Elevated Activity of Oligomycin-Sensitive ATPase in Lymphocytes From Patients With Lung Carcinoma[1] (36226)

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The lymphocytes are engaged in the defense against external and internal invaders of the human body (1). The interaction between lymphocytes and cancer cells has been clearly demonstrated (1–4). The ability of the lymphocyte to adhere to or to penetrate into and remain inside the cancer cell (2, 4) indicates the significance of their relationship. The mechanism of the cytolytic effect of lymphocytes has been discussed extensively (1, 3, 5, 12). It has been demonstrated that lymphocytes from sensitized animals possess a cytolytic effect which is dependent upon a modification of their functional capacity. One of the important functions of the lymphocytes appears to be combination with antigens or antibodies, a reaction which is thought to be followed by a distortion of a recognition site of the cell membrane initiating an intracellular chain reaction beginning with a rise in the activity of a membrane-bound adenosinetriphosphatase (ATPase) (5). It has been reported that the activity of such a Na, K-activated ATPase is elevated in human leukemic leukocytes (6, 7).

The present study was undertaken to determine the activity of ATPase in human lymphocytes from normal individuals and from patients with lung carcinoma.

Materials and Methods. Two groups of patients were used: 1—Histologically proven cases of lung carcinoma; and 2—patients with nonmalignant diseases such as liver cirrhosis (2 cases), pneumonia (2 cases), thrombophlebitis (1 case), occlusion of the femoral artery (1 case), bronchial asthma (1 case), diabetes mellitus (1 case), uterine fibroids (1 case) and tuberculosis of the lung (1 case). Normal individuals were used as a control group.

Thirty milliliter samples of venous blood were drawn in plastic syringes. The blood was mixed with heparin (8 IU/ml) and 0.9% NaCl at a ratio of 1:2. A modification of Boyum's method (8) for separation of lymphocytes was used: a 10 ml mixture of Ficoll 9% and Isopaque 33.9% (v/v 24:10) was layered under 30 ml diluted blood in 50 ml siliconized centrifuge tubes by injection through a long, blunt needle. After centrifugation at room temperature for 20 min at 650*g* the lymphocyte zone was harvested with siliconized Pasteur pipettes and transferred to another tube. The lymphocytes were then mixed with 0.9% NaCl 1:5 and spun for 10 min at 650*g*. The supernatant was discarded and the cell pellet suspended in 5 ml of 0.9% NaCl. To remove contaminating platelets the cell suspension was passed slowly through a 2 ml packed column of glass wool in a plastic syringe. The glass wool was washed with 10 ml saline and the cell suspension centrifuged for 10 min at 650*g*. The supernatant was again discarded and the cell pellet exposed to a hypotonic shock for 30 sec to lyse occasionally present red cells. After a third centrifugation at 650*g* for 5 min the cells were suspended in 3 ml 0.25 *M* sucrose for homogenization. At the same time smears were prepared and stained with Wright's stain to verify the purity of the lymphocyte preparation. Cell counting was performed in each case using a hemocytometer. The lymphocyte preparations contained 98–100% small and medium sized

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lymphocytes, the remainder being polymorphonuclear leukocytes and monocytes.

The homogenization was carried out at 4° with a motor driven tip-serrated teflon pestle in 5 ml tubes for 10 min. To the lymphocyte suspension was added approximately 1 g small glass beads to ensure complete rupture of all cells. The resulting homogenate was centrifuged for 10 min at 1000*g*, and aliquots of the supernatant were used for the enzyme assay. The protein concentration of the homogenates was determined by Lowry's method (11).

The homogenates, containing a heterogeneous microsomal fraction of the lymphocytes, were incubated for 30 min immediately after preparation using the following assay system: 100 m*M* NaCl, 15 m*M* KCl, 6 m*M* $MgCl_2$, 3 m*M* Tris-ATP, in 30 m*M* Tris-HCl buffer pH 7.4. The final volume was 2.5 ml. All incubations were carried out at 37° and the reaction was terminated by adding perchloric acid to achieve a final concentration of 3%.

After 5 min at 0° the precipitated protein was removed by centrifugation and the concentration of inorganic phosphorus in the supernatant determined by the method of Fiske and SubbaRow (9). Enzymatic activity was expressed as μmoles inorganic phosphorus liberated per milligram protein in 30 min and represents the net result after correction for the content of inorganic phosphorus in the lymphocytes and the nonenzymatic hydrolysis of Tris-ATP during the same incubation setup.

A series of experiments was performed, omitting Na and K or adding ouabain, oligomycin, or 2,4-dinitrophenol in order to characterize the ATPase activity in the preparations used.

Results. The average activity of ATPase in lymphocytes from the group of patients with lung carcinoma is shown in Fig. 1. The cancer group exhibited significant elevation of the enzyme activity as compared to the group of normals and the group of patients with nonmalignant diseases ($p < 0.001$). ATPase activity of lymphocytes from normals and from patients with nonmalignant diseases was identical. In the overlapping

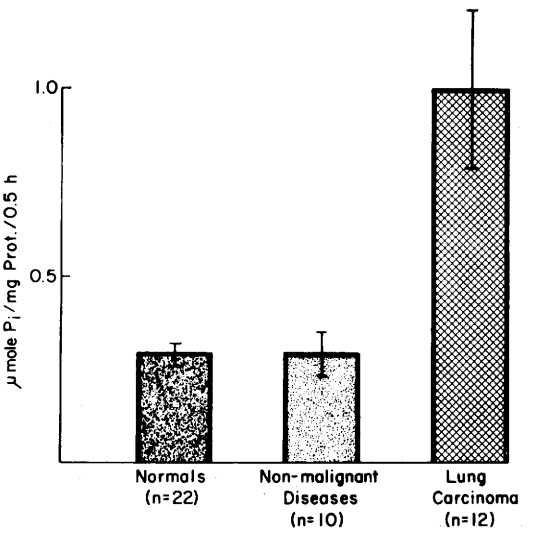

FIG. 1. The ATPase activity in lymphocyte homogenates from patients with lung carcinoma compared to normal controls and patients with nonmalignant diseases. The values are expressed as the means ± SE.

area between the control groups and the lung carcinoma group were 6 patients from the normal group, 4 patients from the nonmalignant group and 5 patients from the cancer group. The highest values in the nonmalignant group were found in lymphocytes from patients with liver disease.

The protein content of the homogenate was proportional to the amount of cells. The average protein content was 33.5 ± 4.5 (SE) $\mu g/10^6$ lymphocytes. This value was the same in all three groups.

The endogenous inorganic phosphorus concentration in the lymphocytes determined without incubation of the cells, was used in each case to correct the total amount of inorganic phosphorus after incubation. Figure 2 shows the average phosphorus concentration in lymphocytes in the three groups. The concentration was higher in the nonmalignant controls and in the cancer group than in the normal controls.

In order to characterize some of the properties of the enzyme activity, several experiments were performed. Table I shows that addition of ouabain to the assay mixture in a concentration of 0.5 m*M* did not produce any

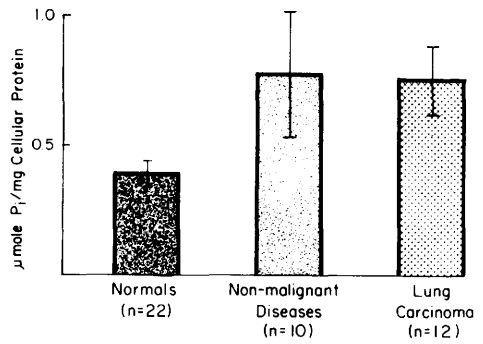

FIG. 2. The concentration of endogenous inorganic phosphorus in lymphocytes from normals, patients with nonmalignant diseases, and patients with lung cancer. The values are expressed as the means ± SE.

effect in either the normal lymphocyte homogenates or in lymphocyte homogenates from patients with lung carcinoma. Omission of Na and K from the assay mixture did not affect the enzyme activity. Addition of oligomycin (20 μg/ml) considerably decreased the activity in all experiments. Incubations in the presence of 2,4-dinitrophenol (0.1 m*M*) increased the enzymatic activity. Total inhibition of the activity was achieved by addition of sodium fluoride (10 m*M*) or by replacement of magnesium chloride with calcium chloride.

Discussion. The results of our studies indicate that elevation of ATPase activity in lymphocytes is demonstrable in patients with lung carcinoma. The observation that morphologically normal lymphocytes from patients with solid tumors exhibit a significantly higher ATPase activity should be given further attention. Specificity of such a finding could be very useful as a diagnostic test and a valuable indicator for the efficacy of a given cancer therapy.

Inhibition of the enzyme activity by oligomycin and stimulation by 2,4-dinitrophenol suggests that the lymphocyte homogenate contained mitochondrial derived ATPase. As shown in Table I, complete inhibition by oligomycin was only achieved in one experiment. The remaining activity in the other experiments could be caused by nonspecific phosphatases.

Previous reports concerning ATPase activity in human lymphocytes are very few. Block and Bonting (6) and Lichtman and Weed (7) studied the activity of Na, K-activated ATPase in normal and leukemic human lymphocytes. In our experiments we failed to demonstrate any Na, K-activated ATPase activity. This could be explained by the different method used for homogenization of the cells. The standard method for homogenization, as well as freezing and thawing, did not disrupt all lymphocytes but left many cells with morphologically intact cell membranes. Only homogenization with glass beads provided complete disruption of all lymphocytes. Such a procedure allows release of histones from fragmented nuclei, which is shown to inhibit the activity of Na, K-activated ATPase (10). The lack of membrane-bound ATPase activity could also be explained by a loss of membranes during centrifugation of the homogenate.

Interaction between the carcinoma cell and the lymphocyte does not seem to be only a

TABLE I. The Effect of Ouabain, Oligomycin, and 2,4-Dinitrophenol on ATPase Activity in Lymphocyte Homogenates.

	Enzymatic activity (μmoles P_i/mg protein/0.5 hr)			
Additions to normal assay	Exp. #65 Normal	Exp. #67 Normal	Exp. #66 Lung carc.	Exp. #68 Lung carc.
None	0.48	0.43	2.01	0.72
Ouabain, 0.5 m*M*	0.44	0.44	2.06	0.93
Oligomycin, 20 μg/ml[a]	0.17	0.12	1.24	0.00
2,4-dinitrophenol, 0.1 m*M*[a]	0.55	0.76	2.27	1.15

[a] Oligomycin and 2,4-dinitrophenol were dissolved in 90% ethanol (v/v) and corrections were made for a slight depression of the enzymatic activity from the ethanol alone.

morphological event. It seems reasonable to believe that the lymphocyte performs a surveillance of the malignant cell. Such an activity could be followed by alteration of the metabolic pattern of the lymphocyte. The elevation of ATPase activity in lymphocytes from patients with lung carcinoma demonstrated in our study could reflect such changes.

Summary. Lymphocytes from patients with lung carcinoma exhibit elevated oligomycin-sensitive ATPase activity. Patients with non-malignant diseases and normal individuals showed identical low activity. The absence of Na. K-activated ATPase has been given an explanation by the procedures used for preparation of the cell homogenate.

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