Primate Spermatogonial Stem Cells Colonize Mouse Testes

Makoto Nagano, John R. McCarrey, and Ralph L. Brinster

Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6009

Department of Genetics, Southwest Foundation for Biomedical Research and Southwest Regional Primate Research Center, San Antonio, Texas 78245-0549

ABSTRACT

In mice, transplantation of spermatogonial stem cells from a fertile male to the seminiferous tubules of an infertile recipient male results in progeny with donor-derived haplotype. Attempts to extend this approach by transplanting human testis cells to mice have led to conflicting claims that no donor germ cells persisted or that human spermatozoa were produced in the recipient. To examine this issue we used the baboon, a primate in which testis cell populations of several ages could be obtained for transplantation, and demonstrate that donor spermatogonial stem cells readily establish germ cell colonies in recipient mice, which exist for periods of at least 6 mo. However, differentiation of germ cells toward the lumen of the tubule and production of spermatozoa did not occur. The presence of baboon spermatogonial stem cells and undifferentiated spermatogonia in mouse seminiferous tubules for long periods after transplantation indicates that antigens, growth factors, and signaling molecules that are necessary for interaction of these cells and the testis environment have been preserved for 100 million years of evolutionary separation. Because germ cell differentiation and spermatogenesis did not occur, the molecules necessary for this process appear to have undergone greater divergence between baboon and mouse.

Sertoli cells, spermatogenesis, testis

INTRODUCTION

The ability to produce spermatozoa in the adult male depends on the presence of spermatogonial stem cells in the seminiferous tubules of the testes. These cells can both self-renew and produce daughter cells that undergo differentiation. Whereas mature spermatozoa of different species have unusual and distinct morphological appearances [1, 2], the differentiation process that produces these mature forms shares many common characteristics among species. Moreover, the similarities appear greatest during the early stages of spermatogenesis. For example, primitive, undifferentiated spermatogonia are remarkably similar in appearance in many species [3–5], and spermatogonial stem cells may share important characteristics [6].

A powerful argument for shared biological attributes of stem cells and primitive undifferentiated spermatogonia from different species comes from testis cell transplantation studies in which it has been found that spermatogonial stem cells of many species have the ability to colonize the mouse testis. When rat and hamster testis cells are transplanted to mouse testes, stem cells generate colonies of donor cell-derived spermatogenesis and spermatozoa in recipients [7, 8], and transplanting cells from mouse to rat results in mouse spermatozoa produced in the rat testis [9]. In addition, transplantation of testis cells to the mouse from nonrodent species, such as rabbit, dog, pig, and bull, results in formation of colonies that consist of undifferentiated spermatogonia on the basement membrane of seminiferous tubules, which are maintained for 6 mo or more [10, 11]. However, differentiation of germ cells toward the seminiferous tubule lumen and generation of spermatozoa do not occur. Recently, it was reported that following xenotransplantation of human testis cells to mice no donor germ cells could be identified in recipient seminiferous tubules [12]. In contrast, Sofikitis and colleagues [13] claimed that transplantation of human testis cells to mouse and rat seminiferous tubules resulted in production of spermatozoa in more than 25% of recipients. These divergent results suggest that primordial germ cells may be either less or more compatible with the microenvironment of rodent seminiferous tubules than has been demonstrated for germ cells from other nonrodent, nonprimate species. Thus, either a pessimistic or optimistic outlook for medical and research application of xenogeneic spermatogonial transplantation is generated.

In a broad context, the possibility of a biological similarity in spermatogonial stem cell characteristics among mammalian species has fundamental implications regarding the effect of evolutionary divergence on all self-renewing cell systems in the body, such as hematopoietic tissue, epidermis, or intestinal epithelium. Previous testis cell transplantation studies involving nonprimate species suggest considerable similarity among stem cells of many species based on their ability to colonize the mouse testis [7–11]. In contrast, the inability of nonrodent germ cells to proceed through meiosis and spermiogenesis suggests that these specialized differentiation processes have experienced greater variation or divergence among species over time [10, 11]. The conflicting results from human testis cell transplantation experiments raise questions regarding this general premise arising from nonprimate experiments.

Evidence from human hematopoietic cell transplantation into immunodeficient mice suggests that human hematopoietic progenitor/stem cells colonize mouse hematopoietic organs and show limited differentiation. Administration of species-specific cytokines or cotransplantation of supporting cells from fetal liver, bone, and thymus is necessary to maintain human hematopoietic stem cells and induce multilineage differentiation in mice [14–17]. A general evolu-
tionary phenomenon may be that stem cells of individual self-renewing cell systems of the body display minimal divergence in basic biology among species, whereas the differentiation stages show more divergent characteristics. The spermatogonial stem cell transplantation system provides an opportunity to test this hypothesis and has several advantages over other self-renewing systems in this regard. The testis is self-contained and readily accessible; individual stem cell colonization events can be enumerated [18–20], the extent and quality of colonies can be measured and evaluated [21–23], and terminal differentiation of transplanted cells produces a single cell type, spermatozoa, with an easily assayed and definitive function.

In order to determine whether spermatogonial stem cells of a primate differed from those of nonprimates in their ability to colonize mouse testes, baboon testis cells were transplanted to testes of immunodeficient nude mice and the recipient tubules were analyzed with immunohistochemistry. The baboon was chosen as a model for primates because testis tissue of several ages could be readily obtained without ethical concerns associated with human testis tissue. In addition, the baboon is closely related to humans, necropsy material is more readily available than from chimpanzee or gorilla, and unlike the macaque, the baboon is a noneseasonal breeder. Therefore, the results of transplantation studies could help to clarify the divergent reports from human experiments, and test in primates the concept that spermatogonial stem cells of many species share important biological properties. Following transplantation of baboon germ cells, areas of colonization were found in recipient tubules for at least 6 mo following transplantation, but differentiation of germ cells into the lumen was not seen, and spermatozoa were not produced. The efficiency of colonization of recipient tubules by primate stem cells appeared similar to that previously found for syngeneic transplantation in the mouse.

MATERIALS AND METHODS

Donor Cell Preparation

Testis cells were obtained from prepubertal (1-yr-old) and postpubertal (7- and 14-yr-old) baboons (Papio hamadryas anubis) at necropsy. Seminiferous tubules and interstitial tissue were initially mechanically dissected away from connective tissues. Cellular dissociation was accomplished by sequential enzymatic digestions [24, 25]. The seminiferous tubules were digested first with 1 mg/ml collagenase (type II, Worthington Biochemical Corp., Lakewood, NJ) at 33°C for 15–20 min. Resulting seminiferous tubule fragments were recovered by decanting the collagenase solution, then treated with 0.5 mg/ml trypsin (Sigma Chemical Company, St. Louis, MO) at 33°C for 15–20 min. DNase I (Sigma) was added to a final concentration of 1 mg/ml and the solution was pipetted repeatedly until a minimum viscosity was achieved. The cells were centrifuged at 600 × g at 4°C for 10 min and resuspended in 12.5 ml Dulbecco modified Eagle medium (DMEM). The resulting single cell suspension of baboon testis cells was frozen by adding an equal volume (12.5 ml) of freezing media [26]. The cell suspension was aliquoted (1 ml/vial), and each aliquot was snap-frozen in liquid nitrogen and stored at −196°C for 70–866 days. The cell suspensions were prepared at the Southwest Foundation for Biomedical Research and shipped overnight on dry ice for injection into recipient mice in Philadelphia.

Recipient Mice and Donor Cell Transplantation

Ncr Swiss nude (nu/nu) mice (Taconic, Germantown, NY), 6–8 wk of age, were treated with busulfan (44 mg/kg) at least 4 wk before donor cell transplantation to destroy endogenous spermatogenesis [27, 28]. Frozen baboon testis cells were thawed and resuspended in DMEM, as described previously [26]. Cell viability after thawing was 50.0% ± 2.4% (mean ± standard error of the mean, n = 14) for postpubertal cells and 71.9% ± 0.5% (n = 3) for prepubertal cells. The concentration of donor cell suspension was 164.7 ± 11.4 × 10^6 cells/ml for postpubertal cells and 44.3 ± 5.6 × 10^6 cells/ml for prepubertal cells, and approximately 10 μl of donor cells were introduced into the seminiferous tubules of a testis by injecting the rete testis of the recipient mouse [29]. All animal experimentation protocols were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania and the Southwest Foundation for Biomedical Research.

Antibody Production

Cells for development of testis- and species-specific antibody were prepared from a 7-yr-old adult baboon as described above. The polyclonal antiserum against baboon testis cells was commercially produced (Covance Research Products, Denver, PA) by immunizing a New Zealand White rabbit [10]. Briefly, 2 × 10^7 baboon testis cells were injected subcutaneously on the back of the rabbit in Freund complete adjuvant on Day 0. Six booster injections were made with 10^7 baboon testis cells in Freund incomplete adjuvant 3 wk apart. Serum was prepared 2 wk after the last immunization. The total immunoglobulin G (IgG) fraction was extracted using the EZ-Sep kit (Pharmacia Biotech, Piscataway, NJ), and purified through HiTrap-Q columns (Pharmacia Biotech). Purity of the IgG was verified by SDS-PAGE and Coomassie Blue staining. Purified IgG was concentrated using CentriPlus spin filters (Amicon, Beverly, MA) and biotinylated by incubation with N-hydroxysuccinimidobiotin (EZ-Link NHS-biotin; Pierce, Rockford, IL) in 0.1 M NaHCO_3 for 4 h at room temperature followed by extensive dialysis against Dulbecco phosphate-buffered saline (D-PBS). Biotinylated IgG was incubated with 2% acetone extract of mouse testis [30] for 4 h at room temperature to reduce nonspecific binding to mouse testis cells.

Analysis

Whole-mount immunohistochemistry using biotinylated rabbit anti-baboon IgG was performed following the protocol of Dobriniski et al. [10]. Recipient mouse seminiferous tubules dispersed with 1 mg/ml collagenase (type IV; Sigma) and 1 mg/ml DNase I (Sigma) were fixed in 4% paraformaldehyde (PFA) for 2 h at 4°C and washed in D-PBS. Samples were incubated at room temperature with 0.1 M glycine in D-PBS and then 0.3% H_2O_2 in D-PBS to block endogenous peroxidase activity. Samples were treated at room temperature in D-PBS that contained 2% skim milk and 0.1% Triton X-100 (PBS-MT), followed by incubation in D-PBS containing 5% normal goat serum, 1% BSA, and 0.1% Triton X-100. Endogenous biotin was blocked using an avidin/biotin blocking kit (Zymed Laboratories, San Francisco, CA). Samples were then processed with biotinylated anti-baboon IgG (5 μg/ml) in D-PBS with 5% BSA at 4°C overnight. Samples were washed twice for 30 min at 4°C and once for 30 min at room temperature with PBS-
FIG. 1. Histological appearance of normal testis of prepubertal (1-yr-old) and postpubertal (7- and 14-yr-old) baboons. Testis of 1-yr-old baboon (a) contains only Sertoli cells (arrows) and spermatogonia (arrowheads) in the seminiferous tubules. In the testes of 7-yr-old (b) and 14-yr-old (c) baboons, all stages of spermatogenesis, from spermatogonia (arrows) to spermatozoa (arrowheads) can be seen in the seminiferous tubules. Hematoxylin-eosin staining. Bar = 30 μm (a) and 75 μm (b and c).

FIG. 2. Detection of baboon germ cells transplanted into recipient mouse testes using baboon-specific antibodies and germ cell specific anti-human RBM antibodies.

a) Whole-mount of recipient mouse seminiferous tubules stained with baboon-specific antibodies and germ cell specific anti-human RBM antibodies. 129Sv × C57BL/6 (129/B6) F1, recipient mouse was pretreated with busulfan to destroy endogenous spermatogenesis [27, 28]. Seminiferous tubules of prepubertal baboon testes contained only Sertoli cells and primitive spermatogonia, whereas in the tubules of adult testes, all differentiating germ cell stages were present (Fig. 1).

Species-Specificity of Antibodies Raised Against Baboon Testis Cells

Because early stages of male germ cell differentiation have a similar morphological appearance among many spe-

RESULTS

Donor testis cells were obtained from prepubertal and postpubertal baboons to determine the effect of reproductive status on colonization potential of spermatogonial stem cell. Donor cells were cryopreserved for 70–866 days before transplantation into recipient testes of immunodeficient mice that had been treated with busulfan to destroy endogenous spermatogenesis [27, 28]. Seminiferous tubules of prepubertal baboon testes contained only Sertoli cells and primitive spermatogonia, whereas in the tubules of adult testes, all differentiating germ cell stages were present (Fig. 1).

MT. The washing process was completed by incubating samples in D-PBS containing goat serum, BSA, and Triton X-100, described above, twice for 30 min at room temperature. Samples were reacted with Z-avidin coupled to horseradish peroxidase at 1:1000 dilution (Zymed Laboratories) at 4°C overnight. After washing five times at room temperature in 0.1% Triton X-100/D-PBS (15 min each), donor baboon cells were visualized using 3-amino-9-ethylcarbazole (AEC, Vector Laboratories, Burlingame, CA). Approximately one-quarter to one-half of a testis was analyzed at a time.

For whole-mount double staining with anti-baboon antibody and rabbit anti-human RNA Binding Motif (RBM) antibody (a gift from D. Elliott), samples were fixed in 4% PFA at 4°C for 2 h followed by sequential incubations in methanol and acetone at −20°C for 30 min each. Using the same blocking and washing procedures described above, samples were incubated with rabbit anti-human RBM polyclonal antibodies [31] and then with goat anti-rabbit IgG coupled to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA), both overnight at 4°C. After extensive washes, samples were processed with biotinylated anti-baboon antibodies as described above. Donor cells were first visualized for RBM using the VECTOR Blue alkaline phosphatase substrate kit (Vector Laboratories), then for baboon-specific antigens with AEC.

Histological sections of adult (7-yr-old) baboon testis were stained to examine the reactivity of anti-human RBM antibodies to baboon germ cells. Paraffin sections of adult baboon testis fixed in Bovin solution were processed as described [31] and cells were visualized using the VECTOR VIP peroxidase substrate kit (Vector Laboratories), which produces a purple color on positive cells. Paraffin sections of normal testes were prepared from 1-, 7-, and 14-yr-old baboons and stained with hematoxylin and eosin. Statistical analyses were performed with Student t-test or ANOVA followed by Tukey HSD multiple comparisons (Systat 7.0; SPSS Inc., Chicago, IL).
cies, a baboon testis-specific polyclonal antibody was produced. To verify the specificity of the antibodies to donor baboon germ cells, mouse recipient tubules microinjected with donor cells were double-stained with baboon-specific antibodies and germ cell-specific anti-human RBM antibodies [31]. The RBM antibody cross-reacts well with nuclei of germ cells in baboon testes and is particularly strong in spermatogonia and early stages of germ cell differentiation (Fig. 2c), whereas the antibody does not cross-react with mouse germ cells (Fig. 2d). When recipient seminiferous tubules were treated with both antibodies, germ cells that were stained with RBM antibodies were also positive for baboon-specific antibodies (Fig. 3, a–c). Although baboon fibroblast-like cells and clumps of intraluminal cells also stained with the baboon antibody, these cells were clearly not on the basement membrane (intraluminal cell clumps) or did not have the appearance of germ cells (e.g., fibroblasts). The double-stained cells were always on the basement membrane as isolated single cells and chains of connected cells, which are known to be a typical cellular arrangement of undifferentiated spermatogonia [32]. Therefore, baboon antibodies were subsequently used to identify donor-derived germ cells.

**Effect of Donor Reproductive Status on Ability of Baboon Germ Cells to Colonize Mouse Testes**

Initially, the colonization and proliferation of prepubertal (1-yr-old) and postpubertal (7- and 14-yr-old) donor baboon testis cells in recipient mouse testes were studied during the first 2 mo after transplantation. Despite significant differences in appearance of germ cells in the prepubertal and postpubertal testes, donor cell-derived colonies in mouse seminiferous tubules appeared similar (compare Fig. 4, a and b with c and d). Transplanted donor germ cells were found on the basement membrane as single cells or chains of two to eight cells, and small groups or clusters of cells were also present. The baboon cells often showed blebs or pseudopod-like cytoplasmic extensions (Fig. 4). To quantify donor stem cell colonization and subsequent proliferation, the number of cell groups (clusters) observed in each recipient testis was counted (Table 1). A cluster was defined as a group of cells consisting of 10 or more cells in a 150-μm length of seminiferous tubule. The age of the donor appeared to have only a small effect on the efficiency of colonization (Table 1, lines 1–4, 7, 8). The number of recipient testes with clusters derived from 14-yr-old testis cells (8/12; Table 1, lines 7 and 8) was about twice that found for 1-yr-old testis cells (4/12; Table 1, lines 1 and 2), but the number of clusters per testis from 14-yr-old testis cells (1.25) was slightly less than that from 1-yr-old testis cells (1.75). The size of the cluster (the number of cells contained per cluster) was about 50% larger (12.3 vs. 18.9) for 14-yr-old than for 1-yr-old donor testis cells. These differences in number and size of clusters were not significant. Therefore, donor testis cell age appeared to have little effect on colonization during the first 2 mo following transplantation. Many more adult cells were available; consequently, postpubertal testis cells were used for subsequent experiments.

**Long-Term Colonization of Mouse Testes by Baboon Germ Cells**

In order to characterize colonization over an extended time, donor cells from a single adult animal (7 yr old) were analyzed at 1–6 mo following transplantation (Table 1, lines 3–6, and Fig. 5). This approach was used to minimize variation that might arise among different donors, and one adult animal provides sufficient cells for many transplantations. The pattern of donor cell colonization observed in recipient testes remained similar up to 6 mo following transplantation, and groups of cells were found on the basement membrane of the seminiferous tubules throughout the period (Fig. 5). These groups of cells had a distinct appearance with individual and connected cells clustered on the basement membrane, suggesting continued proliferation of transplanted donor baboon germ cells.

The length of time following transplantation influenced the degree of recipient seminiferous tubule colonization.
Approximately 43% of recipient testes contained clusters at 1 and 2 mo, but this increased to more than 70% at 3 and 6 mo after transplantation. The average number of clusters per testis significantly increased from 1 to 2 mo following transplantation, from 0.5 to 9.2, and then decreased thereafter to 2.9 at 6 mo. The increase from 1 to 3 mo was highly significant ($P = 0.003$), and the decrease from 3 to 6 mo was just significant ($P = 0.05$). The number of cells in a cluster rose from 11 at 1 mo to about 20 at 3 and 6 mo, suggesting that a steady state of cell proliferation and disassembly had been reached in the colonies that remain after 3 mo. The maximum cell number per cluster observed in a testis was 22 at 2 mo, and 45 and 54 at 3 and 6 mo, respectively. Occasionally, extensive proliferation patterns with many more cells (Fig. 5a) were observed on the basement membrane of mouse seminiferous tubule in the form of individual and two to four associated cells, indicating that undifferentiated spermatogonia have replicated. Out of focus are intraluminal clumps of baboon cells (arrows). Bar = 40 μm (a-c) and 100 μm (d).

**TABLE 1. Baboon germ cell colonization of immunodeficient mouse testes.**

<table>
<thead>
<tr>
<th>Age of donor baboon</th>
<th>Months after transplantation</th>
<th>No. of tests analyzed</th>
<th>No. of clusters per testis$^4$</th>
<th>No. of clusters with clusters$^4$</th>
<th>No. of cells per testis³</th>
<th>No. of cells per cluster$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 yr old</td>
<td>1</td>
<td>2</td>
<td>$1.6 \pm 1.0$</td>
<td>$12.7 \pm 1.5$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>$1.9 \pm 1.2$</td>
<td>$11.8 \pm 1.0$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 yr old</td>
<td>1</td>
<td>8</td>
<td>$0.5 \pm 0.2^a$</td>
<td>$11.0 \pm 1.0$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>$1.0 \pm 0.5^b$</td>
<td>$14.0 \pm 0.9$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>$9.2 \pm 4.0^{c,d}$</td>
<td>$20.2 \pm 1.7$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>$2.9 \pm 0.9^e$</td>
<td>$21.4 \pm 3.7$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 yr old</td>
<td>1</td>
<td>5</td>
<td>$1.2 \pm 0.6$</td>
<td>$18.5 \pm 3.2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>$1.3 \pm 0.4$</td>
<td>$19.3 \pm 5.0$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All donor baboon testis cells were cryopreserved for 70–866 days following enzymatic digestion of seminiferous tubules.

$^b$ A cluster is defined as a group of 10 or more cells in a 150-μm length of seminiferous tubules. All recipient mouse testes contained individual baboon testis cells or small groups of baboon testis cells that were not categorized as a cluster.

$^c$ Based on the number of tests analyzed (mean ± SEM). Because the number of viable cells transplanted in the 1-yr-old group differed from that in the other two age groups (31.9 × 10⁶ vs. 82.4 × 10⁶ cells/ml; see Materials and Methods), the number of clusters for the 1-yr-old group was adjusted by a factor of 2.6 (82.4/31.9).

$^d$ Mean ± SEM.

$^e$ $P = 0.003$.

$^f$ $P = 0.002$.

$^g$ $P = 0.05$. 

**FIG. 4.** Whole-mount detection of donor baboon germ cells using baboon-specific antibodies 1 and 2 mo after transplantation into mouse testes. 

- **a** Germ cells from 1-yr-old baboon 1 mo after transplantation. Donor cells were cryopreserved for 589 days. Baboon spermatogonia in this panel have an irregular shape with cytoplasmic extension (bleb, arrowheads) and cytoplasmic bridge (arrow).

- **b** One-yr-old baboon spermatogonia 2 mo after transplantation. The pattern of cellular group formation is similar to that at 1 mo after transplantation shown in b. c Germ cells from 7-yr-old baboon 2 mo after transplantation. Donor cells were cryopreserved for 584 days. Twelve cells can be seen as a cluster composed of individual and pairs (arrow) of cells. A prominent bleb can also be observed in an individual cell (arrowhead). Note the similarity of cellular group formation to those shown in a and b.

- **d** Germ cells from 14-yr-old baboon 2 mo after transplantation. Donor cells were cryopreserved for 99 days. Twelve cells can be seen as a cluster composed of individual and pairs (arrow) of cells. A prominent bleb can also be observed in an individual cell (arrowhead). Note the similarity of cellular group formation to those shown in a and b.

**DISCUSSION**

Following transplantation of donor stem cells to a recipient, two processes occur. First, donor cells colonize the recipient environment, which involves migration to the appropriate location and survival. Stem cells often reside within a niche that is formed by the basement membrane and supporting cells [33]. Second, donor stem cells must increase in number and differentiate in the recipient environment to ultimately produce terminally differentiated cells. In studying these two processes, spermatogenesis is unique among self-renewing systems of the body because the transplantation technique allows 1) identification of individual colonies, which generally represent the activity of cells.
single stem cells; and 2) quantification of colony differentiation and expansion over time.

Studies using baboon testes from both prepubertal and postpubertal animals clearly demonstrate that stem cells of the baboon, regardless of reproductive age, can accomplish the first important part of the transplantation process. Baboon stem cells recognize mouse Sertoli cells, attach to the cell’s apical surface, and migrate between these cells to reach the basement membrane. This directional movement of stem cells is a complicated process because Sertoli cells are closely connected by intercellular processes to form the “testis-blood” barrier that normally separates the unique antigens of differentiated germ cells that are present in the adluminal compartment of the seminiferous tubule from the immune cells and immunoglobulins of the testis interstitial tissue [34]. Therefore, molecules necessary for recognition of Sertoli cells and migration of baboon stem cells through the intercellular processes to reach the basement membrane must be compatible between baboon and mouse. This is remarkable considering that these species diverged in evolution 100 million years ago [35, 36]. Furthermore, baboon germ cells survive more than 6 mo in the niche formed by mouse testis cells, indicating that appropriate surface molecules and growth factors are available and compatible.

The long survival of baboon germ cells in the mouse testis and formation of clusters may represent a process of slow division of undifferentiated spermatogonia followed by apoptosis of early differentiation stages, a situation that has been described previously [37–39]. For example, the seminiferous tubules in several experimentally produced infertile mouse models (cryptorchidism and vitamin A deficiency) [38, 39] and naturally occurring infertile mutant mice (SteelH and Jsd homozygous mutants) [39] display clusters of cells comprised of single cells and short chains of spermatogonia. Recent evidence suggests that in these situations the undifferentiated spermatogonia replicate, but are unable to proceed to differentiating spermatogonial stages, and undergo apoptosis [39]. The small clusters or colonies of baboon germ cells on the seminiferous tubule basement membrane of the mouse are also similar to the pattern of undifferentiated spermatogonia reported in the rhesus monkey following irradiation [37]. Limited spermatogonial proliferation and decline in cluster numbers over time observed in this study suggest that interactions between baboon germ cells and mouse testis environment may be suboptimal.

An important observation in these studies was the absence of differentiated donor germ cell stages in recipient seminiferous tubules. Thus, no evidence was found among donor germ cells of meiosis or spermiogenesis, the complex transition of germ cells to the unique spermatozoa morphology. These results differ from the report from Sofikitis and colleagues [13], indicating that transplantation of human testis cell suspensions to the seminiferous tubules of mouse or rat resulted in production of human spermatozoa in a significant number (>25%) of recipients. Previous studies have indicated similar results only when rat or hamster testis cells were transplanted to mouse, or mouse testis cells were transplanted to rat [7–9]. These species are all rodents and diverged in evolution only 10–15 million years ago [40]. Differentiating germ cells were never found when testis cells from rabbit, dog, pig, or bull, which have been diverged from the mouse for at least 60 million years [35, 36], were transplanted to mouse recipients [10, 11]. However, spermatozoa on the basement membrane could always be identified in these testis cell transplants from distantly related species.

The results of the baboon testis cell transplantation experiments reported here suggest that the findings of Sofikitis and colleagues with human testis cell transplantation [13] reflect an unusual and unexpected outcome. The baboon and human diverged in evolution approximately 20 million years ago and have been separated from the mouse for a much longer period [36, 41]. Therefore, results following transplantation of testis cells from baboon and human to the mouse are likely to be similar and to mimic the pattern seen for donor cells from other distantly related nonrodent species. It seems unlikely that transplants of human cells to mice would be as successful as was found for transplants from rodents to mice. Additional information describing the techniques used by Sofikitis and colleagues [13] will be especially valuable in clarifying their results. In contrast, the findings of Reis and colleagues [12] may indicate only that they were unable to distinguish small donor germ cell clusters or colonies on the basement membrane in recipient seminiferous tubules without an appropriate antibody or other marker of stem cells and early germ cell stages. In their studies they relied on an antibody against proacrosin, which appears only in late stages (mid-pachytene primary spermatocytes and spermatids) of germ cell differentiation [42].

Finally, in the mouse spermatogonial transplantation system, which has been extensively studied, each colony is generally believed to represent the product of a single transplanted stem cell [18, 19] and the efficiency of colonization by transplanted stem cells has been estimated to be 5% to 10% [18, 19, 23]. If it is assumed that each baboon germ cell cluster identified in the recipient mouse testis represents
a colony from a single stem cell and that the experimental procedure does not selectively affect stem cell survival, an efficiency of colonization might be roughly estimated from the data in Table 1. Approximately 10 μl of baboon testis cells at a concentration of 164 × 10^6 cells/ml (postpubertal) were injected into each testis, a total of 1.64 × 10^7 cells per testis. Because 50% of the cells were dead, about 0.8 × 10^6 viable cells entered the seminiferous tubules of a testis. If the stem cell concentration is about two in 10^4 testis cells, similar to the estimate for mouse [6, 43], then 160 stem cells would have been microinjected into the tubules of each testis. An average of 3.2 germ cell clusters or colonies were found in the recipient testes examined between 2 and 6 mo; therefore, about 1 in 50 (2.0%) of the baboon stem cells injected formed a cluster of primitive germ cells in the mouse that was maintained for 2 or more mo. This value of colonization efficiency could be an underestimate, because donor cells that did not belong to a cluster but were present in all recipient testes are not considered. Thus, the overall efficiency of baboon testis cell transplantation appeared not very different, for the initial colonization process, than has been observed when mouse testis cells were used in syngeneic transplantations.

Long-term maintenance of primitive baboon germ cell clusters in mouse testes clearly demonstrates that antigens, growth factors, and signaling molecules that interact between stem cells and the testis environment have been preserved for 100 million years in these widely divergent species [35, 36]. Together with results from nonprimate testis cell transplantation, these results indicate that similar biological properties have been preserved for spermatogonial stem cells among diverse mammalian species. Because human hematopoietic stem cells also colonize mouse bone marrow and generate differentiated cell lineages when human cytokines or supporting tissue is provided, stem cells of self-renewing tissues in general may share conserved signals and characteristics among species. Efforts to study and understand gene activity in stem cells of the germ line and of other self-renewing systems should be facilitated by this evolutionary conservation. A primary objective in spermatogonial transplantation studies will be to determine factors required to enhance proliferation and differentiation of primate donor germ cell colonies in mouse testes, and the baboon provides a valuable model. The central nature of spermatogonial stem cells and their differentiation process to species continuity makes such studies of high value.

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