Reviewer's report

**Title:** Systemic inflammation links to atherosclerosis via C3aR1; results of a human in vivo LPS infusion study.

**Version:** 1  **Date:** 4 April 2011

**Reviewer:** Xia Yang

**Reviewer's report:**

In this manuscript, Sivapalaratnam et al reported an in vivo screening of monocyte transcriptome changes upon LPS induction and identified a total of 39 genes being differentially expressed at two time points (T=1 and T=4 compared to T=0). This study involves very small sample size and the details of the data analysis are not sufficient to justify the validity of their findings. The conclusion on C3ar1 being the causal gene for the systemic inflammation induced by LPS is purely speculative with no experimental evidence.

**Major compulsory revisions:**

1. The differential hybridization array used in the study is not a commonly used one. Therefore, the data processing steps require more detailed description and explanation. Specifically, the following questions need to be answered: a) what does each of the criteria listed in the first paragraph under Data Analysis on Page 8 means, b) why were such criteria chosen to define the differentially expressed genes, c) why linear mixed model was used and what are the fixed and random effects of the model, d) what FDR cutoff was used and what was the corresponding p values?

2. The previous in vitro study was published as an abstract (reference 11) rather than a full paper. The authors need to report the detailed findings from the in vitro study within this manuscript in order to utilize the results as well as allow the reviewer to evaluate the validity of the in vitro study.

3. The overlap between the in vivo and in vitro studies is very poor. Although this could be a result of the intrinsic differences between the two systems, it is more likely due to the small sample sizes utilized in the studies.

4. It is not clear how and why 10 and 8 arrays were used for 11 LPS and 5 control samples, respectively for T=1 vs T=1 (last paragraph on Page 10). Similarly, why 10 and 4 arrays for T=0 vs T=4. Why certain samples are duplicated or excluded?

5. How were the genes involved in RT-PCR confirmation selected? VCAN does not seem to be in any of the differentially expressed genes (Table 3, 4, Figure 3) and why was it chosen to be validated?

6. The conclusion that C3ar1 induces systemic inflammation was solely based on previous findings on involvement of this gene in inflammation and atherosclerosis. However, neither the previous studies nor the current study provide evidence supporting C3ar1 as being solely responsible for the systemic...
inflammation. The conclusion has no base. In addition, if C3ar1 was involved in the initiation of systemic inflammation, why wasn’t it in the T=1 signature?

Minor revisions:
1. The microarray accession number is missing from the manuscript.
2. It is not clear whether Figure 1 was from reference 17 or from the current study. Need to specify. If from previous study, it does not justify the need to reproduce the figure in the current manuscript.
3. Why there is no error bar associated with the MA results?

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.