Transfer of Signs of Aging to Young Mice by Splenic Lymphoid Cells from Old Syngeneic Donors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 7, pp. 100-102, July, 2007
Original article submitted December 6, 2006

Single intraperitoneal injection of splenic lymphoid cells from 20-month-old mice to 2-month-old syngeneic mice (similarly as 5-day injections of purified brain extract) leads to preterm (4 months earlier than in the control) appearance of aging factor in the blood (the main sign of old age). Combined injections of brain extract and splenic lymphoid cells led to the appearance of aging factor in the blood 5 months earlier than in the control.

Key Words: *aging factor; lymphocytes; transfer of signs of aging*

Splenic lymphocytes acquire morphogenetic activity during repair processes. Adoptive transfer of these lymphocytes to normal recipients causes changes in morphological signs and resumption of protein synthesis associated with regeneration [1]. It was also shown that lymphocytes acquire morphogenetic activity in autoimmune diseases and some non-immune disorders [6]. Adoptive transfer of these lymphocytes to normal recipients induces the appearance of signs and symptoms of the corresponding pathology. However, it remains unclear whether morphogenetic activity of lymphocytes can appear during normal development of the organism.

For answering this question a model of aging process can be used. Aging is a physiological process associated with obligatory progressive accumulation of species-specific thermostable trypsin- and UV-resistant factor in the blood and brain tissue. This factor stimulates proliferation of glial cells *in vivo* and in cell culture [3]. This aging factor (AF) is an inductor of aging: intraperitoneal injection of purified brain extract from old mice accelerates aging of young experimental mice [4]. This fact was confirmed by findings of morphometrical analysis indicating rapid neuronal death and simultaneous rapid proliferation of the glia (gliosis) in the brain cortex of recipients [5]. Under conditions of chronic experiment, the content of AF can be evaluated by the capacity of purified brain extracts and purified serum from experimental animals to stimulate active proliferation of glial cells in culture [3,5].

The aim of this study was to clear out whether lymphocytes acquired morphogenetic activity during aging.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old C57Bl/6 mice (n=40) divided into 4 groups, 10 per group.

Group 1 (control) comprised naturally aging animals. Group 2 animals were daily (for 5 days) injected (intraperitoneally) with 0.5 ml purified extract from the brain of 20-month-old syngeneic mice prepared by the standard method [4]. Group 3 animals received a single intraperitoneal injection of 6×10^6 splenic lymphoid cells in 1 ml from the same donors RPMI. Group 4 mice received a single intraperitoneal injection of 60×10^6 splenic lymphoid cells in 1 ml RPMI and daily (for 5 days) 0.5 ml brain extract from 20-month-old syngeneic mice.

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0007-4888/07/1441-089

Splenic cell suspension was washed 3 times with RPMI and centrifuged at 3000 rpm for 5 min [2]. Activity of AF in recipient serum was evaluated by the intensity of proliferation of cultured EPIT-5 glial cells.

The cells were cultured in growth medium containing DMEM, glutamine (3%), HEPES (3%), FCS (10%), and antibiotics.

Blood samples from live mice were collected into sterile centrifuge tubes and incubated at 37°C for 30 min; after which the clots were detached from the tube walls, the samples were centrifuged for 15 min at 2000 rpm, the serum was collected, diluted 10-fold with distilled water, heated for 30 min at 90°C, and clarified by centrifugation.

Activity of AF in recipient sera was evaluated by intensity of cell proliferation in EPIT-5 culture.

Activity was expressed as the proliferation index (ratio of cell count in experiment to that in the control). To this end, 0.1 ml test serum was added to 1 ml suspension. The mixture was incubated for 72-96 h at 37°C in nitrogen atmosphere, after which the cells in the samples were counted. Cell samples to which 0.1 ml distilled water was added, served as the control.

RESULTS

The data demonstrating the intensity of glial cell proliferation in the culture (proliferation index) under the effect of serum specimens collected at different terms of observation from naturally aging mice and from mice injected with various biological substrates are presented in Table 1.

Activity of serum AF increased during aging in all groups. In group 1, the first significant increase in AF activity was observed at the age of 8 months (6 months after the start of the study). In group 4, activity of AF increased significantly as early as at the age of 3 months (1 month after the start of the study), though injections of brain extract or lymphoid cells alone did not appreciably modify the level of AF. This means that lymphoid cells undergo some changes in the course of aging, as a result of which they acquire capacity to accelerate the appearance of signs of preterm aging by at least 5 months. Activity of AF increased most intensively in the sera of young recipients in comparison with the previous term of observation, its increase was detected 2 months after the start of treatment, but later the increase became slower. One month after the start of treatment, the first deaths were observed in group 4, which was 1 month earlier than in group 2 and 5 months earlier than in group 3.

TABLE 1. Changes in the Cellular Proliferative Activity Index in the Sera of Young Recipients Injected with Brain Extract or Lymphoid Cells from Old Donors, or Combination of Both

Month after injections	Group			
	1 (control)	2	3	4
1	1.00	1.06	1.02	1.21
2	1.09	1.75	1.48	1.90
3	1.08	1.86	1.67	2.26
6	1.24	1.99	1.87	2.49
12	1.71	2.41	2.02	2.72
15	2.2	2.62	2.18	2.92

Comparative analysis of AF content in the blood of recipients injected with brain extract alone and in combination with lymphocytes showed that lymphoid cells from old mice not only accelerated, but also potentiated the effect of injected AF at all stages of observation. The results in group 3 are most interesting. The increase in blood level of AF in these recipients was maximum and manifested 4 months earlier than in naturally aging mice (group 1). The increase in the proliferation index in recipient serum under the effect of lymphoid cells from old mice indicates that their cytogenetic characteristics change with aging, acquiring the capacity to stimulate proliferation of glial cells, similarly as AF. Hence, the data indicate that normal lymphocytes can exhibit morphogenetic activity under certain conditions. The mechanism of these changes in lymphoid cells and their role in modification of AF activity remain unknown. The next step now is to clear out whether lymphoid cells start to produce this factor or they induce its synthesis in other (not identified yet) cells.

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