Abstract

The complement system, consisting of dozens of serum proteins, is a part of our innate immunity and constitutes the first-line defence of the organism against invading pathogens. Its activation can occur via the classical, lectin, or alternative pathway. Mutations in the alternative pathway (e.g., mutations in factor B) are well-known etiological factors of several autoimmune diseases. In contrast, knowledge about mutations in components of the classical/lectin pathway and their role in autoimmune diseases is very limited. My Ph.D. project aimed to functionally analyse and test the potential application of mutants of the early proteins of the complement cascade. The obtained results present a novel application of factor B and C2 mutants, which are components of key enzymatic complexes (convertases) of the alternative and classical pathways of the complement system. Moreover, my results make an important contribution to knowledge about the role of the complement system in the development of autoimmune diseases. In my project, I was able to demonstrate that factor B K323E mutant can be applied as a standard in the assay detecting the pathogenic antibodies, the causative agent of C3 glomerulopathy. In addition, the proposed factor B variant can also serve as a positive control to screen for other factors, including mutations, that alter the activity of the complement system. As part of the project, I performed an extensive functional analysis of C2 variants designed on the basis of mutations identified in patients with nephrological pathologies. Of the mutations analysed, one (S250C) resulted in protein forming a hyperactive and more stable classical convertase, and this effect was more pronounced in the presence of the CD55 inhibitor. Later in the project, I described another case of a patient with C2 mutation S250C and identified another R249C substitution leading to a gain-of-function (GOF) phenotype, indicating the presence of gain-of-function mutational hotspot. Furthermore, I completed the study by proposing a mechanism of how such GOF C2 variants can be involved in the damage of glomerular vascular endothelium during the development of glomerulopathy. The significant increase in C3b deposition on the surface of glomerular endothelial cells after incubation with C-reactive protein and C2 mutant is in agreement with the clinical picture, where the post-infection manifestation of the disease occurs. Importantly, to that moment there was no clinical data on naturally occurring mutations in the C2 protein, thus obtained results may contribute to a better understanding of the etiology of autoimmune diseases, to improved risk assessment, and to the possibility of constructing effective therapy. The next part of the project revealed how to transform a potentially pathogenic factor into a useful tool with therapeutic potential. I confirmed that C2 mutants able to form a classical convertase of the prolonged half-life can enhance the cytotoxic effect of anti-CD20 antibodies on primary leukaemia cells. These antibodies are potent activators of the complement system and are routinely used in the treatment of chronic lymphocytic leukaemia and non-Hodgkin's lymphoma. My studies indicate that such C2 protein not only has the potential to reduce the therapeutic dose of anti-CD20 antibodies and consequently prevent the development of resistance but may also act as a universal supporter of antibodies primarily not inducing the complement cascade. In case of positive results from future *in vivo* studies on an animal model, C2 mutant may become the subject of studies of preclinical character.