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Comparison of Energy Metabolism in Human Normal and Neoplastic (Burkitt's Lymphoma) Lymphoid Cells

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ABSTRACT

In order to detect possible differences in the energy metabolism between normal and neoplastic lymphoid cells, we studied purified normal human lymphocytes (FL) and transformed lymphoblastoid cell lines derived from umbilical cord blood (CL) and compared them to cell lines derived from American Burkitt's lymphoma (BL). The total adenosine triphosphate production rate by these cells was estimated by measuring O_2 consumption and lactic acid production rates. O_2 consumption (nmol/min/mg protein) was 4.9 ± 0.3 (S.D.) in CL, 4.4 ± 0.3 in FL, and 4.9 ± 0.3 in BL. Lactic acid production (nmol/min/mg protein) was 30.9 ± 3.0 in CL, 29.9 ± 3.0 in FL, and 23.4 ± 4.0 in BL. Using these values of O_2 consumption and lactic acid production, the average adenosine triphosphate production rates (nmol/min/mg protein) were calculated to be 60 in CL, 56 in FL, and 53 in BL. We conclude that the BL do not have more aerobic glycolysis than do normal lymphoid cells, suggesting that the lactic acidosis seen in American Burkitt's lymphoma is not due to a preferential glycolytic metabolism of the tumor. More likely, the lactic acidosis is simply due to the large total mass of these neoplastic cells and not due to a modification of their energy metabolism.

INTRODUCTION

Our goal was to determine whether the lactic acidosis observed in Burkitt's lymphoma could be explained by either an abnormally high production of lactate per cell in the tumor or the large tumor mass. An overview of the literature indicates that there is no consensus concerning oxygen consumption, lactic acid production, and ratio of aerobic glycolysis (*i.e.*, production of lactate in the presence of oxygen) to respiration in normal or neoplastic lymphoid cells. Because the absolute values of those measurements are highly dependent on the method used (12), we chose to study both normal and neoplastic lymphoid cell lines by the same method in order to look for differences between them in oxygen consumption and aerobic lactic acid production rates. Most of the work in the literature has been done with peripheral blood leukocytes (2, 3, 9, 16) rather than purified lymphocyte populations and thus is difficult to interpret. We studied freshly obtained peripheral blood lymphocytes for comparison to the Burkitt's lymphoma cells. Because of variability in reported studies on purified lymphocytes (15, 16, 19), however, we were hesitant to base our comparisons on peripheral lymphocytes only. We therefore chose also to study continuous *in vitro* cultures of EBV[2]-transformed lymphoblastoid cells derived from umbilical cord blood. These cells have a normal diploid karyotype and are believed to represent a normal cell counterpart of Burkitt's lymphoma (18).

MATERIALS AND METHODS

Cell Isolation and Culture. Fresh lymphocytes were obtained from heparinized peripheral blood by separation on a Ficoll:Hypaque gradient as described originally by Boyum (7). This technique yields at least 90% lymphocytes. The remaining contaminating cells are monocytes. The CL cell line was derived by transformation of normal umbilical cord blood lymphoblastoid cells with EBV according to methods described previously (18). These cells have a normal diploid karyotype. The BL cell line (CA 46) was derived from the ascites fluid of a patient with Burkitt's lymphoma. Cells present in the effusion were suspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 20% fetal calf serum, penicillin (100 units/ml), and streptomycin (10 μg/ml) immediately after aspiration. A suspension cell line grew after a few hr in culture. This line was characterized as a tumor line by its monoclonality (IgM_κ) and the presence of an 8:14 chromosomal translocation (4, 11). Both cell lines were routinely subcultured twice a week and carried in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% CL or 20% BL fetal calf serum and antibiotics, as above, in a 37° humidified incubator containing 5% CO_2 in air. Previous experiments have established that the cells are in log-phase growth 24 to 36 hr after subculture (17). All cells used in the experiments described here were examined during this time interval.

Oxygen Consumption. Cells were centrifuged for 2 min at 1000 rpm and washed twice in Dulbecco's phosphate-buffered saline supplemented with 15 mM glucose. Aliquots of the cell suspension were placed in an air-tight glass chamber designed to fit a Clarke O_2 electrode and surrounded by a constant-temperature water jacket at 37°. Additions were made through a small hole in the stopper by means of a syringe-mounted PE50 tubing. Before the insertion of the electrode, the preparation was bubbled with a mixture of 95% oxygen and 5% CO_2. The electrode was calibrated at the oxygen tension of room air (0.199 mM O_2 per liter at 1 atmosphere) and at zero oxygen tension in the presence of sodium hydrosulfite. During the measurements, mixing was provided by a micromagnetic stirring bar. The oxygen concentration in the suspension was recorded as a function of time, the slope of the line corresponding to the oxygen consumption rate.

Lactic Acid Measurements. Cells were washed twice in lactate-free Dulbecco's phosphate-buffered saline supplemented with 15 mM glucose. The mixing chamber (Yellow Springs Instrument Co., Yellow Springs, Ohio), containing 3-ml samples, was bubbled with a mixture of 95% O_2 and 5% CO_2 during the entire experimental period and maintained at 37°. During the first 20 min, 200-μl samples were taken every 5 min and added to Eppendorf tubes containing 100 μl of 12% perchloric acid. The tubes were kept on ice for 20 min and then centrifuged (Brinkmann) for 2 min at 3200 rpm; the supernatants were assayed for lactate concentration using the Trizma kit (Sigma Chemical Co.). In the presence of excess NAD and lactic dehydrogenase, the accumulation of β-NADH formed during the conversion of lactate to pyruvate was monitored spectroscopically. Lactate concentration at each time point was determined from standard curves using lactate and NAD^+ standard solutions. The rate of lactate production was calculated from the slope of the line of lactate appearance in the supernatants *versus* time. The slope was determined by linear regression analysis.

Protein Measurements. Protein was measured using the Bio-Rad

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[2] The abbreviation used is: EBV, Epstein-Barr virus.
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Table 1

O_2 consumption and lactic acid and ATP production rates

Values of O_2 consumption rates in the presence or absence of glucose were not different in the 3 cell types. Lactic acid production was similar in CL and FL and slightly but significantly lower in BL. Total ATP production rates were calculated from the O_2 consumption and lactic acid production rates in the presence of 15 mM glucose.

Cell types	O_2 consumption (nmol/min/mg protein)		Lactic acid (nmol/min/mg protein)	Total ATP (nmol/min/mg protein)	% of ATP production from	
	15 mM glucose	0 mM glucose			Respiration	Glycolysis
CL	4.9 ± 0.3[a] (8)[b]	9.0 ± 1.1 (9)	30.9 ± 3.0 (8)	60.2 ± 4.9 (8)	45	55
FL	4.4 ± 0.3 (6)	8.3 ± 0.8 (3)	29.9 ± 3.0 (5)	56.3 ± 4.9 (6)	44	56
BL	4.9 ± 0.3 (6)	9.1 ± 0.8 (7)	23.4 ± 4.0[c] (5)	53.0 ± 6.3 (6)	52	48
p	NS[d]	NS	<0.05	NS		

[a] Mean ± S.D.
[b] Numbers in parentheses, references.
[c] Lactic acid slightly but significantly lower.
[d] NS, not significant.

commercial reagent with bovine globulin as a standard.

All experiments were carried out in triplicate or more. The errors given in the text and Table 1 are S.D.s. A 2-tailed *t* test was used in the statistical evaluation of data, and *p* values <0.05 were taken to indicate statistically significant differences.

RESULTS

Oxygen Consumption. The rates of O_2 consumption (slope of PO_2 *versus* time) in the presence of 15 mM glucose were (nmol/min/mg protein) 4.9 ± 0.3 in CL, 4.4 ± 0.3 in FL, and 4.9 ± 0.3 in BL (Table 1). As shown in Table 1, O_2 consumption in the presence of 15 mM glucose was approximately 50% lower than the O_2 consumption measured in the absence of glucose in both CL and BL (Crabtree effect). It is noteworthy that antimycin A at the concentration of 5×10^{-6} M completely abolished respiration. Lactic acid production (nmol/min/mg protein) also did not differ between normal cultured lymphoid cells (30.9 ± 3.0) and fresh lymphocytes (29.9 ± 3.0) and was slightly lower in cultured tumor lymphoid cells (23.4 ± 4.0) as shown in Table 1. The total ATP production rates from respiration and aerobic glycolysis were calculated from the oxygen consumption and lactic acid production measurements. The calculation was done assuming that, during aerobic respiration, 5.6 ATP molecules are produced per O_2 molecule consumed (14) and that, during aerobic glycolysis, one ATP molecule is produced per lactate and 0.4 ATP molecule is produced per O_2 molecule consumed when glucose is the only substrate. Total ATP production rates were not different in the 3 groups of cells (Table 1).

DISCUSSION

In this paper, we compared the energy conversion pathways in fresh normal lymphocytes (FL), cultured transformed lymphoid cells (CL), and cultured neoplastic lymphoid cells (BL). Burkitt's lymphoma cells are rapidly dividing blastic cells. Therefore, it would be inappropriate to compare them only to nondividing peripheral blood lymphocytes. Thus, we studied EBV-transformed umbilical cord blood lymphoid cells. These cells, although transformed by EBV, will not induce tumors when implanted s.c. in nude mice and represent an EBV-infected cell type which is present in all EBV-seropositive individuals. They closely resemble normal lymphoblasts and are used here as a surrogate for the unknown normal counterpart of Burkitt's lymphoma cells.

Table 2

Relative contributions of aerobic respiration and aerobic glycolysis to ATP production

ATP production was calculated from values of O_2 consumption and aerobic lactic acid production found in the references cited. In all the normal tissues, a significant proportion of ATP is produced through aerobic glycolysis.

	% of ATP production from	
	Respiration	Glycolysis
Tumor tissues		
HTcBH cells (1)	66	33
Ehrlich ascites (25)	64	36
Burkitt's lymphoma cells	52	48
Normal tissues		
Vascular smooth muscle (20)	75	25
Retina (13)	75	25
Pigment epithelium (eye) (13)	50	50
Cornea epithelium (13, 24)	70	30
Kidney medulla (10)	70	30
Lymphoid cells in culture	45	55
Blood lymphocytes	44	56

The total production of ATP is essentially the same in the neoplastic as in the control lymphoid cells (Table 1), indicating that the rate of ATP hydrolysis in the 3 cell types is the same. In all of the 3 cell types tested, the rate of aerobic glycolysis was very high and provided approximately 50% of the ATP produced by the cells. Thus, a high aerobic glycolysis rate was not peculiar to the neoplastic cell line. Indeed, many other normal tissues including vascular smooth muscle (20), retina (13, 24), pigment epithelium of the eye (13), cornea (13), and kidney medulla (10) possess high aerobic glycolytic rates as shown in Table 2. These findings confirm the previous conclusions (21) that the high aerobic glycolytic rates found in cancer cells by Warburg (23) are not necessarily confined to tumor cells. Previous studies have shown that the aerobic glycolytic rate of Burkitt's lymphoma cells is sufficient to explain the observed lactic acidosis in patients (5, 6, 21). Our results are consistent with these findings but also indicate that the rate of aerobic glycolysis of Burkitt's lymphoma cells is no greater than that of normal lymphocytes. Thus, we conclude that the lactic acidosis seen in patients (5, 6, 22) cannot be accounted for by a preferential glycolytic metabolism of the tumor but is due, rather, to the presence of large masses of neoplastic cells producing lactate at a normal rate. In addition, local hypoxia within the tumor could also contribute to lactic acidosis through anaerobic glycolysis (8).

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