EFFECT OF ANTIGEN, INJECTED AT DIFFERENT TIMES OF DAY, ON THE DURATION OF MITOSIS IN CORTICAL CELLS OF THE MOUSE THYMUS

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The effect of antigen on cell proliferation in the cortex of the thymus was studied after injection at times of different mitotic activity in the course of the 24-hour period. The duration of mitosis and the number of cells starting to divide were determined. Immunization with sheep's red cells injected in the morning was shown to have a marked stimulating effect: The number of cells starting mitosis increased and the duration of mitosis decreased (MI_{colch} = 29.79‰ in the control and 47.99‰ in the immunized experimental mice; the duration of mitosis in the control animals was 0.4 h and in the immunized animals 0.24 h). In the animals immunized during the evening no significant changes were observed in these indices compared with intact mice.

KEY WORDS: duration of mitosis; cortex of the thymus; antigen.

The writer previously demonstrated a diurnal rhythm of changes in the mitotic index (MI) of the cortical cells of the thymus and showed that the effect of antigenic stimulation depends on the time of day that the animals were immunized [3].

MI, an index of the proliferative activity of cells, reflects the number of mitoses in an organ at the time of investigation and depends, on the one hand, on the rate at which the cells begin to divide, and on the other hand, on the duration of mitosis (t_m) [7]. Accordingly diurnal changes in MI have been regarded by some workers as the result of changes mainly in the duration of mitosis [6]. Most workers consider that a leading role in the determination of the diurnal rhythm of mitosis is played by the rhythm of entry of the cells into division, although the time of mitosis does not remain constant in the course of the 24-h period [1, 2, 5, 7].

The object of this investigation was to clarify our ideas of the character of cell proliferation in the thymus and on the effect of antigenic stimulation on processes of cell division in that organ by determination of t_m in the thymocytes at different times of day in intact and immunized animals.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino mice weighing 20-25 g, which were divided into four groups (with an average of 25 mice in each group). The animals of groups 1 and 2 (intact and immunized) were killed in the morning (the "morning" group) whereas those of groups 3 and 4 were killed in the evening (the "evening" group). The antigen, sheep's red blood cells, was injected in a dose of 10^9 cells intraperitoneally 4 days before sacrifice of the animals began [4]. Mice of the "morning" groups were immunized at 8 a.m. and those of the "evening" groups at 8 p.m. In each of the four groups 10 mice were given an intraperitoneal injection of colchamine in a dose of 5 mg/kg. The animals of the "morning" groups received this injection at 8 a.m. and they were sacrificed 4 h later, i.e., at noon. Mice of the "evening" groups were given colchamine at 8 p.m. and they were sacrificed at midnight. The remaining mice of each group served as the control and were sacrificed 4-6 at a time at intervals of 2 h (the "morning" group at 8 and 10 a.m. and at noon, those of the "evening" group at 8 and 10 p.m. and at midnight).

Stained films were prepared as described in [4]. Mitoses were counted starting from well-defined prophase and until late telephase, in 18,000-22,000 cells. The duration of mitosis was calculated by the usual equation

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TABLE 1. Changes in MI, MI_{colch}, and t_m in Cortical Cells of the Thymus of Intact and Immunized Animals at Different Times of Day

Group of animals	MI, ‰	MI_{colch}, %	t_m, h
1, 2 (morning, 8 a. m.-noon)			
intact mice	2,99±0,25	29,79±1,46	0,4±0,052
immunized mice	2,84±0,23	47,99±3,97	0,24±0,026
	>0,05	<0,001	<0,011
3, 4 (evening, 8 p. m.-midnight)			
intact mice	2,10±0,29	33,27±2,68	0,25±0,04
immunized mice	1,75±0,15	24,05±3,6	0,3±0,04
	>0,05	0,05	>0,05

$$t_m = \frac{MI \cdot t}{MI_{colch}},$$

where t is the duration of action of colchamine (4 h), MI the mean mitotic index of the control animals in the groups, and MI_{colch} the mitotic index obtained for mice receiving colchamine.

EXPERIMENTAL RESULTS

As Table 1 shows, MI in the cortex of the thymus of the intact mice was higher in the morning than in the evening ($P = 0.046$). This is in agreement with previous observations on the diurnal rhythm of mitosis in mouse thymocytes [3]. The smaller degree of difference between the values of MI in the morning and evening in this investigation than in the previous one [3] can be explained by certain changes in the time of sacrifice of the animals and also, perhaps, by the fact that in the earlier work C57BL mice were used.

Analysis of the results obtained after injection of colchamine into the intact animals at different times of day showed no significant differences in the accumulation of blocked mitoses (MI_{colch} = 29.79 ‰ in the morning and 33.27 ‰ in the evening). Meanwhile, t_m was significantly higher in the morning than in the evening (0.4 and 0.25 h respectively, $P = 0.02$). This suggests that the differences observed in MI of the thymocytes of the intact mice at different times of day were due to the slower course of mitosis in the morning. However, it cannot be stated with confidence that the diurnal rhythm of mitosis depends only on changes in t_m, for the present experiments were carried out not over the whole 24-h period, but only in the morning and in the evening.

The short duration of mitosis in the thymocytes, which varied from 24 min (0.4 h) to 15 min (0.25 h) at different times of day, will be noted. Despite the relatively low values of MI, this confirms the view that the thymus is an organ with high proliferative activity.

The results obtained with the immunized mice showed a significant increase in the number of cells starting mitosis after the morning injection of the antigen than in the intact animals (MI_{colch} = 47.99 and 29.79 ‰ respectively, $P = 0.001$). At the same time a marked decrease in the duration of mitosis from 0.4 to 0.24 h was observed. These changes in the number of blocked mitoses and in the duration of mitosis evidently account for the absence of differences in the values of MI of the thymocytes of the intact and immunized animals during the morning. Nevertheless the increase in the number of cells starting mitosis, accompanied by the acceleration of cell division, suggests that injection of the antigen had a stimulating effect on proliferative processes in the cortex of the thymus.

Injection of sheep's red blood cells in the evening, when minimal values of MI are observed in intact animals [3], caused no significant change in the number of blocked mitoses or in the duration of mitosis. This indicates that the stimulating effect of immunization on proliferation in the thymus depends on the phase of the diurnal rhythm of cell division at the time of injection of the antigen.

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