Author's response to reviews

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

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Author's response to reviews: see over
Re: Successive influenza virus infection and Streptococcus pneumoniae stimulation alter human dendritic cell function (MS: 5875090715261190 version 1)

Dear Prof. Jigisha Patel

We thank the editors and reviewers for their careful review and thoughtful comments on our manuscript. In response to their comments and concerns, we have revised the manuscript. We believe that we have addressed their concerns fully and are submitting the revised manuscript for publication.

Below is a point-to-point response to the comments of the reviewers, associate editor and the editorial requests (verbatim comments in bold) provided along with the location and text of any substantive changes (highlighted with yellow) in our revised manuscript.

REVIEWER 1:
Comments:
The authors have chosen to address the question as to whether dendritic cells contribute to the enhanced inflammatory response during consecutive infection with influenza, followed by S. pneumoniae. This viral-bacterial synergistic infection is a common cause of severe pneumonia resulting in hospitalisations and death. The data presented by the authors indicates that challenge of DCs with influenza virus and pneumococcus resulted in maturation of the DCs, up-regulation of pro-inflammatory cytokines and down regulation of anti-inflammatory mediators in a time and dose dependent manner. The study however is limited to an in vitro viral-bacterial system and provides no mechanistic evidence of pathways activated in the presence of either pathogen. The data presented is novel as it uses human DCs and a human influenza isolate and may contribute to enhancing knowledge in the field of the detrimental effects of the exuberant responses to influenza followed by consecutive infection with bacterial pathogens. However, the authors must firstly address some major issues prior to publication:

Major Revisions
1. In the methods section in the abstract (and in the methods text), the authors mention that
heat killed pneumococcus were used to mimic the viral pneumococcal infection. The results make no mention of whether these treated bacteria were utilized, but instead refer to the number of CFU that each system was exposed to, which indicates live bacteria were used. This needs to be clarified throughout the text as to what exactly was done.

A: We have used heat killed pneumococcus in all the experiments described in the manuscript. The use of the heat killed pneumococcus has been clarified throughout the text in the revised version of manuscript.

2. It is not demonstrated whether the DCs infected with influenza yielded a productive infection. The methods section “virus preparation, titration and infection” indicates that DCs were infected with a particular MOI. However, it has been reported in literature that infection of DCs with some influenzas is abortive. This paper needs an experiment and a results section showing whether the particular strain used to infect DCs yields intact virions, or cannot produce virus. This would be a major consideration when interpreting results in a time course manner as is done by the authors.

A: We performed new experiments to demonstrate whether the infection of the particular strain of influenza virus on MoDCs was abortive or productive. We infected MoDCs at MOI 0.1 for 0hr, 6hr or 24hr, and collected supernatant of the infected MoDCs for virus titre analysis by TCID$_{50}$. No infectious virus was detected, indicating that the infection could not yield intact virions. The figures below show that there is no change in CPE of MDCK cells after 5 days of incubation with supernatants collected from the infected MoDC as compared to PBS control. Accordingly, the following sentence was added in Page 10 in the revised manuscript. “Since most viruses are poor at replication once being internalized by dendritic cell [33], we next investigated whether the influenza infection of MoDCs was productive or abortive. MoDCs were infected with H1N1 at MOI 0.1, no infectious virus was detected in the culture supernatant collected at 0hr, 6hr and 24hr post-infection, which indicated that the infection could not yield intact virions (data not shown).
3. In Figures 2a, 3a, and 4b for each time point listed, comparisons to mock infection must be made each time, not just a single mock infection, figures 3b, 3c, 5 and 6 have done this correctly.

A: In Figure 2a and 3a, the data for mock controls at each time point have been added. In Figure 4b, the mock controls were the samples collected at 0hr after virus infection. Therefore, the label of the axes has been changed to more accurately express the message that the figures aimed to show.

4. Figure 2D, 5d, 5e, 5f, 5g, 5h and 6b are unclear. The way the axes are labeled appears as though every treatment group has also received the indicated CFU of pneumococcus.

A: In Figure 2D, 5D, 5E, 5F, 5G, 5H and 6B, the labels of axes have been modified to improve graphical presentation more clearly.

5. For Figure 1, n=4. How many times was this experiment repeated? Are the results indicative of several experiments? The first paragraph in the results section indicates marked increases, yet does not provide statistics. Figure 1 indicates fold-increase compared to mock control, whilst Figure 3 shows MFI – it would be good to keep a consensus between the graphs.

A: In Figure 1, the experiment was repeated for 4 times. To increase clarity, the legends of figure 1, as well as all the other figures have been changed in the revised manuscript. Figure 1 has also been changed to show MFI in order to keep a consensus between the graphs as suggested.

6. Figure 2C 6hour time point is identical to 2D 5x10^6. Whilst these treatment groups should be identical given the same conditions, were they truly different experiments and warrant graphing twice? Same argument for Figure 5A at 24hrs and 5G at 5x10^6 dose, as well as Figure 6A at 6hr and 6B at 5x10^6 dose.

A: Figure 2C, 6hr time point group was the same as 2D 5X10^6 group. These were the data generated from the same experiment and being presented twice in two different panels for the clear graphical presentation and interpretation. To clarify the way that the data are derived from, a sentence “Please note that some groups of data were presented twice in different panels for easy interpretation” has been added to the legends of all the related figures.

7. The only time the methodology is clearly explained with respect to the different groupings is in the 2nd paragraph of the discussion. In the methods section under the heading “viral bacterial stimulation protocol” it is unclear as to which DC stimulation sentence refers to the concurrent or successive challenge. The sentence beginning with “To examine the dose effect
of….” should include ‘6h post-infection with influenza’ at the end of the sentence. Similarly, “DCs treated with both influenza virus and pneumococcus” should include some terminology that the cells were infected concurrently. Also, there is no mention here as to whether the heat killed bacteria were used in any of the experiments as indicated in “bacterial preparation” and in the abstract.

A: Heat killed pneumococcus was used in all the experiments. To explain how viral bacterial stimulation was performed more clearly, the text under the heading “viral bacterial stimulation protocol” has been completely re-written. Briefly, the viral bacterial stimulation consisted of three groups. In the first group, MoDCs were first infected with H1N1 for 0hr, and then immediately stimulated with heat killed pneumococcus for 24hr. The total incubation time was 24hr. This was the concurrent viral and bacterial challenge. In the second group, cells were infected with H1N1 for 6hr, and then stimulated with heat killed pneumococcus for another 24hr, to make up a total incubation time of 30hr. In the third group, cells were infected with H1N1 for 24hr, and then stimulated with heat killed pneumococcus for another 24hr, to make up a total incubation time of 48hr. The later two groups were the successive viral and bacterial challenge. For revised version, please refer to Page 8.

8. The use of the word “exquisitely” in the first section of the results is incorrect.
A: The word “exquisitely” has been deleted as suggested.

9. In the results section “Successive challenge of influenza virus and pneumococcus dysregulated DC cytokine production” what is meant by negative dose response? What is meant by positive dose response?
A: The negative dose response referred to the observations that the production of TNF-α and IL-12 from MoDCs gradually became significantly lower upon the increasing dose of heat killed pneumococcal challenge alone. The positive dose response referred to the observation that the production IL-10 from MoDCs gradually became significantly higher upon the increasing dose of heat killed pneumococcal challenge alone. To describe these observations more clearly, the corresponding text has been changed to “significant decreasing trend in the production of TNF-α and IL-12” and “significant increasing trend in the production of IL-10” respectively. For revised version, please refer to Page 13.

10. In the 3rd paragraph of the section listed in point 8, there is overuse of the word synergistic and it is often used incorrectly. In this same paragraph, the discussion of the results indicate increasing bacterial dose increased the inflammatory response, whilst virus infection lowered the production of pro-inflammatory cytokines. However, figure 5 shows
the most marked upregulation is when cells were infected with both virus and bacteria and not bacteria alone. It is unclear as to what the authors mean by “inverse dose response” and “direct dose response”

A: The 3rd paragraph has been largely revised to describe data more clearly. Figure 5 was trying to show two main messages: synergistic regulation and non-synergistic regulation. Viral bacterial synergism was observed in the critical 6hr time-frame. Under this time frame, influenza virus and pneumococcus appeared to work together and enhanced the inflammatory response. This was illustrated by Figure 5A-5F. On the other hand, in certain time frame (e.g. 24hr group) when the synergism did not occur, it might appear that the dose of pneumococcus was another important factor to regulate the cytokine response. Under this situation, influenza virus no longer synergized with pneumococcus to promote inflammation. Instead certain cytokines (e.g. TNF-α and IL-6) induced by pneumoocccus were significantly lowered by prior influenza virus infection. This was illustrated by Figure 5G and 5H. The “inverse dose response” referred to the observation that upon the increasing dose of heat killed pneumococcal challenge alone, MoDCs gradually produced less TNF-α with a significant trend. The “direct dose response” referred to the observation that upon the increasing dose of heat killed pneumococcal challenge after influenza virus infection, MoDCs gradually produced more TNF-α with a significant trend. To explain these observation more clearly, the corresponding text has been changed to “significant decreasing trend or significant increasing trend in the production of TNF-α” For the whole revised version, please refer to Page 14-15.

11. In results section, last line of “successive challenge of influenza virus and pneumococcus caused a time related change in DC phenotype: the authors indicate there is a lack of change of DC activation markers with respect to dose, however this statement isn’t clear as to whether it didn’t change from the upregulated state, or whether it was not different from the mock controls

A: The statement referred to the observation that there was no change in the activation markers from the up-regulated state. The statement has been revised to improve clarity. For revised version, please refer to Page 13.

12. Figure 2 indicates a marked upregulation of cell death at 6h post-co-infection with virus and bacteria, and Figure 5 indicates at this same time point and treatment regime that there is a marked increase in TNF, IFN and IL-2. There should be some discussion on the links between these inflammatory pathways and induction of cell death in the DCs and how these mechanisms could affect outcomes in vivo.

A: It is suggested that apoptosis is a strategy for the resolution of inflammatory response [1]. This
is demonstrated in patient with community acquired pneumonia that neutrophil apoptosis could contribute to the resolution of lung inflammation [2]. However, there is no report describing if this is also the case in dendritic cells. On the other hand, intense acute inflammation could cause cell apoptosis [3], and apoptotic cells may in turn activate immune response to promote inflammation [4]. In the in vivo model, TNF-α is reported to block apoptotic cell clearance by alveolar macrophage, and leads to inflammatory response in the lung [5]. It is possible that apoptosis and inflammation may affect each other to cause tissue injury in co-infection. The discussion section has been revised to address the link between inflammation and apoptosis. For revised version, please refer to Page 18-19.

13. If heat treated bacteria were used in the successive and concurrent infection experiments were the results the same as for when live bacteria were used?
A: In our study, heat killed pneumococcus is always used in all the experiments described. On the other hand, if live pneumococcus were used, we predicted that the result might appear very similar to what observed in the study. As both live and heat killed bacteria make use of their cell wall lipoteichoic acid and peptidoglycan for stimulation, which target TLR2. Pneumococcus induces DC apoptosis by two distinct mechanisms: 1) a rapid, caspase-independent mechanism of apoptosis induction, critically dependent on bacterial expression of pneumolysin, and 2) a delayed-onset, caspase-dependent mechanism of apoptosis induction associated with terminal DC maturation [6]. The live bacteria process pneumolyin, which could rapidly induce dendritic cell death. To address the response of MoDC to successive viral bacterial challenge, MoDCs were first infected with H1N1 before challenged with pneumococcus. In this situation, if live bacteria were used, the cell could become dead very quickly before any analysis could be performed. This would definitely make the experimental design and data acquisition difficult. Therefore, this was one of the major considerations of not choosing live pneumococcus in our experiments.

Minor Revisions:
1. 1st line in abstract Background, Introduction and Discussion should be “respiratory disease” not “respiratory diseases”
A: The phase “respiratory diseases” has been revised as “respiratory disease” as suggested.

2. Monocyte DCs are commonly referred to in literature as MoDCs, not MDDCs. This nomenclature should be fixed in the text and wherever DCs are mentioned, it should state MoDCs.
A: MoDCs have been used to describe monocyte derived dendrite cells throughout the revised version of the manuscript as suggested.
3. The authors have often written “treated by” and “infected by”, this should be “treated with” and “infected with”.
A: All the phase “treated by” and “infected by” have been changed to “treated with” and “infected with” throughout the text.

4. 7th paragraph, 1st sentence in the discussion section: plastic means flexible, therefore the sentence needs changing.
A: The sentence has been changed to state that “MoDCs response to pathogen challenge is very plastic”. For revised version, please refer to Page 20.

REVIEWER 2:
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.

A: Yes, in Figure 2, the 6hr time point for $5 \times 10^6$ *S. pneumoniae* was used in both panel C and D. This was also the case in all other similar figures e.g. Figure 5 and Figure 6. These were the same data being presented twice for clear graphical presentation and interpretation. To clarify the way that the data are derived from, a sentence “Please note that some groups of data were presented twice in different panels for easy interpretation” has been added to the legends of all the related figures.

Specific Comments:
On page 4, third paragraph, the first sentence should be re-worded to state “…presenting cells that are highly potent…” for clarity.
A: The sentence has been revised as suggested. For revised version, please refer to Page 4.

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.
A: The sentence has been revised as suggested. For revised version, please refer to Page 7.

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.
A: The sentence has been revised as suggested. For revised version, please refer to Page 10.

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-# levels after sequential exposure beginning lower than *S. pneumoniae* alone before eventually matching and exceeding the levels with *S. pneumoniae* alone could be improved.
A: The paragraph has been largely revised to improve clarity. For revised version, please refer to Page 15.

Sometimes the authors refer to their cells as DCs, and other times as MDCCs. Consistency in this regard would be appreciated.
A: The term “MoDCs” has been used to describe monocyte derived dendritic cells throughout the revised version of the manuscript.
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.

A: The title of all the figure legends has been changed to state what the experiment was designed to demonstrate to achieve consistency.

**REVIEWER 3:**

Comments:

The present study seeks to characterize human DC responses to concurrent or sequential in vitro stimulation of live influenza virus and heat-killed pneumococci. Although there was a detailed description of DC apoptosis, phenotypic maturation, and cytokine responses in a time and dose dependent manner, the significance of these observations is limited by the complexity in rationalizing the timing and magnitude of in vitro stimulation to physiological circumstance. The authors should at least discuss the possible involvement and role of DCs in the context of viral and bacterial co-infection (in vivo) relevant to their experimental approaches and results. In particular,

1. Regarding to time-dependent responses including DC apoptosis (Fig. 2) and pro-inflammation cytokine production (Fig. 5), significant synergistic stimulation was only observed at 6hr interval (with a high dose of pneumococci), but not 0hr or 24hr. Does this point to an insignificant role of DCs in most cases of co-infection (when it happens beyond this narrow interval)?

A: The main message of this study was that successive challenge of influenza virus and pneumococcus on MoDCs induced cell apoptosis and inflammatory response significantly. This observation could suggest the possible contribution of DCs to co-infection. Our study found that synergistic stimulation was observed at the 6hr time interval, but not 0hr nor 24hr time interval. The importance of the critical time frame to allow synergism to occur was consistent with the in vivo mouse co-infection model in which the greatest mortality of sequential co-infection occurred when pneumococcal infection came at 7 days, but not 0-3 days nor 14-21 after influenza virus infection [7]. The scale of the time frame for synergism to occur was of course different between our in vitro study and the in vivo model. In vitro condition was limited that MoDCs have rather a short lifespan. However, in the in vivo co-infection, there was a similar time frame for the synergism. Therefore the significance of DCs in co-infection in vivo remains to be investigated.

2. Increased CD83 and CD86 expression was observed in certain co-stimulated DCs when
compared to these treated with influenza virus alone, but not these treated with pneumococci alone (Fig. 3). In addition, decreased IL-10 production was found in co-stimulated DCs when compared to these treated with pneumococci alone, but not these treated with influenza virus alone (Fig.6). These separated observations do not provide enough support for their involvement in viral and bacterial co-infection.

A: In the successive challenge experiments, there were two pathogens: influenza virus and pneumococcus. The study tried to find out the differential modulation of MoDC functions in viral bacterial challenge, and compared these dysregulated cell functions with MoDCs that had been infected with influenza virus alone or treated with pneumococcus alone. The result found that both influenza virus and pneumococcus were involved in the viral bacterial challenge, but contributed to the dysregulated MoDC functions differently. Influenza and pneumococcus could both contribute to the inflammatory response. For example, there was synergistic up-regulation of inflammatory cytokines when compared to MoDCs infected with virus alone or treated with pneumococcus alone. On the other hand, only influenza virus was involved in the suppression of anti-inflammatory response. For example, the IL-10 induced by pneumococcus alone was down-regulated by the prior influenza virus infection. Also, only pneumococcus was involved in the change in MoDC phenotypes. For example, the up-regulation of CD83 by secondary pneumococcal challenge after virus infection when compared to that of the MoDCs infected with virus alone.

**REVIEWER 4:**

Comments:

This is a very interesting study of the synergistic effects of influenza and pneumococcus on myeloid dendritic cells. Overall, this manuscript is well written and does make a significant contribution to the field of dendritic cell immunobiology. In addition, the co-infection model studied has great clinical significance. There are minor grammatical errors that should be amenable to editorial correction. A few minor essential revisions are detailed below and will add strength and clarity to this very nice study.

**Minor Essential Revisions**

1. Paragraph 2, sentence 1, section (iii) - please briefly define "immunodysfunction"
   A: “immunodysfunction” referred to the dysregulation of immune system components. The word has been changed in the revised text. For revised version, please refer to Page 4.

2. Cell staining and flow cytometry paragraph - It is important to mention which
fluorochromes the anti-CD83 and anti-CD86 antibodies were conjugated to and whether these stains were performed together or individually (were samples stained with the 2 above antibodies and PI in the same tubes?). If the stains were performed in one tube, could the authors show Figures 1 and 2 gated on PI-negative events? This may highlight differential effects of flu and pneumococcus on DC maturation (by excluding apoptotic cells which could alter the flow cytometry data shown).

A: In Figure 1, cells were stained with APC conjugated anti-CD83, PE conjugated anti-CD86 and FITC conjugated anti-MHC-II together in one tube. Cells were stained with PI in another tube separately. Analysis was based on gating from the total cell population because we aimed to examine the overall effect of influenza virus on MoDC maturation in order to choose an appropriate viral infectious dose. In Figure 2, cells were stained with Annexin V and PI in one tube.

3. Figure 1 - Please add statistical comparisons as shown in Figure 2. At what dose of flu does the expression of CD86, CD83, and MHC-II become significantly increased? This will strengthen the data shown.

A: Statistical comparison has been made by one way ANOVA analysis with Bonferroni multiple comparison test as suggested.

4. In Figure 2, the total incubation times are confusing to figure out. While this is clear for Figure 2A and can be found in the figure legend for 2B, it is unclear what the total incubation time for Figure 2C is (since the authors now demonstrate the times post virus infection that pneumococcus is added). Figure 2D total incubation times are mentioned in the figure legend (24 hours). Including a label of both the Times post virus infection AND the total incubation time at harvest would greatly enhance the clarity of this figure (and also other similar figures in the paper, like figure 5) and should be added to the manuscript.

A: The time between the sequential addition of influenza virus and pneumococcus, as well as the total incubation time at harvest were added to all the related figures, legends of the figures and the method section. To explain how the viral bacterial challenge being performed more clearly, the text under the heading “viral bacterial stimulation protocol” has been completely re-written. Briefly, the viral bacterial stimulation consisted of three groups. In the first group, MoDCs were first infected with H1N1 for 0hr, and then immediately stimulated with heat killed pneumococcus for 24hr. The total incubation time was 24hr. This was the concurrent viral and bacterial challenge. In the second group, cells were infected with H1N1 for 6hr, and then stimulated with heat killed pneumococcus for another 24hr, to make up a total incubation time of 30hr. In the third group, cells were infected with H1N1 for 24hr, and then stimulated with heat killed pneumococcus for
another 24hr, to make up a total incubation time of 48hr. The later two groups were the successive viral and bacterial challenge. For revised version, please refer to Page 8.

5. In the results section, second paragraph, the sentence beginning "However, when DCs were exposed to pneumococcus at 24 hr after viral infection, the frequency of apoptotic DCs was substantially reduced" - the authors need to clarify exactly what comparison they are making here. It was substantially reduced compared to the 6 hour co-infected groups? If so, a p value for this comparison should be added.

A: Yes, this aimed to compare the apoptotic frequency of MoDCs treated with both influenza virus and pneumococcus in the 6hr group and 24hr group. However, there was no statistical significance, therefore this sentence was deleted.

6. In the results section, paragraph 2, the last sentence (starts with "Furthermore") is confusing, since the white bars in Figure 2D show a dose-dependent effect but the black bars do not. This sentence should be clarified.

A: This sentence aimed to state that there was a significant decreasing trend of cell apoptosis upon increasing dose of pneumococcal stimulation alone (the significant trend in Fig 2B). However, such decreasing trend of cell apoptosis was lost when the pneumococcal stimulation was preceded by influenza virus infection. Therefore, there was no statistical significance showing the dose dependent effect on the black bars in Fig 2D. To improve clarity, the related text has been largely revised. For the revised version, please refer to Page 11.

7. Last sentence in paragraph 3 of the results - please show the MHC-II data; it is still interesting to show this with the other phenotypic markers, even though the effects on MHC-II were not significant (suggests differential regulation of maturation that will be better displayed by showing this data).

A: A new graph Figure 3D has been added to show the expression of MHC-II during the viral bacterial challenge.

8. In the results section, 6th paragraph I think, sentence beginning "Furthermore, in the regulation of TNF-alpha production induced by pneumococcus alone..." - this sentence is very confusing and should be clarified.

A: To improve clarity, this part has been largely revised. Briefly, this part aimed to state that there was a significant decreasing trend in the production of TNF-α upon the increasing dose of pneumococcal stimulation alone. However, this decreasing trend was reversed to that of a significant increasing trend in the production of TNF-α upon the increasing dose of pneumococcal
stimulation after influenza virus infection. For revised version, please refer to Page 14.

9. In the last sentence of the second-to-last paragraph of the results section (sentence discussing Figure 6B), in order to make the statement that the inhibition of IL-10 was partially rescued, the authors need to compare the black bars of figure 6 with a statistical test (please add p value for this comparison).
A: There is no statistical significance between the black bars. Therefore this sentence has been deleted.

10. In the 4th paragraph of the discussion, the second sentence - the statement that "which can explain the clinical observation that human autopsy material...." is too strong for the data presented. This should be replaced with a statement like "could potentially explain" or "this data is consistent with the findings in human autopsy material", etc.
A: The sentence has been changed as suggested. For revised version, please refer to Page 17.

11. Last sentence of the discussion - change to something like "which could skew developing immune responses toward Th1" to be more clear.
A: The sentence has been changed as suggested. For revised version, please refer to Page 21.

12. Last sentence of Conclusions - change to "Our study contributes to the understanding of the underlying ....." just to be more clear. This is a minor suggestion, but will make this read more clearly. Thanks for your kind consideration.
A: The sentence has been changed as suggested. For revised version, please refer to Page 21.

ASSOCIATE EDITOR’S COMMENTS:
The data are represented in many figures as mean +/- SEM. This is inappropriate as SEM is measure of the precision of the test being utilized and provides no data about the actual biological outcomes. SD, which is a measure of the variability of the biological data, should be used here instead. The authors may wish to review Olsen CH, Infect Immun 2003;71(12):6689-92 or consult a qualified biostatistician for assistance.
A: All the figures have been changed to represent mean +/- SD as suggested.
EDITORIAL REQUESTS:

1) Please can you clarify whether patients provided consent for the blood samples

A: In the study, we recruited blood samples from normal healthy donor but not patients. The blood samples were obtained as buffy coat from healthy donor in accordance with an approved protocol from the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Culster. This information has been mentioned in line 1 to 3 from top, under the heading “In vitro generation of MoDCs” in the Methods section on Page 6.

Yours sincerely,

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