The proper functioning of the immune system requires **perfectly regulated communication** between immune cells and between immune cells and other cell types (e.g. those lining blood vessels or the respiratory tract). This communication is carried by membrane adhesion molecules, as well as soluble mediators (cytokines) interacting with specific membrane receptors. Inflammation often occurs in response to injury and infection and the immune system directs all its stages: from initiation to termination. However, if the trigger of inflammation persists or if some elements of control fail, chronic inflammation may develop, often leading to still incurable, debilitating or serious diseases including rheumatoid arthritis, atherosclerosis and multiple sclerosis. Not without significance is also a mutual relationship between inflammatory diseases and cancer. **ADAM17** (**A D**isintegrin **A**nd **M**etalloproteinase 17), a member of ADAM family, is a ubiquitous membrane enzyme playing an important role both in physiological and pathological inflammation. ADAM17 is a sheddase, i.e. an enzyme that releases biologically active, extracellular domains of numerous membrane proteins, such as: cell adhesion molecules, cytokines, chemokines and growth factors, as well as their receptors from the plasma membrane. ADAM17, via the shedding of membrane proteins, modifies the cell surface and supplements the microenvironment in soluble, diffusing active molecules, thus strongly influencing intercellular communication during inflammation. Proinflammatory effects of ADAM17 are attributed to ADAM17-mediated shedding of a number of proteins involved in the generation and progression of inflammation including one of the most potent proinflammatory cytokines, TNF. **Is it the only mechanism?**

The results of our experiments prompt us to formulate the hypothesis, that ADAM17 promotes inflammation not only by providing soluble mediators but also through sensitizing the cells to proinflammatory stimuli. We found that when we, via genetic engineering, strongly limited the synthesis of ADAM17 in the cells (i.e. we caused so-called *Adam17* silencing) the cells showed very low sensitivity to mediators of inflammation (cytokines and bacterial products) and they did not effectively produce certain "defence" proteins such as inducible nitric oxide synthase. We also observed disturbances in signaling pathways involved in the regulation of the expression of genes important for inflammation. But most intriguingly: *Adam17* silencing was accompanied by changes in DNA methylation pattern, which according to the current knowledge, may significantly affect the patterns and levels of gene expression.

The aim of the project is to elucidate the novel mechanism by which ADAM17 promotes inflammation. To achieve this, we plan to study the effects of *Adam17* silencing in two model cell lines of different origin: P388D1 (monocyte/macrophage cell line) and MC38 (epithelium). Utilizing available to us, modern, high-throughput methods we will analyze the changes in: (***i***) transcriptome (i.e. in the set and the levels of genes being transcribed), secretome (i.e. the set and the levels of secreted and shed proteins), and (***iii***) DNA methylation pattern (showing which CpG dinucleotides in the analyzed DNA are methylated and which are not). We also plan to identify elements of intracellular signaling pathways differently regulated in normal cells and in cells deprived of ADAM17. For the transcriptome analysis we will use the **RNA-seq** technique, which is based on next-generation sequencing and allows for the sensitive and precise quantitative analysis of mRNAs and non-coding RNAs. For the secretome analysis we will use the comparative, quantitative **mass spectrometry (MS) technique – SILAC**. Based on the results of RNA-seq we will select the genes that will be subject to analysis of the DNA methylation pattern. We will also answer the question whether ADAM17 silencing affects the global DNA methylation level as well as the levels and activity of the enzymes responsible for this process. The potential involvement of the genes selected via RNA-seq and MS-SILAC in intracellular signaling pathways will be analyzed using the sophisticated bioinformatics program **Ingenuity Pathway Analysis tool**, which allows for integration of data from multiple analyses. The molecular engineering, as well as the immunochemical methods, will be used to verify the results of RNA-seq and MS-SILAC and for in-depth analysis of signaling pathways. We are confident that this modern and multifaceted experimental approach will lead to achieving the project objectives but will also provide additional benefits: (***i***) the results of the analysis of transcriptomes will be uploaded to the open-access worldwide GEO database and thus may be used by the research community for various bioinformatics analyses, not necessarily concerning ADAM17 activities; (***ii***) the results of the comparative analysis of cell secretomes may help to identify new ADAM17 substrates and therefore provide a new insight in ADAM17 biological roles. These results will also be shared with the scientific community; (***iii***) bioinformatics analysis of transcriptomes and DNA methylation patterns may provide information on possible relationships between signaling pathways, DNA methylation and expression of certain genes that may extend beyond ADAM17 effects.