Modulation of Allergic Response by Gene–Environment Interaction: Olive Pollen Allergy

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Abstract

This article summarizes the most important advances of recent years in the field of gene–environment interaction in allergic response. It specifically examines sensitization to olive pollen as an example of one of the main causes of allergic disease in the Mediterranean area.

The presence of at least 20 proteins with allergic activity has been demonstrated in olive pollen, and 10 of these have been characterized (Ole e 1 to Ole e 10).

Ole e 1, which is considered to be the majority allergen (causing sensitization in more than 70% of patients), has been the subject of many studies looking for risk factors and ways to protect against sensitization. Markers of the major histocompatibility complex and other genetic loci associated with the allergic response have been analyzed using population-based, family-based, and functional approaches, which have revealed the involvement of genetic regulation in this type of response.

Furthermore, evaluation of environmental factors and their relationship with genetic factors is essential when attempting to understand this type of disease. In this review, we provide examples of how exposure to high doses of olive pollen allergen in a specific genetic context can trigger different allergic conditions (from asthma to nonresponse). We stress the importance of evaluating these factors in order to modulate this response correctly.

Key words: Genetic polymorphisms and allergy. Gene–environment interactions. Olive pollen allergy.

Resumen

Este artículo resume los avances más relevantes de los últimos años, relacionados con la interacción genes–medio ambiente en la respuesta alérgica, centrándose en la sensibilización al polen del olivo, como ejemplo de una de las principales causas de patologías alérgicas en los países Mediterráneos.

En el polen del olivo se ha demostrado la presencia de al menos 20 proteínas con actividad alergénica, de las cuales, 10 han sido caracterizadas (Ole e 1 a Ole e 10).

Ole e 1, considerado el alérgeno mayoritario (causante de la sensibilización en más del 70% de los pacientes), ha sido objeto de múltiples trabajos centrados en la búsqueda de factores de riesgo y protección frente a su sensibilización. Marcadores dentro del complejo mayor de histocompatibilidad, así como, el análisis de otros loci genéticos relacionados con la respuesta alérgica han sido analizados, tanto por análisis poblacionales y familiares, como por estudios funcionales, demostrándose la implicación de elementos genéticos reguladores en este tipo de respuestas.

Por otro lado, la evaluación de los factores ambientales y su interrelación con los factores genéticos es un elemento clave para poder comprender este tipo de enfermedades. En esta revisión, se describen ejemplos de cómo la exposición a altas dosis alergénicas de polen de olivo en un contexto genético determinado, pueden ser elementos claves para el desarrollo de distintos tipos de patologías alérgicas (desde el desarrollo de asma a la no-respuesta), destacando la importancia de la evaluación de ambos tipos de factores para poder modular correctamente este tipo de respuestas.

Palabras clave: Polimorfismos genéticos y alergia. Interacciones gen–medioambiente.Alergia al polen del olivo.

Introduction

We review the most relevant advances in gene–environment interaction related to allergy, taking olive pollen sensitization as an example of one of the major causes of allergic disease in Mediterranean countries.

Allergic diseases are an adverse reaction of the immune system against otherwise harmless substances and are characterized by their high complexity. Patients can be asymptomatic or their involvement could be as severe as asthma. The complex nature of the phenotypes involved seems to point to genetic and environmental factors.

Familial aggregation or genetic implication in the development of these diseases is well reported, and experts seem to agree that atopic diseases affect homozygotic twins more than in dizygotic twins [1,2].

Allergic diseases are characterized by a Th2 inflammatory response involving several possible subject-related or antigen-related modulators such as adjuvants, solubility in the microenvironment of mucosa, size of the sensitization agent, mucosal permeability, viral infections, and the greater or lesser ability of effector cells to liberate mediators.

Other factors include atmospheric pollution, exposure to tobacco, lifestyle-related diet and hygiene habits, and maternal effects. The interaction between these factors produces the clinical picture of allergic disease.

During the last 15 years, much has been done to identify protective factors that could increase tolerance to allergens. Research has focused on detecting potential risk factors in the environment and "allergy genes." To date, despite significant findings regarding susceptibility regions and genes, these studies have only provided us with a very limited understanding of asthma and allergic diseases. The failure to replicate the detection of particular loci may be due to a lack of homogenization between studies [3]. Nevertheless, the scientific community agrees on some essential points, eg, the fact that few genes have independent effects, as occurs in Mendelian diseases. The model described by Hersey [4] leaves no doubt that the pathogenesis of complex polygenic disorders depends on multilayered gene–environment interactions over time, although the approaches used to find susceptibility genes, either through linkage or association studies, have for the most part considered 1 gene at a time. Despite this overly simplistic view of the genetics of asthma and atopy, many important discoveries have been made (figure) [5,6]. Here, we summarize the most recent advances in the genetics [7] and environmental aspects of olive pollen allergy.

Olive Pollen Allergy

In the Mediterranean area and in California, the olive tree (*Olea europaea*) is widely distributed and its pollen is one of the most important causes of respiratory allergy [8]. Our group was a pioneer in the characterization of olive pollen antigens [9,10] and, since the 1990s, our work has focused on trying to understand the different aspects of this complex sensitization.

Olive pollen extract has at least 20 protein bands with allergenic activity [11]. The most frequent sensitizing allergen is Ole e 1, which is recognized by more than 70% of patients [9,10]; therefore, our work focuses mainly on this allergen.

Ole e 1 is a heterogenous 18- to 20-kDa protein that has been cloned and whose complete amino acid sequence has been described [12,13]. It is an acidic protein that exhibits 2 variants—glycosylated (20 kDa) and nonglycosylated (18.5 kDa)—of the same 145-residue polypeptide chain. This protein has at least 4 B cell-epitopes [14] defined by mapping with 6 monoclonal antibodies, and 2 regions of approximately 91 to 102 and 109 to 130 amino acids in length have been defined as immunodominant T-cell epitopes [15].

Genetic Restriction

Genetic regulation of IgE responses to specific allergens may differ from that of the general atopic response. Genetic associations are more readily detected with reactions to purified major allergens rather than to complex allergenic sources. The immunogenetic mechanisms underlying the heightened IgE responsiveness observed in atopic diseases may be divided into 2 types: antigen-specific and nonantigen-specific.

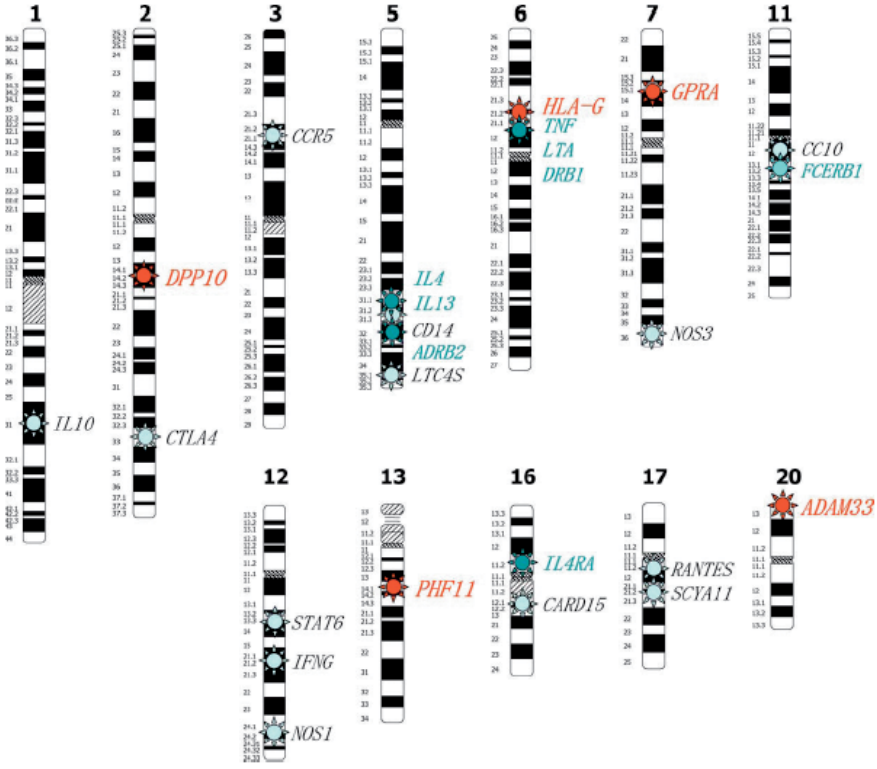
Antigen-specific

The generation of cellular immune response depends upon recognition by the T cell antigen receptors of peptidic fragments derived from processed foreign proteins associated with products of the major histocompatibility complex on the antigen-presenting cell (APC) surface. The implication of HLA class II antigens in the recognition of soluble antigens (such as allergens) led different authors [16,17] to think that this antigenic complex could be the key to the production of specific IgE antibodies.

Our first study of genetic restriction in olive pollen allergy was a case–control study analyzing the possible association between HLA class II antigens and Ole e 1 IgE antibody response [18]. This report showed strong associations between DR7 and DQ2 antigens and the immune response to Ole e 1 in allergic patients and was the first description of a positive response involving DQ alleles. This strong association between DQ2 and anti-Ole e 1 IgE antibody response induces a high relative risk (9.73) and an etiologic fraction of 0.81. We also found a remarkable decrease in the phenotypic frequencies of DR4 haplotypes in allergic patients, suggesting a possible protective role for HLA-DR4 antigen or haplotypes.

It is very difficult to determine whether or not DR7 and DQ2 operate jointly as susceptibility factors for olive allergy, because both these antigens are in linkage disequilibrium. Therefore, we adopted the following 2 approaches [19]:

1. The study of another ethnic group, individuals of Arab origin (in co-operation with C. Geller-Bernstein and Y. Waisel from Tel Aviv University), with presumably distinct linkage disequilibrium between DR and DQ antigens. The Arab patients showed a similar restriction pattern to the Spanish patients. Interestingly, once again, DR4 was under-represented in the allergic patients (not represented in the Ole e 1 responders, present in 44% of the control population), suggesting the possible protective role of this antigen.



Approximate locations of asthma and atopy genes on human chromosomes. Five genes identified through linkage followed by positional cloning studies are shown in red. Twenty-one genes that were identified through association studies and replicated in subsequent studies are also shown. Eight genes that have been replicated in more than 5 studies are shown in blue, and 13 genes that have been replicated in fewer than 5 studies are shown in black (figure from Ober 2005).

2. The analysis of the genetic requirements of the Ole e 1 response, using antigen-specific T-cell lines derived from 2 patients sensitized to *O europaea* and with IgE antibodies to Ole e 1.

Two strategies were used: inhibition of the proliferative response to Ole e 1 with HLA class II specific antibodies and a study of genetic restriction using a panel of histocompatible and histoincompatible B-cell lines transformed with the Epstein-Barr virus as the APC.

Both kinds of assay confirmed that DR7 and DQ2 are involved in the IgE antibody response to Ole e 1, because the inhibition assays showed that T-cell proliferation was inhibited with α-DR and α-DQ monoclonal antibodies and that only when the APCs were DR7 and/or DQ2 were the T-cell lines able to respond to Ole e 1 [19].

However, although these associations have been clearly confirmed, the HLA class II antigens do not seem to be the only element involved in the IgE status of responders vs nonresponders to an antigen.

Non–antigen-specific

Strong candidate genes for atopy have been reported to be located on chromosomes 11q13 [20] and 5q31.1 [21], where high-affinity IgE Fc receptor β-subunit and allergy-associated cytokines (interleukin-4 gene cluster), respectively, have been mapped. Other candidate genes have been found on chromosome 12q13.2-q23.3 [22,23], where the genes of interferon-γ (TH1 cytokine), stem-cell factor, and insulin-like growth factor-1 required for the maturation of mast cells and differentiation of B and T lymphocytes are located, in addition to the β-subunit of nuclear factor-Y, which upregulates the transcription of IL-4 and HLA-D genes, and B-cell translocation gene 1, which negatively regulates cell proliferation mapping in this region. Candidate genes located on chromosome 6 are tumor necrosis factor and lymphotoxin-α and lymphotoxin-β genes, which are potent pro-inflammatory cytokines found in high concentrations in the airways and lavage fluid of asthmatic patients.

In order to find more genes involved in olive pollen sensitization and analyse the response to Ole e 1, we studied 22 nuclear olive pollen-allergic families (n = 88) by analyzing the FcεRI-β exon 7 and intron 2 polymorphisms on chromosome 11 as atopy genes and we typed the DR and DQ antigens on chromosome 6 and the Vα8.1 polymorphisms on chromosome 14 as genes influencing specific IgE response to particular allergens. Finally, the polymorphisms of lymphotoxin-α gene on chromosome 6 were studied as gene influencing non-IgE-mediated inflammation [24]. This work confirmed the previous data reported by population studies that associated the IgE antibody response to Ole e 1 with DR7-DQ2 antigens [18,19] and the association between FcεRI-β and IgE and IgE antibodies against Ole e 1.

Genetics and Environment

Besides Ole e 1, a further 9 olive pollen allergens have been isolated and purified from *O europaea* pollen extract [25]. Studying the relevance of these allergens can help us to design more efficacious diagnostic techniques and therapy.

Although several reports have described the high prevalence of *O europaea*-induced nasal and conjunctival symptoms [8], this pollen may also induce epidemics of asthma exacerbations between late April and early June. However, the threshold level of *O europaea* pollen required to elicit symptoms of seasonal allergic rhinitis is extremely high (around 400 grains/m^3) compared to the 50 grains/m^3 needed by patients who are clinically sensitive to grasses [26]. In some regions of Andalusia (southern Spain), the atmospheric pollen concentration varies between 500 and 1000 grains/m^3 during at least half of the pollination season, with peaks of more than 5000 grains/m^3 by mid-May. For this reason, patients have more severe rhinitis and/or bronchial asthma when they are exposed to these high pollen counts [27]. Therefore, recent evidence suggests a more complex IgE response to the olive pollen allergens in allergic patients from Jaén (with extremely high pollen grain counts) than that observed in patients with pollinosis from areas with a lower count [28]. Our group described this situation by analyzing a population from a region with an extremely high antigenic load and a very high percentage of asthma (74.7%). This could be associated with a "special olive pollen sensitization" as reflected in our results: 4 of the 6 allergens analyzed (Ole e 1, 2, 6, 7, 8, and 10) are major allergens in this population (Ole e 1, 2, 7, and 10), and not in populations with a lower antigenic exposure. Nevertheless, we found a strong association with asthma in Ole e 2- and Ole e 10-sensitized patients. No other allergen in the *O europaea* group behaves in a similar way [29]. In addition, patients who showed Ole e 10 IgE antibodies also had more severe bronchial asthma, because the number of asthma days was significantly higher than in non-Ole e 10-sensitized patients. These data suggested that the existence of anti-Ole e 10 IgE in patients with *O europaea* bronchial asthma would make it possible to identify patients at risk of a more severe disease. Furthermore, the response to Ole e 2 and Ole e 10 was associated with DR7-DQ2 and DR2 (15), respectively. These data suggest that the genetic control of allergen-specific IgE responses in olive allergy may play a role in clinical disease.

We then analyzed the role of genes previously associated with allergy and asthma regulation—IL13 (C-1112T, R130Q), IL4RA (I50V, Q551R), IL5 C–746T, and B2AR (R16G, Q27E)—by examining the interaction between single polymorphisms, genetic haplotypes, and gene–gene and specific clinical parameters (unpublished data). The results showed how some of these polymorphisms are implicated in the clinical phenotype and how some of these polymorphisms act together.

Finally, we also studied the link between high exposure to olive pollen and genetic regulation [30]. Although it is generally believed that allergic sensitization to aeroallergens reflects the degree of exposure, and this also applies to pollen sensitivity, our results are different. We investigated sensitization to *O europaea* in a population of Israeli Arabs who live in a region where olive has been cultivated for centuries. Their houses and courtyards are surrounded by olive trees; therefore, they have been massively exposed to olive pollen from birth. We studied patients with respiratory allergies from Shefaram, a city in the mountains, where the population belongs to 3 subgroups: Druse, Christian, and Muslim. The parameters assessed were clinical diagnosis, skin prick test, total and Ole e 1-specific IgE, and HLA class II genomic typing. Skin prick testing showed that the prevalence of sensitization to olive pollen in the study population was extremely low, especially in the Druse subgroup (12%) compared with the Israeli Jewish population living in olive tree-rich regions (66%). Total IgE levels were high in 35% of the Arab allergic patients and in 50% to 60% of the Jewish allergic patients.

HLA class II genomic typing showed that phenotypic frequency was high for DQ2 and low for DR4 in Arab allergic patients who were sensitive to olive pollen, while in those who were not sensitive to olive pollen, phenotypic typing was high for DR4 and low for DQ2. It therefore appears that DR4 protects against sensitization to olive pollen, whereas DQ2 predisposes to it. More interestingly, Druses showed a particularly low presence of DQ2.

The continuous exposure of this Arab population has apparently lowered their likelihood of becoming sensitive to olive allergens as reported recently by Florido et al [27] for the Spanish population in Jaén. This could be due to the development of tolerance, spontaneous desensitization, or other mechanisms. These results lead us to ask whether it is really "bad hygiene" to inhale pollen early in life and whether early exposure enhances or reduces the risk of developing pollen allergy later in life.

Interestingly, in a preliminary work with the cells of these patients, we have found how peripheral blood mononuclear cells from olive-allergic patients, after stimulation with immunodominant Ole e 1 peptides (P-10, P-12, and P13), produce less IL-10 than non-olive-allergic patients. Olive-allergic patients did not produce IL-10 or IL-2 after stimulation with peptides recognized by non-allergic patients (P-2, P-3), which induced the production of IL-10 in a high percentage of nonallergic patients (unpublished data).

IL-10 is a regulatory cytokine that has been postulated as a possible modulator of allergic response, inducing IgG4 instead of IgE, and directly implicated in the modulation of some of the most important events in the allergic response. We must ask if this lack of response to olive pollen, despite high exposure, is modulated by T cell regulators and try to determine the differential mechanisms implicated in the nonresponse versus response to olive pollen in order to establish which is more important: genetics, the environment, or both.

New approaches, such as microarray analysis (a methodology that enables us to simultaneously study the expression of all genes) combined with different exposure conditions could be interesting for our understanding of this complex response. The next phase of genetic investigation should continue to unravel the nature and overall importance of gene–environment and gene–gene interactions in the development of asthma and allergic phenotypes, disease progression and severity, and response to therapy. Thus, the next 10 years of genetic research in asthma will begin to meet the goals of new molecular medicine [5].

Funding sources

Supported in part by research grants FIS 01/1234, CP05/00183, and CIBERES (ISCIII, 0013) from the Fondo de Investigación Sanitaria (Ministerio de Sanidad y Consumo, Spain) and SEAIC (Research grant 2002). E Llanes, M Chacártegui, and R. Mollá are supported by the Fundación Conchita Rábago, Madrid, Spain.

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