Summary

Studies on the reactivity of human serum antibodies with the bacterial OmpC protein as a marker of humoral immunodeficiency

Gram-negative rods of the *Enterobacteriaceae* family are pathogens that cause diarrhea, typhoid fever, bacillary dysentery and other intestinal diseases. These microbes are a problem, especially in developing countries, where they cause the most fatal infections. Infections usually occur through the oral route through contaminated food and water, but also through personal contact. Therefore, at a time when the level of population migration has significantly increased, we are undoubtedly more exposed to contact with pathogens. Children, the elderly and people whose immune systems are not functioning properly are most often infected. In the effective treatment of bacterial infections, the emergence of strains resistant to antibiotics and other antimicrobial drugs is a huge problem. To undertake effective treatment, it is important to choose the right drugs and start therapy as soon as possible. The methods used in diagnostic laboratories require both financial and time expenditure, which makes the fight against these pathogens much more difficult.

A person with a properly functioning immune system is much better at dealing with pathogens than an individual whose immune system is malfunctioning, and patients suffering from primary immunodeficiencies (PIDs) are more exposed to infections. Primary immunodeficiencies are a group of diseases manifested by defects in the functioning of the immune system of genetic origin. Among PIDs, those associated with antibody deficiency are the most common, with 1 case per 300 to 500 live births.

Due to defects in the immune system, infections develop faster, are more difficult to treat and often recur. The therapy used for antibody deficiencies is based on prophylactic or interventional antibiotic therapy, vaccinations, substitution treatment (with immunoglobulins, complement components, growth factors) or bone marrow transplants. The most effective, least invasive and safest way to fight pathogens is treatment with intravenous immunoglobulin preparations (IVIG – intravenous immunoglobulin) or in the form of subcutaneous injections. Infection prevention and treatment through the supplementation of missing antibodies is a very promising therapy for many patients, including those suffering from infections accompanying primary and secondary immunodeficiencies.

The etiological factor of infections occurring in PID patients are often bacteria from the *Enterobacteriaceae* family, e.g. bacteria of the genus *Shigella*. The cause of this type of infection is *Shigella flexneri* and *Shigella sonnei*, which cause shigellosis, bacillary dysentery,

and colitis. In people with a properly functioning immune system, these infections may resolve spontaneously or after administration of an antibiotic. On the other hand, patients with deficiencies are much more difficult to treat effectively and infections often recur, causing severe inflammation of the mucous membranes of the anus and intestines, accompanied by bloody and purulent diarrhea.

In the effective treatment of many infections, including intestinal infections, it is important to determine the pathogen that is the etiological factor of the infection as soon as possible and to quickly implement the therapy. In the case of patients with PID, we can diagnose the deficiency of specific antibodies before the infection occurs and, by supplementing them, prevent infection, and when it has occurred, thus facilitating treatment.

Among the patients with humoral deficiencies, there are those whose general immunoglobulin levels are at the appropriate level, but persistent, recurrent infections that do not respond to conventional treatment are observed. This clinical picture suggests a deficiency of specific antibodies (SAD), i.e. antibodies directed to a specific antigen/pathogen. The result of the SAD assay must be an anti-OmpC *Shigella flexneri* antibody titre indicating a deficiency.

To develop such an assay, in addition to the native OmpC protein, protein conjugates were synthesized based on the BSA protein and a cyclic/linear peptide containing the binding aminoacids sequence of the OmpC protein. With the help of these three antigens, antibody titers were determined by ELISA in patients with primary immunodeficiencies (PIDs) and patients with recurrent respiratory tract infections (RPIs) and in healthy individuals. Comparative analysis of mean specific antibody titres among the studied groups makes it possible to develop a serological test to diagnose humoral immunodeficiencies.

Previous research conducted by our team allowed us to confirm the presence of anti-OmpC antibodies from *Shigella flexneri* in umbilical cord blood serum. The antibodies found in umbilical cord blood are passed on to the baby by the mother, which may suggest that they are protective. To confirm this thesis, a protection experiment was designed on a mouse model. The antibodies for the animal test were obtained from human/mouse serum by affinity chromatography on immobilized OmpC protein. Animals were passively immunized with human or mouse anti-OmpC antibodies and then infected with *Shigella flexneri* 3a. The obtained results confirmed the protective effect of isolated anti-OmpC antibodies, which is the basis for the development of therapeutic supplementing antibody deficiencies.

Reactivity studies of the native OmpC protein, recombinant OmpC protein and BSA protein conjugates – linear/cyclic peptide containing the OmpC protein binding sequence are an important basis for research on the development of a test for determining specific antibody deficiencies and a therapeutic supplementing these antibodies deficiencies.