Reviewer's report

Title: Monocyte depletion increases local proliferation of macrophage subsets after skeletal muscle injury

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Reviewer: Kelly M McNagny

Reviewer's report:

In this manuscript, Cote and colleagues address the question of M1 and M2 macrophage recruitment versus local proliferation in the repair of muscle damage. The authors use colodronate to deplete 87% of the peripheral monocytes, but find only approximately 50% drop in muscle monocytes which they cite as evidence for local proliferation. They also show a dramatic increase in actively proliferating M1 macrophages (16-fold), which is reduced by 50% with local irradiation. They show a similar effect on M2 macrophages which is only partially impaired with irradiation and conclude that there is both active recruitment and local proliferation of M1 and M2 in response to muscle damage and that these pathways can cross compensate for each other.

While I agree that, at face value, the experiments support the conclusions of the authors, I have several criticisms of this manuscript that I feel need to be addressed:

1) as the authors themselves point out, the conclusions are not completely novel. In addition to the article they cite:


there are a number of other relevant articles including:


The authors should further stress the novelty of there specific observations in relation to this previously published literature.

2) While the experiments support the conclusions of the authors, they are not definitive for a number of reasons. The 13% monocytes that remain after colodronate treatment could still contribute to the local muscle pool. One cannot assume that an 87% drop in blood monocytes will equate to a 87% drop in local recruitment (this is likely not a linear relationship).
In addition, one could argue that the preconditioning with colodronate has enriched the residual population for the highly proliferative monocytes that are recent emigrants from the bone marrow. No Ki678 staining of the bone marrow and peripheral blood population (to match the muscle population) is provided to address this issue.

3) It is also of concern that the 20 Gy local irradiation only depletes the "compensatory local proliferating pool" of M1 macrophages by >50%. I would have expected this dose to ablate the local population completely.

Suggested experiments:

- the authors should provide Ki67 label profiles of peripheral blood and bone marrow monocytes. It would also be much more informative if the authors provided a cell cycle profile of the bone marrow blood and muscle pools using an independent label such as BrdU or EdU pulse labeling.

- the data on monocyte depletion should be expressed over a similar time course to that shown in Fig 2b and c.

- the M1 and M2 data would be stronger with the use of additional markers.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests