EFFECTS OF GENETIC MODIFICATION OF *LACTOBACILLUS CASEI* BL23 CELL WALL ON HUMAN MONOCYTE-DERIVED DENDRITIC CELL FUNCTIONS

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Introduction *Lactobacilli* are commensal bacteria, which can be found in the human gut. They are facultative anaerob, rod-shaped Gram-positive bacteria with pro-biotic potential. Several strains of *Lactobacilli* have attracted intense interest due to their immunomodulatory properties. One of the most extensively studied strain is *Lactobacillus rhamnosus* GG which mediates anti-apoptotic activity in intestinal epithelial cells (IEC). The secreted factors responsible for this effect were indentified as proteins with peptidoglycan-hydrolase (PGH) activity. The two identified proteins are able to stimulate Akt signaling in host IEC, prevent these cells from damage and cytokine-induced apoptosis. Genes encoding homologues enzymes are present in closely related strains of *L. rhamnosus* such as *L. casei* BL23 which was the target of our experimental model.
Methods Monocytes were separated from human buffy coats and differentiated to dendritic cells (DCs) *in vitro* in the presence of GM-CSF and IL-4 for 5 days. *L. casei* BL23 wild type and two peptidoglycan-hydrolase mutant bacteria (Δ2770 and Δ2770/p45) were added to the monocyte-derived DCs (moDCs) for 24 hours. The phosphorylation status of the three major families of mitogen-activated protein kinases (MAPK) in moDCs was studied with MAPK array. Phagocytic activity of the stimulated moDCs was monitored by flow cytometry. Culture supernatants were collected on day 6 of moDC differentation and the concentration of the secreted cytokines was measured by ELISA. The number of IFN-γ and IL-17 producing T cells was detected by ELISPOT assays.
Results Our experimental results showed that the phagocytic activity and the secretion of cytokines increased after bacterial stimulation and the genetic modification of the *L. casei* cell wall could modulate the phosphorylation status of selected MAP kinases. These experiments also revealed that the most efficient uptake by moDCs could be detected with the wild-type bacteria that also triggered the highest cytokine levels secreted by moDCs. *L. casei* BL23 wild-type and PGH negative mutants were able to induce the polarization of T lymphocytes by moDCs to Th1 or Th17 directions.
Conclusions In our *in vitro* culture system we compared the functional activities of live wild-type *L. casei* BL23 bacteria as compared to their peptidoglycan-hydrolase mutants. Genetic modification of the bacterial cell wall modulated the activating potential and the cytokine secretion of moDCs and affected the outcome of T lymphocyte responses.

Poster presentation
Theoretical immunology