Reviewer's report

Title: Total blood lymphocyte counts in hemochromatosis probands with HFE C282Y homozygosity: relationship to severity of iron overload and HLA-A and -B alleles and haplotypes

Version: 1 Date: 31 March 2005

Reviewer: Maria de Sousa

Reviewer's report:

The intriguing question of a possible role for HLA in the interaction of iron and lymphocytes was first addressed experimentally nearly 25 years ago by Bryan et al., (Immunogenetics, 12:129-40, 1981) in an in vitro study of the effect of ferric citrate on responder cells in the MLR. A significant susceptibility to iron treatment was found in A2- subjects, including the alleles A1, A3 and A11. The results were highly reproducible within individuals and between testings. The work was done shortly after HH was found to be in linkage disequilibrium with HLA-A3. As a result, the first in vivo studies of lymphocyte numbers in transfusional and genetic iron overload focused on T-cell set numbers. The first study revealing significant correlations between CD8+ numbers, severity of iron overload and HLA in HH was published by Porto et al., in Hepatology, in 1997, a reference missing in Dr Barton's ms. That study preceeded, however, HFE genotyping. As Barton and co-workers claim justly, the present study is the largest complete study of total lymphocyte numbers, in HFE C282Y homozygous probands, HLA phenotyped and characterized for iron stores. The results illustrate a significant association of a decrease in log total blood lymphocyte count with an increase in units of phlebotomy to induce iron depletion, log serum ferritin concentration and the A*01 B*08 haplotype. This finding is reminiscent and perhaps concordant with earlier studies claiming an association between low CD8+ numbers, HLA phenotype and severity of iron overload. There is not and there should be reference to the relevant paper by Porto et al. published in 1997 (Hepatology). The authors do not discriminate lymphocyte subsets but the association with MHC class I points to the CD8 set as the most likely set involved in the results. In the view of this referee, "the" weakness of the ms resides in the exclusive interpretation as a conclusion that "the severity of iron overload is not the sole determinant of total blood lymphocyte counts". Experimental evidence following the first results with HH patients led to the demonstration of spontaneous iron overload replicating HH in mice lacking beta2microglobulin and MHC class I expression (De Sousa et al., Immunology Letters, 1994, Rothenberg et al., PNAS, 1996) and more recently MHC class I (Cardoso et al., Blood, 2002). Severe iron overload has also been reported in mice double not knock for hfe and rag, lacking lymphocytes (Miranda et al., 2003) all favoring the reverse interpretation that MHC class I and lymphocyte numbers precede the development of iron overload. In summary I would like Dr Barton to include the alternative, albeit intriguing, explanation that lymphocyte numbers are not consequence but intriguingly inextricable association with MHC class I and iron overload of the genetic, not transfusional type. The experimental evidence that Dr Barton knows well is much more in favor of this second explanation. The findings are challenging, point to the A1 region as an important region to explore for regulation of lymphocyte numbers, a region already indicated by the correctly cited work of Cruz et al. The results are of significance for hematologists, immunologists and iron overload specialists. It should be published appropriately by Biomed after Dr Barton and co-workers are invited to consider inclusion of the alternative interpretation referred to above and consideration of the significance of his results with HLA-A for other group's results with CD8. The implications of the alternative interpretation are not just "theoretical". Low total lymphocyte numbers (in this case) or low CD8+ lymphocyte numbers, have been a decisive element in the assessment of the nature of the follow-up offered to HH patients and identified as one of those much sought after modifiers of clinical expression of disease.
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I have the healthiest competing interest in relation to this paper. My group is grateful to Dr Barton for having become a worthy competitor for a basic question crossing the fields of immunogenetics, basic immunology and iron homeostasis. A rather lonely position practically held exclusively by us until publication of this work.