Reviewer’s report

Title: COPD exacerbation severity and frequency is associated with impaired macrophage efferocytosis of eosinophils

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Reviewer: Caroline Owen

Reviewer’s report:

General comments

The authors have studied blood and sputum leukocytes from a cohort of COPD patients and controls to determine impaired macrophage efferocytosis of eosinophils correlates with clinical outcomes in COPD. Their results show that impaired efferocytosis of eosinophils measured in sputum macrophages correlates with exacerbation frequency and severity (assessed by reductions in FEV1 during exacerbations) but does not correlate with COPD severity (assessed by GOLD stage or SGRQ, CRQ, or VAS scores). Studies of monocyte-derived macrophages (MDM) from COPD patients confirm that those subjects having impaired macrophage efferocytosis in vivo have impaired uptake of apoptotic eosinophils by MNM in vitro.

Strengths of the paper are that it focuses on eosinophils, an understudied cell type in COPD. In addition, the authors have correlated macrophage efferocytosis with a broad range of parameters in COPD patients and the patient population has been well characterized. The authors demonstrate a novel and interesting link between impaired macrophage efferocytosis and exacerbation frequency and severity.

Weaknesses of the paper are that there is a lack of clarity and incomplete descriptions of some of the methods and results. Also, the controls are significantly younger than the COPD patients which could influence the results. It should be possible to add results from in vitro studies from older controls to address this issue. Additional statistical analyses, revisions, and additions to the Discussion section are needed as outlined below.

Major compulsory revisions:

1. Macrophage red hue:
   a. The authors should make it clear in the introduction and methods section that low macrophage red hue is indicative of reduced efferocytosis of eosinophils and provide the range of % red hue that represents normal versus low values for this readout upfront in the Introduction section. Unless the reader has read the prior JACI from this group, it is difficult to interpret the results section as this information is only provided in the discussion.

   b. In the legend to Fig. 2b, the authors state that macrophages in subparts A and
D. have purple cytoplasm and cells in C and D have light blue cytoplasm. I think this statement is incorrect and should be revised to state that cells in A and C have blue cytoplasm and cells in B and B have purple cytoplasm.

c. How were the cells stained to measure red hue? Although the method has been published, it is not a commonly used technique. It would be helpful to the reader to add some brief details to the Methods and figure legend.

2. COPD cohort:
b. The COPD patients were assessed every 3 months, but for how long were they followed to assess exacerbation frequency and severity (line 91)?
c. The authors studied 103 patients. However, the numbers listed in lines 107-111 adds up to 104 rather than 103.

3. In vitro studies of MDM:
a. The authors should provide information on the GOLD stage of the COPD patients that were recruited for the in vitro studies in Table 3.
b. Did MDM efferocytosis correlate with COPD severity as assessed by GOLD stage in these in vitro studies?
c. Table 3 shows a highly significant P value for the age comparison. The authors should include results of cells isolated from older versus younger healthy controls to make sure that age does not affect uptake of eosinophils by MDM especially as leukocyte function is known to decline with increasing age.
d. Did the use or dose of ICS influence MDM efferocytosis in the COPD subjects?

4. Figure 3:
a. Can the authors provide a better quality image of the cells in Fig 3A? The green fluorescence is not very intense in the image provided.
b. The authors should make it clear in the results text and figure legend that the 4 COPD groups studied are the same as those shown in Fig. 2 (i.e. determined by their sputum macrophage characteristics).
c. Is the mean value for group A lower than those for the other COPD groups in Fig. 3b? The authors should provide p values for the pair-wise comparisons in the results section. The number of subjects studied is small. The authors should make sure that the sample size is adequate to detect a significant difference.

5. Discussion:
a. The authors should discuss potential mechanisms by which eosinophil uptake by macrophages might be impaired in COPD lung and how this process might increase exacerbation frequency and severity.
b. The authors could speculate why the relationship between MDM efferocytosis and exacerbation frequency was lost when subjects were followed for > 1 year.

Minor essential revisions:
1. Additional information is needed in the Methods section:
   a. The authors should provide the names of antibiotics and antifungal reagents added to the media and the concentrations tested (in micrograms/ml) rather than as “%”.
   b. In which medium were the eosinophils suspended for induction of apoptosis?
   c. Instead of “fed” can the authors use “add” on line 140 and in the section below?
   d. On line 140, please add the incubation temperature and medium used in the cell based assays in addition to the incubation time.

2. For the hypothesis outlined in lines 75-79, can the authors be more specific? E.g. qualify “related” and “associated” by either “directly” or “indirectly” so that the hypothesis is clearer to the reader.

3. The authors should order the sub-parts of Fig. 3 in the order that they are arranged in the order that they are described in the text.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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.