

ANSWERING REVIEWERS



January 7, 2015

Dear Professor Campitstol,

Please find enclosed the edited manuscript in Word format (file name: 11991-revised MS) and figures (power point format: 11991-revised Figs).

Title: Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus

Authors: Junichi Fujii, Toshihiro Kurahashi, Tasuku Konno, Takujiro Homma Yoshihito Iuchi

Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 11991

We are sincerely grateful for kind criticisms that helped us to improve the manuscript. We have amended the manuscript according to the reviewers' advices. Our responses follow the Reviewers' comments as below.

Reviewer 1

The review article entitled "Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus" by Fujii et al. concisely introduces abundant information about mouse models developing anemia, autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) from the viewpoint of oxidative stress. Kidney is an important organ playing an essential role in erythropoiesis by producing erythropoietin. Therefore, chronic kidney disease causes renal anemia by reducing erythropoietin production. Lupus nephritis is one of serious symptoms of SLE. However, this article focuses on the hypothesis that oxidative stress induces AIHA and SLE, and thus new information about renal function or renal pathology in these diseases is limited.

The authors should add description regarding renal function for the audience of this journal. In addition, because lots of abbreviated words are frequently used, general readers would be confused.

Response: We focused on anemia and related autoimmune diseases in this review article because it was the proposed theme by the Editor.

By following readers' interest, however, we have added a new paragraph under heading "*Potential roles of oxidative stress in lupus nephritis*" with additional 5 references.

The authors should make an effort to lessen abbreviations.

Response: We have used genetic names for some enzymes to concise the text, so that many terms are not simple abbreviations. However, according to the reviewer's comment we spelled out some of them for readers' convenience.

Other concerns are also listed below.

Fig. 1 Although Hb-O₂ is thought to be oxyhemoglobin (Fe(II)), there is no description in the text. In addition, oxyhemoglobin (Fe(II)-heme), which can interact with oxygen, is completely different from MetHb (Fe(III)-heme), which cannot interact with oxygen, but it is confused which molecule is oxidative form. Therefore, the authors should explain about that and draw the figure clearly. The oxidative form (MetHb) should be also drawn at the right side to make it correspond to the upper figure and Fig. 2.

Response: We explained oxyhemoglobin in the text as well as legend for Fig. 1. We also have added MetHb, which increases in the NZB mouse RBC during aging, to Fig. 2 according to the comment.

Fig. 2 Because the triangle showing “Oxidative damage” is downward to the right, it seems like that oxidative damage decreases. The triangle of “oxidative damage” should be drawn as well as that of “Autoantibodies”.

Response: Thank you for pointing out. We have rearranged Fig. 2 according to the comment.

Fig. 3 and the legend, This figure and the legend are hard to be understood. Does ROS inhibit Lyp-SOH or a lymphocyte signal? The inhibition mark is OK? or it should be an arrow? How does DNA interact with these molecules in the lymphocytes? The authors should redraw Fig. 3 and explain the meaning of this figure more clearly.

Response: We have redrawn Fig. 3 and explained more clearly in the legend. “DNAs in nuclei” have been replaced with “signal” for clarity.

Page 8, line 3, The reference [41] is not correct. I think it is “Li, Y. et al. Nature Genet. 11, 376-381 (1995)”.

Response: Thank you very much for kindly pointing out. We have corrected the reference 41.

Page 8, line 10, Prx deficiency; An abbreviation of peroxiredoxin is Prx or Prdx? It is written “Prdx” in other places.

Response: Thank you very much for pointing out. We have corrected the abbreviation.

Page 11, line 7, What is “Ro60”? The authors should explain about this.

Response: Ro60 is the 60-kDa autoantigen that is one of targets of autoimmunity in both SLE and Sjögren’s syndrome. We have added the explanation according to the comment.

Page 12, line 5 up, Check the follow sentence. A comma before LYP is needed, or put LYP in a parentheses. PTPN22 encodes lymphoid tyrosine phosphatase LYP, which

Response: Thank you very much for pointing out. We have put Lyp in parentheses.

Page 12, line 3 up, What are “TCR” and “PEST domain”?

Response: TCR is the abbreviated name of T-cell receptor and commonly used without definition in immunology. However, we have defined the abbreviation at the first appearance here.

PEST domain is the domain rich in proline-glutamate-serine-threonine and undergoes rapid degradation. We have added explanation.

Page 12, 13, 14 and Fig. 3 Lyp is LYP?

Response e: In the field of genetics, gene names are differentially expressed; lower case letters for human and large case letters for mouse. However, we have used the name with lower case letters only throughout the manuscript to avoid confusion in this article.

Reviewer 2

The manuscript by Junichi Fujii et al. discussed the association between oxidative stress and SLE pathogenesis based mainly on the genetic and phenotypic characteristics of NZB and NZW mice and provide insight into the mechanism of SLE pathogenesis. The data are new, there are some concerns.

General comments

1. In abstract, the author introduced the mechanisms of erythropoiesis and anemia, and then mentioned the C57BL/6 mice model under superoxide dismutase (SOD1) deficiency. It is better to make a transition between these two parts for better understanding.

Response: We have added a sentence “Elevated reactive oxygen species (ROS) result in the sulfhydryl oxidation and hence are another potential cause for anemia.” as the transition.

2. In Perspectives, it is better to make more discussions about the potential of antioxidant therapy, such as the potentially adverse, which will provide more information and attract more attention.

Response. We have discussed more on the subject.

3. Figure 1 is complicated and not clear, please improve it.

Response. We have improved Fig. 1 by simplifying it as much as we could.

We hope that the revised manuscript is now suitable for publication in *World Journal of Nephrology*, and we look forward to hearing decision from you at your earliest convenience.

Sincerely yours,

Junichi Fujii, Ph.D.

Professor

Department of Biochemistry and Molecular Biology,
Graduate School of Medical Science, Yamagata University,
2-2-2 Iidanishi, Yamagata 990-9585, JAPAN

Tel. +81-23-628-5227 Fax +81-23-628-5230

E-mail: jfujii@med.id.yamagata-u.ac.jp

