Reviewer’s report

Title: The protective effect of recombinant lactococcus lactis to Clostridium difficile infected animal model

Version: 3 Date: 25 November 2012

Reviewer: Martijn P Bauer

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Major compulsory revisions
1. My most important comment on this manuscript is that it is insufficiently clear what the main hypothesis of this study is. If I interpret the text correctly, the authors use a Syrian hamster model of Clostridium difficile infection (CDI) to investigate whether Lactococcus lactis, used as a probiotic, can be used to prevent CDI, and whether two recombinant strains of Lactococcus lactis, expressing some Clostridium difficile protein (part of toxin A (TcdA)?), can be used to induce (humoral) immunity against TcdA and thus have a preventive effect against CDI in addition to the probiotic effect. Nowhere in the manuscript are these two parts of the main hypothesis distinguished explicitly and related to previous studies. Furthermore, the manuscript does not mention where the recombinant strains of L. lactis come from, how they were constructed, what protein they express and what the difference is between secreted L. lactis and membrane-anchored L. lactis. There even is no reference to previous literature. In spite of this lack of clarity, the experiments appear scientifically sound.

2. In addition to this lack of clarity concerning the main hypothesis, parts of the description of the methods are unclear. How does gastric perfusion in the animal experiment work? Was the ELISA to determine antibodies against the carboxyl terminus of TcdA standardized? What were the details of the ELISA? What were the details of the neutralization assay? What was the concentration of TcdA? How were serum and TcdA mixed and in what proportion? How long were they incubated together before being applied to the cells? Which cells were used? How many cells were used and to what volume of serum/ TcdA mix were they exposed? When was cytopathic effect evaluated and what criteria were used? What are dead cells in the flow cytometry experiment? Cells that could be stained by PI?

3. The abstract suffers from the same lack of clarity. For example, a serum IgA titer is mentioned, but it is not clear from the abstract whether this is total IgA or IgA directed at a specific antigen.

4. The discussion lacks focus and is unclear, too. A large part consists of references to historical studies on CDI in humans and hamsters, but these studies are not relevant to the main study hypothesis. Another part consists of theoretical considerations on findings from preliminary studies, of which no data are shown. This should neither be part of the discussion. What I would like to read about in the discussion are strengths and weaknesses of the study,
comparison with other studies on probiotics and vaccines for CDI, and the implications of this study, but that information is lacking from the study.

Minor essential revisions
1. In the introduction, it is stated that CDI leads to billions of economic losses (in China, globally? in dollars or renminbi?), that the incidence in China has increased and that L. lactis is safe and effective (when it is applied in what way?). These statements should have references.
2. PLA = People’s Liberation Army?
3. The numbers of the days of the animal experiment are not used in a consistent way. Please use one reference point, for instance the start of the administration of L. lactis of the challenge with C. difficile, and refer to the figure with the timeline early on. It is difficult to distill the course of events during the animal experiment from the text.
4. Page 10, line 6: ‘The incidence of diarrhea was statistically significant among all groups.’ What does this mean? What is the null hypothesis?
5. The figures should indicate where the statistically significant differences are. That would be much clearer for the reader than from the text.
6. Page 12, line 3: ‘but fungi a found’. I suppose something is missing from this sentence. Why is it relevant that fungi are found from the stool?
7. What is degree III dysbacteriosis? Please give a reference.
8. Page 12, second paragraph: It should once more be mentioned against what epitope of TcdA the antibodies are directed.
10. Table 4: Tables should be self-explanatory, so it should be stated again that each group consisted of 8 animals. ‘Most at day 2’ How many is most?
11. Figures B and C do not add much to figure A and could be deleted.
12. Figure E is not self-explanatory. How long after the challenge?

In conclusion, the results of this study are probably interesting, but, written with such lack of clarity, this cannot be judged properly.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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.