Reviewer’s report

Title: Nasal double DNA adjuvant induces salivary FimA-specific secretory IgA antibodies in young and aging mice and blocks Porphyromonas gingivalis binding to a salivary protein

Version: 0 Date: 10 Apr 2019

Reviewer: Lesley Bergmeier

Reviewer's report:

General comments.

This is an interesting paper suggesting a novel double adjuvant might be useful in inducing Fimbriae specific antibodies in the oral cavity in a mouse model.

There are some areas where the authors are not clear on the methods used and some of the text is confusing. The paper could be greatly improved by making the materials and methods section more detailed and thus causing less confusion to the reader.

For example: The authors are using a gst-fusion protein of rFimA produced in E. coli, but it was not clear if this is the full-length protein or a fragment. Fig 1 suggests it is 41kDa but there is no indication if this is the fusion protein. Gst has a molecular weight of about 26kDa so that would mean a fragment size of about 15kDa. I was confused as the papers to which they refer (refs 25,26) suggest that protein has a molecular weight of between 37-38 with variants at 43-48kDa. While Cai et al 2019 (ref 12) show a band at about 60kDa. This would mean that the rFimA-gst fusion protein should have a mol wt of at least 60kDa. Why did the authors not use anti gst antibodies to determine the mol wt of the fusion protein and to look for any breakdown products?

It is not until the results section that it becomes clear that the gst has been cleaved from the fusion protein.

The overall M&M is rather sloppy.

The Authors use ELISA to detect IgG and IgA antibodies and seem to assume that the latter are SIgA but do not give evidence of secretory component.

As far as the ELISPOT is concerned direct secretion from the lymph node derived B cells might be expected to show dimeric IgA but since the antibodies acquire the secretory component prior to passage though the epithelium, the authors might be more cautious in the assumption of SIgA in saliva- without a demonstration of SC.
There is evidence in the literature that antibodies to a variety of P.ging proteins can be induced in both IgG and IgA compartments and their function can be contradictory in terms of protection or enhancement of progressive periodontal disease. Some of the data presented suggest that that IgG antibodies might interfere with the ability of IgA to inhibit adhesion to Statherin- but this is not very well explored, and the conclusions are confusing.

In the present state the paper needs to be revised with attention to detail of the materials and methods and a better description of the fusion protein.

Specific comments:

Abstract:

Page 2: Line 16: This line does not make sense. Should perhaps read "which were preincubated with saliva from which IgG antibodies had been removed using protein G column"

Background: Good description of the literature but the paper would benefit from comments on what is known about IgG/IgA antibodies in perio.

Materials and methods:

Page 6 line 9. The authors mention that the fusion protein is purified on a Glutathione Sepharose 4B gel- implying that it is a full-length fusion protein. Gst+ rFimA should be>60kDa but Fig 1b and 1c has the fusion protein at 41kDa- this needs to be explained/clarified.

Page 7, line 7: Do the authors have data on the induction of DCs with DA alone in this series of experiments. This would appear to be a control that would be important for the cellular aspects of the study.

Page 7 line 20: The color reaction--- What substrate was used? OD415 - this seems an odd OD for an HRP substrate. More detail is required here.

ELISPOT analysis: Have the authors looked at Total AFCs in the lymph nodes? Why was anti-gst not used to look at the immunogenicity of the fusion protein. Not enough detail.

FACs analysis: I would like to see an example FACs plot. What gating strategy was used? It could be given as a supplementary Fig.

Blocking assay:
This is a crucial functional assay for this study but is very poorly described. It is not at all clear what the readout for the assay is. ATP in live bacteria? It would be impossible for anyone to use this assay as there is not enough detail of how it was done.

Page 9 line 6: What is the read out for the assay.

Results:

Page 9 line 20- the use of "Prescision (? Precision) Protease to purify the rFimA is not mentioned in the M&M. This part of the methodology should be in M&M. It is crucial to the clarity of the paper in terms of the purity of the rFimA.

Page 11 line 7: What tissues are included in this - Waldeyers ring? Tonsils?

Page 11 line 23. This is difficult o to follow as the assay and the readout are not clear.

Discussion:

Page 12 line 24- SLGs have higher IgA AFCs than SMGs.

Page 12 line 26- contradiction? SMGs are the major mucosal effector tissues in the oral cavity.

This is a very good point that this study indicates that SMG and SLG are both part of the effector sites- this would be supported by SLIT therapy studies where IgA is induced in SLGs.

Conclusion: Brief and to the point.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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