Reviewer's report

Title: Anti-TNF Inhibits The Airways Neutrophilic Inflammation Induced By Inhaled Endotoxin in Human.

Version:3 Date:7 May 2014

Reviewer:Brian Leaker

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General comments

I have concerns over the sputum methodology and definition of cell types. Also the data could be presented in a much simply manner than currently shown.

It might be better to present a re – analyses of the data as there are clear errors at present (see below). A simple Box and whiskers plot to compare each group might be a better statistical method of demonstrating the differences in cell counts and demonstrating outliers.

Sputum and LPS methods

The usual method is to perform the induced sputum collection at 6 hours when the cell counts are maximal post stimulation

• The choice of the lower dose of LPS in the methods (page 6) and discussion (page 11) are included in the manuscript and therefore the description should extend to should also include this point regarding the timing of sputum collection

• The cells described present in sputum are macrophages and not monocytes. The authors need to be clear that the cell counts were made by a experienced cytologist who did indeed recognise and count macrophages. Further sputum lymphocytes are unusual although present in small numbers sputum; I think we should seek reassurance that these so called lymphocytes are not in fact respiratory epithelial cells.

• Table 1. The method states are healthy non smoking subjects, yet they produced on average 7g sputum. In our experience normal healthy subjects produce far less sputum than this (range 200 – 500 mg sputum only). Only COPD subjects produce sputum in such quantities. The sputum characteristics are not pre-defined at baseline. It would be normal practice to defined sputum samples that were suitable for inclusion in the study (ie minimum sputum weight; cell viability; sputum neutrophil count and absence of overt clinical infection 9100% sputum neutrophilia would suggest clinical infection and subject exclusion).

• In these studies using normal healthy subjects it is rare to find that all subjects produce sputum on demand on three consecutive occasions; normally there would be a requirement to enrol 40-50 subjects to ensure that 30 subjects
completed all their sputum collections. Hence 49 subjects were enrolled to give 30 subjects who produced sputum at baseline. The one would expect that there would be several subjects who could not produce sputum at subsequent visits post randomisation. This data is not described except for the TNF group Fig 5 scatter plot where the n=10. What is the data form the other groups and were there missing data. If so how was this data analysed given the small numbers (n=10 or less)

• The methods describe selecting the sputum plugs so the weight isn’t from saliva. Also baseline values for the groups for sputum weight and cells counts are higher in the controls compared to the drug group. Is there an explanation for this? Further if subjects were randomised one might have expected that all sputum produces should be equally distributed across the three groups. Given the significant differences in basal cell counts how can the subsequent differences be adjusted for this apparent bias

• Need to add n values in the figure legends. Of the three groups, Fig 5 shows only the TNF data (n=10) in a scatter plot of sputum data at all visits. All three groups should be shown in a similar scatter plot or indeed a Box and whiskers plot

• The normal method for expression of sputum is cells/gm of sputum. Given sputum was produced in such large quantities it is odd that Fig 5 shows graph of cells / mcg sputum weight. For clarity, it is preferable for the axis on the absolute cell count graphs to be expressed as simple raw numbers or as per gram sputum, not log per ul sputum.

• Figure 5d. Linear regression line is incorrectly fitted. It is not representative of the r value.

• Figure 2. The key of what each bar represents must be added to the legend.

• Minor typo on page 9 last sentence ‘pourcentage’. ; pp 12 simvatstatine

Introduction;
First para seems superfluous;
Discussion
Pp 10 3rd para
Should state reproducibility (not repeatability); this data is not shown for controls (see point above regarding Fig 5 scatter plot data).

The reproducibility should be stated in the results section with a similar scatter plot for n=10 subjects across the three different time points for sputum collection