EFFECT OF IMMUNODEFICIENCY AND IMMUNOSTIMULATION ON DEVELOPMENT OF EXPERIMENTAL TUBERCULOSIS

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A previous investigation [10] showed that the functional state of the T system of immunity in experimental tuberculosis is largely determined by the hormonal activity of the thymus, as shown by the lower intensity of the tuberculosis lesion in guinea pigs following transplantation of the thymus into them by the diffusion chamber method. These results are in agreement with those obtained in thymectomized mice infected with tuberculosis [1].

After thymectomy in adult rats their immunologic potential is known to fall progressively [13].

The object of this investigation was to study the course of experimental tuberculosis in the presence of a varied degree of deficiency of the T system of immunity and correction of the immunodeficiency by administration of the thymus preparation thymalin (a basic polypeptide consisting of 38 amino acids) [6].

EXPERIMENTAL METHODS

The experimental scheme is illustrated in Fig. 1. Experiments were carried out on 180 female albino rats, thymectomized (experimental) and intact (control). Thymectomy was performed on the animals at the age of one month, by a surgical method under ether anesthesia. Completeness of removal of the thymus was verified macroscopically at autopsy on the animals and by histological examination of the cellular tissue of the anterior mediastinum. Rats with residual thymus tissue were rejected. Rats with different degrees of development of immunodeficiency, i.e., 3, 6, and 9 months after the operation (age groups 1, 2, and 3), were used for infection. Each group was accompanied by control animals of corresponding age. A culture of *M. tuberculosis* strain bovis 109 was injected subcutaneously in a dose of 1 mg per rat. Two months after injection half of the thymectomized rats began to receive intramuscular injections of thymalin three times a week in a dose of 1.5 mg/kg body weight (14 mg per course of treatment). The other half of the thymectomized animals received injections of the same volume of solvent. The peripheral blood was investigated before infection and again 2 and 4 months after infection. The number of leukocytes and the blood formula, determined in films stained by Pappenheim's method, and lymphocytology also was carried out. The peripheral blood leukocyte migration inhibition test to PPD was carried out in capillary tubes by Artemova's method [2] 2 and 4 months after infection. All animals of a given age group were killed with ether 4 months after infection. The lung lesions found at autopsy were assessed in accordance with a 4-point system [5]. The results of examination of the peripheral blood and inhibition of leukocyte migration were subjected to statistical analysis by the Student–Fisher method [9] and the indices for the lung lesions were analyzed by a nonparametric method using the Wilcoxon–Mann–Whitney U criterion [3].

EXPERIMENTAL RESULTS

The results showed that the severity of the course of tuberculosis in the experimental rats was significantly determined by the intensity of the immunodeficiency: In groups 1 and 2 (3 and 6 months respectively after the operation) the index of lung involvement in the

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TABLE 1. Indices of Lymphocytometry in Control and Thymectomized Rats Differing in Their Degree of Immunodeficiency (M ± m)

Experimental conditions	Time of investigation, months	Group 1			Group 2			Group 3		
		large	medium	small	large	medium	small	large	medium	small
Control	0	1381±308	9990±1479	3116±610	858±111	7100±695	3239±249	1172±127	3621±344 †	2015±214 †
	2	819±99	8702±950	3719±438 †	1629±260	7969±748 †	2353±356	1317±250	4361±655	1462±172 †
	4	1182±152	5863±429	2570±441	802±96	5610±600	1075±164 †	1570±254	6589±892	921±127 †
Thymectomy	0	1465±168	7002±961	1772±291	836±138	4668±375*	1448±281	1142±169	1956±184*	473±100*
	2	793±139	7731±1200	2425±442*	1220±266	4495±1184*	1365±320	1166±262	3525±641	495±73*
	4	1021±161	4664±590	1401±379	1095±200	5138±748	629±84*	1519±196	5006±498	459±189*
Thymectomy+ thymalin	4	1502±284	6164±705	1867±392	765±182	2633±245 ‡	508±119*	859±134 †	3184±239 ‡	505±135*

*Differences significant compared with control.
†Differences significant compared with group of thymectomized animals without treatment.
‡Differences significant compared with control and with group of thymectomized animals without treatment.

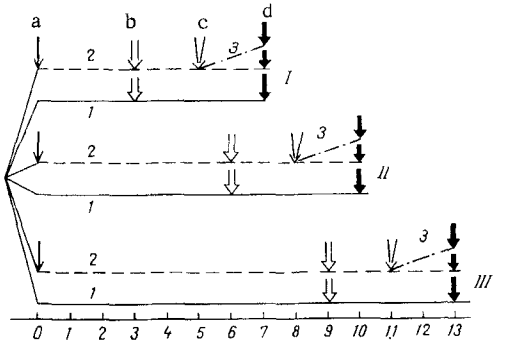

Fig. 1. Experimental scheme. I-III) Age groups 1, 2, and 3 respectively. 1) Intact control rats; 2) thymectomized rats; 3) thymectomized rats treated with thymalin. a) Thymectomy; b) infection; c) beginning of treatment; d) autopsy. Numbers below indicate time after thymectomy (in months).

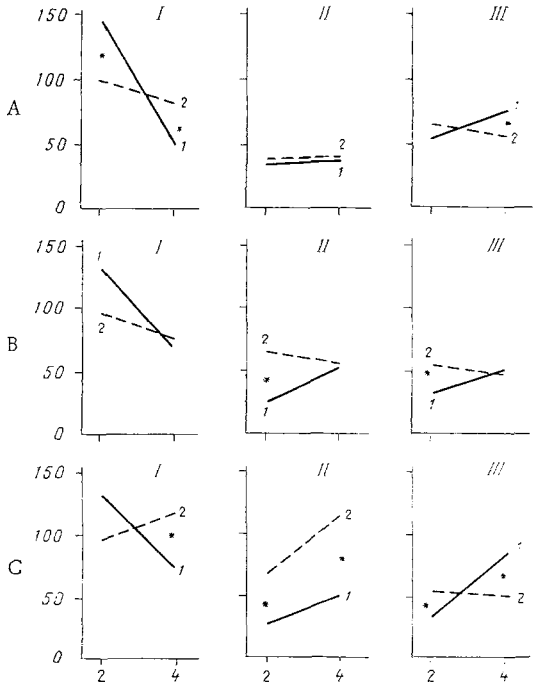

Fig. 2. Changes in migration activity of peripheral blood leukocytes (spontaneous and in response to PPD) in control and thymectomized rats with different degrees of immunodeficiency. Abscissa, time after infection (in months); ordinate, migration (in conventional ocular micrometer scale units). A) Control animals; B) thymectomized animals; C) thymectomized rats treated with thymalin. I-III) Groups 1, 2, and 3 respectively. 1) Spontaneous migration; 2) migration in response to PPD. Asterisk indicates significance of differences.

thymectomized animals, both treated and untreated, did not differ significantly from the level of involvement in the control rats. In the thymectomized rats of group 3 (9 months after the operation), on the other hand, the index of involvement was 2.0 (1.0 in the corresponding control; $P = 0.05$). Injection of thymalin into the thymectomized rats of this group significantly reduced the intensity of the tuberculosis lesion (index of involvement 0.8). Comparison of the indices of lung involvement in the control animals and in those of age groups 1, 2, and 3 did not show any significant difference (0.8, 0.8, and 1.0 respectively).

Inhibition of immunologic reactivity in the thymectomized rats was confirmed by the data of lymphocytometry [7]. The results in Table 1 show a sharp fall in the level of small lymphocytes in the thymectomized rats, most marked in age group 3. The age decrease in the number of small lymphocytes in the control animals was by a much lesser degree. Injection of thymalin into the thymectomized rats did not change the number of small lymphocytes in any age group compared with that found in thymectomized untreated animals, but it was accompanied by a fall in the level of the population of large and medium lymphocytes.

Thymectomy led not only to quantitative changes in the peripheral blood lymphocyte subpopulation, but also to disturbance of some of their functional parameters; in particular, lymphokines production during stimulation by PPD and the migration properties of the peripheral blood leukocytes were modified.

Spontaneous migration of peripheral blood leukocytes is known to correlate with the level of functional activity of the T system of immunity [8]. Analysis of spontaneous migration in the control and thymectomized animals of all age groups showed a sharp decline in migration activity, due evidently to the development of the tuberculous lesion, and was

most marked in the rats of group 2. Injection of thymalin, while reducing the severity of the tuberculosis in the animals of group 3, at the same time caused a significant increase in spontaneous leukocyte migration.

The development of tuberculosis not only inhibits spontaneous migration of leukocytes, but also completely blocks lymphokine production by lymphocytes *in vitro* in response to PPD in thymectomized untreated rats of all groups 4 months after the beginning of the disease (Fig. 2). Two months after infection, in response to PPD the thymectomized rats of groups 2 and 3 showed significant stimulation of migration, evidently due to the production of migration-stimulating factor by the lymphocytes. The cause of the switch from lymphokine production in response to PPD *in vitro* during the development of tuberculosis in the control and thymectomized rats was evidently a difference in the degree of sensitization and the predominant response of the T and B lymphocyte subpopulations. According to data in the literature [11], PPD is a mitogen for the B population even in unsensitized animals. The possibility cannot be ruled out that when rats treated with thymalin developed sensitization the response of the B population to stimulation by PPD *in vitro* is effected through the thymalin-stimulated helper subpopulation of T lymphocytes.

The similarity between responses to PPD in the rats of group 3, which were thymectomized and treated with thymalin, and the control rats, and also potentiation by thymalin of responses typical of the control nonthymectomized animals, will be noted.

Variations in the response of the control rats to PPD observed in the course of development of tuberculosis are in harmony with the contradictory results of investigations of inhibition of peripheral blood leukocyte migration in clinical practice [4, 12].

The results of this investigation thus indicate that the development of experimental tuberculosis depends on the degree of intensity of the immunodeficiency, and ability of the lymphocytes to form lymphokines in response to PPD. Injection of thymalin into immunodeficient rats led to restoration of the animals's immunologic reactivity, as reflected in the results of lymphocytometry and data on the functional properties of the lymphocytes obtained in the leukocyte migration inhibition test, and it reduced the severity of the tuberculosis process.

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