Reviewer's report

Title: Successive influenza virus infection and Streptococcus pneumoniae stimulation alter human dendritic cell function

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Reviewer: Victor Huber

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Comments for the authors of BMC Infectious Disease Manuscript:
The authors of the BMC Infectious Disease Manuscript: Successive influenza virus infection and Streptococcus pneumoniae stimulation alter human dendritic cell function, have designed experiments to address the role of the dendritic cell at the early stages of super-infection, with regard to apoptosis, cytokine production, and cell surface receptor expression. Specifically, they expose human monocyte-derived macrophages to infectious influenza virus and heat-killed pneumococcus to mimic the early stages of these super-infections ex vivo. They evaluate both the time after influenza inoculation and the dose of pneumococcus as factors that mediate dendritic cell responses. The responses by dendritic cells that are specifically monitored are apoptosis, expression of cell surface receptors (CD83, CD86, and MHC II), and expression of cytokines (TNF-#, IL-6, IFN-#, IL-12, and IL-10). Using multiple permutations of their test conditions, the authors conclude that early during super-infection there is an upregulation of pro-inflammatory cytokines by dendritic cells, which likely contributes to this disease in the living host. This manuscript was well-written, the data were clearly presented, and the conclusions represent an important contribution to the literature with regard to evaluation of an under-studied cell type in the early stages of super-infection. This reviewer would like to see the following comments addressed before acceptance of the article for publication.

General Comments:
The data are clearly presented within this manuscript, and as presented the findings were very easy to interpret. One suggestion I would make is that in some of the figures, it appears that the same groups are represented in two separate panels (specifically Figures 2 and 6), and it is unclear whether these data are from separate experiments, or from the same experiment. Specifically, in Figure 2, the 6 hour timepoint for 5 X 10⁶ S. pneumoniae is seen in both panels C and D. Please indicate whether these are two repeats of the same experiment, or representation of the same data twice, within the results section and/or the figure legends. This type of data representation makes it very clear for the reader, but it should be noted how the data are derived.

Specific Comments:
On page 4, third paragraph, the first sentence should be re-worded to state
“…presenting cells that are highly potent…” for clarity.

On page 7, second line from the top, the sentence should be re-worded to state “…were washed before being infected with liver H1N1…” for clarity.

On page 9, first paragraph of the Results section, the second sentence should read “Gating on the total DC population at 24 hr after…” for clarity.

On page 13, in the paragraph describing the results presented in Figure 5, the wording of this paragraph could use significant editing for clarity. Specifically, the statement regarding TNF-α levels after sequential exposure beginning lower than S. pneumoniae alone before eventually matching and exceeding the levels with S. pneumoniae alone could be improved.

Sometimes the authors refer to their cells as DCs, and other times as MDCCs. Consistency in this regard would be appreciated.

Some of the figure legends simply state what the experiment was designed to demonstrate (“Figure 1 Dose response of DC's to influenza virus”), while other tend to draw conclusions from the data presented (“Figure 2 Successive challenge of influenza virus and pneumococcus induced greater DC apoptosis”), and some consistency would be appreciated.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Needs some language corrections before being published

**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