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SIGNR3-dependent immune regulation by Lactobacillus acidophilus-Surface layer protein A in colitis

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 10 November 2014

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see, the referees find your analysis interesting and insightful. Referees #1 and 2 raise only very minor concerns, while referee #3 has more significant ones. I anticipate that you should be able to address the specific points well enough. I do see that the general comments raised by referee #3 might involve more work to resolve, but I also do think that they are valid and that they would nicely extend the work. I am happy to discuss them further if needed.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the concerns raised. I should add that it is EMBO Journal policy to allow only a single round of revision, and that it is therefore important to resolve the issues raised at this stage.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

REFEREE REPORTS

Referee #1:

In this study, Lightfoot and colleagues report about the ability of the S-layer glycoprotein SlpA of the GI/probiotic species Lactobacillus acidophilus (La) to protect against experimental colitis in mice through interaction with the C-type lectin receptor SignR3 (homolog to human DC-SIGN). Specifically, using a recombinant strain of La that expresses SlpA only (but not SlpB, SlpX and lipotechoic acid) or purified SlpA, the authors show in two models of T cell- and chemical-induced colitis that:
- SlpA induces the recruitment of regulatory T cells (IL-10- and TGF-b-expressing FoxP3+CD4+ Tregs) and impairs the recruitment of pathogenic Th1 and Th17 cells in the colon;
- SlpA ameliorates disease in term of tissue pathology, body weight, colitis-associated diarrhea, and impairs local production of inflammatory mediators, such as IL-1b and TNF;
- The GI microbiota is shifted towards more Bacteroidetes and less Verrucomicrobia in treated mice, which translates into healthier epithelia (as measured by tight junction proteins for example);
- Among the 8 DC-SIGN homologs, only SignR1 and SignR3 are induced in the colon of La-SlpA treated mice;
- SlpA binds SignR3, but not SignR1;
- Production of inflammatory mediators (e.g. IL-1b) and recruitment of Tregs is dysregulated in La/SlpA-treated SignR3-null mice;
- Accordingly, the ability of La/SlpA to protect against experimental colitis is lost in SignR3-null mice, which translates into increased pathology and increased recruitment of neutrophils, IL-1b-producing DCs and macrophages and pathogenic RORgt+FoxP3+CD4+ Tregs in the colon.

Comments:
The paper is excellently written and easy to follow; the experiments have been designed appropriately together with appropriate controls and the overall dataset is most convincing. The overall message will be of high interest for a large audience, ranging from immunologists interested in innate immunity and inflammation to those interested in probiotics-mediated treatment of IBD and other inflammatory disorders.

I have no further (even minor) comment that could help further improving the manuscript.

Referee #2:

This is an interesting study in which it is a profound tolerizing effect of the interaction between SIGNR3 with SlpA in inflammatory bowel disease. The interaction with SlpA has strong influence on the induction of Treg, antiinflammatory phenotype of T cells, also alters composition of microbiota, and intestinal barrier function. The SlpA has shown to have this strong potential. The experimental setups are very solid for the proof if this molecular interaction with it functional consequences.
The only thing I miss is in the discussion to link to the human setting, what molecules systems may give similar effects. Do they think that DC-SIGN has similar tolerogenic potential in the gut? Moreover the authors should explain a little bit more on the different SIGNR molecules and why they think that only SIGNR3 is involved in the interaction with SlpA. Is this due to same glycan specificity as DC-SIGN. Might also other Signs be involved, how is it with the expression of others Signs in the gut.

Referee #3:

The manuscript submitted by Lightfoot and colleagues describes experiments identifying bacterial strains that modulate host immunoregulatory signals to maintain gut homeostasis. An engineered Lactobacillus acidophilus, strain NCK2187 expressing only SlpA protects against gut inflammation in a murine model of induced colitis through its interaction with the C-type lectin, SIGNR3. The study demonstrates that SlpA binding to SIGNR3 dampens mucosal inflammation and reduces dysbiosis in the setting of a T cell transfer model of colitis in mice.

General Comments:
1. An isogenic mutant strain deficient only in SlpA should be employed as a negative control in all of the experiments.
2. To support the generalizability of the observations, other widely employed and reproducible animal model of gut inflammation should be tested. The effects of SlpA delivery by NCK2187 in an infectious murine model of colitis would be relevant.
3. The potential significance of the findings relates to using such a genetically engineered L. acidophilus strain to regulate host immune responses in human inflammatory diseases, such as Crohn's disease and ulcerative colitis. To demonstrate the relevance of SlpA binding to a C-lectin receptor in human disease, the human counterpart should be expressed in mammalian cells and in the murine gut to show whether SlpA also binds to DC-SIGN (Int Immunol 20001;13:1293-90).

Specific Comments:
1. Figure 1C: variance around the mean (eg. SEM) should be included. The legend for Figure panel D should indicate the N value. Not all of the CFU/ml values need to be listed on the y-axis.
2. Figure 2: A-C: Statistical differences and N values should be provided. The word "colon" can be removed from the top two graphs as this is indicated in the legend.
3. Figure 3: the time line of oral gavages can be removed, because it is described in the legend. In the first section of panel C showing gross morphology, an indication of what each colon represents and a scale bar should be included.
4. Figure 4A: for clarity, it should be indicated that gene expression is relative to the untreated, control group.
5. Figures 5C and D: purified SlpA should be tested and the data included here.
6. Figure 5: expression of SIGNR1 and SIGNR3 in CHO-S cells should be included. Alternatively, it could be added as a Supplementary Figure.
7. Figure 5: additional complementary experimental approaches, such as surface plasmon resonance, atomic force microscopy and fluorescence spectrometry, would provide additional information about the interactions of SlpA with SIGNR3. Similar experiments should also test the binding of SlpA to DC-SIGN.
8. Figure 5: For clarity, the panel which is currently referred to as "Fig. 5C, right" in the Results (page 8) and in the figure legend should be referred to as Fig. 5D (with Fig. 5D then relabeled as Fig. 5E).
9. Figure 6A: for consistency, the results of the untreated and PBS groups should be included. Statistical differences and P values should be indicated on the graph presenting changes in body weight and panel F. The day on which colonoscopies were performed should be indicated in the legend for panel D.
10. Figure 7A: statistical differences and P values should be provided for the various study groups presented.
11. Page 8, line 11: the word "cannot" should be replaced with "does not".
12. Expanded view Figure 1: The primer sequences for Ltb4r1 should be included in expanded view Table 6.
13. Expanded view Figure 1B: A legend should be included, with an indication that gene expression is relative to the untreated, control group.

1st Revision - authors' response 23 December 2014

Referee #1:

In this study, Lightfoot and colleagues report about the ability of the S-layer glycoprotein SlpA of the GI/probiotic species Lactobacillus acidophilus (La) to protect against experimental colitis in mice through interaction with the C-type lectin receptor SignR3 (homolog to human DC-SIGN). Specifically, using a recombinant strain of La that expresses SlpA only (but not SlpB, SlpX and lipotechoic acid) or purified SlpA, the authors show in two models of T cell- and chemical-induced colitis that:
- SlpA induces the recruitment of regulatory T cells (IL-10- and TGF-b-expressing FoxP3+CD4+ Tregs) and impairs the recruitment of pathogenic Th1 and Th17 cells in the colon;
- SlpA ameliorates disease in term of tissue pathology, body weight, colitis-associated diarrhea, and impairs local production of inflammatory mediators, such as IL-1b and TNF;

- The GI microbiota is shifted towards more Bacteroidetes and less Verrucomicrobia in treated mice, which translates into healthier epithelia (as measured by tight junction proteins for example);
- Among the 8 DC-SIGN homologs, only SignR1 and SignR3 are induced in the colon of La-SlpA treated mice;
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Comments:
The paper is excellently written and easy to follow; the experiments have been designed appropriately together with appropriate controls and the overall dataset is most convincing. The overall message will be of high interest for a large audience, ranging from immunologists interested in innate immunity and inflammation to those interested in probiotics-mediated treatment of IBD and other inflammatory disorders.

I have no further (even minor) comment that could help further improving the manuscript.

We are deeply grateful for the comments and complements regarding our manuscript. Such encouraging comments sustain our efforts to continue our mechanistic work, which may result in a potential therapeutic platform. Humbly, thank you!

Referee #2:

This is an interesting study in which it is a profound tolerizing effect of the interaction between SIGNR3 with SlpA in inflammatory bowel disease. The interaction with SlpA has strong influence on the induction of Treg, antiinflammatory phenotype of T cells, also alters composition of microbiota, and intestinal barrier function. The SlpA has shown to have this strong potential. The experimental setups are very solid for the proof if this molecular interaction with it functional consequences.
The only thing I miss is in the discussion to link to the human setting, what molecules systems may give similar effects. Do they think that DC-SIGN has similar tolerogenic potential in the gut?
Moreover the authors should explain a little bit more on the different SIGNR molecules and why they think that only SIGNR3 is involved in the interaction with SlpA. Is this due to same glycan specificity as DC-SIGN. Might also other Signs be involved, how is it with the expression of others Signs in the gut.

We greatly appreciate all of the comments we have received from this highly respected reviewer. Accordingly, we have followed his advice and added more information regarding the aforementioned subject. In our revised manuscript, we demonstrate that CHO cells expressing human DC-SIGN bind to SlpA-coated beads, which is a human analogue of SIGNR3 (Fig. 5), as requested by the reviewer. This interaction suggests that our responses induced via SIGNR3 binding might promote similar regulatory responses in humans through DC-SIGN, likely due to the same glycan specificity.

Referee #3:

The manuscript submitted by Lightfoot and colleagues describes experiments identifying bacterial strains that modulate host immunoregulatory signals to maintain gut homeostasis. An engineered Lactobacillus acidophilus, strain NCK2187 expressing only SlpA protects against gut inflammation in a murine model of induced colitis through its interaction with the C-type lectin, SIGNR3. The study demonstrates that SlpA binding to SIGNR3 dampens mucosal inflammation and reduces dysbiosis in the setting of a T cell transfer model of colitis in mice.

General Comments:

1. An isogenic mutant strain deficient only in SlpA should be employed as a negative control

in all of the experiments.
Response: For years, it has been attempted to create a strain that does not express SlpA. Due to plasmid pressure however, the applied molecular approach failed to generate any positive outcome. To overcome this issue, we successfully demonstrate that the purified SlpA induces immune regulation, while LTA alone elicits pathogenic inflammation that exacerbates disease progression. Additionally, the same trend was observed with the wild type parent expressing SlpA and LTA as demonstrated in this and other manuscripts.

2. To support the generalizability of the observations, other widely employed and reproducible animal model of gut inflammation should be tested. The effects of SlpA delivery by NCK2187 in an infectious murine model of colitis would be relevant.
Response: We believe we have provided sufficient evidence using the two animal models previously presented to highlight the ability of NCK2187 and its purified SlpA to induce immune regulation, resulting in the amelioration of colonic disorders (e.g., colitis). Nonetheless, we now also provide new data dealing with the Citrobacter rodentium infection model that generally demonstrates the same trend of the regulatory role of NCK2187-SlpA. We hope that this will be sufficient to satisfy the high expectations of this highly respected reviewer.

3. The potential significance of the findings relates to using such a genetically engineered L. acidophilus strain to regulate host immune responses in human inflammatory diseases, such as Crohn's disease and ulcerative colitis. To demonstrate the relevance of SlpA binding to a C-lectin receptor in human disease, the human counterpart should be expressed in mammalian cells and in the murine gut to show whether SlpA also binds to DC-SIGN (Int Immunol 20001;13:1293-90).

Response: Again, we clearly show that only SIGNR3 binds to SlpA. To show the same trend, we now also demonstrate the binding of SlpA to its human counterpart DC-SIGN. Please see Figure 5.

Specific Comments:

1. Figure 1C: variance around the mean (eg. SEM) should be included. The legend for Figure panel D should indicate the N value. Not all of the CFU/ml values need to be listed on the y-axis.
Response: Done! Appreciated!
The graph shown in C does include the mean +/- SEM; however, due to the scale of the y-axis and the reproducibility of the results, the variance around the mean cannot be appreciated in the graph. The y-axis values have been changed to not include all of the CFU/mL, as suggested by the reviewer. The N value for the in vitro study shown in D has been included in the legend as requested.

2. Figure 2: A-C: Statistical differences and N values should be provided. The word "colon" can be removed from the top two graphs as this is indicated in the legend.
Response: Sure! Appreciated!
The statistical differences and N values described applied to the entire figure. This has now been specified in the legend. The word "colon" has been removed from the figure as suggested.

3. Figure 3: the time line of oral gavages can be removed, because it is described in the legend. In the first section of panel C showing gross morphology, an indication of what each colon represents and a scale bar should be included.
Response: Sure, and appreciated!
The time line has been removed as suggested. Panel C, showing the gross morphology of the colon of the mice relative to each other, follows the same color key described in the first section of C, which is above each colon accordingly.

4. Figure 4A: for clarity, it should be indicated that gene expression is relative to the untreated, control group.
Response: Sure!

This information has been included in the figure legend.

5. Figures 5C and D: purified SlpA should be tested and the data included here.
   Response: As in other steady-state studies (see figure 2), purified SlpA was not included, instead, this additional control group was reserved for the disease models employed herein, as they were deemed more significant to demonstrate its immunomodulatory role.

6. Figure 5: expression of SIGNR1 and SIGNR3 in CHO-S cells should be included. Alternatively, it could be added as a Supplementary Figure.
   Response: Done!
   Please see amended figure 5.

7. Figure 5: additional complementary experimental approaches, such as surface plasmon resonance, atomic force microscopy and fluorescence spectrometry, would provide additional information about the interactions of SlpA with SIGNR3. Similar experiments should also test the binding of SlpA to DC-SIGN.
   Response: Thank you for this comment, however we don't currently possess the capacity to perform this experiment. Please accept our apologies!

8. Figure 5: For clarity, the panel which is currently referred to as "Fig. 5C, right" in the Results (page 8) and in the figure legend should be referred to as Fig. 5D (with Fig. 5D then relabeled as Fig. 5E).
   Response: Sure! Thanks!
   The suggested relabeling of the figure was completed.

9. Figure 6A: for consistency, the results of the untreated and PBS groups should be included. Statistical differences and P values should be indicated on the graph presenting changes in body weight and panel F. The day on which colonoscopies were performed should be indicated in the legend for panel D.
   Response: Of course! Thanks
   The suggested changes to the Figure were made. The day when the colonoscopies were performed was included in the legend.

10. Figure 7A: statistical differences and P values should be provided for the various study groups presented.
    Response: See expanded view table 6 for statistical differences among the treatment groups in WT mice. No differences were observed in KO mice as can be noted in the figure.

11. Page 8, line 11: the word "cannot" should be replaced with "does not".
    Response: Done!

12. Expanded view Figure 1: The primer sequences for Ltb4r1 should be included in expanded view Table 6.
    Response: We apologize about that. The sequence is now included. Thanks!

13. Expanded view Figure 1B: A legend should be included, with an indication that gene expression is relative to the untreated, control group.
    Response: Sure!
    This information has been included in the figure legend.

Accepted 16 January 2015

Thank you for submitting your revised manuscript to The EMBO Journal. Your study as now been re-reviewed by referee #3. As you can see below, this referee appreciates the added data provided and supports publication in The EMBO Journal. I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a very nice paper

REFEREE REPORT

Referee #3

The authors have responded positively to most of the comments and suggestions provided previously by two of the reviews, including new data that provides relevance to humans. As a result, the revised manuscript is improved compared with the previous submission.

