ANSWERING REVIEWERS

ESPS Manuscript NO: 20353

Title: Migration of bone marrow progenitor cells in the adult brain of rats and rabbits

Author: Donnahue Dennie, Jean-Pierre Louboutin, David S Strayer

Dear Editor,

We would like to thank the Reviewers for their useful comments and hope that the manuscript is now suitable for publication in the *World Journal of Stem Cells*. The answers to the Reviewers comments are presented on the following page.

Please note that following a comment from one Reviewer, the title of the manuscript is now: "Migration of bone marrow progenitor cells in the adult brain of rats and rabbits".

Answers to Reviewers

Reviewer 1

1. "I have only two minor suggestions. One is that it would be better if authors would write additional comments regarding the characters of the bone marrow cells that produced brain progenitor cells. Although authors properly mentioned hematopoietic stem cells and bone marrow stromal cells, there are still other types of cells showing multipotency including MUSE cells (Wakao S et al., Proc Natl Acad Sci U S A. 2011;108:9875-9880) and mesenchymal stem cells (MSC) (Houlihan DD et al, Nat Protoc. 2012; 7:2103-2111, Niibe K et al., PLoS One. 2011;6:e17610). "

We described bone marrow cells that can produce brain progenitor cells in a better way. We also introduced a paragraph dedicated to MUSE and MSC cells. We also cited the References provided by the Reviewer.

2. "In page 22, line 10, the sentence "However the ultimate proof that …" should be corrected as "However, the ultimate proof that …".

The grammatical error has been edited.

Reviewer 2

1. "In fact, most of the references are seven or more years old. Has the field not progressed during that time?"

Recent references have been cited.

2. "Also, as the authors point out, the experiments they review from their work does not address the characterization of the population(s) of cells that lead to the phenomena described occurring in the brain. The potential information that exists in this area should be addressed."

We introduced several paragraphs dedicated to iPS cells, MUSE and MSC cells.

3. “The authors present the viewpoint that negative results reported elsewhere in transplantation experiments may relate to the time interval involved between introduction of the cells and analysis of the results. A critical question not addressed is if full maturity of the cells takes place and what type of neurons these might be.”
The Reviewer is right concerning the differences that we observe with other results of the literature. They might be due to the full maturity of the cells, the molecular marker that is used, and the type of neurons. This is now introduced in the text.

4. “As the authors point out, the trans-differentiation mechanisms are not known in any detail. Expansion of information or speculation in this area would contribute significantly to the paper. Homing mechanisms might be further examined at a molecular level, as could the differentiation process itself. There is little information presented on these issues.”
Several paragraphs concerning homing and transdifferentiation of HSCs have been added.

5. “A major portion of the paper is devoted to CCR5 effects or related ones, and this subject is presented as a major issue in the concluding remarks. However, this issue is not even mentioned in the abstract.”
The effects of CCR5 are now mentioned in the Abstract.

6. “Fig 2 deserves additional explanation in the legend (“LV,” “C cells”).”
Figure 2 has now additional explanations regarding the types of cells.

Reviewer 3
1. “Providing a list of abbreviations would help, or at least make sure that all acronyms are properly deciphered. There are a few minor editorial suggestions provided below…”.
Acronyms have been deciphered. Typos have been corrected. We followed the editorial suggestions made by the Reviewer.

Reviewer 4
1. “An introductive paragraph governing the content should be placed before the first sub-headline.”
An introductive paragraph is now placed before the first sub-headline.

2. “Rephrase the subheading: Current “Gene delivery to bone marrow progenitor cells” doesn’t reflect its content.”
Subheading has been replaced by “Bone marrow progenitor cells in adult neurogenesis”.

3. “The manuscript addresses their approach that injection of SV(RevM10.AU1) into the rabbit bone marrow (BM), transgene- positive cells were seen in the dentate gyrus (DG) (Fig. 6). Some places state rats: Clarification is needed for different species..”
The study has been performed in the rat as well as in the rabbit. This point is now clarified in the introductive paragraph.

4. “Table title should be placed on the top of a table, not below the table as shown now. Try to revise the table titles as much summarized and descriptive as possible.”
Titles are now placed at the top of the Tables.

5. “Restructure the abstract to cover all contents, to be concise.”
The Abstract has been restructured.

6. “Rephrase the title to point out it’s about rat brain only, not human.”
The study has been performed in the rat as well as in the rabbit, as it is written in the text: “Similar results were seen in the rat (Figures 4B-C,5) and the rabbit (Figure 6). It is now clearly indicated in the Title.

7. “Page 2, Abstract: “Permanent” should be referred to “long-term.”
“Permanent” has been replaced by “long-term”.

8. “Elaborate more on “an increase of BM progenitor cells migrating towards the brain following experimental seizure” for its possible mechanism of migration and engraftment. E.g., what’s cytokine/chemokine profile upon experimental seizure. CCR5 ligand production?”
CCR5 is only a receptor for CCR5. Different cytokines and chemokines have been reported to be increased after experimental seizures. It is known that receptors for IL-1 and TNF-a are upregulated rapidly during seizures (4). The magnitude of seizure activity impacts on the inflammatory responses that follow seizures (5-7). Microglial activation, and production of IL-1b, IL6, TNF-a and free radical species, directly affect the process of post-seizure neurogenesis (5, 8) and the survival of the neurons that are produced as a result (6, 9-11). We added the previous paragraph in the Conclusion section.
We have also added the following paragraph concerning the production of CCR5: “ KA-induced increases in production of CCR5 ligands and ICAM-1 within blood vessels suggests that CCR5+ cells may be increased in the hippocampi of KA-treated rats, compared to control animals. We therefore enumerated CCR5+ cells in different areas of the hippocampi of rats injected with KA or vehicle-only. No CCR5+ cells were detected in control rat hippocampi. In contrast, CCR5+ cells were significantly more abundant throughout the hippocampi in KA recipients. In hippocampi of rats injected with KA, CCR5 was expressed mainly by lymphocytes, monocytes/macrophages, microglial cells, to a lesser extent by neurons, and rarely with astrocytes”.

9. “Fig. 2: What is LV? It needs fully describing all 4 types cell in figure legend, not in text.”
“LV” is lateral ventricle. The different types of cells are now described in the legend of Figure 2.

10. “Fig. 4: legend “Bone marrow derived cells can migrate to the rat hippocampus” -- “Is it injured hippocampus” or normal one? It’s well know stem cells (MSC) attracted to injury, not normal tissue.”
In Figure 4, BM-derived cells migrated to uninjured/normal hippocampus. Subsequent experiments show that migration of BM stem cells to the hippocampus is increased by experimental seizure. However, in the absence of injury, there is a small proportion of hippocampal cells deriving from the BM.

11. “Given redundancy of cytokines and chemokines, cell membrane chemokine receptor, CCR5, is just one aspect.”
We agree with the Reviewer that there is a redundancy of cytokines and chemokines and that CCR5 is just one aspect of the story. However, we cannot investigate the role of all chemokines/cytokines in one paper. Furthermore, we did not have vectors directed against other chemokine receptors. We now mention this redundancy in the Conclusion section.

12. “Cite more literature for the past 5 years.”
More references from the past 5 years have been added.

13. “Page 22: “We reported above that permanent BM-directed gene transfer using recombinant SV40-derived vectors led to expression of the genes delivered to the BM in mature neurons, and thus without CNS lesion.” That’s not logical. “delivered to the BM in mature neurons”????? “
We agree that the sentence might have appeared ambiguous. The genes delivered to the bone marrow (BM) progenitor cells, either FLAG or AU1, by SV40-derived vectors are expressed in BM progenitor cells, then in circulating cells, then in the brain, firstly in immature cells expressing nestin and doublecortin, and finally in mature neurons expressing NeuN. We changed the sentence in a way it does not appear ambiguous anymore.

14. “Overall, only structural analysis (location of cells, markers) is shown, how about the function?”
There are important functional effects linked to the reduction of CCR5 and the modulation of neuroinflammation on neurogenesis in the context of excitotoxicity. The present paper is a Review and we did not present all the results we previously published in the initial version. We describe here that SV40-based gene delivery of RNAi targeting CCR5 to the BM results in downregulating CCR5 in circulating cells. Consequently, the inhibition of interactions between CCR5 on peripheral blood mononuclear cells (PBMC) and CCR5 ligands in vessels prevents BBB disruption after KA. One of the most striking effects is the diminution in the number of seizures in the animals receiving the vector. The decrease of leukocyte-vascular interaction affects vascular permeability, thus, infiltration of parenchyma by inflammatory cells, and reduces neuroinflammation. Subsequently, our data suggest that CCR5 plays a role in the synergistic interactions between leukocyte adhesion, endothelial activation, BBB leakage and seizure activity.
This paragraph is now part of the Conclusion and a paragraph concerning the effects of the reduction of CCR5 on the number of seizures is also mentioned in the main text.

Respectfully.

Best regards.

Jean-Pierre Louboutin, MD, PhD