CELL PROLIFERATION IN MOUSE TISSUES AFTER THYMECTOMY AND T-ACTIVIN ADMINISTRATION

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UDC 612.112.12-06:612.438

KEY WORDS: cell proliferation; thymectomy; T-activin.

Lymphoid cells have been shown to take part in the regulation of repair processes [3]. Lymphocytes, it is considered, can carry morphogenetic information and, first, can stimulate entry of cells into the mitotic cycle after partial loss of an organ, and second, can give the signal for proliferation to end when the regenerating organ has reached its original weight [4].

We know that five days after splenectomy mitotic activity declines in a tissue of ectodermal origin, namely the corneal epithelium, and synchronization of cell division is disturbed during the 24-h period [6].

In the investigation described below cell proliferation was studied in mouse tissues on another model of immunodeficiency, namely at different times after splenectomy, and also after immunocorrection with the thymus preparation T-activin (fraction AFT-6). T-activin is known to restore many functions of the T system of immunity [1, 2, 5].

EXPERIMENTAL METHOD

Experiments were carried out on 243 male CBA mice weighing on average 20-22 g. The mice were divided into three groups: 1) control: mock thymectomy was performed on some of the animals of this group; 2 and 3) animals undergoing thymectomy by means of the OKh-1 electric suction device, and mice of group 3 received T-activin in a dose of 5 μg per mouse 1 week before the experiment. The mice were killed at different times after the operation or administration of T-activin. To determine mitotic activity (MA) the animals received an intraperitoneal injection of a solution of demecolcine in a dose of 5 mg/kg 3 h before sacrifice. In the experiments to determine the circadian rhythm of proliferation after T-activin administration, besides demecolcine, [^{3}H]thmyidine also was injected, in a dose of 1 μCi/g, 1 h before sacrifice. The 24-hourly fraction of DNA-synthesizing cells also was determined in this same experiment by injection of [^{3}H]thymidine every 4 h for 24 h. The index of c-mitoses (MI_{col}) was determined on total preparations of the cornea and the index of labeled nuclei (RI) in the interfollicular zone of the cortex and in the paracortical zone was determined on histological sections of the inguinal lymph nodes. Both these zones are known to be thymus-dependent [9]. MI_{col} and RI were expressed in promille. The results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

The results of investigation of MA during the 24-h period in the corneal epithelium 9 days after thymectomy are given in Fig. 1. The ordinary circadian rhythm of MA was found in the control mice with maximal values between 3 and 6 a.m. and minimal between 3 and 9 p.m. The time course of MI_{col} during the 24-h period in the corneal epithelium of the thymectomized mice on the 9th day after the operation was generally similar to the control, but the rise of MA between 9 p.m. and midnight was delayed. The total number of corneal epithelial cells entering into mitosis during the 24-h period was 32.28% in the control but 26.41% (or 81.8% of the control value) in the experiment. The cell renewal time was increased correspondingly from 3.1 to 3.8 days.

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 102-104, January, 1985. Original article submitted November 23, 1983.

 0007-4888/85/9901-0126$09.50

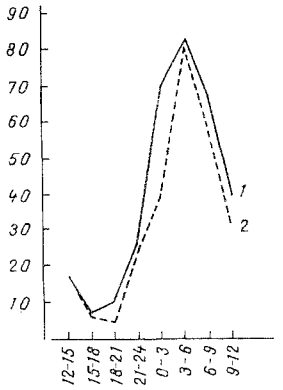

Fig. 1

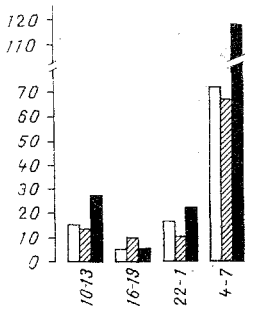

Fig. 2

Fig. 1. Circadian rhythm of MA of corneal epithelium of mice after thymectomy. Abscissa, clock time; ordinate, MA (in %). 1) Control, 2) thymectomy.

Fig. 2. Circadian rhythm of MA of mouse corneal epitheleium after T-activin administration. Unshaded columns — control, obliquely shaded — thymectomy, shaded black — thymectomy + T-activin. Remainder of legend as to Fig. 1.

Thymectomy performed on adult animals thus leads to a decrease in the intensity of cell proliferation in the epithelial tissues and to a disturbance of the rhythm of proliferation soon after the operation. It will be noted that during the short times after thymectomy which were investigated only the population of short-living T_1 lymphocytes of the animals could have decreased. It may therefore be assumed that the funtion of regulation of cell proliferation in nonlymphoid tissues is performed by precisely this fraction of T lymphocytes.

Changes in MA after administration of T-activin to thymectomized and control mice were opposite in character. Table 1 gives the results of determination of MA in the corneal epithelium of mice 5 weeks after thymectomy and 1 week after a single dose of T-activin. In this experiment the animals were given demecolcine at 3 a.m. and were killed at 6 a.m. As the results showed, T-activin increased MA. An even greater increase in the mitotic index was observed in mice with an intact thymus. It could therefore be postulated that immunologically active thymus factors stimulate proliferation of epithelial cells directly, as has been described for lymphokines [11]. However, this suggestion is contradicted by the similarity of the changes in proliferation taking place after thymectomy and splenectomy. T-activin probably activates lymphocytes to interact with epithelial cells, and this is reflected in an increase in MA of those cells.

However, activation of cell proliferation by thymus factors is not direct proof of their regulatory function relative to physiological regeneration of the tissues. All the data so far available, considered as a whole, indicate that the regulatory role of a particular factor can be accepted only if it participates in the formation of the rhythm of physiological function [7, 8]. Accordingly, in another experiment the circadian rhythm of MA in the corneal epithelium and of the number of DNA-synthesizing cells in the target tissue (the thymus-dependent zone of the inguinal lymph nodes) was studied during a 24-h period in mice thymectomized 5 months before the experiment. T-activin was given 1 week before the animals were sacrificed.

Synchronization of proliferation in the lymph nodes of thymectomized mice was found to be reduced during the 24-h period (Table 2). The amplitude of fluctuations of RI fell from 2.0 in the control to 1.7 after thymectomy. After injection of T-activin the amplitude of fluctuations of RI during the 24-h period increased to 5.1, with minimal values at 7 a.m. (4.2 %) and maximal at 1 a.m. (20.0 %). The difference is significant ($P = 0.03$). Under these experimental conditions the mean values of RI in the 24-h period for all three groups did not differ significantly. This may be because of the long intervals between taking the material from the animals. However, comparison of values of the 24-hourly fraction of DNA-

TABLE 1. MA in Corneal Epithelium of CBA Mice under Normal Conditions, after Thymectomy, and after T-Activin Administration

Procedure	MI_{col}, ‰	% of control	P compared with control
Mock thymectomy (control)	88,4±7,6	—	—
Thymectomy	96,5±5,8	109,2	>0,05
Thymectomy+ T-activin	112,3±7,3	127,0	0,047
Mock thymectomy+ T-activin	134,5±8,9	152,1	<0,001

TABLE 2. Circadian Changes in RI in Interfollicular and Paracortical Zones of Inguinal Lymph Nodes of Mice after Thymectomy and T-Activin Administration

Clock time	Control	Thymectomy	Thymectomy+ T-activin
1 p.m.	18,7±2,3	12,0±1,6	8,6±0,5
7 p.m.	9,94±1,0	9,37±1,3	4,2±0,2
1 a.m.	9,11±1,0	12,55±2,4	20,01±2,8
7 a.m.	13,5±1,4	7,37±3,9	3,95±1,7
Mean value for 24-h period	12,8±2,0	10,64±1,2	9,2±2,1

synthesizing cells in the control and experimental mice revealed differences. RI for thymectomized mice was 55.2%, but after administration of T-activin it was 65.2%, or 18.1% higher.

The amplitude of the circadian fluctuations of MI_{col} in the corneal epithelium (Fig. 2) fell from 14.7 in the control to 6.7 after thymectomy and rose to 22.1 after T-activin administration. The general level of proliferation also increased. The aggregated value of MI_{col} for the 24-h period in the corneal epithelium and the 24-hourly fraction of DNA-synthesizing cells were both higher than in the control. One other fact must be taken into account. Some workers have expressed the view that after complete development of the peripheral lymphoid itssue animals become resistant to thymosin [10]. The present experiments showed that a lymphocyte function such as the regulation of proliferation remains sensitive to T-activin, an immunoactive factor of the thymus.

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