Reviewer’s report

Title: Eosinophils in anti-neutrophil cytoplasmic antibody associated vasculitis

Version: 0 Date: 21 Sep 2018

Reviewer: Angelo Calado

Reviewer's report:

In this manuscript, the authors proposed to contribute to the understanding of the role of eosinophils in AAV (anti-neutrophil cytoplasmic antibodies associated vasculitides). For such, eosinophils from AAV patients were characterized in what concerns their function and activation in comparison with cells collected from healthy blood donors (HBD). Overall, the authors were able to conclude that the lower numbers of blood circulating eosinophils present in AAV patients in comparison to HBD cells presented: (i) altered surface marker expression consistent with their activation and (ii) decreased capacity to produce ROS. In addition, the authors were also able to show that stimulation with anti-neutrophil cytoplasmic antibodies (ANCA) enhanced extracellular trap formation in AAV eosinophils which was not observed in cells from HBD.

In my opinion, this manuscript is well written and is presented in a clear manner. Authors have clearly posed an important and well-defined scientific question and consistently, have designed and conceived suitable and well controlled experiments for addressing it. The obtained data are original and sound and have enabled the authors to withdraw relevant insights on the contribution of eosinophils to AAV. Still, this manuscript will greatly benefit from additional improvements that are subsequently explained and discussed below.

Major points:

1) It is not clear why the authors have not included any patient with EGPA (eosinophilic granulomatosis with polyangiitis) in their study, taken that this particular subtype of AAV is manifested with eosinophilia in the blood and in tissues. Anyway, and having this in mind, I would suggest that any conclusion withdrawn in this manuscript should be particularly ascribed to the disease subtypes analysed rather than being generally ascribed to AAV in general.

2) The data in this manuscript shows that a decreased number of eosinophils is observed in the blood of AAV patients in comparison to HBD. This result should be carefully and thoroughly analysed in comparison with what has been published previously regarding AAV in general, and specifically, in the different subtypes of AAV diseases.

3) The manuscript should as well include a figure with the data on the corticosteroid treatment in AAV patients for a better assessment of the obtained results and of the data analysis already included in the manuscript.
4) The analysis of an extensive panel of cellular markers enabled the authors to conclude that blood eosinophils from AAV patients are activated. In the manuscript, it is however not clear how the authors reached such conclusion. In my opinion, this should be thoroughly outlined and discussed in the manuscript. Importantly, the authors should as well compare their data with what has already been published previously for AAV patients in general as well as for specific subtypes of AAV diseases.

Minor points:

1) The section of Methods will clearly benefit from several changes.

   - Authors should indicate which antibodies (with references) were used for the several applications, namely flow cytometry and immunofluorescence staining.
   - A summarized protocol should be included for the flow cytometry analysis.
   - It is also not clear whether the same method was used for the isolation of eosinophils from all the blood samples collected. This issue should be better clarified.
   - In the protocol for the induction of eosinophil extracellular traps, the authors forgot to include the addition of IgG from HBD (that is referred to having been used in the Results section).
   - A brief description of how imaging of immunofluorescence staining was performed should as well be included.
   - The section of the Methods should as well contain a brief explanation of how neutrophil isolation was performed.

2) The title of the 2nd part of the section of Results should be revised. As it is, the title does not illustrate what has been done in this part of the manuscript or what were the conclusions of it.

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 a 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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