Author's response to reviews

Title: Functional activation of proline-rich tyrosine kinase2 (PYK2) in peripheral blood mononuclear cells from patients with systemic lupus erythematosus

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Version: 4 Date: 21 September 2009

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Version: 3 Date: 2009-9-15

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Object: MS: 1131451551273331- Functional activation of proline-rich tyrosine kinase2 (PYK2) in peripheral blood mononuclear cells from patients with systemic lupus erythematosus.Dr Meiying Wang et al.

Thanks for your consideration of our manuscript. We have studied their comments carefully and have made correction which we hope meet with their approval.

1) Because of lymphocytopenia, monocytes are commonly overrepresented in SLE, which may be an explanation for the findings on Western blots. Optimally, this would be resolved by pure lymphocyte preparations. Alternatively, immunocytochemistry, which is not terribly useful for proving a quantitative point, could be used to show that the staining is clearly in lymphocytes only, and discuss this accordingly.

Systemic lupus erythematosus (SLE) is an autoimmune disease with a broad spectrum of clinical and immunological abnormalities. Numerous aberrations of the immune system have been reported in SLE, Included in these abnormalities are factors such as changing in the expression of co-stimulatory molecules and defects in apoptosis, leading to the accumulation of autoreactive T and B cells. What is more important, some studies reported an increase in lymphocytes in patients with systemic lupus erythematosus, and a high degree of activation.
PBMCs isolated from peripheral blood by Ficoll–Paque gradient centrifugation contain 90-95% lymphocyte, and PBMCs lysates proteins are mainly from lymphocyte protein. we admit that we did not re-blot with markers for monocytes and/or T cells although perhaps we should have done. This was due to lack of research funds to support. we hope these do not affect our interpretation of the result.

2) Proliferation of lupus T cells has been found diminished by many groups. This makes the last Figure difficult to understand and also casts doubt on whether the effect of TyrA9 on lymphocyte proliferation is a good argument in SLE.

Regards the issue about proliferation of lupus T cells, we disagreed the referees to some extent.

SLE is characterized by profound immune alterations that lead to activation of autoreactive T and B cells and to the generation of a variety of autoantibodies (Abs) to nuclear antigens (Ags). Proliferation and activation of autoreactive T and B cells plays a pivotal role in the pathogenesis of this disease. Furthermore, many groups have reported that proliferation and activation of lupus lymphocytes were increased. Our result demonstrate that the PBMCs from SLE patients exhibit both an increased activation and a heightened function of PYK2.

3) Inhibitors are almost never perfect in their specificity, and the authors should discuss limitations of such approach.

Following the suggestion of the referees, a discussion of the limitations of TyrA9 action on lymphocyte proliferation has been included(page 17, the last paragraph, “Taking into account the limitations that chemical inhibitor of Pyk2 kinase activity is almost never perfect in its specificity, in subsequent experiments, we are going to use RNA-mediated interference,Pyk2-deficient PBMCs and dominant negative Pyk2 mutants to confirm the exact function of Pyk2 in regulating SLE PBMCs proliferation”)

Minor

4) The authors clearly perform multiple comparisons. In essence, there are two ways to do that correctly, namely by either correcting for the number of tests (Bonferroni), or, if the statistics are meant descriptively only, by stating so.

Thank you for giving us a good advice on statistical methods. But, because these do not affect our interpretation of the result, we don’t changes the statistical methods.

5) There is a small problem with the numbers in Table 1: 5+30 (male/female) is not 36, and 2+11 is more than 12. It thus appears that one patient was regrouped, but the table was not worked through completely.

The authors are grateful to the referees for pointing out their errors. These have now been corrected on table 1.

Additionally, We add an author named Hongsheng Sun, who has added and operated additional experiments at the referee’s suggestion,and has revised the manuscript.