Lactococcus lactis-HSP65 induces IL-9 production, mast cell gene expression in the colon andcontrols the type 1 diabetes in an experimental model.

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Type 1 Diabetes (T1D) is considered an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta cells, leading to insulin secretion deficiency and chronic hyperglycemia. Alterations in the intestinal barrier, in the mucus production, and the composition of the gut microbiota are strongly associated with the development of T1D due to exacerbated immune activation, which promotes inflammation and autoimmunity. Probiotics such as the *Lactococcus lactis* have therapeutic potential by modulating the gut microbiota and immune response. Heat shock protein 65 (HSP65), plays an important anti-inflammatory role in several models of autoimmune diseases. Here, we evaluated the effects of *L. lactis* expressing HSP65 on IL-9 expression, in the mucus production and mast cell markers expression in colon of mice with T1D induced by 40 mg/kg of streptozotocin (STZ). The recombinant probiotic was cultured in M17 agar medium and $1x10^9$ CFU was administered by gavage to mice daily before and during STZ induction, totaling 10 days, and every other day after the end of disease induction for an additional 10 days. We observed that *L. lactis*-HSP65 reduced the incidence of T1D and the ameliorated the hyperglycemia, and wild *L. lactis* and *L. lactis* HSP65 (LL-HSP65) significantly increased mucus production in the colon of STZ-induced T1D mice. Although we found no significant differences in the gene expression of *Muc1* and *Muc2*, we observed an increasing trend for in the LL-HSP65 group. Quantitative analysis demonstrated a significant increase in mucus in the *L. lactis*- and LL-HSP65 groups when compared with groups that did not receive the recombinant probiotic. In addition, we observed a significant increase in IL-22 gene expression in both groups that received probiotics when compared with STZ group, but no statistic differences in protein production. IL-9 production was significantly higher in the *L. lactis*-HSP65 group when compared with STZ group, suggesting an important role of this cytokine in the protection against T1D onset. We also analyzed the expression of *Mmcp1* and *Mmcp4* genes, and we observed that both genes were increased in the colon of diabetic mice that received *L. lactis*-HSP65, suggesting the presence and/or activity of mast cells in the colon. In conclusion, the probiotic *L. lactis*-HSP65 protects against T1D development, increases IL-9 and mast cell gene expression in the colon of diabetic mice. These results show that *L. lactis*-HSP65 can significantly improve intestinal barrier function and be an innovative and promising approach to controlling hyperglycemia and limiting the onset of T1D.

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