Determination of cytotoxic activity of rabbit blood lymphocytes on the example of MTT assay

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Introduction

Lymphocytes are agranular leukocytes that perform the function of recognizing foreign proteins (antigens) and participating in the body's immune response, including the destruction of tumor cells, cells infected with viruses and other damaged cells of the body. To assess the effectiveness of the functional state of lymphocytes, among other things, determine the level of their cytotoxic activity.

Materials and methods

MTT assay is used to quantify colorimetric proliferative and cytotoxic activity of mammalian cells. The method is based on the fact that mitochondrial enzymes (dehydrogenases) of the electron transport chain in viable cells in which mitochondrial activity is preserved, convert the substrate added to cell culture — MTT-tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) yellow — in crystalline MTT-formazan purple.

Results and discussion

MTT assay is one of the ways to assess the activity of the immune process — cytolysis of target cells. Under influence of antibodies (and other opsonins) this process in the body is more active. The greater this cytotoxic activity, the higher the body's resistance to infections and atypical cells. Therefore, this indicator of the functional activity of lymphocytes has not only diagnostic, but also prognostic value in the treatment of animals.

Lymphoid cells were obtained by gradient centrifugation method of a suspension of rabbit spleen cells on a phycol-verograph (ρ=1,077). The reaction itself is as follows: 0.1 ml of a suspension of standardized target cells (CT) (sarcoma-37 cells at a concentration of 30 thousand/ml) is applied to the well of the microplate and then 0.1 ml of a suspension of test lymphoid cells at a concentration of 100 thousand/ml. The ratio of target cell (CT) to effector cell (CE) is 1:3. The suspension is incubated in a thermostat for 18 hours under conditions of 5% CO_2, 100% humidity and +37°C. After incubation, 0.02 ml of MTT solution at a concentration of 5 mg/ml is added and incubated for 4 hours under conditions of 5% CO_2, 100% humidity and +37°C, then washed twice with saline at 4000 rpm for 15 minutes. After the last wash, 0.14 ml of 50% DMSO solution is added to dissolve the formed MTT formazan. The optical density of the samples is measured at λ=540 nm. All samples must be repeated in at least three replicates.

$$\frac{OD_e + OD_t - OD_{e+t}}{OD_e + OD_t} * 100\%$$

OD_e — optical density in samples with CE
OD_t — optical density in samples with CT
OD_{e+t} — optical density in the experimental series

Conclusions

Determination of cytotoxic activity and intensity of cytolytic potential of lymphocytes by MTT assay allows to assess not only the state of the body's immune system, but also the ability to determine tactics and provide a forecast for the treatment of animals with various infectious and oncological pathologies.