Mutations of early proteins of the complement cascade – functional characteristics, therapeutic potential and applications.

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Complement convertases are enzymatic complexes which play a critical role in propagation and amplification of the complement cascade. Under physiological conditions convertases decay within minutes after being formed. Prolongation of their half-life due to presence of autoantibodies preventing convertase dissociation results in pathogenic conditions often manifested by renal diseases. Recently our group has developed a new method for a direct measurement of convertase activity in a patient's serum. Having numerous advantages over traditional methods, one drawback is connected to the batch-to-batch variability of rabbit erythrocytes employed in the procedure. Their quality and condition influence the vulnerability to complement and makes the comparison of the data from different experiments problematic. Abovementioned problems demand application of internal standard in each experiment. Obtaining a defined preparation of autoantibodies is complicated due to ethical and practical considerations.

The aim of the research was to obtain a compound which would mimic pathogenic autoantibodies in convertase assay. Employing HEK293 Freestyle eukaryotic expression system we produced several mutants of factor B, which were previously described in a literature as the gain-of-function variants. In order to simplify the purification process all proteins were 6xHis-tagged at C-terminus and such modification did not influence their specific activity as proven by comparison with purified, native protein isolated from serum.

Two of the tested mutants, namely K323E and D279G showed the dominant character and a significantly prolonged convertase half-life when added to normal human serum. We conclude that K323E and D279G are easy to express variants of factor B, which can be used as positive controls in the functional assays measuring the activity of complement convertases in full serum.

KSZTAŁCIMY NAJLEPSZYCH *– kompleksowy program rozwoju doktorantów, młodych doktorów oraz akademickiej kadry dydaktycznej Uniwersytetu Gdańskiego. Zad. 2. Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS)*

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EUROPEAN UNION
EUROPEAN
SOCIAL FUND

Project co-funded by European Union under the European Social Fund