ANALYSIS OF IMMUNOMODULATING PROPERTIES OF CYTOCHALASIN B INDUCED MEMBRANE VESICLES, ISOLATED FROM HUMAN MELANOMA CELLS IN VITRO

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1. Introduction

Membrane vesicles are membrane-bound structures of various sizes and are secreted by different types of cells. The number of naturally produced membrane vesicles by cells is low. Cytochalasin B is a cell-permeable mycotoxin that has a stimulating effect on the formation of induced membrane vesicles. Cytochalasin B induced membrane vesicles (CIMVs) of tumor cells, due to their ability to fuse with recipient cells through endocytosis and release their contents into the cytoplasm of recipient cells, are considered as a promising vector for targeted delivery of various antitumor agents. CIMVs isolated from tumor cells carry the same antigens on the surface as the parent cells, so they are a promising source of tumor antigens for presentation to the cells of the immune system. In this way CIMVs of tumor cells can be used to develop therapeutic vaccines for the treatment of cancer. The immunomodulatory properties of membrane vesicles consist in the genetic modification of parental tumor cells with the interleukin 2 (IL-2) gene, which is one of the most effective cytokines that induce tumor-specific immune responses. The aim of the study was to analyze the interaction of CIMVs isolated from human melanoma cells with human peripheral blood mononuclear cells (PBMC) in vitro.

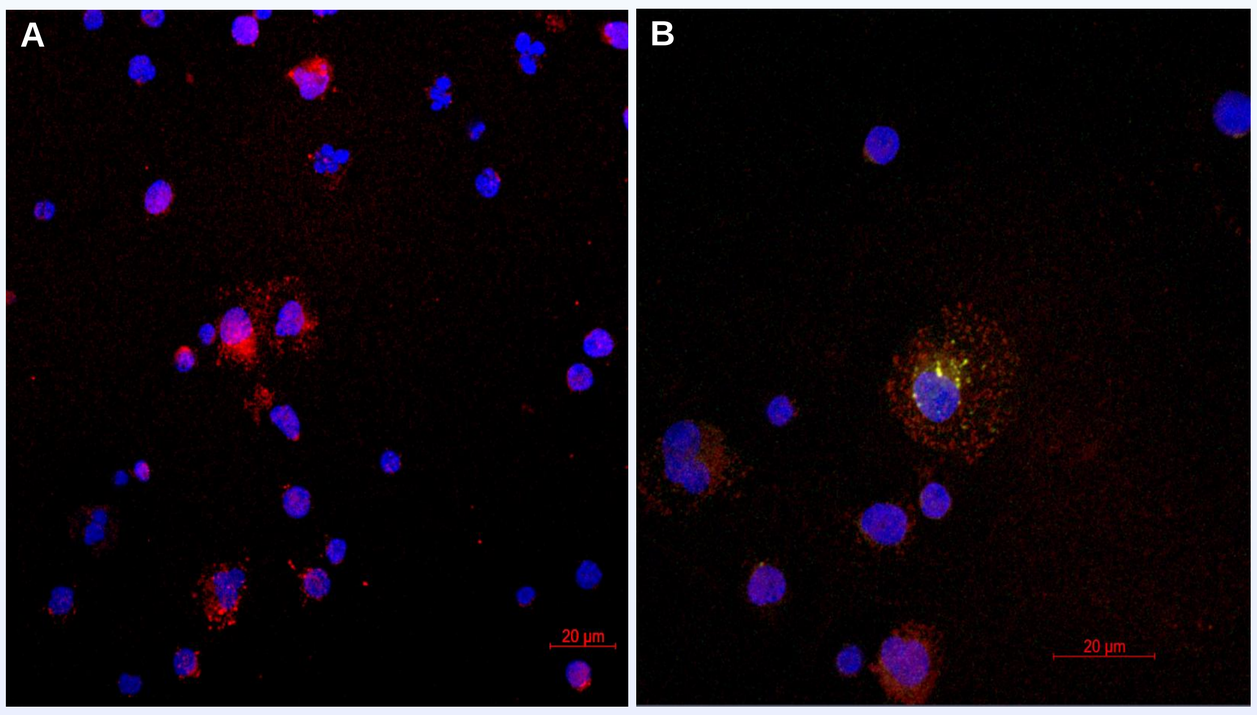
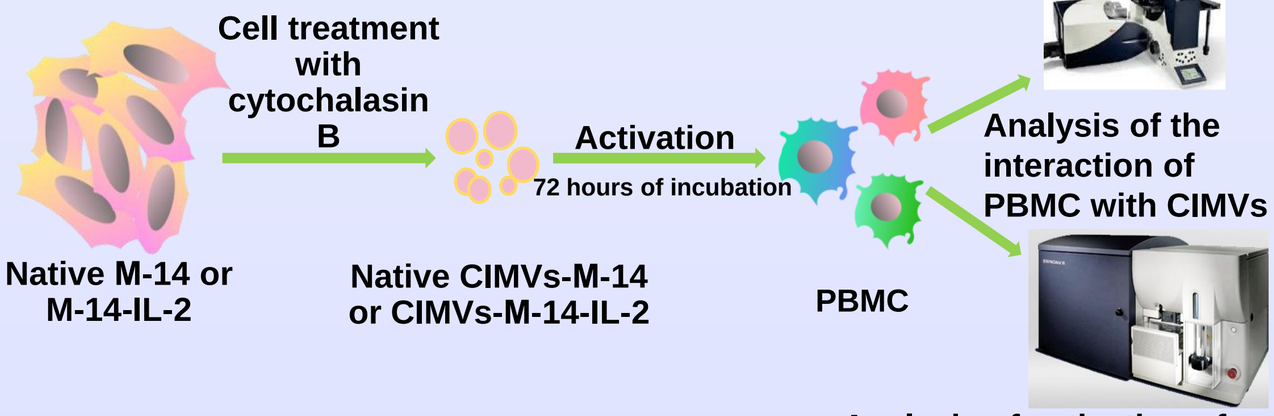


Fig. 2: Confocal microscopy of PBMC (AlexaFluor 647 / Dapi) after incubation with CIMVs (FITC). A – Native PBMC (40x); B – PBMC + CIMVs (63x)

2. Materials and methods



Analysis of activation of PBMC after cultivation with CIMVs

3. Results and discussion

We have shown that CIMVs of native M-14 cells stained with Vybrant™ DiO Cell-Labeling Solution fluorescent dye are able to fuse with PBMC in co-culture, which leads to their active interaction and exchange of cytoplasmic membrane components. The difference between control T-lymphocytes without the addition of CIMVs and T-lymphocytes culturing with CIMVs by 4,3% was noted. But the difference in population of granulocytes was higher - by 27,5%. This can be explained by the ability of granulocytes to phagocytosis.

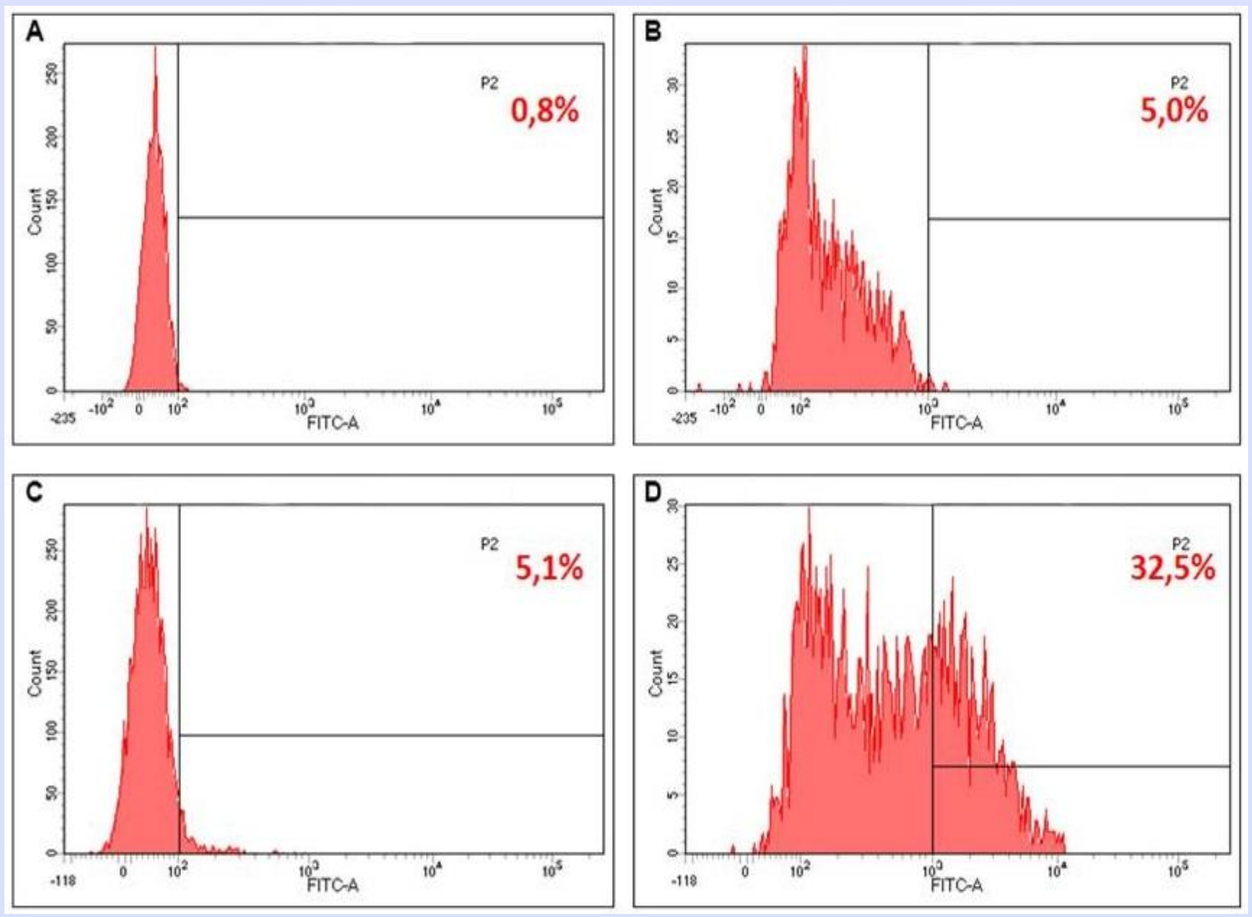
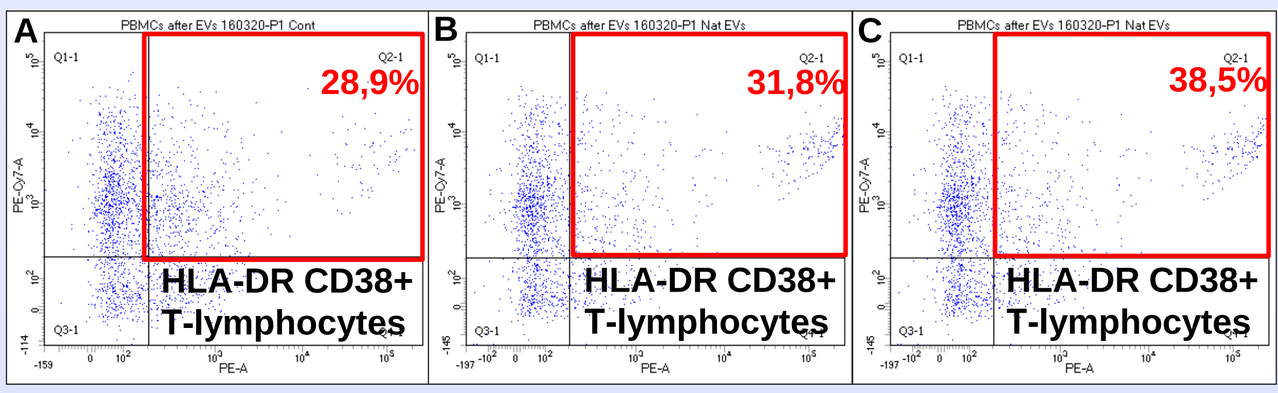
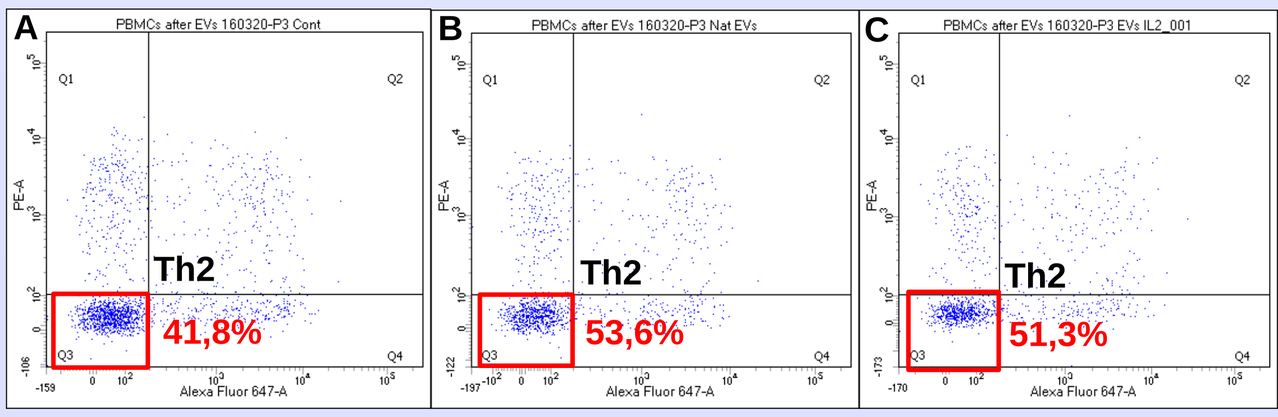
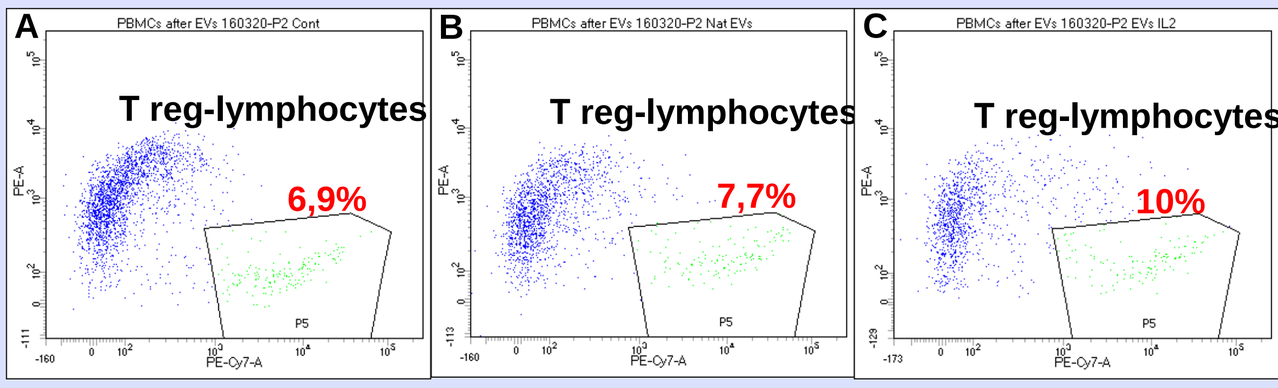


Fig.1: Flow cytometry of T-lymphocytes and granulocytes after cultivation with CIMVs of M-14 cells. A – T-lymphocytes control; B – Granulocytes control; C – T-lymphocytes + CIMVs; D – Granulocytes + CIMVs.

It was also shown using flow cytometry that the addition of CIMVs of native M-14 and M14-IL2 cells at a concentration of 145 µg/ml increases the number of HLA-DR CD38+ T-lymphocytes compared with the control by 3% and 9,6%, respectively. An increase of T-helpers 2 (Th2) by 11,8% and 9,5% was also noted. There was an insignificant increase in the population of T-regulatory cells compared with the control by 0,8% and 3,1%, respectively. There were no statistically significant differences in the natural killer population.







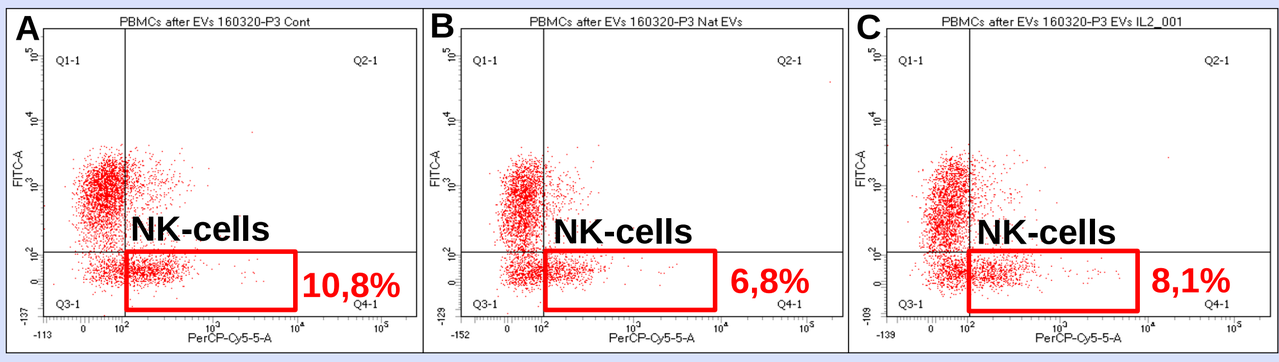


Fig. 3: Cytofluorometric analysis of PBMC populations after interaction with CIMVs of M-14 cells. A – Native PBMC; B – PBMC + CIMVs (145 µg/ml); C – PBMC + CIMVs-IL-2 (145 µg/ml)

4. Conclusion

Thus, due to the ability to present tumor antigens to cells of the immune system and activate the antitumor immune response, CIMVs of tumor cells are a promising object for the development of therapeutic antitumor vaccines. However, further studies are needed in this area to study the mechanisms of interaction of CIMVs with immune cells and possible ways of modulating the immune response.

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