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Immunophenotypic and Morphometric Evaluation of Bone Marrow Macrophages Culture Stimulated with Sodium Aminodihydrophthalazinedione *In Vitro*

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Morphometrical and immunophenotypical characteristics of macrophage cell cultures obtained from rats bone marrow were evaluated. Obtained cell cultures were stimulated with macrophages activator aminodihydrophthalazine-

dione (ADPN) *in vitro* for 24, 48 and 72-hour. Cells, nucleus, cytoplasm area were measured and nuclear cytoplasmic ratio (NCR) were calculated. Proliferative activity was assessed by the presence of the Ki-67 protein. The phenotype of macrophages was determined by the expression of the CD163 and F4/80 receptors. The content of the growth factor TGF- β in the cytoplasm of macrophages was estimated. Stimulation of macrophages by 50 $\mu\text{g}/\text{mL}$ ADPN promoted an increase in the number of CD163⁺ cells and content of this marker with an increase of cultivation time. On the contrary, the action of 100 $\mu\text{g}/\text{mL}$ ADPN leads to an increase in the proportion of F4/80⁺ cells in the culture and to an increase of the marker content with an increase of cultivation time. The accumulation of TGF- β occurs during 48 h of cell cultivation under the action of 50 $\mu\text{g}/\text{mL}$ ADPN. Increased amounts of the factor persist for 72 hours. 100 $\mu\text{g}/\text{mL}$ ADPN enhanced the formation of TGF- β within 48 h, and inhibited it in 72 h in the presence of the substance. When ADPN is added to the culture of monocytes, the maturation of macrophages is accelerated. Moreover, the substance also has a pronounced dose-dependent effect on bone marrow macrophages.

Keywords: macrophages, bone marrow, sodium aminodihydrophthalazinedione