EXPERIMENTAL WORKS

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THE RESTORATION OF HYBRIDOMA CELLS LINES AFTER UNSUITABLE STORAGE

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Cryopreservation stands as a cornerstone in the long-term storage of cells, including hybridoma cells crucial for various research and biotechnological applications. Typically, cells are preserved by freezing them in liquid nitrogen at ultra-low temperatures, typically around −196 °C [1, 2]. This meticulous process ensures their stability and viability for future research endeavors or industrial applications.

However, during the devastating war resulting from the savage Russian invasion, Kyiv, Ukraine's capital, found itself partially encircled by Russian forces. This aggressive move disrupted the infrastructure essential for maintaining vital cell collections. The siege on Kyiv left the hybridoma cell collection in a state of turmoil, deprived of the liquid nitrogen necessary for proper storage and preservation [3, 4].

Consequently, a significant portion of the cells had to be stored at −80 °C, a compromise that has already led to the loss of part of our cell collection.

Aim. In light of this dire situation, the primary objective of this study was to devise optimal conditions for the restoration and recovery of hybridoma cells that produced antibodies targeting protein C. By identifying effective restoration protocols, we aimrd to mitigate the impact of compromised storage conditions and ensure the continued utility of these invaluable cell lines for ongoing research and biomedical applications.

Methods. For cultivation, a clone of cells producing IV-6A antibody that was frozen in the liquid nitrogen, but was transferred to the freezer −80 °C on 01.03.2022. Cells were cultured in RPMI medium (Sigma) that contained 10% of Fetal Bovine Serum (FBS). Three variants of FBS were used: FBS, sterile-filtrated, suitable for cell culture, suitable for hybridoma (F7524, Sigma), USA; FBS, sterile-filtrated, suitable for cell culture, suitable for hybridoma (F2442, Sigma), USA; Fetal Bovine Serum, One Shot format, Glibco, USA (A5256701,ThermoFisher). Cell viability was monitored using a light microscope (Carl Zeiss Jena, Germany) under magnification ×200. After cultivation and accumulation of antibody in cultural liquid cells were spun-down and antibody was obtained by affinity chromatography on Protein G Sepharose (Sigma). ELISA using recombinant protein C, kindly provided by Dr Olexandr Korchynski, checked the activity of antibody in the culture liquid and purified antibody. HRP-conjugated anti-mouse antibody was purchased from (Sigma).

Results. Samples of hybridoma cell lines that were stored in the freezer −80 °C during 6 months were unfrozen according to the standard procedure and cultivated in RPMI medium. Different types of bovine serum were used to select the most appropriate for cells restoration.

The viability of hybridoma cells cultured in RPMI medium with F7524 and F2442 decreased on day 4 of cultivation, and dead cells appeared. Hybridoma cells cultured in RPMI medium with A5256701 had high viability and grew exponentially. The transparent live cells and black dead cells were observed using microscopy on ×200 (Fig. 1). Therefore, only RPMI medium with A5256701 was suitable for the cultivation of hybridoma cells after unsuitable storage.

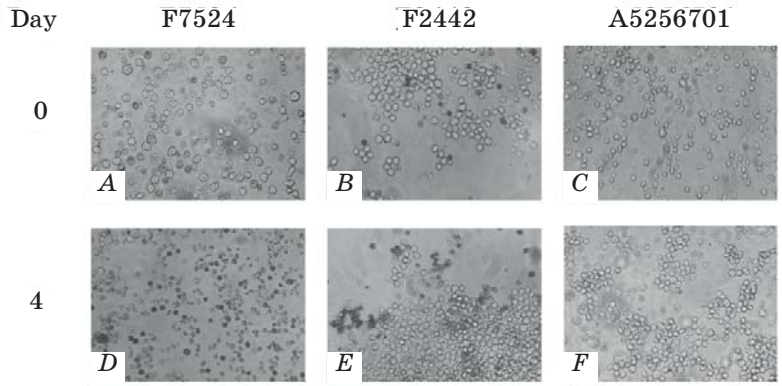

Fig. 1. **Clone of cells producing IV-6A antibody that are cultured in different mediums**
A, D — hybridoma cells which cultured in RPMI medium with Fetal Bovine Serum, sterile-filtrated, suitable for cell culture, suitable for hybridoma (F7524, Sigma), USA; *B, C* — hybrid cells which cultured in RPMI medium with Fetal Bovine Serum, sterile-filtrated, suitable for cell culture, suitable for hybridoma (F2442, Sigma), USA; *C, F* — hybrid cells which cultured in RPMI medium with Fetal Bovine Serum, One Shot format, (A5256701, ThermoFisher), Glibco, USA; *A–C* — hybridoma cells on day zero of cultivation; *D–F* — hybridoma cells on the fourth day of cultivation

ELISA demonstrated that hybridoma cells cultivated in RPMI medium with Fetal Bovine Serum, One Shot format, (A5256701, ThermoFisher) preserved the ability to produce monoclonal antibody IV-6A specific to recombinant full-length protein C (Fig. 2).

Monoclonal antibody IV-6A produced by the restored cell line was purified by affinity chromatography on Protein G Sepharose. The activity of the purified antibody was determined by ELISA. It was confirmed that the antibody was not present in the non-binded fraction of culture medium (Fig. 3).

The activity of the purified antibody that produced by the restored hybridoma cells was compared to the reference antibody which were obtained before full-scale invasion. We observed the less intensive binding of newly obtained antibody compared to the reference one, however the difference was not critical (Fig. 4).

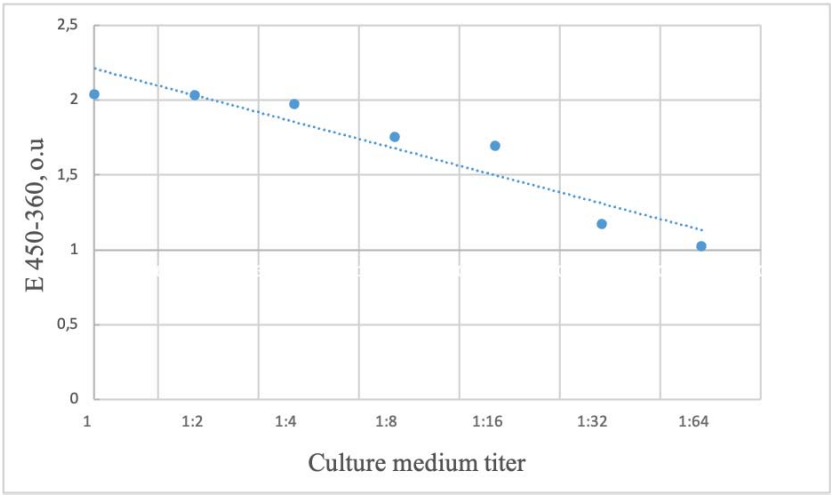

Fig. 2. **ELISA of binding of antibody IV-6A to recombinant full-length protein C produced by hybrid cells which cultured in RPMI medium with Fetal Bovine Serum, One Shot format, (A5256701, ThermoFisher)**

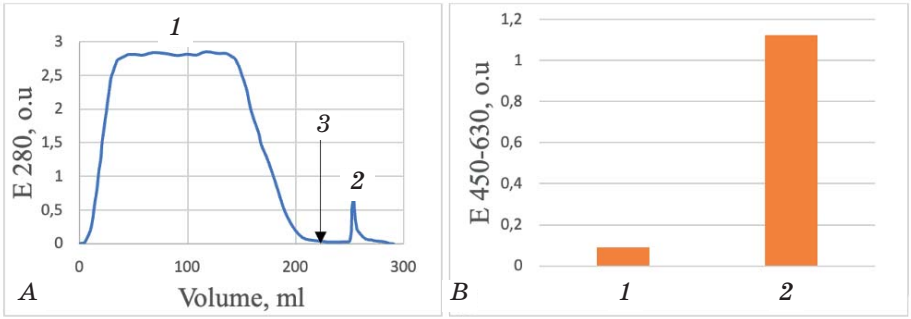

Fig. 3. **Purification and characterization of monoclonal antibody IV-6A from the culture medium of the corresponding hybridoma:** *A* — chromatogram of purification of monoclonal antibody IV-6A by affinity chromatography on a column with Protein G Sepharose: *1* — non-binded fraction; *2* — bound fraction; *3* — elution with 0,1 M Glycine-HCl buffer, pH 3.0; *B* — ELISA of non-binded fraction of culture medium and purified monoclonal antibody IV-6A: *1* — non-binded fraction; *2* — purified monoclonal antibody

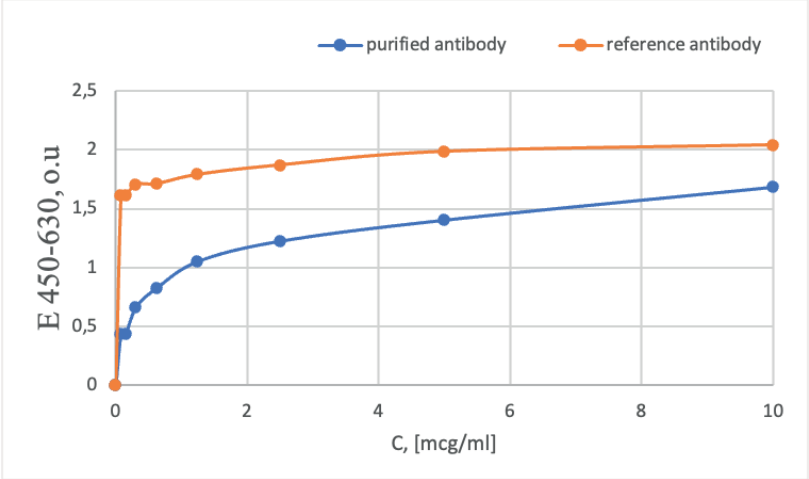

Fig. 4. **ELISA of binding of purified antibody IV-6A and the reference antibody**

Discussion. Use of different sources of fetal bovine serum allowed to select the best medium for the restoration of hybridoma cells that were stored at –80 °C during 6 months. We were able to restore the stable cell line and to obtain the monoclonal antibody able to bind protein C. Such antibody is of interest for the development of test-system for quantification of protein C in blood plasma.

Protein C is an anticoagulant protein that is crucial for keeping the balance between coagulation and anticoagulation. Protein C deficiency leads to the development of thrombotic complications. Therefore, accurate diagnostic measurement of PC activity is essential for patients with cardiovascular diseases [5].

Conclusions. We managed to grow hybridoma cells capable of producing monoclonal antibodies specific to protein C, despite the fact that the cells were forced to be stored in the wrong conditions for a long time. Antibody that has high specificity and affinity for protein C can be used to develop immunochemical test systems for the detection of this fast-reacting protein for diagnostic purposes.

Key words: hybridoma cells, monoclonal antibody, protein C, ELISA.

Authors' contribution

KB was in aim for cell cultivation and manuscript preparation; OK performed ELISA; KK performed chromatography; OG prepared the manuscript and analyzed data; DK maintained the study and orchestrated the work on it.

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Conflict of interest. Authors declare no competing interests.

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REFERENCES

1. *Ishizaki T., Takeuchi Y., Ishibashi K., Gotoh N., Hirata E., Kuroda K*. Cryopreservation of tissues by slow-freezing using an emerging zwitterionic cryoprotectant. *Scientific reports*. 2023, 13(1): 37. https://doi.org/10.1038/s41598-022-23913-3
2. *Gustafsson B*. Cryopreservation of hybridomas. *Methods in molecular biology (Clifton, N.J.)*. 1990, 5: 619–621. https://doi.org/10.1385/0-89603-150-0:619
3. Rebuilding Ukrainian science can't wait — here's how to start. *Nature*. 2023, 614(7949): 593–594. https://doi.org/10.1038/d41586-023-00505-3
4. *Ladokhin A. S*. Ukrainian science in the context of its anticolonial struggle. *BBA advances*. 2023, 3: 100093. https://doi.org/10.1016/j.bbadva.2023.100093
5. *Hryshchuk V.I., Zhernosekov D.D., Bereznytskyi G.K*. Comparative characteristics of C protein purification methods. *Visnyk of Taras Shevchenko Kyiv National University*. 2010, No. 13: 39–43.