Sepsis is a state of immediate threat to life developing as the consequence of bacterial infection. During sepsis, a severe immune system response leading to impaired functioning of tissues, organ dysfunction and septic shock resulting in death is often observed. Sepsis is not an independent disease entity. It is currently defined as a systemic inflammatory response syndrome (SIRS) caused by infection. It is established that sepsis is induced by pro-inflammatory factors released from the bacterial cell wall, causing a strong host immune system response. Depending on the pathogen causing infection, this group of pro-inflammatory entities can include lipid A and lipopolysaccharide (LPS) from Gram-negative bacteria, or lipoteichoic acid (LTA) – the main Gram-positive endotoxin. Through TLR-mediated mechanisms they lead to the release of immune modulators, i.e. substances responsible for the development of systemic inflammation at the cellular/tissue level. It was shown that some local inflammation states, such as pulmonary infections, intra-abdominal, kidney and oral / gastrointestinal tract infections, often result in release of pathogens into the blood and the subsequent development of sepsis. Sepsis is a challenging state with high risk of death in hospital intensive care units, hematological oncology and geriatrics departments. It is estimated that the mortality of patients diagnosed with sepsis ranges from 20 to 40 percent. Moreover, statistical data shows an increase in the number of patients affected by systemic infections. It is assumed that the rise in morbidity is contributed to by an aging population, growing prevalence of chronic diseases, (including immune disorders) and inappropriate use of antibiotics, leading to the development of resistant bacterial strains and fungi. Importantly, the mortality among patients diagnosed with septic shock is relatively high and the treatment is long-term and costly.

Therefore, sepsis should be considered as an emerging clinical problem with a real need for pursuit of new agents, characterized by an ability to inhibit biological activity of pro-inflammatory factors released from pathogens and the possibility of use in sepsis treatment. Strong scientific data collected so far established that plasma gelsolin (pGSN) might be employed to develop a possible treatment for this otherwise very deadly condition. pGSN is present in various body fluids, and its blood concentration under physiological conditions ranges from 200 to 300 μg/ml. Gelsolin is also present inside the cells as a cytoplasmic product of the same gene. GSN belongs to the family of actin binding proteins - actin is the predominant cytoplasmic protein and gelsolin is responsible for binding and severing actin filaments. GSN is composed of 730 amino-acids, which are organized into six identical segments G1-G6, where each corresponds to a different function. Cytoplasmic gelsolin, by interacting with actin filaments, regulates the structure of the cytoskeleton organization, which determines alterations in its shape, and is involved in different cellular functions such as chemotaxis and secretion. GSN preferentially interacts and binds lysophosphatidic acid (LPA), LPS, LTA, shphingosine 1-phosphate (S1P) and platelet-activating factor (PAF). This phenomena is probably caused by the structural similarity of these molecules to the intracellular molecule regulating actin-binding ability of gelsolin i.e. phosphoinositide (PIP, PIP2) and LPA. Different studies performed so far have shown that in the course of various diseases characterized by an immediate threat to life (critical conditions) or a chronic inflammatory process, a decrease of gelsolin blood concentration causing dysfunction of the blood actin buffer is observed. A reduced plasma gelsolin concentration in acute respiratory distress syndrome has been detected, and when the level of gelsolin falls below 30% of its concentration detected in healthy subjects, the life of affected subject is at high risk. A decrease in gelsolin blood concentration was observed in sepsis, myocardial infarction, serious injuries and severe blood parasitoses (e.g. malaria). These findings emphasize a key role of this protein in different conditions with a direct threat to life. Recent animal studies have shown that administration of exogenous gelsolin to animals with induced septic shock significantly reduced mortality. However, the mechanism of such phenomena is still unknown. There is also no data describing GSN distribution in different body compartments, both under physiological conditions and during septic shock. The correlation between low blood levels of gelsolin and risk of death in life-threatening conditions indicates that gelsolin possesses a high potential for use as a prognostic marker. It is hypothesized that administration of exogenous gelsolin might minimize the symptoms and consequences associated with the described clinical states associated with hypogelsolinemia.

The aim of this project is to investigate the ability of pGSN and gelsolin-derived peptides to control and prevent development of inflammation (immunomodulatory and anti-inflammatory properties), and to perform bactericidal activity during sepsis, in which a decrease in blood GSN concentration is observed. The project will enable the identification of gelsolin ligands, other than previously described actin, from blood samples taken from healthy subjects and patients with diagnosed sepsis. Additionally, evaluation of the immunomodulatory activity of gelsolin and gelsolin delivered peptides and pGSN isolated from blood by analyzing the activation of signaling pathway (NF-kB) and cytokine expression after stimulation with bacterial products (LPS, LTA, heat inactivate bacteria E. coli or S. aureus) will be performed using purified human blood neutrophils and cell culture systems. Bactericidal effect of phagocytic cells (able to absorb different microorganisms) arising partly from the activation of nitric oxide synthase type 3 (NOS3), and anti-inflammatory properties (eg. the production of inflammatory mediators) will be evaluated as well. We hypothesize that the immunomodulatory properties of gelsolin might significantly reduce an inflammatory response that develops during sepsis. The proposed study will allow for understanding the mechanism of action of gelsolin and the identification and biochemical analysis of ligands bound by this protein. Carrying out experiments on animals (rats and mice) will confirm the in vivo antimicrobial activity of exogenous gelsolin to treat septic shock induced by selected bacteria, as well as in the presence of bacterial LPS. Evaluation of the biodistribution will help to assess and identify the pharmacological parameters of GSN, which are essential for GSN description as a therapeutic agent. In addition, due to an observed recent progress in the development of therapeutic (DDS - Drug Delivery System) and diagnostic methods (separation and imaging techniques), which focus on the implementation of nanotechnology, this project will assess the potential use of magnetic nanoparticles as gelsolin and gelsolin derived peptide carriers.

The basic component of the DDS includes using an appropriate carrier that should not be toxic, should bind the drug properly, making its release possible at the target site and keeping within the therapeutic concentration range. We expect that the use of nanoparticles as a GSN carrier will significantly increase LPS / LTA binding efficiency via modification of their solubility, retention time and penetration through biological barriers. Bioavailability will be enhanced as well by the above-mentioned mechanisms. In effect, MNPs functionalized with GSN or GSN-delivered peptides might be characterized by higher therapeutic efficacy. They can be potentially used to generate nanosystems for body fluid

purification from LPS / LTA.

The research proposed in this project is projected for 36 months. During this project a number of biochemical and physicochemical methods will be applied which will enable for the implementation of project objectives. In the first stage the exploration of new ligands binding to gelsolin will be performed. This will be achieved by isolation and purification of GSN from blood samples collected from healthy volunteers, patients with septic shock and from animal serum using chromatographic techniques. Mass spectrometry (MS), nuclear magnetic resonance (NMR) and spectroscopy methods including UV and FT-IR techniques will be implemented. To evaluate immunomodulatory activity of gelsolin and gelsolin delivered peptides as free molecules or immobilized shells on the magnetic nanoparticle surface, molecular biology techniques (microarray profiling of the cytokine expression and electrophoresis) will be performed. Bactericidal effects of gelsolin will be evaluated using analysis of pathogen survival in the presence of increasing concentrations of the protein (CFU assay). Additionally, effects of GSN on phagocytosis in human monocyte culture of cell line U937 will be performed by using microscopy analysis. In order to determine the pharmacological parameters of gelsolin, a protein will be functionalized by fluorescent dye (DYE-800CW). Biodistribution of GSN-DYE-800CW will be assessed in real-time by near-infrared imaging technique, and the change in brightness over time will be registered.

The generated data will allow for the identification of gelsolin's ligands in normal and septic blood. In addition, the project will allow for the identification pGSN's mechanism of action, including immunomodulatory, anti-inflammatory and bactericidal properties. We hypothesize that the immunomodulatory properties of gelsolin might significantly reduce the inflammatory response underlying septic shock. Therefore, the proposed study will allow us to understand gelsolin's mechanism of action, pharmacological properties and identification/biochemical analysis of ligands bound by this protein which might provide tools to describe new targeted therapeutic methods of diseases characterized by immediate threat to life.