A Method for Cellular Stimulation of Thyroid Transplant for Correction of Thyroid System Function

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The efficiency of autotransplantation of the thyroid tissue into the greater omentum was demonstrated in experiments on rats. High viability of the transplant and restoration of the hormonal thyroid activity were attained after a single injection of the cell suspension of autologous adherent bone marrow cells directly into the transplant.

Key Words: *transplantation; thyroid; stem cells; endocrine system*

Transplantation of the thyroid is a method for restoration of the thyroid function after thyroidectomy [1,2]. However, measures aimed at immune selection and antigen-specific immunosuppression are very complex and expensive, which implies the prospects of autotransplantation [1-3]. On the other hand, it is often difficult to obtain morphologically and functionally intact tissue in a volume sufficient for transplantation.

Recent data on the properties of stem cells open new vistas for preserving the endocrine function of the thyroid gland after transplantation of thyroid tissue fragments by stimulating recovery of the transplant morphology and functional activity by autologous adherent bone marrow cells (AABC) [4-8].

Here we studied the effect of AABC on restoration of the histological structure and endocrine function of the thyroid gland after transplantation of its fragment into the greater omentum.

MATERIALS AND METHODS

The experiments were carried out on 150 certified male Wistar rats (300-350 g) from Breeding Center of the Department for Experimental Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center.

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Experimental animals were subjected to thyroidectomy under ether narcosis and subsequent transplantation of a 4-5 mm^3 tissue fragment (groups 1 and 3) or homogenate (groups 2-4) into the greater omentum. AABC suspension, prepared as described below was injected to rats of experimental groups 3 and 4 (directly into the transplant). The control group consisted of 20 intact animals.

For isolation of bone marrow adherent fraction, the whole bone marrow was suspended in 1 ml medium (95% RPMI-1640 and 5% FCS), filtered through a capron filter, and washed by centrifugation at 1500 rpm for 10 min. The suspension of cell elements in 5 ml medium was incubated in a Petri dish for 90 min in a CO_2 incubator at 5% CO_2 and 37°C. Nonadherent cells were removed and adherent cells were separated from plastic with 0.25% trypsin and 0.02% Versene (1:1) for 15 min under the same conditions. After repeated washout the cells were suspended to a needed concentration and injected into the transplant (500,000 cells in 0.2 ml medium).

The function and morphology of the transplanted tissue was evaluated 30 days after surgery. The rats were decapitated under ether narcosis. Serum levels of thyrotropin (TSH), thyroxin (T4), triiodothyronine (T3), and their free fractions (T4fr, T3fr) were measured by enzyme immunoassay using TiroidIFA-TTG-1, TiroidIFA-tiroksin-01, TiroidIFA-svobodnyi T4 (Alkor-Bio), and SvT3-IFA (Khema) kits. For morphological study 5-μ paraffin sections across the entire transplant

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fixed in 10% formalin were made by the standard method and stained with hematoxylin and eosin. Morphometry included evaluation of the area of follicles and colloid (×160).

The results were statistically processed using Wilcoxon—Mann—Whitney nonparametric *U* test.

RESULTS

Evaluation of the functional competence of the transplant showed a significant decrease in serum TSH concentration in animals of all experimental groups in comparison with its basal values, which attests to the development of hyperthyroid reaction after thyroid tissue transplantation irrespective of the applied method (Table 1). However, the total serum concentration of T3 was normal, while T4 concentration was significantly lower in experimental animals compared to controls. Hence, changes in TSH content by the hyperthyroid type were presumably caused by increased levels of free fractions of thyroid hormones. Further studies confirmed significant increase in T3fr level in groups 1 and 2 and T4fr level in groups 3 and 4 in comparison with the control ($p<0.05$; Table 1).

Comparative analysis of the efficiency of transplantations of the fragment of the thyroid gland and its homogenate without cellular stimulation revealed increased serum T3fr concentration in group 1 and T4fr concentration in group 2 (Table 1). Presumably, the increased concentration of T4fr was determined by gradual release of the content of destroyed thyrocytes from transplanted homogenate, while T3fr was produced mainly in preserved and *de novo* formed transplant follicles. There were no significant differences in the hormonal status between group 3 and 4 animals, which can be due to the normalizing effect of injected AABC on transplant functioning (Table 1).

T3 is physiologically more important thyroid hormone, and hence, the increase in its concentration in the absence of AABC stimulation (groups 1 and 2) attests to more pronounced hyperthyroid reactions in these groups in comparison with groups 3 and 4. Since T3fr level in groups injected with AABC into the transplants was normal, we can speak about the formation

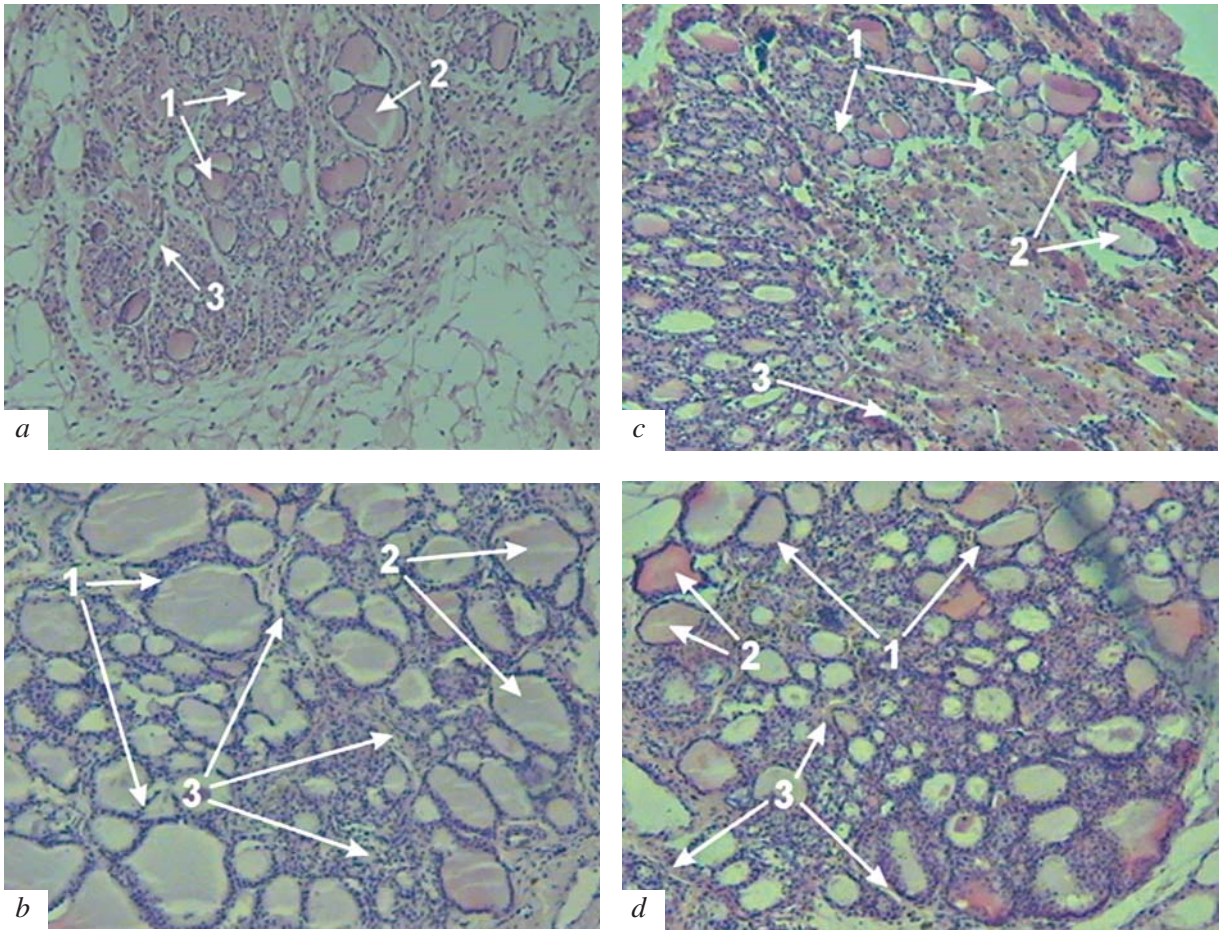

Fig. 1. Thyroid tissue after heterotopic transplantation into the greater omentum 30 days postoperation. *a*) transplanted thyroid tissue fragment; *b*) transplanted thyroid tissue fragment+single injection of AABC; *c*) transplanted thyroid tissue homogenate; *d*) transplanted thyroid tissue homogenate+single injection of AABC. *1*) follicle; *2*) colloid; *3*) blood vessel.

TABLE 1. Thyroid Hormonal Status of Rats Subjected to Thyroidectomy and Thyroid Tissue Transplantation into the Greater Omentum with Injection of Adhesive Bone Marrow Cells into the Transplant, 30 Days Postoperation ($X \pm m$)

Group	T3fr, pmol/liter	T4fr, pmol/liter	T3, nmol/liter	T4, nmol/liter	TSH, mU/liter
Control	5.46±0.27	16.28±0.33	1.97±0.13	65.74±0.64	2.36±0.19
1 (tissue fragment)	10.34±1.14*+o	15.18±2.66+o	3.14±0.46*+o	37.32±5.37*	0.02±0.01*
2 (homogenate)	7.14±0.38*+	28.63±2.81*	2.02±0.09	46.11±5.71*	0.02±0.01*
3 (tissue fragment+AABC)	4.71±0.05*	29.00±4.07*	1.73±0.21	42.96±5.24*	0.01±0.01*
4 (homogenate+AABC)	4.68±0.10	37.04±1.98*	1.83±0.20	38.84±3.71*	0.03±0.01*

Note. $p<0.05$ compared to: *control, +corresponding AABC group, ocorresponding homogenate group.

of a state close to euthyroid, which can be due to high degree of adaptation of transplanted tissue and hence, less pronounced hyperfunction of this tissue against the background of cellular stimulation. The possibility of AABC differentiation into functionally competent thyroid tissue, which is confirmed by the findings of a comparative morphological study (Fig. 1), is also worthy of note.

Examination of thyroid transplant sections (Fig. 1) using a computer-assisted image-analysis system showed that stimulation of the transplant with AABC led to the formation of large thyroid follicles with high content of brightly colored colloid. The follicles on preparations from groups 3 and 4 animals are stratified; sclerosis and lymphocyte and macrophage infiltration are less pronounced than without bone marrow cell transplantation. Focal infiltration and active neoangiogenesis confirm functional competence of the transplant.

Hence, our experiments on rats proved viability of the thyroid tissue fragment and homogenate, transplanted into the greater omentum. The histological structure and functional activity of the thyroid can be restored under conditions of heterotopic transplantation by stimulation of the transplant with injection of autologous adherent bone marrow cells.

REFERENCES

1. G. A. Bozhok, N. M. Alabedal'karim, and E. I. Legach, *Transplantologiya*, **5**, No. 1, 88-92 (2004).
2. T. P. Bondarenko, G. A. Bozhok, N. M. Alabedal'karim, *et al.*, *Ibid.*, **4**, No. 4, 60-63 (2003).
3. O. S. Popov, V. V. Udut, D. S. Titov, *et al.*, *A Method for Prevention of Postoperative Hypothyrosis* [in Russian], Patent of the Russian Federation No. 2161917.
4. V. I. Shumakov, N. A. Onishchenko, M. E. Krasheninnikov, *et al.*, *Vestn. Transplantol. Isskusstv. Organov*, No. 4, 7-11 (2002).
5. C. Curtillet, P. Cuadras, E. Dore, *et al.*, *Arch. Pediatr.*, No. 11, 1326-1332 (2004).
6. R.-Yi. Lin, A. Kubo, G. M. Keller, and T. F. Davies, *Endocrinology*, **144**, No. 6, 2644-2649 (2003).
7. M. Matsumoto, H. Ishiguro, Y. Tomita, *et al.*, *Pediatr. Int.*, No. 3, 291-295 (2004).
8. T. Saito and S. Minieshi, *Eksper. Onkol.*, **23**, 4-10 (2001).