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CHANGES IN SOME IMMUNOLOGIC AND BIOCHEMICAL PARAMETERS INDUCED IN GERMFREE ANIMALS BY T-ACTIVIN

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The microflora has a considerable influence on the formation of physiological and immune reactions and on metabolic functions of the host organism [9, 11]. The use of germfree animals as models of immunodeficiency states has assumed great importance in connection with the study of the mechanisms of action of immunomodulating agents.

Several investigations aimed at studying the effect of the microbial factor on the defensive systems of the body have been undertaken on germfree animals [4, 13]. So far, however, the state of activity of the enzymes of xenobiotic metabolism and the state of immunoreactivity, including natural cytotoxicity, have received little study. The immunomodulator T-activin is known to have a many-sided influence on functioning of the T-system of immunity [1]. This preparation has been successfully used in the treatment of various immunodeficiency states [5].

The aim of this investigation was to study the effect of the thymus preparation T-activin on activity of enzymes of xenobiotic metabolism (EXM) and on cell-mediated cytotoxicity — two important mechanisms of defense of the body which realize their action without antigenic stimuli.

EXPERIMENTAL METHOD

Noninbred guinea pigs were obtained from the Central Laboratory Animals Nursery, Academy of Medical Sciences of the USSR. Young germfree guinea pigs were obtained by hysterotomy, using the gnotobiotic operating isolator system of the Research Laboratory of Experimental Biology and Medicine, Academy of Medical Sciences of the USSR. The animals were reared up to the age of 8 days with observance of the rules of a germfree technique in soft plastic isolators

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TABLE 1. Effect of T-Activin on Enzyme Activity in Germfree Guinea Pigs

Enzyme	Germfree animals		Conventional animals (n = 18)
	control (n = 10)	T-activin (n = 8)	
Cytochrome P-450, nmoles/mg	0,50 ± 0,03	8,88 ± 0,14*	1,18 ± 0,30
b_5, nmoles/mg	0,50 ± 0,08	0,55 ± 0,06	0,946 ± 0,12
NADPH-cytochrome C-reductase, nmoles/mg/min	372,5 ± 81,6	499,0 ± 44,3	694,4 ± 23,4*
Benzpyrene hydroxylase, nmoles/mg/min	173,7 ± 21,4	190,5 ± 49,2	371,1 ± 161*
	23,5 ± 3,5	28,5 ± 9,0	54,3 ± 6,3*
Epoxide hydrase	9,25 ± 0,6	14,3 ± 0,6*	20,8 ± 4,4*
Glutathione-S-transferase, nmoles/mg/min			

Legend. *p < 0.05 compared with control.

(Czechoslovakia). The animals were fed for 2 days after birth on an autoclaved L-477 diet, and then changed over to natural food, sterilized by irradiation in a dose of 2.5 Mrad. Throughout the experiment sterility of the germfree animals and their surroundings was monitored. Young conventional guinea pigs were used as objects for comparison. They were obtained by natural birth and also reared up to the age of 8 days, but with their mothers under ordinary conditions.

Germfree animals were divided into two groups. The guinea pigs of group 1 received T-activin in a dose of 5 μg per animal by intraperitoneal injection 3 times at intervals of 24 h. The animals of group 2 (control) received physiological saline. The conventional animals were divided in a similar way. All animals were killed simultaneously 24 h after the last injection of T-activin. The liver and peripheral blood were taken for investigation. The liver was used to isolate microsomes by differential centrifugation. The cytochrome P-450 concentration was measured spectrophotometrically by the method in [12]. Benzpyrene hydroxylase and NADP-cytochrome C-reductase activity was measured by the methods in [7] and [10] respectively, glutathione-S-transferase by the method in [8], and epoxide hydrase by the method in [6]. Peripheral blood lymphocytes were isolated in a Ficoll–Hypaque gradient. Activity of antibody-dependent killer cells (KC) was estimated by the ability of the lymphoid cells to lyse target cells (sheep's red blood cells), treated with antibodies [2]. The results were expressed as a cytotoxic index (CI), in percent.

EXPERIMENTAL RESULTS

EXM activity in the liver microsomes differed significantly in conventional and germfree guinea pigs. It will be clear from Table 1 that the cytochrome P-450 concentration in the germfree animals was only 40% of that in the conventional animals. Activity of benzpyrene hydroxylase and epoxide hydrase was considerably reduced (by more than half) and a significant fall in glutathione-S-transferase activity also was observed in the germfree animals.

Germfree animals are known to differ from conventional in their much lower level of certain factors of natural immunity, dependent on previous microbial stimulation. These factors include lyxozyme, properdine, and functional activity of macrophages, which may be studied by various tests [4].

These results showing a decrease in EXM activity in the germfree animals may be evidence of an inducing effect of the nonpathogenic microbial flora on EXM in the internal organs. During ontogeny antigenic stimulation evidently plays an important role not only in maturation of the immune system, but also in the formation of the system for biotransformation of foreign compounds.

The experiments showed that T-activin (Table 1) has a stimulating action on several EXM. The concentration of cytochrome P-450 was considerably increased (by 1.8 times), activity of NADP-cytochrome C-reductase and of glutathione-S-transferase was raised, and a tendency was observed for activity of benzpyrene hydroxylase and epoxide hydrase to be increased. Meanwhile, T-activin had no significant action on these enzymes in conventional animals. At the

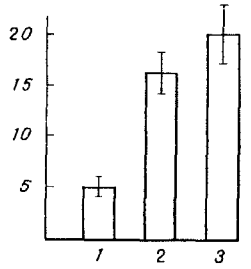

Fig. 1

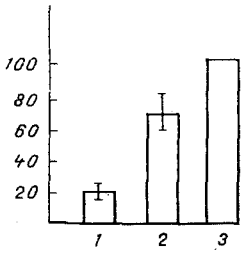

Fig. 2

Fig. 1. Changes in glutathione-S-transferase activity in lymphocytes of germfree guinea pigs under the influence of T-activin. Ordinate, activity (in nmoles/ mg/min). Here and in Fig. 2: 1) intact germfree animals, 2) germfree animals receiving T-activin, 3) conventional animals; short vertical lines denote mean error for three experiments; number of germfree animals 18, of conventional animals 18.

Fig. 2. Antibody-dependent cellular cytotoxicity of germfree animals before and after injection of T-activin (in %) relative to level of KC activity in conventional animals).

same time, we know that T-activin had a marked stimulating effect on glutathione-S-transferase activity in lymphocytes of the germfree animals (Fig. 1). It can be tentatively suggested that this was connected with T-lymphocyte activation. The writers showed previously that activity of this enzyme is found mainly in the T-cell subpopulation of immunocytes [3].

KC activity of the germfree animals was found to be lower than that of conventional animals. Meanwhile, under the influence of T-activin this parameter rose until it reached about 70% of its value in normal animals (Fig. 2).

The suggested model can probably be used to obtain proof that the formation of the immune system and of the system for biotransformation of foreign substances are ontogenetically linked processes. The study of the action of peptide immunomodulators, such as T-activin, can provide a new approach to the study of the mechanisms of interaction of the two leading systems maintaining homeostasis, and also to the specific correction of various immunodeficiency states.

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