SUMMARY

Yersinia enterocolitica O:3 (YeO3) is a gram-negative rod-shaped bacterium capable to grow in a wide range of temperature: from 0 to 44°C. It is an etiological factor of intestinal infections in humans as well as facultative intracellular pathogen and causes yersiniosis. Acute infection can occur after contaminated blood transfusion, due to ability of the bacterium to grow in a wide range of temperature. *Y. enterocolitica* O:3 express a broad spectrum of virulence factors, such as carbohydrate surface antigens: lipopolysaccharide (LPS), enterobacterial common antigen (ECA), and presumably exopolysaccharide (EPS). Moreover, it carries a virulence plasmid *pYV*, which provides genes responsible for crucial adhesins and outer membrane proteins expression important for *Yersiniae* pathogenicity.

Outer membrane vesicles (OMVs) belong to virulence factors of Ye and are poorly characterized for this species. OMVs carry pathogen associated molecular patterns (PAMPS) such as LPS, peptidoglycan, nucleic acids, which are recognized by pattern recognition receptors (PRR), for example Toll-like receptors (TLR) presented on the surface of immune system cells. As a result of these interactions with innate immune system proinflammatory mediators and cytokines are secreted. Overactivation and homeostasis dysregulation as a result of infection can cause systemic inflammatory response syndrome (SIRS) and sepsis. Complement system is one of a major component of innate immune response. Its activation during infection evokes pathogen opsonization (by antibodies, MBL collectin and ficolins), what helps to eliminate pathogen, however its uncontrolled activation leads to a development of SIRS, sepsis and septic shock. Nowadays there are limited number of publications reporting complement activation by OMVs regarding temperature-regulated structure of LPS.

This PhD project was a part of OPUS 16 grant implemented by a consortium of Institute of Medical Biology PAS in Łódź, Institute of Immunology and Experimental Therapy, Polish Academy of Science in Wrocław, and University of Silesia in Katowice. The major aim of the project was OMVs characterization and investigation of the interaction with human complement system. It is investigation the basic hypothesis, that the OMVs of the selected bacterial species could serve as a "shield" for bacteria from the complement system of the infected host as well as an activator of the inflammatory response. *Y. enterocolitica* O:3 has been chosen as the model microorganism due to its LPS having unique scheme and temperature-

regulated structure, particularly the lipid A. Moreover a wide library of mutants of YeO3 with different LPS chemotypes (S, Ra, Re and Rd1) were available.

In preliminary studies, LPS and ECA structures were verified for four strains of chemotypes S, Ra, Re and Rd1. Unidentified glycoforms of the inner core regions of LPS were detected, composed of a increased number of Kdo residues (Re chemotype) and a increased number of heptose residues (Rd1 chemotype). Additionally, in the case of Re chemotype, the presence of a relevant amount of ECA_{PG} was detected. An important aspects of this studies were analyses of lipids A structures in both, the LPS isolated from bacteria and the LPS present in OMVs. The lipid A region of LPS is a key activator of immune system cells via TLR4. Preliminary results were also obtained suggesting the production of EPS by YeO3 with the most probable structure $[\rightarrow 2)$-α-D-Man-$(1\rightarrow 3)$-α-D-Man-$(1\rightarrow 6)$-α-D-Man-$(1\rightarrow]_n$

The highlights of the doctoral project was the selection and optimization of OMV isolation and purification procedures. The obtained OMVs were characterized by physicochemical techniques - DLS and NTA, TEM microscopy, mass spectrometry and NMR spectroscopy. It was shown that *Y. enterocolitica* O:3 releases OMVs, and their number and size are dependent on the culture temperature (37, 22 and 4°C) and LPS chemotype. We compared LPS structures, the main virulence factor, in the bacterial preparation and OMVs. It was shown that OMVs isolated from each chemotypes activate complement system, particularly the lectin pathway, which is activated by mannose binding lectin (MBL). We have identified the EPS, the polymer of mannose, as the ligand recocnised by human MBL. This EPS is present on the OMV surface or coextracted with the vesicles. Proteins, DNA and mannans derived from LB culture medium were excluded as ligands for MBL. Isolated YeO3 OMV were not recognized by ficolins.

We have demonstrated that isolated OMVs and LPS derived from smooth strain of YeO3 (S) activate the THP-1 cells to express mRNA for the IL-8, IL-10, IL-6 and TNF-α genes. The strongest activity against THP-1 cells was observed for OMVs isolated from bacterial cultures carried out at lower temperatures: 22°C and 4°C. The correlation between described biological activity *in vitro* and the structure of lipid A in LPS present in OMV preparations was observed. OMVs were able to inhibit the killing activity of NHS serum against YeO3 bacteria, where the key role in protection was provided by the number of secreted OMVs, as well as the presence of O-specific polysaccharide in LPS on their surface. Furthermore, the studied vesicles isolated from the smooth strain (chemotype S) administered intraperitoneally to BALB/c mice,

entered the circulation and accumulated in organs, with the liver being the main site of accumulation of labeled OMVs was the liver, followed by the lungs, kidneys and lymph nodes. The obtained results confirm the research project hypothesis that *Y. enterocolitica* O:3 OMVs may serve as a "shield" for the bacteria protecting from the complement system and constitute a strong activator of the inflammatory response, which is important for a better understanding of the development of infections and sepsis.