Reviewer's report

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

Version: 3 Date: 29 October 2012

Reviewer: Elizabeth Mann

Reviewer's report:

Major compulsory revisions:

-The novel findings of this study are that the membrane anchored L.lactis group was more effective at preventing C.diff infection compared to secreted L. lactis group - this is actually very interesting and clinically relevant but has not been mentioned in the abstract or until the very end of the paper. In my opinion this should be the main highlight of the study. The LL-pNBCL2003 (membrane anchored) group prevented symptoms, however authors have stated in discussion this is due to L. lactis being taken in by M cells of the intestinal wall and presentation to the immune system but there is no evidence in the results to confirm this so this is based on speculation.

-Question posed by authors is not well defined. It is stated in the discussion that Lactobacillus lactis can prevent and treat C.diff associated diarrhea from other studies, though unclear from discussion whether this refers to humans or animals (not sure if reference 13 is correct either). In which case, if L.lactis has already been shown to be effective for C.diff treatment and prevention, what is the novel impact of this research and the relevance of using animal models? what is the clinical relevance - to vaccinate patients at high risk of C diff infection?

-Abstract does not get main message across about what is important and novel about this research to the field of gastroenterology. The groups needs to be explained better and there is no mention of the Clostridium A toxin treatment group in methods of abstract but then it is referred to in the results in abstract. What is this group? Are the "cells" referred to intestinal epithelial cells? Determine whether focus is on treatment or vaccination as they are two different approaches.

-Methods confusing for a non-specialist in animal models of infection and vaccine/vectors. This is a general scientific gastro journal but there is no description of how the groups were specifically defined e.g. how were the empty L.lactis plasmid group and secreted L.lactis etc groups generated and what does this mean?

- Layout of results confusing, include statistics and pathology into well-defined, headed sections stating what findings are.

-In general confusing referring to hamster groups by their codes, much clearer to
call them "L.lactis secreted" or "membrane bound" etc.

Discussion included very little discussion of the actual results and implications of results of this study but went into a lot of detail about the implications of research from literature that was not entirely relevant. There is no mention of the epithelial cell apoptosis or the mRNA expression of adhesion markers and cytokines, even though it would appear there is less IL-6 and ICAM-1 with the membrane anchored group. Is this significant? Unclear. What are the implications of this? Microbial translocation? There is no mention of why this study is novel and relevant to the field of research; nor much discussion on the use of probiotics on GI infections, particularly C.diff. One of the most striking and relevant results from literature is surely is that faecal transplants (replacement of gut microbiota with "healthy microbiota") can be effective for C.diff treatment in humans. As mentioned above, the novel findings of this study are that membrane anchored L. lactis is more effective at preventing C.diff infection than secreted - the discussion should revolve around this as a vaccine approach and speculate why this might be, and find evidence from the literature to support this. E.g. M-cell section is logical speculation but there is no evidence from this study supporting this. What about the literature? Could the mechanisms involve action of L.lactis on antigen presenting cells of the immune system, making them more able to sample C.diff antigen?

In general, there are a few sentences throughout that are not finished or do not make sense.

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

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

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests