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Effect of Autologous Sera on Mitogenic Stimulation of Peripheral Blood Lymphocytes of Mini-Pigs Chronically Intoxicated with Alcohol

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There are no statistically significant differences in the stimulation index for purified lymphocytes of control mini-pigs and mini-pigs with chronic alcohol intoxication. Autologous sera of control and experimental animals strongly suppress mitogen-induced blast transformation of lymphocytes without death of these cells. There are no statistically significant intergroup differences in the absolute number (per mm^3) of T lymphocytes in peripheral blood.

Key Words: *blast transformation; lymphocyte; autoserum; alcoholism*

At the present time, when characterizing the function of human and animal immunocompetent cells, more and more researchers are including in the test systems elements of the microenvironment of these cells; this is done not only to study the potential activity of various karyocytes represented by "washed" cells, but also to tackle this problem in respect of the whole organism [1,3]. This is logical, since, particularly in pathology, serum (plasma) contains a great number of diverse factors that may modulate the immune response [1,6,7,13,14]. The use of purified compounds is not always well-advised, and we think that the utilization of an individual's serum or plasma in a test system is the most appropriate (at least at the first step) when the functional parameters of immunocompetent cells are being assessed.

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Our aim was to study the response of peripheral blood lymphocytes obtained from adult mini-pigs intoxicated with alcohol to the T-cell mitogen concanavalin A (ConA) in the reaction of blast transformation (BT), using washed lymphocytes and autologous sera in the test system.

MATERIALS AND METHODS

Mini-pigs (n=22) were divided into 4 groups: group 1 consisted of mini-pigs given alcohol for 3 years, group 3 pigs received alcohol for 5.5 years, and groups 2 and 4 served as the control. Chronic alcohol intoxication was modeled according to the scheme developed in the Laboratory of Experimental Biological Models (Russian Academy of Medical Sciences): alcohol (25% aqueous solution) was included in the diet from the age of 4 months in a dose of 2.5-3.0 g/kg body weight once daily 5 times a week. The blood concentration of ethanol, determined spectroscopically with Boehringer Mannheim kits, was 1.5-2.0 mg/% in the control animals and 70-120 mg/% in experimental animals, which corresponds to medium-degree alcohol intoxication of humans. Lymphocytes were isolated from peripheral blood on a Ficoll-Verografin gradient (d=1077) [5]. T cells (E-RFU) were identified by spontaneous rosette formation with sheep erythrocytes [9], which is an adequate test T lymphocytes of mini-pigs [8]. A mixture of acridine orange and ethidium bromide was added to the cell suspensions at the end of the reaction to differentiate living (emerald-green) and dead (orange-red) lymphocytes. The blast transformation reaction was carried out using the conventional procedure. Plastic dishes were incubated at 37°C in an atmosphere containing 5% CO_2. Concanavalin A (Flow, 5 μg per 100,000 lymphocytes) was used as mitogen. The reaction lasted 48 h. Complete culture medium (CCM) consisted of medium RPMI-1640 (Flow), 10% fetal calf serum (Flow), 10 mM HEPES buffer (Flow), 2 mM L-glutamine (Flow) and 40 μg/ml gentamicin. Tritiated thymidine (1 μCi) was added 4 h before the end of the reaction. Cells were transferred onto fiberglass filters and washed with 5% trichloroacetic acid and absolute ethanol. The intensity of β-radiation (cpm) was measured in an LKB β-counter using liquid scintillator (toluol, PPO and POPOP). Each test was performed in triplicate. The reaction was also performed in the presence of 50% (100 μl) fresh inactivated autologous serum. Cell viability was controlled throughout the experiment. In the control and experiment it was more than 85%. The stimulation indexes (SI) were calculated as follows:

$$SI_1 = \frac{[(\text{cpm})\ \text{lymphocytes+mitogen+CCM}]}{[(\text{cpm})\ +\ \text{lymphocytes without mitogen}\ +\ \text{CCM})]},$$

$$SI_2 = \frac{[(\text{cpm})\ \text{lymphocytes}\ +\ \text{autologous serum}\ +\ \text{CCM}]}{[(\text{cpm})\ \text{lymphocytes}\ +\ \text{CCM}),},$$

$$SI_3 = \frac{[(\text{cpm})\ \text{lymphocytes}\ +\ \text{mitogen}\ +\ \text{autologous serum}\ +\ \text{CCM})]}{[(\text{cpm})\ \text{lymphocytes}\ +\ \text{autologous serum}\ +\ \text{CCM}]}.$$

T cells were counted in thousands per mm^3 blood. Histological liver preparations were obtained by routine methods and stained with hematoxylin and eosin [4]. The results were analyzed by conventional methods of variational statistics and Student's t test.

RESULTS

Our results (Table 1) indicate that SI_1, the parameter most frequently used in studies of mitogenic stimulation of lymphocytes, is practically the same (p<0.05) in control and experimental ani-

TABLE 1. Parameters of the BT Reaction and the Number of T Cells in the Peripheral Blood of Mini-Pigs Chronically Intoxicated with Alcohol and in Control Animals ($M \pm m$)

Group of animals	T cells	IS_1	IS_2	IS_3
1 (3 years of alcohol consumption)	1320±186 (n=6)	33.8±6.3* (n=6)	1.4±0.5 (n=6)	8.8±3.8 (n=6)
2 (control for group 1)	2121±928 (n=4)	30.0±10.7* (n=4)	0.9±0.2 (n=4)	2.8±0.9 (n=4)
3 (5.5 years of alcohol consumption)	1528±346 (n=6)	41.3±15.0* (n=7)	1.2±0.3 (n=7)	4.0±0.5** (n=7)
4 (control for group 3)	2401±446 (n=5)	39.0±15.6* (n=5)	1.2±0.5 (n=5)	1.3±0.9 (n=5)

Note. n = number of animals; p<0.05: * vis-a-vis IS_3; ** vis-a-vis control.

mals. This implies a high potential ability of washed lymphocytes from mini-pigs chronically intoxicated with alcohol to respond to ConA. It should be mentioned that the absolute number of T cells in the wells was almost the same. Our findings conflict with the published data, particularly with those obtained on human lymphocytes, which indicate that in alcoholism BT in response to a T-cell mitogen is strongly inhibited in parallel with pronounced T leukopenia [7,10,12]. However, neither leukopenia nor BT inhibition was observed when the maintenance of laboratory animals was thoroughly controlled [11,12]. We think that good maintenance, a balanced diet, and pure ethanol (rather than the surrogates often consumed by alcoholics), and the absence of social stress account for our results. In addition, it has often been emphasized in the literature that the pronounced shifts in the immune response observed in alcoholics occur against the background of liver cirrhosis [12]. In our experiments, the animals receiving alcohol during a 3-year period developed chronic persistent hepatitis, while the primary stages of cirrhosis were observed after a 5.5-year period of alcoholization. It is noteworthy that in both the control and experimental groups IS_3 markedly decreased after the addition of autologous sera, the decrease being greater in the control group, particularly in the animals who had been given alcohol during a 5.5-year period ($p<0.05$). By the end of the BT reaction, cell viability in all wells was not less than 85%. The decrease in IS_3 under the influence of autologous serum from mini-pigs intoxicated with alcohol can be explained by the fact that both ethanol and its first metabolite acetaldehyde inhibit the BT reaction in response to mitogens *in vitro*, which is not accompanied by cell death [10,13,15]. However, if this occurs in the experimental group, then why does autologous serum dramatically inhibit the BT reaction in the control group? The possibility that this effect is species specific and the mechanisms responsible for this effect will be studied in our future investigations. From the results obtained here it can be concluded that: 1) the level of BT of washed lymphocytes in response to ConA does not differ from that displayed by the lymphocytes of control mini-pigs; 2) sera obtained from mini-pigs chronically intoxicated with alcohol and from control mini-pigs markedly suppress the mitogenic response of lymphocytes, which is not accompanied by their death; 3) sera of experimental and control mini-pigs do not change the spontaneous proliferation of lymphocytes; 4) There are no statistically significant differences in the absolute number of T cells in one mm^3 of peripheral blood obtained from experimental and control animals, but only a tendency toward a decrease in this parameter in chronically intoxicated animals.

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