Reviewer’s report

Title: Comparison of a Microsphere-based Platform with a Multiplex Flow Cytometric Assay for determination of Circulating Cytokines in the mouse.

Version: 0 Date: 07 Oct 2016

Reviewer: Amado Zurita-Saavedra

Reviewer’s report:

Summary:

Stricker-Krograd et al. used multiplex flow cytometry (through the BD Accuri C6 system) and Luminex (Myriad RBM) to measure and compare the concentrations of 7 inflammatory mediators (Th1/Th2/Th17) in plasma of mice treated with LPS in the presence or absence of dexamethasone. The authors identified some differences and similarities in the measurements between the two approaches.

Significance and Novelty:

* This is a technical paper of potential practical relevance for investigators working on animal models of inflammation and/or immune modulation.

* If the main goal is to propose the use of multiplex flow cytometry as an alternative to ELISA or bead-based assays, the authors must discuss the expected advantages and limitations of the assay and its relative value.

Comments

Major issues (In no particular order)

1. Discuss the expected advantages and limitations of the flow cytometry assay compared to the Luminex-based. Why are these two approaches relevant and why were these two specific vendors chosen?

2. Results. Comparative levels of the selected cytokines… The statements that the BD CBA assay was more reliable for measurement of IL-4, TNF and IFNg require more detailed explanations of the technique used and the results obtained, quoted by the authors as “gold standard” of the response of those markers to LPS. Why are the results of those references more trustworthy than Myriad's?

Minor issues
1. Title. Please consider changing "multi-analytes platform", used by Myriad, for a more generic "bead- or microsphere-based" reference.

2. Abstract. Please clarify in the results section why IL-17A was a sustained responder.

3. Methods, Plasma preparation and analysis. Please clarify whether the acquisition of approximately 200 events in the flow cytometry approach is representative enough.

4. Methods, Levels of selected cytokines... The information presented on the concentrations of standards for the BD CBA assay is repeated in the text.

5. Methods, Levels of selected cytokines... The statement that "the background of cytokine levels is non-existent" in untreated mice is inaccurate. Rather the assay is not sensitive enough. Was the Luminex tried on these samples?

6. Table 3. Please explain what 0.8a (IFNg 2h BD CBA) refers to.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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