Author's response to reviews

Title: Interleukin-10 polymorphisms in Spanish IgA deficiency patients: a case-control and family study

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Author's response to reviews: see over
Madrid, April 18th, 2006

Dear Editor,

Please find enclosed the revised version of our manuscript “Interleukin-10 polymorphisms in Spanish IgA deficiency patients: a case-control and family study” with the changes in response to the reviewers. A detailed point-by-point reply to them could be found below.

We think the final version of the manuscript has improved after the reviewer’s suggestions and hopefully it will be suitable for publication.

Best regards,

Alfonso Martínez, PhD
Responses to reviewer: Dr. Schroeder. We thank this reviewer for his kind comments on our manuscript.

Responses to reviewer: Dr. Kube.

Association studies of a candidate gene deal with an increase/decrease of disease susceptibility in a population, depending on the frequency of genetic markers in patients and healthy controls. Replications in independent cohorts contribute to the firm establishment of the gene as a new risk factor for the disease. Our aim with this paper was to investigate the role of the IL-10 gene as putative IgA deficiency predisposition factor, as only another report with questionable power had been published on this topic. We selected the genetic markers based on previous studies performed by us in other autoimmune diseases and also used by others. They extend through a 4 Kb region upstream the transcription start site. The two microsatellites are very informative, as both present several alleles, and together with the three SNPs aid in the definition of the haplotypes in this locus. These haplotypes cover the variability within this 4 Kb region, where other polymorphisms have been described.

There have been a number of functional studies of the IL-10 promoter in the literature. Several SNPs, not only 2849, have been claimed to be associated with different levels of cytokine production. Moreover, haplotypes defined by IL10R and IL10G microsatellites have been also related to changes in the production levels of this cytokine (Eskdale J, Gallagher G, Verweij CL, Keijser V, Westendorp RG, Huizinga TW: Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998, 95(16):9465-9470).

In a paper published some years ago (Gibson A, Edberg J, Wu J, Westendorp R, Huizinga T, and Kimberly R: Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus J Immunol, 2001, 166: 3915–3922), a detailed analyses with several SNPs was performed. As can be observed in their Figure 2, the variability recovered by those SNPs is lesser than that described by us. Our choice of three SNPs and two microsatellites maximizes the genetic information obtained from our sample. No positive association (after appropriate correction) was observed either in the case-control study for any allele or analyzing the transmission of the extended haplotypes formed by the three SNPs and the two microsatellites. As the IL-10 distal promoter polymorphisms are located in the 4 Kb region studied, one can assume that a putative effect of any of them on disease susceptibility would be detected by the haplotype transmission test. Provided that a positive association is found, functional studies help to explain the mechanisms involved
in the effect described (increase/decrease in protein levels) and to identify the etiologic polymorphism. In this case, as no difference between patients and healthy subjects could be detected, we consider that there is no need for further analyses. The results of our study evidence a negligible role of the IL-10 gene on susceptibility to IgA deficiency, and lack of clinical data prevents us from testing on the severity of the disease.

The approval of this study by the Ethics Committee of the Hospital Clínico San Carlos and its compliance with the Helsinki declaration are indicated in the Methods section of the manuscript (page 5, line 6).

The microsatellites are described as it is customary in the IL10 literature, rather than in terms of the number of repeats. However, the allele G12 has 24 CA repeats, as the referee correctly points out. It has already described that alleles shorter than G7 or R2 are extremely infrequent (see, among many other papers, Eskdale J, Gallagher G, Verweij CL, Keijser V, Westendorp RG, Huizinga TW: Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998, 95(16):9465-9470).

Table 2 describes the number of subjects presenting each of the listed proximal promoter haplotypes. As homozygous individuals are counted only once, the numbers do not add up to twice the total of patients/controls; however, phenotypic frequencies (also known as carrier rates) are generally accepted in genetic studies.