ABSTRACT FOR GENERAL PUBLIC

Helicobacter pylori (*H. pylori*) is a bacterium that colonizes gastric mucus layer of more than half of human population. Majority of *H. pylori* infections remain asymptomatic. However, in 10-15% of cases, it leads to a development of gastric inflammation, gastric and duodenal ulcers or even gastric cancer. The course of *H. pylori* infection depends on the bacterial antigens as well as individual predispositions of a host. Genetic and archeological studies showed that *H. pylori* accompany humans hundreds thousands of years. Thus, it is no surprising that the bacterium "*knows*" how to use its structures to manipulate the immune cells to remain unrecognized and avoid eradication. Lipopolysaccharide (LPS) of *H. pylori* is a unique cell wall component of this bacterium, due to differences in chemical structure such as low acylation and phosphorylation, long fatty acid chains and presence of Lewis^{XY} determinants. All these features make the *H. pylori* LPS less recognizable by immune cells and thus it possess a weak potential to stimulate immune cells or may even inhibits their activity. The results obtained by our research team indicate that the LPS of *H. pylori* may inhibit macrophage maturation, affect cytotoxic activity of natural killer cells, and interfere with lymphocyte proliferation. Microbial glycolipids such as LPS, were considered less immunogenic and poorly recognized by more complex receptors. The discovery of iNKT cells and their ligands revolutionized the potential of glycolipids as immune modulators. This small sub-population of cells combines the features of innate and acquired immunity which allow recognizing and responds to glycolipid antigens by TCR Vα24Jα18 receptor, which binds sugar residues of glycolipids. LPS of *H. pylori*, due to its unique structure and the presence of sugar and lipid moieties, meets the criteria to become a newly characterized ligand for iNKT cells and CD1d of antigen presenting cells such as dendritic cells (DC). Thus, likely it is recognized by the receptors of iNKT cells and influence their activity thereby determining the course of infection. Knowledge on the participation of iNKT cells in *H. pylori* infections is very poor, and particularly the phenomenon of iNKT-*H. pylori* LPS interactions has not been studied to date.

Thus, the objective of this project is to search for iNKT cells ligand among various forms of *H. pylori* LPS (with or without Lewis antigenic determinants) and to evaluate its interaction with TCR Vα24Jα18 receptor and to describe their cellular consequences. In addition, since in *H. pylori* infected subjects immune cells remain under influence of *H. pylori* components *in vivo*, including its LPS, we want to compare the number, phenotype and activation of peripheral iNKT cells between *H. pylori* infected and uninfected individuals.

The study will include 50 donors, divided into a group of *H. pylori* infected and uninfected individuals, classified based on serological assays, urea breath test and medical interview made by clinician gastroenterologist. In a first step, using flow cytometry techniques we will examine the phenotype and number of peripheral iNKT cells of both study groups. Then, by using fluorescently labeled dextramer-CD1d technique we will examine binding of different forms of *H. pylori* LPS to CD1d molecules and detect its recognition by iNKT cells by flow cytometry. Then on semi-cellular level we will check if the binding result with cell activation (iNKT cells and mDC) measured by selected cytokine quantification. Using technique of surface plasmon resonance we will be able to show the strength and kinetics of ligand-receptor binding. Finally, mixed culture model of iNKT cells with DCs pulsed with LPS of *H. pylori* will reveal whether, the antigen recognition leads to changes in intracellular and surface expression of activation markers and by confocal microscopy we will visualize the direct/indirect interaction between these cells by creating *immune synapse*.

The chemical structure of *H. pylori* LPS including long fatty acid chains and the presence of sugar moieties with fucose residues within Lewis^{XY} antigens makes this glycolipid a potential ligand for the iNKT cells, however this aspect have not been evaluated yet in the context of *H. pylori*. Therefore our project will explain the role of iNKT cells in the immune responses triggered by *H. pylori* and will lead to an identification of the *H. pylori* glycolipid ligands of iNKT cells and explore the nature of DC-iNKT cell interactions. The obtained results will bring us closer in understanding how the components of *H. pylori* modulate immune cells which further may lead to a development of glycoconjugate therapeutic/prevention agents.