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Professors J. Campistol and Dr. A. Mandal
Editors-in-Chief
World Journal of Nephrology

12th January 2016

Dear Dr. Campistol and Dr. Mandal,

Re: Manuscript ID 02007936: C5b-9 does not mediate tubulointerstitial injury in experimental acute glomerular disease characterized by selective proteinuria

I am very grateful for the Reviewer's comments regarding this manuscript. The manuscript has now been thoroughly revised according to the Reviewer comments and also additional comments from the Editorial Office. Hopefully the revised manuscript is now acceptable for publication in the *World Journal of Nephrology.*

Please find attached:

1. Point-by-point response to the Reviwers comments
2. Clean version of the revised manuscript
3. Tracked version of the revised manuscript
4. Signed pdf of the "Statement"

I look forward to hearing from you and thank you for considering this manuscript

Yours sincerely,

Gopala K. Rangan
Associate Professor in Medicine, Westmead Institute, University of Sydney
Staff Nephrologist, Westmead Hospital, Western Sydney Local Health District, Sydney

REVIEWER #1

1. page 10, line 17: "Table 2" should be changed to "Table 1"
Author response: Corrected

2. page 10, line 20: "Table 3" should be changed to "Table 2"
Author response: Corrected

3. page 10, line 22: "Table 4" should be changed to "Table 3"
Author response: Corrected

4. Table 4, PON Day 4, C6-, Interstitial ED-1: The authors described "21.0 1.8+" here. What is "+"? No explanation was found in the table.
Author response: Corrected

5. Table 4, PON Day 8, C6+, Protein cast: The authors described "1.1 0.3#" here. The authors defined that"#" represented $P<0.05$ when compared to PON Day2, C6+. However, data of protein cast of PON Day 2, C6+ was absent in Table 4.
Author response: Corrected

REVIEWER #2

1. The authors should furthermore explain the findings that in C6+ rats with PON, the tubulointerstitial expression of C5b-9 was increased and localized predominantly to the basolateral surface of tubular epithelial cells, whereas it was undetectable in C6- animals. Could the C6- animals model be a null-immunogenicity animal?

Author response: The PVG C6 deficient rat strain was a chance discovery made by Leenaerts et al. in 1994 who first described the specific defect in the complement system in these animals. The key abnormality is a partial and isolated deficiency of C6 due to a spontaneous autosomal recessive genetic defect. Leenaerts et al. (1994) found that the abnormality was restricted to PVG rats obtained from Bantin and Kingman (Fremont, CA, USA) and not present in rats from other animal vendors (Harlan SD, Harlan Olac, others). In the C6 deficient strain the activation of the complement system proceeds normally to the level of C5, and thus the generation of opsonic C3b and chemotactic C5a are not known to be affected. The C6 deficient strain are not susceptible to immunocompromised infection, have normal T-cell responses (Merten et al. 1998) and their tissue antigenic expression and immunity are identical to the C6 sufficient PVG strain. The exact mechanisms underlying the tubulointerstitial localisation of C5b-9 in the basolateral region in C6+ rats with PON are not clear but previous evidence would suggest that it is a consequence of non-immunological mediated tubular epithelial cell injury, intra-renal complement synthesis and presumably a sequela of interstitial oedema and inflammation in this model, similar to that which occurs in unilateral ureteric obstruction, streptozotocin-induced diabetes and cyclosporin nephropathy. The tubular epithelial cell injury and interstitial inflammatory response in PON is postulated to be secondary to "proteinuria-induced tubular epithelial cell injury" rather than immunologic factors (Eddy et al. 1989). The PON model does not show evidence of tubulointerstitial rat IgG or circulating antibodies to BSA (Eddy et al. 1989).

This additional explanation has been added to the Discussion of the revised manuscript.

2. Except minimal change dieses, most GN-related proteinuria is non-selective proteinuria. Is the conclusion limited to pathophysiology of MCD only? The author should clarify the point.

Author response: Bearing in mind the limitations of the animal model, we believe that the results are most likely to apply to patients with minimal change disease only.
We have included an extra paragraph in the Discussion to highlight this point.

3. Many GNs are accompanied "intrinsic factors" and leading to proteinuria, in contrast to overflow proteinuria. I doubt that the protein-overload nephropathy (PON) is appropriate for the nephropathy work-up

Author response: We agree that the PON model has limitations and have included a paragraph to discuss the limitations of the data.

REVIEWER #3

I wonder that although the data are non-parameteric and authors used Kruskall wallis test for test of difference, yet they express central values as means. Median in these situation is of utmost importance.

Author response: Median values and interquartile ranges are provided in Tables 1 to 4.

REVIEWER #4

One concern in accepting the finding as applicable to patients with proteinuria as the Discussion did not include either validation of the role of C5b-9 in rodent kidney disease nor was there evidence that rat exposure to bovine serum albumin - injected into study rats to simulate protein release in human kidney disease - reproduces the environment present in human proteinuria. Indeed, there are numerous examples of satisfactory treatments of induced renal diseases in rodents that are ineffective when tested in patients with kidney disorders so that this issue should be raised and discused in the present report. Overall, an interesting and potentially important study that requires addition of discussion of the probability that its findings in rodents may transllate into a therapy for proteinuric patients.

Author response: We agree that the PON model has limitations and have included a paragraph to discuss the limitations of our data.

REVIEWER #5

1.Title : The author eventually result showed that C5b-9 does not mediate tubulointerstitial injury in acute glomerular diseases characterized by selective proteinuria. We suggest that author revised the title.

Author response: Thank you for the suggestion. We have modified the Title as suggested.

2. Material and method: I am not certain whether are there congenital kidney disease in (PVG) rats and C6 deficiency rats? Please provide proper data. For example, urinary protein test.

Author response: This is an important question. In previous studies have thoroughly examined and characterised the renal structure and function of PVG rats, and there is no evidence of congenital renal disease in these rats. Please see References #39, #16, #17, #31 and #32. In addition, in the current study, no abnormalities were detected in the control group without PON. The latter also included both C6+ and C6- animals.

3. The description of results is very poor, which is one of the main weaknesses of the manuscript.

Author response: We have reviewed the description of the Results and have revised the section, also taking into account the comments of Reviewer #1.

4. In this paper, most of the references of partial old, you have better take references in recent five years.

Author response: Thank you for this suggestion. The manuscript has been extensively revised and evidence on the role of properdin, heparin sulphate and the results of murine knockouts have been included in both the Introduction and Discussion.