Intracellular sensing of beneficial bacteria: an alternative probiotic therapy approach?

Elaina M. Maldonado, Carlos Maluquer de Motes, and Jorge Gutierrez-Merino

School of Biological Sciences, University of Surrey, Surrey GU2 7XH, UK

Background

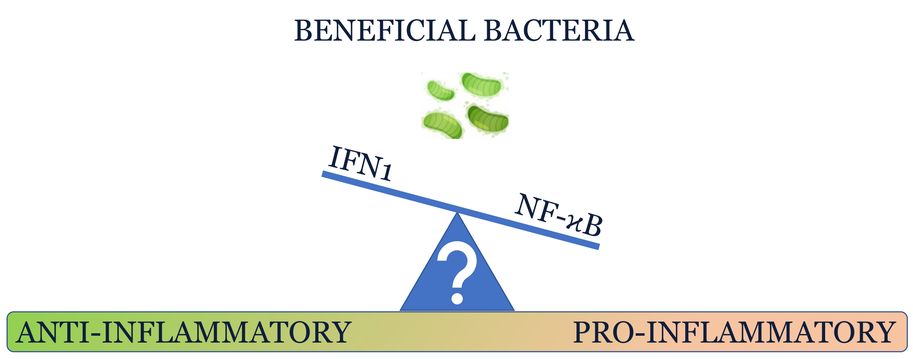
Probiotics have been explored as an alternative therapy for inflammatory gut disorders to help restore the microbiota and appropriate immune responses for decades. Yet, it has proven difficult to utilize this therapy effectively for all, e.g. Crohn's disease.

Current research has made the ability of intestinal microbiota to modulate innate immune responses well known, yet the underlying mechanisms remain elusive.

Previous research as shown beneficial bacteria activate anti-inflammatory type 1 interferon (IFN1) production via intracellular sensors, STING and MAVS.[1,2] This response appears to be dependent on live bacteria, rather than dead bacteria.

Research Question

How are anti-inflammatory IFN1-inducing beneficial bacteria sensed by the innate immune system?

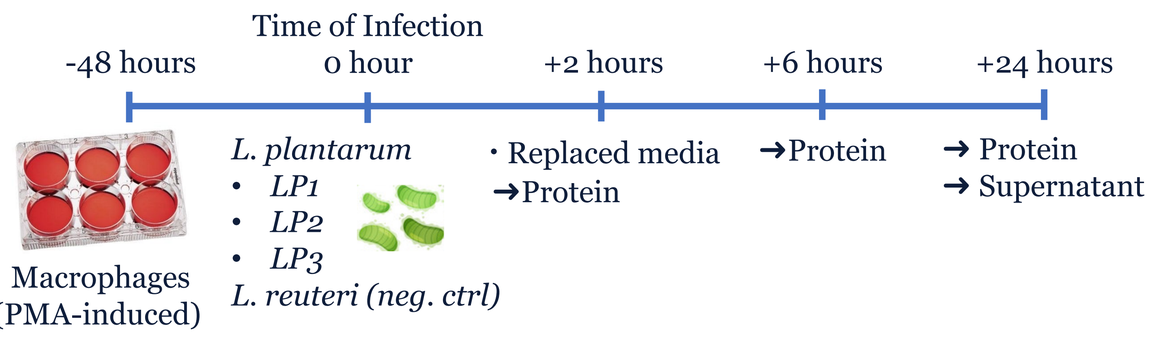


Methods

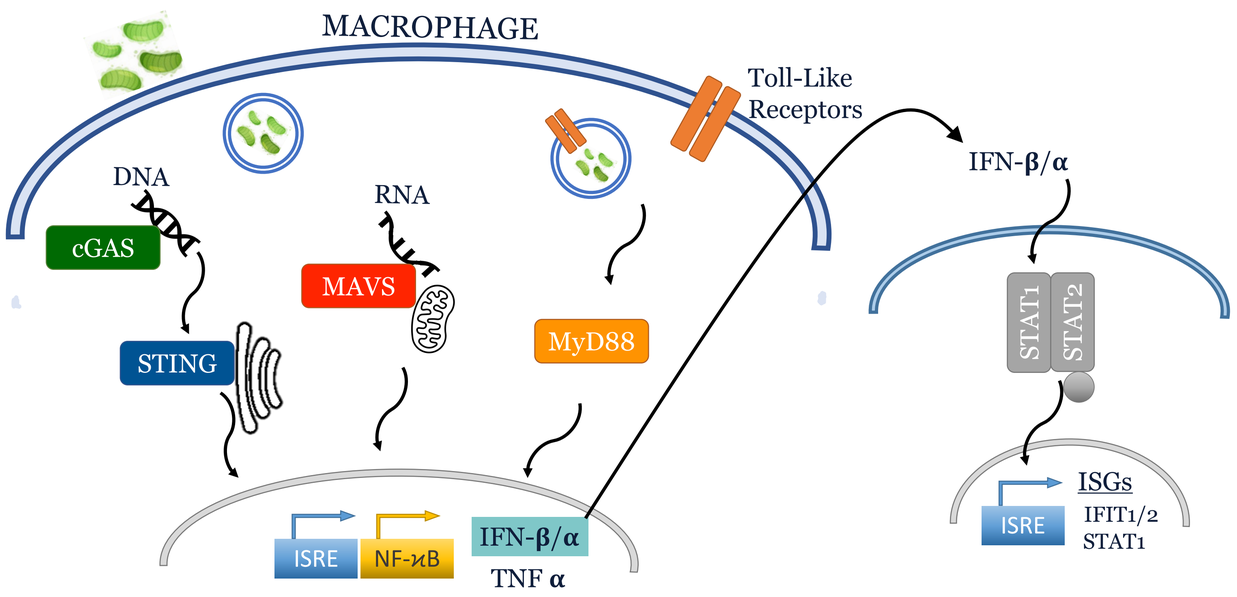
After screening several live and heat-killed lactic acid bacteria strains[1], we identified three *Lactiplantibacillus plantarum* strains that induced a low, moderate and high IFN1 response while failing to activate significant pro-inflammatory NF-κB levels.

Here, THP-1 knockout cell models with a luciferase-reporter background were utilized to monitor interferon stimulated gene (ISG) proteins, IFIT1/2 and STAT1 via luciferase-based reporter assay and phospho-blotting, respectively.

Experimental Set-Up



IFN1 – Innate Immune Signaling

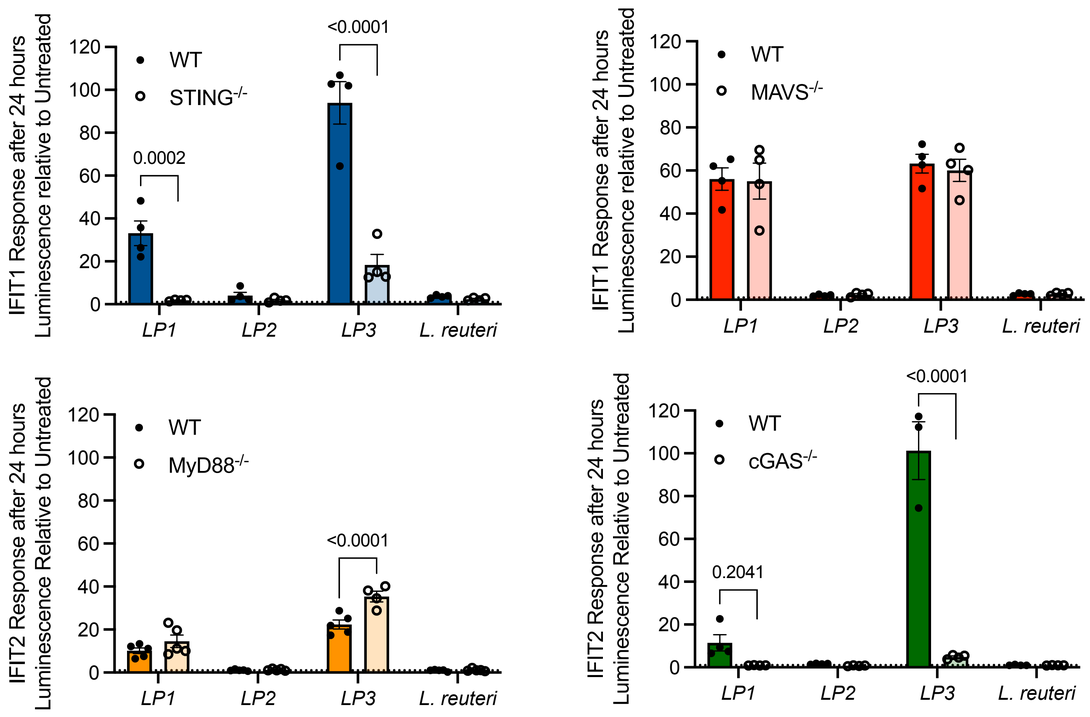


Results

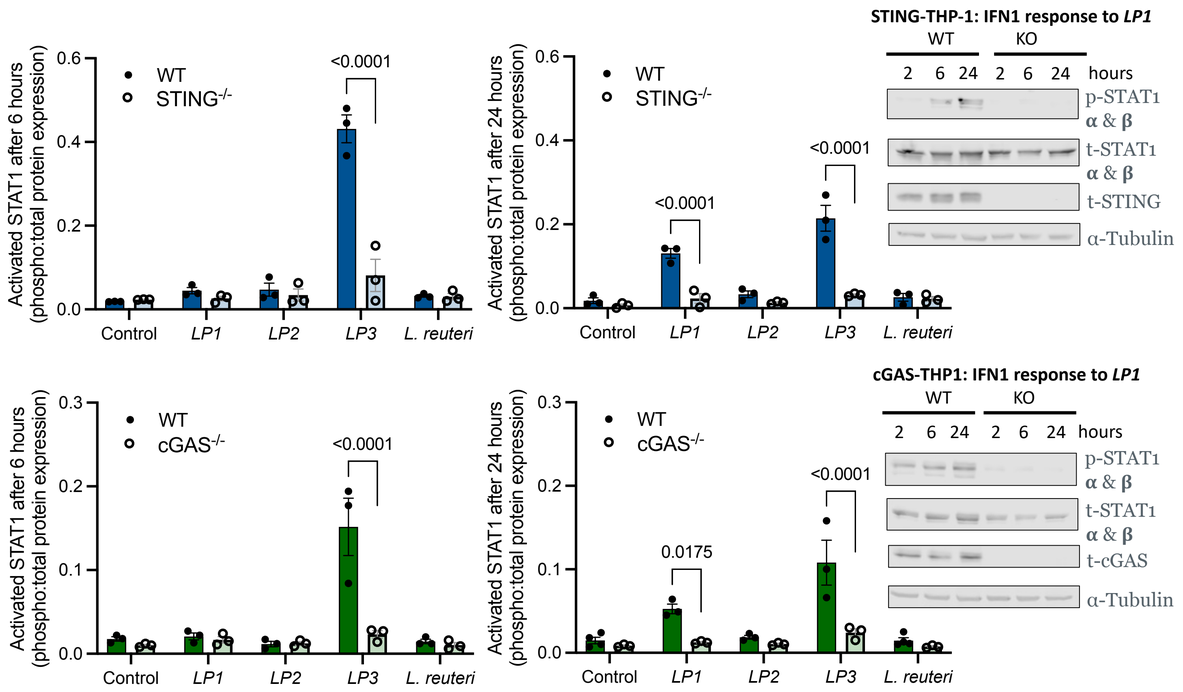
STING, MAVS, MyD88 and cGAS knockout luciferase-based reporter cell lines were treated with live *LP1-3* and negative control, *L. reuteri*. Luciferase-based assays and phosphoprotein blotting detected nucleic acid intracellular sensor STING and DNA sensor cGAS as critical players in the IFN1 response to *LP1* and *LP3*.

Meanwhile, RNA sensor MAVS and toll-like receptor adaptor MyD88 were not essential for the *LP1*- and *LP3*-induced IFN1 response.

IFIT1/2 Immune Response



STAT1 Phospho-Kinetic Response



Data shown as individual data points, n= 3-5, mean ± SEM as bar graphs. Analysis by two-way ANOVA, Šidák post hoc tests, and P-values of significance are shown above.

Conclusion

- Lactic acid bacteria strains were identified to induce a high IFN1:low NF-κB response that is dependent on intracellular sensors, cGAS and STING.
- IFN1 responses were strain specific as found by early and late IFN1 induction by *LP3* and *LP1*, respectively.
- Future work will include studies in primary immune cells, cytokine profiling, and to identify critical features of *LP*s to understand why they are sensed in this way.
- This work aims to exploit the induction of anti-inflammatory pathways by specific probiotic strains for difficult-to-treat inflammatory gut disorders.

References

1. Gutierrez-Merino, *et al.* 2020, Gut Microbes, 11:4, 771-788.
2. Si, *et al.* 2021, Gut. doi: 10.1136/gutjnl-2020-323426