

Response to Reviewer 3 Comments

Point 1: Show the detection histograms of leukocyte populations, when the cells are extracted with Ficoll by centrifugation, the cell layer contains lymphocytes and monocytes, the population of neutrophils sediments together with the erythrocytes.

In phagocytosis experiments, the only population that phagocytoses is that of monocytes, therefore the value that is observed is a value of monocytes. This problem can be solved in several ways

1 Lysing peripheral blood with commercial erythrocyte lysing solution, in that case FSC vs SSC morphology will clearly separate lymphocytes, monocytes and neutrophils.

2 Separating the population with Ficoll but adding a pan-leukocyte antibody such as CD45 (or the equivalent in their study) or a monocyte-specific antibody.

In Figure 9 A1 and B1 of morphology, it is not possible to distinguish which population is lymphocytes and which population is monocytes.

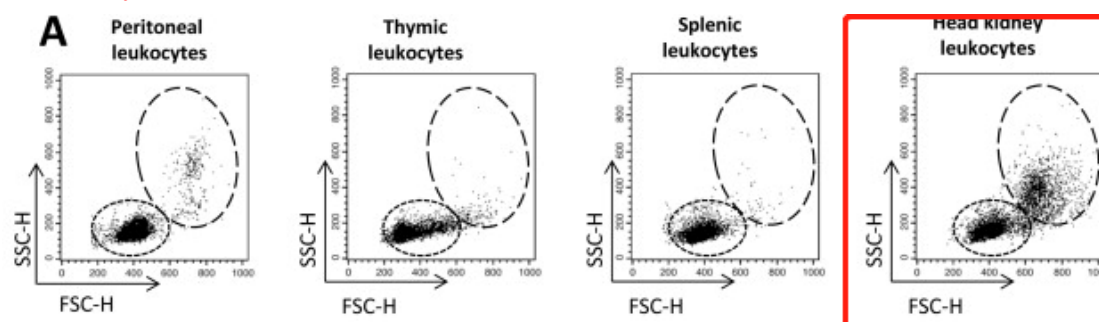
If instead of working with mononuclear cells isolated with Ficoll we work with lysed blood only erythrocytes and keep the leukocytes alive, we will clearly have the three leukocyte populations, the neutrophils are recovered, which is important in the case of phagocytosis and oxidative burst.

Response 1:

Dear reviewer:

Thank you very much for your hard work in reviewing this article, which greatly helped us to improve the manuscript. According to your valuable comments, we have tried our best to improve the manuscript with some modifications in revised manuscript.

Your suggestions are very valuable for us to isolate and clearly distinguish the leukocytes. We completely agree with your perception that the isolated cells only contain lymphocytes and monocytes, the neutrophils sediments will together with the erythrocytes, when the cells are extracted with Ficoll. However, in this research, we isolated the leukocytes from head kidney of largemouth bass by leukocytes isolation kit of fish tissues (WBC1080FZ, TBD, China), instead of Ficoll. And the result (Figure 9A1, B1) of our isolation was consistent with rainbow trout (*Oncorhynchus mykiss*)[1] (Red box shown in blow), which contains two populations including lymphoid gate (FSC-H^{low}, SSC-H^{low}) and myeloid cells (FSC-H^{high}, SSC-H^{high}). Thus, the method of isolation of leukocytes can still support the argument of this article. In our further study, we will take your advice to isolate the leukocytes.



It is well recognized that "professional phagocytes", including macrophages/monocytes, neutrophils, and dendritic cells. Recent studies also have identified the potential for the phagocytosis of lymphocytes cells in reptiles[2], mice[3,4], humans[5] and teleosts[6]. And the purpose of this study is to investigate the effects of Fc γ R on phagocytosis of leukocytes. Thus, in Figure 9 A1 and B1, we gated the whole isolated leukocytes for analysis.

Reference:

1. Korytář T, Jaros J, Verleih M, et al. 2013. Novel insights into the peritoneal inflammation of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* 35 (4):1192-9. doi:10.1016/j.fsi.2013.07.032
2. Zimmerman LM., Vogel LA., Edwards KA., et al., 2010. Phagocytic B cells in a reptile. *Biol. Lett.* 6, 270-273. <https://doi.org/10.1098/rsbl.2009.0692>
3. Gao J., Ma X., Gu W., et al., 2012. Novel functions of murine B1 cells: Active phagocytic and microbicidal abilities. *Eur. J. Immunol.* 42, 982-992. <https://doi.org/10.1002/eji.201141519>.
4. Nakashima M., Kinoshita M., Nakashima H., et al., 2012. Pivotal advance: Characterization of mouse liver phagocytic B cells in innate immunity. *J. Leukoc. Biol.* 91, 537-546. <https://doi.org/10.1189/jlb.0411214>
5. Zhu, Q., Zhang, M., Shi, M., Liu, Y., et al. 2016. Human B cells have an active phagocytic capability and undergo immune activation upon phagocytosis of *Mycobacterium tuberculosis*. *Immunobiology.* 221, 558-567. <https://doi.org/10.1016/j.imbio.2015.12.003>
6. Li J, Barreda DR, Zhang YA, et al. 2006. B lymphocytes from early vertebrates have potent phagocytic and microbicidal abilities. *Nat Immunol.* 7 (10):1116-24. doi:10.1038/ni1389

Point 2: In Figure 4C, the quality of the photo has to be improved since it is not observed that there are 42.78% of positive cells as shown in Figure 4D.

Response 2: Thanks for your good suggestion. The quality of Figure 4C and Figure 4D has been improved.

Point 3: In line 288 replace a flow cytometry with by flow cytometry.

Response 2: The sentence in line 288 has been revised as "The phagocytic ability of leukocytes was analyzed by flow cytometry".