Author's response to reviews

Title: Fox smell abrogates the effect of herbal odor to prolong mouse cardiac allograft survival

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Version: 4  Date: 21 February 2014

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

Please find the attached manuscript, “Fox smell abrogates the effect of herbal odor to prolong mouse cardiac allograft survival” which we are submitting for consideration for publication as a “Research article” in *Journal of Cardiovascular Surgery*.

According to the two reviewers’ advices, we revised our article and added the comments to the manuscript. We appreciate the constructive comments of your reviewers, which have helped us to improve our manuscript. Each of these comments is addressed below:

**Author’s response to reviews**

**Title:** Fox smell abrogates the effect of herbal odor to prolong mouse cardiac allograft survival

**Authors:**
- Xiangyuan Jin (jinxiangyuan@hotmail.com)
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**Version:** 2 Date: 21 February 2014

**Author’s response to reviews:**

**Reviewer's report**

**Title:** Fox smell abrogates the effect of herbal odor to prolong mouse cardiac allograft survival

**Version:** 2 Date: 26 November 2013

**Reviewer:** James George

**Reviewer's report:**

General Comments: This manuscript tests the interesting hypothesis that olfactory sensing of Tokishakuyaku-san (TJ-23), or one or more of its components, can affect the duration of orthotopic cardiac allograft survival in a mouse model. This is a well characterized system and, given the strain combinations, a stringent test of the ability of a given compound to modulate vascularized solid organ allograft rejection. Overall, the data presented to show that exposure to TJ-23 modulates allograft survival are convincing and there is reasonable
evidence to suggest that it could be mediated by regulatory T cells. The central issue that dampens this reviewer's enthusiasm for the paper is the contention that the mechanism of action involves olfactory sensing. The description of the experimental system suggests that there is considerable respiratory exposure to the chemical compounds under test. To try to differentiate between simple respiratory exposure and olfactory sensing, the authors took the reasonable approach of using animals that were thought to have their olfactory sensing apparatus disabled, but there is unfortunately no data presented in which the technique to disable olfactory sensing is validated. The central premise of the entire manuscript rests on this point, and the only data are presented in figure 1d. It is particularly important given that TJ-23 is also effective via oral administration. This problem is mitigated somewhat by the use of sham operated controls, which allows one to conclude that, at the very least, there may be a brain-mediated mechanism involved. If this is a standard model in the field of olfactory sensing, with which I am unfamiliar, then I stand corrected.

Thank you for your reminder.

In the preliminary experiment, we gave CBA mice a cotton bud, which was soaked in TMT, and tested the avoidance response time. The avoidance response time of naïve CBA mice is 1.64±0.25 s, and that of olfactory dysfunction CBA mice is 7.54±4.25 s (P <0.05). Moreover, we also dissected the skulls of naïve and olfactory dysfunction CBA mice. Here we will show you the photos:

<table>
<thead>
<tr>
<th>Naïve CBA</th>
<th>Olfactory dysfunction CBA mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Naïve CBA" /></td>
<td><img src="image2.png" alt="Olfactory dysfunction CBA mice" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Surgical scar" /></td>
<td><img src="image4.png" alt="Surgical scar" /></td>
</tr>
</tbody>
</table>

Surgical scar
We think olfactory bulb excision is a standard method in surgery. However, there are knockout mice whose olfactory sensing apparatus were disabled. Because the knockout mice were too expensive, we used the mice which induced by olfactory bulb excision instead of knockout mice. Moreover, we did not give oral administration of TJ-23 to olfactory dysfunction CBA mice.

**Specific comments:**

It is a little unclear what "Fox smell" means. Do the authors mean the smell of the animal or do they mean some scent associated with forkhead transcription factors?

Thank you for your reminder.
The “Fox smell” means the odor of TMT which is secreted from the anal gland of fox and induces aversive behavior and fear responses in mice.

Page 3: "...are converted to different topographical maps in the olfactory bulb...". This sentence is constructed in a somewhat confusing manner. I assume that the authors mean that the signals from the nasal epithelium map to different areas of the olfactory bulb depending on the compound?

Thank you for your reminder.
According to the reference [2], “Innate versus learned odour processing in the mouse olfactory bulb” (Nature 2007;450:503-508), we suppose that the sentence means what you had said.

The authors properly cite their methodology for the MLC, but it would be helpful to at least tell the reader what detection method was used, otherwise the optical density measurements in the data cannot be evaluated. Was it MTT or something else?

Thank you for your suggestion and reminder.
We have added the sentence “Proliferation was assessed by using an Enzyme-linked Immunosorbent Assays (ELISA) for bromodeoxyuridine incorporation (Biotrak, version 2, Amersham, Little Chalfont, United Kingdom [UK]) according to the manufacturer’s instructions” to paragraph 8, line 3 of
"Methods".

When performing adoptive transfer experiments using sorted cells, it is quite important to know what the purity of the sorts were prior to the transfer. The Miltenyi kits can generate excellent results, but the purity of the sorts that one obtains with them can be quite variable when using magnetic sorting techniques of this type. The authors should indicate the purity of their sorts and, most importantly, whether there were any differences in the relative purity between groups.

Thank you for your suggestion and reminder. Each sorted cell was detected and counted by FACS and the purities were 95~98%. Moreover, there were not any differences between groups.

The manuscript has been reviewed and approved by all authors. The paper has not previously been published and is not currently under consideration by another journal. The authors report no conflicts of interest.

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Thank you for your consideration.

Sincerely,

Xiangyuan Jin, MD
Teikyo University, Japan