Eliminating acute lymphoblastic leukemia cells from human testicular cell cultures: a pilot study

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Objective: To study whether acute lymphoblastic leukemia (ALL) cells survive in a human testicular cell culture system.
Design: Experimental laboratory study.
Setting: Reproductive biology laboratory, academic medical center.
Patient(s): Acute lymphoblastic leukemia cells from three patients and testicular cells from three other patients.
Intervention(s): Acute lymphoblastic leukemia cells were cultured alone or in combination with testicular cells, at various concentrations, in a system that has recently been developed to propagate human spermatogonial stem cells.
Main Outcome Measure(s): Viability of ALL and testicular cells during culture was evaluated by flow cytometry using markers for live/dead cells. Furthermore, the presence of ALL cells among testicular cells was determined by highly sensitive (1:10,000 to 1:100,000 cells) patient-specific antigen-receptor minimal residual disease polymerase chain reaction. The presence of spermatogonia at the end of culture was determined by reverse transcription–polymerase chain reaction for ZBTB16, UCHL1, and GPR125.
Result(s): The ALL cells cultured separately did not survive beyond 14 days of culture. When cultured together with testicular cells, even at extremely high initial concentrations (40% ALL cells), ALL cells were undetectable beyond 26 days of culture. Reverse transcription–polymerase chain reaction confirmed the presence of spermatogonia at the end of the culture period.
Conclusion(s): Our pilot study shows that the described testicular cell culture system not only allows for efficient propagation of spermatogonial stem cells but also eliminates contaminating ALL cells. (Fertil Steril® 2014;101: 1072–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Fertility preservation, childhood cancer, leukemia, spermatogonial stem cell, human testis

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The survival rate of children with cancer has continuously improved over the past few decades, and the 5-year event-free survival rates are currently approximately 80% (1–3). Gonadal damage is a relatively common consequence of the treatments used to cure pediatric cancer, and many cancer survivors are sterile (4, 5). Currently, in prepubertal boys with cancer, testicular biopsies taken before the onset of cancer

treatment are being cryopreserved in several centers to preserve fertility (6–9).

Future use of this tissue will involve thawing of the biopsy, followed by in vitro propagation of spermatogonial stem cells (SSCs) and autotransplantation of these cultured cells when infertility has manifested itself (10). We recently described a testicular cell culture system that enables propagation of adult and prepubertal human SSCs necessary for efficient transplantation (11, 12).

One of the most important safety issues that still needs to be addressed is the elimination of remaining malignant cells in testicular biopsies in the case of nonsolid tumors. The most common nonsolid cancer in prepubertal children is acute lymphoblastic leukemia (ALL), constituting up to 40% of the main tumors in children under the age of 14 years (13). In a single report infiltration of leukemic cells in the testis as proven by histologic examination was described in up to 30% of boys with ALL (14). Although precise data are not available, the percentage of leukemic cells in the testis of boys with ALL is unlikely to exceed 1% of all cells (15). A few studies have attempted to eliminate malignant cells from mouse, nonhuman primate, and human testicular cell suspensions (16–20). These studies are all based on cell sorting techniques and show contradicting results.

To investigate the persistence of contaminating malignant cells in the previously described culture system (11, 12), we cultured ALL cells separately and in combination with testicular cells in various concentrations and used highly sensitive methods to detect the presence of ALL cells at various time points during culture.

MATERIALS AND METHODS

Cell Preparation and Culture

Malignant cells were obtained by bone marrow aspirations from three patients (SQ8512, SQ9610, and SQ11485) with B-cell ALL during their diagnostic process. The percentages of ALL cells before and after cryopreservation in these cell suspensions were evaluated according to their specific phenotypes and percentage of epitope expression, as determined by flow cytometry with a panel of multiple antibodies (CD1, -2, -3, -5, -10, -19, -20, and -34) (21, 22). This percentage, which varied between 77% and 94%, was used to correct for the number of ALL cells to reach the desired percentage of living ALLs cells at the start point of any culture (i.e., 0, 0.04%, 0.4%, 4%, and 40%). The numbers of ALL and testicular cells were also corrected for viability using trypan blue staining at the start of every culture, so that the ratios described always reflected the percentage of vital and alive cells.

Testicular cells were isolated from the donated testis tissue from one adult man who underwent castration as part of the prostate cancer treatment (URO0126) and from two prepubertal boys with Hodgkin lymphoma who stored their testis biopsy before chemotherapy (URO0113 and URO0114, 8- and 6.5-year-old boys, respectively) (12). Samples were used for research after receiving oral informed consent from the adult man and written informed consent from the parents of the prepubertal boys (12). According to Dutch law, ethics committee approval was not required, because anonymized tissue samples (URO0126, SQ8512, SQ9610, and SQ11485) were used. The local institutional review board (Avicenna Research Institute) approved collection and use of URO0113 and URO0114 samples in this research. Pathologic evaluations of the testicular biopsy from the included boys with Hodgkin lymphoma showed no evidence of malignant cells. To increase the number of testicular cells, these cells were cultured for 3–5 weeks before initiation of the experiments in our recently established testicular cell culture condition based on supplemented StemPro medium (#10639-011, Invitrogen) in noncoated dishes as previously described (11, 12). Testicular cells were then cocultured with ALL cells at various concentrations (percentage viable ALL/testicular cells: 0, 0.04%, 0.4%, 4%, and 40%) in the same condition as culturing the testicular cells alone. The medium was refreshed every 3 to 4 days with centrifugation to also collect floating cells. When cells became 90%–100% confluent, cells were passaged (average 1:6) using trypsin ethylenediaminetetraacetic acid (0.25%) (Invitrogen) and transferred to new dishes in a concentration of 10,000–15,000 cells per cm^2. During each passage, surplus cells were used for DNA and RNA isolation. Because at least 35 days of culture are required to propagate enough SSCs for autotransplantation, we cultured the ALL/testicular cell mixes of all three patients for 24–52 days.

Viability of ALL and Testicular Cells during Culture

To examine viability of ALL cells from three patients (SQ8512, SQ9850, and SQ11485) during culture for a period of 14–16 days, an equal amount of cells was plated in each well of a 48-well plate (30,000 cells per well). During this 14–16-day culture period, every 24 hours cells from three wells were harvested by cell scraping (#3010, Corning) and evaluated by flow cytometry with a live–dead kit based on Calcein AM and Ethidium H1 staining according to manufacturer's instructions (MP 3224, Invitrogen). As a control, parallel cultured human testicular cells (URO0126) were used.

Minimal Residual Disease Polymerase Chain Reaction to Trace ALL Cells

To detect the leukemic cells in the cocultures with testicular cells, a leukemia-specific polymerase chain reaction (PCR) was developed for each patient on the basis of patient-specific antigen receptor rearrangements present in the leukemic cells, as previously described (23–25). Moreover, to minimize the theoretical risk of false negativity by the outgrowth of subclones of leukemic cells during the culture period, two patient-specific antigen receptor targets were selected for each patient.

Deoxyribonucleic acid (DNA) from ALL cells mixed with testicular cells before and during culture was extracted using the QIAamp DNA Mini Kit (51306, Qiagen) (26) with an elution volume of 55 μL instead of 200 μL to increase the concentration of DNA. Extracted DNA was measured by the fluorometer Qubit (Invitrogen) using the DNA HS assay kit (Q32854, Invitrogen). Polymerase chain reaction was performed as previously described (27), using the ABI PRISM