A Study on Pollen Allergens in China[1]

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Objective Allergic disease caused by airborne pollen is a major health problem in China. Intensive study on pollen allergens can be of great help for preventing and treating pollinosis. Four aspects of the study on pollen allergens in China including major allergic pollen in our country, analysis and purification of pollen allergen composition, recombinant pollen allergens and clinical application of pollen allergens are described in this paper.

Key words: Airborne pollen; Allergens; Clinical application; Pollinosis; Recombinant allergens

Allergic diseases are common in clinical practice and are considered as an important health problem by WHO. Among the numerous allergens, pollen from grasses and trees is the most important inhalant allergen. The majority of flowering plants are insect-pollinated and are insignificant as the cause of allergic diseases. It is the wind-pollinated plants that are most important for pollinosis. The airborne pollens of these plants have their own characteristics to facilitate wind dispersal. They commonly have a large quantity and tend to be small, dry, and smooth and the exine of them may have buoyancy aids, such as air sacs, as in the case of many conifers[1]. These airborne pollens are the major inhalant allergens, and they can elicit type I hypersensitivity such as asthma, rhinitis, and hay fever which result from a serial of complicated immune disorder in atopic individuals who contact with pollen allergens frequently[2]. According to the investigation, there are about 10 000 000 patients with pollinosis in China. The prevalence rates vary from 0.5% to 1%, and even reach 5% in high-risk areas[3]. Atopic diseases have been increasing in recent years. For this reason, allergenic pollens have been paid great attentions for a long time in our country. In this paper, four aspects about the study on pollen allergens in China will be presented.

Major Airborne Allergic Pollen in Different Areas of China

Plant species and their blooming seasons vary greatly between regions because of differences in topography, climate and vegetation, and the complex patterns in the abundance and mixture of airborne pollen[4]. Information about the concentration and periodicity of pollen types is not only useful for interpreting symptoms of seasonal allergic rhinitis and seasonal asthma, but is also helpful for the allergic patients when they travel to other regions.

The investigations on pollens have been carried out in many cities of our country such as Shanghai, Guangzhou, Nanchang, Xinjiang, Tianjin, and others since Zhang investigated the pollens in Beijing for the first time in 1960[5]and the detailed investigations were done by Ye *et al.*[6]. The differences in geography, climate, and vegetation result in the diversification in plant species and flower seasons in different regions of China. Different areas have their own sensitizing pollens[5]. For example, according to the studies on pollen spreading in the spring of 1985 and 1992 in Qiqihaer city[7], some pollens belonging to Elm, Poplar, Goosefoot, and Crop have been confirmed to be allergenic, and these kinds of pollen are exactly dominant species in that city. In Wuhan city, as Platanus have been planted as green trees since the 1950s, they have become the dominant plant and their pollen has been the main allergenic agent in spring. Moreover, in the same region, the major allergenic pollen also changes in different seasons. The investigation data show that in Linyi city, which lie in the southern part of Shandong province, *Humulus* pollen is the main allergen in autumn and summer and the highest record happens between August 24 and September 5. Pine pollen and Poplar pollen are main causes of spring pollinosis, and their highest records appear from March 10 to 15[8]. In the Lanzhou region, willow pollen and walnut pollen are

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the major pollen in early spring, but in late spring pine pollen and locust pollen become the dominant one, and in summer and autumn, Artemisia pollen is the dominant one[9].

The major allergic pollens in different regions are summarized in Table 1. From Table 1, we can see that plants of Artemisia have widespread distribution and are the important grass pollen allergen in every region of our country; plants of *Humulus*, *Ambrosia*, and *Amaranthaceae* are also widespread. Plants of *Artemisia Humulus*, *Ambrosia*, and *Amaranthaceae* are the major grasses that cause late summer or autumn seasonal pollinosis[10]. As to the tree pollen, the principal tree in Northern China is the cold-resistant tree, so the pollen of Populus becomes the dominant sensitizing pollen. Temperate and semi-tropical plants are mainly distributed in Central and Southern China, for example, pollens of Broussonetia, Melia, and Casuarina plants are the sources of main tree allergic pollen.

TABLE 1

The Major Sensitizing Pollens in China

Region	Major Sensitizing Pollens	
	Tree Pollens	Grass Pollens
Eastern	Moraceae, Platanus, Ulmus L, Pterocarya	Humulus L,Artemisia L, Ricinus
Central	Platanus, Cupressaceae	Artemisia L, Ambrosia L
Southern	Melia, Broussonetia L, Casuarina	Amaranthaceae, Artemisia L, Ambrosia L
Northern	Populus L, Platanus L, Pinaceae	Artemisia L, Cannabis, Humulus L
Northeastern	Populus L, Ulmus L, Pinaceae	Artemisia L, Ambrosia L, Cannabis, Humulus L
Southwestern	Ulmus L, Salix L, Pinaceae	Cannabis, Gramineae, Artemisia L, Ricinus, Amaranthaceae
Northwestern	Ulmus L, Populus L, Salix L, Pinaceae	Artemisia L, Chenopodiaceae, Amaranthaceae

Because most plants flower between March and October, there are two peaks of pollen counts in one year. One is from March to May and the other from July to October. The first peak is mainly due to tree pollens, while the second peak is mainly caused by grass pollens. The peaks of airborne pollen count are consistent with the clinical fact that the seasons of pollinosis are concentrated on spring and autumn.

Analysis and Purification of Pollen Allergen Composition

Pollen contains many components such as pigment, fat, amylase, and proteins, but only a small portion of the proteins has the ability to interact with the human immune system to cause allergic symptoms. The major allergens of pollen are usually water-soluble proteins or glycoproteins of molecular masses from 10-70 kDa. Nowadays, in China, preparations used for allergen detection *in vivo/vitro* or immunotherapy are crude pollen extracts, which are ill-defined mixtures of allergenic and non-allergenic components and the amount of allergen can vary markedly. When administrated for immunotherapy, serious anaphylactic reaction may probably occur in super hypersensitive patients. On the other hand, non-allergenic components will influence specificity and sensitivity of the diagnostic tests[11]. Therefore, it is vital to isolate, identify, and purify the major allergens that have good bioactivity.

Most domestic researchers make use of chromatography to isolate the components of crude pollen extracts, and then analyze the molecular weight of different proteins by SDS-PAGE, and determine the activity of different proteins with WesternBblot and skin test in the patients of allergic rhinitis and asthma, Through the three steps above, the major and minor allergens can be determined. In China, *Brassica* pollen[12], French *Firmiana* pollen[13], *Humulus* pollen[14], *Platanus hispanica* pollen[15], *Artemisia* pollen[16-17], and some other pollen have been purified and analyzed. But only with this step, the quality of allergen is far sufficient to meet the demand of biotechnology of drug production and clinical application. For example, Huang[12] succeeded to have identified the main pollen allergen component of rape by purification and allergenicity test, but the component could be isolated into two isoelectric point belts after isoelectric focusing electrophoresis test. Compared with the methods mentioned above, two-dimensional electrophoresis (2-DE) has higher resolution. For example, Zhang studied the possibility of two-dimensional electrophoresis in protein analysis of *Artemisia* allergen extract, and found four obvious bands in SDS-PAGE, but 85 protein components in 2- DE[18]. Now this technology is ever widely used in the

protein field.

There is no general rule for isolating every protein because of the difference in contents, physical, and chemical characters. The allergen of high purity may be obtained by recombinant DNA technology.

Study on Pollen Recombinant Allergen

The international or internal standard of allergens requires that the amount of specific allergen protein should be measured, or the measured protein should be purified and characterized[19]. But it is difficult and expensive to purify the natural allergen from pollen. Compared to natural allergen, recombinant allergens clearly offer numerous advantages, such as high yields, invariable production conditions, easier purification and standardization. Many researches have shown that most of recombinant allergens have the same or similar bioactivity as their natural counterparts[20]. So, recombinant allergens have gradually become a research focus in allergy study.

The molecular cloning of allergen genes is the foundation for the study of recombinant allergens. There are two commonly used techniques. One is constructing a cDNA expression library and then screening the libraries use sera from allergic patients containing specific IgE. Liu constructed a cDNA expression library of *Artemisia apiacea* pollen[21] and isolated a major allergen named Art a 1 from the cDNA library. After over expression in *E.coli*, a 22.7 kD recombinant protein was highly purified through one-step affinity chromatography and the immunoassay showed that this recombinant allergen had good IgE-binding capacity[21] (unpublished data). The other one is isolating single band of the allergen and analyzing its amino acid sequence, then cloning the correspond gene with primers designed base on the N-terminal and C-terminal sequence of the allergen. The disadvantages of these two methods are complicated and time-consuming. How to simply and rapidly clone the allergen gene from non-study species becomes an issue of concern. Tao *et al.* designed degenerate primers on the basis of the bioinformatic analysis of numerous allergens available from the database, and obtained full-length cDNA from *humulus* and short ragweed pollen by Touchdown-gradient PCR. Sequence analysis showed that these new genes shared as high as 79%-85% homology with well-known allergen profilins[22-23]. It is obvious that bioinformation provides a shortcut to analyze recombinant allergens.

The key step to produce recombinant allergens of high quality is to choose the suitable expression system. The most commonly used host is *E. coli*. High-level expression of correctly folded and soluble proteins may be achieved with some allergens, whereas others are expressed as insoluble inclusion bodies (IB). The latter often have the advantage that quite pure protein preparations can be obtained from the washed IB, but an in vitro solubilisation/refolding strategy has to be established to recover the recombinant allergen in a soluble form. Eukaryotic expression systems such as the yeast Pichia pastoris can modify and correctly fold the expression protein.

Clinical Application of Pollen Allergen

The pollen extracts can be used for specific diagnosis of allergic diseases. The diagnostic methods include *in vitro* and *in vivo* test. Chinese physicians frequently utilize *in vivo* test, especially skin test because of its high specificity and sensitivity although it may brings discomfort to patients. Nowadays, some major hospitals spend a lot of money on the Pharmacia CAP auto detection system for in vitro IgE test. But generally determination of specific serum IgE is considered less sensitive than skin prick tests[24].

The pollen extracts are also available for specific immunotherapy (SIT). Many years of clinical trial has confirmed that the treatment of the patients with the increasing doses of pollen crude extracts can achieve good effects before the arriving of the peaks of floating pollen. During 1999-2000, the anti-epidemic station of Zibo city treated 864 patients of allergic rhinitis with pollen allergen extracts, and the results showed that the effective rate of experiment group was 76.7% and that of the control group was 24.0% ($P<0.01$)[25]. The therapeutic effects of SIT had been evaluated in other regions of China, and the effective rate ranged from 70% to 80%. SIT can reduce the symptoms of pollinosis, especially in preventing the transition from allergic rhinitis to allergic asthma. The study on SIT with purified pollen allergen also has been reported. During 1999-2001, Sun randomly divided 86 patients with seasonal *humulus* pollen allergic asthma into two groups: observation group (group A) and control group (group B), which were administered SIT with purified and crude *humulus* pollen allergens, respectively. The study revealed that the general effect of SIT with purified allergens in group A was 90.7% (39/43), while that with crude allergens in group B was 79.1% (34/43) ($P<0.05$). The patients' CD_4 and CD_4/CD_8 decreased significantly and CD_8 increased significantly after SIT, especially in group A. Obviously, the effects of purified *humulus* pollen allergens in SIT were higher than that of crude allergens[26]. Compared with pollen crude extracts, recombinant pollen allergens will strongly improve *in vitro* as well as *in vivo* diagnosis of pollen allergy because of their high purity. Some research groups in

our country are performing skin prick tests or intradermal tests with recombinant pollen allergens to evaluate their allerginicity and clinical doses. Current studies showed that when recombinant pollen allergens were used for skin test, the concentrations ranging from 5 to 100 ug/mL could achieve good diagnostic effect[27]. The diagnostic sensitivity can be increased significantly by using panels of recombinant allergen (cocktail) which cover the most important allergen molecules present in a natural allergen extract.

Generally, SIT with recombinant allergen does not lead to an increased risk of side effects, and would allow higher dose administration of allergen comparing with pollen extracts. Some researchers have combined recombinant allergens with certain adjuvants in order to achieve good effects for SIT. The commonly used adjuvant is $AL(OH)_3$, which not only releases drugs slowly but also down-regulates TH_1. Other approaches to SIT are the use of other adjuvants including allergens coupled to interleukin-12, interleukin-18, and lectins.

PROSPECT

In a word, the study on pollen allergen in China has been far behind that in the advanced countries such as USA and European countries. For example, there are more than 700 kinds of pollen allergen genes which have been registered in the NCBI database. Among them, only a few are registered by Chinese researchers. To our joy, however, recently many research groups have taken more effort in this field. It is believed that allergens microarray chip and standardized allergen preparation with our own copyright will be invented in the near future.

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