Author's response to reviews

Title: Transplantation of Endothelial Progenitor Cells in Treating Rats with IgA Nephropathy

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Version: 3
Date: 13 February 2014

Author's response to reviews: see over
Jonathan Barratt

1. No other animal organs are described in the paper- I would like to see if the EPC entered any other organs, also did they enter non-diseased kidneys? In Fig 4 it is unclear whether "normal" is pre- injection of EPC or a normal kidney exposed to EPC -the authors need to clarify.

2. Was there any sign of other organ dysfunction in these rats-either due to the induction of IgAN or as a result of EPC injection?

3. Figure 6 is too small as presented to really discriminate any differences between groups- images need to be magnified significantly.

Laureline Berthelot

1. The authors claims that transplantation of endothelial progenitor cells treats rat IgA nephropathy, but they did not show IgA deposits after transplantation. I expect that there is no change in term of IgA deposits and IgA serum levels after transplantation. These results must be

The authors thank the reviewer for this comment. EPC migration in the kidney and liver tissue was also investigated in the control and EPC treated rats. We observed clear EPCs in the kidney. EPC-positive signal could been detected in the liver tissue. However, the number of specimens which we reserved was insufficient for statistical research. We will add the picture in fig4. In Fig 4 the "normal" is pre-injection of EPC. It has been reworded to “pre-injection”.

Liver function after the induction of IgAN and EPC injection was investigated as well in present study. Result showed that Alanine amino transferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl-transferase (GGT) were no significant changes in these rats. Detailed information has been added in the method, results, and also discussion section of revised manuscript.

Image with higher magnification has been added.

Thank you for your suggestions. Direct immunofluorescence (FITC-conjugated rabbit anti-rats IgA antibody) was used to visualize the IgA deposition in the glomeruli both in control and EPCs treated rats. Semi-quantitative analysis of the fluorescence signal was performed to
2. The authors must moderate their conclusion, this transplantation attenuate renal injury in IgA nephropathy but not IgA deposits. So, they must change their title and modify the discussion part. This treatment could be useful in addition to other therapies. Moreover, the authors must add liver or lung histological analysis.

3. The authors did not precise when we inject the cells: is it preventive or curative intervention? At the onset of IgA nephropathy induction? This information can also change the conclusion.

Hiroyuki Inoshita

I strongly doubt that this rat model is used as IgAN model. Is this model already established as human IgAN model? Probably, IgA deposition and glomerular damage in this model is occurred by liver dysfunction due to CCL4, as you know CCL4 is generally used for experimental liver cirrhosis. Interstitial nephritis and glomerular damage might be directly affected by CCL4 and/or LPS injection. If the authors still would like to use this model, they should illustrated the effects of EPCs on IgA deposition. Decreased immunofluorescence was observed in transplantation group compared with untreated animal. Detailed information has been added in the 6th paragraph of the result section with red color.

The authors thank the reviewer for this comment. This model was established by USA+ LPS+ CCL4. It is true that CCL4 may result in liver cirrhosis. We are aware of this. To minimize the effect of CCL4 on livers, working dosage of CCL4 was reduced to one third of its common use. What ‘more, subcutaneous instead of Intraperitoneal injection. was selected. At same time, only two intravenous injection of LPS at the 6th, 8th week is less likely to

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EPCs was injected 3 days after the induction of IgAN. So, it could considered as a curative intervention. See details in the “Transplantation of EPCs” part of the method.
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<td>2.</td>
<td>According to the comment in #1 above, the authors should change the title which is misleading for me. For example, “Transplantation of endothelial progenitor cells in treating rats manifesting secondary IgA nephropathy-like phenotype”. The authors also should revise their Introduction, Methods, and Discussion in terms of secondary IgAN.</td>
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<td></td>
<td>Thank you for your suggestions. We have change the expression into “BSA, LPS, and CCL4-induced IgAN” in the introduction. Revision and explanation has been made in the section of Methods, and Discussion.</td>
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<td>3.</td>
<td>The authors should show electron microscopic study for glomeruli from EPCs group at pre- and post transplantation. How about electron dense deposit in mesangial area which is frequently observed in human IgAN? How about podocytic foot process effacement and endothelial cell damage?</td>
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<td>It’s really a regret that electron microscopic study is not involved in our present study. Instead, immunohistochemistry staining was performed to illustrate the deposit in the kidney after injury. This study is still going on. In our future work, we will further investigate the damage in podocytic foot process and endothelial cell using electron microscopy.</td>
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<td>4.</td>
<td>The authors claim that endothelial cell damage in the rat model was improved by transplantation of EPC.</td>
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<td>We are so sorry to miss this information in previous manuscript. Actually we collected the urine of all...</td>
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<td>They should show grade of hematuria (using dip sticks, hemoglobin ELISA, or something like that) at pre-transplantation, 3, 7, and 14 days after transplantation because hematuria generally indicates endothelial cell damage in glomerulonephritis.</td>
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<td>5.</td>
<td>In Subjects and Methods, Animal section, the author must state adequate reference instead of reference No.8 (Lou T et al. Nephrology 2006) which does not describe how to make the rat-model.</td>
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