Reviewer’s report

Title: Eosinophils in anti-neutrophil cytoplasmic antibody associated vasculitis

Version: 1 Date: 16 Nov 2018

Reviewer: Angelo Calado

Reviewer's report:

In this manuscript, the authors present data that contribute to the understanding of the role of eosinophils in AAV (anti-neutrophil cytoplasmic antibodies associated vasculitides), and more specifically in granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). For such, eosinophils from patients of these AAV subtypes were characterized in what concerns their surface marker expression and function in comparison with counterparts collected from healthy blood donors (HBD). The authors were able to conclude that lower numbers of blood circulating eosinophils were present in GPA and MPA patients in comparison to HBD cells. Importantly, eosinophils from patients further exhibited: (i) altered surface marker expression; (ii) decreased capacity to produce ROS and (iii) enhanced extracellular trap formation upon stimulation with anti-neutrophil cytoplasmic antibodies (ANCA), what was not observed in cells from HBD.

As I had previously stated in my review of the first submitted manuscript, this report is well and clearly written. The scientific question here addressed is a relevant one in the field of vasculitis, as it may critically contribute for better understanding the contribution of eosinophils to GPA and MPA. Moreover, it has been addressed via well designed and controlled experiments yielding original and sound data. Despite this, I had several concerns regarding the previously submitted manuscript. These were all clearly and satisfactorily answered by the authors in this current version.

However, there are some details that in my opinion will still need to be corrected. Specifically, I call the authors' attention for the following corrections:

- In the Conclusions, 2nd line, consider replacing "indicating" by "suggesting", to be consistent with the results interpretation throughout the manuscript.

- In page 6, line 12, consider replacing "Duplicate cells" by "Doublets".

- In the legend of Figure 6, line 12, replace "lysin" for "Poly-L-Lysin".

In addition, throughout the manuscript, one encounters some typographical and grammatical errors that should as well be corrected. Do please consider a careful revision of the manuscript text. Below, among several of the possible corrections, I outlined a few:

- In page 4, line 10, consider replacing "including" for "factors".
- In page 7, line 14 of the section of "Isolation of blood eosinophils and neutrophils", replace the sentence "Viability at isolation was determined Trypan blue" by "Viability at isolation was determined with Trypan blue".

- In page 14, line 11, the expression "from patients" is repeated twice consecutively in the text.

- In page 14, lines 14-16, consider merging the two sentences contained in the following excerpt "An important feature of eosinophils is the binding of antibodies and of complement proteins via specific receptors. Events that will induce degranulation and antibody mediated cellular cytotoxicity and eventually killing of invading microbes or host cells [25]." into just one sentence such as, "An important feature of eosinophils is the binding of antibodies and of complement proteins via specific receptors, events that will induce degranulation and antibody mediated cellular cytotoxicity and eventually killing of invading microbes or host cells [25]."

- In the legend of figure 2, indication of panel C is missing.

- In the legend axis of figure 5, "Ostim" should be replaced by "Unstim". Moreover, this abbreviation should be explained in the Figure legend.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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