Reviewer's report

Title: The protective effect of recombinant Lactococcus lactis oral vaccine on a Clostridium difficile-infected animal model

Version: 5 Date: 6 March 2013

Reviewer: Martijn P Bauer

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The manuscript has become much more readable after the modifications. However, I have some more comments.

Minor revisions

In general

The terms ‘secreted plasmid’ and ‘membrane-anchored plasmid’ suggest that the plasmid is secreted and membrane-anchored, whereas this happens to the protein for which the plasmid codes. It would be clearer if the name were changed to ‘secreted protein plasmid’ or the groups were numbered or something like that.

Why was cwaM6 chosen as one of the genes in the vaccine with cell wall-anchored proteins?

What could be the advantage of membrane-anchored proteins as vaccine over secreted proteins? Have the authors tried to demonstrate secreted proteins in stool?

In the Discussion, the authors only refer to one article on humoral immunity in CDI in animals, whereas there is a lot of literature on the role of the antibody response in humans. The manuscript would improve if this literature would also be taken into account.

Abstract

‘Is an important pathway’ or, rather, ‘may be an effective strategy’.

Introduction

Can a reference be given for the statement that CDI incidence is increasing in China?

Please spell out CLDM the first time it is used.

2.2

‘Modification’ instead of ‘medication’.

2.4

‘Manufacturer’ instead of ‘manufacture’.
How was cytopathic effect scored in the neutralisation assay?

3.1; last line
One ‘no’ should be removed.

3.2
I do not understand the scores for the membrane-anchored group. Neither do I understand why only the scores for this group are given. The figures are clear enough by themselves. Were any of these differences statistically significant?

4.2 & 4.3
The authors state in paragraph 4.2 that because their animal model had been treated with clindamycin after vaccination, recombinant L. lactis could not have acted as a probiotic. My interpretation is that they assume all L. lactis are killed by the clindamycin treatment. Is the MIC for clindamycin of this strain of L. lactis known? Furthermore, this assumption could be stated more explicitly. On the other hand, in the next paragraph, the authors explain the milder clinical course of the animals that were given L. lactis with an empty plasmid by its acting as a probiotic. They seem to assume that some L. lactis survive clindamycin treatment.

The figures still have the names of the hamster groups used in the previous version. Please change.

Could statistically significant differences be indicated in all figures?

It is difficult to read fig. 9.

**Level of interest:** An article of importance in its field

**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.