CHANGES IN THE DIURNAL RHYTHM OF MITOTIC ACTIVITY OF LYMPHOCYTES FROM THE THYMUS AND PERIPHERAL LYMPH GLANDS AFTER IMMUNIZATION

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Diurnal fluctuations in the mitotic index of the thymocytes and lymphocytes from the inguinal lymph glands from C57BL mice immunized with sheep's erythrocytes were studied 2 and 4 days after injection of antigen. No marked response to the antigenic stimulus two days after injection was observed in the cells of these organs. After 4 days there was a significant decrease in the mitotic index of the lymphocytes in the lymph glands, while in the thymus, proliferative processes were stimulated during the morning.

The function of the thymus in the neonatal period is considered to be the formation of lymphoid tissue, and the thymus is also considered to be responsible for the immunological reactivity of the organism [12].

The role of the thymus in sexually mature animals was in doubt for a long time, and it is only recently that investigations have shown that this organ throughout life performs an important function in the regeneration of lymphoid tissue and maintenance of a constant population of small lymphocytes in the peripheral lymphatic system [5, 8, 9, 10, 11, 17]. It also participates in the immune response of the adult organism to introduction of foreign protein [7, 13, 15].

With these facts in mind, it is interesting to study the pattern of cell division in the thymus and in the thymus-dependent peripheral lymphoid tissue [14, 16] during exposure to a specific antigen.

In a previous investigation [1] the writer showed that immunization leads to an increase in proliferative activity in the thymus 4 and 10 days after injection of sheep's erythrocytes, which is accompanied by an increase in the titer of humoral antibodies.

The object of the present investigation was to study the effect of antigen on diurnal changes in the mitotic index (MI) of cells in the cortex of the thymus and lymphocytes from the interfollicular cortical zone of the inguinal lymph glands in the early stages after immunization.

EXPERIMENTAL METHOD

Experiments were carried out on 168 male C57BL mice weighing 16-18 g, divided into three groups: 1) control mice, 2 and 3) immunized animals sacrificed 2 and 4 days after a single intraperitoneal injection of 10^9 sheep's erythrocytes. The mice were decapitated in groups of 7 at a time at different times of day at intervals of 3 h. Animals sacrificed in the morning (at 4, 7, and 10 A.M. and 1 P. M.) were immunized at 8:30 A.M., while the "evening" groups (4, 7, and 10 P.M., 1 A.M.) received their injection of antigen at 8:30 P.M.

All the investigations were thus carried approximately 48 (group 2) and 96 h (group 3) after immunization. The value of MI was determined in the cortex of the thymus and in the cortical layer of the inguinal lymph glands. Specimens were made and the materials treated by the method described previously [1].

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TABLE 1. Changes in MI (in %) of Thymocytes and Lymphocytes of Immunized Animals at Different Times after Injection of Antigen

Time when mice sacrificed	Thymus				Lymph glands			
	group 1 (control)	group 2 (2 days after immunization)	group 3 (4 days after immunization)	P for comparing groups 1 and 3	group 1 (control)	group 2 (2 days after immunization)	group 3 (4 days after immunization)	P for comparing groups 1 and 3
4 P.M.	2.8	2.8	2.4	—	0.31	0.30	0.13	0.022
7 P.M.	2.5	2.7	2.1	—	0.24	0.24	0.07	0.005
10 P.M.	1.8	2.3	2.3	—	0.25	0.12	0.09	0.032
1 A.M.	1.9	2.5	1.7	—	0.20	0.17	0.11	—
4 A.M.	2.3	2.3	3.5	0.023	0.17	0.17	0.15	—
7 A.M.	2.3	2.2	3.2	0.058[1]	0.26	0.20	0.11	0.028
10 A.M.	2.8	3.0	3.4	—	0.37	0.42	0.10	0.001
1 P.M.	3.7	2.6	3.4	—	0.49	0.40	0.21	0.001
Mean value of MI	2.4	2.6	2.5		0.28	0.25	0.12	
	10 A.M.–1 P.M. P=0.004 1 P.M.–4 P.M. P=0.010	10 P.M.–10 A.M. P=0.035	10 P.M.–4 A.M. P=0.069 1–4 A.M. P=0.002 1–7 A.M. P=0.004 (10 P.M.+1 A.M.) (4 A.M.+7 A.M.) P=0.001		10 A.M.–1 P.M. P=0.069 7 A.M.–1 P.M. P=0.011 4 A.M.–1 P.M. P=0.001 1 P.M.–4 P.M. P=0.056 1 P.M.–7 P.M. P=0.002	7 A.M.–10 A.M. P=0.069 4 A.M.–10 A.M. P=0.028 10 P.M.–10 A.M. P=0.011	10 A.M.–1 P.M. P=0.021 1 P.M.–4 P.M. P=0.071 1 P.M.–7 P.M. P=0.005 7 P.M.–4 A.M. P=0.034	

[1]For 4 and 7 A.M. together, P=0.001 for groups 1 and 3; for 7 and 10 A.M. together, P=0.011 for groups 1 and 3.

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that cortical cells of the thymus and thymus-dependent zone of the lymph glands are characterized by a diurnal rhythm of mitotic activity, MI reaching a maximum between 10 A.M. and 1 P.M. and a minimum between 10 P.M. and 4 A.M. (group 1). The value of MI for the thymocytes was 5–7 times higher than for the lymphocytes.

In group 2, MI of the thymocytes was less than in group 1 at 1 P.M. (P=0.019), whereas at other times no significant differences were found. Changes in MI of the thymocytes during the 24-h period were not significant in group 2. MI values for the lymph glands of animals of groups 1 and 2 were almost identical, except at 1 and 10 P.M., when the number of mitoses in the immunized mice was a little lower.

More marked changes in cell division were observed in the organs in group 3. MI in the thymus of these animals was lower than in the controls in the early morning from 4 to 7 A.M. Activity of cell division in the lymph glands was lower at nearly all times of sacrifice.

Changes arising in thymocytes in response to injection of antigen have been described [2, 3]. Cells of thymic origin, when injected into irradiated syngeneic animals, respond by increased mitotic activity to immunization of the recipient [4]. The results of the present investigation, demonstrating an increase in cell proliferation in the cortex of the thymus in the morning hours, 4 h after immunization, are in agreement with these other findings. They also confirm the results of the earlier investigations [1]. This conforms completely with the view that the thymus contains antigen-reactive cells [13, 15], which proliferate actively after injection of foreign protein into the organism and interact with the bone-marrow precursors of antibody-forming cells.

The perifollicular zone and the deep cortical substance of the lymph glands are known to be densely populated by small lymphocytes and to belong to the thymus-dependent lymphoid tissue [14, 16]. The inductive role of small lymphocytes in the immune response has been demonstrated by the work of Gowans et al. [6]. These cells are capable of reacting with antigen and of being converted into cells with different morphological characteristics. The decrease in MI of the small lymphocytes of the inguinal lymph glands observed in the present experiments may perhaps be connected with the involvement of these cells in the changes induced by injection of antigens.

The study of the diurnal changes in activity of cell division in the cortex of the thymus and the thymus-dependent zone of the inguinal lymph glands thus showed that four days after injection of the antigen, cell proliferation was intensified in the thymus during the morning. In the lymph glands, cell division was depressed in response to injection of antigen throughout the 24-h period 4 days after immunization.

LITERATURE CITED

1. V. V. Sinel'shchikova and A. A. Burkin, Byull. Éksperim. Biol. i Med., No. 5, 80 (1971).
2. M. M. Black and H. R. Ansley, J. Cell Biol., 26, 202 (1965).
3. E. P. Cohen, J. Mayer, and K. Freidman, J. Exp. Med., 130, 1161 (1969).
4. A. J. S. Davis, E. Leuchars, V. Wallis, et al., Transplantation, 4, 438 (1966).
5. A. Globerson and M. Feldman, Transplantation, 2, 212 (1964).
6. J. L. Gowans, D. D. McGregor, D. M. Cowen, et al., Nature, 196, 651 (1962).
7. J. M. Johnston, Fed. Proc., 29, 429 (1970).
8. G. Linchet and A. M. Cuvelier, Path. et Biol., 17, No. 78, 365 (1969).
9. D. Metcalf, Nature, 208, 1336 (1965).
10. J. F. Miller, Nature, 208, 1337 (1965).
11. J. F. Miller, Nature, 208, 1332 (1965).
12. J. F. Miller and P. Ducor, The Biology of the Thymus [Russian translation], Moscow (1967).
13. J. F. Miller, Jap. J. Bact., 24, 417 (1969).
14. D. M. V. Parrott, M. A. B. de Sousa, and J. East, J. Exp. Med., 123, 191 (1966).
15. G. M. Shearer and G. Cudkowicz, J. Exp. Med., 130, 1243 (1969).
16. D. E. R. Sutherland, M. F. McKneally, M. J. Kellum, et al., Internat. Arch. Allergy, 38, 6 (1970).
17. R. B. Taylor, Nature, 208:1334 (1965).