Author's response to reviews

Title: A new mouse model for renal lesions produced by intravenous injection of diphtheria toxin A-chain expression plasmid

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Dr. Emma Parkin
Editorial Administrator, BMC Journals

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Dear Editor;
In response to your e. mail dated on 9 January, 2004, I revised the manuscript entitled, A new mouse model for renal lesions produced by intravenous injection of diphtheria toxin A-chain expression plasmid (Number: 332893022467234), for publication in BMC Nephrology.
As pointed out by the referee, I improved several sentences which will be seen in the revised manuscript and the answer for the referee below.
I wish to appreciate your kind treatment on this matter and also the referee for his/him kind criticism on this manuscript.
If you feel inconvenience in these materials sent, please let me know. I am ready to respond it soon.
Thank you again for your effort.

Sincerely yours,

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Answer to the referee;

Minor essential revisions
A) Minor essential revisions (such as missing labels on Figures, or the wrong use of a term, which the author can be trusted to correct).

As pointed out by the referee, I improved all the matters as possible (see the revised text).

B) Major compulsory revisions (that the author must respond to before a decision on publication can be reached)

1) Is there much variation for kidney disease parameters among different individuals?

Of 8 kidneys (derived from 6-repeated introduction of DT-A expression plasmid/lipid complex; examined on day 4) tested, all specimens exhibited mesangial proliferation (tightly associated with matrix deposition), glomerular lobulation, loss of Bowmans space at the level of H-E staining. Inflammatory response was sometimes (2/8) observed. At EM level, formation of focal deposits was remarkable. This was observed in all specimens (2 kidneys from different mice) tested. These abnormalities disappeared on weeks 3 and 5 after final introduction of the DNA/lipid complex. All H-E-stained specimens tested (8 kidneys) sampled at week 3 exhibited almost recovery of glomerulus. These are mentioned in the revised text. In my impression, these abnormalities appear not to fluctuate among different individuals. This is probably because we employed repeated (up to 6 times) introduction of the DNA/lipid complex, which may cause relatively uniform transfection of a target tissue rather than single (or few) gene delivery.

2) The authors show that the CAG promoter and EGFP were predominantly located in glomerular epithelial cells, but the alteration of glomerular components were only mesangial cells and GBM, not glomerular epithelial cells. The author should explain it.

This point is quite reasonable. As mentioned in the revised text (the middle to the bottom in page 17), the damaged cells would have been removed from the affected area and immediately replaced by proliferating cells such a mesangial cells. We examined the specimens sampled only at 4 days and weeks 3 and 5 after final DNA introduction. I guess that by 4 days those ablated cells are rapidly removed, and therefore it may be difficult to detect such damaged glomerular epithelial and endothelial cells at EM level. In the next step of experiment, it may be necessary to examine the specimens sampled at time-points before day 4 (for example, day 0 and day 2 after final introduction of DNA/lipid complex)

Finally, I added a new figure as (f) in Figure 1C. This is because the glomerular endothelium is the second cell that pCAG/DT-A is introduced and expressed, although its expression level appears low.