ROLE OF THE THYMUS IN REGULATION OF PERIPHERAL T-CELL ACTIVITY DURING SPONTANEOUS CARCINOGENESIS IN MICE

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The development of malignant neoplasms is known to be accompanied by involution of the cortical layer of the thymus [7, 8]. It has been shown on a model of transplantable tumors that involution of the thymus is connected primarily with migration of immature cortical thymocytes into peripheral lymphoid organs [11]. Tanaka and co-workers showed that the release of cortical cells from the thymus in the early stages of tumor growth is linked with the appearance of specific suppressor T cells in the spleen of tumor-bearing mice, blocking antitumor immune reactions and stimulating tumor growth [10]. On the basis of these results, the widely held views on functional depression of the thymus during tumor growth can be re-examined [2, 13], and the thymus can be regarded as an organ playing an active role in the regulation of relations between the immune system and the tumor. Support for this hypothesis also is given by data on the stimulating effect of the thymus on the development of spontaneous neoplasms in mice of lines highly susceptible to cancer [1]. In this connection it is interesting to study functional activity of the thymus cells and the mechanisms of their role in the regulation of immunity during spontaneous carcinogenesis.

EXPERIMENTAL METHOD

Intact female A/Sn and C57BL/6 mice of three age groups (2, 6, and 12-14 months), and also A/Sn and C3H/He mice with spontaneous mammary gland adenocarcinomas (age 10-14 months) were used. The thymus and spleen were removed from the animals and suspended under aseptic conditions. Spontaneous proliferation of thymocytes was assessed on the basis of incorporation of ^{3}H-thymidine in a 14-16-h culture with cells in a concentration of $2 \cdot 10^6$ cells/ml. Alloantigen-stimulated T-cell proliferation in mixed lymphocyte culture (MLC) was assessed by the method in [6] with minor modifications. As stimulating cells we used splenocytes from DBA/2 mice, treated with mitomycin C ("Serva," West Germany, 20 μg/ml). To induce nonspecific suppressor T cells, thymus and spleen cells ($2 \cdot 10^6$ cells/ml) were incubated for 2 days in the presence of concanavalin A ("Serva," West Germany; 10 μg/ml) in a 24-well planchet ("Costar," USA). Activity of con A-induced suppressor cells was estimated by inhibition of proliferation of syngeneic splenocytes in MLC. Lymphocytes were cultured in medium RPMI 1640 with the addition of 10% embryonic serum, 20 mM HEPES, 2 mM L-glutamine, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and 40 μg/ml gentamicin. At the end of culture the cells were sedimented on glass fiber filters. Radioactivity incorporated into the acid-insoluble fraction was measured on a Mark III scintillation counter. The number of T lymphocytes in the spleen of the mice was determined in the cytotoxic test using anti-Thy-1,2-serum [9]. The electrophoretic mobility of the thymus cells was measured on a "Parmoquant-2" automatic microscope ("Carl Zeiss," East Germany). The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The experiments showed that the cell content of the thymus did not differ significantly in A/Sn and C3H/He mice with spontaneous neoplasms (weight of tumor not over 2-3 g) and in intact animals of the same age, and amounted on average to $(18\text{-}21) \cdot 10^6$ cells. No significant changes likewise were observed in spontaneous proliferation of the thymocytes (Table 1). A definite tendency was noted for the proliferative response of the thymus and spleen cells in mixed lymphocyte culture to fall

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TABLE 1. Parameters of Functional Activity of Thymus-Dependent Lymphocytes in A/Sn and C3H/He Mice with Spontaneous Tumors

Parameter, $\times 10^3$ cpm	A/Sn		C3H/He	
	control	tumor	control	tumor
Spontaneous proliferation of thymocytes	53,2±1,8	67,4±6,9	79,1±4,9	73,2±2,1
Proliferation of thymocytes in MLC	4,2±0,5	3,0±0,3	3,3±0,3	2,3±0,2*
Proliferation of splenocytes in MLC	19,7±1,3	16,4±1,7	18,2±1,3	14,6±1,6

Legend. *$p < 0.05$ compared with control.

TABLE 2. Age Changes in Functional Activity of Thymus-Dependent Lymphocytes in A/Sn and C57BL/6 Mice

Parameter, $\times 10^3$ cpm	Line of mice	Age of animals (months)		
		2	6	12
Spontaneous proliferation of thymocytes	A/Sn	51,7±2,7	49,4±1,8	50,6±1,7
	C57Bl/6	46,3±3,4	52,1±3,4	45,0±3,9
Proliferation of thymocytes in MLC	A/Sn	1,61±0,10*	2,68±0,30	4,22±0,46*
	C57Bl/6	1,36±0,05	1,96±0,26	2,34±0,44
Proliferation of splenocytes in MLC	A/Sn	13,7±1,0	20,1±1,4*	18,4±0,8*
	C57Bl/6	11,6±1,2	12,7±0,6	8,2±0,9

Legend. Asterisk denotes significance of differences between lines of mice of the same age group, $p < 0.05$.

(Table 1). However, this was most probably due, not to depression of functional activity of the mature T lymphocytes reacting in MLC, but to a decrease in their relative number. In particular, the percentage of Thy-1^+ splenocytes, against the background of general hyperplasia of the spleen, was 26.5 ± 1.9% in A/Sn mice wtth a tumor, compared with 34.5 ± 2.5 in the control group ($p < 0.02$). No significant changes in functional activity of the con A-induced suppressor T cells likewise were found in the spleen, in which it was 22 ± 3.5% in A/Sn mice with spontaneous tumors and 26 ± 1.4% in animals of the control group. Consequently, the appearance of spontaneous mammary gland neoplasms in mice of high-cancer lines is not accompanied by significant disturbances of function of the thymus-dependent component of the immunity system.

It can be tentatively suggested that the process of spontaneous carcinogenesis, linked in this case with vertical transmission of an oncovirus, determines changes in the thymus and T-dependent immunity long before the appearance of tumors. To test this hypothesis we compared age changes in the number and functional activity of thymus cells and peripheral T lymphocytes in mice of the high-cancer line A/Sn and of the C57BL/6 line, resistant to development of spontaneous mammary gland tumors and other neoplasms (age up to 118 months). The investigations showed that a gradual decrease in the cell content of the thymus was observed in the animals with age, and was significantly more marked in the high-cancer mice. Involution of the thymus in A/Sn mice was linked with a parallel increase in the T-cell population in the spleen (Fig. 1). Meanwhile, against the background of age-induced hypoplasia of the thymus, a high level of spontaneous proliferation of thymocytes was maintained (Table 2). These circumstances suggest that in mice of the high-cancer line migration of cells from the thymus into peripheral lymphoid organs, especially the spleen, takes place.

The proliferative response of the thymus and spleen cells to alloantigens in MLC increases with age in A/Sn mice. In C57BL/6 mice a tendency was noted for activity of thymocytes to increase, but no such changes were found in the spleen (Table 2). The changes observed may be partly associated with a change in the population structure of the lymphoid organs. For instance, according to the data of analytical cell electrophoresis, an increase in the relative percentage of mature thymocytes, with high mobility in an electric field, is observed in A/Sn mice: 10.1 ± 1.2, 13.0 ± 1.4, and 14.8 ± 1.9% respectively at ages of 2,

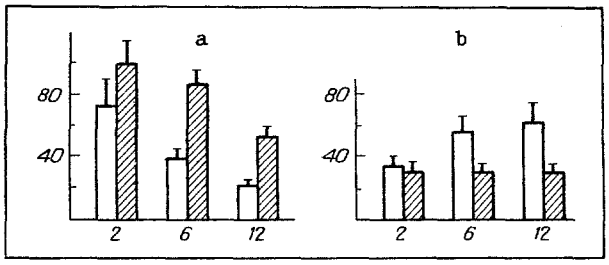

Fig. 1. Changes in number of thymocytes (a) and T-lymphocytes in the spleen (b) with age in A/Sn and C57BL/6 mice. Abscissa, age of animals, months; ordinate, number of cells, $\times 10^6$; unshaded columns — A/Sn mice, shaded columns C57BL/6.

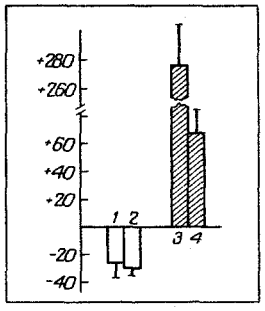

Fig. 2. Effect of con A-induced immunoregulatory thymus and spleen cells of A/Sn and C57BL/6 mice on proliferation of syngeneic splenocytes in MLC. Abscissa: 1) A/Sn thymocytes, 2) A/Sn splenocytes, 3) C57BL/6 thymocytes, 4) C57BL/6 splenocytes; ordinate, index of activation (+) or suppression (−).

6, and 12 months. The relative percentage of T lymphocytes in the spleen of A/Sn mice in the period from 2 to 6 months rose from 23.1 ± 2.0 to 33.4 ± 2.4% ($p < 0.01$), and this was accompanied by a simultaneous increase in the proliferative response of the splenocytes in MLC. Meanwhile considerable differences in functional activity of spleen cells of mice of the opposite lines between the ages of 6 and 12 months were observed with a similar relative content of T lymphocytes, namely about 30% on average. Consequently, aging mice of the high cancer line were distinguished by a comparatively high level of response of their T lymphocytes to alloantigens.

Comparison of the results relating to population and functional changes in the thymus and spleen suggests that migration of immature cortical thymocytes was intensified in A/Sn mice. Precursors of suppressor T cells, which have an inhibitory effect after activation by con A, are found among immature thymocytes [5]. The present investigation showed that thymus and spleen cells of A/Sn mice, preincubated with con A, do in fact inhibit the proliferative response of syngeneic splenocytes to MLC. Meanwhile, thymocytes and splenocytes of C57BL/6 mice, on the other hand, stimulated activity of syngeneic T lymphocytes (Fig. 2). A similar trend in con A-induced immunoregulatory effects in mice of opposite lines is exhibited also at ages of 2 and 12 months.

Thus in the period before the appearance of spontaneous tumors, and after their appearance, the thymus-dependent component of immunity in A/Sn and C3H/He mice is in a relatively highly active state. On the other hand, it is very likely that increased migration of immature thymocytes (precursors of suppressor T cells) takes place into the spleen of animals with a high risk of cancer, and this can be regarded as one of the mechanisms regulating the immune response to a tumor. As a rule, activa-

tion of suppressor cells of varied specificity does in fact take place in the early stages of tumor development [3, 4]. The immunosuppressive properties of immature thymocytes and their ability to stimulate tumor growth in vivo have been demonstrated [12]. A connection has been established between the appearance of specific suppressor T cells in the spleen of tumor-bearing mice and intensive migration of cortical cells from the thymus [10]. Considering some common properties of specific and con A-induced suppressors (dependence on the presence of the thymus, Lyt $\varnothing 1^+2^+$ phenotype of the precursors, etc.) [5], it seems probable that the data cited in the literature and our own results reflect the basically single mechanism of involvement of the thymus in specific regulation of the immune response during tumor growth.

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