Regeneration Potential of Transplanted Adult Mouse Sertoli Cells

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The regeneration potential of differentiated Sertoli cells subjected to thermal treatment was studied by the method of cell transplantation. Cells from mice with artificial cryptorchism (1.5 months after fixation of the testes in the body) and after culturing (10 days, 37°C) were transplanted. Transplantation of Sertoli cells from 2-3-month-old and 2-day-old mice served as controls. The cells were transplanted into the testes of recipient mice, from which sex cells and Sertoli cells were removed by busulfan and cadmium salt treatment. Adult mouse Sertoli cells exposed to thermal treatment exhibited much higher regeneration potential than intact cells. Two months after transplantation, mature Sertoli cells subjected to thermal treatment populated the recipient testicular tubules, formed new tubules, and in some cases supported the development of sex cells similarly as immature cells from newborn mice.

Key Words: *Sertoli cells; cell transplantation; cryptorchism; Sertoli cell culture*

Somatic Sertoli cells (SC) located on the basal membrane of testicular tubules (TT) play the key role in spermatogenesis. They form a niche for spermatogonial stem cells (SSC), are a part of the blood-testis barrier, support and feed sex cells, and are involved in the paracrine and endocrine regulation of the spermatogenic process. The death of SC is the beginning of destruction of the entire system of male sex cell development.

Fetal undifferentiated SC form TT in the course of gonadal development. Neonatal SC transplanted together with SSC into the recipient gonads free from their own sex cells and SC populate the recipient TT, form new tubules, and maintain the spermatogenic process. Adult animal SC are incapable of all these [11], presumably because normally they are highly differentiated in adult mammals with season-independent reproduction and are incapable of proliferation. However, proliferation of adult mammalian SC was observed in chemical mutagenesis in mice [1,2], in some spermatogenesis abnormalities in humans [8], and in culturing of mature mouse and human SC [3]. Recent data indicate that mature SC resume the expression of cytokeratin 18 (marker of undifferentiated SC) under conditions of spermatogenesis disorders in heat shock [12] and cryptorchism [13], as well as during culturing [3]. In addition, SC repair DNA double breaks after radiation exposure [3,4], which is untypical of undividing differentiated cells; they also express a set of proliferation inhibitors ($p27^{kip1}$) [5] and stimulants (ID proteins) [6]; imbalance in the production of these factors can result in cell transition to divisions [3,7].

These data suggested that adult animal SC, stimulated to division, have the same regeneration potential as the neonatal SC. We carried out this study to verify this hypothesis. Thermal exposure of adult mouse SC (experimental cryptorchism and culturing of SC at 37°C) was selected as the stimulus.

MATERIALS AND METHODS

Hybrid $(CBA \times C57Bl/6)F_1$ were selected as recipients, transgenic ROSA26 mice derived from C57Bl/6 strain

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and carrying *E. coli LacZ* gene expressed in all body cells served as donors. The animals were kept under standard vivarium conditions with free access to water and food.

In order to evaluate regeneration characteristics of transplanted SC, the gonads of 2-3-month-old recipient mice were released from their own sex cells and SC as described previously [11]. The animals were intraperitoneally injected with busulfan (a cytotoxic agent; Sigma) in a dose of 40 mg/kg, diluted in a mixture of dimethylacetamide, PEG, and distilled water (1:2:2). After 1 month, when no sex cells were left in the testes after busulfan treatment, 13-14 μl saline containing cadmium salt $3CdSO_4 \times 8H_2O$ (Sigma) in a concentration of 0.23 mg/ml was injected directly into TT. As a result, 2-3 weeks after cadmium sulfate injection only "skeletons" were left in the greater part of recipient TT, while others retained some SC, some of them desquamated. Cell suspensions were transplanted into these TT, some transplanted cells got into the interstitium through holes in TT. Solutions and cell suspensions were injected into TT as described previously [10].

In experiment 1, the cell suspensions from the gonads of 4-month-old mice with cryptorchism (1.5 month after cryptorchism induction) were injected into recipient mouse gonads. Bilateral cryptorchism was induced by fixing the testes to the fat or to the tunica albuginea at the inner side of the peritoneum just below the thoracic cage. In experiment 2, adult mouse SC were transplanted after 10-day culturing at 37°C. Isolation of SC into culture and their *in vitro* maintenance have been described previously [3].

Control series included transplantations of gonadal cells from 2-3-month-old intact mice (negative control) and from 2-day-old mice (positive control). Gonadal cell suspensions were prepared as described previously [10]; cell concentrations in the suspensions were 2×10^7 cell/ml; 15 μl suspension was injected into each testicle.

Two months after transplantation the recipients were sacrificed, the gonads were removed and fixed in 4% paraformaldehyde for 1.5 h, after which donor cells were identified by β-galactosidase (*LacZ* gene product) activity [9]. After staining the gonads were postfixed in 10% paraformaldehyde; serial paraffin sections (7 μ) were sliced and poststained in 1% neutral red.

RESULTS

Two colonies of donor cells were found in 1 of 6 gonads of recipients of cells from adult intact animals. These colonies were located on the recipient TT basal membrane and, judging by the morphology and location, were groups of SC or SC and spermatogonia. Some donor sex cells desquamated and filled the TT lumen (Fig. 1, *1*, *2*).

Vast areas occupied by donor cells were seen in the recipient interstitium (Fig. 1, *3*) and host TT (Fig. 1, *4-7*) in all cases after transplantation of gonadal cells from 2-day-old mice. Judging by the location, morphology, and structure of perinuclear heterochromatin, numerous differentiated donor SC, but not sex cells, were seen on the TT basal membrane (Fig. 1, *4-7*). In the interstitium, the transplanted cells formed structures similar to the sexual cords and neonatal TT, with SC on their basal membranes and in many cases spermatogonia (Fig. 1, *8*). Numerous interstitial donor cells were seen around the recipient TT. Virtually complete absence of differentiating sexual donor cells in the recipient gonads 2 months after transplantation of newborn mouse gonadal cells was presumably due to the fact that a great part of cells after transplantation got into the interstitium where SSC died.

Colonies of donor cells were found in 3 of 5 recipient gonads 2 months after transplantation of cell suspensions from cryptorchid gonads, the number of these colonies being comparable to that in the positive control. Some transplanted SC formed groups in the interstitium, the greater part of them were found on the host TT basal membrane (Fig. 1, *9*, *10*). The spermatogenic process restarted in some recipient TT originating from the transplanted SSC: spermatogones, spermatocytes, and spermatides expressing β-galactosidase were found; some SC supporting this spermatogenesis was also of donor origin (Fig. *1*, *11*, *12*).

After transplantation of cultured (10 days) SC from adult mice donor cells were detected in 2 of 5 recipient gonads, the counts of these cells being less than after transplantation of SC from cryptorchid testes. Small structures (Fig. 1, *13*) formed by transplanted SC and peritubular muscle cells, resembling small tubules (minitubules), were found in the host TT lumens. Some donor SC were located on the recipient TT basal membrane (Fig. 1, *14*), others formed structures similar to the forming TT in the interstitium (Fig. 1, *15-17*).

Hence, the results of transplantation of mature SC exposed to thermal treatment indicate that these cells, similarly as neonatal SC, populate the recipient TT, form new tubules, and in some cases maintain the development of donor sex cells. Presumably, the increase of SC regeneration potential in experiment in comparison with intact adult mouse SC was due to transition of these cells to proliferation and due to changes in the expression of genes determining the maturity of these cells.

The study was carried out on the common equipment of Institute of Developmental Biology.

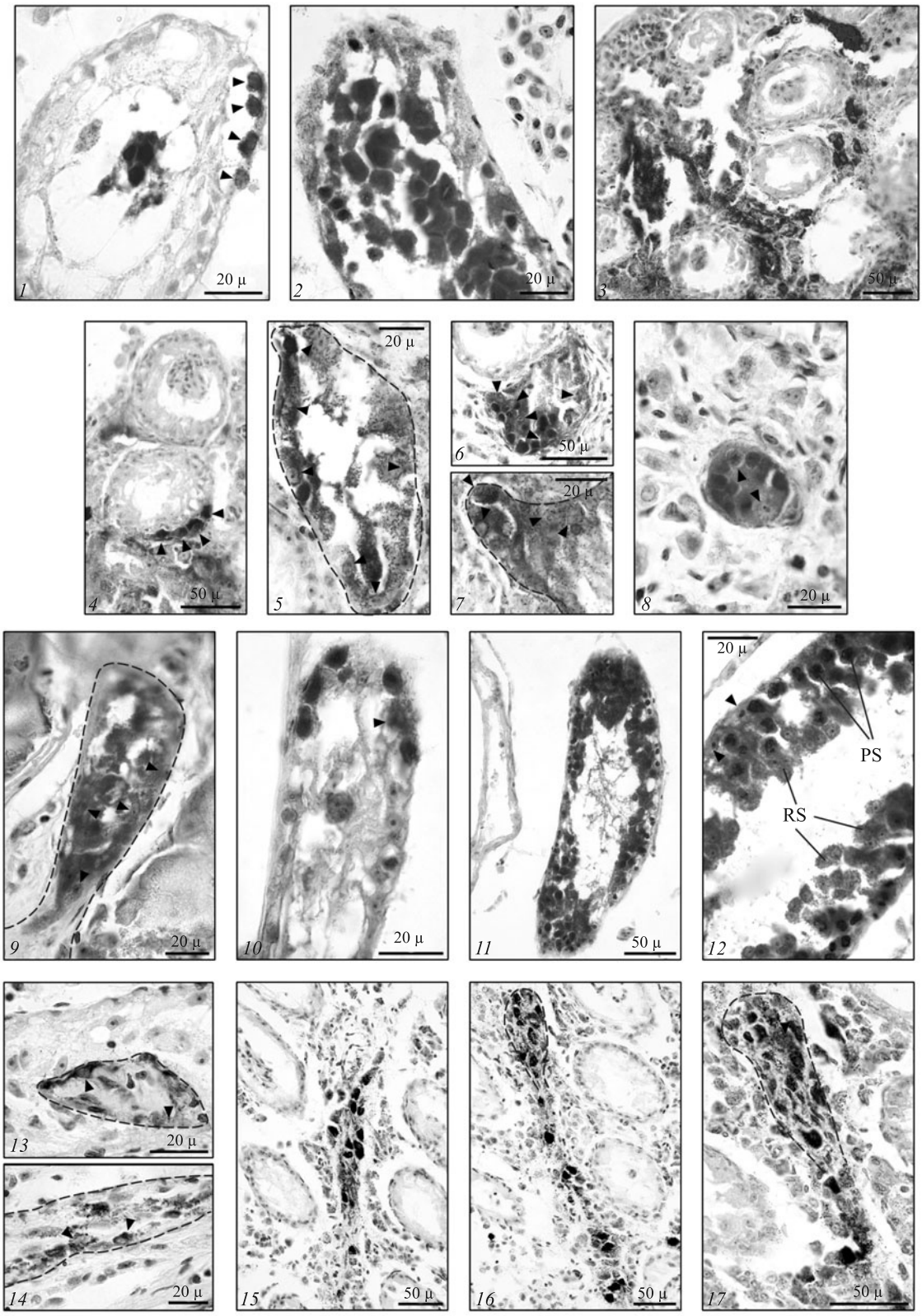

Fig. 1. Histological sections of recipient mouse gonads 2 months after SC transplantation. *1*, *2*) cell donors, 2-3-month-old ROSA26 mice (negative control); *3*, *8*) cell donors, 2-day-old ROSA26 mice (positive control); *9-12*) cell donors, ROSA26 mice with induced cryptorchism; *13-17*) transplantation of cells from 10-day SC culture, derived from 2-3-month-old ROSA26 mice. Triangular arrows: donor SC nuclei; PS: spermatocytes at the pachytene stage; RS: round spermatide nuclei; dotted line shows the recipient TT border (*5*, *7*, *9*, *14*) and the minitubules formed by donor cells (*13*, *16*, *17*).

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