Heat Production as a Quantitative Parameter for Cell Differentiation and Cell Function

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Heat production was measured in relation to cell differentiation and phagocytic function using cells of human monocyte-histiocyte cell line U937. U937 cells differentiated monocytic phagocytes when cultured with lymphokine. Heat production increased as result of differentiation and phagocytosis. An important finding was that the heat increase in differentiated cells and during phagocytosis was directly proportional to the concentration of lymphokine. This strongly suggested that heat production is a quantitative parameter not only for cell differentiation but also for phagocytic function. The measurement of heat produced by mammalian cells can therefore be used to quantitate the differentiation and function of cells.

(Key words: Heat production, Cell function, Cell differentiation)

INTRODUCTION

Morphological, biochemical and functional changes have been measured to determine cell differentiation (1, 4). It was previously shown (6) that heat production is a good parameter for monitoring the cell cycle, and it may also be a good parameter for analyzing cell differentiation as well as cell function. Heat production was measured to analyze differentiation and function of U936 cells (5). The cells were able to differentiate to monocytic phagocytes when cultured with lymphokine.

MATERIALS AND METHODS

U937 cells, kindly given by Dr. Taniyama of the Japanese National Institute of Health, were maintained in RPMI 1640 supplemented with 10% fetal calf serum at 37°C in a CO2 incubator. Supernatants of phytohemagglutinin (PHA, Difco PHA-P)-stimulated lymphocyte culture were used as a source of lymphokine, the supernatant contained 4μl/ml of PHA and 20mM HEPES. For U937 cell differentiation, 10, 20 and 50 v/v % lymphokine was added to the culture, while an equivalent amount (10, 20 and 50 v/v %) of control medium was added to control cells. The control medium contained 4μl/ml of PHA and 20mM HEPES. The culture time varied from 1 to 3 days. After cultivating the cells for an appropriate time, the cells were collected, centrifuged and resuspended in fresh medium. The cells were adjusted to 2 × 10^6 per ml and the degree of differentiation was tested as described below. The monocytic phagocytes were counted under a microscope using an excess amount of autoclaved Candida albicans in the presence of 0.1% nitroblue tetrazolium (NBT) and 5% fresh human serum as opsonin. A cell was defined as a phagocytic cell when C. albicans was seen to be engulfed and was stained blue due to reduction of NBT. Heat production of cells was measured by a stopped-flow procedure as described earlier (6) and heat production during phagocytosis was measured by the same procedure but the cells
were mixed with an excess amount of *C. albicans* in the presence of 5% fresh serum just before measurement using the ESCO 3000 thermoactive cell analyzer. The results were presented as:

(1) heat production (µWatts, µW/10^6 cells) of cells in Fig.1;

(2) differential heat production (d heat, µW/10^6 cells) in Fig.2a;

(3) additional heat production (A-heat, µW/10^6 cells) in Fig.2b.

Differential heat production was calculated by subtracting the heat of control cells from the heat of differentiated cells and additional heat production was calculated by subtracting the heat of differentiating cells from heat production during phagocytosis.

RESULTS

U937 cells differentiated to phagocytic cells when cultured with lymphokine, which was confirmed by phagocytosis of *C. albicans* in a microscopic examination as described in Materials and Methods, while few phagocytic cells were found among control cells. It was not-
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Fig. 2a  Differential heat production. Fig. 2b  Additional heat production of cells in various culture conditions. d heat and A-heat were plotted against the concentration of lymphokine on day 1 indicated as ○—○, day 2 as ●—● and day 3 as □—□.

ed that the clumping of cells occurred both in cultures with lymphokine and control medium. The degree of clumping seemed dependent on the concentration of PHA but PHA did not alter the growth rate of cells.

Heat production of $1 \times 10^6$ cells cultured with lymphokine and control medium, and during phagocytosis of *C. albicans* are shown in Fig.1. It was evident that the differentiated cells always produced more heat than control cells. In control cells the more the control medium added, the less heat produced. In differentiated cells, a further increase in heat production was observed during phagocytosis of *C. albicans*, while there was absolutely no heat increase recorded in control cells. A decline in heat production in the cells cultured with 50% lymphokine for three days was noted, and this decline was more obvious during phagocytosis.

Increases in heat production of differentiated cells as well as during phagocytosis appeared to depend on the concentration of lymphokine and the culture period. When differential and additional heat production, i.e. d heat and A-heat, were plotted, d heat and A-heat were directly proportional to the concentration of lymphokine(Fig. 2a and 2b) except for the results obtained from cells cultured for three days with 50% lymphokine.

DISCUSSION

Differential and additional heat production are clearly proportional to concentrations of lymphokine up to 50% as long as U937 cells were cultured within a limited time, 2 days in this case. The reason why less heat production was recorded in cells cultured with 50% lymphokine for 3 days was that these cells were
probably in poor condition because differentiated cells can only remain alive for a limited period of time. It is therefore reasonable to conclude that differential and additional heat production are quantitative parameters for cell activity and cell function.

To obtain reliable results, corresponding control cells must be selected. In these experiments, U937 cells in maintenance culture condition were not used as control cells; cells cultured with equivalent concentrations of PHA and HEPES were chosen. The reason was that although morphology and growth rate were almost identical, the cells with PHA clumped and the degree of clumping was dependent of the PHA concentration. Thus, the cells with PHA differed from the U937 cells in maintenance culture. Interestingly heat production seems to detect this difference since the cells with PHA produced less heat than U937 cells in maintenance culture. Therefore, U937 cells in maintenance culture could not be used as control cells in these experiments. U937 cells were used as a model for differentiation since they are known to differentiate to phagocytic cells and their function can be easily assayed using C. albicans (5). Even though a number of reports (2) describing calorimetric application in cells have been published, this field of study has unfortunately not been sufficiently exploited. From the above mentioned results, it was evident that calorimetry is a very useful method for monitoring the activity, function and condition of mammalian cells.

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REFERENCES