Specific Features of the Immune Response in Old Recipient Mice to Cell Therapy

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Implantation of somatic cells from autopsy specimens (15-18 weeks gestation) had a modulatory effect on immunogenesis in old mice. Activation of the immune system in recipient animals after administration of somatic cells reflects a multistage influence of test preparation. The observed changes were reversible. A progressive decrease in activation of the immune system in recipient mice was not accompanied by the development of pathological changes. Repeated implantation was required to maintain these processes.

Key Words: *immune system in old mice; somatic cells*

Aging is associated with the replacement of the parenchyma in various organs by connective tissue. This process is accompanied by a decrease in functional activity of all systems in the organism. The immune system has a major role in this process. Age-related involution of the thymus and suppression of thymic hormone production were described previously [1]. Much attention is paid to drug treatment and tissue therapy under conditions of aging and change in histophysiology of organs. Tissue therapy is extensively used in a variety of diseases. Tissue products are obtained from animal tissues and, more rarely, from human tissues. Fetal tissues have several advantages over tissues of the adult organism. They are characterized by rapid growth, expression of many genes [2], and high concentration of growth factors [6]. These tissues are used for the therapy of various diseases. The effects of fetal tissues are mediated by specific and nonspecific mechanisms [3,5]. A specific action is associated with the substitution effect of fetal tissues. A nonspecific action results from activation of the immune system in recipients and is poorly understood. It remains unclear whether immune organs in old people with age-related involution can exhibit a positive response to implantation of donor tissues (*i.e.*, applicability of tissue therapy in gerontology). This question is of considerable importance due to a high ratio of elderly people in Russia.

Here we studied the response of immune organs in old recipients to administration of cryopreserved preparations of somatic cells (PSC), but not of living fetal tissues from the donor.

MATERIALS AND METHODS

Experiments were performed on 30 CBA mice weighing 30 g and aging 30 months (which corresponded to 75-80 years of human life). PSC were isolated from autopsy specimens (15-18 weeks gestation) and stored at -196°C until implantation. PSC in a single sub-tolerance dose (nontoxic for animals) was injected subcutaneously into the thigh of recipients [7]. The animals were killed on days 7, 14, 42, and 64. The thymus and spleen were isolated and weighed. Blood leukocyte count was estimated by routine tests. Specimens of the thymus and spleen

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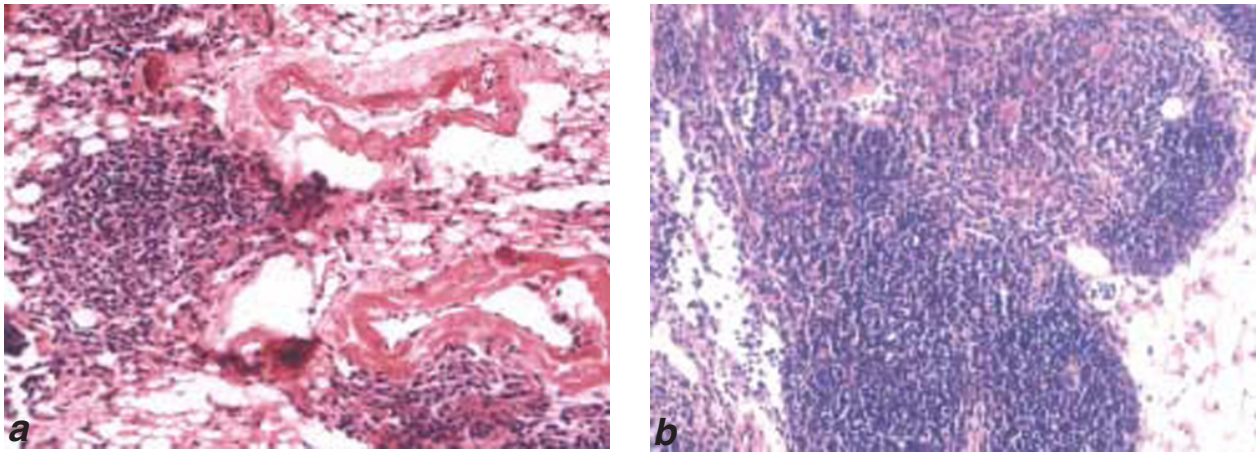

Fig. 1. Thymus of an old CBA mouse. Control: no division of the thymus into lobules (*a*). Day 42 after treatment with PSC: division of the parenchyma into the cortex and medulla (*b*). Here and in Fig. 2: hematoxylin and eosin staining (*70).

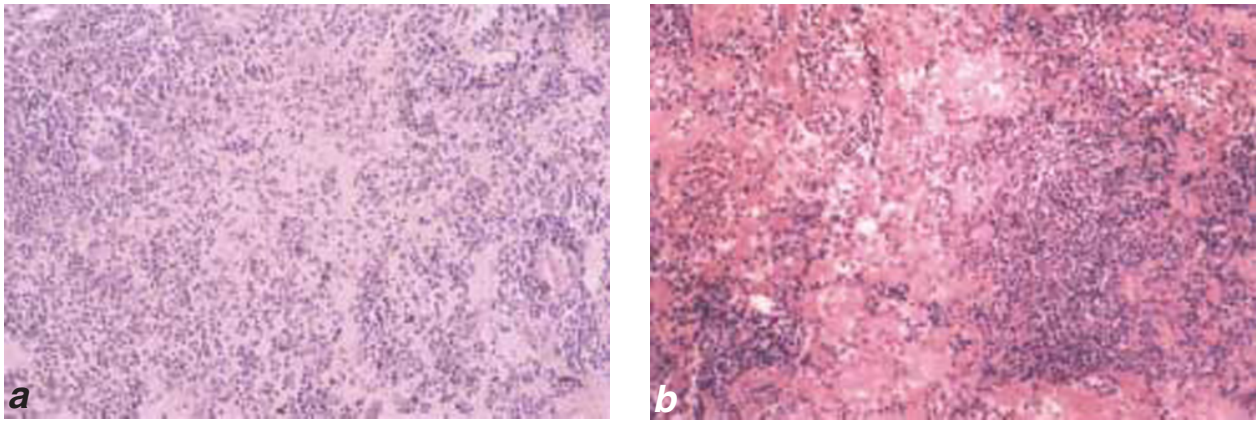

Fig. 2. Spleen of an old CBA mouse. Control: absence of lymphoid follicles (*a*). Day 42 after treatment with PSC: clear division into the white and red pulp (*b*).

were fixed and subjected to histological treatment. The samples were stained with hematoxylin and eosin, Schiff reagent, and Brachet-Pearl's dye.

The mitotic index of cells and total number of T lymphocytes, B lymphocytes, and macrophages (in percent) were measured in the thymus. T lymphocyte count was determined with rabbit antiserum to mouse brain tissue. The number of mouse B lymphocytes was estimated from the presence of surface immunoglobulins. The area of the white and red pulp and total number of T lymphocytes, B lymphocytes, and macrophages were measured in the spleen.

TABLE 1. Cytological Characteristics of the Thymus in Old CBA Mice after Treatment with PSC (*M*±*m*)

Time after PSC treatment, days	Weight of the thymus, mg	Lymphocyte count in the thymus, $\times10^6$	T cells, %	T cell count in the thymus, $\times10^6$	B cells, %	B cell count in the thymus, $\times10^6$	Macrophage number in one field of view	Lymphocyte mitotic index
Control (*n*=4)	14.7±3.7	10.0±4.0	93.0±0.6	9.6±4.1	1.7±0.3	0.2±0.1	4.3±0.7	0.2±0.2
7 (*n*=3)	15.0±2.5	22.7±6.7	89.0±1.6	19.9±28.0	2.1±0.3	0.40±0.03	7.6±0.7	0.70±0.05
14 (*n*=3)	9.0±1.0	11.8±2.5	88.0±8.5	5.4±0.8	2.0±0.6	0.20±0.08	7.0±0.6	1.6±0.4
42 (*n*=3)	10.0±1.5	9.0±3.5	88.0±1.0	5.3±3.3	2.0±0.3	0.2±0.1	8.3±1.1	1.7±0.1
64 (*n*=4)	13.5±1.5	7.3±3.5	95.0±5.0	7.1±3.8	2.0±1.0	0.07±0.03	5.0±0.8	0.80±0.09

Note. Here and in table 2: *n*, number of animals.

TABLE 2. Cytological Characteristics of the Spleen in Old CBA Mice after Treatment with PSC in Sub-Tolerance Doses (*M*±*m*)

Time after PSC treatment, days	Weight of the spleen, mg	Lymphocyte count in the spleen, $\times 10^6$	T cells, %	T cell count in the spleen, $\times 10^6$	B cells, %	B cell count in the spleen, $\times 10^6$	Macrophage number in one field of view	Area of the white pulp, %	Area of the red pulp, %
Control (*n*=4)	146.0±30.2	235.3±40.5	26.0±1.5	61.9±7.0	20.0±2.3	47.3±7.2	3.7±1.8	4.2±0.5	95.8±0.7
7 (*n*=3)	87.3±5.8	166.0±21.0	25.7±3.8	39.9±3.3	26.1±2.7	43.2±0.6	16.3±4.1	52.4±2.5	47.6±2.5
14 (*n*=3)	120.3±12.4	197.3±35.0	26.0±5.0	46.9±4.0	22.7±2.6	43.4±6.7	20.0±4.5	52.3±7.4	47.3±7.4
42 (*n*=3)	101.0±20.3	216.0±53.0	30.0±1.5	65.5±16.5	26.0±4.0	66.5±16.5	28.7±4.1	63.2±2.3	36.8±2.3
64 (*n*=4)	118.6±16.5	153.0±13.0	17.5±0.5	26.8±5.0	24.3±3.6	36.7±0.5	13.3±3.8	57.4±5.2	42.6±4.2

The concentration of hormones in PSC was measured by a highly sensitive enzyme immunoassay with Amersham kits. The results were analyzed statistically.

RESULTS

Old mice were characterized by involution of the thymus [1]. Cytoarchitectonics of the thymus in old mice differed from that in young animals. Injection of PSC in the sub-tolerance dose was followed by significant changes in the thymus of old mice. The number of lymphocytes in the thymus was shown to increase on day 7 (including T cells, Table 1). These changes were accompanied by an increase in the number of macrophages. Lymphocyte count in the thymus decreased on days 14 and 42 (Table 1). These observed changes were probably related to migration of lymphocytes into the peripheral regions. A histological study of the thymus showed that the parenchyma is clearly divided into the cortex and medulla (as differentiated from control specimens, Fig. 1). On the 64th day, cytoarchitectonics of the thymus in old mice was similar to that in young animals. This period was characterized by the appearance of individual lymphoid follicles with no light centers.

Administration of PSC was also followed by significant changes in the spleen of old mice. A histological study of samples from 2-year-old control mice revealed a sharp decrease in the area of the white pulp. Lymphoid nodules were often undetected (Fig. 2). We found a small number of megakaryocytes. Treatment with PSC was followed by a significant increase in megakaryocyte number in the spleen of old mice. The intensity of myelopoiesis in the spleen is reduced in old mice. PSC had a normalizing effect on this process. Lymphoid nodules (follicles) were formed in the splenic parenchyma. These structural units of the white pulp are necessary for the immune response. The weight of the spleen decreased on day 7 (as compared to the control, Table 2). Small lymphoid nodules with no light centers were revealed in the pulp (Fig. 2). The red pulp and white pulp of the spleen were clearly separated from each other. The presence of free iron crystals reflects erythrocyte hemolysis. The total number of T lymphocytes decreased, while the ratio of B lymphocytes increased (Table 2). The weight of the spleen increased on day 14 after PSC injection. The white pulp was presented by well-developed lymphoid nodules with no light centers. The area of the white pulp in treated mice was greater than in control animals. The red and white pulp was well defined. Structural characteristics of the

spleen in treated mice were similar to those in young animals (Fig. 2). An enzyme immunoassay showed that PSC contains more than 10 hormones, including follicle-stimulating hormone (PSH), luteinizing hormone (LH), thyrotropic hormone (TTH), ST-3, ST-4, *etc*. The hormonal composition and qualitative parameters of autopsy specimens at this stage of gestation were described previously [4].

Our results indicate that the therapeutic agent PSC is a well-balanced composition of hormones and proteins [4], which provides the synchronous process of organogenesis. Implantation of PSC to animals with age-related involution of organs has a strong modulatory effect on the system of immunogenesis in old recipients. This treatment is followed by an increase in the lymphocyte mitotic index in the thymus and spleen and activation of cell migration from organs to peripheral regions. Variations in the count of T lymphocytes and B lymphocytes were most significant. Lymphocyte count sharply decreases in 2.5-year-old animals. The white pulp with lymphoid follicles is undetected in the spleen. The structure of organs returns to normal after PSC implantation. These changes are probably related to the presence of specific immunomodulatory hormones and proteins in PSC. These compounds contribute to a sharp decrease in the rate of aging. The observed processes are adaptive, but not destructive. The tissue extract has no toxicity. Structural characteristics of the thymus and spleen in old mice of the treatment group do not differ from those in young animals.

Activation of the immune system in recipient animals after treatment with PSC extract reflects a multistage influence of test preparation. The observed changes progressively increase on days 7-14. The effect of this preparation is most pronounced on days 14-40. The reduction of reversible processes in recipient animals occurs up to the 64th day and is not accompanied by pathological changes. Repeated implantation is required to maintain these processes.

REFERENCES

1. T. Makinodan and E. Yunis, *Immunology and Aging* [in Russian], Moscow (1980).
2. V. S. Repin and G. T. Sukhikh, *Medical Cell Biology* [in Russian], Moscow (1996).
3. O. P. Ryabchikov, L. V. Kuznetsova, S. V. Nazimova, *et al*., *Byull. Eksp. Biol. Med*., Suppl. 1, 156-158 (1998).
4. G. T. Sukhikh, *Ibid*., Suppl. 1, 3-13 (1998).
5. *Transplantation of Human Fetal Tissues and Cells. Collected Works of the International Institute of Biological Medicine*, Eds. G. T. Sukhikh and A. N. Erina [in Russian], Moscow (1996).
6. S. V. Shirshev, *Uspekhi Sovrem. Biol*., No. 4, 350-363 (2003).
7. P. Schmid, *Zell-Therapie. Ein Schritt in die Zukunft der Medizin*, Stuttgart (1992).