Author’s response to reviews

Title: Lactobacillus paracasei feeding improves the control of secondary experimental meningococcal infection in flu-infected mice

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Author’s response to reviews:

Dear Professor Zaraket

We would like to thank you for handling our manuscript and we thank the Reviewers for their comments and suggestions.

We modified the manuscript according to the comments of the Reviewers and here we provide point by point answers on how we dealt with these comments.

Sincerely Yours

Muhamed-Kheir TAHA

Technical Comments:

- Please include email addresses of all authors.

Response of the authors: The emails of all authors were included.
Reviewer reports:

Reviewer 1.

Hazem Ghoneim (Reviewer 1): The authors of the manuscript titled "Lactobacillus paracasei feeding improves the control of secondary experimental meningococcal infection in flu-infected mice" investigated whether feeding mice with Lactobacillus paracasei as a probiotic treatment would improve the outcome of secondary meningococcal infection after primary influenza infection. They showed that the oral consumption of Lactobacillus increased the numbers of some inflammatory myeloid populations in the co-infected lungs with lower meningococcal load at 24 h post-infection. I have the following comments/questions for the authors:

1- Although the survival rate of the co-infected mice is slightly improved with Lactobacillus feeding on day 9 p.i, it seems to arise from better disease symptoms during primary influenza infection rather than the control of secondary meningococcal infection (Fig. 1). Does Lactobacillus feeding affect the clearance rate of meningococcal infection without primary influenza infection?

Response of the authors: The Reviewer is right. Lactobacillus feeding may affect the clearance rate of meningococcal infection in two nonexclusive manners:

1- Better clearance and control of primary infection

2- Better modulation of immune response

We modified the sentence lines 270-277 to clearly state this action. Unfortunately, the mice are not susceptible to meningococcal infection without a prior flu infection. Indeed, we have devolved this model of flu-meningococcal sequential infection using the epidemiological association between flu and meningococcal infection (See Alonso et al., FEMS Microbiology Letters 222 (2003) 99-106). In all cases our data underline the role of Lactobacillus feeding in the early activation of the pro-inflammatory cytokines recruitment of immune cells in the lungs in the control of infections allowing better control of the flu infection. However, our data further
show that at day 9 there were higher levels of neutrophils, inflammatory monocytes and DCs. This also is expected to allow better control of the secondary bacterial infection.

2- The authors suggested that the beneficial effect of this probiotic treatment might be related to enhanced function of alveolar macrophages. Although they gated for alveolar macrophages in the supplemental figure, they did not show any data on the numbers of alveolar macrophages in different treatment groups or assess their phagocytic function.

The response of the authors: We first would like to underline that the feeding with L. paracasei recruited higher number of alveolar macrophages (AM) at day 0 prior to flu infection (see our previous published work: Ref15). So the role of alveolar macrophage is mainly to better control the flu infection. The levels of AM is reduced by the depletion of the AM by the flu infection as has been also shown by the Reviewer (Ref 22). Later during the infection, our data presented in figure 4 (numbers of different cell types in the two groups) showed that in the L.paracasei-fed group, there were higher levels of other immune cells including neutrophils, inflammatory monocytes and DCs that may have contributed to better control the secondary meningococcal infection (See lines 270-273).

3- Previous studies have demonstrated that lung-resident alveolar macrophages are depleted during influenza infections breaking innate immune defense against bacterial superinfection. The authors need to discuss how probiotic treatment may modulate this defect in anti-bacterial immunity during influenza infection.

The response of the authors: The Reviewer raised an important and highly relevant point. Our aim in this work was to show that feeding with Lactobacillus paracasei CNCM I-1518 improves the control of secondary infection after a primary flu infection. Our data are in favor of a better control of the secondary infection that is due mainly to better control of flu infection by preventing/reducing the depletion of immune cells. We reported higher recruitment of AM after lactobacillus feeding at day 0 prior to flu infection (Ref15 Belkacem et al., 2018 PLOSONE). We added a paragraph 273-277). Our data are in agreement with the work of the Reviewer on the
association between the early depletion of alveolar macrophages in the lungs and lethal pneumococcal pneumonia in flu-infected mice (REF Ghoneim et al., 2013 J. Immunol.). We also added this reference.

4- Several typo errors need to be checked, such as lines 138-139, 176, 219, 282, and 299.

Response of the authors: The response of the authors: The typo were corrected

Reviewer 2

Dear authors,

The work you presented in this manuscript is very interesting and well done. I just signaled some grammatical errors and I recommend correcting them for perfection of your manuscript.

We thank the Reviewer, Dr. Farida Bendali, for her comments. We performed the changes she requested that are indicated in blue within the revised version.

Keywords (homogenate the style)

Mice instead of mice

Infection instead of infection

Response of the authors: The Keywords were homogenized

Background

Lines 54-56: Sentence too long, it should be reformulated

Response of the authors: The sentence was reformulated and shortened

Line 99: In the beginning of a paragraph, the name of the bacterial species should be fully written. To write Lactobacillus instead of L
Response of the authors: “L” was replaced by “Lactobacillus”

Line 99: …………………under aerobiosis instead of in aerobiase

Response of the authors: “in aerobiase” was replaced by “under aerobiase”

Line 100: Indicate the duration and temperature of the centrifugation Lines 100-108: throw all the manuscript, the material (centrifuge, spectrophotometer…) mark, city and country of the supplier should be indicated.

Response of the Authors: We added the duration, temperature, mark, city and country for the centrifugation. We also screened the manuscript to add the information for the materials used in this work.

Line 105: Significance of GCB agar medium?

Response of the authors: GCB is a medium is manufactured by Difco and is a specific medium for Neisseria: Gonococcal (GC) Medium Base Gonococcal. We added this information in the revised version of the manuscript lines 106-107.

Line 106: To use ([H3N2]),

Response of the authors: We performed the change as requested by the Reviewer.

Lines 113, 115,342 and 359: Pasteur Institute instead of Institut Pasteur

Response of the authors: The name Institut Pasteur is used with no changes in both English and French.

Line 113: ad libitum (latin) stands for “at liberty”

Response of the authors: ad libitum (Latin) stands for “at liberty”
Line 119: with (200 ml) with .. ???Not clear

Response of the authors: We changed the sentence to “200 µl containing 2x10^8 colony forming unit, CFU/mouse) of L. paracasei CNCM I-1518 or 200 µl PBS (control)”

Line 120: CFU/ml Line 122: CFU/ml? What is the volume used?

Response of the authors: it’s the number of CFU/mouse in a volume of 200 µl. As we changed the sentence in the precedent line, the is now clearly indicated.

Line 140: The sacrifice method used?

Response of the authors: The mice were were euthanatized at the end of experiment by injection of high dose of pentobarbital.

Line 140: After perfusion: with what ??

Response of the authors: we indicated that the perfusion was performed with 3 ml of PBS to wash out blood.

Line 158: 5000xg (duration and temperature ?)

Response of the authors: We added the duration and the temperature (for 10min at 4°C)

Line 159: for cytokine were assays. To delete were

Response of the authors: “were” was deleted
Lines 161 and 229: of total proteins instead of total protein….. The quantification of total proteins

Response of the authors: The changes were performed as requested by the Reviewer.

Line 168: ACK buffer (Gibco)???

Response of the authors: We clarified the name and the source of the buffer. ACK (Ammonium-Chloride-Potassium) Lysing Buffer (Fisher Scientific, Graffenstaden, France).

Line 169: the LSR Fortessa (BD Biosciences)???

Response of the authors: we added the name of the city and country for this equipment (Flow Cytometer LSRFortessa LSRFortessa, BD Biosciences, San Jose, CA, USA)

Results

Line 193: after H3N2 influenza virus infection : repetition

Response of the authors: we deleted the repetition.

Discussion

Line 268: feeding mice with

Response of the authors: we replaced “feeding mice of“ by “feeding mice with”

Line 289: was reported to decrease in the number of S. pneumonia
Response of the authors: we replace the sentence by “reported to decrease the number of S. pneumoniae”

Figure 1. Part A

107/mouse instead of 2.108 CFU/mouse

Response of the authors: The gavage with Lactobacillus paracasei was performed with 2*108CFU/mouse. It is the infection with Neisseria meningitidis that was performed with 107CFU/mouse

Bioluminescent Neisseria meningitidis instead of NM

Response of the authors: We replaced NM by Neisseria meningitidis.