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Asmaa A Eltaramsy



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Metabolic changes in naïve and polyclonal activated T-cell from naïve mice

Mohamed L. Salem¹, Tarek M. Mohamed², Mona M. Hegazi¹, Asmaa A Eltaramsy¹

¹Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt

²Biochemistry Department, Faculty of Science, Tanta University, Egypt

ABSTRACT

We have found previously that early activated T cells show higher activities and antitumor effects than late activated T cells upon their adoptive transfer. However, the exact mechanism is not clear. This study aimed to investigate the impact of polyclonal activation on the metabolic signature of T cells from naïve mice. Splenocytes of Swiss albino mice were harvested and cultured at 2.5×10^6 /ml in T25 flasks in presence of (Con-A) and IL2 for 24h or 72h. Then, cells were harvested and processed to determine activities of hexokinase (HK), phosphofructokinase (PFK), lactate dehydrogenase (LDH) and glucose 6 phosphate dehydrogenase (G6PD). The obtained data showed that activity of enzymes in activated cells was increased after 24h and 72h activation as compared to naïve cells, when and supernatant their level at 72h activation was higher than 24h activation. Comparison of enzymes activity between cells and supernatant showed higher activity of HK, G6PD and LDH in cells, While PFK activity was higher in supernatant after 72h. These results indicate that upon the early activation, naïve T cells have higher glycolytic pathway than late activation. These results have a great implication in adoptive T cell therapy.

Keywords: Metabolic, changes, naïve, T cells

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Correspondence to

Asmaa A Eltaramsy, M.Sc.

Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt

Tel: +201277355835

Email: laialy1985@gmail.com

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INTRODUCTION

Adoptive cell transfer (ACT) is an effective therapy for patients with certain types of cancer and chronic infectious disease (Lee et al., 2017). ACT enhances the activity of the immune cells by isolating them from the endogenous environment of the tumor bearing host. The anti-tumor cells are cultured *ex vivo* to activate and expand them numerically prior to infusion to the syngeneic tumor-bearing host for therapy (Mule et al., 1984). In spite of these advantages there are some challenges faces these approaches as T cell are often close to the end of their lifespan after *in vitro* expansion, which reduces their functional capacity and persistent after transfer back into the patient. So, it is important to understand how T lymphocytes alter their metabolism during activation.

Mitogenic activation by Con A leads to activation similar to antigenic stimulation, including cell growth (blastogenesis) and proliferation. T lymphocytes must rapidly

respond, by shifting, from quiescent state to highly proliferative state (Hume et al., 1987 and Lochner et al., 2015). As a result, the metabolic requirement of T cell increased dramatically upon activation to support biosynthesis of intracellular constituents including cell membrane, nucleic acid and protein. In resting T cells, consumption of glucose and other essential nutrients at low rate are to maintain normal housekeeping function (Loftus and Finlay, 2016). On other hand, after activation lymphocyte increase aerobic glycolysis more than energy efficient oxidative phosphorylation. At the end, metabolism must meet cell function demand to grow and rapid proliferation.

Hexokinases (HKs) catalyze the first step of glucose metabolism. Glucose transported through glucose transporters (GLUTs) on the plasma membrane is phosphorylated by HKs to produce glucose-6-phosphate (G-6P) (Rose and Warms, 1967). The key rate-limiting step in glycolysis is the phosphorylation of fructose 6-

phosphate by 1-phosphofructokinase (PFK), generating fructose 1,6-bisphosphate. This is also the step that fully commits glucose to glycolysis. PFK-1 activity is controlled by allosteric regulators, and one such regulator is fructose 2,6-bisphosphate, the product of PFK-2 activity and a potent activator of PFK-1 (Van Schaftingen, 1981).

The pentose phosphate/hexose monophosphate shunt pathway is an alternative metabolic pathway for glucose breakdown. Upon transportation of glucose into the cell via glucose transporters, the enzyme hexokinase converts glucose to glucose-6-phosphate. Glucose-6-phosphate can then be metabolized further via glycolysis or the pentose phosphate pathway (Chaneton and Gottlieb, 2012). The pentose phosphate pathway synthesizes precursors for nucleotide biosynthesis and generates NADPH (Kruger and von Schaewen, 2003). Pan et al. (1991) suggested that elevation of LDH isoenzymes is generally related to cell proliferation. Human lymphocytes have shown an increase in respiration and also produced lactate when stimulated by the mitogen phytohemagglutinin (Roos and Loos, 1973). In this study, we aim to address the activity of HK, PFK, LDH and G6PD in T cells harvested from naïve mice and after culture with mitogenic activator (Con-A/IL2) for 24 and 72 hr. of culture.

MATERIALS AND METHODS

Mice

Adult female Swiss albino mice (18-20 grams) were purchased from Theodor Bilharz institute (Giza, Cairo, Egypt). Animals were kept in clean and dry plastic cages as 6 mice per cage under normal laboratory conditions of temperature and humidity. They were fed with rodent pellets and had free access to water. In this study, mice care was done in accordance to the guidelines of the use of experimental animals in research at Zoology Department, Faculty of Science, Tanta University, Egypt.

Chemicals

RPMI- 1640 (Lonza) with L-glutamine media supplemented with 10% heated inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin. Concanavalin A (Con A), Fructose6phosphate barium salt, phosphate

buffer (PBS), Ammonium-Chloride-Potassium buffer (ACK) from Jack bean Sigma, Aldish, St Louis Mo. USA company, stored at -20°C. Interleukin-2 (IL-2): purchased from R&D company stored at (-20°C). NADH and Pyruvic acid from Oxford, ATP and NADP from Alpha, NAD from AppliChem. Glucose6phosphate was purchased from Serva.

Lymphocytes purification

Spleen was harvested from naïve mice. They were passed through 70µm sterile nylon cell strainer (falcon, Franklin Lakes) and mashed to obtain splenocytes. The red blood cells were then ruptured by sterile Ammonium-Chloride-Potassium buffer ACK buffer. Cell viability was assessed by trypan blue stain and counted by hemocytometer. Cell suspension was then adjusted to concentration 2.5×10^6 cells/ml for tissue culture uses.

In vitro activation of lymphocytes

Splenocytes were transferred into T-25 culture flasks containing 10 ml of fresh RPMI 1640 media. Cell final was adjusted to 2.5×10^6 cells/ml. Cells were cultured with Con A ($2.5 \mu\text{g/ml}$) and (20 ng/ml) IL-2 (Diaz-Montero et al., 2009) at 37°C 5% CO₂ humidity incubator. Cells were harvested after 24 h. and 72 h of incubation.

Cell preparation for enzymes assay

Harvested lymphocytes were washed once in PBS, and resuspended at a concentration of 2×10^6 cell/ml in PBS. The cell suspension was frozen at -80°C. Cells were disrupted by four freeze-thaw cycles. For the removal of crude debris, lysate was centrifuged for 10 min at 3000xg and supernatant was carefully collected.

Enzymes assay

Activity of Glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) was determined according to Ben-Bassat, (1980) method, activity of Lactate dehydrogenase (LDH, EC. 1.1.1.27) was determined according to Fritz et al. (1970) method. Activity of Hexokinase (HK, EC. 2.7.1.1) was determined according to Brandstrup et al. (1957) method and activity of Phosphofructokinase (PFK, EC. 2.7.1.11) was determined according to Gottschalk and Kemp (1981).

Statistical analysis

Data were analyzed using Graph pad instate software (San Diego, CA, USA). The obtained data were expressed as Mean \pm SD. The significance of difference among the various treated groups and control were analyzed by means of one- way ANOVA followed by Tuke to compare all pairs of columns. Significance was recorded when p-value was < 0.05

RESULTS

Activity of HK in naïve and activated T cells

Activity of HK was significantly increased after 24h activation by 4.4 folds compared to naïve cells and also significantly increased after 72h activation by 1.24. Its activity. However, was decreased by 1.24 folds compared to cells after 24h activation (Figure1). Activity of HK after 72 h stimulation in the supernatant increased by 1.86 folds compared to 24 h activation while decreased by 1.28 compared to its activity in cells. (Figure 1). In contrast, activity of HK in supernatant after 24h activation showed a significant decrease by 2.98 folds compared to the 24 h activity in cells and also its activity after 72 h activation in supernatant was increased by 1.28 folds compared to 24h activation (Figure 1).

Activity of PFK in naïve and activated T cells

Activity of PFK was significantly increased after 24h activation by 2.66 folds compared to naïve cells. It also increased after 72h activation by 1.54 folds, while decreased by 1.72 folds compared to 24 h activation (Figure 2). As shown in Figure 2 activity of PFK after 72h activation in supernatant showed significant increase by 3.64 folds compared to 24h, and significant increased by 11.55 folds compared to activity of 72h activation in cells. Activity of PFK in supernatant after 24h activation increased by 1.8 compared to the 24h activity in cells (Figure2).

Activity of LDH in naïve and activated T cells

Activity of LDH significantly increased by 10.25 folds after 24h activation compared to naïve cells and showed significant increase by 7.7 folds after 72h of activation. It decreased, however, by 1.2 compared to 24h after activation (Figure 3).

Activity of LDH after 72h activation in supernatant was increase by 1.45 folds compared to that of 24h activation (Figure 3). LDH activity after 24h activation in cells showed significant increased by 1.7 folds compared with supernatant. Its activity after 72h in cells decreased by 1.46 compared with supernatant (Figure 3).

Activity of G6PD in naïve and activated T cells

Activity of G6PD significantly increased by 3.54 folds after 24h activation compared to naïve cells and it increased by 1.84 folds after 72h activation while decreased by 2.2 compared to 24h activation (Figure 4). Activity of G6PD after 72h activation in supernatant increased by 2.18 compared to that of 24h after activation, and also increased by 1.43 folds compared to the activity of cells after 72h activation (Figure 4). G6PD activity in supernatant after 24h was decreased significantly by 2.94 folds compared to its activity in cells (Figure 4).

DISCUSSION

In this study, the changes in cellular metabolism during lymphocyte activation was studied with a particular emphasis is on glucose metabolism. The activities of HK, PFK, LDH and G6PD were determined in both naïve and activated T cells and in both cells and supernatant. Phosphorylation of glucose to glucose-6-phosphate traps glucose in the cell in addition it is considered as an initial substrate for glycolysis. In the absence of stimulation, the primary function of T cells is antigenic surveillance, which requires relatively small amounts of energy in the form of ATP (Beier et al., 2015).

HK activity after 24h activation in cells showed a significant increase by 2.94 folds compared to that in this supernatant while its activity after 72h in cells was decreased by 1.43 compared to supernatant. At the same time, activity of HK in supernatant after 24h activation showed a significant decrease by 2.98 folds compared to the 24h activity in cells. These present data are in agreement with the previous study by Marko et al, (2010) showed that upon activation, HK activity in T cells is highly upregulated. They added that after the uptake of glucose into the

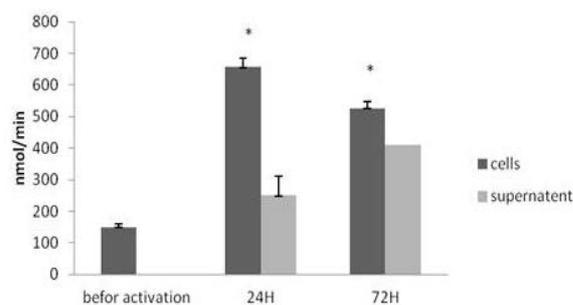


Figure 1. Hexokinase activity in both lymphocytes and their supernatant before and after activation for 24h and 72h in naïve mice, * $p < 0.05$.

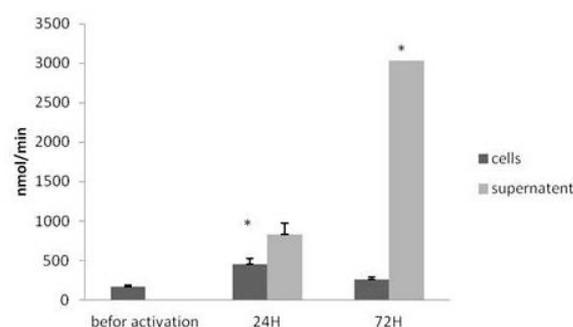


Figure 2. PFK activity in both lymphocytes and their supernatant before and after activation for 24h and 72h in naïve mice, * $p < 0.05$.

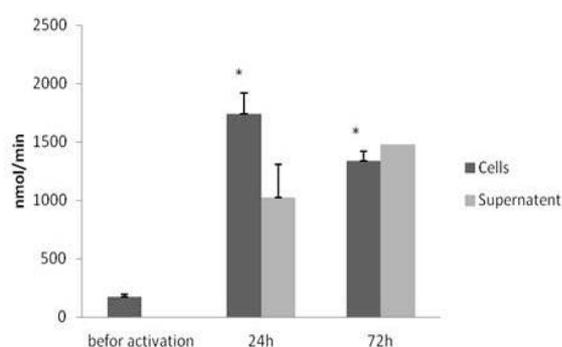


Figure 3. LDH activity in both lymphocytes and their supernatant before and after activation for 24h and 72h in naïve mice, * $p < 0.05$.

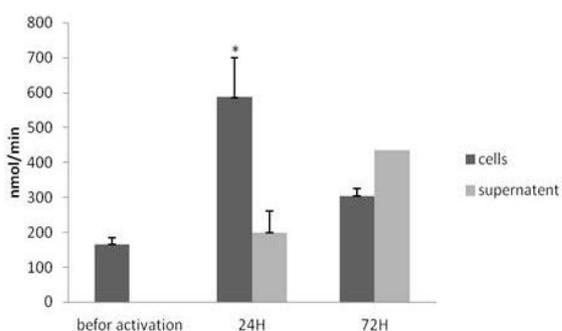


Figure 4. G6PD activity in both lymphocytes and their supernatant before and after activation for 24h and 72h in naïve mice, * $p < 0.05$.

cell, the HK enzymes commit it to metabolic processing by phosphorylation to glucose-6-phosphate, which can either enter glycolysis or the pentose phosphate pathway.

The activity of PFK significantly increased by 2.66 folds after 24h activation compared to naïve cells and it increased by 1.54 folds after 72h activation, while decreased by 1.72 folds compared to 24h activation. While activity of PFK after 72h activation in supernatant showed significant increase by 0.27-fold compared to 24h, and significant increased by 11.55 folds as compared with activity of 72h activation in cells. Alternatively, the shift to glycolysis could result from a primary stimulation of glucose uptake and catabolism that exceeds the cell's demand for glucose-derived macromolecular precursors or NADH, which is used to produce ATP through oxidative phosphorylation (Roos and Loos, 1973). Lactate production revealed that unstimulated T cells generate high percentage of ATP via oxidative phosphorylation with glycolysis only providing low percentage (Guppy et al., 1993). Activity of LDH significantly increased by 10.25 folds after 24h activation compared to naïve cells and showed a significant increase by 7.7 folds after 72h activation while it decreased by 1.2 folds compared to 24h after activation. Activity of LDH after 72h activation in supernatant increased by 1.45 folds compared to 24h after activation. LDH activity after 24h activation in cells showed a significant increase by 1.7-fold compared to supernatant activity. Current data is agreement with a previous study which reported that within hours of stimulation, lymphocytes begin to increase glucose uptake up to forty- or fifty-folds and to secrete most of the glucose-liberated carbon as lactate (Coloff et al., 2011). Upon activation of lymphocytes, the energy produced by glycolysis in resting cells is not sufficient, therefore, lymphocytes undergo a metabolic shift to increase glycolytic rate, which leads in turn to lactate production (Maciver et al., 2008).

G6PD is a key and initiating enzyme of the pentose-phosphate pathway (PPP). (Salvemini et al., 1999). Activity of G6PD significantly increased by 3.54 folds after 24h activation compared to naïve cells and increased by 1.84 folds after 72h activation while decreased by

2.2 compared to 24h after activation. G6PD activity in supernatant after 24h was decreased significantly by 2.94 folds compared to its activity in cells. G6PD initiate (PPP) pathway this is important for cells proliferation as it provides nucleotides and aromatic amino acids synthesis, also reducing equivalents in the form of NADPH that is important to maintain the pool of reduced glutathione, as well as to support lipid and cholesterol synthesis.

In conclusion, the present study shows that early activation for 24 h has higher glycolytic activity than late activation. These results recommend that short activation is more suitable for adoptive T cell therapy (Wang et al., 2011). Our conclusion may explain why adoptive transfer of early activated T cells show higher activities and anti-tumor effects than those of late activation T cells as we previously reported.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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