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Культивування та кріоконсервування протозойних кишкових паразитів

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Cultivation and Cryopservation of Protozoan Intestinal Parasites

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Among servicemen in the Northern region of Ukraine, intestinal infections are most often caused by *Blastocystis sp.* (5.5 %) and *Dientamoeba fragilis* (4.6 %). In general, the cultivation and long-term storage of strains of protozoan intestinal parasites are carried out to study their virulent potential, the pathogenesis of the diseases they cause, to determine the sensitivity of pathogen cultures to drugs, to obtain parasite antigens for diagnostic purposes, *etc.*

Primary cultures of *Blastocystis sp.* (n = 5) and *D. fragilis* (n = 3) were grown from feces of patients with symptoms of gastrointestinal diseases on RPMI 1640 nutrient medium (Biovest International, Inc.) with added ampicillin (12 mg/ml), streptomycin (4 mg/ml) and heat-inactivated horse serum (10 % (vol/vol)) (hereinafter NM RPMI 1640) in screw-cap tubes (16 × 100 mm) with 5 ml of this medium. Cultivation was performed at 37°C under anaerobic conditions for 4 days. The total concentration and percentage of live cells in parasite cultures were counted daily in a hemocytometer using the trypan blue dye exclusion test (0.4 % solution). The grown suspensions of *Blastocystis sp.* and *D. fragilis* supplemented with DMSO and glucose up to a final concentration of 5 % of each ingredient were subjected to cryopreservation. To evaluate the survival of parasite cells, the aliquots frozen in liquid nitrogen were inoculated after 3 months.

There was established that NM RPMI 1640 is quite suitable for the cultivation of *Blastocystis sp.* and *D. fragilis.* Indices of generation time, maximum concentration of viable cells (trophozoites) and the day of its achievement are as follows: (17.8 ± 2.5) h, (56.6 ± 9.0)×10^5 cells/ml and the day 3 when growing cultures of *Blastocystis sp.*; (11.5 ± 1.2) h, (3.9 ± 1.8)×10^5/ml and the day 2 when growing *D. fragilis.* Trophozoites of *Blastocystis sp.* and *D. fragilis* grown on NM RPMI 1640 retain their characteristic morphological and tinctorial properties, that allows them to be accurately identified by commonly used microscopy methods. After cryopreservation, the growth of 4 isolates of *Blastocystis sp.* (80%) and only 1 culture of *D. fragilis* (20%) was obtained.

The composition of NM RPMI 1640 provides high productivity in *Blastocystis sp.* and *D. fragilis* cultivation with achievement in stationary growth phase of cell concentration of these parasites at least 10^6/ml and 10^5/ml, respectively. However, the method of cryopreservation of *Blastocystis sp.* and *D. fragilis* cultures in NM RPMI 1640 needs improvement to increase the survival efficiency of parasite cells during their long-term storage.

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Експериментальне обґрунтування ефективності біопрепарату на основі сироватки пуповинної крові для корекції аутоімунного тиреоїдиту

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Experimental Justification of Effectiveness of Cord Blood Serum-Derived Biological Product for Correction of Autoimmune Thyroiditis

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The relevance of the work is related to the urgent need to develop effective drugs of biological origin for the treatment of autoimmune diseases, in particular, autoimmune thyroiditis (AIT). It is of interest to study the possibility of using cryopreserved biological products, in particular from cord blood serum (CBS) to correct thyroid function and restore the body's natural tolerance during AIT development, since it is known that such a severe disease as hypothyroidism develops against the background of long-term autoimmune aggression, which provokes not only the disorders of the endocrine system, but the body as a whole.

The research was conducted in male rats with induced AIT. The administered experimental drugs were as follows: CBS – intramuscularly with a course of 10 injections every other day, at a rate of 0.1 ml of diluted solution per 100 g of body weight; the comparative drug was a synthetic analog of thyroid hormone (LT4) administered orally at a dose of 10 μg/kg body weight in a 2% starch solution for 10 days, daily.

The results of immunological studies were evaluated in the model and after its correction. It was noted that CBS administration reduced the levels of TG antibodies already in the early stages of the post-study period. The total T-lymphocytes (CD3) and T-suppressors (CD8) increased, while the number of T-helper cells decreased relative to the indices of rats with AIT. The immunoregulatory index (IRI) was within control values.

After one month of observation, the effect of LT4, in conjunction with CBS, normalized the balance of specific lymphocyte subpopulations in the blood of rats with AIT. Within three months after the end of AIT induction, there was a slight increase in the level of CD3,8 subpopulations, significant elevation of CD16, 22, and a tendency towards increased IRI.

Administration of CBS for six months led to the restoration of immune system tolerance to thyroid tissue. The levels of TG antibodies were halved, indicating suppression of autoimmune aggression. This was confirmed by an increase in CD3, 16, 22. IRI remained within the physiological norm.

In the comparison group of animals receiving LT4, IRI was within control limits, but some groups of T-lymphocytes remained potentially higher than control levels, including B-lymphocytes. All this may indicate the activity of plasma cells and the presence of a high level of antibodies produced by these cells.

Thus, the cell-free biologic of cord blood serum shows potential efficacy for correcting autoimmune diseases, including autoimmune thyroiditis.