Reviewer's report

Title: Nanoparticle uptake by airway phagocytes after fungal spore challenge in murine allergic asthma and chronic bronchitis

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Reviewer: Rakesh Kumar

Reviewer's report:

In this manuscript, the authors have examined the uptake of nanoparticles by macrophages following phagocytosis of fungal spores, as well as by other inflammatory cells. The macrophages were derived both from normal control mice and from animals in which allergic airway inflammation or a COPD-like disease state had been induced.

There are several issues of concern with respect to the experimental approach and the interpretation of the data. These are listed below.

Major Compulsory Revisions:

1. The basis for undertaking the work and the underlying biological question are not clearly defined or articulated. The authors state in the introduction that uptake of nanoparticles appears to occur by quite a different process to phagocytosis. Why then is there a need to assess the effect of phagocytosis of fungal spores on the uptake of nanoparticles? This is never explained -- the authors simply state that knowledge about this is lacking, but do not present an argument for biological relevance. Presumably the experimental design of exposure to nanoparticles after phagocytosis of spores, rather than concurrent exposure, was based on a desire to avoid passive carriage of nanoparticles on the surface of the spores, but this is not explained either.

2. There seems to be some misrepresentation of what the experiments are actually assessing. In the abstract the authors refer to common ambient air microparticles and again in the discussion they refer to previous challenge with ambient air particles. However, they did not test ambient air particles, most of which are carbon particles with adsorbed metals and organic components on their surface. Rather, they studied just one kind of fungal spore which may be present in the ambient air in some environments. No explanation is offered for why they chose this particular fungal species, other than to indicate that it is an example of Basidiomycetes, the spores of which they state are the major portion of the spore load in ambient air. What proportion of the particle load in ambient air is fungal spores? What proportion of that spore load is Calvatia excipuloformis spores? The relevant context is not provided.

3. Similarly, the authors repeatedly refer to chronic inflammatory lung diseases and in the Conclusion suggest that they have studied models of the two most relevant such diseases. However, one of the models employed is of acute
allergic airway airway inflammation, not of chronic challenge.

4. All of the ex vivo studies examined cells which had first been exposed to fungal spores for 2 hours. Were there any experiments in which cells were not exposed to any microparticles prior to challenge with nanoparticles? And were there any experiments in which cells were exposed to relatively inert particles of similar size to the spores? Such controls would seem to be essential to interpret the data.

Minor Essential Revisions:

5. Although the morphological studies have been carefully performed and the data are clearly presented, the authors place what seems to be a great deal of emphasis on a ~1.5 micron change in mean diameter of macrophages between Scnn1b-Tg mice and WT mice, which they suggest is indicative of activation of these cells. While the size difference is statistically significant, the authors do not present any basis for believing that such a modest increase in cell diameter, in cells that can change size dramatically following phagocytosis, is biologically important or associated with demonstrable activation of e.g. cytokine production or some other relevant marker. Some clarification or re-wording is required.

6. The authors present but do not comment upon the result that nanoparticles were taken up by a higher percentage of macrophages from naïve C3H/C57BL mice than from sham-sensitised BALB/c mice. Do they believe this to be a strain difference? Is it really relevant? If not then this could be deleted.

7. Much of the arithmetic around ratios of vesicle to particle diameter (pages 12-13) seems to amount to presentation of the same result in different ways. The authors could seek to simplify this.

Level of interest: An article of limited interest

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.