Dissertation abstract

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Determination of serological specificity of human antibodies
binding to lipopolysaccharides of S and R forms of *Proteus mirabilis* O3

Rheumatoid diseases are an example of civilization diseases with multifactorial causes such as environmental impact, genetic predisposition, or human microbiome. The latter represents antigens that, through molecular mimicry, can contribute to the development of an inflammatory response induced by a humoral response and the formation of antibodies. As a research hypothesis, it was assumed that one of the elements of antibodies associated with the presence of opportunistic bacteria are antibodies against bacteria from the genus *Proteus.* These bacteria are the natural microflora of humans, but they are also a frequent cause of urinary tract infections. It is assumed that *Proteus* protein and sugar antigens may contribute to the formation of antibodies that may be responsible for inflammatory reactions through the molecular mimicry. An important element in the development of rheumatoid diseases are antigen-antibody immune complexes. Therefore, the study of these complexes is an important element that supplements knowledge about the causes of rheumatoid diseases. The research hypothesis was verified by the following specific objectives:

1. An attempt to determine the presence of antibodies in the sera of patients that bind to lipopolysaccharide antigens. As a research model, lipopolysaccharides of the smooth form and two Ra and Re mutants were chosen, which allows to verify which endotoxin fragment is responsible for binding of antibodies. To confirm the specificity of the reaction, synesthetic antigens that are part of the O-specific part of the lipopolysaccharide *Proteus mirabilis* O3 will be used.
2. Determination of the presence of antibodies that bind to a fragment of protein antigens that is a fragment of urease. Synthetic peptides and their modifications will be used here.
3. Formation of antigen-antibody immune complexes with sugar antigens using ellipsometry and atomic force microscopy techniques.

The study showed that both patients with rheumatoid arthritis and healthy persons (blood donors) have antibodies recognizing the lipopolysaccharide of the smooth form Proteus

mirabilis S1959 as well as the synthetic fragment of this lipopolysaccharide, which is the lysine and galacturonic acid residue. The highest antibody titers were noted for the Ra form containing no O-specific part. Because a large portion of the lipopolysaccharide is not substituted with O-specific chains, this may indicate a strong exposure of the core antigens and thus the ease of generating antibodies. It is also not excluded that the more hydrophobic form of the Ra-type lipopolysaccharide has greater adjuvanticity, which generates more antibodies. The lowest number of antibodies in the sera of patients and healthy persons was recorded for the form of a rough Re type mutant. The study also showed that by adsorption, at least some of the antibodies that recognize lipopolysaccharide, also recognize type I collagen in which the lysine residue is exposed. This type of antibodies common to lipopolysaccharide and binding to collagen may cause an autoimmune reaction through the Fc fragment. Such antibodies can activate the immune system, complement system or macrophage cells.

This paper attempts to develop / use a new panel of tools to study lipopolysaccharide, allowing precise measurement of physico-chemical interactions and visualization of antigen-antibody reactions. Using the method of ellipsometry and atomic force microscopy, it was possible to confirm that the thickness of the lipopolysaccharide nanolayers corresponds to the chemical structure. Correspondingly, the thickness of the layer was dependent on the amount of O-specific part. Data from ellipsometry have been confirmed by atomic force microscopy where, using the PicoTrec technique, ie. a chemical rod, the sites for combining antibodies with antigen were determined.

As shown by the results of the work, these methods can be used to develop tools that enable precise measurements of physical interactions and chemical and visualization of the antigen-antibody reaction. In the future, these techniques may be used, for example, to study the binding of lipopolysaccharide to effector cells of the immune system (e.g. macrophages, lymphocytes). In the next stage, maybe it is possible to search for inhibitors of inflammatory reactions in endotoxemia or chronic diseases caused by molecular mimicry.

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