Author's response to reviews

Title: Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage

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Author's response to reviews: see over
Dear Dr. Maria Merrie Jul Ladag and Dr. Hayley Henderson
Senior Executive Editor
BioMed Central
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27 February 2014

Dear Editor:
I would like thank you very much for considering our revised manuscript entitled “Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage” (MS: 180700656113352).

I am now submitting the manuscript as revised in the light of 2 reviewers suggestions. We have modified the text as suggested by the reviewers.

I hope the new revised version of the manuscript will be now considered suitable for publication in your journal BMC Nephrology and look forward to hearing from you in due course.

Thank you for your consideration.

Sincerely yours,

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Reviewer's report
Title: Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage
Version: 2 Date: 18 December 2013
Reviewer: Michael Robson

Reviewer's report:
In this paper, the presence of complement components in the urine of patients with proteinuric renal disease is studied, and in vitro experiments look at complement deposition on a proximal tubule cell line. Properdin (used at physiological concentrations) increases C3 and MAC deposition due to NHS, and also increased C3 but not MAC deposition when added to properdin depleted serum.

Major compulsory revisions.
Some clinical details of the patients should be included. A breakdown of the diseases, and age/sex relative to controls for example.

In the Methods, we have added a breakdown of the diseases and age/sex relative to controls (Page 3, Line 30).

The authors state in the text that adding properdin to 5% PDS in 7(d) increases MAC deposition. It does not as there is no significant difference. It is either statistically significant or not. If the authors wish to suggest a trend, they could include a precise p value.

In Figure 6, the depositions of MAC on PTECs incubated with 5% PDS after preincubation with P were not statistically significant; however, we did find a tendency for an increase ($p = 0.0556$) compared with those after incubation with 5% PDS without preincubation with P. We have added a precise $p$ value ($p = 0.0556$) in the Figure 6 and in the Figure legends (Figure 6; Page 17, Line 25).

Why is there data for 5% and 25% NHS but only 5% PDS ? Data for 25% PDS should be added for completeness, as it looks like it may not have fitted in otherwise.

As you pointed out, we have added the figures to show the enhancement effects of P for complement AP activation on PTECs incubated with 25% PDS as well as 5% PDS. We
have to apologize and correct the text in our manuscript. First, we didn’t show and discuss the figures in the addition of 25% PDS due to inconvenience of slight staining of P. We unexpectedly detected slight P staining on PTECs incubated with 25% PDS but not with 5% PDS. We previously considered the possibility that PDS was not completely depleted of P in the serum and then even a very small amount of P could bind to PTECs and might activate AP and PDP leading to the depositions of MAC. In this time, to confirm this speculation, we tried to measure levels of P in 25% PDS whether P were contained or not by ELISA kit (Human Properdin KIT, Hycult Biotech, Uden, Netherlands). Then we could detect not significant but a very small amount of P in 25% PDS, 5.77 ng/mL, one thousandth of a physiological concentration of P (4–6 µg/mL). Those might cause the unexpected P staining on PTECs. Then we have added the figure (in Figure 6) and the text in the Results (Page 8, Line 29), in the Figure legends (Figure 6; Page 17, Line 21) and in the Discussion (Page 10, Line 26).

Second, we previously considered that there was no significant difference between 25% PDS with P preincubation and 25% PDS without P preincubation, especially without assessing the detail of depositions of C3 and MAC and performing quantitative analysis. However, as showed in Figure 6, the depositions of C3 were significantly increased \((p=0.0079)\) and those of MAC were not significant but relatively increased \((p=0.0556)\) in the addition of 25% PDS with P preincubation compared with 25% PDS without P preincubation. Those results reminded us that it was very important, identical to the results of 5%PDS and supportive to the enhancement effect of P in AP activation. Then we have added the figure (in Figure 6) and the text in the Results (Page 8, Line 30), in the Figure legends (Figure 6; Page 17, Line 22) and in the Discussion (Page 10, Line 21 and 27).

The authors state in the text (last paragraph) that serum decreases PTEC viability. It doesn’t – the data in figure 8 show no effect, and only a decrease when properdin is added to NHS. This is entirely in keeping with no effect on the morphology shown in figure 1. In fact the pictures in figure 1 should be moved and included with figure 8. We should also be shown what the morphology is when viability is down with added properdin.

As you pointed out, there was no significant decrease in PTEC viability incubated with up to 25% NHS. We checked the morphology when the viability was decreased with added P. There were no significant morphological changes in PTECs. Accordingly, we have added a new figure 7. In our study, although the activation of AP was ineffective in
morphology, a functional effect was observed with preincubation with P (Discussion: Page 10, Line 32).

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests
Reviewer's report
Title: Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage
Version: 2
Date: 19 December 2013
Reviewer: Neil Sheerin

Reviewer's report:
The authors describe a series of experiments supporting their hypothesis that properdin is involved in the activation of the alternative pathway of complement on renal tubular cells. Generally the work is well performed and clearly presented.

My main concern is the lack of novelty. Much of what is presented in the paper is already known for example complement products are found in the urine of proteinuric patients, tubular cells activate complement in a properdin-dependent manner.

There are numerous reports of complement products that are observed in the urine of proteinuric patients. However, most of the previous reports were researches on U-complement components in patients with single renal diseases such as IgA nephropathy, membranous nephropathy and others or studies that evaluated either urinary (U)-properdin (P) or U-factor H (fH) in patients with proteinuric renal diseases. The subjects of our research ranged from those with a variety of renal diseases, including nephrotic syndrome, to those with a normal range of urinary protein. Furthermore, we focused not only on P but also on fH, including combinations of P, fH and MAC, in the simultaneous evaluations of urine sample from patients with every single renal disease. We considered the subjects of our researches were not yet discussed, especially in the balance between P and fH. There were problems with our expressions in our previous submission, so thank you for bringing it to our attention. We have added the relevant text in the Background (Page 3, Line 9) and Discussion sections (Page 9, Line 24).

One outstanding question is whether the tubular injury is due to complement activation. Multivariate analysis should be performed on the data to see whether the correlation between urinary complement protein concentration and markers of tubular injury (such as NAG) that is not explained by the relationship with proteinuria.
We assessed whether the tubular injury may be due to complement activation.

a. First, we checked the relationship between markers of tubular injury (B2MG and NAG) and proteinuria. There were positive correlations between urinary protein levels and B2MG ($p = 0.001$) and NAG ($p < 0.0001$) levels. We have added this data in the Results section (Page 7, Line 28).

b. Furthermore, as you pointed out, we performed multivariate analyses using multiple regression. The results showed the relationship between the U-complement components and tubular damage markers. U-B2MG was correlated with U-P, and NAG was correlated with U-protein and U-P. U-P was the only factor that was associated with both B2MG and NAG. We have added this data in the Results section (Page 7, Line 29) and Table 2 (Page 15).

Following on from this I would be interested to know more about the different patient groups, particularly the patients with Minimal Change Disease. You would not expect significant tubular injury in this group. Do urinary complement levels reflect this?

We rechecked the data to assess whether U-complement levels reflected tubular injury in patients with minimal change disease. We have accordingly added the data in the Discussion section (Page 9, Line 12).

Minimal change nephrotic syndrome (MCNS) is one of the representative diseases that clinically showed nephrotic levels of urinary protein. In contrast, histologically, there was no major damage in the glomeruli and tubulointerstitium. In our report, even in patients with MCNS and decreased renal function, there was a tendency for high levels of U-tubular damage markers, U-protein and U-complement proteins. Although there were no high levels of tubular damage markers and U-complement components in patients without decreased eGFR in MCNS, this result suggested that relationships among high levels of U-protein, U-complement components and U-tubular damage markers existed, even in patients with MCNS. Therefore, as U-complement components and tubular damage markers increase in patients with MCNS and advanced renal dysfunction, the levels of U-complement components and tubular damage markers may correlate with U-protein levels.

Minor points:
1. The physiological concentration of FH is given, but not of properdin. This should be added.
We have added the physiological serum concentration of properdin in the Results section (Page 8, Line 8) and referenced the paper in References (No 21; Page 13, Line 25).

2. It is not clear how the deposition of complement proteins on cells was quantified. Was some form of image analysis software used?

In the Methods (Page 6, Line 32), we have added the image analysis software.

3. Fig 6. No factor H staining is seen on PTEC incubated with 25% serum, which would contain FH at a concentration that clearly binds to PTEC when isolated protein is used.

With regard to the staining of factor H in the new Figure 5:
   a. We have to apologize about the staining of fH on PTECs incubated with 25% NHS being scarce on Figure 6 in our previous submission. Although the staining of fH incubated with 25% NHS was stronger than that with 5% NHS, we substituted the image with a new image of fH incubated with 25% NHS in Figure 5.
   b. Furthermore, we tried to determine the serum concentration of NHS that would allow fH to clearly bind to PTECs. The concentration of NHS at which fH clearly bound to PTECs was from 35% (data not shown).

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests