Present state of Japanese cedar pollinosis: The national affliction

Takechiyo Yamada, MD, PhD,[a] Hirohisa Saito, MD, PhD,[b] and Shigeharu Fujieda, MD, PhD[a] *Fukui and Tokyo, Japan*

Seasonal allergic rhinitis (SAR) caused by Japanese cedar pollen (JCP; ie, sugi-pollinosis) is the most common disease in Japan and has been considered a national affliction. More than one third of all Japanese persons have sugi-pollinosis, and this number has significantly increased in the last 2 decades. In our opinion the reason why sugi-pollinosis became a common disease in the last half century is the increased number of cedar pollens, with global climate change and forest growth caused by the tree-planting program of the Japanese government after World War II playing substantial roles; dust storms containing small particulate matter from China might also contribute to the increased incidence of sugi-pollinosis. To help minimize their symptoms, many Japanese wear facemasks and eyeglasses at all times between February and April to prevent exposure to JCP and aerosol pollutants. Forecasts for JCP levels typically follow the weather forecast in mass media broadcasts, and real-time information regarding JCP levels is also available on the Internet. Because a large amount of JCP is produced over several months, it can induce severe symptoms. Japanese guidelines for allergic rhinitis recommend prophylactic treatment with antihistamines or antileukotrienes before the start of JCP dispersion. Additionally, sublingual immunotherapy will be supported by health insurance in the summer of 2014. However, many patients with sugi-pollinosis do not find satisfactory symptom relief with currently available therapies. Collaboration between scientists and pharmaceutical companies to produce new therapeutics for the control of sugi-pollinosis symptoms is necessary. (J Allergy Clin Immunol 2014;133:632-9.)

Key words: *Seasonal allergic rhinitis, Japanese cedar, global climate change, prophylactic treatment, alternative complementary treatments*

Allergic rhinitis (AR) represents a global health care problem that greatly affects daily activity, work productivity, learning, sleep, and quality of life (QOL) in persons of all ages. In the Allergic Rhinitis and its Impact on Asthma study, AR is divided into 2 categories: intermittent or persistent disease.[1] However, many otorhinolaryngologists in Japan use a perennial allergic rhinitis (PAR) and seasonal allergic rhinitis (SAR) classification system.[2] The major allergen contributing to SAR in Japan is pollen from the Japanese cedar (*Cryptomeria japonica*; ie, sugi). SAR is caused by Japanese cedar pollen (JCP; ie, sugi-pollinosis) and was first reported in 1963.[3] During the height of the allergy season (between February and April), a large number of patients with sugi-pollinosis experience more severe symptoms for longer periods of time compared with other pollen allergies (Fig 1, *A*). This might be because JCP is dispersed in large quantities over long distances (>100 km in some cases) and can remain airborne for more than 12 hours (Fig 1, *B*).[4] Furthermore, pollen from the Japanese cypress *(Chamaecyparis obtusa)*, which also causes SAR, is dispersed in April and May, immediately after the release of JCP. Because Japanese cypress pollens are considered to contain several components that cross-react with JCP, 70% of patients with sugi-pollinosis also experience SAR caused by Japanese cypress pollen. Therefore allergic symptoms can last for as long as 4 months, from February to May, with some variation caused by annual climate differences.

A meta-analysis of 38 reports representing 27 prevalence subgroups and 134 sensitization rate subgroups showed that the prevalence of sugi-pollinosis increased 2.6-fold between 1980 and 2000.[5] The prevalence of sugi-pollinosis was 19.4% of the Japanese population in 2001.[6] We conducted a survey of 1540 persons aged 20 to 49 years in Fukui City between 2006 and 2007 that indicated the positive rate of serum JCP-specific IgE was 56.3% and the prevalence of sugi-pollinosis was 36.7%.[7] Additionally, the International Study of Asthma and Allergies in Childhood showed that Tokyo schoolchildren have an extremely high prevalence of SAR.[8,9] Specific to Japan, SAR-JCP is now called a national affliction. Manufacturers and

From [a]the Department of Otorhinolaryngology–Head & Neck Surgery, University of Fukui, and [b]the Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo.

Supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Health, Labour and Welfare (H23-Immunology-general-006) and from the Japanese Ministry of Education, Culture, Sports, Science and Technology (23390397, 22659306).

Disclosure of potential conflict of interest: T. Yamada is employed by the University of Fukui and has received research support from the Japanese Ministry of Health, Labour, and Welfare. H. Saito is employed by the National Center for Child Health and Development; has received research support from the Japanese Ministry of Health, Labour, and Welfare; has received payment for lectures from Teijin Pharma, Shiseido, Merck Sharp and Dohme K.K., Taiho Pharmaceutical, Nippon Boehringer-Ingelheim, Ono Pharmaceutical, GlaxoSmithKline K.K., Pfizer Japan, and Novartis Pharma K.K.; has received payment for manuscript preparation from Taiho Pharmaceutical; has received payment for development of educational presentations from Shimane University and Toho University; and has received travel support from Shimane University Japanese Society of Allergology, the Japanese Society of Pediatric Allergy & Clinical Immunology, and Pfizer Japan. S. Fujieda has received research support from the Japanese Ministry of Health, Labour, and Welfare, the Japanese Ministry of Education, Culture, Sports, Science and Technology, Ono Pharmaceutical, and Merck Sharp & Dohme K.K.; has consultant arrangements with Kyorin Pharmaceutical; is employed by the University of Fukui; and has received payment for lectures from Kyowa Hakko Kirin, Ono Pharmaceutical, and Merck Sharp & Dohme K.K.

Received for publication July 17, 2013; revised November 6, 2013; accepted for publication November 7, 2013.

Available online December 19, 2013.

Corresponding author: Shigeharu Fujieda, MD, PhD, Department of Otorhinolaryngology–Head & Neck Surgery, University of Fukui, 23-3 Matsuoka-Shimoaizuki, Eiheiji, Yoshida, Fukui 910-1193, Japan. E-mail: sfujieda@u-fukui.ac.jp.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.11.002

Abbreviations used
AD: Asian dust
apoA-IV: Apolipoprotein A-IV
AR: Allergic rhinitis
GWAS: Genome-wide association study
JCP: Japanese cedar pollen
PAR: Perennial allergic rhinitis
PM: Particulate matter
PM2.5: Particulate matter less than 2.5 μm in diameter
QOL: Quality of life
SAR: Seasonal allergic rhinitis
SLIT: Sublingual immunotherapy
Sugi-pollinosis: Seasonal allergic rhinitis caused by Japanese cedar

retailers are set to tap into a soaring demand for medications and related items, and the market for JCP prescription drugs has soared to 200 to 300 billion yen per season (2.1-3.2 billion US dollars). Also, the government will be required to take effective actions.

In this review we introduce sugi-pollinosis, the national affliction of Japan, with the intention of informing allergists about the spectrum of symptoms and treatment options available for patients with sugi-pollinosis.

PUTATIVE TRIGGER FACTORS, ENVIRONMENT, AND PATHOLOGY FOR THE INCREASED PREVALENCE OF SUGI-POLLINOSIS

In Japan forests cover approximately 25 million hectares (ie, 66% of the total area of Japan). More than half of these trees were planted from the early 1950s to the early 1970s, and according to the Forestry Agency of Japan, an estimated 4.6 billion of these are Japanese cedar trees, covering nearly 18% of the total land area of Japan. The sugi trees are extremely straight and tall, making them ideal construction materials, but after wood tariffs decreased in 1964, imported wood put the sugi foresters out of business, and most sugi trees have been abandoned and grow taller and produce more pollen each year. With the exception of Hokkaido and Okinawa islands, this yellow-green dust is scattered throughout Japan. Airborne JCP levels have been monitored in Sagamihara hospital (Kanagawa, Japan) since 1965. JCP counts can vary significantly from year to year because of weather conditions; however, the total JCP counts from 1995 to 2013 have been significantly greater than those in the initial period from 1965 to 1994 ($P < .05$; Fig 2, *A*).

Epidemiologic studies have demonstrated that global climate change correlates with the number of symptomatic pollen-induced respiratory allergies and allergic diseases.[10,11] One of the fundamental effects of climate change is the potential for shifts in flowering phenology and pollen production associated with warmer seasonal air temperatures. As such, the length of the sugi-pollinosis season has increased since 1995.[12] Although the average global temperature has only increased by approximately 0.6°C in the 20th century, climate change in Japan has been more severe, with temperatures increasing by an average of 1.15°C in the past 100 years. Fig 2, *B*, shows the annual temperature change since 1960 in Japan.

Cedar pollen is released from male flowers on sugi trees (Fig 1, *C*). Hot summers usually affect sugi trees, promoting flower bud development and increasing pollen production; meanwhile, cool summers have opposite effects. Ito et al[13] investigated the correlation between total JCP count and the previous years' summer weather conditions from 1987 to 2006. The annual cumulative level of airborne JCP was significantly related to the mean temperature and sunlight hours in late July before the start of the pollen season (see Table E1 in this article's Online Repository at www.jacionline.org). The mean temperature in July during the 20th century has also significantly increased in Japan. Average temperatures in the Fukui area from 1974 to 1993 and 1994 to 2012 were 25.1°C ± 0.3°C and 26.2°C ± 0.3°C, respectively ($P < .05$; Fig 2, *C*). The weather of late winter and early spring was not correlated with JCP counts; however, temperatures in January and February did influence the start of sugi pollen production and the pollen season (data not shown).

JCP counts were significantly associated with the prevalence of sugi-pollinosis. The mean JCP count in the mountainous area of Akita prefecture was 2 times higher than that in the coastal area of Akita from 1996 to 2006 (see Table E2 in this article's Online Repository at www.jacionline.org). The prevalence of sugi-pollinosis in children (age, 10-11 years) in 2006 was higher in mountainous areas than in coastal areas, although the prevalence of PAR was not different between the 2 areas (see Table E3 in this article's Online Repository at www.jacionline.org). The positive rate of serum anti-JCP–specific IgE in the mountainous area was also higher than in that in the coastal areas, but the positive rate of anti-mite IgE did not increase in the mountainous area.[14]

Asian dust (AD) and urban particulate matter (PM) are risk factors for sugi-pollinosis. AD storms originating in the deserts of Mongolia, northern China, and Kazakhstan are seasonal phenomena that affect much of Eastern Asia, including Japan, and occasionally spread around the globe, affecting the United States as well.[15] The number of spring dust storms has increased in the last 13 years. The frequency of the storms combined with increases in airborne pollution has led to an increase in the adverse effects of the storms. AD contains pollutants, such as sulfur dioxide and nitrogen dioxide, which stimulate immune cells through oxidative stress, enhancing inflammation-related cytokines.[16] AD enhanced nasal allergic reactions induced by repeated JCP administration in guinea pigs.[17] In fact, allergic symptoms have increased during AD storm events in Japan and Taipei.[18,19] Administration of AD plus allergen induced allergen-specific IgE production in mice,[20] suggesting that AD can bind to JCP and induce JCP sensitization in nonatopic or unsensitized atopic subjects.

The main pollutant in Japanese cities is fine PM. Particulate matter less than 2.5 μm in diameter (PM2.5) is frequently reported in spring. PM2.5, a component of AD, induced asthma and enhanced sneezing and rhinorrhea in a manner of type I allergy.[21] Beijing has also recorded its worst levels of air pollution in recent years, and the onset and sensitization of sugi-pollinosis could be easily induced by PM2.5 from China. Environmental authorities in Japan, the United States, and other nations have adopted strict regulations to control PM levels. A wave of criticism, both at home and abroad, prompted Chinese officials to set their own standards in February 2012; however, air quality in China still remains an issue.

GENETIC FACTORS

Genome-wide association studies (GWASs) and meta-analyses of GWASs have shown both common and distinct pathways that

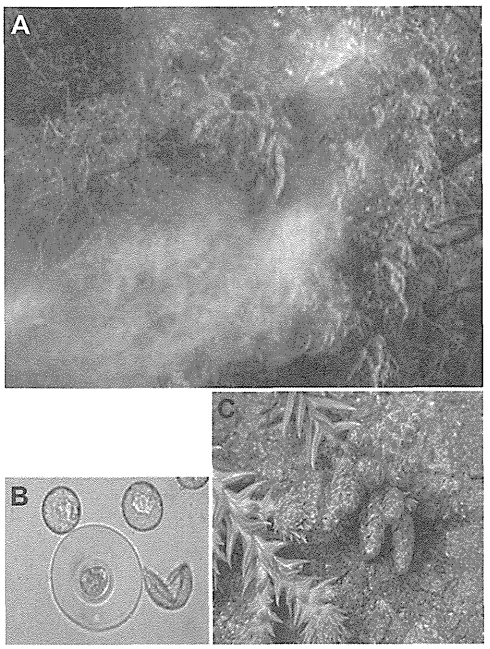

FIG 1. The blooming Japanese cedar tree. **A,** Yellow-green pollen is scattered from male Japanese cedar flowers. **B,** JCP (magnification ×400). **C,** Male *(right)* and female *(left)* flowers of Japanese cedar.

might contribute to asthma and allergic diseases.[22,23] The first GWAS of asthma identified a novel asthma susceptibility locus on chromosome 17q21 including the *ORMDL3* genes.[24] Five polymorphisms in the *ORMDL3* gene are significantly associated with sugi-pollinosis.[25] Additionally, the matrix metalloproteinase 9 gene is involved in the pathogenesis of AR and asthma. The matrix metalloproteinase 9 gene confers susceptibility to sugi-pollinosis in children and might be associated with sensitization processes.[26] One of the polymorphisms in the gene for the IL-4 receptor α chain, the Ile50 allele, might be involved in both sugi-pollinosis and atopic dermatitis.[27] IL-33 (an IL-1–like cytokine) is a ligand for IL-1RL1, an important effector molecule of the T_H2 response. Serum levels of IL-33 are significantly higher in patients with sugi-pollinosis than in their nonallergic counterparts. In a genetic association analysis we found a positive association between the polymorphism of IL-33 and sugi-pollinosis.[28]

Complement systems are known to play an important role in allergic diseases. Decay-accelerating factor, which is involved in the regulation of the complement system, is one of the genes involved in conferring susceptibility to AR and sugi-pollinosis. Low levels of decay-accelerating factor might be associated with the enhanced specific IgE response that occurs in patients with allergic diseases in the Japanese population.[29]

Microarray analysis showed that JCP exposure increased IL-17 receptor β RNA expression in patients with sugi-pollinosis.[30]

ASSESSMENT OF SEVERITY

Nasal symptom scores are assessed with a grading system that includes sneezing, rhinorrhea, and nasal congestion in Japan. Symptom scores are graded from 0 to 4. Grading of sneezing, rhinorrhea, and nasal congestion are evaluated based on the frequency of sneezing (number per day), frequency of nasal blowing (number per day), and duration of mouth breathing, respectively (Fig 3).[31] Total nasal severity of sugi-pollinosis is evaluated by using the grading scores of nasal obstruction and sneezing/rhinorrhea as very severe, severe, moderate, and mild symptoms. According to Fig 3, of 795 patients with sugi-pollinosis, 22.6%, 29.4%, 31.3%, and 13.9% had very severe, severe, moderate, and mild symptoms, respectively.[32] Ninety-one percent of patients with sugi-pollinosis are classified as having moderate-to-severe AR.[32]

ENVIRONMENTAL EXPOSURE UNITS

There are 4 environmental exposure units in Chiba, Tokyo, Wakayama, and Osaka City to investigate the effectiveness of medicine or devices on sugi-pollinosis (Fig 4).[33]

ELIMINATION AND AVOIDANCE OF ANTIGENS

The most effective means of decreasing allergic inflammation reactions is avoidance of the aeroallergen.[1] Although the complete avoidance of pollen is impossible because of its ubiquitous nature, patients with sugi-pollinosis often wear protective facemasks between February and May (Fig 5); these masks have a significant protective effect on nasal JCP invasion.[34] This has created a large market for an array of related devices. Forecasting daily JCP dispersal conditions can help patients decide which prevention measures to take to protect them from pollen inhalation (see Fig E1 in this article's Online Repository at www.jacionline.org). Using a service unique to Japan that has not been adopted in other countries, the amount of airborne JCP is automatically counted with equipment set up on the roofs of 1000 buildings and connected to the Internet (see Fig E2 in this article's Online Repository at www.jacionline.org). The information is gathered from these sites and can be accessed for free using the Internet service. Furthermore, the success of these automatic pollen counters has led to their use to assess levels of other pollens, such as Urticaceae, Poaceae, and *Ambrosia* species.[35]

Ventilation systems can be equipped with appropriate filters to avoid drawing JCP into the house and car. Novel air-purification systems using positively and negatively charged cluster ions have been developed to create comfortable indoor living environments. Treatment with positive and negative cluster ions significantly decreased the *in vitro* and *in vivo* antigenicity of atomized JCP.[36] Treatment with low-concentration hypochlorous acid generated by means of electrolysis is also an effective method for significantly reducing the immunogenicity of JCP.[37]

PHARMACOTHERAPY

Therapy for sugi-pollinosis is chosen based on severity (Fig 3) and disease type (Fig 6).[2,38] Because the severity of pollinosis markedly changes with the amount of dispersed pollen, the severity of a patient's symptoms is determined during a medication evaluation at peak pollen dispersal times and also takes into account the amount of dispersed pollen. Nasal antihistamine and mast cell stabilizers were not used for 1 decade in Japan.

Because there are a large number of patients with severe or very severe sugi-pollinosis, Japanese guidelines recommend the prophylactic treatment of sugi-pollinosis before pollen release.[2] Prophylactic treatment with oral antihistamines (olopatadine) significantly suppressed nasal symptoms by 40% and was

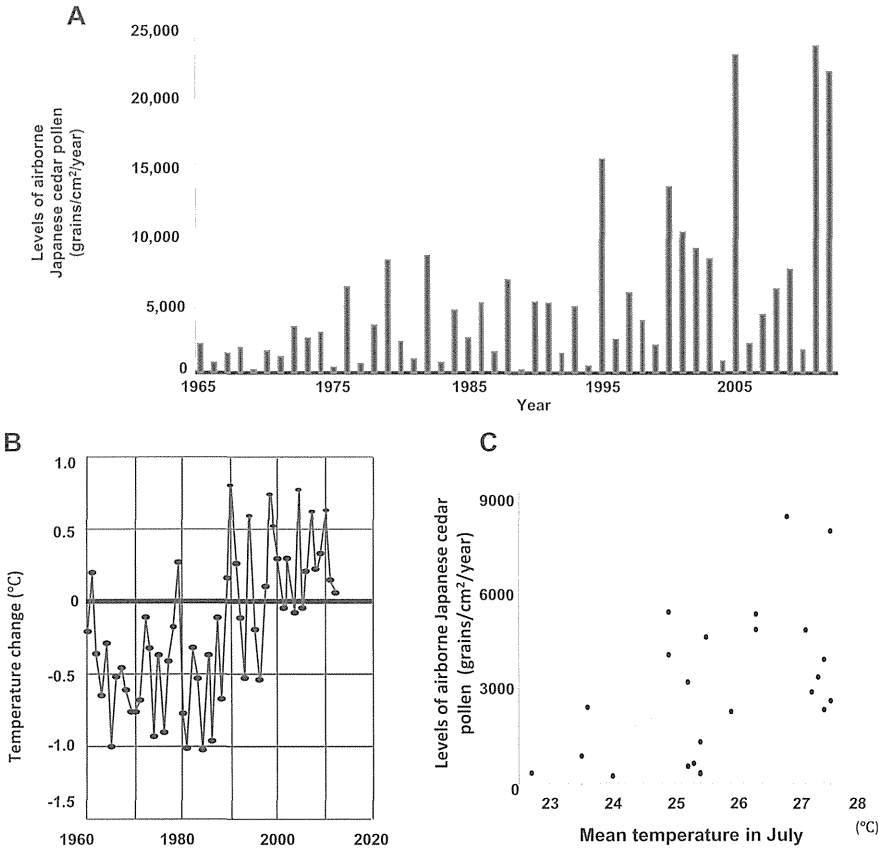

FIG 2. Correlation between increased JCP counts and climate change. **A,** Annual JCP counts from 1965 to 2013 in Kanagawa prefecture. **B,** Annual temperature change in Japan compared with baseline (mean temperature from 1960 to 2012). Data were provided by the Japan Meteorological Agency. **C,** Correlation between annual cumulative levels of airborne JCP and the mean temperature in Fukui, Japan. The mean temperature was taken in late July before the pollen season and from 1988 to 2012 (Spearman rank correlation coefficient = 0.531, $P = .0067$). Pollen counts were determined from daily data with the Durham sampler (the standard gravity slide sampler) by counting the JCP particles dropped onto glass microscope slides.

associated with a high level of QOL during the peak of the JCP season in a randomized, double-blind, placebo-controlled study.[39]

Prophylactic administration of antileukotriene (pranlukast) 1 to 2 weeks before or at the start of the JCP season significantly reduced nasal symptoms by 50% at the peak of pollen dispersal compared with placebo.[40] Additionally, antileukotrienes reduces nasal congestion and allergic inflammation in patients with sugi-pollinosis.

Intranasal corticosteroids are the most effective drugs for controlling AR. In a double-blind, randomized, placebo-controlled study with mometasone furoate nasal spray (MFNS) as a prophylactic treatment for sugi-pollinosis, no worsening occurred in the MFNS group, whereas the placebo group showed a significant worsening of symptoms after the start of the continuous dispersion.[41] The 12-week mean total nasal symptom score in the prophylactically treated group was significantly lower than that in the postonset-treated group (which reduced the symptoms by 61%).[42] Intranasal corticosteroids should be administered prophylactically; with the addition of an oral antihistamine, they might improve outcomes in patients with severe sugi-pollinosis.[43] However, many Japanese persons prefer not to use intranasal corticosteroids because of personal issues associated with using nasal sprays, including their smell.

IMMUNOTHERAPY

Antigen-specific immunotherapy can change the natural course of AR and is recognized as a curative treatment without impaired performance. In the 1970s, subcutaneous immunotherapy for sugi-pollinosis was performed at university hospitals and medical clinics.[44] However, in the 1980s, the development of second-generation antihistamines and intranasal corticosteroids gradually decreased the frequency of application of subcutaneous immunotherapy. This decrease was also attributable to the fact that JCP extracts were not standardized until 1999.

In 2004, a multicenter, double-blind, randomized, placebo-controlled, parallel-group study of sublingual immunotherapy (SLIT) demonstrated the safer and more beneficial effects of immunotherapy for sugi-pollinosis than pharmacotherapy alone.[45,46] The mean of the daily total symptom scores was significantly lower in the SLIT group than in the placebo group. The QOL score in the SLIT group was almost half that in the placebo group.

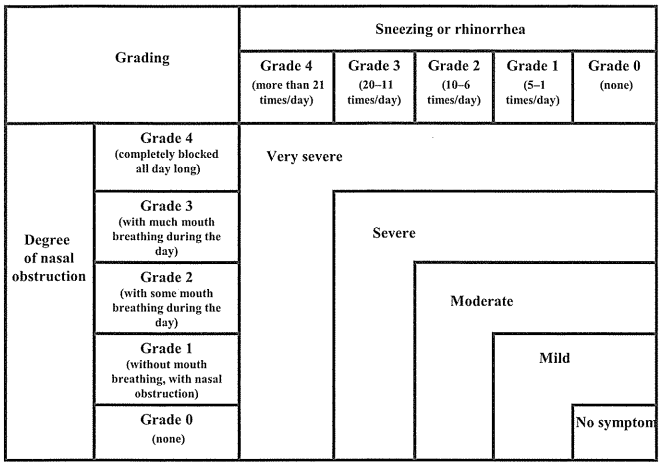

FIG 3. Classification of the total severity of nasal symptoms of AR. Grading of sneezing, rhinorrhea, and nasal obstruction is evaluated based on the frequency of sneezing or the frequency of nasal blowing per day and the duration of mouth breathing. High grading scores are selected from sneezing or rhinorrhea. Uncontrollable severe symptoms are classified as very severe.

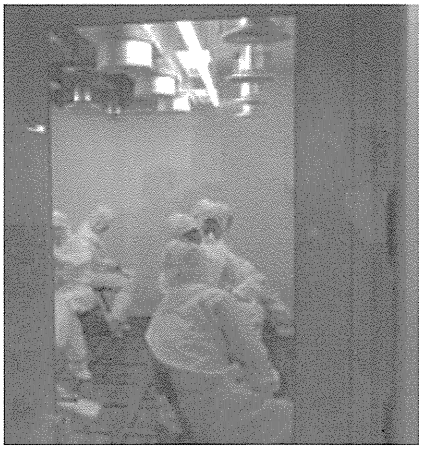

FIG 4. An environmental exposure unit.

Sera from 25 patients with sugi-pollinosis in a double-blind, randomized, placebo-controlled study for SLIT were analyzed by using 2-dimensional electrophoresis.[47] Sixteen proteins were found to be differentially expressed during the pollen season. Among the differentially expressed proteins, serum levels of apolipoprotein A-IV (apoA-IV) were significantly increased in SLIT-treated patients but not in placebo-treated patients. Higher levels of apoA-IV induction were correlated with lower clinical symptom–medication scores and better QOL scores in the case of SLIT-treated patients. The amount of histamine released from basophils *in vitro* was significantly reduced after addition of recombinant apoA-IV in the medium.[47] SLIT increased IL-10 production by monocytes and T cells in patients with sugi-pollinosis.[48] ApoA-IV and IL-10 might become clinical markers for the evaluation of the effectiveness of SLIT for sugi-pollinosis.

Uses of antigen-derived peptides that retain immunogenicity (but are insufficient in length to cross-link IgE on mast cells or basophils [immunotherapeutic peptides]) are a promising strategy

FIG 5. Persons on a train platform in a large Japanese city wearing facemasks during the JCP season.

for improved immunotherapy, and this concept has been applied to a variety of allergens. Cry-consensus peptide for sugi-pollinosis contains 6 major human derived T-cell epitopes. In an AR mouse model Cry-consensus peptide markedly inhibited Cry j 1–induced sneezing, eosinophil infiltration, and eosinophil peroxidase activity in nasal tissue.[49] Human immunodominant T-cell epitopes of the Cry j 1 molecule are being studied for peptide-based immunotherapy in patients with sugi-pollinosis.[50]

ANTIBODY THERAPY

Omalizumab, a recombinant, humanized, anti-IgE mAb, has been shown to be effective for the treatment of SAR.[51] A randomized, placebo-controlled, double-blind study was conducted in Japanese patients with a history of moderate-to-severe sugi-pollinosis. The primary and all secondary efficacy variable scores were significantly lower in the omalizumab group than in the placebo group.[52] Retreatment with omalizumab is effective and safe when readministered in the second JCP season.[53]

PROBIOTICS

Probiotics, including lactobacilli and bifidobacteria, might prevent several allergic diseases. Japanese persons are very interested in probiotics for the self-treatment of AR. A double-blind, placebo-controlled trial with lyophilized powders of *Bifidobacterium longum* BB536 for the treatment of sugi-pollinosis during the height of the pollen season indicated that BB536 intake alleviated subjective symptoms, reduced prescription of allergic medicines, and significantly suppressed the increase of plasma thymus and activation-regulated chemokine during the pollen season.[54] In another study BB536 reduced nasal symptoms from early allergic reactions in patients with sugi-pollinosis exposed to JCP in an environmental exposure unit outside of the normal JCP season.[55] Oral administration of heat-killed *Lactobacillus gasseri* OLL2809 reduced nasal

<table>
<tr><td>Severity</td><td>(prophylactic treatment)</td><td>Mild</td><td colspan="2">Moderate</td><td colspan="2">Severe or very severe</td></tr>
<tr><td>Types</td><td></td><td></td><td>Sneezing rhinorrhea</td><td>Nasal blockage or combined</td><td>Sneezing rhinorrhea</td><td>Nasal blockage type or combined</td></tr>
<tr><td rowspan="5">Choice of therapy</td><td>Oral histamine H_1 antagonists (2^{nd} generation) or Oral Th2 cytokine inhibitors or Oral leukotriene receptor antagonists or Oral Prostaglandin D2/ Thromboxane A2 receptor antagonists</td><td>Oral histamine H_1 antagonists (2^{nd} generation) + Eye drops, + Intranasal corticosteroid (when needed)</td><td>Oral histamine H_1 antagonists (2^{nd} generation) + Intranasal corticosteroid</td><td>Oral leukotriene receptor antagonists + Oral histamine H_1 antagonists (2^{nd} generation) + Intranasal corticosteroid</td><td>Intranasal corticosteroid + Oral histamine H_1 antagonists (2^{nd} generation)</td><td>Intranasal corticosteroid + Oral leukotriene receptor antagonists + Oral histamine H_1 antagonists (2^{nd} generation)</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>+ Intranasal vasoconstrictor nose spray 7 to 10 days + oral corticosteroid 4-7 days (when needed, at the start of treatment)</td></tr>
<tr><td></td><td colspan="3">Histamine H_1 eye drops antagonists or mast cell stabilizer</td><td colspan="2">Histamine H_1 eye drops antagonists, mast cell stabilizer, or steroids</td></tr>
<tr><td></td><td colspan="3"></td><td colspan="2">Operation for cases of nasal blockage type with nasal deformities.</td></tr>
<tr><td colspan="6">Specific immunotherapy</td></tr>
<tr><td colspan="6">Avoidance and elimination of antigens</td></tr>
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FIG 6. Algorithm for the treatment of sugi-pollinosis recommended by Japanese guidelines.[2]

symptoms and JCP-specific IgE levels.[56] Additionally, oral administration of *Lactobacillus paracasei* strain KW3110 decreased total symptom scores and serum eosinophil cationic protein levels and improved QOL scores at the start of the JCP production season.[57]

In addition, dietary intervention with nondigestible prebiotics might be effective for allergic diseases.[58] Administration of β-1, 4 mannobiose decreased sneezing frequency, histamine release, and IL-4 production in a sugi-pollinosis mouse model, suggesting a potential molecular therapeutic supplement in clinical trials for sugi-pollinosis.[59,60]

FLAVONOIDS AND TEA

An appropriate intake of flavonoids might constitute a dietary preventative or therapeutic strategy for allergic diseases because flavonoids, which are abundant in plant foods, possess antioxidants and antiallergic activities. Analyses of structure-activity relationships of 45 flavones, flavonols, and their related compounds showed that luteolin, ayanin, apigenin, and fisetin were the strongest inhibitors of IL-4 production by basophils in mice because of their inhibitory action on the activation of nuclear factors in activated T cells and activator protein 1.[61] Additionally, prophylactic ingestion of enzymatically modified isoquercitrin significantly decreased levels of ocular symptoms and medication scores in patients with sugi-pollinosis in a double-blind, placebo-controlled study.[62]

Self-care with Ten-Cha (*Rubus suavissimus*, sweet Chinese tea) is the most common alternative complementary treatment for AR in Japan. Ten-Cha extract inhibited histamine release from rat peritoneal mast cells, as well as calcium ionophore–induced vascular permeability. However, the effects of drinking Ten-Cha beverages on sugi-pollinosis symptoms were considered low in an unblinded study.[63] Nevertheless, Ten-Cha has sold very well during JCP seasons.

CONCLUSION

In Japan, 69.7% of the adult population has positive results for one of 7 aeroallergen-specific IgEs (JCP, 2 types of mite, ragweed, orchard grass, and *Aspergillum* and *Candida* species), and the prevalence of patients with AR is 44.2%.[7] In response to the increasing demand for AR relief, second-generation antihistamines have been approved for sale as over-the-counter medications. However, present therapies still do not offer sufficient relief for patients with sugi-pollinosis. In addition, the Japanese government has reduced the budget for AR research because they consider more lethal diseases, such as cancer, to be a more serious threat than sugi-pollinosis. Therefore cooperation between scientists and pharmaceutical companies will be needed to find new treatments that better control AR and its symptoms.

We thank Professor K. Akiyama for providing data on JCP counts from 1965 to 2013 and Y. Ishikawa, M. Imamura, and H. Tsuchiya for counting JCP in Fukui.

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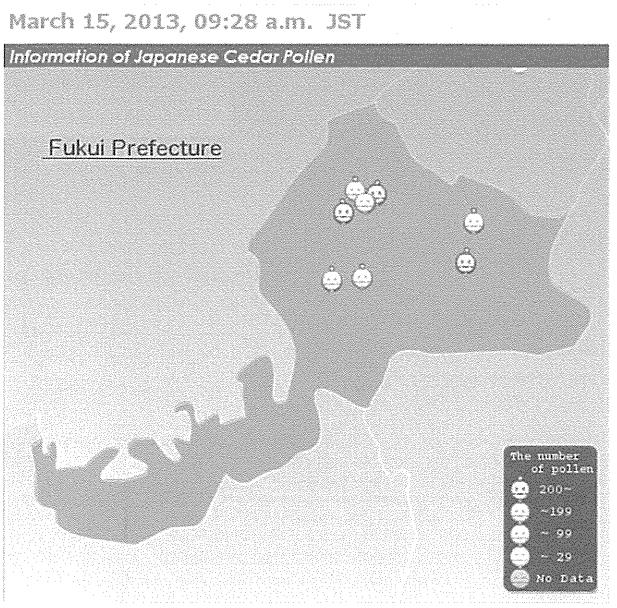

FIG E1. Forecasting daily JCP counts can be seen in television or newspaper accounts after a weather forecast during the JCP season. This picture shows JCP dispersal conditions in Fukui prefecture. *Red symbols* indicate a large amount of JCP would disperse in that area today.

FIG E2. Automatic apparatus for measuring JCP levels. The pictured equipment automatically counts the amount of airborne JCP and transfers the data to a central office through telephone lines. This machine is based on a new laser particle counter methodology to measure the optical properties and hydrodynamic characteristics of pollen.

TABLE E1. Correlation coefficient matrix of JCP counts with the previous year's summer weather data, 1987-2006[13]

Period	Maximum temperature	Mean temperature	Sunlight hours	Rainfall	Relative humidity
June 21-30	0.124	0.062	0.283	−0.627†	−0.364
July 1-10	0.357	0.382	0.257	−0.264	−0.158
July 11-20	0.805†	0.781†	0.661†	−0.555*	−0.526*
July 21-31	0.637†	0.777†	0.682†	−0.386	−0.510*
August 1-10	0.517*	0.470*	0.428	−0.540*	−0.402
August 11-20	0.540*	0.539*	0.695†	−0.412	−0.467*
August 21-31	−0.216	−0.116	−0.208	0.200	0.234

*$P < .05$.
†$P < .01$.

TABLE E2. JCP counts in Akita prefecture, 1996-2006[14]

	JCP count (grains/cm^2/y)	
Year	**Coastal area**	**Mountainous area**
1996	20	80
1997	420	1280
1998	590	1090
1999	440	330
2000	3910	4850
2001	500	1200
2002	200	1560
2003	1190	4140
2004	530	1870
2005	2580	5730
2006	2460	4780
Mean	1167	2446*

*$P < .05$ compared with JCP counts at coastal areas.

TABLE E3. Prevalence of allergic disease and positive rate of allergen-specific IgE in the children of Akita prefecture (10-11 years old of age)[14]

	Prevalence in percentage (no. of children)			
Disease	**Coastal area (n = 156)**	**Mountainous area (n = 183)**	**Odds ratio (95% CI)**	***P* value**
Perennial AR	42.9 (67)	48.1 (88)	1.2 (0.8-1.9)	.4
Sugi-pollinosis	5.8 (9)	13.7 (25)	2.6 (1.2-5.7)	.02
Asthma	10.3 (16)	11.5 (21)	1.1 (0.6-2.3)	.8
Eczema	13.5 (21)	8.7 (16)	0.6 (0.3-1.2)	.2
	Positive rate of IgE			
Allergen	**Coastal area (n = 156)**	**Mountainous area (n = 183)**	**Odds ratio (95% CI)**	***P* value**
Mite	48.1 (75)	50.8 (93)	1.1 (0.7-1.7)	.7
JCP	20.5 (32)	41.5 (76)	2.8 (1.7-4.5)	<.0001
Wormwood	7.1 (11)	6.0 (11)	0.8 (0.3-2.0)	.8

Journal of Dermatological Science 75 (2014) 75–81

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Invited Review Article

A nucleic acid-based medication for allergic skin diseases

Hiroo Yokozeki *

Tokyo Medical and Dental University, Graduate School, Department of Dermatology, 113-8519 1-5-45, Yushima, Bunkyo-ku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 6 February 2014
Accepted 11 March 2014

Keywords:
Atopic dermatitis
Nucleic acid-based medications
Decoy
Contact hypersensitivity

ABSTRACT

Among allergic skin diseases, atopic dermatitis is the most difficult to cure. In the majority of patients, atopic dermatitis can be easily controlled by treatment based on three therapeutic approaches: avoidance of precipitating factors, skin care, and medication. In some adult patients, however, severe atopic dermatitis is refractory to treatment, and no fundamental effective treatment modality has yet been established for such cases. Chronic contact dermatitis without an identified causative hapten is also considered an allergic skin disease that is difficult to cure. Topical nucleic acid-based medications are currently being applied clinically, and an ointment containing nuclear factor-κB decoy oligodeoxynucleotides (hereafter referred to as Decoy) has reached clinical trials. In addition, synthetic double-stranded DNA with high affinity for signal transducers and activators of transcription 6 (STAT6) introduced *in vivo* as a decoy *cis* element to bind the transcriptional factor and block the activated gene that contributes to the onset and progression of atopic dermatitis functions as an effective therapeutic agent. We also introduce another STAT1 decoy treatment, cytosine-phosphate-guanine-ODN or STAT6 small interfering RNA therapy, for allergic skin diseases.

Contents

1. Introduction

Among allergic skin diseases, atopic dermatitis is the most difficult to cure, but it can be controlled in the majority of patients by proper skin care, medication, and avoidance of allergens. Some adults present with severe atopic dermatitis that is refractory to treatment, however, and an effective treatment modality has yet to be developed. Although novel strategies for the treatment of atopic dermatitis are currently under development, including anti-immunoglobulin E (IgE) antibody therapy (omalizumab), none has reached the clinical application stage. Chronic contact dermatitis without evidence of the haptens responsible for the condition is also difficult to cure. Nucleic acid-based topical medications are currently being investigated, and an ointment containing nuclear factor (NF)-κB decoy oligodeoxynucleotides (ODNs, hereinafter referred to as Decoy) has gone to clinical trials. We recently developed a decoy for signal transducer and activator of transcription 6 (STAT6), a transcriptional regulator with an

* Tel.: +81 3 5803 5282; fax: +81 3 5803 0143.
E-mail address: 3064derm@tmd.ac.jp

http://dx.doi.org/10.1016/j.jdermsci.2014.03.003
0923-1811/

important role in the physiologic actions of interleukin (IL)-4 and IL-13, that inhibits STAT6 from binding to its *cis*-elements, and demonstrated that the STAT6 Decoy suppresses skin inflammatory reactions in mouse models of atopic dermatitis [1,2]. The STAT6 Decoy ointment was effective against atopic dermatitis in some patients [3]. Small interfering RNA (siRNA) therapy targeting STAT6 has also been developed, and is effective against contact dermatitis and allergic rhinitis [4]. A STAT1 Decoy was also reported to be useful in the treatment of contact dermatitis [5]. Unmethylated cytosine-phosphate-guanine (CpG) dinucleotides of microbial DNA sequences activate Toll-like receptor (TLR) 9. Previous studies demonstrated that ODNs containing CpG in specific base sequence motifs (CpG ODNs) can reproduce the majority of immunomodulatory effects induced by bacterial DNA. Many of the complications of allergic diseases, including atopic dermatitis, are primarily caused by T helper type 2 (T_h2) cell-type responses. CpG ODNs induce T_h1 and T-regulatory (T_{reg}) cell-type cytokines, which suppress the T_h2 response [6,7]. Here we discuss therapeutic strategies using nucleic acid-based medications, such as Decoys, CpG ODNs, and siRNAs, for allergic diseases, including atopic dermatitis and chronic contact dermatitis.

2. Theory/mechanism

Decoys belong to a group of nucleic acid-based medications and antisense ODN, and exert their actions via the regulation of gene expression by competitively inhibiting the binding of specific transcriptional regulators to their binding sites (*cis*-elements; Fig. 1). Decoys have a number of advantages over other therapeutics. The use of Decoys eliminates the need for cloning transcriptional regulators. Because transcriptional regulators occur as families that share common features, Decoys inhibit the binding of several transcriptional regulators to their sites. Further, Decoys inhibit the expression of a wide range of target genes [8]. Therefore, compared to antisense nucleotide therapy, Decoy therapy is considered more effective for regulating gene expression. The siRNA therapy, which targets cellular messenger RNA (mRNA), has been used for the treatment of malignancies [9] (Fig. 1).

2.1. NF-κB Decoy for atopic dermatitis

NF-κB is an important cellular signaling molecule that regulates gene expression in response to inflammatory cytokines such as IL-1 and tumor necrosis factor-α. Inhibition of this signaling molecule alleviates inflammatory reactions. Steroid hormones inhibit NF-κB from entering the nucleus and binding to DNA by boosting the expression of inhibitory κB. Morishita et al. developed a 20-base pair Decoy containing a specific sequence for binding to NF-κB (CCCTAAAGGG), and demonstrated in mouse models that the Decoy can be used to treat ischemic heart disease [10]. Further, topical application of an ointment containing a NF-κB Decoy alleviated the symptoms of atopic dermatitis in a mouse model of the disease [11].

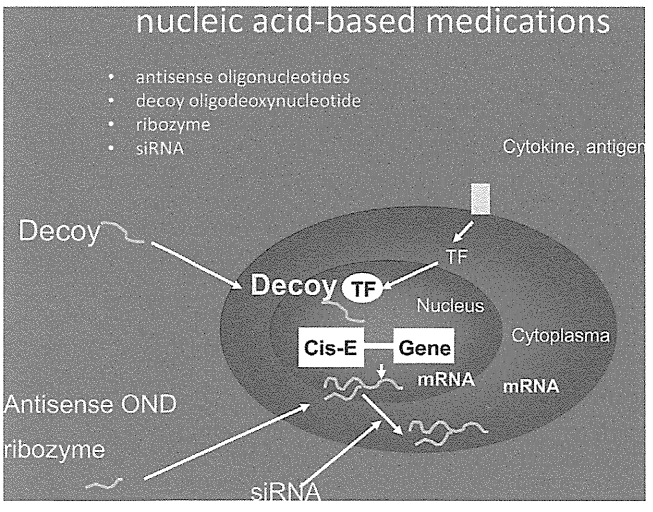

Fig. 1. Mechanism of action of decoy oligodeoxynucleotide (Decoy): The nucleic acid decoy competitively inhibits specific transcriptional regulators from binding to their binding sites (*cis*-elements) in the nucleus to suppress the expression of genes that would be activated by such binding.

2.2. Inhibition of late-phase reactions and contact hypersensitivity (CHS) by a STAT6 Decoy

To understand how the STAT6 Decoy affects the induction of late-phase reactions mediated by antigen-specific IgE in mice, we subcutaneously injected the Decoy into the ears of mice sensitized by an intravenous injection of anti-dinitrophenyl-IgE antibody [1]. To compare the reactions between STAT6 Decoy-treated mice and scramble (in which the same nucleic acids as in the Decoy are inserted randomly) Decoy-treated mice, dinitrofluorobenzene (DNFB) was applied to initiate an auricular swelling reaction after subcutaneous administration of the Decoy into the auricles of the sensitized BALB/c mice. Although no differences in the IgE-mediated auricular swelling reactions were observed during the early phase (1 h after the application of DNFB), late-phase reactions that occurred 24 h after the application of DNFB were weaker in the STAT6 Decoy-treated mice than in the scramble Decoy-treated mice [1]. This finding suggested that the STAT6 Decoy was effective against late-phase IgE-mediated reactions. To elucidate the mechanism of inhibition of the inflammatory reactions, the kinetics of cytokines in the local auricular skin at the initiation site were analyzed at the mRNA and protein levels [1]. Our results revealed that in STAT6 Decoy-treated mice, infiltration of (T_h2) cells, which produce IL-4 and IL-5, was suppressed in the local skin where late-phase reactions were induced by antigen-specific IgE [1]. The findings also suggested that IgE-mediated late-phase reactions required STAT6, which plays an important role in the signal transduction of IL-4. Based on the histopathologic finding that the infiltration of eosinophils and neutrophils was inhibited in the STAT6 Decoy-treated mice, STAT6 might have an important role in the production of chemokines such as eotaxin in mast cells and fibroblasts [1]. Targeted disruption of the STAT6 DNA binding activity by a STAT6 Decoy blocked the IL-4-derived Th2 cell response *in vitro*. A STAT6 Decoy inhibited acute and chronic CHS *in vivo* in mouse models of CHS or atopic dermatitis [2]. Although a number of important issues, including the safety and side effects, were not addressed in that study, gene therapy using STAT6 Decoys appears to be promising for the treatment of atopic dermatitis. STAT6 is thought to play important roles in the development of contact dermatitis, atopic asthma, and allergic rhinitis. Therefore, STAT6 Decoys may be useful for treating these conditions.

2.3. STAT1 Decoy treatment for contact dermatitis

Contact dermatitis is a common disease that can be effectively treated by topical corticosteroid treatment and removal of haptens. Chronic contact dermatitis, however, is often difficult to cure. CHS, clinically known as allergic contact dermatitis, is a delayed-type hypersensitivity reaction in the outermost layers of the skin triggered by hapten-specific T cells [12]. In experimental CHS, the cutaneous proinflammatory response to the sensitizing agent depends on the haptenated antigen and is regarded a cellular immune response mediated either by interferon-γ-producing T_h1 cells and T cytotoxic type 1 (T_c1) cells or IL-4- and IL-10-producing

T_h2 cells [13]. The proinflammatory response is caused by the combination of a small number of key transcription factors, such as STAT1, that regulate proinflammatory gene expression in psoriasis. Increased expression of STAT1 and STAT1-dependent proinflammatory gene products are found in a T_h1/T_c1-biased rodent model of CHS [14]. Advanced treatment strategies focus on small-molecule and nucleic acid-based drugs. Because oral bioavailability is limited, topical administration of these drugs is preferred. The barrier properties of the skin, however, might impede intradermal delivery. A Decoy is a short double-stranded DNA molecule that readily penetrates the skin because of active transcellular transport. These molecules mimic the genomic binding site of their target transcription factor and block the expression of their target genes. Hagener et al. verified the broad anti-inflammatory effects of a STAT1 Decoy in different *in vivo* models of various diseases [15,16]. The STAT1 Decoy was reported to be effective against CHS [5]. Wagner et al. found that topical application of a STAT1 Decoy was as effective as a mild hydrocortisone, an ultrapotent topical corticosteroid, or a highly active topical immunosuppressive, and that the STAT1 Decoy suppressed the cutaneous inflammatory reaction in animal experimental models of CHS. The STAT1 Decoy also showed high efficacy. Thus, Wagner et al. concluded that blocking STAT1 using STAT1 Decoys is a beneficial option for the treatment of inflammatory skin diseases because it is more effective than state-of-the-art dermal anti-inflammatory drugs and less prone to side effects [5].

2.4. CpG ODN treatment for atopic dermatitis

As described above, ODNs containing CpG in specific base sequence motifs (CpG ODNs) activate TLR9. Previous studies demonstrated that CpG ODNs reproduce the majority of the immunomodulatory effects induced by bacterial DNA (Fig. 2) [17]. Therapeutic application of TLR9 modulation has been extensively explored in recent years. Many studies are being conducted to assess the safety and efficacy of TLR9 agonists developed for the treatment of atopic and infectious diseases. Studies using murine models revealed that treatment with CpG ODNs prevents the development of atopic asthma [17]. Clinical trials are currently underway to determine the efficacy of CpG ODNs in the treatment of atopic diseases [17]. In this section, we focus on the therapeutic application of CpG ODNs in allergic diseases. CpG ODNs can be used alone or as an adjuvant to immunotherapy to treat these disorders [17]. Dynavax Technologies collaborated with AstraZeneca to create CpG ODNs for asthma therapy, and this collaboration led to the identification of a lead compound, AZD1419, for initiation of Investigational New Drug Application (IND)-enabling preclinical studies in 2002. Cytos Biotechnology focused on CpG ODNs for allergy immunotherapy. In a double-blind, randomized, placebo-controlled phase 2 clinical trial, however, it was found that the allergen was not required to produce the clinical benefit of an A-class CpG ODN (CYT003-QbG10: CpG ODNs) administered to patients with allergic rhinoconjunctivitis [18]. In this trial, CYT003-QbG10 was subcutaneously injected once a week into patients with house dust-mite allergy [18]. The CpG ODN treatment was well tolerated and allergy symptoms were significantly less severe in patients treated with a high dose of CYT003-QbG10 [18].

In addition to the many positive results, there have been some failures, indicating the limitations of some of these therapeutic approaches. A clinical trial of CpG ODN therapy for the treatment of atopic dermatitis has not been conducted. Kim et al., however, demonstrated that topical application of CpG ODNs markedly suppresses the symptoms of atopic dermatitis in skin lesions of NC/Nga mice. Compared with untreated skin, skin lesions treated with CpG ODNs had a significantly reduced thickness and attenuated acanthosis and hyperkeratosis [19]. Immunohistochemical staining performed after the treatment of skin lesions with CpG ODNs revealed a decrease in the number of infiltrating T cells and cells producing T_h2 cytokines, such as IL-4, IL-10, and interferon-α. Serum IgE and IgG1a levels were also reduced, indicating an improvement in the condition. Consistent with previous reports, in NC/Nga mice, a one-time topical application of ODNs on skin lesions resulted in systemic penetration within 24 h of treatment, a desirable treatment property for clinical use. CpG ODN ointment would be useful for the treatment of atopic dermatitis.

2.5. Suppression of allergic reactions by siRNA

Three candidate STAT6 siRNAs (STAT6 siRNA 1, 2, and 3) were recently evaluated for their ability to treat CHS and allergic rhinitis. All three siRNAs effectively inhibited the expression of STAT6 protein in fibroblasts [4]. STAT6 siRNA 3, which inhibited the expression of STAT6 most efficiently, was selected for *in vivo* studies. Subcutaneous administration of STAT6 siRNA into the auricle significantly suppressed CHS to trinitrochlorobenzene (Fig. 3) [4]. Similar results were obtained when sensitivity to DNFB and oxazolone were analyzed (Fig. 3) [4]. Histopathologic examination revealed that STAT6 siRNA markedly inhibited dermal edema and dermal cell infiltration (Fig. 3) [4]. The effects of STAT6 siRNA were also evaluated in mouse models of allergic rhinitis. In mice sensitized to ovalbumin, rhinitis was induced by daily administration (by inhalation) of ovalbumin from days 21 to 27 [4]. Starting from the day after the reaction was induced, STAT6 siRNA (3 nmol/day) was administered for 3 days into the nasal cavity to evaluate its therapeutic effects. Administration of STAT6 siRNA markedly reduced the frequency of sneezing and nasal rubbing behavior (Fig. 4) [4]. The decreased number of eosinophils infiltrating the nasal mucosa confirmed that the siRNA suppressed the inflammatory reactions. These findings indicated that the use of STAT6 siRNA provides significant degree of relief from CHS and allergic rhinitis. Future development of gene therapy targeting STAT6 is eagerly awaited.

CD86, a crucial co-stimulatory ligand on dendritic cells, is a target of siRNA therapy that is being developed for the treatment of atopic dermatitis. Blockade of the CD86 pathway inhibits antigen-specific T-cell responses in murine T_h2-mediated allergic disease models, including atopic dermatitis [20]. CD86 and CD80 share two functionally opposing receptors, CD28 and CD152. CD86 is induced earlier and expressed at higher levels on antigen-presenting cells than CD80. CD80 binds CD152, however, with higher affinity than CD86. Although CD80 compensates for the function of CD86 in its absence, the CD86–CD28 co-stimulatory pathway appears to contribute primarily to the activation of antigen-specific T cells by antigen-presenting cells. We reported that CD86 has crucial roles in atopic dermatitis in humans and mice [21–23]. In murine T_h2-mediated allergic disease models, blockade of the CD86 pathway interferes with antigen-specific T-cell responses [23]. Recently, Azuma et al. reported that topical application of a CD86 siRNA efficiently inhibited CHS and markedly decreased the numbers of infiltrating $CD86^+$ or major histocompatibility complex class II-positive cells in the murine ear skin and that the CD86-expressing dendritic cells in regional lymph nodes were also significantly decreased [24,25]. These authors reported that the silencing of CD86 in local dendritic cells inhibits dendritic cell recruitment to the skin and their subsequent migration toward regional lymph nodes, resulting in reduced antigen-specific local inflammation [24,25]. This study demonstrated the therapeutic efficacy of the CD86 siRNA in NC/Nga mice, a model of atopic dermatitis [24,25]. Targeting the expression of CD86 in cutaneous dendritic cells using CD86 siRNA appears to be a promising strategy for the treatment of allergic skin disease.

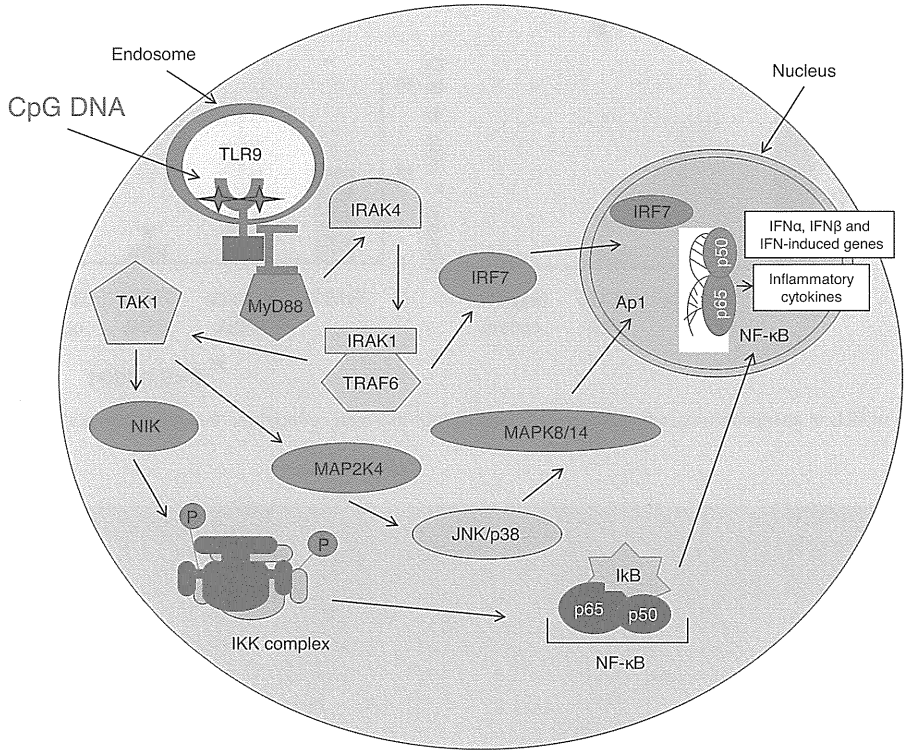

Fig. 2. Toll-like receptor (TLR) 9 signaling pathway: cytosine-phosphate-guanine (CpG) oligodeoxynucleotides (ODNs) are internalized into endosomes and interact with endosomally located TLR9. Intracytoplasmic activation signal transduction occurs following the interaction between CpG ODN and TLR9. Recruitment of myeloid differentiation primary response gene 88 (MyD88) is initiated, and the MyD88-TLR complex promotes association with interleukin (IL)-1 receptor-associated kinase (IRAK) 4. Activation of IRAK4, in turn, induces the hyperphosphorylation of IRAK1, which induces tumor necrosis factor receptor-associated factor (TRAF) 6 to interact with the complex. TRAF6 activates transforming growth factor-β-activated kinase (TAK) 1 and this leads to mitogen-activated protein kinase (MAPK) kinase 4 (MAP2K4)-mediated activation of both MAPK8 and MAPK14 and inhibitor of nuclear factor (NF)-κB kinase (IKK) complexes, resulting in the upregulation of transcription factors, including NF-κB and activator protein-1 (AP1). This activation in turn activates pro-inflammatory cytokine genes.

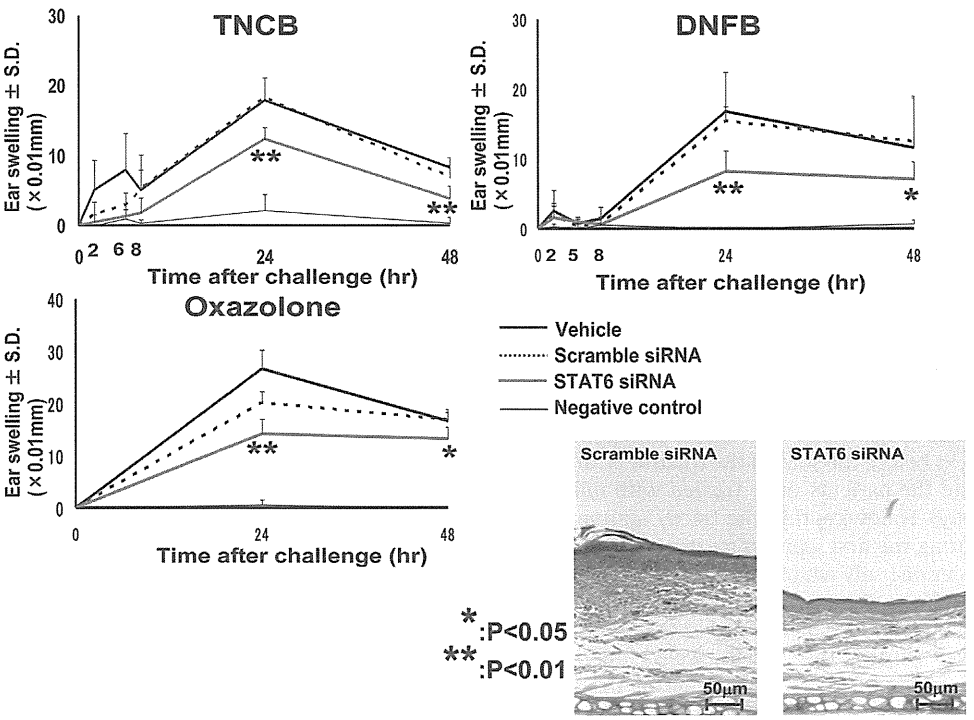

Fig. 3. Contact hypersensitivity reactions induced by the application of various haptens were significantly inhibited by subcutaneous injection of STAT6 siRNA into the auricle. DNFB, Dinitrofluorobenzene; TNCB, trinitrochlorobenzene.

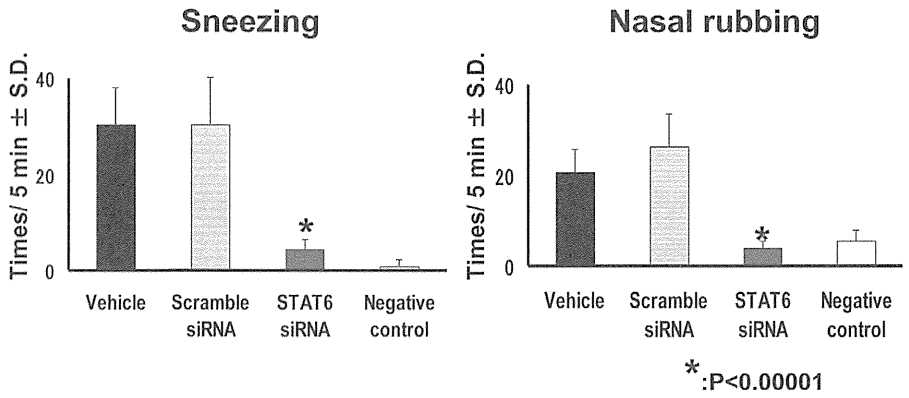

Fig. 4. In mouse models of allergic rhinitis, administration of STAT6 siRNA markedly reduced the frequency of sneezing and nasal rubbing.

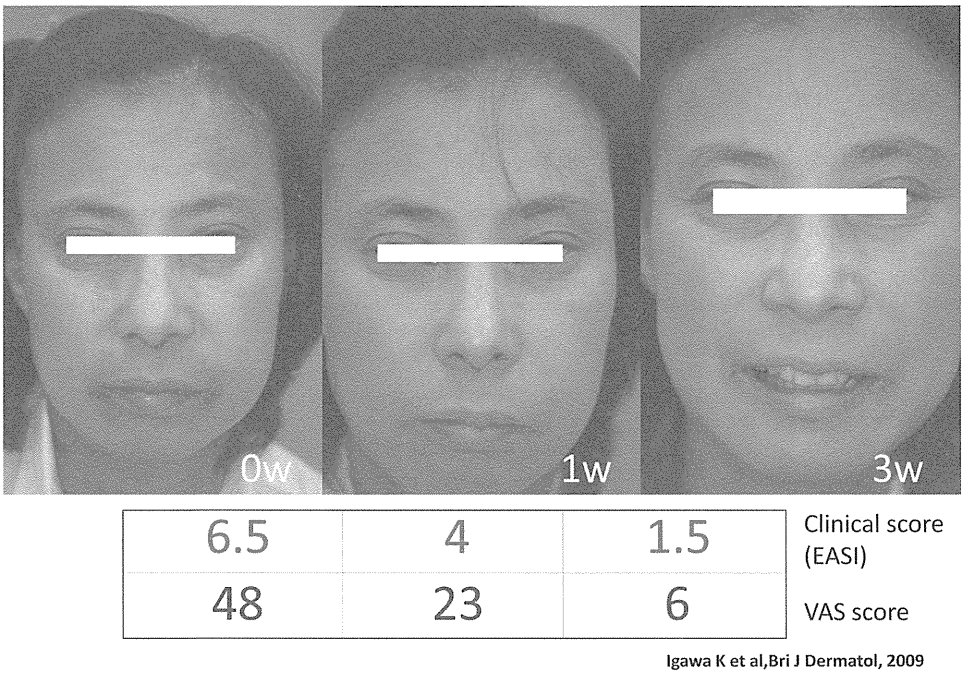

Fig. 5. Clinical scores: The effects of topical application of STAT6 Decoy ointment for refractory facial erythema in patients with atopic dermatitis were assessed using procedures approved by the university ethics committee. Marked amelioration of the erythema and pruritus was observed after application of the ointment in patients with atopic dermatitis. Photographs show the clinical manifestations of a patient who showed a complete response.

3. Actual procedures, techniques, targets, outcomes, and long-term prognosis

The efficacy of a 2% NF-κB Decoy ointment was evaluated in a clinical trial conducted in 10 adult patients with severe atopic dermatitis who visited the Department of Dermatology at Hirosaki University School of Medicine Hospital. In this study, patients between 20 and 65 years of age with severe atopic dermatitis were treated [26]. Two weeks before the start of the treatment, all drugs were discontinued and the patients were treated with only base (white petrolatum jelly). Following this, the Decoy ointment was applied once daily during the first week of treatment. The second week was a washout period (only white petrolatum jelly) designed to observe the clinical effects. During the third week, the 2% NF-κB Decoy ointment was applied twice daily, once in the morning and once in the evening. During the fourth week, only white petrolatum jelly was applied. The total duration of the clinical trial was 1 month. The ointment was effective in 6 of 7 patients with facial inflammation, whereas none of the patients experienced relief from skin lesions on the trunk [26]. Subsequently, a phase II clinical trial was conducted in Japan to examine the effects of the 2% NF-κB Decoy ointment on the facial lesions of adult patients with severe atopic dermatitis. Although analysis of the data from 162 patients revealed no statistically significant differences in the skin symptom scores, a tendency towards improved scores was observed in the moderate-dose group. Based on an analysis of 155 patients, excluding those who violated the protocol, the skin symptom scores showed significant improvement in the moderate-dose group as compared to the placebo group. Although there were no safety issues, the results pointed to the need for further improvement in absorptivity.

The effects of topical application of a STAT6 Decoy ointment for the treatment of refractory facial erythema in patients with atopic dermatitis were assessed. Marked amelioration of the erythema and associated pruritus was observed after application of the ointment in 7 patients (Fig. 5). Notably, relief from pruritus occurred early after the start of the treatment (Fig. 5) [3]. The safety and efficacy of the STAT6 Decoy must be evaluated further, however, in a larger sample size. The long-term outcomes of these treatments remain to be investigated.

4. Outlook and future direction

Nucleic acid-based medicines such as Decoys, CpG-ODNs, and siRNAs are effective for the treatment of facial lesions with erosions in adults suffering from severe atopic dermatitis and chronic contact dermatitis. Decoys, CpG ODNs, and siRNAs with a molecular weight of approximately 20,000 to 120,000 do not readily pass through even a disrupted corneal barrier in patients with atopic dermatitis and chronic contact dermatitis. The success of the Decoys, CpG ODNs, and siRNAs used in the clinical trials described here is attributable to the thin facial skin and lesions with erosions. For broader clinical application of the Decoys, CpG-ODNs, and siRNAs, a delivery system that will allow these molecules to pass through the corneal barrier must be developed. Delivery of macromolecules into cells and tissues is challenging. Small-interfering RNAs are potential therapeutics for various dermatologic diseases, including psoriasis, atopic dermatitis, contact dermatitis and skin cancer. Their utility is limited, however, by their low absorption across the stratum corneum into the viable skin cells. Recently, Hsu et al. addressed this problem using a peptide identified by phage display, termed skin penetrating and cell entering (SPACE) peptide. The ability of the SPACE peptide to deliver siRNA was tested *in vivo* using two targets, IL-10 and glyceraldehyde 3-phosphate dehydrogenase. Conjugation of the peptide to siRNA led to their enhanced absorption into the skin and knockdown of the corresponding target protein [27].

We previously reported that the STAT6 decoy improved CHS and atopic dermatitis in a mouse model using liposome-mediated transfection with HVJ (hemagglutinating virus of Japan; Sendai virus) [4]. Kaneda et al. showed that HVJ fused with the cell membrane at a neutral pH, and HN and F, the fusion proteins of the virus, contribute to this fusion [28]. For fusion-mediated gene transfer, DNA-loaded liposomes were fused with UV-inactivated HVJ to form the fusion liposome, HVJ-liposome. Fusion-mediated delivery protects the molecules incorporated in the liposome from degradation by endosomes and lysosomes before reaching the cytoplasm. Based on this concept, the HVJ envelope vector was developed using inactivated HVJ particles, and these vectors have been utilized for gene therapy. A tissue-targeting HVJ envelope vector was also constructed [28]. These delivery vectors might also be useful for gene therapy in the near future [28].

Although more detailed studies are needed, gene therapies to control NF-κB, STAT6 and STAT1, and TLR-9 expression may represent valid treatment strategies, not only for atopic dermatitis, but also for atopic asthma and allergic rhinitis. Further developments in this field are anticipated.

Acknowledgments

We thank Ms. Yu Kawamura for her excellent technical assistance. This work was partially supported by the Cosmetology Research Foundation and grants (25461665) from the Ministry of Education, Japan.

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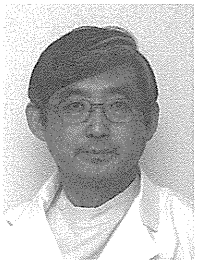

Hiroo Yokozeki M.D., Ph.D. is Professor and Chairman, Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan since 2005. He graduated from Tokushima University School of Medicine in 1980 and earned PhD degree from Osaka University in 1886 as a graduate student of Prof. Shigeharu Sano under the supervision of Prof. Kiyoshi Nishioka. In 1984–1986 he studied physiological and biochemical sciences of sweat gland and sweat as a Research Fellow at Department of Dermatology, University of Iowa, Iowa city, Iowa, USA under the supervision of Prof. Kenzo Sato. In 1986–1988, he studied immunology as a Research

Fellow at Department of 1st Dermatology, Vienna University, Vienna, Austria under the supervision of Prof. Georg Stingle.
In 1988–1990. He worked as Assistant Professor at Department of Dermatology, Kitasato University, Sagamihara city, Kanagawa. In 1991–1993, he worked as Assistant Professor at Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, and he worked in 1993–1996, as Junior Associate Professor, in 1996–2004, as Associate Professor, in 2005-Present, as Professor and Chairman, Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University.
His research specialty is immunodermatology especially he is studying the pathogenesis of contact hypersensitivity and atopic dermatitis and his research subspeciality is physiological study in sweat gland. As a dermatologist he specialized in allergic skin diseases and disorders of sweating (hyperhidrosis and hypohidrosis).