

## **The influence of humic substances and based on them bionanomaterials with silver nanoparticles on the type of immune response**

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Introduction. Screening studies that allow identifying leading molecules are of great importance in the creation of new medicinal substances. One of the very important parameters of a medicinal substance is also its effect on the type of immune response. Macrophages, which are heterogeneous and universal cells and can undergo both phenotypic and functional changes in response to microenvironment signals, are very effectively used for these purposes. The type of developing immune response depends on the type of macrophage activation in the first phase of immune inflammation: by producing IL-12 or IL-10, they contribute to the development of Th1 or Th2 type of immune response.

The goal of this study is a comparative study of the humic ligands influence and created on their basis bionanomaterials with silver nanoparticles (AgNPs) on the type of immune response in a culture of mouse peritoneal macrophage cells.

Materials and Methods. A study was conducted of humic ligands, as well as bio-nanomaterials with AgNPs synthesized on their basis (HS-AgNPs). Among the basic matrices were humic substances (HS) from various types of peat and coal, as well as coal HS modified with various quinones. These bionanomaterials were synthesized in the Laboratory of Natural Humic Systems of the Faculty of Chemistry, Lomonosov MSU.

In immunotropic activity study C57BL/6 female mice aged 8-10 weeks were used. Peritoneal macrophages were harvested by lavaging the mouse peritoneal cavity with ice-cold physiological saline. After 48 hours of incubation, nitric oxide (NO) production was assessed based on the nitrite content in the supernatant, cell proliferation, and arginase activity. Nitrite levels in the cell supernatants were determined by the Griess reagent method (Sigma-Aldrich, USA). Arginase activity was assessed in the macrophages' lysates using method of urea concentration determination using the Urea-450 test system (Bio-La-Test, Czech Republic) according to the protocol provided with the test system, applying the ChemWell 2910 analyzer (Awareness Technology, USA) at a wavelength of 540 nm.

Results. It was established that some HS samples promote the antigen-presenting cells polarization according to the classical type (M1) by increasing the activity of NO synthase and inhibition arginase. Moreover, all these samples don't have a toxic effect on cells. The functions of macrophages (M1), which are traditionally associated with such activation as phagocytosis of microorganisms, microbicidal activity, induction of inflammation, antitumor activity, are stimulated by Th1 cytokines, primarily IFN- $\gamma$ , as well as IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-8, IL-12. But, some HS-AgNPs samples on the contrary, activate the alternative properties of macrophages (M2). Moreover, almost all of these samples, cause a significant inhibition of the proliferation of the tested cells. The properties of M2 activators are aimed at the formation of the extracellular matrix, repair and remodeling of tissues, suppression

of inflammation, stimulation of vascular formation, phagocytosis of apoptotic cells, synthesis of anti-inflammatory cytokines - IL-10, TGF- $\beta$ , IL-4, IL-1ra.

Conclusion. The conducted screening study made it possible to identify leading molecules for the development of medicinal products with antibacterial, anti-inflammatory and wound-healing properties.

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