European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201646297

Jakob Zimmermann, Thomas Hübschmann, Florian Schattenberg, Joachim Schumann, Pawel Durek, René Riedel, Marie Friedrich, Rainer Glauben, Britta Siegmund, Andreas Radbruch, Susann Müller and Hyun-Dong Chang

High-resolution microbiota flow cytometry reveals dynamic colitis-associated changes in fecal bacterial composition

European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201646297

Jakob Zimmermann, Thomas Hübschmann, Florian Schattenberg, Joachim Schumann, Pawel Durek, René Riedel, Marie Friedrich, Rainer Glauben, Britta Siegmund, Andreas Radbruch, Susann Müller and Hyun-Dong Chang

High-resolution microbiota flow cytometry reveals dynamic colitis-associated changes in fecal bacterial composition

European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201646297

Jakob Zimmermann, Thomas Hübschmann, Florian Schattenberg, Joachim Schumann, Pawel Durek, René Riedel, Marie Friedrich, Rainer Glauben, Britta Siegmund, Andreas Radbruch, Susann Müller and Hyun-Dong Chang

High-resolution microbiota flow cytometry reveals dynamic colitis-associated changes in fecal bacterial composition

European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201646297

Jakob Zimmermann, Thomas Hübschmann, Florian Schattenberg, Joachim Schumann, Pawel Durek, René Riedel, Marie Friedrich, Rainer Glauben, Britta Siegmund, Andreas Radbruch, Susann Müller and Hyun-Dong Chang

High-resolution microbiota flow cytometry reveals dynamic colitis-associated changes in fecal bacterial composition

European Journal of Immunology

High-resolution microbiota flow cytometry reveals dynamic colitis-associated changes in fecal bacterial composition

Jakob Zimmermann, Thomas Hübschmann, Florian Schattenberg, Joachim Schumann, Pawel Durek, Marie Friedrich, Rainer Glauben, Britta Siegmund, Andreas Radbruch, Susann Müller & Hyun-Dong Chang

Correspondence: Dr. Hyun-Dong Chang, German Rheumatism Research Center Berlin (DRFZ), an institute of the Leibniz Association, Berlin, Germany

---

Review Timeline:	Submission date:	11-Jan-2016
	First Editorial decision:	04-Feb-2016
	First Revision received:	10-Feb-2016
	Accepted:	19-Feb-2016

Handling Executive Committee member: Dr. Steffen Jung

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision - 04-Feb-2016

Dear Mr. Zimmermann,

Manuscript ID eji.201646297 entitled "High-resolution flow cytometry to determine dynamic changes in the fecal microbiota", which you submitted to the European Journal of Immunology, has been reviewed.

The comments of the referees are included at the bottom of this letter. Although the referees have recommended publication, some revisions to your manuscript have been requested. Therefore, I invite you to respond to the comments of the referees and revise your manuscript accordingly.

You should also pay close attention to the editorial comments included below. **In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.**

If the revision of the paper is expected to take more than three months, please inform the editorial office.

European Journal of Immunology

Revisions taking longer than six months may be assessed by new referee(s) to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology. We look forward to receiving your revision.

Yours sincerely,
Laura Soto Vazquez

on behalf of Dr. Steffen Jung

Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu

****

Reviewer: 1

Comments to the Author

The manuscript by Zimmermann et al describes a new flow based methodology to assess changes in fecal microbiota composition over time. Current state of the art technology for assessing microbiota composition in the intestine involves 16s rDNA or metagenome sequencing of the microbiota, which is both time consuming and, at least currently, not readily available to many laboratories. The authors use flow cytometry, based on light scattering and DNA content, to assess intestinal microbial diversity in the steady state and in the T cell transfer colitis model. The experiments appear well performed and the conclusions well founded. Given the observation that some gates may contain different dominating genus in the steady state and inflammatory setting it seems unlikely that this technology will replace the need for sequencing for those interested in more detailed analysis. Unfortunately the manuscript does not utilize this technology to answer any novel questions, and the microbial analysis is only performed in a single model, limiting its implications. This said the technology as described appears novel, relatively easy to set up and should be of broad interest to researchers wishing, in a quick way, to assay overall alterations in microbiome composition.

European Journal of Immunology

Reviewer: 2

Comments to the Author

This very nice and interesting work describes a rather neat flow cytometry-based method for microbiota profiling (developed by the same group) that many in the field are not yet aware of and may be highly interested to establish. The method appears robust and fairly easy to establish. Major advantages are high speed, cost-efficiency and high throughput. The possibility of dense sampling and rapid analysis of the resulting (potentially big) data sets is highly advantageous for the monitoring of highly dynamic changes and allows for responding quickly to the results in result-driven experimental designs and studies, for which the established gold-standard metagenomics sequencing methods tend to be too slow, too labour intensive and too costly. This method can serve as a an alternative where applicable as well as a complementary approach in combination to established sequencing methods. It is highly likely that others in the field will soon start evaluating the method, which will reveal its full potential and limitations.

The data and their interpretation are scientifically sound and clear. I have only rather minor points that should be addressed:

1. Although the authors adequately refer to their nature protocols paper for further technical details, it may help many readers to briefly state the minimal required flow cytometer specifications (for the benefit of those who do not have access to the same model that the authors used for this study) in the Methods section.

2. Materials and Methods, section “Mice”• . For the scope of this paper, it may add valuable information if additional information on the “health/hygiene status”• of the mice used and analyzed in this study would be given. “Pathogen-free conditions”• is not very specific. For instance, where were the mice originally sourced from (supplier, and of which SPF quality)? Were they maintained in individually ventilated cages?

3. The title could be more specific. Suggestion: "High-resolution flow cytometry to determine dynamic colitis-associated changes in the fecal microbiota"

European Journal of Immunology

First Revision – authors' response 10-Feb-2016

Reviewer: 2

This very nice and interesting work describes a rather neat flow cytometry-based method for microbiota profiling (developed by the same group) that many in the field are not yet aware of and may be highly interested to establish. The method appears robust and fairly easy to establish. Major advantages are high speed, cost-efficiency and high throughput. The possibility of dense sampling and rapid analysis of the resulting (potentially big) data sets is highly advantageous for the monitoring of highly dynamic changes and allows for responding quickly to the results in result-driven experimental designs and studies, for which the established gold-standard metagenomics sequencing methods tend to be too slow, too labour intensive and too costly. This method can serve as an alternative where applicable as well as a complementary approach in combination to established sequencing methods. It is highly likely that others in the field will soon start evaluating the method, which will reveal its full potential and limitations.

The data and their interpretation are scientifically sound and clear. I have only rather minor points that should be addressed:

1. Although the authors adequately refer to their nature protocols paper for further technical details, it may help many readers to briefly state the minimal required flow cytometer specifications (for the benefit of those who do not have access to the same model that the authors used for this study) in the Methods section.

Response: As we did not systematically test the minimal technical requirements for our study, we cannot give any information on the minimally required flow cytometer specifications. We did, however, test the method on two other cytometers from other suppliers with similar results. We have added the information in the materials and methods section (page 11 of supporting information) for our recommendations for the minimal requirements.

2. Materials and Methods, section "Mice". For the scope of this paper, it may add valuable information if additional information on the "health/hygiene status" of the mice used and analyzed in this study would be given. "Pathogen-free conditions" is not very specific. For instance, where were the mice originally sourced from (supplier, and of which SPF quality)? Were they maintained in individually ventilated cages?

Response: We have now added the information in the materials and methods section. The mice were purchased from Charles River and housed in individually ventilated cages. The SPF status was according to FELASA standards with positive testing for Helicobacter bilis (H. bilis), H. hepaticus, H. typhlonicus, murine norovirus, and Pneumocystis murina but negative for any obligate mouse pathogens.

3. The title could be more specific. Suggestion: "High-resolution flow cytometry to determine dynamic

colitis-associated changes in the fecal microbiota"

Response: We thank the reviewer for his suggestion. We have changed the title of our manuscript as suggested by reviewer 2.

Second Editorial Decision - 15-Feb-2016

Dear Mr. Zimmermann,

It is a pleasure to provisionally accept your manuscript entitled "High-resolution flow cytometry to determine dynamic colitis-associated changes in the fecal microbiota" for publication in the European Journal of Immunology.

For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Laura Soto Vazquez

on behalf of
Dr. Steffen Jung

Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu