EFFECT OF A LYMPHOCYTIC CHALONE-CONTAINING PREPARATION ON MITOTIC ACTIVITY OF MOUSE THYMOCYTES IN VIVO

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It has been shown that many endogenous tissue-specific inhibitors of cell proliferation (chalones) can detain cells in the late G_1- or G_2-phase of the mitotic cycle and thus prevent their entry into the phase of DNA synthesis or into mitosis [4]. Most studies of biological properties of lymphocytic chalones have been concerned mainly with the G_1 chalone [3]. There is no information in the literature on the inhibitory action of lymphocytic chalone-containing substance (LCCS) on the entry of cells into mitosis from the G_2-phase.

The aim of this investigation was to determine whether the chalone-containing substance prepared from the spleen has an inhibitory action in vivo on the passage of thymus cells from the G_2 phase into mitosis.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male (CBA × C57BL)F_1 mice weighing 17-18 g. LCCS was obtained from the spleens of noninbred albino rats weighing 170-180 g, previously washed with physiological saline, by the method of saturation of an aqueous extract of the tissue with 96% ethanol from 55 up to 81% [1]. LCCS was injected intraperitoneally at 12 noon in a dose of 5 mg per mouse in 0.5 ml of physiological saline. Control mice received an intraperitoneal injection of 0.5 ml of physiological saline. The inhibitory activity of LCCS was judged by changes in the mitotic index (MI) in the thymus of the mice 15, 30, 45, and 60 min after injection of the preparation in experiment 1 and 30, 45, 60, and 90 min after injection in experiment 2. MI was determined in promille after analysis of 10,000-20,000 thymus cells from each animal. At each time of the experiment five animals were used. Films prepared from the thymus were fixed twice with methanol, hydrolyzed for 4 min in 1 N HCl at 56°C, washed with water, and stained with methylene blue. The numerical results were subjected to statistical analysis by the Fisher–Student test. Differences were considered significant at the $p \leqslant 0.05$ level.

TABLE 1. Effect of LCCS on MI of Mouse Thymocytes (M ± m)

Time of testing, min	MI, ‰		% of inhibition	p
	control	experiment		
Experiment 1				
15	3,9±0,10	5,0±0,33	—	0,024
30	5,0±0,75	3,5±0,35	27	0,022
45	5,6±0,47	1,6±0,10	67	0,001
60	4,3±0,10	2,2±0,51	54	0,001
Experiment 2				
30	5,9±0,38	4,3±0,56	27	0,034
45	5,7±0,39	2,3±0,44	61	0,001
60	5,6±0,61	3,8±0,39	36	0,040
90	6,0±0,35	5,9±0,35	—	—

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EXPERIMENTAL RESULTS

The results in Table 1 show that 15 min after injection of LCCS into the animals MI rose. After 30 min a distinct fall of MI was observed, which was most marked after 45 min; after 60 min inhibition of entry of the thymocytes into mitosis still continued, but was weaker than at the previous time of the experiment (Table 1). After 90 min MI of the thymocytes of animals receiving LCCS returned to the control level (Table 1).

The duration of the G_2-phase of the mitotic cycle of mouse thymocytes is known to be about 45 min [2, 5]. On the basis of the fact that delay of entry of cells into mitosis under the influence of LCCS develops over a period of 30-60 min and is reversible in character, it can be concluded that the preparation used gives a G_2-chalone effect, expressed as a specific action on the passage of thymocytes from the G_2-phase into mitosis.

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AGE CHANGES IN IMMUNE CYTOSIS OF MOUSE CELLS WITH STREPTOLYSIN-O-INDUCED CYTOGENETIC DISTURBANCES

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With age the number of cytogenetically changed cells in man and animals increases [6, 10], as also does the sensitivity of the cells to the action of mutagens [7, 9]. It was shown previously [3] that in connection with these age changes there is a very close relationship between the state of the T system of immunity and the level of cytogenetic disturbances in patients with influenza and in mice immunized with measles vaccine. It has been suggested that since the immune system exercises control over genetic homeostasis in vivo [2], when the function of the immune system declines with age, accumulation of cytogenetically changed cells must evidently take place on account of both spontaneous and induced mutagenesis. Meanwhile it is logical to suppose also that the observed effects may be due to weakening of immune antiviral defense during aging, and this may lead to the accumulation of virus-induced cytogenetically changed cells in the body.

The aim of this investigation was to answer the question whether age differences exist in the control of genetic homeostasis during infectious mutagenesis. Experiments were carried out on the basis of a method of studying cytolytic activity of lymphocytes relative to cytogenetically changed cells in culture, developed previously [4].

EXPERIMENTAL METHOD

Streptolysin O, a toxin of *Streptococcus haemolyticus* [5], was used as the mutagenic factor. Pure-line BALB/c mice, closely inbred for five generations, were used. The mice were aged 1-10, 180-190, and 360-380 days. After decapitation of the mice the kidneys were removed and cell cultures prepared from them by the usual method [1]. The cultures were "infected" with streptolysin-O in a dose of 0.1 ml of the standard dilution to 1 ml of culture medium. A standard solution of the preparation was used. After contact with the culture for 8 h the streptolysin-O was washed off with medium 199, and 24 h later splenic

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