Toll-like receptors (TLRs), including TLR3 (Toll-like receptor 3), are a family of proteins found in cells. Every pathogen (bacteria, virus, fungus etc.) has a specific molecular "identity card", which the immune system recognizes. The "identity card" is the pathogen molecular pattern that is recognized by TLRs at the early stage of innate immune response. Hence, TLRs play a very important role in most species by recognizing pathogens. TLRs initiate an appropriate immune response to invasion by infectious agents and therefore play the role of molecular controllers retaining intruders identity. TLR3 identifies the double-stranded ribonucleic acid (dsRNA), an intermediary during replication of many viruses. TLR3 belongs to the subfamily of TLRs, which are found in endosomes – vesicles inside the cell. Recognition of dsRNA activates TLR3 and leads to a signaling cascade, which stimulates production of type I interferons and pro-inflammatory cytokines. These cytokines are responsible for activation of the host antiviral response.

TLR3 is expressed in many cells including brain cells such as astrocytes, oligodendrocytes, neurons and microglial cells. Genetic studies of animals and humans with the deficiency of this receptor revealed that **TLR3 is essential in defending the body against herpes virus infection (*herpes simplex virus 1*, HSV-1)**, particularly against encephalitis caused by this virus. The deficiency of functional TLR3 was the first of the TLR deficiencies to be discovered in humans. The discovery established that proper TLR3 functioning is essential for effective antiviral response against HSV-1 in the central nervous system (CNS). Hence, thorough investigation of immune mechanisms governing the control of HSV infection in the CNS is justified.

TLR3 is located in endosomes, where ESCRT-0 (endosomal sorting complex required for transport-0), a protein complex consisting of Hrs and STAM, is also located. ESCRT-0 is responsible for reconstruction and circulation of cell membranes, from which endosomes are built. In addition, ESCRT-0 directs proteins and/or various cell receptors tagged with ubiquitin ("tag" predestining them for degradation) to endosomes.

Previous research on ESCRT-0 showed that TLR3 may co-precipitate with STAM and that silencing the TLR3 expression decreases the expression of Hrs and STAM in mouse microglial cell line C8D1A. There is also evidence that treatment of cells with a TLR3 ligand – poly(I:C) (polyinosinic-polycytidylic acid) leads to phosphorylation and activation of Syk kinase, which in turn activates Hrs. If the kinase mediates activation of ESCRT-0 by TLR3 (that is, if the activated receptor induces kinase' phosphorylation), then the activation of the complex by TLR3 can lead to the transport of ESCRT-0 into endosomes.

The primary aim of the project is to **characterize the molecular mechanisms by which ESCRT-0 regulates the antiviral activity of the TLR3 upon binding to the corresponding ligand**. Experiments will be conducted on a mouse microglial cell line, because human microglial cells of non-tumor origin are currently unavailable. TLR3 is expressed in both human and mouse microglial cells and shows many structural and functional similarities among different species. Consequently, it is assumed that the mouse cell model will faithfully and accurately reflect the TLR3 regulatory mechanisms in human cells.

The experiments will determine Hrs/STAM phosphorylation and ubiquitination profile after TLR3 stimulation and carefully examine TLR3 ubiquitin conjugation. Concurrently, studies will be conducted to measure Syk kinase activity in cells, followed by *Syk* gene silencing and assessment of its role in Hrs/STAM activation. Further, TLR3 expression as influenced by ESCRT-0 will be determined. Also, it is expected that silencing of Hrs and/or STAM gene expression will reveal the impact of ESCRT-0 on the synthesis of transcription factors responsible for antiviral response in cells. Ultimately, the project will determine whether the disruption of Hrs/STAM influences the antiviral responses, in which TLR3 takes part.

Results obtained in this research will be the first step in evaluating the role of ESCRT-0 in the innate immune response in CNS after TLR3 activation with a specific ligand. It will be possible to ascertain the extent of ESCRT-0 regulation of the TLR3 function and whether protein interaction requires the trafficking of ESCRT-0 to endosomes. Knowledge gained from these studies will help to identify the signaling pathways that may play a role in antiviral responses and therefore, have a therapeutic relevance. Precise recognition of TLR3-associated signaling pathways can be a key element in developing signaling modulation of the receptor in humans or animals, particularly with defects in these signaling pathways. The proposed research may be helpful in obtaining advanced scientific tools and making them available for international research teams. The obtained data may become an area of interest for groups of scientists studying the therapeutic possibilities in viral infections. This may also be important in other processes, since TLR3, beyond the antiviral defense, is either related to neurogenesis or may be involved in pathogenesis of neurodegenerative diseases.