ABSTRACT
of the dissertation for the degree
Doctor of philosophy (Ph.D.)
6D060700-Biology

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Investigation of myeloid-derived suppressor cells in different experimental models of chronic inflammation

General description of the thesis. Dissertation is devoted to a comprehensive study of the functional and phenotypic characteristics of myeloid-derived suppressor cells (MDSCs) in different experimental models of chronic inflammation and their combinations, as well as to a study of possible pharmacological ways of MDSC elimination.

Relevance of the topic. According to the clinical data, chronic inflammation benefits the onset and progression of many diseases including cancer. It activates cells with immunosuppressive functions or stimulates their accumulation in the inflammatory site *via* production of large amounts of proinflammatory mediators. Increased numbers of suppressor cells downregulate functions of effector cells of the immune system. As a result, the immune system is altered which attenuates tumor immune surveillance and favors tumor progression and metastasis. Another factor that affects inflammatory processes and activates the development of cancer is disruption of circadian rhythms (light-at-night, night-shift work, sleep disorders, etc.). It is assumed that disrupted circadian rhythms also result in the development of an immunosuppressive environment which increases the risk of cancer, adversely affecting the regulation of inflammatory signals and immune responses.

MDSC population is one of the major cellular components of the tumor immunosuppressive microenvironment. Physiologically immunosuppressive MDSCs are naturally generated during pregnancy in order to protect the fetus from the attacks by mother's immune cells, as well as during tissue regeneration after injuries to suppress the acute inflammatory process. They also participate in the maintenance of the homeostasis in healthy organism. However certain chronic diseases such as cancer hijack the immunosuppressive potential of MDSCs to overcome the immune surveillance and use these cells for their own favor.

MDSCs have been most investigated in cancer biology. They have been found to weaken anti-tumor immunity and decrease efficacy of immunotherapy in animal models and cancer patients. High levels of MDSCs in the blood of cancer patients have been linked to poor outcome. Despite the significant findings on MDSCs in cancer biology, the role of MDSCs in chronic inflammation is poorly understood. The majority of studies on MDSCs use established animal cancer models, which do not provide adequate information of the possible involvement, characteristics, and the role of MDSCs in the

initial steps of chronic inflammation. Studies also assume that chronic circadian rhythm abnormalities lead to immune effector cell dysfunctions by increasing the production of inflammatory factors (TNFα and IL-6). However, the effect of disrupted circadian rhythms on immunosuppressive cells, including MDSCs, has not been studied. It is important to investigate MDSCs that can alter immunological homeostasis and increase risk of cancer.

In recent years, researchers have been trying to find effective strategies to modulate or eliminate MDSCs in cancer. Most common ways of addressing this issue is to use various antineoplastic drugs that have been used in the clinical oncology. In spite of some success in MDSC elimination, the potential off-target effects of antineoplastic drugs leave some uncertainties for their application to cancer patients or patients with chronic diseases. Therefore, it is urgent to develop and evaluate cell-specific cytotoxic agents for effective MDSC eliminations.

Considering the above-mentioned issues, a thorough study of functional and phenotypic characteristics of MDSCs in chronic inflammation and the role of MDSCs triggered by chronic inflammation in the development of cancer is an important aspect of immunological research, which will help to gain more insights into the mechanisms underlying various inflammatory diseases and discover new ways to treat them effectively.

The goal of the study is to investigate phenotypic and functional characteristics of MDSCs in experimental models of chronic inflammation and to develop an effective pharmacological way of targeted MDSC elimination.

To achieve the goal the following **tasks** have been designated:

1 To study phenotypic and functional characteristics of MDSCs in the adjuvant arthritis in an animal model of chronic inflammation;

2 To study the effect of light-at-night stress on phenotypic and functional characteristics of MDSCs in the animal model of chronic inflammation;

3 To study the role of MDSCs induced by chronic inflammation on the growth of a transplanted tumor;

4 To develop a pharmacological approaches to targeted elimination of MDSCs.

Objects of the research. MDSCs derived from the mouse spleen and bone-marrow.

Subject of the study. Functional and phenotypic characteristics of MDSCs in chronic inflammation.

Methods. Animal models, isopycnic centrifugation on the density gradients of percoll, trypan blue exclusion test, cell culture, CFSE proliferation assay, MTT assay, immunomagnetic cell separation, flow cytometry, enzyme-linked immunosorbent assay, spectrophotometry, gelfiltration.

Scientific novelty of the work:

- For the first time, the increased proportion of $CD11b^{+}Ly6G^{high}$ granulocytic and $CD11b^{+}CD49d^{+}$ monocytic MDSC subpopulations with immunosuppressive potential in the mouse model of chronic adjuvant arthritis has been shown.

- Increased accumulation of $CD11b^{+}CD49d^{+}$ and $CD11b^{+}Ly6G^{high}$ MDSC subpopulations induced by light-at-night in mice with chronic inflammation was shown.

- The promotion of the transplanted tumor growth by MDSCs induced by chronic inflammation has been demonstrated.

- The role of exogenous TNFα in augmenting the MDSCs' immunosuppressive potential against T cells and induction of CD62L expression by MDSCs has been identified *in vitro.*

- It was shown that accelerated growth of a transplanted tumor under chronic inflammatory condition was accompanied by increased numbers of CD62L-expressing MDSCs.

- Specific binding of alpha-fetoprotein (AFP) by mouse G-MDSCs and M-MDSCs has been demonstrated.

- Selective elimination of MDSCs by AFP-daunorubicin (AFP-DR) cytotoxic conjugate in tumor-bearing mice which resulted in tumor growth inhibition and upregulation of NK cell levels has been shown *in vitro* and *in vivo*.

The theoretical significance of the work. The obtained results deepen our understanding of the cellular mechanisms involved in the development of chronic inflammation and tumor growth linked to chronic inflammation. Identification of the phenotypical profile of MDSC subpopulations increased in chronic inflammation, as well as the role of these cells in the activation of tumor growth may be used for the future development of approaches to immunotherapy of chronic inflammatory diseases and cancer. The data on the effect of light-at-night on MDSC induction allow us to take a new insight into the increased incidence of chronic inflammatory and oncological diseases observed in people with disturbed circadian rhythms and provide new directions in the prevention and treatment of cancer and chronic disorders.

Practical value of the work. Dissertation work has practical value. We showed that the developed AFP-based cytotoxic conjugate is an effective anti-tumor immunotherapeutic preparation. Its effect is linked to the elimination of immunosuppressive MDSCs. These findings are of interest for clinical immunologists, as well as oncologists involved in the development of novel therapy for cancer patients. As a part of this work, a patent for a method of detection of MDSCs binding AFP has been obtained (Patent of RK, No. 32074, 18.04.2017).

The main results of the research and conclusions:

1 Modeling of local chronic inflammation in mice (adjuvant arthritis) is accompanied by an increase in the levels of granulocytic ($CD11b^{+}Ly6G^{high}$) and monocytic ($CD11b^{+}CD49d^{+}$) myeloid-derived suppressor cells (MDSCs) in the spleen, this indicates that MDSCs are an important component of chronic inflammatory process.

2 Light-at-night stress causes an increase in the level of both granulocytic and monocytic MDSCs expressing the molecules CD62L and CD195 responsible for the migration of MDSCs into the spleen and inflammatory site. At the same time, the level of pro-inflammatory (IL-6) and anti-inflammatory (TGFb) cytokines increases in the

blood. Light-at-night stress amplifies MDSC accumulation in animals with adjuvant arthritis.

3 Local sterile chronic inflammation leads to acceleration of tumor growth, accompanied by increased levels of MDSCs and increased levels of proinflammatory cytokines S100 and TNFα. MDSCs are characterized by increased expression of CD62L. We found that TNFα enhances expression of CD62L by MDSCs generated from bone marrow *in vitro* and suppressor activity of MDSCs against $CD8^+$ T lymphocytes.

4 The oncofetal protein, alpha-fetoprotein, was first found to bind selectively to subpopulations of myeloid-derived suppressor cells. The chemical conjugate of alpha-fetoprotein with daunorubicin has a selective cytotoxic effect on a subpopulation of monocytic MDSCs *in vitro*, and when administered *in vivo* significantly reduces the level of MDSCs, increases the level of NK cells, and decreases the growth of subcutaneous Ehrlich carcinoma which results in an increase in the survival rate of tumor-bearing mice.

All the designated tasks in the dissertation have been completed.

The main statements for the thesis defense:

1 Chronic inflammation induces accumulation of suppressive MDSCs.

2 Light-at-night stress increases the level of MDSCs with migratory potential due to enhanced expression of adhesive molecules CD62L and CD195.

3 MDSCs induced by chronic inflammation accelerate tumor growth.

4 Proinflammatory cytokine TNFα enhances suppressive activity and expression of the adhesive molecule CD62L by MDSCs *in vitro*.

5 AFP specifically binds to MDSCs obtained from tumor-bearing mice.

6 AFP-DR conjugate exerts a selective cytotoxic effect on M-MDSC subpopulation *in vitro*.

7 AFP-DR conjugate is able to selectively eliminate MDSCs in tumor-bearing mice which correlates with decreased tumor growth and increased NK cell levels.

Level of research of the topic. Research in the dissertation is performed at the cellular and molecular levels using experimental models.

Personal contribution of the author. The author independently conducted the literature review on the topic of the research, experiments, statistical processing and analysis of the obtained results, as well as writing and design of the thesis.

Connection with the plan of basic scientific work. The thesis is performed within the projects: 244/GF3 «Effect of elimination of myeloid-derived suppressor cells by cytotoxic alpha-fetoprotein conjugates on antitumor immunity and tumor growth in experiment» (2013-2015 yy) and AP05131710 «Pharmacological approaches to target myeloid-derived suppressor cells (MDSCs) for suppression of chronic inflammation as a stimulant of tumor growth in experimental models» (2018-2020 yy) Ministry of Education and Science of the Republic of Kazakhstan.

Approbation of the work. Materials of the dissertation were presented and discussed at the following international scientific conferences:

- International Scientific Conference of Students and Young Scientists “Farabi Alemi”, 14-16 April, 2015 Almaty, Kazakhstan;

- International scientific-practical conference “Modern problems of biotechnology: from laboratory researches to production”, within the III international Farabi readings, 5-6 April, 2016, Almaty, Kazakhstan;

- International scientific conference “Biomedical innovation for healthy longevity”, 25-28 April, 2016 St. Petersburg, Russia;

- IV International Scientific Conference of Students and Young Scientists “Farabi Alemi”, 10-11 April, 2017, Almaty, Kazakhstan;

- V International Scientific Conference of Students and Young Scientists “Farabi Alemi”, 10-11 April 2018, Almaty, Kazakhstan;

- International Scientific Conference “Biological Markers in Fundamental and Clinical Medicine”, 31 October - 2 November, 2018, Prague, Czech Republic;

- International scientific conference “Actual problems of cell biology and cell technology”, 8-11 October, 2019, St. Petersburg, Russia.

- International scientific conference of young scientists “Fundamental research and innovation in molecular biology, biotechnology, biochemistry”, 28-29 November, 2019, Almaty, Kazakhstan.

Publications. The results of the thesis are reflected in 14 publications, including 3 articles in international journals indexed by *Web of Science* and *Scopus*; 4 articles in the domestic journals recommended by the Monitoring Committee on Education and Science of the Republic of Kazakhstan; 3 abstracts in the materials of international conferences held in foreign countries; 4 abstracts in the materials of international conferences held in the Republic of Kazakhstan. Also 1 domestic patent for invention was received.

The structure of the dissertation. The dissertation was written on 120 pages including adjustment, abbreviations, introduction, literature review, research materials and methods, research findings and their discussions, summaries and 267 references. The dissertation contains 46 figures, 1 table and 2 appendix.