993).

* Genetic factors

Allergenic sensitivity is genetically determined. Cooke & Vanderveer (1916) reported that allergy runs in families.
Heritability of serum IgE concentrations ranges from 50-84% (Hamburger et al., 1973; Bazaral et al., 1974). The overall regulation of atopic diseases include at least one antigen-nonspecific gene that is not HLA-linked (Hopp et al., 1984). The major antigen specific control appears in HLA-linked genes (Levine et al., 1972). Hopkins (1990) suggested the non-MHC linked genetic control of IgE synthesis, in which the inheritance of atopy is related to a gene on chromosome 11q.

**Environmental factors**

Environmental factors are very important in enhancing the genetic potential for expression of a high IgE response. The most important factor is allergen exposure (Pepys, 1973; Peat, 1994). Although farms and trees frequently occur in villages and suburbs, pollen allergy is higher in urban and industrial areas (Panzani et al., 1986; Ishizaki et al., 1987). This suggests that environmental factors, apart from the presence of pollen, are perhaps related to pollution, which increase pollen allergy.

D'Amato (1991) reported that air pollution can increase the incidence of allergy. SO\textsubscript{2} and NO\textsubscript{2} have also been reported to increase asthma in crowded cities (Prin, 1990; Cerceau-Larrival et al., 1991). Majd & Ghanati (1995) reported that the environmental pollution changes the relative exine mineral amount of pollen, which enhances pollen allergenicity.

**Allergy Diagnosis**

The diagnosis of allergic disorders have been mainly based on skin tests. The idea of skin testing came from the cutaneous test introduced by von Pirquet (Feinberg, 1946). Although intradermal skin testing is more reliable and sensitive, skin-prick testing is more convenient and has less chance of systemic reaction (Heijjaouri et al., 1992, Lin et al., 1993). Another important in vivo test is direct challenge of the mucosa of affected subjects. However, as the method is time consuming and hazardous, its clinical practice is restricted (Davis & Corrado, 1985).

A range of in vitro techniques are also used in allergy diagnosis, based on measuring the concentration of allergen specific IgE in a patient’s serum. Such tests include the radio allergosorbent test (RAST), introduced by Wide et al. (1967). The enzyme linked immunosorbent assay (ELISA), developed by Engvall & Perlman (1971), is easier cheaper, and non-hazardous in relation to RAST.
Allergenic Pollen

The allergenic potential of pollen has been confirmed by a large number of clinical and immunochemical studies. The principal airborne allergenic pollen grains causing respiratory allergy in different regions of the world are tabulated below.

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Workers</th>
<th>Principal Allergenic Pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>Bosquet et al., 1984</td>
<td>Poaceae</td>
</tr>
<tr>
<td>Japan</td>
<td>Shilda &amp; Wagatatsuma, 1968</td>
<td>Artemisia, Cryptomeria, Erigeron, Pennisetum</td>
</tr>
<tr>
<td></td>
<td>Ishizaki et al., 1987</td>
<td></td>
</tr>
<tr>
<td>Mediterranean Region</td>
<td>Eriksson et al., 1987</td>
<td>Artemisia, Olea, Parietaria, Poaceae</td>
</tr>
<tr>
<td></td>
<td>Ford et al., 1986</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D'Amato &amp; Spieksma, 1991</td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>Harfi et al., 1992</td>
<td>Phoenix dactylifera</td>
</tr>
<tr>
<td></td>
<td>Kwaasi et al., 1993</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>Ordman, 1970</td>
<td>Cupressus, Cyperus, Poaceae, Prosopis</td>
</tr>
<tr>
<td>Sweden</td>
<td>Eriksson et al., 1984</td>
<td>Betula</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Kalliel &amp; Settipane, 1988</td>
<td>Betula, Pinus</td>
</tr>
<tr>
<td></td>
<td>Clayton et al., 1989</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Newmark &amp; Itkin, 1967</td>
<td>Acer, Alnus, Ambrosia, Betula, Artemisia,</td>
</tr>
<tr>
<td>of America &amp; Canada</td>
<td>Lewis et al., 1984</td>
<td>Cynodon, Juniperus, Morus</td>
</tr>
<tr>
<td></td>
<td>Daniel et al., 1984</td>
<td>Parthenium hysterophorus</td>
</tr>
<tr>
<td>US Gulf Coast</td>
<td>Wedner, 1989</td>
<td></td>
</tr>
</tbody>
</table>

The first study on pollen allergy in India was made by Shivpuri et al. (1960), and later continued by his coworkers (Shivpuri & Parkash, 1967; Shivpuri et al., 1979). They studied the allergenic pollen of Delhi metropolis, which included Prosopis, Ageratum, Allanthus, Cassia, Cenchrus and Cheno-Amaranthaceae.

The widespread aeroallergens from southern India included Cassia, Ageratum, Salvador, Ricinus, Albizia and Artemisia (Acharya, 1980; Agashe & Anand, 1982). The pollen grains of Parthenium hysterophorus (Subbarao et al., 1985) and Casuarina equisetifolia (Agashe & Soucenadin, 1992) were identified to cause allergy in Bangalore.
The important aeroallergens from Calcutta and suburbs include the pollen grains of *Lantana, Cucurbita, Cocos, Azadirachta* and *Phoenix*. (Chanda et al., 1978; Banik & Chanda, 1992; Karmakar and Chatterjee, 1994; Gupta-Bhattacharya et al., 1994).

The principal allergenic pollen grains of Central India are *Parthenium, Cassia, Azadirachta* and *Brassica* (Tiwari, 1970; Chaubal & Gadve, 1979).

The allergenic pollen taxa differ with climato-ecological zones, so that proper identification of plant species causing pollinosis in different regions is very important for allergy diagnosis, therapy and should be considered for proper environmental planning.

**Pollen Allergen Standardization, Isolation, Purification and Characterization**

Allergen extracts have been used to diagnose and treat allergy for a long time. Despite their use on millions of patients annually, little is known about their composition. The remarkable heterogeneity and batch-to-batch variation has often resulted in unreliable diagnosis, ineffective therapy and other side effects. Generally, allergens are standardized *in vivo* by the wheal diameter in skin tests (Aas, 1980; Dreborg, 1989). *In vitro* standardization is generally performed by RAST, ELISA, EUSA & RASl inhibition, basophil histamine release, immunoblot or immunoelectrophoresis (Arbesman et al., 1977; Dreborg & Frew, 1993). Differences in allergenic activity depend on several factors, including time of collection (Larson & Gleich, 1975; Singh et al., 1993), storage (Sridhara et al., 1992) and protease activity (Valenta et al., 1991).

In 1978, the Allergen Standardization Committee of the International Union of Immunological Studies (IUIS) undertook the production of a range of allergenic extracts that would meet specifications for international standard preparation (WHO, ECBS 1978). Several pollen allergens had already been standardized internationally (Helm et al., 1984; Amtzen et al., 1989). The drawback of standardization using the principal allergenic components is that, minor components, which may sometimes be important in the development of allergic diseases, are ignored (Platts-Mills & Chapman, 1991). However, the use of this allergen in analysis after standardization had resulted in a four-fold elevation of activity (Dreborg & Einarsson, 1992).

During last few decades, the principal allergenic components of a large number of pollen grains have been identified, isolated and purified. These have included *Dactylis*
glomerata (Ford et al., 1985), Secale cereale (Westphal et al., 1988), Cynodon dactylon (Shen et al., 1988), Artemisia vulgaris (Nielsen & Paulsen, 1990), Phoenix dactylifera (Harfi et al., 1992), Olea purpurea (De Cesare et al., 1993) Paristaria officinalis (Kahlert et al., 1996) and Mercurialis annua (Valverdu et al., 1997).

There have been few attempts at studying the principal allergenic pollen components in India. Pollen extracts of Xanthium strumarium (Jaggi & Gangal, 1987), Cocos nucifera (Jaggi et al., 1989), Parthenium hysterophorus (Subbarao, 1984; Sriramarao et al., 1993), Holoptelia integrifolia (Malik et al., 1991), Ricinus communis (Singh et al., 1993) and Azadirachta indica (Karmakar & Chatterjee, 1994) from different parts of India have been standardized.

Following the biochemical characterization and standardization, several pollen allergens have been cloned and sequenced using the techniques of molecular biology (Lauzurica et al., 1988; Rafner et al., 1991; Suphioglu & Singh, 1995; Helm et al., 1996). This has allowed allergens to be produced as recombinant proteins, expressed in bacteria or yeast cells (Hirschwehr et al. 1993). The study on crystal structures of plant profilins, representing cross-reacting allergens for almost 20% of pollen allergic patients (Fedorov et al., 1997) from different families showed the presence of a solvent filled pocket located near its active site, which may be responsible for its allergic cross reaction (Thorn et al., 1997).

**Allergen Nomenclature**

The rapid expansion of allergen research has resulted into the formation of a growing family of isolated allergens from a range of sources. To avoid misinterpretation, a systematic nomenclature was needed and it has been suggested that purified allergens must be named according to strict guidelines of International Union of Immunological Society's (IUIS) Allergen Nomenclatural Sub-Committee approved by the World Health Organization (WHO, 1994).

An allergen is named according to the accepted taxonomic name of their source as follows:

The first three letters of the generic name, followed by the first letter of specific name and an arabic number representing the order of identification, e.g., Lol p 1 refers to the first pollen allergen identified from Lolium perenne.
To avoid ambiguity, where the generic and specific names give rise to same name for an allergen, an additional letter can be added, e.g., Ves v 5 and Ves vi 5 for allergens from respectively Vespula vulgaris and Vespula vidua, where the allergen from V. vulgaris had been characterized before V. vidua. Partially purified or non-purified allergens are designated by arabic numerals starting from greatest anodic mobility (Marsh, 1986). However, this could still lead to confusions, for example, Lol p 11 and Phl p 11, both carry the same number but one is a trypsin inhibitor and other a profilin.

The term "isoallergen" has been used to denote one of a group of allergens from a specific source with experimentally distinguishable immunologic properties but closely related physico-chemical characters. They usually differ from one another in isoelectric focussing (pl), because of differences in protein amidation, carbohydrate moieties or genetic factors (Johanson & Marsh, 1965; Marsh, 1975), eg., Phl p V from Phleum pratense, Amb a I from Ambrosia, Ole e I from Olea europea, all are found to separate into two fractions in isoelectric focussing (Rafner et al., 1991). Such allergens could be designated in the order of decreasing pl, e.g., Amb a vA (pl 9.6) and Amb a vE (pl 8.5) from Ambrosia according to IUIS.

Localization of Pollen Allergen

In the 1970's, it was thought that the allergens were stored in the exine/intine of pollen grains (Knox et al., 1970) because of its instantaneous release in contact with water. Later Dumas et al. (1984) suggested that the rapid liberation of allergenic protein of pollen is related to their function in pollen germination. Singh et al. (1991) and Suphioglu et al. (1992) detected allergens in rye grass and other grass pollen grains in the amyloplast. Later Taylor et al. (1994) detected allergens in cytoplasm and starch granules. The association with carbohydrates perhaps suggest that some poller allergens are glycoproteins. In Betula, Gorte et al. (1993) found the allergen in cytoplasm, whereas El-Ghazaly et al. (1996) detected them specifically in starch granules by using monoclonal antibodies.

Cross Reactivity of Allergenic Pollen

In clinical and immunological studies, pollen-allergic patients have rarely been found to be sensitive to a single pollen type. Sensitivity has frequently been reported to a range of pollen taxa from grasses, weeds and trees even when some of the pollens were not encountered (Hemmens et al., 1988; Pham et al., 1994). The presence o
shared allergenicity and antigenicity among taxonomically related pollen types is well-established (Wodehouse, 1971; Baldo et al., 1982; Eriksson et al., 1987; Sridhara et al., 1995). The cross-reactivity generally results from the presence of homologous or similar proteins, like the group I and group V allergens in grasses (Esch & Klapper, 1987; Matthiesen & Lowenstein, 1990), profilin in grasses and other families (Vallier et al., 1992) and group I allergens from tree pollen (Ipsen & Hansen, 1991). Taxonomically diverse pollen types have also been reported to show cross-reactivity (Cornfold et al., 1990; Pham & Baldo, 1995).

Further studies showed that there is a 40.1-81.7% and 60.6-95.5% sequence identity among amino acid and nucleotide sequences respectively from Groups I and V allergens from canary grass and some other grass pollen (Suphioglu & Singh, 1995). In case of Lol p 1 (a major allergen from Lolium perenne) and bromelin (ceseine proteinase), which were found to be allergenically cross reactive, the effect of deglycosylation in such cases shows evidence of the role of carbohydrate in cross-reaction (Pike et al., 1997).

In the Indian context, studies on shared allergenicity and antigenicity in different pollen taxa were reported by Sridhara et al. (1995) for grass pollen. Application of such common and cross-reactive allergens that are predominantly responsible for sensitization may be helpful in the treatment of allergic patients sensitive to an array of pollen taxa.

Management of Pollen Allergy

For pollen allergy, as for all allergic disease, it is essential to make patients aware of the causes of the allergy and seasonal occurrence of the allergens. A general awareness can be communicated through various media. Pollen control ordinances have been found helpful in decreasing the incidence of respiratory allergy in Tucson, Arizona (Sneller et al., 1993). Another approach to the control of allergic disease is the symptomatic treatment using anti-allergic drugs including antihistamines, steroids, non-steroid mast cell stabilizers (e.g., chromoglycate) and bronchodilators, e.g., drugs with adrenoreceptor agonistic and cholinoreceptor antagonistic action, theophylline. Despite regular medication, some patients continue to suffer from troublesome symptoms. For them, allergen immunotherapy can be useful. The efficacy of such therapy has been confirmed by numerous clinical studies (Frankland & Augustin, 1954; Viander & Koivikko, 1978; Varney et al., 1991) and it was found that long term treatment for
3-5 years results in a more sustained effect than a short term immunotherapy (Hedlin 1995). Recently double blind placebo controlled trials have been performed using partially purified and standardized grass pollen allergen on hay fever patients (Bosquc et al., 1989) which decreased overall symptoms and medication was thus avoided. Hakansson et al. (1997) reported that immunotherapy prevents priming of eosinophil adhesion during pollen season in the patients sensitive to birch pollen due to the shift from production of eosinophil priming agents to neutrophil priming agents. However, this type of treatment can elicit severe side effects (Lockey et al., 1987).

Vrtala et al. (1997) suggested the use of recombinant allergen in nonanaphylactic but epitope containing converted forms in the development of a safe and specific immunotherapy.

**Aims and Objectives of the Present Study**

The present study was undertaken with the following aims and objectives:
1. To study the prevalence and seasonal & diurnal changes in the frequency of common airborne pollen grains in a suburban area of Greater Calcutta Metropolis.
2. To identify the allergenic pollen grains in the study area.
3. To study the allergenic significance of particular pollen types by clinico-immunological tests.
4. To isolate, purify and characterize, at least partially, the major allergenic component of particular pollen types.
5. To study the presence of shared allergenicity and antigenicity among different pollen taxa of the same family.
6. To quantify the allergens and antigens originating from an important airborne pollen during the pollen season using immunochemical techniques.