

Trends in Food Allergy Research

JUNKO SHIMADA (*Life Science and Medical Research Unit*)*

HIROYUKI YANO (*Affiliated Fellow*)**

KOKO MIZUMACHI (*Affiliated Fellow****)



1 Introduction

The incidence of allergic diseases such as food allergy, atopic dermatitis, bronchial asthma and cedar pollinosis continues to increase, mainly in developed countries, and has grown into a serious problem worldwide. In particular, food allergy, often developed during infancy, can affect a child's growth or trigger an “allergic march,” i.e., the progressive development of various allergies. Consequently, early prevention and treatment are recommended for food allergy.

The incidence of food allergy continues to increase year by year. In 1997, a questionnaire survey conducted by the Ministry of Health and Welfare (currently Ministry of Health, Labour and Welfare) revealed a high incidence of food allergy not only among children (8.6% among children aged 3) but also among adults (9.3%) (Food Allergy Study Group Report for 1997, 1998). Conventionally, food allergy developed during childhood has been the major concern, but an increasing number of adults are developing allergies to seafood and fruit, and cross-reactivity (i.e., reactivity against structurally common antigens) with cedar pollen is suggested. Thus, we should urgently study and understand food allergy and find fundamental solutions.

The second Science and Technology Basic Plan (approved at the Cabinet meeting on March 30, 2001) depicts the “realization of a nation securing a comfortable, safe and high quality of life” as one of the three “pictures of the nation and of the

science and technology policy to be attained.” Research and development of food products that are effective against food allergy can contribute to realizing such a nation and should therefore be promoted.

The development of allergic diseases seems to involve complex interactions between genetic factors and diet and other environmental factors. We must consider the genetic diversity of the human race when conducting research and development in this area.

This article summarizes the status and mechanism of food allergy and presents trends in research on hypoallergenic and immunosuppressive food products.

2 Food allergy

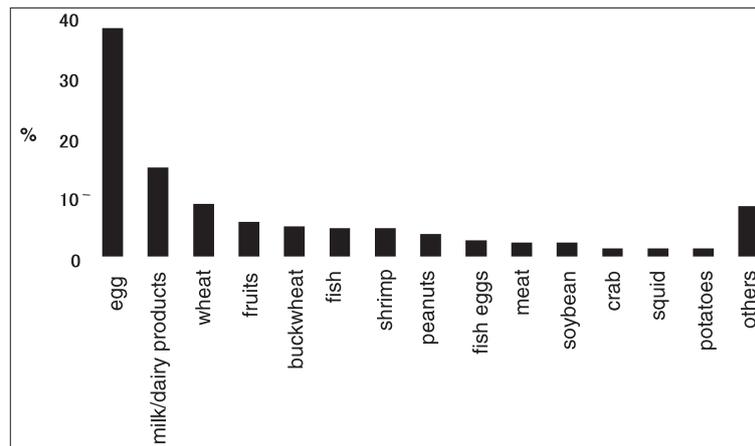
2-1 What is food allergy?

Food allergy is an exaggerated immune response induced by the misrecognition of orally ingested food as a foreign substance. It is a hypersensitive reaction that damages the self, with symptoms that can include diarrhea, abdominal pain, hives and eczema. Severe cases of food allergy can induce anaphylactic shock*¹, which may lead to death. People show allergies to different kinds of food and experience different symptoms depending on their physical condition. Moreover, food allergy can also be induced through cross-reaction*². Complex interactions between the body and allergens (substances causing allergy) result in a variety of responses and symptoms, making the prophylaxis and

** Hiroyuki Yano National Agricultural Research Center of the National Agriculture and Bio-oriented Research Organization <http://narc.naro.affrc.go.jp/>

***Koko Mizumachi National Institute of Livestock and Grassland Science of National Agriculture and Bio-oriented Research Organization <http://nilgs.naro.affrc.go.jp/>

Figure 1 : Foods that can cause allergy



Based on a survey conducted in 2001 on 2,434 subjects who visited medical institutions for symptoms developed within 60 minutes after food ingestion.
Source: Reference^[4].

treatment of food allergy difficult.

The development of food allergy involves both genetic and environmental factors. Genes involved in the immune system show genetic diversity, and a high degree of genetic polymorphism, which is responsible for our individual constitutions, is found between Japanese people and Western people^[1-3]. Infectious disease history during childhood, exposure to environmental pollutants, antigen levels and diet are possible environmental factors related to allergy. In addition, the hygiene hypothesis^{*3} suggests that the increase in allergic diseases can be attributed to the decreased incidence of infections during childhood due to improvements in living standards and public health.

2-2 Food allergens

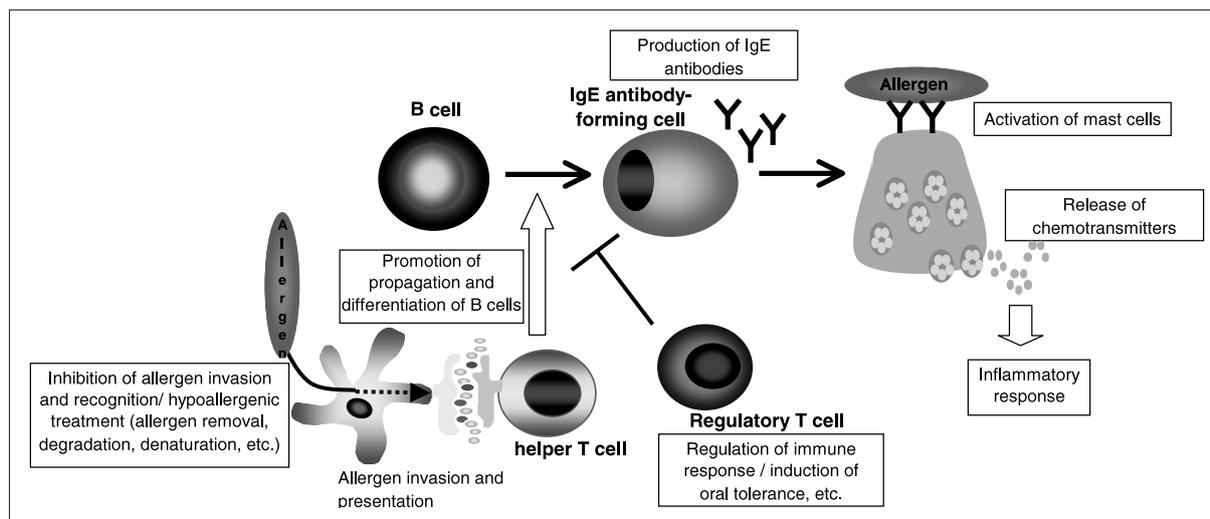
Food allergy is caused by various foods including eggs, milk, wheat, beans, buckwheat, fruit, seafood and meat (Figure 1)^[4]. The kinds of food causing allergy vary by age; eggs and milk are the most common allergens among infants, but most overcome these allergies as they grow up. Meanwhile, food allergies in adults are mainly caused by seafood or fruit.

To prevent exposing people allergic to specific food allergens to health risks, labeling has become mandatory for food products containing such allergens. The food labeling system came into force in April 2002, based on the Food Sanitation Law revised in April 2001 in response

to “A report on the discussion concerning the food labeling system (1998).” According to the incidence and severity of allergies, foods are categorized into two groups; labeling is compulsory for the first group of five foods (eggs, milk, wheat, buckwheat and peanuts) and encouraged for the second group of nineteen other foods (abalone, salmon roe, prawn, oranges, crab, kiwifruit, beef, walnuts, salmon, mackerel, soybeans, chicken, pork, matsutake mushrooms, peach, yam, apple and gelatin). A report suggesting the addition of bananas to the second group was submitted in July 2004. Despite the establishment of this compulsory labeling system, allergen contamination during manufacturing and accidents due to oversight caused by confusing labels have been reported. Thus, it is essential to popularize the system, improve the distribution channels that provide the necessary information to the people who need it, and develop techniques for detecting contamination with allergens.

2-3 History of food allergy research

Food allergy has been recognized since pre-Christian times; Lucretius of Greece once said, “One man’s food might be another man’s poison.” In the 18th and 19th centuries, wheat asthma and hay fever (a kind of pollenosis) were recognized among bakers. In the early 20th century, Pirquet proposed the concept of allergy (defined as “a temporarily, quantitatively and qualitatively altered capacity of the body to react

Figure 2 : Immune mechanism underlying immediate allergy

to external antigens”), a rather broad definition covering the entire immune response. The current definition of allergy is much narrower: “a disease mediated by the immune response induced by an otherwise harmless antigen.”

In Japan, the Japanese Society of Allergology was established in 1952 and started research on the diagnosis and treatment of food allergy.

2-4 Mechanism of the immune response

The food we eat is digested and absorbed by our stomachs and intestines. Food contains an enormous variety of foreign proteins, which are foreign substances (i.e., not “self”) to our bodies, but usually, inappropriate immune reactions are suppressed by the gastrointestinal immune system or the mucosal immune system in the gut. The failure of this system can result in allergic reactions.

(1) Mechanism of allergy development

Most cases of food allergy are the ‘immediate’ type in which symptoms develop within 2 hours after the causal food has been ingested. Food allergens absorbed through the digestive tract induce immediate reactions in the alimentary canal mucosa, since this is where they make direct contact with the body. This leads to cardinal symptoms such as vomiting, abdominal pain and diarrhea. In addition, the allergens absorbed through the digestive tract are systemically delivered via the blood vessels to induce symptoms such as rhinitis in the respiratory tract or symptoms such as

hives and angioedema on the skin. They also seem to be involved with the development of atopic dermatitis or anaphylactic shock, which is accompanied by systemic symptoms such as breathing difficulty and decrease in blood pressure, and damages various organs.

Such symptoms are the result of the inflammatory response induced by the reaction between the allergens and immunocompetent cells. Allergens ingested and absorbed by the body are taken up by the antigen-presenting cells, which are then recognized by T-cells. The T-cells activate the B-cells and convert them into the IgE antibody-forming cells that produce the IgE antibodies that bind to the mast cells. Allergenic stimulation induces degranulation, i.e., the release of chemotransmitters such as leukotriene and histamine from the mast cells, and these chemotransmitters trigger an inflammatory response, inducing the allergic reaction.

Healthy individuals are equipped with certain mechanisms that suppress the inflammatory response. For example, the Th1/Th2 balance (balance between two types of T-cells, Th1 and Th2) is adequately maintained in healthy individuals to suppress IgE antibody production; however, in patients with food allergy, the Th1/Th2 balance shifts toward Th2-dominant immunity, allowing IgE antibody to proceed out of control.

(2) Oral tolerance

Food contains a vast range of foreign proteins, which are foreign substances (i.e., not “self”) to

our bodies. The antigenic regions of proteins are mostly degraded by digestive enzymes during food digestion, but a small proportion may be absorbed by the body without losing antigenicity. Nevertheless, our bodies do not produce excessive immune responses against the orally ingested, digested and absorbed food antigens. This phenomenon is called oral tolerance^[5,6].

People have made practical use of oral tolerance from time immemorial. Japanese lacquer craftsmen eat the lacquer to prevent lacquer poisoning. In experiments using mice or guinea pigs, the oral administration of an antigen before the subcutaneous or intraperitoneal injection of the same antigen can suppress immunoglobulin production and anaphylactic shock.

Oral tolerance is an excellent system for obtaining the required nutrients without inducing an excessive immune response against food antigens that are otherwise foreign substances to our bodies. In patients with food allergy, this system may fail to function properly for a number of reasons. Therefore, it is necessary to understand the mechanism of oral tolerance and find an effective approach for inducing the phenomenon. However, a complete understanding of the mechanism has been hindered because of the variety of contents in our digestive tracts, i.e., various microbes and miscellaneous antigens derived from food and other things, as well as the variety of immunocompetent cells involved in oral tolerance.

(3) Mucosal immunity in the gut

The digestive tract forms “an exterior environment within the body”; it is continuously exposed to large quantities of foreign substances such as food and microbes via the mucosa. It can be considered to be a huge immune organ that acts in the front line of the biological defense system. In adults, the total area of the intestinal mucosa is 300-400 m² (1.5 times larger than a tennis court), and the intestines hold a total population of more than 10¹⁴ microbes (about 1 kg). The microbial composition can change depending on factors such as race, food habits, age, physiological/pathological status, stress and

drug use. The microflora in the intestine (gut microflora) are significantly affected by the host's nutritional status, physiological functions, age, carcinogenesis, immune system, infection history, etc. It is also reported that gut microflora differ between children with and without allergy.

The digestive tract distinguishes useful substances, e.g., food or microbes that normally inhabit the intestine, from harmful substances, e.g., pathogenic microbes. Based on this distinction, it prevents invasion by, or eliminates antigens harmful to the body and promotes absorption of the required nutrition.

In patients with food allergy, the digestive tract cannot distinguish between useful and unwanted substances properly. Some food ingredients or microbes have been reported to enhance the making of proper distinctions, but further research is required to understand the detailed mechanism and to specify effective food ingredients and microbes.

3 Trends in research and development for overcoming food allergy

The development of food allergy could be prevented by blocking any of the steps in the allergic reaction sequence, from allergen absorption to inflammatory reaction. For example, food allergy can be prevented by blocking allergen invasion/recognition, T-cell activation, binding of allergens with IgE antibodies or release of chemotransmitters.

The first step towards conquering food allergy is understanding the mechanism of the immune response and finding ways to block the allergic reaction induced by the allergens that have entered the body. Meanwhile, to prevent allergens from entering the body, research has been conducted on removing or reducing allergens in food products. Furthermore, the use of food to enhance the immunoregulatory function inherent in the body has also been studied.

Of the various research efforts aimed at overcoming food allergy, this chapter describes the food-oriented approach, starting from research and development on hypoallergenic food developed by breaking the antigenic structures

in food. Hypoallergenic food products have been developed to prevent allergic reactions by blocking the incorporation of allergens into the body. Thus, research related to anti-allergic food, which prevents or suppresses food allergy, is introduced from the viewpoints of immunoregulation and immunosuppression.

3-1 Trends in research and development of hypoallergenic food

Currently, food allergy is mainly treated symptomatically through medication and removal of the causal food. However, the elimination of allergen-containing food from the diet of growing infants is undesirable, as it may lead to nutritional deficiency or developmental disturbance. The development of hypoallergenic food through the degradation or denaturation of allergens is essential to prevent nutritional disorders or growth disturbance and to maintain a rich and varied diet. Therefore, it is required that hypoallergenic food is nutritionally identical to normal food and shows minimum allergenic activity.

(1) Hypoallergenic food developed to date

To date, several methods for reducing allergens in crops or food products have been developed and applied to commercial products. One such example is “Fine Rice,” which has been developed through joint research between Shiseido, the Faculty of Agriculture of The University of Tokyo and the School of Medicine of Yokohama City University, and was commercialized in 1991. Fine Rice has been produced through the protease treatment of rice, which has led to the decomposition of globulin, an allergenic protein. The product was approved by the Ministry of Health and Welfare as the first “food for specified health uses” in June 1993 and as “food for medical purpose” in June 1997.

Another example of hypoallergenic rice is that developed by Mitsui Toatsu Chemicals, Inc. (currently Mitsui Chemicals, Inc.) and the National Institute for Agro-Environmental Sciences, in which the expression of allergenic proteins has been suppressed by genetic engineering. This hypoallergenic rice reached the stage of open-field cultivation by 1995 but has

still not been commercialized.

The development of hypoallergenic crops has also been attempted by screening a variety of mutants produced through radiation mutagenesis for one lacking allergen-encoding genes. The National Agricultural Research Center for Tohoku Region has developed a hypoallergenic soybean variety named “Yumeminori” lacking two of the three major allergens found in soybeans.

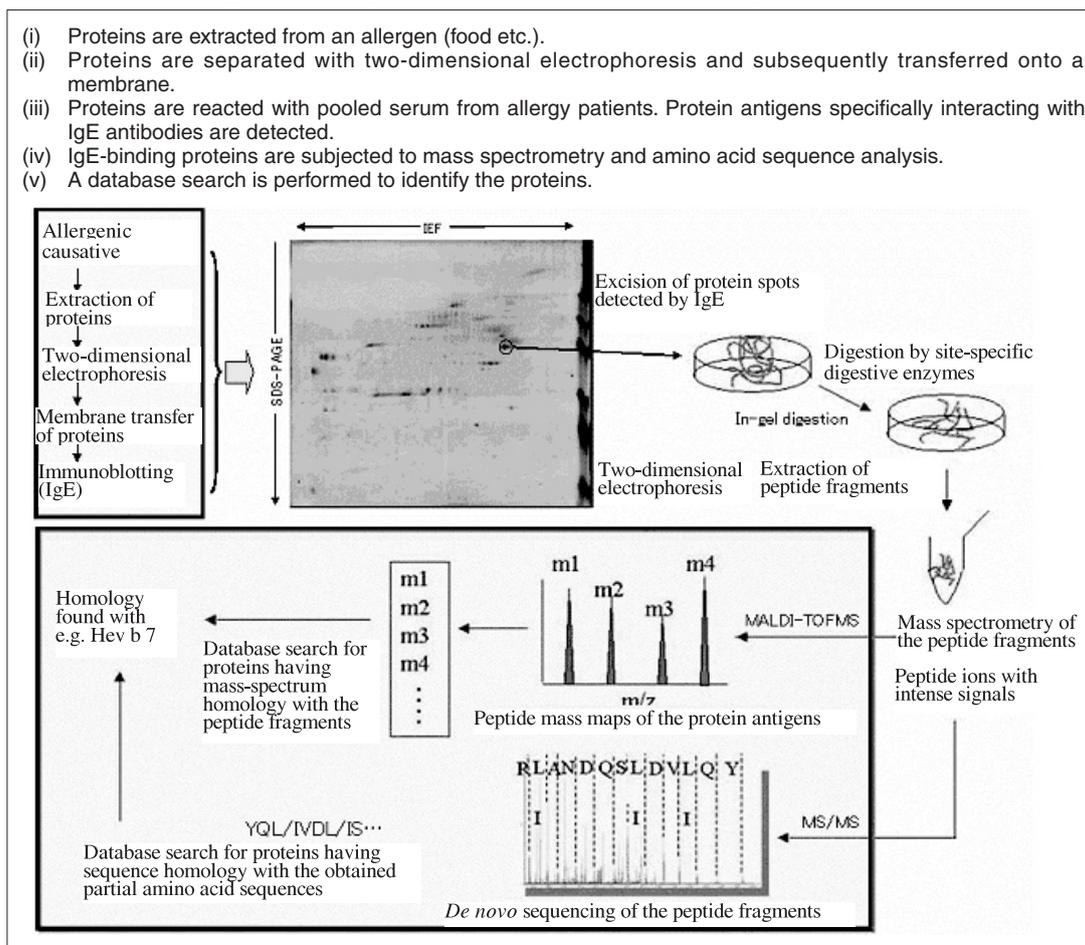
Nevertheless, the above-mentioned methods for developing hypoallergenic food have their individual limits; each protease can digest only a limited number of allergens, and the use of proteases with wide substrate specificities could lead to decreased nutritional value due to the loss of useful proteins or reduction of the product value due to rice grain damage. The genetic engineering-based approach cannot be applied to crops containing several allergens because it requires individual engineering of the genes corresponding to each allergen. The radiation mutagenesis-based approach also has its limits; the chance of damaging allergen-encoding genes is low, and evaluation can only be performed on known allergens.

(2) Allergen studies based on proteome*⁴ analysis

There have also been attempts to develop hypoallergenic food by breaking the chemical structures commonly found among allergenic foods. For example, Buchanan et al. (University of California) reduced the allergenicity of the major allergen in cereals or milk by modifying the structures of the allergy-inducing domains in the allergen using thioredoxin, a reductase that mediates the cleavage of disulfide bonds^[7]. Moreover, disulfide bonds in allergen proteins in barley seeds were broken in genetically modified barley plants that express thioredoxin in their storage organs (edible parts)^[8]. Thioredoxin is widely distributed among various organisms, so its application to food should involve little risk to health. The enzyme should serve as an important tool for developing hypoallergenic food.

Several allergens can be reduced at once by breaking the structures they share in common that cause allergy. To expand the application of this approach to other allergens, structures

Figure 3 : Allergen studies based on proteome analysis approach



Source: Reprinted from reference^[9] with permission

common to other allergens must be identified, and methods for their safe destruction must be established. As a consequence, proteome analysis has been adopted for the comprehensive detection of allergens.

This approach can detect allergens by comprehensively analyzing the proteins that react with the antibodies found in the serum of patients with allergy. Figure 3 shows an example of this approach.

This method is potentially applicable to the screening of allergens, including food and house dust, and its future looks promising. Structural analysis of the detected allergens and identification of their common molecular structures should contribute to the development of hypoallergenic food. Such structures can be detected efficiently by performing structural analysis on a large number of allergens and establishing a searchable database.

3-2 Trends in research and development of anti-allergic food

In addition to the development of hypoallergenic food, the use of food to achieve immunoregulation and immunosuppression has been studied. Anti-allergic food prevents or controls food allergy by inducing oral tolerance or utilizing gut immunity and anti-allergic food components.

(1) Anti-allergic food based on oral tolerance induction

Oral tolerance is a phenomenon in which proteins in orally ingested food do not induce excessive immune responses, despite the fact that food has a vast amount of antigenic substances containing foreign proteins. Food allergy is induced when a specific allergen is orally ingested and absorbed by the body via the digestive tract. Japanese lacquer craftsmen eat lacquer to prevent lacquer poisoning, but eating the causal food does not prevent food allergy. To

apply oral tolerance to preventing food allergy, the causal food must be processed in a certain way before being ingested.

It has been reported that oral tolerance to a certain antigen is induced by the protein itself or by a peptide of the protein. In other words, it may be possible to suppress an allergic reaction by inducing oral tolerance using a peptide that reacts with the T-cell but does not bind to the IgE antibody involved in the allergic reaction.

In mice, this method has successfully induced oral tolerance to milk or egg allergens^[6,10].

Although many steps remain before this technique can be applied to human beings, such as determining the appropriate peptides and their optimum doses, these peptides potentially serve as allergy-preventive food that also provides antigen-specific immunotherapy.

(2) Anti-allergic food based on mucosal immunity in the gut

Probiotics are defined as living microbes that improve the microflora in the intestine (gut microflora) and are beneficial to the host, or as food containing such microbes. Microbes such as lactic bacteria in cheese, yogurt and other fermented dairy products improve the storage quality and taste of milk and its nutritional value through proteolysis, lactose degradation and vitamin synthesis. They also improve the gut microflora and have other health effects such as intestinal regulation, normotension and immunostimulation. Moreover, substances such as oligosaccharides enhance the propagation of probiotics.

Recently, probiotics have attracted attention for their effect of enhancing the immunoregulatory function of the digestive tract and suppressing or ameliorating allergic symptoms. There is a difference in gut microflora between people with and without allergy, and smaller numbers of lactobacillus, a kind of lactic bacteria, have been found in the former compared to the latter group. This report has triggered a series of research projects on probiotics including lactic bacteria and the development of probiotic-based products with anti-allergic effects. The consumption of a lactic bacterium called lactobacillus CG by pregnant women and their babies reduced the

incidence of atopic dermatitis among these infants at the age of two^[12]. In addition, various lactic bacteria and bifidus bacteria have been confirmed to exert anti-allergic actions such as reducing IgE antibodies in mice and humans. Scientific demonstration of the anti-allergic actions of probiotics has enhanced the development of probiotic-related products.

These microbes potentially inhibit the development of allergy through their active interaction with the body. However, their effects are strain-specific, and their mechanisms are poorly understood. Their application requires further research, including evaluation of their safety.

(3) Use of anti-allergic components

One way to prevent the development of food allergy is to suppress the production or action of chemotransmitters such as histamine and leukotriene that trigger the inflammatory response in the allergic reaction sequence. The production or action of chemotransmitters is called anti-allergic action. While hypoallergenic food involves antigen-specific suppression, anti-allergic action works in a non-specific manner, suppressing allergic reactions independent of the allergen type.

Many food components are known to have anti-allergic actions. For example, highly unsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, which are abundant in fish, suppress leukotriene production, and tea polyphenol suppresses histamine and leukotriene release. Anti-allergic actions have also been reported in tea catechin and caffeine. Additional anti-allergic components have been identified in various foods, including flavonoids, sesamin and perilla leaf extract.

Anti-allergic activity levels differ among the above-mentioned food components and also according to the variety of crop and when they are harvested. For instance, methylated catechin is a tea component showing a strong anti-allergic action, but its content differs among tea varieties (oolong tea, black tea or green tea); little is found in the green tea variety, "Yabukita," but it is abundant in dong-ding oolong and "Benifuuki," a variety developed for black tea. Moreover,

the catechin content changes according to the timing of the harvest or during tea processing. Meanwhile, the anti-allergic activity is substantially the same in the leaf extracts of green and red perilla.

Some anti-allergic components are known to act synergistically with others; for example, the combination of sesamin contained in sesame, and-tocopherol, a Vitamin E contained in vegetable oils and fats, exerts a strong anti-allergic effect.

To make effective use of the anti-allergic actions of food consumed daily, there must be further discussion of ways to achieve adequate intake and the synergistic actions of different food components, based on the facts that anti-allergic activities differ according to the variety of crop and when it is harvested, and that the level of active components can be altered by food processing.

4 | Conclusions

This article has introduced research and development in hypoallergenic food and the use of food to prevent and control allergy.

Hypoallergenic food is essential for those who have already developed food allergy, from the standpoint of preventing the development of allergic reactions and improving their diet. It also eliminates the risk of nutritional disorders caused by the long-term elimination of food during childhood. Nevertheless, further research is required for developing hypoallergenic food effective against various allergens while maintaining taste and nutritional value.

As for research on food that prevents and controls allergy, the mechanism for oral tolerance induction and interactions between gut microflora and other food antigens in the digestive tract remains to be understood. There are great expectations for such food, as it can directly interact with the body to suppress allergic reactions.

The development of allergic diseases involves complex interactions between genetic factors and diet and other environmental factors. The genes involved in the immune system show

significant genetic diversity. A high degree of genetic polymorphism, which is responsible for our individual constitutions (SNP = single nucleotide polymorphism), can be found between Japanese people and non-Japanese people. Thus, it is essential to consider the genotypes and diets of the Japanese for developing anti-allergic food effective in preventing or treating allergy.

Future tasks in food allergy research include characterizing food allergens, establishing allergenicity evaluation systems, understanding the mechanism of food allergy development, and confirming the relationship between food allergy and environmental factors. While these tasks are certainly important, it is even more important to establish a system for assessing the efficacy and safety of newly developed food products that are effective against food allergy. Moreover, it is important to find appropriate ways to apply the knowledge gained, to improve the lives of patients with food allergy.

Pharmaceutical products have evaluation systems for assessing their efficacy and safety prior to approving them as pharmaceuticals. However, food products effective against food allergy are not regarded as pharmaceutical products, so they lack equivalent systems. Allergenic foods that are harmless and beneficial to healthy individuals may be harmful or lethal to certain other individuals, which makes evaluation difficult and hinders the establishment of evaluation systems. Some animal models have been suggested, but symptoms are not developed clearly enough to validate their use. However, even if efficacy and safety could be assessed in mice or other experimental animals, the model could not be directly applied to human beings. Thus, it is important to establish a system for evaluating efficacy and safety in humans.

Future progress in research and development requires not only setting individual research tasks but also discussing the evaluation of the efficacy and safety of anti-allergic foods and their application to allergic diseases. Therefore, cooperation with the medical profession is essential for the research and development of food products.

Glossary***1 Anaphylactic shock**

An uncontrollable allergic reaction that occurs after exposure to a causal allergen. It is associated with acute, systemic, serious, sometimes life-threatening symptoms such as convulsions, breathing difficulty and decrease in blood pressure.

***2 A cross-reaction**

A reaction between an antigen and an antibody raised against a different but structurally similar antigen. For example, a person who is allergic to chicken eggs may also develop allergic symptoms when exposed to other avian eggs.

***3 Hygiene hypothesis**

A hypothesis proposed in 1989 by Strachan. It was based on a 23-year follow-up survey conducted on 17,414 English subjects for the retention ratios of allergic diseases, the number of family members and the number of siblings. Subjects with more siblings had lower retention ratios of bronchial asthma and eczema, and subjects with later birth orders showed greater suppressive effects against an atopic disposition. Based on these results, Strachan proposed that the increase in allergic diseases can be attributed to the decreased incidence of infections during childhood due to improvement in living standards and public health. A great deal of evidence supporting this hypothesis has been found through epidemiological studies, among which are findings on the Th1/Th2 balance. During the fetal and neonatal periods, immune response relies primarily on the Th2 system, but later, the Th1 system evolves in response to stimulation by infections or harmless microbes, establishing a good balance between the Th1 and Th2 systems. Less exposure to microbes during childhood induces a Th2-dominant immune system, which is more vulnerable to allergic diseases.

***4 Proteome**

A proteome is the entire set of proteins expressed in a certain cell or tissue.

Proteome analysis consists of two steps, i.e., protein separation and identification. Usually, the proteins are separated by two-dimensional electrophoresis, and target proteins are identified by mass spectrometry.

References

- [1] Shirakawa T et al. "Atopy and asthma: genetic variants of IL-4 and IL-13 signalling" *Immunol. Today*, 21: 60-64. (2000)
- [2] Eerdewegh PV et al. "Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness." *Nature*, 418: 426-430. (2002)
- [3] Vercelli D, "Genetic polymorphism in allergy and asthma" *Curr. Opin. Immunol.*, 15: 609-613. (2003)
- [4] Takanori Imai, "Current status of immediate-type food allergy in our country". *Journal of Food Allergy Study Group*, Vol. 2 (1), pp.2-6. 2002 (in Japanese)
- [5] Strobel S and Mowat AM, Oral tolerance. *Immunol. Today* 19: 173-181 (1998)
- [6] Koko Mizumachi and Jun-ichi Kurisaki, "Mechanisms of oral tolerance and its clinical applications." *Research Journal of Food and Agriculture*, 24 (5), pp.25-30. 2001 (in Japanese)
- [7] Buchanan BB et al. "Thioredoxin-linked mitigation of allergic responses to wheat." *Proc. Natl. Acad. Sci. USA* 94: 5372-5377. (1997)
- [8] Wong JH et al. "Transgenic barley grain overexpressing thioredoxin shows evidence that the starchy endosperm communicates with the embryo and the aleurone" *Proc. Natl. Acad. Sci. USA* 99: 16325-16330. (2002)
- [9] "Allergenomics (rapid and comprehensive analysis of allergen protein candidates)": <http://dmd.nihs.go.jp/latex/allergenomics-J.gif>
- [10] Mizumachi K. et al. "Oral tolerance induced in mice by T cell epitope peptides of betalactoglobulin" *Immunol. Cell Biol.* 75 (supplement 1) A24. (1997)
- [11] Kalliomaki M. et al. "Probiotics in primary prevention of atopic disease : a randomized placebo-controlled trial" *Lancet* 357: 1076-1079. (2001)

Other References, etc.

- Edited by Zenro Ikezawa, "Development of Hypoallergenic Food" CMC Publishing Co., Ltd. 1995 (in Japanese)
- Edited by Hiroshi Kiyono, Hiromichi Ishikawa and Hiroshi Nagura, "Mucosal immunity —the intestine serves as the control tower in immunity, Nakayama Shoten Co., Ltd. 2001 (in Japanese)
- Charles A. Janeway, Jr. et al., "Immunobiology-the immune system in health and disease" 6th Edition, Garland Science Publishing, 2005

(Original Japanese version: published in February 2005)
