

Lactococcus lactis expressing HSP65 regulates the immune response and protects against the development of type 1 diabetes in a TLR2-dependent manner

JEFFERSON ELIAS-OLIVEIRA¹, VANESSA FERNANDES RODRIGUES¹, ÍTALO SOUSA PEREIRA¹, SARA CÂNDIDA BARBOSA¹, MELISSA SANTANA GONSALEZ MACHADO¹, JÉSSICA ASSIS PEREIRA¹, THAÍLLA CRISTINA FARIA PACHECO¹, ANA PAULA MASSON¹, DANIELA CARLOS¹

¹. FMRP - USP, Ribeirão Preto Medical School, University of São Paulo, Av. Bandeirantes, 3900 - Campus USP, Ribeirão Preto - SP, 14049-900;

Heat-shock proteins (HSPs) are molecular chaperones that help maintain cellular homeostasis under stress by assisting in protein folding and degrading misfolded proteins. Heterologous HSPs, particularly HSP65, have shown potential as therapeutic tools in autoimmune diseases by modulating immune responses. When administered to the intestinal mucosa, HSP65 induces an anti-inflammatory response, reducing the severity of conditions like arthritis and atherosclerosis. Type 1 diabetes (T1D), characterized by the autoimmune destruction of pancreatic β cells and resulting hyperglycemia, remains a challenge to manage. Novel strategies that target immunological mechanisms, such as HSP-based therapies, may control autoimmune diseases and mitigate their complications, advancing treatment possibilities for these diseases, including T1D. Here, we evaluated the role of TLR2 in the protective effects of *L. lactis* expressing HSP65 in T1D experimental models (CEUA 007/2020). HSP65 expression was induced in *L. lactis* (NCD02118) by xylose in M17 culture medium. In prophylactic-therapeutic models using mice with streptozotocin (STZ)-induced or spontaneous T1D (NOD mice), *L. lactis*-HSP65 reduced hyperglycemia and disease incidence. The reduction in hyperglycemia in the group receiving *L. lactis*-HSP65 when compared to the diabetic group receiving only PBS was also observed in the glucose tolerance test (GTT). Additionally, we observed that *L. lactis*-HSP65 preserved insulin expression, reduced the degree of insulinitis and decreased serum IgG anti-HSP65 levels in the serum of mice with STZ-induced diabetes. Wild-type and recombinant *L. lactis* increased the expression of Il22, Reg3g and Ocln genes in the colon, whereas only *L. lactis*-HSP65 increased ZO-1, suggesting that both strains appear to improve intestinal barrier function. We observed that *L. lactis*-HSP65 increased the gene expression of Ido, Aldh1a2, Pd11, Tgfb and Irf8 in the colon, which are markers of tolerogenic DCs. *L. lactis*-HSP65 increased cDC1 XCR1⁺ TLR2⁺ and PD-1⁺ Tregs in cecal lymph nodes, and TGF- β production in colon when compared with STZ+PBS group. No differences were observed in the cytokines IL-10, IFN- γ and IL-17 in the colon of mice that received probiotic. In pancreatic lymph nodes, we observed that *L. lactis*-HSP65 increased LAP⁺ T regs, in association with elevated levels of IL-10 and TGF- β in the pancreas. In TLR2-deficient mice, *L. lactis*-HSP65 did not effectively control hyperglycemia or disease incidence, correlating with reduced cDC1 XCR1⁺ and Tregs and lower IL-10 production. *In vitro*, we observed that TLR2- or IL-10-deficient bone marrow-derived dendritic cells (BMDCs) showed impaired Treg induction. These findings demonstrate that *L. lactis*-HSP65 protects against T1D via a TLR2/IL-10-dependent mechanism involving immunoregulatory Tregs and tolerogenic dendritic cells.

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International Symposia

Beneficial Microbes

March 27th and 28th, 2025

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Khan, F.U.; Khongorzul, P.; Raki, A.A.; Rajasekaran, A.; Gris, D.; Amrani, A. Dendritic Cells and Their Immunotherapeutic Potential for Treating Type 1 Diabetes. *Int. J. Mol. Sci.* 2022, 23, 4885. <https://doi.org/10.3390/ijms23094885>

Acknowledgements: The authors thank Wander Cosme Ribeiro da Silva (FMRP-USP) and Denise Brufato Ferraz (FMRP-USP) for their technical support.