culture and transplantation.

Spermatogonial Stem Cell Transplantation

Since the spermatogonial stem cell cannot be identified unequivocally by morphological, antigenic, or biochemical criteria, a population of donor testis cells was prepared by an enzymatic procedure previously described,[1] which results in enrichment of the germ cell components. The suspension of testis cells was then injected directly into the lumen of the seminiferous tubules of a recipient mouse with blue dye as a tracer. By microinjecting several tubules of a testis, 70%-100% of the surface tubules can be filled with the cell suspension. Because the tubules follow a tortuous course within the testis, filling of surface tubules reflects the degree of total filling achieved. Two alternative injection methods can be used. In 1 approach, the cells are injected directly into the rete testis; in the second approach, the injection pipette is inserted into an efferent duct.[3] Currently, the number of stem cells contained in any injected cell population cannot be determined, because there are no unique identifying criteria for these cells. However, a mouse testis is believed to contain approximately 2 stem cells in 10^4 cells, and cell concentrations of 1-2 $\times$ 10^8 cells/ml can be injected. Since the seminiferous tubules of a recipient mouse testis have a volume of approximately 10 μl,[3] probably only 200-400 stem cells are likely to be injected into the tubules of a testis in a typical experiment, unless some form of enrichment is attained.

In the initial studies, donor cells isolated from the testes of mice (C57BL/6 $\times$ SJL) were designated ZFlacZ, because they were hemizygous for a transgene with a zinc finger (ZF) promoter fused to the *Escherichia coli* β-*galactosidase gene (lacZ)*. In the testes of these mice, round spermatids and later stages of spermatogenesis stain blue following incubation with substrate.[4, 5] Donor cells from these mice were injected into testes of (C57BL/6 $\times$ SJL) F_1, recipient males that had been treated with busulfan (40 mg/kg) 4-6 weeks previously to destroy endogenous germ cells. The testes of recipient males were stained with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) 90-120 days following cell transplantation to determine the extent of colonization.[4, 5] Donor cell-derived spermatogenesis is clearly visible as blue areas in recipient seminiferous tubules after staining. Recently, these researches have begun experiments using the transgenic mouse line, B6,129-TgR(ROSA26)26Sor, from Jackson Laboratory (ROSA26) to supply donor cells with the immunologically compatible (129SV $\times$ C57BL/6) F_1 male as a recipient. In ROSA26 mice, many cell types produce β-galactosidase (βgal) and can be stained blue with Xgal. In adult testes, all stages of spermatogenesis stain blue, including spermatogonia and stem cells.[2] Analysis of donor cell colonization using ROSA26 mice has several advantages compared to using ZF*lacZ* donor cells. For example, donor stem cell expansion can be studied in detail immediately following ROSA26 cell transplantation, whereas X-gal staining only identifies late stages of spermatogenesis when ZF*lacZ* cells are used. In addition, quantitative analysis of colonization of recipient testes can be made more quickly for ROSA26 than for ZF*lacZ* donor cells following transplantation. While the appearance of transgene-dependent staining in the testes of recipient mice is clearly an indication of donor cell-derived spermatogenesis, in the best transplants, progeny carrying the transgene will be produced by the recipient.[4]

Following microinjection of donor cells into the seminiferous tubules of a testis, the cells remain widely dispersed in the recipient seminiferous tubules during the first week after microinjection. Donor cells are distributed randomly in the lumen, but some are found close to or on the basement membrane. Since only approximately 2 of 10^4 cells injected are likely to be stem cells, a few of the cells near or on the basement membrane are probably stem cells. By 4 weeks after transplantation, the location of stem cell colonization can be identified as patches of blue cells that are distributed along the tubules. These patches consist of a network formed by chains of cells as they divide on the basement membrane. Some of these cells represent new stem cells, which extend the area of colonization along the basement membrane; the remaining cells of the network are likely to be early stages of spermatogonial division and differentiation. Areas of the tubule outside the patches contain very few blue cells. A possible ex-