CHANGES OF ENZYME ACTIVITIES RECOGNIZED IN LYMPHOCYTES FROM PATIENTS WITH CARCINOMA OF THE GASTRO-INTESTINAL TRACT*

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Summary

Adenosine triphosphatase (ATPase) activity and acid phosphatase activity in lymphocytes from patients with carcinoma of the gastrointestinal tract were determined in order to investigate whether or not changes in these enzyme activities have any relation to the immune reactivity of carcinoma-bearing patients. In patients with a performance status of more than 60%, the mean value of the total ATPase activity in lymphocytes differed little from that in controls, but the mean value of the oligomycin-sensitive ATPase activity decreased as compared to that in the controls. On the other hand, the mean values of the activities of both free and total acid phosphatase in lymphocytes increased as compared to those in controls. The mean values of the activities of both ATPase and acid phosphatase in lymphocytes from patients whose performance status was less than 50% decreased as compared to those from controls and patients whose performance status was more than 60%. The change of the activities of both ATPase and acid phosphatase in lymphocytes has relation to that of the immunological parameters of the patients with carcinoma of the gastrointestinal tract. These results indicate that both ATPase and acid phosphatase in lymphocytes may play an important role in the immune mechanism.

Key Words: adenosine triphosphatase, acid phosphatase, peripheral lymphocytes, immunological parameter, gastrointestinal carcinoma.

Interaction between lymphocytes or macrophages and tumor cells is an essential component in the host defense against the growth of tumor cells. During the process of lymphocyte-mediated target tumor cell destruction, a sequence of biochemical change in host lymphocytes may occur. However, the biochemical change in lymphocytes which reflects the change of the immune reactivity of the carcinoma-bearing host has not been elucidated well clinically. It has been demonstrated, in experimental studies using tumor-bearing animals, that a correlation exists between elevation in the activities of ATPase and acid phosphatase (acid Pase) in spleen lymphocytes and the activation of the host defense mechanism against tumor growth10–12. This experimental
fact led us to elucidate the activities of ATPase and acid Pase in lymphocytes from carcinoma-bearing patients with different performance status (PS) in order to investigate whether or not the change of these enzyme activities has relation to that of the immune reactivity of patients with carcinoma. Gastric carcinoma has long been the major cause of cancer mortality in Japan, so patients with carcinoma of the gastrointestinal tract (GI carcinoma) were selected for the study. This paper shows that the change of the activities of ATPase and acid Pase in lymphocytes from patients with GI carcinoma has relation to that of the immune reactivity of patients with GI carcinoma.

Materials and Methods

Subjects and source of lymphocytes: One hundred and sixty patients with GI carcinoma were selected as subjects. Twelve patients had esophageal carcinoma, 98 had gastric carcinoma, 10 liver carcinoma, 18 pancreas carcinoma, and 22 colorectal carcinoma. They were diagnosed by barium study, fiberoptic endoscopy, angiography, CT scan, and histopathological examination by biopsy and operation or autopsy. The patients consisted of 89 males and 71 females from 32 to 70 years of age. Their Karnofsky performance status (PS) is shown in Table 1. On the other hand, 50 normal individuals (25 individuals for ATPase determination, 25 individuals for acid Pase determination) as well as 50 patients with non-malignant disease of the gastrointestinal tract (25 patients for ATPase determination, 25 patients for acid Pase determination) were selected as controls. Twenty patients had gastric ulcer, 12 had chronic pancreatitis, 8 gastritis, 6 hepatitis and 4 rectal polyp. The normal individuals were 28 males and 22 females from 26 to 48 years of age, and the control patients were 26 males and 24 females from 25 to 60 years of age.

Isolation of lymphocytes and preparation of enzymes: Heparinized venous blood was obtained from the patients and the normal individuals. Mononuclear cells were separated on the Ficoll-Conray density gradient described previously. The mononuclear cells (lymphocytes and monocytes) were washed with saline solution. Over 90% of these cells were lymphocytes. Erythrocytes were lysed by osmotic shock for 1 min. After being washed, the cells were finally suspended in 0.25 M sucrose solution to make a $1 \times 10^7$ cells/ml suspension (for the ATPase determination) and to make a $2 \times 10^6$ cells/ml suspension (for the acid Pase determination), respectively. The cell suspension was homogenized 5 times by using an ultrasonic sonifier for 10 sec each. The whole cell homogenate was used as crude enzyme for the ATPase assay. On the other hand, for the acid Pase assay, the cell homogenate was centrifuged at 4°C, at 280 g for 10 min, to remove nuclei. The supernatant was centrifuged again at 9000 g for 10 min. After centrifugation, the precipitate was used as the mitochondrial fraction and the supernatant as the microsomal fraction. The mitochondrial fraction and the microsomal fraction were suspended, respectively, in 0.25 M sucrose solution to contain the respective constituents of mitochondria and microsome corresponding to $2 \times 10^6$ lymphocytes in 1 ml.

Determination of enzyme activity: The ATPase activity was determined by the modification of Skou's method.

A) Total ATPase activity: 1.0 ml of crude enzyme was mixed with 2.0 ml of substrate solution containing 3 mM ATP, 100 mM NaCl, 15 mM KCl, 6 mM MgCl$_2$ and 30 mM Tris HCl buffer (pH 7.4), and this reaction mixture was incubated at 37°C for 20 min. After incubation, the reaction was stopped by adding 2.0 ml of ice-cold 6.75% perchloric acid. The precipitated protein was removed by filtration, and