Despite the development of novel antibiotics and introduction of protective vaccines, chronic bacterial infections associated with biofilm formation remain a major unsolved clinical problem (relevant for e.g. cystic fibrosis, chronic sinusitis and otitis, non-healing wounds, biofilm on implanted biomaterials). If the bacteria infecting the wound or mucous layer are not eliminated (by the immune system or antibacterial substances such as antibiotics) within the first few hours of infection, they can survive by forming so called biofilm (biological film). This microbial "community" formed by numerous bacteria of one or several species is surrounded by polymeric substances produced by these microbes. Bacterial biofilm facilitates adhesion of bacteria and hinders both antibiotic penetration and bacterial phagocytosis by infiltrating neutrophils and macrophages. The following development of chronic inflammatory response leads to a tissue damage due to the presence of both toxic bacterial products and activated neutrophils (we have coined them BANs – biofilm-associated neutrophils). In cystic fibrosis, aggravation of respiratory tract inflammation has been shown to correlate with numbers of infiltrating neutrophils.

Scientific goals of the project focus on answering the following questions:

- How does biofilm microenvironment of various bacteria (e.g. *P. aeruginosa* and *S. aureus* – major pathogens in cystic fibrosis) activate neutrophils and whether their harmful activity (production of proinflammatory mediators without real antibacterial activity) can be neutralized?
- Could proper macrophage 'training' increase the efficacy of innate immunity mechanisms that are usually ineffective in chronic infections associated with biofilm formation?

We are planning to:

- Isolate high biofilm-producing strains of *P. aeruginosa* and *S. aureus* strains from patients with severe cystic fibrosis.
- Investigate whether the selected bacteria and components of their biofilms can polarize neutrophils into BAN phenotype.
- Evaluate whether macrophage training with β-glucan (polarization into bacteria-killing cells) can limit/suppress biofilm formation in mice infected with the selected strains of *P. aeruginosa* and *S. aureus.*

The proposed research is an innovative attempt to take advantage of macrophage memory – a recently discovered property of macrophages that was previously only attributed only to lymphocytes – in treatment of chronic 'neutrophil' inflammation associated with biofilm formation. Since both active and passive immunization (using antibodies) do not yield desired therapeutic effect in treatment of diseases such as cystic fibrosis, development of methods for regulation of defense mechanisms seems an attractive opportunity for creating an alternative treatment strategy. Further studies are necessary to confirm whether macrophage training *in vivo* could be used for therapy of chronic biofilm infections of various etiologies.