Author's response to reviews

Title: IL-13 expression by blood T cells and not eosinophils is increased in asthma compared with non-asthmatic eosinophilic bronchitis

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Author's response to reviews: see over
Dear Editor,

Re: MS-3068455226306878 IL-13 expression by blood T cells and not eosinophils in asthma compared to non-asthmatic eosinophilic bronchitis

Thank you for giving us the opportunity to resubmit a revised manuscript. A point-by-point response to all the comments is detailed below. We feel the review process has improved our manuscript, which now includes new data and hope that you consider it now suitable for publication in your journal. The additional work was largely undertaken by Vijay Mistry and therefore he has been included in the list of authors.

Reviewer 1

C1 The authors present the patients’ data in Table 1. These data should be briefly described in the text of the Results.

R1 We have described the table in the results section.

C2. In Table 1 the subjects are divided into normal, mild/moderate and severe asthmatics and eosinophilic bronchitis groups. The authors should present the T cell IL-13 expression data according to this stratification too.

R2 We have highlighted the severity of asthma in figure 1.

C3. The authors mention that eosinophil count had no correlation with airway function (AHR). It would be important to show that airway function correlated (or not) with intracellular IL-13 expression. The actual data should be presented.

R3. This data was already presented, but we have reworded this section in the results to clarify this point.
C4. It appears that severe asthmatics had greater numbers of neutrophils than the rest of the groups. The significance of this finding needs to be explained.

R4. The neutrophil number was not significantly increased in severe asthma. Previous observations have suggested that a sputum neutrophilia is a feature of severe disease. Although we agree that this is an important area of research we do not feel it is central to this manuscript and have therefore not expanded on this issue in the discussion.

C5. Figure 2: The authors should have used normal (non-asthmatic) serum in the same dilution as controls and should show those data too.

R5. We have added this new data to the methods, results and figure.

C6. Page 11 line 186: the rational for treating the cells with IL-17 should be described for the readers.

R6. We have expanded on the rationale for using IL-17E (IL-25) in the methods.

C7. Figure 3: the authors should have included serum samples from the eosinophilic bronchitic patients.

R7. Serum from the non-asthmatic eosinophilic bronchitis group were not tested and are no longer available and therefore we have not added this data.

C8. IL-13, IL-4, IL-5 and GM-CSF are missing from the cytokine profile the authors show in Figure 3. These are very important cytokines because their genes are located in the same cluster on chromosome 5 together and they play a central role in the pathogenesis of asthma as well as of tissue eosinophilia. Measurements of protein levels for these cytokines are necessary in order to demonstrate whether differential expression profile exists for the mainly pro-eosinophilic (IL-5, GM-CSF) and pro-atopic/asthmatic (IL-4, IL-13) cytokines.

R8. We recognise that IL-4, 5, and 13 are Th2 cytokines and are implicated in the pathogenesis of asthma. GM-CSF was not part of the panel of mediators measured. However, none of the cytokines measured was differentially expressed between asthma and healthy controls and therefore cannot explain the priming effect of the asthmatic serum. For this reason we do not feel that extending the figure to include all 18 mediators adds to the manuscript.

**Reviewer 2**

C9 The existence of an activator of the IL-13 pathway in asthmatic serum is not indicated by the current data shown in Fig. 2. This experiment should be performed by comparing sera from asthmatics and healthy donors. In addition, for accurate assessment of the potential of asthmatic serum to augment IL-13 production, the data of unstimulated cells with and without serum should also been included.
We have added the data for co-stimulation of PBMC with mitogen and non-asthmatic serum (see R5). Unstimulated cells do not produce sufficient IL-13 to be detected and therefore this data is not included.

As the result that the expression of IL-13 was not different in eosinophils among three groups is one of the precious novel findings in this study, these data should be shown as figures like Fig. 1b and 1c.

We have added these new figures, extended the results section and added to the discussion.

The experimental procedure is not clear. Was IL-17E added in the presence or absence of serum and/or PMA? Why did authors not show the data of anti-IL-17E antibody? A graphical display of these data as Fig. 2 is desirable.

We have extended the methods section. We have not added the IL-17 data to the figures as we feel this negative data does not add to the manuscript.

In Table 1, the meaning of the number followed by the number with parenthesis in the row of Age, FEV1%, FEV1/FVC and Neutrophils and a term “N/A” should be clarified. The description in line 334 “**p<0.05 vs. control & EB” is not correct in the case of Disease Duration in which the data of control subjects is N/A. Also in this line, the symbol “^” is not appeared in the Table 1.

We have clarified all of these issues in the table.

In Methods section, the procedure of the mesoscale system should be described.

We have added more detail about the Mesoscale system to the methods section.

The meaning of “p=0.007, p<0.05” is not clear.

This corresponds to the Kruskal-Wallis and post-hoc Dunn’s pairwise comparison. This is now clarified in the text.

Yours sincerely,

Chris Brightling