The primary goal of this project is to extent current state of knowledge considering mechanisms regulating development of autoimmune demyelination. Process of treatment diseases with autoimmune background, into which multiple sclerosis (*Sclerosis Multiplex*, SM) is included, concentrates on attempts to mitigate their course due to the fact that their pathogenesis remains unknown. It is assumed that significant impact on disease induction have many factors such as: genetic predispositions, deficit of vitamin D which can be related to latitude and exposure to sun or stress. In autoimmune demyelination we observe degradation of myelin sheaths of neurons which leads to neurons necrosis. This process is caused by infiltration to central nervous system (CNS) through blood-brain barrier lymphocytes CD4+ T helper (CD4+ T cells, Th), mainly autoreactive population Th17 and Th1 which recognize myelin antigen as menacing. Recent studies emphasize important role of epigenetic mechanisms in regulation of differentiation o CD4+ T cells. In our previous research we indicated increase in expression of three miRNAs miR-21, miR-155 and miR-301a in CD4+ T cells population. We also revealed the mechanism in which miR-301a regulates Th17 differentiation by inhibition of Pias3 expression which is an inhibitor of Stat3 – key transcription factor for Th17. MiRNAs are short RNA transcripts, 18-22 nucleotides in length which do not code proteins. They are characterized by posttranscriptional mRNA regulation mainly by translation inhibition of proteins or mRNA degradation.
In this project we plan to focus on continuing research on the function of miRNAs, in particular miRNA-155 by the regulation of gene family of heat shock protein 40 (Hsp40) in the process of differentiation of CD4+ T cells and the course of autoimmune demyelination.

Proposed model of research in this project has an innovative trait. In preliminary studies we have shown a significant increase in expression of miR-155 in CD4+ T cells responsive to myelin antigen. The results of this experiment obtained *in vitro* will be confirmed in vivo on a mouse model of SM- experimental inflammation of the brain and spinal cord (experimental autoimmune encephalomyelitis, EAE). It was observed that the course of the disease in mice with genetically blocked expression of miR-155 (miR-155-/-) was significantly milder than in control mice (miR-155+/+). In order to study the mechanisms regulating EAE by miR-155 we plan to investigate the expression of both strands of miR-155: miR-155-3p, and miR-155-5p in the two main cell populations of inflammatory CD4+ T cells and macrophage/microglia using molecular biological techniques such as RT-qPCR both relative and absolute quantitative assessment. In the next step we will investigate the functional importance of both strands of miR-155: miR-155-3p and miR-155-5p in the CD4+ T cells with vectors which enhance the effect of the individual threads (mimics), then we will measure the expression of marker genes for individual T helper cells populations. Vectors which modify the expression of both strands of miRNA-155 will be delivered into cells using electoporation techniques. In the next phase of the project we will examine functionality of both strands: miR-155-3p and miR-155-5p in the process of blocking the expression of Dnaja2 and Dnajb1 by transfection of CD4+ T cells against a given thread with antagomirs. The results of this experiment will be verified with luciferase reporter system. To check the significance of gene expression Dnaja2 and Dnajb1 in CD4 + T cells will undergo transfection with plasmids that enhance the expression of Dnaja2 or Dnajb1 or both genes together. The protein profile showing the response of cells to genetic modification will be investigated with Western Blot technique. The results of in vitro studies will be verified by a series of experiments in mice immunized towards EAE. We assume that the results of research conducted within this project will broaden the state of knowledge of the mechanisms underlying autoimmune demyelination process and will potentially contribute to the improvement of new gene therapy in the future.

The subject of this project correlates with current of research on autoimmune demyelination carried out in the Laboratory of Neuroimmunology in Department of Neurology, Medical University of Lodz for many years. Several scientific reports indicate a link between the expression of miR-155 and the process of differentiation of T cells. MiR-155 was one of the first miRNA which increased level of expression was observed in T cells in response to TCR stimulation. In spite of numerous studies carried out on the miR -155 functionality of the role of the different strands of miR-155, miR-155-3p, and miR-155-5p in the CD4+ T cells in response to antigen myelin has not yet been verified.