Spectrum of Allergens and Allergen Biology in India

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Keywords

Allergens · India · Molecular allergology · Recombinant allergens · IgE epitope · Allergy diagnosis · Transgenic biosafety · Allergen specific immunotherapy

Abstract

The growing prevalence of allergy and asthma in India has become a major health concern with symptoms ranging from mild rhinitis to severe asthma and even life-threatening anaphylaxis. The “allergen repertoire” of this subcontinent is highly diverse due to the varied climate, flora, and food habits. The proper identification, purification, and molecular characterization of allergy-eliciting molecules are essential in order to facilitate an accurate diagnosis and to design immunotherapeutic vaccines. Although several reports on prevalent allergens are available, most of these studies were based on preliminary detection and identification of the allergens. Only a few of these allergen molecules have been characterized by recombinant technology and structural biology. The present review first describes the composition, distribution pattern, and natural sources of the predominant allergens in India along with the prevalence of sensitization to these allergens across the country. We go on to present a

comprehensive report on the biochemical, immunological, and molecular information on the allergens reported so far from India. The review also covers the studies on allergy-related biosafety assessment of transgenic plants. Finally, we discuss the allergen-specific immunotherapy trials performed in India.

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Introduction

Allergic diseases have become a major health concern worldwide, with India being no exception. Currently, more than 25% of the total population of India is sensitized with different forms of allergens [1]. Major sources of allergen in this subcontinent are the pollen grains, fungal spores, foods, insects, and dust mites [2, 3]. Common symptoms among allergy sufferers in India include bronchial asthma, atopic rhinitis, dermatitis, urticaria, oral and gastrointestinal symptoms, ocular allergy, and even life-threatening anaphylaxis [4]. Various reports based

Edited by: H.-U. Simon, Bern.

on hospitalization data from different parts of India have shown a sharp increase in the prevalence rate of IgE-mediated atopic disease in the last few decades [5]. India, being a land of diverse climates, vegetations, populace, and food habits, has been reported to have a broad range of allergens starting from inhalants to ingestants and contactants. Despite having such a serious health risk, the clinical as well as basic research on “Molecular Allergology” in India has never been given that much importance. This has rendered the accurate diagnosis and therapeutic intervention of allergy quite difficult in this country. While in western countries the recombinant allergen-based advanced diagnostic tools like ImmunoCAP [6] and allergen microarray [7] have revolutionized molecular diagnosis, India is still behind, relying on the century-old skin prick test using crude allergen extracts [8, 9]. Moreover, it is clear that the allergen extracts in India are not standardized and no action plan has been established for improving the quality of diagnostic reagents. In addition, there are economic constraints for introducing the allergen manufacturing industry from abroad to improve the situation due to high tax rates and legal requirements for company licensing. There are also only a few reliable sources of high-quality allergenic source materials. Apart from diagnosis, western countries have witnessed a quantum leap in the therapeutic measures of allergy within the last few decades. These therapeutic measures refer to immunotherapy with hypoallergenic variants, allergen-derived peptides/fragments, and genetically engineered allergen derivatives [10]. On the other hand, such advanced immunotherapeutic tools are particularly sparse in India. The present review aims to shed light on the allergen repertoires that contribute to the pandemic dimension of IgE-mediated disorders in India, and how this information can be effectively utilized in translational research to face the health concerns of the huge Indian population.

Allergy and Allergen Research in India

Prevalence of Allergic Diseases in India

Asthma and upper respiratory tract disorders are the prevalent manifestations of chronic allergy, affecting a substantial population of all ages in all parts of India. They pose a significant socioeconomic burden as a result of a reduced quality of life. The most recent and revised estimate on the global epidemiology of atopic asthma under the Global Burden of Disease project suggested that around 334 million people are asthma sufferers [11].

Keeping pace with the global epidemiology, the occurrence of the allergic disorder in India is also increasing at an alarming rate as more than 25% of the Indian population suffer from various forms of allergy [12]. A hospitalization-based survey carried out in India showed that 20–30% of the population suffers from allergic rhinitis and 15% of them developed atopic asthma [13, 14]. In addition to asthma, the other forms of allergy, such as atopic rhinitis, anaphylaxis, drug, food and insect allergy, eczema, urticaria, and angioedema, were also on a rising trend in terms of prevalence as well as severity in India. A multicentre population study performed by Jindal et al. [15] in different parts of India, named the Indian Study on the Epidemiology of Asthma, Respiratory Symptoms and Chronic Bronchitis (INSEARCH), revealed that 20–30% of the Indian population was clinically diagnosed as allergic (Fig. 1). The study also estimated the frequency rate of asthma, chronic bronchitis, and allergic rhinitis to be 2.05, 3.5, and 3.3%, respectively. This study also comprised of a population suffering from a paediatric allergy in which 3.3% of children aged 6–7 years and 5.6% of children aged 13–14 years were diagnosed with allergic rhinoconjunctivitis. Another multicentre study performed by the Asthma Epidemiology Study Group of the Indian Council of Medical Research observed the prevalence of allergic rhinitis in approximately 20% of the population in India [16, 17]. The World Allergy Organisation (WAO) estimated that the pollen allergy prevalence of the whole population ranges between 10 and 40% [18].

Major Sources of Allergens of India and Environmental Factors Favouring Their Growth

Various cross-sectional studies have provided a better understanding of the relationship between allergen exposure, allergic sensitization, and clinical observations. This has led to the identification of the major sources of allergens in India. Moreover, clinical and biochemical reports are available on cross-IgE reactivity between pollen-spore, pollen-food, fungi-dust mite, and so on. India is a subcontinent with variable climatic conditions [19]. Entire northern and north-eastern parts are situated in the foot hills of the Himalayas, the coastal continental parts are surrounded by seas and oceans (Bay of Bengal, Indian Ocean, and Arabian Sea), and the central part is divided into north and south regions by the Vindhya rocky mountains. Airborne pollen as well as fungal flora and their concentration therefore largely vary in the different ecozones and seasons depending upon the flowering seasons and meteorological factors [20, 21]. Several investigators

Fig. 1. Prevalence of allergy and atopic asthma in India and the location of different study centres (adopted from Jindal et al. [15] with slight modifications).

have carried out extensive work to make a pollen and pollination calendar of the various zones of India. An All India Coordinated Project on Aeroallergens and Human Health sponsored by the Ministry of Environment and Forests of the Government of India has been completed [13]. Based on the frequency of skin test and IgE-serology, certain allergenic sources have been designated as major allergens. These major pollen and fungal allergens from 18 different places have been identified, quantified, and partially characterized. The major allergenic pollen grains reported so far from different parts of India are listed in Table 1. The Centre for Biochemical Technology under the Council for Scientific and Industrial Research, India, published a book with pollen calendars of 12 different

states in India [22, 23], providing information on the pollen seasons of various allergenic plants of India.

Elevated temperatures and higher CO₂ levels have resulted in an increase in the pollination cycle of weeds, thereby increasing the pollen load in the air [24]. An association between thunderstorms and asthma-related hospitalization has long been reported by several investigators, ascertaining the possibility of asthma outbreak as a result of osmotic rupture of the pollen and subsequent release of the respirable allergenic particles, popularly known as thunderstorm asthma [25]. In India, a sharp rise in asthma-related morbidity during the period of monsoon could be attributed to this phenomenon, although it has not been thoroughly investigated. Apart

Table 1. List of plant families and species producing allergenic pollen grains reported from various parts of India by different researchers

Region	Family	Species
North India	Asteraceae, Arecaceae, Cheno/Amaranthaceae, Poaceae	<i>Holoptelea integrifolia</i> , <i>Rumex acetosa</i> , <i>Ailanthus excelsa</i> , <i>Eucalyptus</i> spp., <i>Ricinus communis</i> , <i>Casuarina equisetifolia</i> , <i>Putranjiva roxburghii</i> , <i>Cassia</i> spp., <i>Quercus</i> spp. <i>Pinus</i> spp. <i>Cedrus deodara</i> , <i>Platanus orientalis</i> , <i>Narcissus</i> spp., <i>Salix</i> spp.
Eastern India	Cheno/Amaranthaceae, Poaceae, Asteraceae	<i>Trema orientalis</i> , <i>Areca catechu</i> , <i>Cocos nucifera</i> , <i>Borassus flabellifer</i> , <i>Phoenix sylvestris</i> , <i>Azadirachta indica</i> , <i>Acacia auriculiformis</i> , <i>Cassia</i> spp., <i>Cycas circinalis</i> , <i>Madhuca indica</i> , <i>Pongamia pinnata</i> , <i>Peltophorum pterocarpum</i> , <i>Delonis regia</i> , <i>Solanum sisymbriifolium</i> , <i>Xanthium strumarium</i>
South India	Cheno/Amaranthaceae, Cyperaceae, Poaceae	<i>Casuarina equisetifolia</i> , <i>Parthenium hysterophorus</i> , <i>Spathodia campanulata</i> , <i>Cocos nucifera</i> , <i>Eucalyptus</i> spp., <i>Peltophorum</i>
Western India	Poaceae, Cheno/Amaranthaceae	<i>Azadirachta indica</i> , <i>Parthenium hysterophorus</i> , <i>Moringa oleifera</i> , <i>Prosopis julifera</i> , <i>Casuarina equisetifolia</i> , <i>Eucalyptus</i> spp., <i>Sisymbrium</i> spp.
Central India	Poaceae, Asteraceae, Apocynaceae, Cyperaceae, Cheno/Amaranthaceae	<i>Rosa</i> spp., <i>Ricinus communis</i> , <i>Ailanthus excelsa</i> , <i>Holoptelea integrifolia</i> , <i>Cicer aurietinum</i> , <i>Argemone mexicana</i> , <i>Cocos nucifera</i> , <i>Hibiscus</i> spp.
Families are listed in case the pollens in isolated form were difficult to identify at the genus/species level.		

from pollen grains, the fungal spores, insects, and dust mites have also been considered as major contributors of the aeroallergen load in the indoor environment [26–28]. In fact, the aerial concentration of fungal spores and fragments are significantly higher than that of the pollen grains [29, 30]. It is considered that the tropical and subtropical climatic conditions of India along with the high humidity are most favourable for the growth and propagation of microbes like moulds [13]. Indoor dampness was found to exacerbate the severity of mould asthma [31]. In addition, the low level of sanitation, rapid urbanization, and deforestation are the other key factors contributing to the allergen distributions in ambient environments [32].

The Major Route of Exposure to Allergens

Almost all the routes of exposure to allergens have been observed with similar frequency among the atopic population of India, such as inhalation, ingestion, and contact. Respirable airborne particles, including airborne pollen grains, insects, mites, and fungal spores, were well investigated and established as important causes of pollinosis, asthma, and ocular allergy in this country [33]. In addition, certain components of dust mites and moulds have been found to act as contact allergens leading to skin-related diseases [34]. The food

allergens are also quite predominant sensitizers in India as a large variety of foods are consumed across the country [35].

Molecular Allergology in India

Recent advances in molecular biology have contributed significant progress in the field of allergen biology or more precisely the “molecular allergology.” Considering the steady rise in the preponderance of asthma and other allergic diseases, India should now focus on designing tools and strategies for the improvement of appraisal and management of allergy. Recombinant allergens were shown to have great utility in allergen characterization and understanding the immune mechanism of IgE-mediated disease. In addition to that, recombinant purified allergens have shown promise in designing improved therapeutics in the near future. Until now, more than 100 such molecular allergens have been characterized from different parts of the western world and are being used in clinical practices as diagnostic antigens [36–38]. These advancements in biomedical technology are heading towards establishing “precision medicine” for allergy treatment in which “personalized immunotherapeutics” will be available based on the disease phenotype of the indi-

Table 2. List of certain major allergens reported from India and approved by the IUIS with official names

IUIS name	Organism	Route of exposure	Biochemical name	IgE epitope	Cross-reactivity
Epi p 1	<i>Epicoccum purpurascens</i> (fungus)	inhalation	serine protease	no	no
Cur l 3	<i>Curvularia lunata</i> (fungus)	inhalation	cytochrome c	⁵ QGDAKKGANLFKTRC ¹⁷ TRCAQCHTLK ²⁵ LKAGEGNKIGPE ⁵⁴ TDANKQKGIEWNHDT ⁷⁴ ENPKKYIPGTK ⁹⁰ LKKPKDRNDLI	no
Pen i 1	<i>Penaeus indicus</i> (prawn)	food	tropomyosin	⁵⁰ MQQLENDLDQVQESLLK ¹⁵³ FLAEEADRK	no
Rhi o 1	<i>Rhizopus oryzae</i> (fungus)	inhalation	aspartic protease	⁴⁴ TGEYLTQKYFNSQRNN ³¹¹ GAEKNWAGQYVVDCKN and one mAb-4C3 recognisable conformational epitope	Bla g 2
Cat r 1	<i>Catharanthus roseus</i> (pollen)	inhalation	cyclophilin	no	Mala s 6 and Asp f 11
Rhi o 2	<i>Rhizopus oryzae</i> (fungus)	inhalation and contact	cyclophilin	no	no
Coc n 1	<i>Cocos nucifera</i> (pollen)	inhalation	vicilin	no	no
Alt a 13	<i>Alternaria alternata</i> (fungus)	inhalation	glutathione-S-transferase	fragment (1–50 aa); T21 and T27 are critical residues	GST from <i>Aspergillus</i> , <i>Epicoccum</i> , <i>Curvularia</i> , and <i>Cladosporium</i>
Par h 1	<i>Parthenium hysterophorus</i> (pollen)	inhalation	defensin	no	50-kDa potato tuber lectin
Per a 10	<i>Periplaneta americana</i> (insect)	inhalation	serine protease	²³⁰ CAEQGYPGVYS ¹⁹⁹ QQGGKDACQGDSG ¹⁷⁵ SRSECNQAYS DY	no

The IgE epitope information of allergens is highly patient specific. Here, the data have been retrieved from published literature in which the work was conducted in India.

vidual patient [39]. As compared to such tremendous development in the field of molecular allergology worldwide, there have been only a few reports on the molecular characterization of allergens prevalent on the Indian sub-continent [2, 3]. It has resulted in the unavailability of the advanced multiplexed assay system to evaluate the complexity of the IgE repertoire present in the sera of allergic patients of Indian origin. As a consequence, allergists are still relying upon outdated, less-characterized, and less-standardized allergen extracts [40]. Since pharmaceutical-grade recombinant allergen preparation is relatively

expensive, the current focus in India should be to ensure the availability of high-quality allergen sources and allergen extracts. Some of the well-characterized allergens from India with official IUIS designations are summarized in Table 2. It is noteworthy to state that all the entries in the WHO/IUS allergen database should not be strictly considered as the genuine sensitizers as some of these proteins are thought to display IgE reactivity simply due to cross-reactivity and therefore are not clinically relevant. Accurate characterization of novel allergens is an important aspect of molecular allergology. It refers to a

multistep discovery approach consisting of serodetection, identification, purification, and finally the molecular characterization of individual allergen molecules (i.e., components) from a particular source. In the following section, we shall discuss step-by-step the currently available reports on various allergens based on molecular allergological studies performed in India.

Serological Detection and Purification of Allergens from Natural Sources

Pollen Allergens

Based on clinico-immunological studies, important allergenic pollen allergens of India have been identified [41–50]. In 1873, Dr. D.D. Cunningham, a British physician, carried out the first systematic aerobiology over the Presidency Jail at Calcutta, the then Capital of India using an Aeroscope apparatus. His work was quite comprehensive, and published in the form of a book entitled *Microscopic Examination of Air* [51, 52]. It was one of the pioneering works on atmospheric biopollutants recognized by the whole world. The work on pollen allergy in India was initiated in the 1950s in Delhi by an Indian physician, Dr. D.N. Shivpuri. In 1980, aerobiologists and allergologists from different parts of India assembled to attend the workshop on “Modern Trends in Aerobiology with Particular Reference to Plant Pathology and Medicine” held at Bose Institute, Calcutta, where the Indian Aerobiological Society (IAS) was formed and started functioning from January 31, 1980. After that, the significance of aerobiological studies and the importance of pollen and spores as aeroallergens were eventually realized and such investigations were undertaken by scientists in many laboratories of India. In view of the climate, topographical, and ecological diversities, the country was divided into 5 biozones, namely Eastern, Western, Northern, Southern, and Central regions. Hence, reports on pollen allergy in India are quite uneven (Table 1), and it is imperative to identify pollinosis-causing plants from every region, and also to prepare extracts from them for biochemical analysis and clinical diagnosis.

Clinical studies based on pollen allergens were performed at various medical centres in all the 5 biozones of our country under the All India Coordinated Project (AICP) on Aeroallergens and Human Health sponsored by the Ministry of Environment and Forests, Government of India [53, 54]. The works revealed important allergenic pollen from various regions in India. The pollen antigens were prepared and tested on atopic patients. In the northern city of Chandigarh, skin sensitivity was found to be highest against *Rumex acetosa* and *Ailanthus*

excelsa (17.6%), followed by *Trewia nudiflora* (9.7%), *Argemone mexicana* (9.5%), and *Cedrus deodara* (9.3%). In Delhi, 12.6% of the atopic population showed skin positivity to *Amaranthus spinosus*, 8.5% to *Populus deltoides*, and 7.5% to *Dodonea viscosa* and *Bauhinia variegata*. Pollen extract of *Imperata cylindrica* was partially characterized to detect the seroreactive proteins by TLIEF and SDS-PAGE followed by ELISA and IgE-immunoblots, which identified 7 major IgE-reactive components [55]. The monoclonal antibodies developed against group 1, 4, and 5 allergens of *Phleum pratense* pollen extract identified certain new homologs of group 5 and group 4 allergens in *Imperata cylindrica* pollen extract. A 67-kDa major cross-reactive allergen was purified from this pollen, which upon CnBr treatment generated 3 IgE-reactive peptide fragments [56].

In Eastern India, extensive research on pollen/spore allergy were initiated at Bose Institute, Kolkata. In Kolkata megacity, 28.8% of the patients were found to be sensitized to the pollen allergens of *Solanum sisymbriifolium*, 21.1% to *Crotalaria juncea*, and nearly 18% each to *Ricinus communis* and *Ipomoea fistulosa* [20]. In later decades, advanced immunobiochemical techniques such as RAST, column chromatography, and IgE-inhibition assays were performed to detect and purify the pollen allergens.

The Palmae (Arecaceae) family comprises 4 commonly cultivated palms of West Bengal state of India, and the pollen grains of these species were found to be highly cross-reactive as evident from immunoblot inhibition experiments [57, 58]. Out of 70 patients tested for skin reactivity against these 4 pollen extracts, Betel nut (*Areca catechu*) exhibited the maximum percentage (48.5%) of positive cutaneous responses followed by coconut (*Cocos nucifera*; 45.7%), Date palm (*Phoenix sylvestris*; 42.85%), and Palmyra palm (*Borassus flabellifer*; 38.5%). Subsequently, immunobiochemical analyses were performed by several workers on individual palm species to characterize the major allergens. In preliminary screening, 2 major IgE-reactive bands, one 33 kDa and the other 66 kDa, were detected in date palm (*P. sylvestris*) pollen, both of which were purified partially by a Sephacryl S-200 gel filtration column [59]. Later, in a more comprehensive study [60], the allergenomics of date palm pollen was carried out, which reported 12 different IgE-reactive components from the pollen proteome of this species. These immunoreactive proteins were subsequently identified as isoflavone reductase, beta-conglycinin, S-adenosyl methionine synthase, 1,4-glucan synthase, and beta-galactosidase by mass spectrometry and de novo sequenc-

ing. The pollen grains of *B. flabellifer* have been profusely found in the air. In eastern India, almost 32% of seasonal pollinosis patients were sensitized with this pollen allergen. A 90-kDa glycoprotein was detected in *B. flabellifer* pollen extract after immunoblotting with patients' sera [61]. This native protein was purified to homogeneity by size exclusion chromatography and then characterized for immunoreactivity. Six IgE-reactive proteins from pollen extract of *A. catechu* were detected by immunoblotting. The extract was fractionated into an ion exchange and gel filtration column to separate the IgE-reactive components. Two IgE-binding proteins with a molecular weight of 48 kDa and 118 kDa were obtained in a particular fraction, and the 48-kDa allergen was found to be cross-reactive with other palm allergens [62]. Interestingly, certain pollen allergens were also observed to cross-react with the extract of betel nut fruit extract. Coconut is highly cultivated in India, and its pollen grains are carriers of inhalant allergens. A comprehensive immuno-proteomic analysis was performed with its pollen grain, which detected 14 distinct IgE-reactive proteins [63]. All these 14 allergens were identified by extensive mass spectrometry followed by de novo sequencing. The 4 major coconut pollen allergens were identified as vicilin-like protein, isoflavone reductase, alcohol dehydrogenase, and enolase. The vicilin-like protein was a glycoprotein and was purified in natural form using a concanavalin 4B affinity column followed by cation exchange and gel filtration. The purified major allergen was tested for its allergenic activity by histamine release assay [63]. Gulmohar (*Delonix regia*) and Radhachurha (*Peltophorum pterocarpum*) are 2 popular roadside ornamental trees of the Leguminosae (Caesalpiniaceae) family, and both were identified as important sources of inhalant allergens during their flowering season, sensitizing around 30% of the pollen-allergic population [64]. In *Delonix* sp., a 66-kDa glycoprotein was detected as a major allergen and purified to homogeneity [65]. This allergen, along with 3 other proteins of this pollen, showed cross-reactivity with *Peltophorum* allergens. In *Peltophorum* sp., a 28-kDa IgE reactive band was found to be the major allergenic component [65]. Papaya (*Carica papaya*) is a plant cultivated mainly for its edible fruit; however, its pollen grains contribute significantly to the aeroallergen load in the outdoor air. In a study, a high molecular weight (approx. 100 kDa) major allergen with esterase activity was detected from its pollen [66]. Another study performed abroad showed the presence of IgE-reactive proteins in its fruit [67]. Therefore, it can be anticipated that papaya is a triggering factor for pollen-fruit cross-sensitization and suf-

ferance to oral allergy syndrome. Sunflower (*Helianthus annuus*) is a widely cultivated oil-yielding plant in India. Farmers and residents (21% of the population) near sunflower fields were found to experience seasonal rhinitis frequently. A recent immuno-proteomic investigation [68, 69] identified a panel of 7 new allergens from its pollen. The major allergens were identified as 2 isoforms of pectate lyase showing significant homology with Amb a 1.2 and one cysteine protease. The pectate lyase was found to be a glycoprotein, which upon deglycosylation resulted in a significant reduction in IgE binding. Further verifications are warranted to check whether this IgE-binding is due to a genuine sensitization or simply due to cross-reactivity with other pectate lyases of Asteraceae origin.

From Southern India, feverfew weed (*Parthenium hysterophorus*) has been reported as an important source of aeroallergens [70, 71]. In Bangalore, Rao et al. [72] reported allergenicity to *P. hysterophorus* pollen extracts among 34% allergic rhinitis and 12% bronchial asthma patients. Gupta et al. [73] reported that pollen of this species contains 3 glycoprotein allergens with molecular weights of 28, 31, and 45 kDa. The major allergen Par h 1 [74] was identified as an extensin, a class of hydroxyproline-rich cell wall glycoprotein with the sugar moiety showing a strong affinity towards the IgE antibody. Par h 1 displayed significant cross-reactivity with a 50-kDa tuber lectin of potato. In addition, the certain other pollen allergens from *Parthenium* sp. displayed cross-reactivity with ragweed allergens. *Lantana camara* is a roadside shrub dispersing allergen-containing pollen in the surrounding locality. A proteomic investigation identified a number of new allergens from this species, including pathogenesis-related Thaumatin-like protein and ATP synthase beta-subunit as major allergens [75].

Fungi

In India, the humid climate is considered as ideal for the growth of fungi. Hence, allergic disorders elicited by moulds are quite frequent in almost every part of India, and allergy-related hospitalization with typical indoor symptoms have been recurrently recorded [76–78]. Until now, 112 fungal genera are supposed to be the source of allergens. So far, 107 allergens from 28 different genera have been approved and named by the IUIS, and some of them are from India [77]. Most of the allergy-eliciting indoor moulds reported from different Indian megacities were filamentous and present in the air around the year. Extracts of the *Fusarium solani* culture filtrate, mycelium, and spores were analysed by immunoblotting with sera from asthmatics that led to the identification of 23 new

allergens [79]. A 45-kDa cross-reactive protein displayed IgE reactivity with all of the test sera (i.e., 100% frequency) and thus is considered as a major allergen [80]. Upon CnBr treatment, the major allergen released a peptide fragment IV-1 that displayed IgE reactivity and thus is considered as the epitope-containing region of this allergen. However, this fragment was unable to release histamine from sensitized basophils [81]. The culture filtrate contained 18 extracellular allergens, and this was higher than the number of antigens detected in the culture filtrate of *F. equiseti* and *F. moniliforme* [82]; 14 of these 18 allergens of *F. solani* displayed shared antigenicity with the allergens from *F. equiseti* and *F. moniliforme*, as observed in immunoblot inhibition. Different species of *Aspergillus* are highly predominant in the ambient air of India and are major contributing factors for the development of asthma as they discharge a huge number of allergen-containing spores [83]. Two separate immunoproteomic investigations [84, 85] were carried out in which several new, as well as previously unreported immunoreactive molecules, were identified from the intercellular as well as extracellular (secretome) proteome of *A. fumigatus*. Most of these antigenic molecules displayed strong IgE and IgG responses in asthmatics and allergic broncho-pulmonary aspergillosis (ABPA) patients. ABPA patients are most often diagnosed as having high titres of IgE antibodies to some proteins as well as IgG antibodies. The disease is a complex immune reaction. In many cases it is not possible to separate which proteins cause allergy, and which are bystander antigens. It is also not clear whether any of those proteins have 2 or more active IgE epitopes, and are capable of stimulating basophil activation in ABPA subjects. The proteins should clearly be demonstrated to elicit reactions before they are included as recombinant immunotherapeutic agents. *A. tamarii*, another source of allergens, was found to provoke an allergic reaction among 22% of fungal-allergic patients. Six major allergens were identified from this species through immunoblots [86], 3 of which were found to cross-react with *A. flavus* and 1 with *A. niger*. Next to *Aspergillus* sp., *Rhizopus* and *Epicoccum* are 2 other fungal genera, which have been investigated in detail to establish their role in causing respiratory allergy among the Indian population. A detailed proteomic study identified 14 novel spore-mycelial allergens from *R. oryzae* through mass spectrometry [87]. A major allergen from this species, designated as Rhi o 1, was characterized as a 44-kDa aspartic protease with an N-terminal 20-amino acid (aa)-long signal peptide. Rhi o 1 was purified from this mould and characterized for its immunological properties [88].

Another species of this genus is *R. nigricans*, which has frequently been reported as a fungal sensitizer from north Indian cities like Delhi. An immunochemical study detected 31 distinct antigens present in this extract [89]. The allergenic components were purified from this species through column chromatography and FPLC. Two major allergens were identified as having molecular weights of 12 and 14 kDa, both of which were glycoproteins. Four important allergenic molecules were identified from the proteome of *Epicoccum purpurascens* through MALDI TOF/TOF analysis [90]. One major allergen from this species is a 33-kDa glycoprotein with serine protease activity. This allergen was later purified, immunologically tested, analysed for its biological activity, and given the name Epi p 1 [91, 92]. It was experimentally shown that the protease activity of Epi p 1 was necessary to induce a strong allergic response in mice as the inactivated form of this protease allergen was not capable of inducing an inflammatory response in a mouse model [93]. It was anticipated that Epi p 1 exhibited its allergenic activity through the detachment of epithelial cells from the lung lining. Interestingly, Epi p 1 displayed cross-IgE reactivity with other fungal species like *A. fumigatus*, *Curvularia lunata*, *Cladosporium herbarum*, *Alternaria alternata*, and *F. solani*. Another major allergen from this species was a 12-kDa protein, which showed 70% diagnostic sensitivity among the corresponding patient population [94]. This 12-kDa allergen was purified and tested for its immunoreactivity, basophil activation capacity, and lymphoproliferation capacity. *C. lunata* is another important sensitizer for the atopic population of India. The antigenic extract of this mould was first standardized in order to optimize the production of allergens [95]. It was found that semisynthetic media such as Sabouraud broth was ideal for the cultivation of *C. lunata* for this purpose. Some of its allergens displayed considerable cross-reactivity with *A. alternata*, *E. nigrum*, and *A. fumigatus* when crude extracts of these fungi were used to perform ELISA inhibition and immunoblot inhibition assay [96]. A 31-kDa glycoprotein of *C. lunata* was identified as a serine protease and purified as its major allergen. The allergen was given the official designation of Cur l 1 and the purified molecule was studied variously to characterize its biological activity [97]. Another species of this genus, *C. pallescens*, has been reported as an important respiratory sensitizer from eastern India. An extensive proteomic investigation [98] was carried out that led to the identification of certain novel allergens from this species, such as Brn-1, vacuolar protease, and fructose-bisphosphate aldolase. Although several IgE-reactive fungal proteins

have been reported so far, the fungi are still some of the most difficult to judge. Some of these fungal species (or taxonomically related) are the normal inhabitants of the skin, intestine, genital tract, and airway. Many of the fungi also have alternative life stages, such as a hyphal stage, unicellular yeast-like stage, and spores. Allergen manufacturing companies often do not have extracts that exactly simulate the material to which the patients are naturally exposed. Even certain researchers have identified allergens simply based on the apparent molecular weight in SDS-PAGE and immunoblots. Hence, additional investigations are needed to understand the relevance of the individual proteins elicited.

Food

India is popular for varied cuisines and food habits across the country. A large number of vegetables, staple crops, sea foods, poultry, and dairy products are consumed as daily foods. Some of these foods have frequently been reported as causing adverse allergic reactions, with symptoms ranging from oral allergy syndrome to life-threatening anaphylactic shock. The most common types of food allergy prevailing among the Indian population include legume allergy, prawn allergy, eggplant allergy, milk allergy, and egg allergy [99]. Kidney bean (*Phaseolus vulgaris*), a common source of plant protein, has been found to sensitize 22% of the food-allergic population in Delhi [100–102]. It caused a severe reaction in the gastrointestinal tract of a mouse model with a concomitant increase in type-1 hypersensitivity biomarkers. Two major IgE-binding phytohemagglutinins were identified as possible allergens from the seed, which upon sensitization in a mouse model exhibited significant allergic reactions and basophil activation. A total of 6 IgE-binding proteins were detected by immunoblotting. In addition to the phytohemagglutinin (phaseolin), the other 2 important allergens are alpha-amylase inhibitor precursor and group-3 late embryogenesis abundant protein. Most of these kidney bean allergens showed cross-reactivity with other legumes. Some of these proteins displayed considerable cross-reactivity with the allergens from peanut, black gram, lentil, pea, pigeon pea, and chick pea. The 31-kDa phytohemagglutinin was later purified and tested for its allergenic potential. This allergen being a glycoprotein, exhibited no change in IgE-reactivity after periodate modification as well as pepsin digestion, suggesting a highly stable IgE epitope. However, in another study on 3 different legumes, namely kidney bean, black gram, and peanut, a significant reduction in IgE antibody binding as well as allergenic activity were

observed in the soluble and insoluble fractions of these seeds when treated with a combination of heating and gamma irradiation [103]. Although pepsin alone was not able to ameliorate the allergenic properties of legume allergens, it was observed that certain drastic treatment of legume allergens with a cocktail of strong proteases like Flavourzyme® and ALCALASE® (Sigma) resulted in a strong reduction in IgE binding [104]. Black gram is one of the major staple foods in India, and 1.7% of nasobronchial allergy patients were found to be sensitized to black gram extracts. A number of IgE-reactive components of different molecular weight were detected in black gram seed, some of which retained IgE reactivity after heat treatment [105]. A 28-kDa major glycoprotein allergen was isolated and characterized from this legume seed, which displayed partial sequence homology with a Rho-specific inhibitor of transcription termination [106]. The degraded product (16 kDa) from the purified allergen after SGF (pepsin) treatment remained IgE reactive after 15 min of digestion. However, after 5 min of SIF (pancreatin) treatment, the digested fragment lost its IgE reactivity. In addition to this study, a mouse model of black gram allergy [107] revealed the appearance of allergy-related clinical symptoms in lung and gastrointestinal tissues along with the upregulation of allergy-associated markers. A similar study was conducted on red gram (*Cajanus cajan*) allergens [108], where certain new allergens were detected through immunoblotting with Indian sera such as β -conglycinin, vicilin, proteins similar to Len c 1, Len c 2, and Ara h 1. Certain previously unidentified novel red gram allergens were also identified through mass spectrometry. A 26-kDa chickpea allergen was identified as plant albumin [109], which reacted with IgE antibodies of peanut-allergic subjects. A comparative study on the stability of SGF-treated allergens from most of the widely consumed legumes revealed that the majority of the allergens remained IgE reactivity after digestion, which was in agreement with the previous findings on the high prevalence of legume allergy within the Indian population. Other than legumes, rice (*Oryza sativa*) is the largest grown staple crop in India. It showed a 12% positive cutaneous test within the food-allergic population. An immunoblot analysis with the protein extract from boiled rice displayed 4 IgE-reactive components [110]. The 24-kDa chitinase has recently been reported as a major allergen from this crop [111]. Among the solanaceous vegetables, the eggplant has been studied by certain Indian researchers to identify its allergy-eliciting components. Interestingly, the harvested fruits of eggplants, after prolonged storage, were found to contain a high content of

histamines as a metabolic byproduct [112]. In many cases, skin test with this histamine-containing extracts led to a false positive diagnosis. Moreover, it is also not clear whether the itching symptoms are caused by the residual pesticides applied to eggplant during farming. Despite these findings, certain serological investigations identified certain allergens, including one lipid transfer protein from this vegetable [113], and were found to be concentrated in the peel and seeds.

Hilsha (*Tenualosa ilisha*) and pomfret (*Pampus argenteus*) are 2 widely consumed sea fish in India. Immunoblot analyses detected the presence of a 50-kDa IgE-reactive band commonly present in the muscle extract of these fish [114]. However, the major IgE-reactive bands of these 2 carps were heat labile. On the other hand, the allergens detected in the cooked muscle of bhetki (*Lates calcarifer*) and mackerel (*Rastrelliger kanagurta*) fish displayed strong IgE recognition even after boiling [115].

Insects

Allergy to insects is prevailing in areas with poor sanitation systems. In India, cockroach allergens have been considered as one of the triggering factors for the development of atopic asthma. The most commonly found species of cockroach is the American cockroach (*Periplaneta americana*). In a preliminary study, it was shown that the commercial extracts had comparatively fewer antigens than the freshly prepared extracts from adult insects and nymphs [116]. In immunoblots with cockroach-sensitized asthma patients, the freshly prepared laboratory extracts displayed the presence of 9 major IgE-reactive bands. The major 28-kDa (Per a 10) allergen from this insect was purified by affinity column and was identified as a functionally active serine protease [117]. Purified natural Per a 10 exhibited strong IgE reactivity within 80% of cockroach-sensitized patients as well as basophil degranulation and lymphoproliferation. It was shown that the protease activity of Per a 10 was crucial for activation of alveolar epithelium (A549 cell line) via PAR-2 receptor cleavage to expose the ligand peptide 'SLIGKV' that ultimately led to the mobilization of the intracellular calcium level and production of proinflammatory cytokines like IL-6, IL-8, and GM-CSF [118]. This proteolytic activity modulated the dendritic cells towards the Th2 phenotype by upregulation of CD86 and IL6, but down-regulated IL12 secretion [119]. The protease activity of natural Per a 10 was also important to initiate the allergic reaction at the mucosal surface. It increased the permeability of lung epithelium by the disintegration of cell junction proteins to enhance the migration of monocyte-

derived dendritic cell precursors [119]. The functional property of native Per a 10 was also responsible for dislodging the CD23 and CD25 from the surface of PBMC, purified B cells and purified T cells. Hence, in addition to the usual IgE-mediated immune response, the intrinsic enzymatic property of natural Per a 10 allergen was also found to be crucial for augmenting airway inflammation by several means.

Recombinant Allergens

As already mentioned, the reports on recombinant allergens from India are really very sparse as compared to European and North American countries. Until now, the majority of the works reported on prevalent allergens from the Indian subcontinent describe the preliminary identification and purification from natural sources. Still, only a few important respiratory allergens that have been successfully cloned to produce recombinant versions are listed in Table 1. A 54-kDa subtilisin-like serine protease allergen from *C. lunata* fungus was first detected by IgE-immunoscreening of an expression library and then cloned into an expression vector to produce recombinant Cur l 4 [120]. The rCur l 4 reacted with serum IgE from 70% of *Curvularia*-sensitized patients and thus represented a major allergen of this species. This recombinant allergen was able to degranulate the sensitized basophils and showed inhibition of 65% IgE binding to the plate-bound crude extract of the fungus. Another recombinant allergen from this species is rCur l 2, which was identified as an enolase [121]. The rCur l 3 exhibited IgE reactivity as well allergenic property and a certain degree of cross-reactivity with Cla h 6. *A. alternata* is an important source of indoor fungal allergens and a 26-kDa glutathione-S-transferase allergen, known as Alt a 13, was reported as a recombinant allergen [122]. The full-length cDNA coding for rAlt a 13 was isolated by immunoscreening of the phage expression library and then expressed in *Escherichia coli*. The rAlt a 13 was properly folded as the natural Alt a 13 and exhibited basophil activation as well as PBMC proliferation. From eastern India, Rhi o 1 was reported as the most prevalent fungal allergen from *R. oryzae*. The full-length gene along with the signal peptide was cloned and expressed in a bacterial system [88]. rRhi o 1 also displayed similar immunological and biological properties as well as a similar folding pattern like native Rhi o 1. The rRhi o 1 showed a certain degree of cross-reactivity with the German cockroach allergen rBla g 2. A fungal cyclophilin, Rhi o 2, has also been enlisted in IUIS allergen database [unpubl. data], and this recombinant cyclophilin displayed basophil activation as evident from RBL de-

granulation and CD203c upregulation. Another 18-kDa cyclophilin allergen, Cat r 1, was reported from periwinkle pollen (*Catharanthus roseus*), which was highly cross-reactive with other cyclophilins, such as rAsp f 11 and rMala s 6 [123]. The rCat r 1 was properly folded and also retained allergenic activity as evident from RBL assay. Although cyclophilin has been reported to be IgE reactive, this is not likely to represent an important allergen as the pollen grains of periwinkle are not essentially airborne. The cockroach allergen Per a 10 was also expressed as a recombinant allergen [119, 124]. The rPer a 10 was IgE-reactive; however, it was observed that the protease activity in rPer a 10 was somehow attenuated, which resulted in reduced allergenic properties. In later studies analysing the cellular basis of Per a 10-mediated allergy, the nPer a 10 was preferred to rPer a 10. A recombinant food allergen was purified from rice as a 24-kDa chitinase [110]. The recombinant rice chitinase displayed allergenic activities in vitro and was considered as a marker allergen for the diagnosis of rice allergy among Indians. Taken together, these 8 recombinant allergens have been reported so far from India.

Structural Biology and Epitope Mapping

The pollen cyclophilin Cat r 1 is so far the only allergen from India whose structure has been solved by NMR spectroscopy [125, 126]. The Cat r 1 structure showed the characteristic cyclophilin fold consisting of an internal beta-barrel with alpha-helices on its either side. In earlier in silico analyses with cyclophilin allergens [127], it was found that the cyclophilins contained 2 conserved patches on its surface. This observation was later corroborated in the solved 3D structure, where a high degree of conserved antigenic surface was identified, which perhaps was responsible for the high degree of cross-reactivity. In an earlier report, it was stated that the plant cyclophilins were different from other cyclophilins due to the presence of a loop, which upon deletion could lead to loss of IgE binding property. Hence, this loop was considered as important for mediating cross-reactivity among the plant cyclophilins. However, in this report on Cat r 1, a loop deletion mutant of the allergen was generated, which displayed IgE binding as strongly as the intact Cat r 1. In addition to this cyclophilin, a Bet v 1 homolog, known as T1 protein, was reported from *C. roseus*. Because of a high degree of sequence similarity between T1 and Bet v 1, researchers were interested in investigating its allergenicity. However, the T1 protein turned out to be a naturally occurring non-allergenic member of the Bet v 1 family, which, in spite of having 35–85% sequence similarity, did

not show IgE-mediated cross-reactivity with other members of the Bet v 1 family [126, 127]. The overall fold of the T1 model exhibited resembled that of the Bet v 1 group. Comparing the allergenically potent dimeric structure of Bet v 1 with the non-allergenic T1 monomer, the BV16-binding surface, and the P-loop seemed to be of less importance for Bet v 1-mediated in vivo cross-linking of IgE. The absence of cross-IgE reactivity between Bet v 1 and T1 resulted in the lack of surface conservation leading to conformational changes as well as electrostatic properties [128]. On the other hand, certain changes in the sequence, which might interfere in the dimerization of this protein, perhaps resulted in the loss of allergenic activity. Structure-based IgE-epitope localization has also been performed on the allergenic osmotins. The osmotin protein, as already described earlier, was found to be a potent IgE-binding protein and was considered unsuitable for developing transgenics [129, 130]. In silico epitope analyses of the 3D structure of osmotin by generating a homology-based model revealed certain linear as well as conformational regions on its surface, which was presumed to be responsible for antibody binding. The in silico prediction was later validated by site-directed mutagenesis of specific residues that resulted in a reduction in IgE binding as well as the allergenic properties in the in vivo mouse model. The importance of computational tools for the accurate prediction of epitopes has been well established in certain important allergens reported from India. Homology modelling of a fungal alcohol dehydrogenase allergen revealed the presence of a GroES-like catalytic domain connected with the Rossmann superfamily domain via an alpha helix. The on-line epitope prediction servers predicted 6 putative B-cell epitopes for this allergen out of which 1 peptide displayed IgE binding [131, 132]. This peptide had the sequence E(X)₂GGP(X)₃KK, which was conserved in the sequences of pathogenesis-related proteins and a high electronegativity. A similar approach was taken for Cur l 3, in which certain B-cell and T-cell epitopes were predicted [132]. All the predicted B-cell epitopes exhibited IgE binding, while the T-cell epitopes displayed a strong lymphoproliferative response. Surprisingly, one peptide which was originally predicted and validated as a T-cell epitope was found to have IgE reactivity comparable to that of the already identified IgE epitopes. This cryptic epitope of Cur l 3 stimulated IL4 secretion from stimulated PBMC culture and served as a potential candidate for the diagnosis of *Curvularia*-mediated allergy. The fungal aspartic protease Rhi o 1 displayed a certain degree of cross-reactivity with Bla g 2. It was observed that the anti-Bla g 2 mAb 4C3 resulted in

partial inhibition of IgE-binding to Rhi o 2 [88]. Molecular docking analysis revealed the presence of a similar 4C3 binding site on both Rhi o 1 and Bla g 2, which perhaps gave rise to cross-reactivity. However, this cross-reactive mAb 4C3 recognizable epitope was not clinically relevant as it was observed that IgE-binding to Rhi o 1 was primarily determined by linear stretches, which were predominant antigenic determinants of Rhi o 1. A combinatorial approach consisting of computational prediction and experimental validation led to the identification of 216 mer peptides of Rhi o 1 as probable IgE-binding regions [132]. Interestingly, these 2 peptides were mapped on 2 termini of the Rhi o 1 structure; however, in the folded conformation these 2 regions come in proximity to form a large antigenic cluster. Hence, these 2 peptides were actually presented in a conformational manner on the Rhi o 1 surface. An IgE epitope was also identified in the rice chitinase allergen by following the above-mentioned bioinformatic strategy [110]. A heat-stable 34-kDa tropomyosin, Pen i 1, was identified as the major allergen from a native south Indian shrimp species (*Penaeus indicus*). In this allergen, the epitope mapping was carried out by enzymatic digestion of the allergen followed by IgE immunoscreening of the digested products [133]. In this way, 2 regions (residues 50–66 and 153–161) were detected as probable IgE-binding regions of this allergen.

Allergenicity Assessment of Transgenics for Food Safety

Given the scale of India's agro-economy, biotechnological innovations are imperative for crop improvement. Several transgenic approaches are being carried out to enable the crops and vegetables to gain superior traits for combating stress factors. However, the safety level of these transgenics must be carefully qualified through repeated experimentations to ensure that the foreign gene product in the genetically modified (GM) crop does not lead to any adverse reactions in consumers [134]. Until now, no predominant food crops such as mustard, maize, and soybean have received regulatory GM approval in India. In 2008, the Indian Council of Medical Research, Government of India, adopted a safety evaluation procedure nearly identical to the Codex Alimentarius Guideline, 2003. Here, we will review some of the works in which the safety levels, in terms of allergenicity of the transgenic products of Indian origin have been experimentally assessed. Choline oxidase (codA), an abiotic stress tolerant gene isolated from *Arthrobacter globiformis*, was used to develop transgenic mustard to perform better under salinity and cold stress. The codA protein,

despite having a 6-aa-long stretch identical to the latex allergen Hev b 6, displayed no IgE-binding in vitro. Moreover, the codA protein was degraded completely after SGF digestion and elicited no allergic responses in either human subjects or mouse models [135]. Hence, the allergy-related biosafety level of the stable transgenics harbouring the bacterial codA gene was confirmed. Tobacco osmotin is a multifunctional PR-5 family protein used for developing transgenic crops with osmotolerance as well as being resistant to fungal attack. However, it was observed that the aa sequence of osmotin shared considerable homology with known allergens [136]. In addition, the IgE antibodies present in the sera of food allergy patients (e.g., apple and tomato) reacted with the osmotin protein in immunoblots among allergic subjects. Moreover, the protein was found to be highly resistant to pepsin digestion and heat treatment. In a later study, 3 linear stretches of peptides (57–70 aa, 72–85 aa, and 147–165 aa) were identified in osmotin sequence through in silico followed by immunobiochemical analyses that strongly reacted with IgE antibodies and hence served as possible IgE epitopes. In addition, another conformational IgE epitope was also identified on this protein [137]. Mutational changes in the critical residues of these epitopes led to a significant reduction in IgE binding. Hence, the unsuitability of the osmotin gene for developing transgenics was established, and instead the engineered hypoallergenic version of the protein was ascertained as a potential candidate for biotechnological applications. The leaf agglutinin of garlic (*Allium sativum*), popularly known as ASAL, was identified as a potent insecticidal protein. The gene encoding ASAL was used for developing agriculturally important transgenics crops with resistance to sap-sucking insects. For better effectiveness of ASAL, a mutant version of this protein was later developed, known as mASAL, which also displayed similar insecticidal efficiency like the wild-type counterpart. Both of these proteins were tested through in silico and immunobiochemical analyses following FAO-WHO guidelines. The findings of these experiments demonstrated that either ASAL or mASAL were devoid of any allergenic properties (i.e., no sequence homology with known allergens, no allergic inflammation in a murine model, and no IgE reactivity as intact or digested protein), and hence could be safely utilized in transgenic applications [138, 139]. The defensins and defensin-like proteins have been reported as potent allergens from various sources, like peanut, olive pollen, and mugwort pollen. However, a newly reported defensin-like protein from *Rorippa indica*, known as RiD protein, was found to have insecticidal properties and there-

fore was used to develop transgenic mustard with aphid resistance. Like the previously mentioned ASAL protein, the RiD protein was also tested for allergenic activity and was found to be biologically safe in terms of triggering allergic reactions [140]. Hence, it is expected that the Government of India will keep following the ICMR guidelines (2008) along with field test data to demonstrate that the products would remain viable without presenting any potential risks to the consumers.

Allergens for Specific Immunotherapy

The methodology of allergen-specific immunotherapy (SIT) has witnessed a slow transition from the use of crude extract to the use of purified natural and recombinant allergens for advancing immunotherapeutic trials. Conventional SIT involves the repeated administration of escalating doses of crude allergen extracts over a prolonged period of 2–3 years or even longer. The purified allergen (in natural or recombinant form) was preferred over the unreliable extracts in which marker allergens are less abundant. However, the purified native allergens always pose a risk of IgE-mediated side effects during the challenge. Since unmodified recombinant allergens have immunological features similar to their natural counterparts, these molecules also have the risk of eliciting adverse systemic reactions as a consequence of either IgE or T-cell reactivity. It was therefore considered necessary to use recombinant allergen derivatives with reduced allergenic activity or allergen-derived peptides for treatment [141].

A 2-year-long double-blind placebo-controlled trial of immunotherapy with standardized *P. sylvestris* pollen extract in respiratory patients sensitive to this pollen was conducted in a public hospital of Kolkata [142]. It demonstrated a significant decrease in disease severity, specific IgE, and a concomitant increase in FEV and IgG titres amongst the patients undergoing the trial. In order to evaluate the efficiency of recombinant allergens in SIT, a recombinant version of a fungal allergen was used in a mouse model to treat *Alternaria* allergy. Two substitutional mutations in the IgE-reactive fragment of Alt a 13 resulted in a mutant with reduced IgE binding and reduced allergenic activity [143]. Stimulation of PBMCs from *Alternaria*-sensitized patients with this mutant revealed a retained T-cell reactivity with reduced production of IL-4. The IgE epitopes along with the critical residues of Rhi o 1 were mapped, and alanine substitutions were introduced into it in order to make it non-functional. The resulting mutant version of Rhi o 1 appeared to be hypoallergenic in function but retained significant im-

munogenicity. By virtue of its immunogenicity, the hypoallergen induced high titre of blocking antibody to neutralize the parental allergen. Hence, the hypoallergenic Rhi o 1 was found to be a noteworthy step towards the development of an effective patient-tailored vaccine to reduce the burden of the fungal allergy [144]. Osmotin is a PR protein used for generating transgenics with biotic and abiotic resistance. However, earlier studies established the role of osmotin in eliciting allergic responses. Certain IgE-binding peptides were identified and mapped on this protein. It was observed that single and double point mutations on these peptides resulted in a significant decrease in the antibody-binding capacity of these peptides. A mouse model of immunotherapy was designed in which repeated challenge with 4 different hypoallergenic peptides led to the skewing of T cell-mediated immune responses in these mice from Th2 to a tolerogenic Th1 phenotype as evident from elevated levels of IFN- γ and IL10. Hence, it was concluded that immunotherapeutic provocation with osmotin-derived hypoallergenic peptides could induce “anergy,” and thus increase tolerance to osmotin consumption [137]. A peptide-based immunotherapeutic approach was also exemplified in fungal allergen Cur l 3, in which allergen-derived peptide P6, P10, and 3 modified versions of P6 were used for SIT. SIT with a double point mutant of P6 (in cysteine and histidine residues), referred to as P6.2, resulted in a decreased IgE level, increased level of blocking antibody IgG2a, and elevated level of IL10 and IFN- γ . Overall, the P6.2 peptide was observed to be a non-allergenic but immunogenic peptide with immunotherapeutic potential to treat mould allergy. Another study on SIT with cockroach allergen Per a 10 has been reported. In this study, an inactive protease version of Per a 10 was used. The challenge resulted in the induction of a significant level of tolerance as evident from improved histopathology in the airway and increased level of tolerogenic cytokines [145]. A similar approach was also taken where SIT challenge was performed with cockroach extract on human subjects instead of using purified allergen or allergen-derived peptides in a mouse model. After a period of 1 year, a significantly increased level of allergen-specific protective antibody IgG4 was observed in the sera of the active SIT group. On the other hand, a modest reduction in the intensity of the specific IgE level was observed [146]. One of the primary concerns for the use of purified allergens in immunotherapy is that the population of people allergic to any one allergenic source usually includes people with different protein sensitivities. Thus, it is not possible to accurately predict which proteins would be the targets

Fig. 2. Recent trends in allergen research in India as evident from the number of publications available in NCBI PubMed as of September 2017. **a** Number of papers per year on the topic “Allergens from India” published and indexed in the PubMed database in the last 17 years (2001–2017). **b** Total number of papers published from India during the last 17 years (2001–2017) on 4 different types of allergen sources.

of immunotherapy unless sensitivities are determined first. Also, there are problems of unavailability of purified allergens in India, for which high-quality standardized extracts can also be beneficial and safe if the protocols used by allergists practicing in Europe, the USA, and Japan are followed. A number of experimental protocols are using various methods of administration (SIT, OIT, and dermal application) with good results. Immunotherapy success varies markedly with patients and the reagents. Modified allergen immunotherapy (AIT) extracts are commonly used in Europe, and a few in the USA. Some modified allergens have even been proven to be harmful. Peptide therapy has had some success, but the trials were not sufficient to prove efficacy. It is important to note that animal model trials for immunotherapy have not been found to be highly successful in predicting human outcomes. Standardization of allergen extracts have been attempted since the 1980s in some countries, with improved success for major allergenic sources, such as house dust mite, cat dander, short ragweed, and some grasses. However, in India, no effective strategy for the improvement of SIT tools has been initiated. It is important to first standardize the allergen extracts under regulatory and expertise supervision. Then it is possible to optimize the doses, adjuvants, and the modifications of the immunogen specific for the subject.

AIT in India

AIT has been in vogue in several countries for the past 2 decades [147–151]. It is the only causal effective therapy in allergic diseases so far and may be described as the pro-

cess of causing immunomodulation (shift from Th2 to Th1) in IgE-mediated diseases such as allergic rhinitis, rhinoconjunctivitis, allergic asthma, atopic dermatitis, and venom hypersensitivity. There are sufficient numbers of clinical trials worldwide to prove its effectiveness and safety. In India, however, AIT is still a work in progress [152]. Despite its awareness, the country lacks well-equipped allergy centres with trained clinical allergists.

The first and undoubtedly the most important step in designing a successful AIT protocol is identification of the specific offending allergen in a particular patient. This can sometimes be extremely difficult due to cross-reactivity between allergens. Furthermore, India, as a country, is unique in that the aeroallergens change approximately every 200 km. Additionally, the diverse soil conditions (rural or urban) and environment (coastal or hill area) affects the protein content as well as the antigenicity of the allergens. This makes standardizing allergen extracts for skin testing or specific serum IgE tests a major challenge in India, and until high-quality allergen extracts are available the clinical history of symptoms on exposure to the allergen in question remains crucial in planning AIT.

The allergen extracts that have been evaluated in India so far include house dust mites, pollen, horse dander, insects (cockroach, mosquito, and housefly), and mixes of allergen extracts. Clinical trials in asthma and rhinitis, although few in number and variable in duration (6 months to 15 years), design, as well as allergens, have demonstrated improvements in terms of clinical parameters (symptom scores), reduction in IgE, elevation of specific IgG in the post-therapeutic period, improvement in forced expi-

ratory volume in 1 s (FEV₁), and quality of life [153–162]. Immunotherapy with mixes of allergen extracts has shown variable clinical responses. In fact, mere inclusion of all allergens to which IgE antibodies are present, without establishing the possible clinical relevance, make AIT less effective [157]. Recently, a single clinical trial used AIT successfully in conjunction with anti-IgE therapy. However, further clinical trials in this area are required in India.

Conclusions

Although various aspects of allergen biology have been carried out in India, a significant lacuna still exists in terms of understanding the molecular nature of allergens prevalent in India. Most of the reports were primarily based on serological detection and purification of the allergens. However, for a better understanding of the molecular basis of immune recognition of allergens, it is crucial to elucidate the primary to the tertiary structure of the allergens. The more recombinant allergens that are reported, the more the multiplex molecular diagnosis will be improved. In India, allergen research has always received a marginal position compared with other cutting-edge immunological research. We have performed text mining of PubMed abstracts followed by detailed manual curation of the allergen-related literature published from India. A comparative analysis of this literature revealed an increasing trend in pursuing various aspects of molecular allergology over the last 2 decades in India, as shown in Figure 2a. We have also classified the reports

based on different sources of allergens such as “pollen,” “fungi,” “insects,” and “foods.” It was observed that the reports on fungal allergens assumed the highest number, followed by food and pollen allergens, as shown in Figure 2b. This observation was in agreement with the high degree of preponderances of fungal allergy on the subcontinent. Insect allergens occupied the lowest number of reports. However, the clinical importance of insect allergy in India should not be underestimated. The preliminary clinical and hospitalization data revealed a significant level of asthma-related morbidity due to dust mites. Surprisingly, to the best of our knowledge, there has not been any report on the molecular and structural nature of dust mite allergens from India. In most of the cases, the ImmunoCAP-based diagnosis of mite allergy is based on the extracts and purified allergens isolated from other countries. However, we strongly believe that in India the ambient aerosol contains a largely different composition of “tropical” dust mite species and the allergen molecules from these species must be effectively explored. Taken together, our review strongly argues in favour of intensifying the molecular research on the allergen repertoire of India as seriously as that being done in cancer research and research on various infectious diseases. The funding agencies of India should focus on sponsoring such research to control the pandemic spread of this disease in this developing nation.

Disclosure Statement

The authors declare no conflicts of interest regarding the contents of this review article.

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