Reviewer’s report

Title: Oral treatment with Lactobacillus Rhamnosus attenuates behavioral deficits and immune changes in chronic social stress

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Reviewer: Antonio Teixeira

Reviewer's report:

General comments:

This is an interesting paper on the role played by microbiota in behavioral changes. More specifically, the authors evaluated whether the treatment with a Lactobacillus could influence stress-induced behavioral changes. They found that besides influencing a specific set of behavior treatment changed the investigated immune parameters. The results are original, but a mechanistic insight for the observed effect is missing. A more comprehensive approach on the possible mechanisms involved in the effect of Lactobacillus treatment would add significantly to the study. This is particularly important due to the fact that the treatment did not alter significantly the diversity of gut microbiota or fecal metabolites. Stress was the most important factor underlying gut microbiota change.

Specific comments:

Introduction

"The critical role of this community [gut microbial community] in the regulation of diverse physiological functions, including immunity, is well established, as is its bidirectional influence on the central nervous system." Consider rephrasing this statement as there are controversial issues regarding the cross-talk between gut microbiota and CNS/physiological systems.

"Given microbial regulation of host signaling [correct to 'signaling'] at the mucosal interface between microbiota and host, disruptions in this community may lead to systemic changes in peripheral signals [15,16]. Such systemic immune dysregulation has also been implicated in psychological stressors and psychiatric disorders [12,17]." Consider rephrasing this statement as peripheral signals are not limited to immune ones.

Methods:

Why did the authors wait 2 days after behavioral assessment end to euthanize the animals?
Results:

"Neither stress nor treatment altered the expression of corticotropin-releasing factor receptor type 1 or type 2, or the glucocorticoid receptor in the frontal cortex." The lack of effect of stress on CRH is somehow unexpected. Do the authors have any explanation for this? Why did the authors choose the frontal cortex instead of other brain areas like hippocampus? Besides CRH and GABA, other neural pathways should have been investigated.

Regarding the influence of stress and treatment on immune cells phenotype, why did the authors evaluate only two cell lineages?

Clarify what fecal metabolites and related pathways were altered by Lactobacillus treatment in mice subjected to chronic stress.

Discussion

Some parts of the Discussion are highly speculative and not supported by the provided data. For instance, when discussing the findings on T regs, the authors mention "a counteractive response to such pro-inflammatory shifts". Empirical data on enhanced inflammation in the current protocol (even simple measures of inflammatory mediators) could give more support to authors' hypothesis.

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

Yes

**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

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