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Abstract of the doctoral dissertation

Department of Microbiology and Parasitology, Jan Kochanowski University in Kielce

mgr Katarzyna Durlik-Popińska

Supervisor: prof. dr hab. Wiesław Kaca

Additional supervisor: dr Paulina Żarnowiec

Characteristics of rheumatoid arthritis patients' antibodies binding to lipopolysaccharides *Proteus mirabilis*

Rheumatoid arthritis (RA) is an autoimmune systemic inflammatory disorder. RA is a serious medical, social and economic problem. The complex and unclear etiology makes prophylaxis, diagnostics and pharmacotherapy difficult, leading to serious health effects of patients. It is believed that RA is developed in predisposed individuals by a combination of genetics, epigenetic modifications, and environmental factors such as smoking, or viral and bacterial infections. Understanding the factors and mechanisms responsible for the onset and course of the disease is one of the main goals of research in RA.

One of the bacteria associated with the development of RA is *Proteus mirabilis*. Like other gram-negative bacteria, *P. mirabilis* is characterized by the presence of an immunogenic lipopolysaccharide (LPS) covering the surface of the cell wall. However, the importance of specific anti-LPS P. mirabilis antibodies in the study of the influence of bacteria on the development of RA is controversial, as these antibodies can also be detected at a high level in healthy people. Nevertheless, the work so far has focused on testing the level of anti-LPS antibodies. There is no information about the properties of the detected antibodies which, in conditions of disturbed homeostasis of the immune system, can promote the development of the disease.

The aim of this dissertation was to characterize *P. mirabilis* LPS binding antibodies in terms of quantity, class, binding strength, cross-reactivity and the ability to activate complement after binding to the antigen

125 human sera belonging to patients suffering from RA and other rheumatic diseases, and from healthy blood donors were used in the study. Moreover, during the research, 24 different antigens in the form of lipopolysacchardides, type I collagen and synthetic haptens corresponding to the LPS fragments of *P. mirabilis* were used. The experimental part of the work was conceptually divided into several stages. The first, concerning the analysis of biological material in the form of endotoxin preparates and sera, was aimed at qualitative analysis of the used antigens and the characterization of sera in terms of the presence of autoantibodies. In this stage, the following techniques were used: infrared spectroscopy, polyacrylamide gel electrophoresis and serological methods. The obtained information allowed for a reliable analysis of the generated results in the further parts of the work. In the

next stage, an attempt was made detect anti-LPS *P. mirabilis* antibodies in the tested sera and the class of detected immunoglobulins was determined. The last stage of the work was to assess the binding strength of anti-LPS antibodies in the sera of RA patients, the ability to cross-react and the potential for complement factor C3b deposition on purified antibodies immune complex.

Using of ELISA, Western blot and Dot blot techniques, it was shown that, depending on the test performed, the level of detected antibodies binding to LPS *P. mirabilis* O3 was similar or higher ($p < 0.05$) compared to patients with other rheumatic diseases and blood donors. The level of antibodies was individual and did not depend on the health status of the blood donor and the level of autoantibodies ($p > 0.05$). The analysis of the obtained data allowed not only to assess the level of serum reactivity with the tested LPS, but also to determine the appropriate relationships. The detected antibodies belonged mainly to the IgG class ($p < 0.05$), but their amount was nevertheless correlated with the level of IgM antibody response ($p < 0.05$). A positive correlation was shown between the serum reaction with the synthetic Lys-GalA-PAA hapten and other LPS containing lysine residue. In addition, the age of the blood donor and the treatment regimen also had an impact on the serum response.

Despite a tendency indicating a lower binding strength of anti-LPS *P. mirabilis* O3 antibodies derived from the sera of RA patients, statistical analysis did not show a difference in their avidity and affinity compared to healthy blood donors. The analysis of the affinity chromatography results showed higher level of antibodies binding to LPS *P. mirabilis* O3 in the group of RA patient sera. Moreover, the statistical analysis showed a correlation between the concentration of purified antibodies and the presence of disease markers ($p < 0.05$).

Studies had also shown that immune complexes of purified antibodies can bind C3b factor, and the level of deposited protein depends on the level of reaction with the antigen and its type, as well as the presence of autoantibodies.

Based on the analysis of the obtained results, it can be concluded that the level of anti-LPS *P. mirabilis* O3 antibodies is not associated with the occurrence of RA. Antibodies detected in RA patients tend to have decreased binding strength and increased cross-reactivity compared to healthy blood donors. Purified antibodies against LPS *P. mirabilis* O3 show the ability to activate complement during the reaction with type I collagen, which may contribute to the aggravation of the disease.