Author's response to reviews

Title: Membranoproliferative glomerulonephritis with predominant IgG2 and IgG3 deposition in a patient with IgG4-related disease

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Author's response to reviews: see over
Dear Dr. Henderson

MS 2045305693170769R1 “Membranoproliferative glomerulonephritis with predominant IgG2 and IgG3 deposition in a patient with IgG4-related disease” by Ueki K et al.

Dear Drs. Henderson and Marchbank (reviewer)

Thank you for a careful review of our manuscript, and giving us several significant comments. Your suggestions have been incorporated into this revised submission. We feel that this revision has enabled us to get our message across much more effectively. The modifications of the manuscript are highlighted in yellow, and a point by point response to all concerns are as follows;

**Major revisions**

1. Can the authors provide the IgG1, IgG2 and IgG3 serum levels as they have done for IgG4? It is useful to determine the level of IgG4 skew in comparison to IgG2 and IgG3 since these are found predominately in the glomerular staining.

**RESPONSE:** The level of the serum IgG4 subclass (1110 mg/dL) was absolutely high, and it was highly probable that this patient had IgG4-RD. Kidney biopsy showed IgG4-positive plasma cells in the interstitium, whereas predominant IgG2 and IgG3 deposition in the glomeruli. On admission, we only measured IgG4 level, but we preserved the patient’s sera. We have performed additional measurement of all IgG subclasses, and added the data in the manuscript (page 7, lines 101-104). Interestingly, IgG2 and IgG3 levels were also elevated that is compatible with IF findings in the glomeruli.

2. Could C3Nef and anti-FH autoantibody data be included?

**RESPONSE:** As reviewer suggested, it is important to demonstrate the presence or absence of C3 nephritic factor and anti-factor H autoantibody. Unfortunately, the measurements of those factors are not commercially available and we cannot measure in our lab neither. Since immunofluorescence study revealed both immunoglobulin and complement depositions in the glomeruli, we guess MPGN in this patient was not C3 glomerulopathy associated with those factors.

**Discretionary revisions**

1. The authors indicated the sub-endothelial electron dense deposits in figure 3D, I find this
useful. I think it would be helpful to illustrate a clear example of an eosinophil and plasma cell and describe further how these are easily confirmed as such by the staining.

**RESPONSE:** In the interstitium, we recognized the significant infiltration of eosinophils and plasma cells on the conventional H&E stained section, then we have changed photomicrograph of Figure 1B with highest magnification.

2. Do the authors have any double staining that demonstrates the Plasma cells are IgG4 expressers, and not IgG2/3 expressers.

**RESPONSE:** It is important to demonstrate that the most interstitial plasma cells are IgG4 expressers. Actually, it is difficult to perform double-staining because both IgG and IgG4 show cytoplasmic pattern. Most pathology lab including our institute perform the staining using serial sections. Then, we added photomicrographs of CD138 and IgG4 in minor salivary gland, and IgG and IgG4 staining in the kidney interstitium as new Figure 4A-4D.

I hope that you will find this revision to be satisfactory.

Best regards,

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