Development and Future of Allergy Diagnostic Reagents for Screening Test

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OVERVIEW: Allergy tests consist of skin testing and in vitro testing. Currently, in vitro testing is more prevalent because it is easy to use and less burden on the patient's body compared to skin testing. Out of the in vitro testing methods, measuring specific IgE antibodies in the blood is used most widely because it is a simple method that provides quantitative result. The first big step in allergy treatment is to identify the allergen. To identify an allergen, specific IgE antibody tests are gaining a lot of attention. Hitachi Chemical Co., Ltd.'s allergy diagnostic reagents can measure 26 allergen-specific IgE antibodies from 200 μL of blood serum. Now we are developing new reagents that increase the number of measurable allergens in a 200-μL sample to 34 and to shorten the measurement time by over half. Research is advancing to discover allergy onset-related genes. In the future ethical and social policies to govern allergy gene testing will be formulated and allergy gene screening will likely become a mainstream diagnostic tool.

INTRODUCTION

IN recent years, the number of people who suffer from allergies has increased. In Japan, over half of men and women of all ages have some form of allergy-related ailment. Unfortunately, allergy disease has no established method of treatment. A person who has an allergy must suffer through long periods with little more than instructions not to come into contact with the allergy-causing allergen and a prescription for an antihistamine or an anti-allergy medicine.

When allergy symptoms appear, it is essential to perform appropriate testing and to identify the causal allergen. Once identified, it is important to eliminate any environmental or lifestyle factors that may induce allergic reaction.

Allergy testing includes skin tests, measurements of the chemical mediators like histamine and leukotriene, and blood tests that measure specific IgE antibodies. In most cases, doctors measure specific IgE antibodies in the blood because the burden on the patient is light, the measurement is easy to make, and the results produce quantitative values (see Fig. 1).

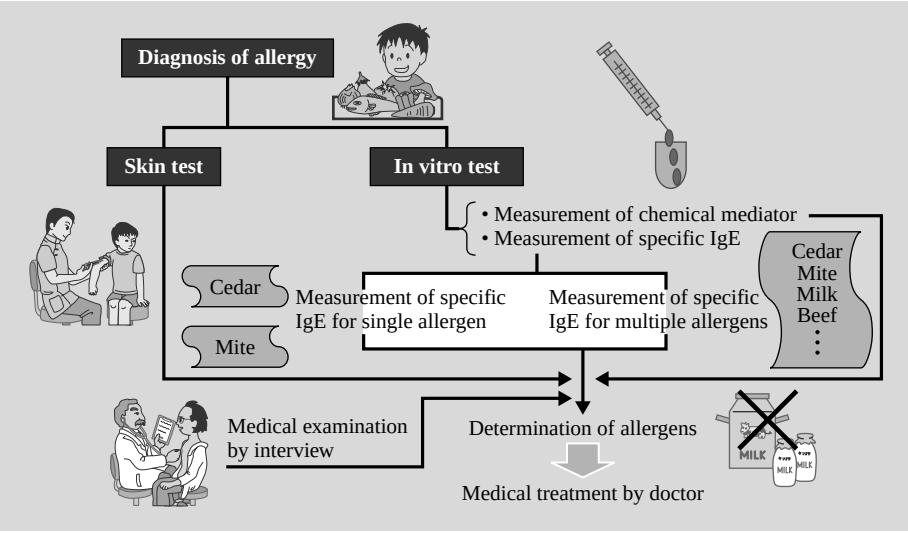

Fig. 1—Allergy Testing Overview. Allergy testing is broadly divided into skin tests and in vitro tests. Between the two types, multiple-item testing of specific IgE antibodies is the most widely used screening method.

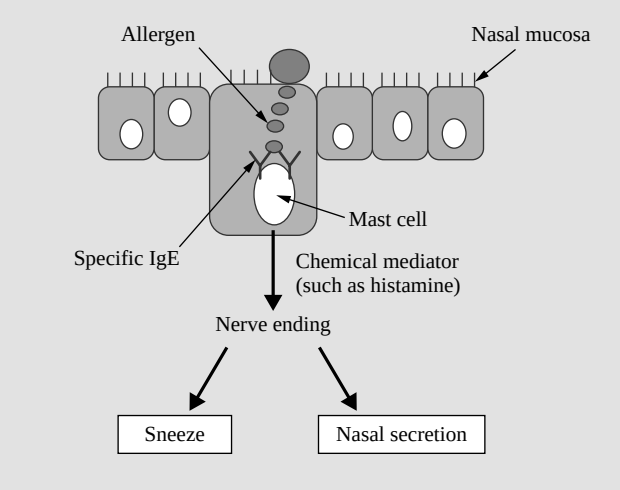

Fig. 2—Allergy Onset Mechanism.
Specific IgE antibodies on mast cells and basophils bind with allergen and the cells become active. The activated cells release chemical mediators like histamine. The mediators stimulate the nerve endings, which cause sneezing and runny nose.

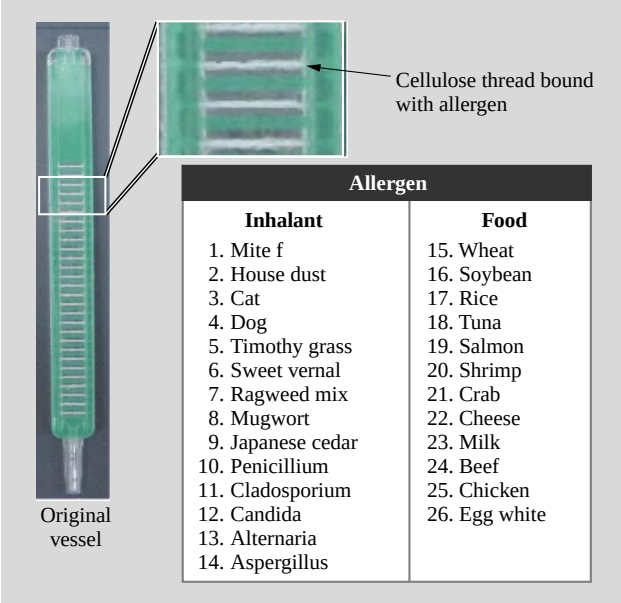

Fig. 3—Reagent Overview.
Cellulose thread is used to immobilize allergens. In 200 μL of serum, 26 allergen-specific IgE antibodies can be measured.

Hitachi researches and develops allergy diagnostic reagents and markets reagents that measure specific IgE antibodies.

Following is a discussion of current allergy testing and future outlook with an emphasis on allergy diagnostic reagents.

ALLERGY TESTING

Allergy Onset Mechanism

As an example of an allergy onset mechanism, we will take a look at hay fever. Normally, when pollen comes into contact with nasal mucous membrane, protein on the outer membrane of the pollen starts to diffuse after a couple of seconds. After a couple of minutes, protein on the inner membrane diffuses from the germ pore of the pollen[1]. Cedar pollen has no germ pore, so it simply swells as it absorbs water; then as it ruptures, allergen is eluted out. The eluted allergen is present on the outermost layer of the nasal mucous membrane. On this layer it reacts with specific IgE antibodies on mast cells and on basophils, which activates them. The activated cells release chemical mediators like histamine and leukotriene. These mediators stimulate nerve endings, which cause sneezing, runny nose and tearing (see Fig. 2).

Allergy Testing Reagents

Among allergy test methods, intradermal and scratch tests involving the skin carry the risk of shock. To lower the burden on the patient as much as possible, in vitro testing is widely used. In vitro measurement is divided into two methods: chemical mediator measurement and specific IgE antibody measurement. However, to measure a chemical mediator, cells must be stimulated by allergen in advance so that histamine is released. This process takes a lot of time and trouble.

On the other hand, to measure specific IgE antibodies, a doctor only needs to collect blood from a patient and separate out the serum. This process is very easy to do and thus widely used.

The measuring reagents for specific IgE antibodies are divided into two types: single-item test and multiple-item test. In a single-item test, a doctor first interviews a patient to estimate the cause of the allergy then he selects and measures allergens one by one using a reagent. In a multiple-item test, a doctor can measure many allergens at the same time. This latter type is often used for screening. In allergy diagnostics, identifying the causal allergen through a medical examination interview is very important. However, measuring specific IgE antibodies through screening is a very effective supplementary method.

ALLERGY SCREENING

Hitachi Allergy Diagnostic Reagents

The reagents developed by Hitachi for allergy screening can measure multiple items. In 200 μL of serum, 26 allergen-specific IgE antibodies can be measured (see Fig. 3).

No.	Allergen	Value	Class
Inhalant			
01	Mite f	OVER	3	* * * *
02	House dust	31.70	3	* * * *
03	Cat	41.20	3	* * * *
04	Dog	41.10	3	* * * *
05	Timothy grass	OVER	3	* * * *
06	Sweet vernal	65.80	3	* * * *
07	Ragweed mix	8.74	1	* *
08	Mugwort	46.10	3	* * * *
09	Japanese cedar	2.89	1/0	*
10	Penicillium	2.60	1/0	*
11	Cladosporium	1.81	1/0	*
12	Candida	2.58	1/0	*
13	Alternaria	2.66	1/0	*
14	Aspergillus	5.00	1	* *
Food			
15	Wheat	8.03	1	* *
16	Soybean	10.70	1	* *
17	Rice	34.10	3	* * * *
18	Tuna	0.00	0	
19	Salmon	0.72	0	
20	Shrimp	2.14	1/0	*
21	Crab	2.52	1/0	*
22	Cheese	0.30	0	
23	Milk	0.89	0	
24	Beef	0.82	0	
25	Chicken	1.78	1/0	*
26	Egg white	5.09	1	* *

Fig. 4—Reagent Measurement Result.
Antibody concentrations for each allergen are divided into five classes (0, 1/0, 1, 2, and 3).

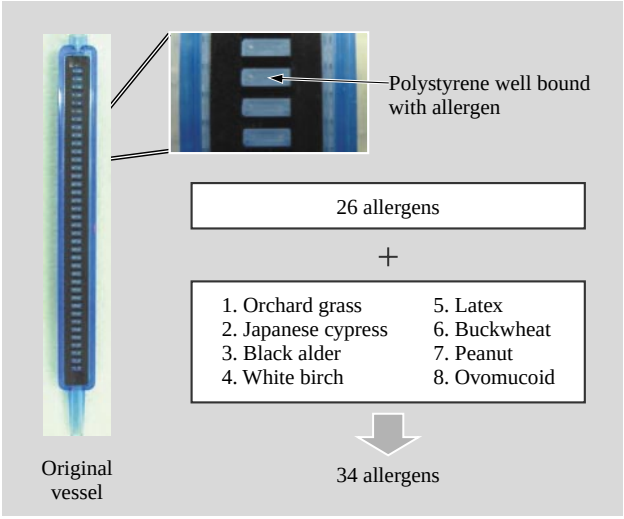

Fig. 5—New Allergy Diagnostic Reagent Overview.
Polystyrene is used to immobilize allergen. In 200 µL of serum, 34 allergen-specific IgE antibodies can be measured.

The 26 allergens are divided into an inhalant type (such as cedar and Mite f) and a food type (such as shrimp and soybean). These 26 items cover nearly all allergens and provide sufficient screening performance.

The measurement principle incorporates ELISA (enzyme-linked immunosorbent assay) method. Cellulose threads inside a specially designed reaction container called an original vessel are used to immobilize each allergen. The first reaction involves patient serum and the second reaction involves peroxidase-labeling anti-human IgE antibody. If an allergen-specific IgE antibody is present in the serum, a complex is formed that contains allergen, specific IgE antibody, and peroxidase-labeling anti-human IgE antibody. Luminol and hydrogen peroxide are added to this complex to cause a chemiluminescent reaction. The specific IgE antibody concentration is then calculated from the luminescence units. The measurement result is converted to an easy-to-understand visual format that shows comparative allergen strength (see Fig. 4).

A single measurement reagent requires more blood samples as the number of tests increase, which can become problematic when measuring allergens in a child who can only give a limited amount of blood. Hitachi's allergy diagnostic reagents have a big advantage in their ease-of-use with children. However, changes in living environments are producing new allergens that affect people. To identify new allergens, allergy clinics are now demanding screening reagents with even better performance.

In response, Hitachi has begun developing an allergy diagnostic reagent system that can test up to 34 types of allergens in a 200-µL blood serum sample.

New Allergy Diagnostic Reagents

The eight newly developed allergens (orchard grass, Japanese cypress, black alder, white birch, latex, buckwheat, peanut, and ovomucoid) bring the total number of measurement items to 34 (see Fig. 5).

During development of the new reagents, the cellulose threads were changed to solid-phase polystylene, which increases the quantity of immobilized allergen, and both sensitivity and dynamic range performance were improved. Improving sensitivity drastically reduces the measurement time from one day to six hours. To reduce the amount of blood serum required for testing, the original vessel's design must be as small as possible. However, a smaller volume causes washing efficiency to drop. So the vessel cannot be smaller than 200 µL. Finally, it was decided to dilute the serum, combine it with an enhancing agent, and inject it into the original vessel. This solution made it possible to measure 34 allergens using 200 µL of serum.

FUTURE ALLERGY TESTING

Microchip Allergy Diagnostic Reagents

In the future, integration onto a microchip will enable an allergy diagnostic reagent system to have smaller specimens with more allergen items and shorter test times. As in the case of immuno-chromatography, in the future, doctors will increasingly demand reagents that are low priced and easy to use.

Allergy-related Genes

In recent years, a completely different type of method than traditional allergy testing has begun to attract attention. Atopic dermatisis and bronchial asthma allergies are similar to diabetes and hypertension in that they are multifactorial hereditary diseases. From early on, doctors have understood the important role genetic and environmental factors play in the onset of multifactorial hereditary diseases. In contrast to a typical hereditary disease, a person may have a gene that easily causes a certain disease, but due to environmental factors the disease may not appear. The onset of an allergy may involve several hereditary factors such as the production and the action of an IgE antibody or a cytokine (an information mediator between cells such as interleukine). And multiple genes may participate in the adjustment to an inflammatory reaction.

With the recent sequencing of the complete human genome, large-scale SNP (single nucleotide polymorphism) analysis has become possible along with systematic related analysis. SNP is a variant in the DNA at one site that occurs once every 1,000 base pairs. This variation is thought to be the cause of individual difference among people. SNP analysis is used to quantify specific SNP expression differences between a disease group and a non-disease group. Expression differences lead to further analysis to locate the gene that causes the onset of an allergy disease.

Two methods have been developed to find the causal gene of a disease. One method is the candidate gene approach, which selects a candidate gene based on past knowledge of related diseases. The other method is linkage disequilibrium mapping, which determines the site of the disease susceptibility gene from linkage disequilibrium strength. The candidate approach has been used to shed light on many genes, such as interleukine 4 and 13, related to the onset of allergies. On the other hand, the Riken SNP Research Center is advancing linkage disequilibrium mapping on a large scale. Researchers are showing how SNP can be used for disease onset risk diagnostics and appropriate drug use diagnostics. Ethical and social policies to regulate allergy onset-related gene diagnostics have not been formulated yet. So clinical application is still on the horizon. However, in the future, gene diagnostics will likely become a mainstream tool.

CONCLUSIONS

This paper has described the allergy onset mechanism and testing methods. Allergies, allergens, and allergy sufferers are all likely to increase in the future. To aid sufferers, allergy screening is gaining a lot of attention. Doctors are highly anticipating the arrival of an allergy reagent system that can measure up to 34 allergens in a single 200-μL-serum sample. Hitachi aims to successfully complete development and bring a product to market.

In the future, Hitachi would like to turn its attention to allergy onset-related gene diagnostics.

REFERENCE

(1) T. Sawazaki et al., “Comparison of Antigenicity of Japanese Cedar Pollen (51 Varieties),” *Hitachi Chemical Technical Report* **28**, pp. 41–44 (1997).

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