

Update in the understanding of genetic predisposition to olive pollen sensitization

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Olive tree pollen is one of the most important causes of allergy in the Mediterranean countries. Although the importance of this pollen provoking respiratory allergy is well established and documented, during the last decades, olive trees have been widely planted for ornamental purposes originating a new source of urban air pollution. This fact is one important reason why studies on olive pollen allergy are needed, in order to have more information about the prevention and possible modulation of this kind of sensitization. This article summarizes the advances in the study of genetic restrictions implicated in the susceptibility to the sensitization against the major allergen from this pollen: Ole e 1.

Asthma and allergies are complex diseases involving genetic as well as environmental factors. The final clinical picture results from the interaction between genetic factors and environmental stimuli, and in addition, variables such as age, concurrent infections, and physiological factors, could promote differences in susceptibility.

Despite this complexity, the allergic diseases are considered as an excellent model for the analysis of the genetic basis of the human immune response (1). The principal advantages are that they are common diseases with increasing prevalence and in addition, they have a well-defined clinical picture and the causative allergens may be identified and characterized so the immune response to them can be identified.

The allergic reaction appears to require the interaction of environmental factors with a series

of steps, which are genetically regulated. Genetic regulation of IgE responses to specific allergens may differ from that of the general atopic response. Probably, genetic associations are more readily detected with reactions to purified major allergens rather than complex allergen sources. The immunogenetic mechanisms underlying heightened IgE responsiveness seen in atopic diseases may be divided into two types: (a) antigen-specific and (b) nonantigen-specific (2–4).

(a) Antigen-specific: The generation of cellular immune response depends upon recognition by the antigen receptors on T cells (TCR) of peptidic fragments derived from processed foreign proteins, associated with products of the major histocompatibility complex (MHC) on the antigen presenting cells (APCs) surface. Due to the implication of HLA class II antigens in the recognition of soluble antigens (such as the allergens), different authors (5–7) thought that this antigenic complex could be the key for the production of specific IgE antibodies. In this respect, mainly two chromosomes have been studied, chromosome 6p21.3 and chromosome 14q11.2. On chromosome 6p21.3, genes that encode MHC class II molecules (HLA-D) are located. Several reports have demonstrated the association between IgE antibody response to highly purified allergens, and specific DR and DQ alleles, revised by Blumenthal (8). However, although these associations are clearly confirmed, the HLA class II antigens do not seem to be the

only element implicated in the IgE status 'responders vs nonresponders' to an antigen.

Finally chromosome 14q11.2, where the T-cell receptor α chain locus is located, has been associated with specific IgE antibody response (9).

(b) *Nonantigen-specific*: Strong candidate genes of atopy have been proposed to be located on chromosome 11q13 (10) and 5q31.1 (11) 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 localized on chromosome 12q13.2-q23.3 (12,13) where the genes of Interferon- γ (TH1 cytokine), stem-cell factor (SCF) and insulin-like growth factor-1 (IGF1) required for the maturation of mast cells and differentiation of both B and T lymphocytes, are located. Also, NFYB (β -subunit of nuclear factor-Y, which up-regulates the transcription of IL-4 and HLA-D genes) and BTG1 (B-cell translocation gene 1, that negatively regulates cell proliferation) mapping in this region.

Other candidate genes located on chromosome 6 are Tumor Necrosis factor (TNF) and Linfoxin (LT) α and β genes, potent pro-inflammatory cytokine that is found in increased concentration in asthmatic airways and in lavage fluid from asthmatic lungs (14).

Allergy to olive pollen

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 (15). The major allergen of this pollen, Ole e 1, is a protein of 18–20 kDa molecular mass (16,17), of a heterogeneous nature, that has been cloned and the complete amino acid sequence described (18,19). Ole e 1 is an acidic protein that exhibits two variants of the same 145-residue polypeptide chain, glycosylated (20 kDa) and nonglycosylated (18.5 kDa). This protein has at least four B cell-epitopes (20) defined by mapping with six mAbs, and two regions around 91–102 and 109–130 aa have been defined as immunodominant T-cell epitopes (21).

Genetic restriction of olive pollen response

Our group was one of the pioneers in the olive pollen antigens characterization (16,17) and since the 90s we have focused our works in the understanding the Ole e 1 antibody response. We have

analysed different aspects: B- and T-cell epitopes characterization (20,21), and genetic restrictions in the Ole e 1 IgE antibodies response.

Besides Ole e 1, several IgE-binding proteins have been detected in the saline extract of this pollen (16,22), and some of them have been purified and characterized: Ole e 2 (23), Ole e 3 (24), Ole e 4 and 5 (25,26), Ole e 6 (27), Ole e 7 (28), Ole e 8 (29) and Ole e 9 (30).

This report summarizes the results obtained in the genetic restriction field.

The genetic studies in the response to allergens has been focused in two ways: first, by population or familiar studies (important for establishing the association or prevalence of an antigen in relation to a particular disease and the inheritance transmission) and secondly, by studying the genetic restriction using specific T-cell antigen (31,32).

Our first study in the genetic restriction of Ole e 1 IgE antibody response was a case-control design study (33). In this report, DNA typing by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) and RFLP (restriction fragment length polymorphism) showed strong associations between DR7 and DQ2 antigens and the immune response to Ole e 1 in allergic patients, being the first time that a positive response was described in relation with DQ alleles.

Genomic DNA typing (HLA-DRB and DQB loci) was performed in 40 unrelated patients with seasonal allergic pollenosis who had specific IgE antibodies against Ole e 1, detected by double antibody radio-immunoassay (DARIA). Phenotypic frequencies were compared with those of 179 healthy unrelated individuals.

Significant increases in the phenotypic frequencies of DR7 (pf = 67.5% vs 31.3% in the control population, pc = 0.0023, RR = 4.56) and DQ2 (pf = 90.0% vs 48.0%, pc = 0.0003) were found, indicating an association between DRB1*0701/2, DQB1*0201 alleles and the IgE antibody response to Ole e 1. This was the first report of an association between HLA-DQ genes and the positive response to an allergen. This strong association between DQ2 and IgE antibody response anti-Ole e 1, induces a high relative risk (9.73) and an etiologic fraction of 0.81. The highest total and specific IgE values were found in the DR7-DQ2 group, in contrast with the nonDQ2 group.

A remarkable decrease in the phenotypic frequencies of DR4 haplotypes in allergic patients was found. Although its significance is borderline (uncorrected $P < 0.05$), allergic patients sensitized to olive, but nonresponders to Ole e 1 (with no detectable specific IgG nor IgE) show a DR4

frequency close to that of the control group. These data suggested a possible protective role for HLA-DR4 antigen or haplotypes. This kind of association between nonresponders and HLA-DR4 has been described for the mountain cedar allergy in the US (34) and for Japanese cedar (*Cry j 1*), that cross-reacts with mountain cedar (35). There is also an association between the IgE response to Jun a 1 (major allergen from mountain cedar) and HLA DR7 in allergic patients (36).

The occurrence of a particular DR subregion associated with a particular DQ subregion on the same chromosome is much more frequent than would be expected by chance, this phenomenon is called linkage disequilibrium. When this situation occurs and the corresponding haplotype is associated with a genetically restricted response, it must be determined whether both these antigens are implicated or if the presence of one of them is a statistical artefact. Some investigators suggest that the best solution is to increase the sample size or to investigate other ethnic groups with a presumably different genetic background, until a significant number of patients possessing only one of these factors is found (37).

In our study, DR7 and DQ2 are two antigens in linkage disequilibrium, as DR7 and DQ2 operating jointly as susceptibility factors for the allergy to the *Olea europaea* pollen cannot be excluded. Cellular experiments with PBLs derived from olive allergic patients point to an implication of both antigens being the inhibition produced by the antiDQ – practically 100% in the case of a DR7-DQ2 homozygous patient (33).

Due to this linkage disequilibrium, we decided to solve the question, by two approaches (38):

(1) The study of another ethnic group, individuals of Arabic origin (in cooperation with C. Geller-Bernstein and Y. Waisel from Tel Aviv University), with a presumably distinct linkage disequilibrium between DR and DQ antigens. Genomic DNA typing was performed in 46 subjects (allergic and nonallergic) by RFLP and results showed that patients with specific IgE antibodies α -Ole e 1, were DR7 and/or DQ2. The Arabic patients showed a similar restriction pattern to the findings in the Spanish patients.

Interestingly, again DR4 was underrepresented in the allergic patients (not represented in the Ole e 1 responders, present in 44% in the control population), suggesting the possible protective role of this antigen.

(2) The analysis of the genetic requirements of Ole e 1 response, using T-cell lines specific for this antigen, derived from two patients sensitized to

Olea europaea and with IgE antibodies to Ole e 1. Two kinds of strategies were carried out:

(a) Inhibition of the proliferative response to Ole e 1 with HLA class II specific antibodies.

(b) Study of the genetic restriction using a panel of histocompatible and histoincompatible B-cell lines transformed with Epstein Barr Virus (EBV) as APC.

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

In a recent study (39), carried out in 22 nuclear olive pollen allergic families ($n = 88$), to increase the study of genetic restriction in olive pollen sensitization and analyse the response against two well characterized allergens of this pollen – Ole e 1 and Ole 3 – we analysed some of the possible genetic restrictions: the atopy genes, the Fc ϵ RI β exon 7, and intron 2 polymorphisms on chromosome 11. As genes influencing specific IgE response to particular allergens we typed the DR and DQ antigens on chromosome 6, and the V α 8.1 polymorphisms on chromosome 14. Finally, the polymorphisms of LT α gene on chromosome 6, were studied as gene influencing nonIgE mediated inflammation.

This work confirmed by family studies the previous data reported by the study of populations that associated the IgE antibody response to Ole e 1 with DR7-DQ2 antigens (33,38). Here, the presence of DRB1*0701 was significantly associated with high levels of IgG antibodies to Ole e 1 and DQB1*0201 with IgG α -Ole e 1 and IgE α -Ole e 1.

In spite of the low number of patients with significant levels of IgE α -Ole e 3 ($n = 17$), the presence of DQB1*0201 was statistically associated with the IgE antibody response to Ole e 3. This similarity in genetic restrictions could indicate similarity at a molecular level between Ole e 1 and Ole e 3, but sequence homology has not been reported (18,19,23).

In a previous report (40), we selected two families composed of five members (parents, two olive pollen-sensitized siblings and one nonallergic sibling) and studied the variability in the V β chain of TCR, by flow cytometry. We used 14 monoclonal antibodies that recognized CD3, CD4, CD8, TCR $\alpha\beta$, V β 2/CD3, 13.3, 17, 3, 5(a), 5(b), 5(c), 6(a), 8(a) and 12(a). The results showed that there are no differences in the repertoire of V β chain of TCR between 'responders' and 'non-responders' to Ole e 1. These data agree with some

reports that describes the lack of a link between V β chain of TCR and specific IgE antibody response to allergens.

However, a link between IgE responses to major allergens and the TCR A/D locus has been reported (9). This locus is located on different chromosome to the V β chain and the germline HLA-DR and TCR-A interaction in restricting the response to exogenous antigen has been demonstrated, being described as the interaction between V α 8.1 polymorphism and HLA-DRB1*1501 in patients allergic to *Dermatophagoides* (41). For this reason we analysed this polymorphism in our population but we did not find any association with the antigenic responses studied. This result did not exclude the V α chain implication in our response. Due to the fact that our HLA class II associations (with IgE α -Ole e 1 and Ole e 3) are related with other alleles (DRB1*0701 and DQB1*0201) it is reasonable to assume that the TCR polymorphism implicated in our specific response could be different.

Analysis of two noncoding RsaI polymorphisms (exon 7 and intron 2) within the Fc ϵ RI- β gene showed that there are significant associations between this gene and the antibody response observed in olive allergic patients. We found that allele 1 of Fc ϵ RI- β RsaI (exon 7) is associated with total serum IgE levels as well as with *O. europaea* specific serum IgE levels and with, Ole e 1 specific IgG and IgE levels. Fc ϵ RI- β RsaI (intron 2) was significantly associated with *O. europaea* specific serum levels and with Ole e 1 specific IgG and IgE antibody levels.

These results are in accordance with several reports (10,42–47) that describe linkage between coding and noncoding polymorphisms within the beta chain of the high-affinity receptor and the serum total IgE, skin prick test responses to allergens, bronchial hyper-reactivity, asthma and atopic dermatitis. However, recently, Castro et al. (48)

described a lack of association between atopy and RsaI polymorphism within intron 2 of the Fc ϵ RI- β gene in a Spanish population. Like our results they found no differences in the genotypic frequency distribution of atopic and nonatopic populations, however, in contrast with our work no other allergic traits were studied to determine possible associations (total IgE, RAST, specific IgE).

Finally, we analyzed the NcoI polymorphisms in the Lymphotoxin alfa gene as a gene influencing nonIgE mediated inflammation. Allele 1 of a NcoI polymorphism in the LT α gene has been associated with the level of tumor necrosis factor secretion (49) and this pro-inflammatory cytokine is increased in asthmatic airways (14).

In our study there was no association between the polymorphisms tested and the allergic traits analysed. This result is in agreement with a work that established an association between the increased secretion of TNF and the HLA-DRB1*03 genotype (50), because our HLA genetic restriction is not related with this genotype.

This study showed the complexity of the allergy response to olive pollen extract. Our results confirm the hypothesis that there are multiple genetic influences in olive pollen allergy.

In summary, we have verified the association between Ole e 1-specific response and DQB1*0201 by different approaches, as well we found the association between this HLA-class II antigen and the IgE antibody response to Ole e 3. The association between Fc ϵ RI- β and IgE antibodies against Ole e 1 was demonstrated.

In conclusion, our studies emphasizes the importance of characterizing the population to be studied, as well as suggesting the implication of multiple genetic influences in olive pollen allergy response.

Further studies are required for a better understanding of this complex response, in order to improve the diagnosis and therapy of this disease.

References

1. MARSH DG, ZWOLLO P, ANSARI AA. Toward a total human immune response fingerprint: the allergy model. *Adv Bioscience* 1989; **74**: 65.
2. MARSH DG, MEYERS DA, BIAS WB. The epidemiology and genetics of atopic allergy. *N Engl J Med* 1981; **305**: 1551–1559.
3. GAUCHAT JF, HENCHOZ S, MAZZEI G, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993; **365**: 340–343.
4. BRADDDING P, FEATHER IH, HOWARD PH, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992; **176**: 1381–1386.
5. LEVINE B, STEMBER R, FOTINO M. Ragweed hay fever. Genetic control and linkage to HLA haplotypes. *Science* 1972; **178**: 1201.
6. BLUMENTHAL MN, AMOS DB, NOREEN H, MENDELL NR, YUNIS EJ. Genetic mapping of Ir locus in man: linkage to second locus of HLA. *Science* 1974; **184**: 1303–1304.
7. MARSH DG, HSU SH, ROEBBER M, et al. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. Response resulting primarily from natural antigenic exposure. *J Exp Med* 1982; **155**: 1439–1451.
8. BLUMENTHAL MN. Searching for the asthma and allergy genes. The current status of our knowledge. *ACI News* 1995; **7**: 15–18.
9. MOFFATT MF, HILL MR, CORNELIS F, et al. Genetic linkage of the TCR- α/δ region to specific

- Immunoglobulin E responses. Lancet 1994; **343**: 1597–1500.
10. COOKSON WCOM, SHARP PA, FAUX JA, HOPKIN JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 1989; **1**: 1292–1295.
 11. MARSH DG, NEELY JD, BREAZEALE DR, et al. Linkage analysis of IL4 and journal chromosome 5q31.1 markers and total serum Immunoglobulin E concentrations. Science 1994; **264**: 1152–1156.
 12. BARNES KC, NEELY JD, DUFFY DL, et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: Evidence from Afro-Caribbean and Caucasian populations. Genomics 1996; **37**: 41–50.
 13. NICKEL R, WAHN U, BARNES K, et al. Evidence for linkage of chromosome 12q15-q24.1 markers and high total serum IgE concentrations in children of the German Multicenter Allergy Study (MAS'90). J Allergy Clin Immunol 1997; **99**: S476.
 14. VIRCHOW JC, WALKER C, HAFNER D, et al. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. Am J Respir Crit Care 1995; **151**: 960–968.
 15. BOUSQUET J, GUERIN B, HEWITT B, LIM S, MICHEL FB. Allergy in Mediterranean area III. Oleaceae pollens. Clin Exp Allergy 1995; **15**: 439–448.
 16. LAUZURICA P, GURBINDO C, MARURI N, et al. Olive (*Olea europaea*) pollen antigens. I, Immunochemical characterization by immunoblotting, CRIE and immunodetection by a monoclonal antibody. Mol Immunol 1988; **25**: 329–335.
 17. LAUZURICA P, MARURI N, GALOCHA B, et al. Olive (*Olea europaea*) pollen allergens. II. Isolation and characterization of two major antigens. Mol Immunol 1988; **25**: 337–344.
 18. VILLALBA M, BATANERO E, LÓPEZ-OTÍN C, et al. The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea Europaea*). Pollen 1993; **216**: 863–869.
 19. VILLALBA M, BATANERO E, MONSALVE RI, GONZÁLEZ DE LA PEÑA MA, LAHOZ C, RODRÍGUEZ R. Cloning and expression of Ole e I, the major allergen from Olive tree pollen. J Biol Chem 1994; **269** (21): 15217–15222.
 20. MARTÍN-OROZCO E, CÁRDABA B, DEL POZO V, et al. Epitope mapping, cross-reactivity with journal Oleaceae pollens and ultrastructural localization. Int Arch Allergy Appl 1994; **104**: 160–170.
 21. CÁRDABA B, DEL POZO V, JURADO A, et al. Olive pollen allergy: searching for immunodominant T-cell epitopes on the Ole e 1 molecule. Clin Exp Allergy 1998; **28**: 413–422.
 22. WHEELER AW, HICKMAN BE, FOX B. Heterogeneity of major allergen from olive (*Olea europaea*) pollen. Mol Immunol 1990; **27**: 631–636.
 23. LEDESMA A, RODRÍGUEZ R, VILLALBA M. Olive-pollen profilin. Molecular and immunologic properties. Allergy 1998; **53**: 520–526.
 24. BATANERO E, VILLALBA M, LEDESMA A, PUENTE XS, RODRÍGUEZ R. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. Eur J Biochem 1996; **241** (3): 772–778.
 25. BOLUDA L, ALONSO C, FERNÁNDEZ-CALDAS E. Purification, characterization, and partial sequencing of two new allergens of *Olea europaea*. J Allergy Clin Immunol 1998; **101** (2 Part 1): 210–216.
 26. MARTÍNEZ A, ASTURIAS JA, PALACIOS R, et al. Identification of a 36-kDa olive-pollen allergen by *in vitro* and *in vivo* studies. Allergy 1999; **54**: 584–592.
 27. BATANERO E, LEDESMA A, VILLALBA M, RODRÍGUEZ R. Purification, amino acid sequence and immunological characterization of Ole e 6, a cysteine-enriched allergen from olive tree pollen. FEBS Lett 1997; **410**: 293–296.
 28. TEJERA ML, VILLALBA M, BATANERO E, RODRÍGUEZ R. Identification, isolation, and characterization of Ole e 7, a new allergen of olive tree pollen. J Allergy Clin Immunol 1999; **104**: 797–802.
 29. LEDESMA A, VILLALBA M, RODRÍGUEZ R. Cloning, expression and characterization of a novel four EF-hand Ca (2+)-binding protein form olive pollen with allergenic activity. FEBS Lett 2000; **466** (1): 192–196.
 30. HUECAS S, VILLALBA M, RODRÍGUEZ R. Ole e 9, a major olive pollen allergen is a β -1,3-glucanase. Isolation, characterization, amino acid sequence and tissue specificity. J Biol Chem 2001; **276**: 27959–27966.
 31. O'HEHIR RE, MACH B, BERTE C, et al. Direct evidence for a functional role of HLA-DRB1 and DRB3 gene products in the recognition of Dermatophagoides spp (house dust mite) by helper T lymphocytes. Int Immunol 1990; **2**: 885–892.
 32. HIGGINS JA, LAMB JR, MARSH SGE, et al. Peptide induced nonresponsiveness of HLA-DP restricted human T cells reactive with Dermatophagoides spp (house dust mite). J Allergy Clin Immunol 1992; **90**: 749–756.
 33. CÁRDABA B, VILCHES C, MARTÍN E, DE ANDRÉS B, DEL POZO V, HERNÁNDEZ D, et al. DR7 and DQ2 are positively associated with Immunoglobulin-E response to the main antigen of olive pollen (Ole e 1) in allergic patients. Human Immunol 1993; **38**: 293–299.
 34. REID MJ, NISH WA, WHISMAN BA, et al. HLA-DR4 associated nonresponsiveness to mountain cedar allergen. J Allergy Clin Immunol 1992; **89**: 593–598.
 35. MATSUSHITA S, MUTO M, SUEMURA M, SAITO Y, SASAZUKI T. HLA-linked nonresponsiveness to Cryptomeria japonica pollen allergy. I. Nonresponsiveness is mediated by antigen-specific suppressor T cell. J Immunol 1987; **138**: 109.
 36. BUDENS RD, STANSTNY P, SULLIVAN TJ. Studies of the immunochemistry of mountain cedar allergy. J Allergy Clin Immunol 1989; **83**: 490 (Abstract).
 37. SVEJGAARD A, RYDER LP. HLA and disease associations: detecting the strongest association. Tissue Antigens 1994; **43**: 18–27.
 38. CÁRDABA B, DE PABLO R, VILCHES C, et al. Allergy to olive pollen: T-cell response from olive allergic patients is restricted by DR7-DQ2 antigens. Clin Exp Allergy 1996; **26**: 316–322.
 39. CÁRDABA B, CORTEGANO I, FLORIDO F, et al. Genetic restrictions in olive pollen allergy. J Allergy Clin Immunol 2000; **105**: 292–298.
 40. CÁRDABA B, JURADO A, DEL POZO V, et al. Family study of olive allergy: HLA class II, TCR and T-epitope mapping. Genetics and specific immune response in

- allergy and asthma. In: CHARROS D, editor. Proceedings of the 12th International Histocompatibility Workshop. EDK France, 1996: 863–866.
41. MOFFATT MF, SCHOU C, FAUX JA, COOKSON WOCM. Germline TCR-A restriction of immunoglobulin E responses to allergen. *Immunogenetics* 1997; **46**: 226–230.
42. PALMER LJ, PARÉ PD, FAUX JA, et al. FcεRIβ polymorphism and Total serum IgE levels in endemically parasitized Australian Aborigines. *Am J Hum Genet* 1997; **61**: 182–188.
43. COX HE, MOFFATT MF, FAUX JA, et al. Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. *Br J Dermatol* 1998; **138**: 182–187.
44. YOUNG RP, LYNCH J, SHARP PA, FAUX JA, HOPKIN JM. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J Med Genet* 1992; **29**: 236–238.
45. SHIRAKAWA T, AIRONG L, DUBOWITZ M, et al. Association between atopy and variants of the β subunit of the high-affinity immunoglobulin E receptor. *Nat Genet* 1994; **7**: 125–130.
46. SANDFORD AJ, SHIRAKAWA T, MOFFATT MF, et al. Localization of atopy and the beta subunit of the high affinity IgE receptor (FcεRI) on chromosome 11q. *Lancet* 1993; **341**: 332–334.
47. HILL M, COOKSON WOCM. A new variant of the beta subunit of the high-affinity receptor for immunoglobulin E (Fc-epsilon-RI-beta E237G)-associations with measures of atopy and bronchial hyper-responsiveness. *Hum Mol Genet* 1996; **5**: 959–962.
48. CASTRO J, TELLERIA JJ, BLANCO-QUIROS A, LINARES P, ANDION R. Lack of association between atopy and Rsa I polymorphism within intron 2 of the Fc (epsilon) RI-beta gene in a Spanish population sample. *Allergy* 1998; **53** (11): 1083–1086.
49. MESSER G, SPENGLER U, JUNG MC, et al. Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-β gene correlates with a variant amino acid in position 26 and a reduced level of TNF-β production. *J Exp Med* 1991; **173**: 209–219.
50. JACOB CO, FRONEK Z, LEWIS GD, KOO M, HANSEN JA, McDEVITT HO. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor α: Relevance to genetic predisposition to systemic lupus erythematosus. *Proc Natl Acad Sci USA* 1990; **87**: 1233–1237.