Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy

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Objective: To assess Sertoli cell involvement in postchemotherapy azoospermia.

Design: Case report.

Setting: Teaching hospital.

Patient(s): A 31-year-old azoospermic man who underwent cancer cytotoxic chemotherapy for non-Hodgkin’s lymphoma at 13 years of age.

Intervention(s): Testicular biopsy specimens were obtained for sperm recovery in preparation for intracytoplasmic sperm injection. The biopsy specimens were evaluated by quantitative immunohistochemistry for the immature Sertoli cell markers cytokeratin 18 (CK-18) and D2-40.

Main Outcome Measure(s): Extent of immature Sertoli cells.

Result(s): A fraction of Sertoli cells (13%) in the atrophic tubules of this patient reexpressed the intermediate filament protein CK-18, which is normally absent after puberty, but not the D2-40 antigen, an Mr 40,000 a-linked membrane glycoprotein, whose loss of expression at puberty marks an irreversible step in Sertoli cell maturation. Tubules with normal spermatogenic progression lined by Sertoli cells negative for CK-18 were also observed.

Conclusion(s): A fraction of Sertoli cells of this patient initially progressed to full maturation at puberty and reverted to a dedifferentiated state marked by reexpression of CK-18 as a consequence of chemotherapy. This inactivation of Sertoli cells caused by the cytotoxicity of the chemotherapeutic drugs may have contributed to the spermatogenic impairment and resulting infertility. (Fertil Steril 2004;81:1391–4. ©2004 by American Society for Reproductive Medicine.)

Key Words: Sertoli cell dedifferentiation, cancer cytotoxic chemotherapy, cytokeratin-18, D2-40, nonobstructive azoospermia

Male infertility is a recognized complication of cancer cytotoxic chemotherapy (CCT), and interest in this association has increased considerably. More information about the mode of action of anticancer drugs on spermatogenesis is urgently needed.

Human seminiferous tubules contain both somatic cells (Sertoli and myoid) and germ cells. Sertoli cells play a key role in triggering and regulating spermatogenesis. Cytokeratin intermediate filaments are a marker of Sertoli cell differentiation. Cytokeratin-18 (CK-18) is expressed in Sertoli cell cytoplasm of the male fetus but not in adulthood; the expression of CK-18 gradually disappears between birth and puberty (1, 2). The predominant intermediate filament expressed in Sertoli cells at all ages is of the vimentin type, indicating the mesenchymal origin of these cells (3).

To evaluate the functional state of Sertoli cells associated with spermatogenic dysfunction, we studied the expression of CK-18 as a marker of immature Sertoli cells in a man with postchemotherapy azoospermia. In addition to CK-18, we examined the expression of the D2-40 antigen, another marker of Sertoli cell differentiation. This marker, a Mr 40,000 O-linked membrane sialoglycoprotein defined by the monoclonal antibodies D2-40 and M2A (4), is expressed in prepubertal, but not adult, Sertoli cells. Loss of D2-40 expression at puberty marks an irreversible step in Sertoli maturation.
Evaluation of Sertoli cell involvement in postchemotherapy azoospermia by using the combination of the differentiation markers CK-18 and D2-40 has not been previously reported.

CASE REPORT

The status of Sertoli cell differentiation was evaluated in a 31-year-old azoospermic man with testicular damage after CCT. He had received multiple courses of combination CCT, including vincristine, methotrexate, cyclophosphamide, doxorubicin, and N,N 1,3-bis-(2-chloroethyl) N-nitrosourea (BCNU), for treatment of non-Hodgkin’s lymphoma at 13 years of age. Other than his history of CCT, no additional clinical findings could account for his infertility. The study was approved by the local institutional review board in accordance with the Helsinki Declaration of 1975.

Bilateral biopsies were obtained to isolate viable sperm for in vivo fertilization (IVF) using the ICSI procedure. Viable sperm were isolated from both testicular biopsies. Immunohistochemistry was performed using mouse monoclonal antibodies to CK-18 (Clone: DC10, 1:50 dilution), vimentin (Clone: V9, 1:500 dilution) (both antibodies from DAKO, Copenhagen, Denmark), and D2-40 (4), 0.1 μg/mL. Briefly, 3-μm sections of paraffin-embedded tissue fixed in Bouin’s reagent were immunostained by the labeled-streptavidin-biotin (LAB-SA) method. Before immunostaining for CK-18 or vimentin, heat-induced antigen retrieval was performed by controlled microwave pretreatment at 97°C for 10 minutes in 10 mM citrate buffer, pH 6.0. For immunostaining, all incubations were performed at room temperature.

The sections were first preincubated in methanol containing 3% H2O2 for 5 minutes to inactivate endogenous peroxidase, followed by a 10-minute incubation with a nonimmune serum blocking solution (Histostain plus kit; Zymed, San Francisco, CA), after which they were incubated for 1 hour with the primary antibodies at the respective concentrations already specified. The sections were then incubated sequentially with other components of the same kit (Histostain), specifically a prediluted biotinylated secondary antibody for 10 minutes, followed by horseradish peroxidase-conjugated streptavidin (HRP-SA) for 10 minutes. The immunoreaction was visualized by an HRP-based chromogen/substrate system, including diaminobenzidine (Liquid DAB substrate kit; Zymed). For D2-40, a similar protocol was used, as described previously elsewhere (4), without prior heat-induced epitope retrieval. Morphologic evaluation revealed the presence of mixed histologic patterns in adjacent seminiferous tubules, ranging from qualitatively normal spermatogenesis, to spermatocyte arrest, to Sertoli cell–only tubules. Minute foci of qualitatively normal spermatogenesis were also interspersed throughout both biopsies. Expression of the CK-18 marker in Sertoli cells was only detected in the tubules showing impaired spermatogenesis, either partial arrest of spermatogenesis or a Sertoli cell–only pattern (Fig. 1A).

**Figure 1**

Immunohistochemical staining for CK-18 and D2-40. In all cases, antibody binding was visualized using diaminobenzidine as a substrate to generate a brown color. Sections were counterstained with Mayer’s hematoxylin, which stains nuclei blue. (A, B) Testicular biopsy from our patient with postchemotherapy azoospermia. (A) Immunostaining for CK-18: Sertoli cells in tubules with impaired spermatogenesis are immunopositive (arrow). (B) Immunostaining for D2-40: Sertoli cells are entirely immunonegative. (C) Testicle of a 4-year-old male, immunostaining for D2-40: Sertoli cells are uniformly immunopositive. (A–C: Bar indicates 100 μm.)
Quantitative immunohistomorphometric measurements, performed as previously described elsewhere (5), identified normal spermatogenesis over areas representing 10% of the biopsies from the right and left testes; over the remaining areas, representing 90% of the biopsies, impaired spermatogenesis was found. We detected CK-18 immunopositive Sertoli cells over 13% of the areas of impaired spermatogenesis. All the Sertoli cells were immunonegative for the expression of the D2-40 antigen (see Fig. 1B). In a control section, immature prepubertal Sertoli cells of a 4-year-old male expressed the D2-40 antigen (see Fig. IC). All Sertoli cells expressed vimentin, irrespective of their state of differentiation or associated spermatogenic impairment (data not shown).

DISCUSSION

The current study identified dedifferentiated Sertoli cells positive for CK-18 in the testicular biopsy samples of a man with post-CCT azoospermia.

The maturation state of Sertoli cells varies in cases of spermatogenic impairment, depending on the condition (5, 6). Acquired spermatogenic impairment is characterized by a mixed histologic pattern, including areas of atrophy and focal spermatogenesis, and is generally associated with Sertoli cells that exhibit an undifferentiated prepubertal stage of development (5, 6). On the other hand, congenital spermatogenic impairment associated with a deletion in the Y chromosome azospermia factor (AZF) region (5), and a pairing defect in spermatocyte chromosomes is associated with fully differentiated, mature, Sertoli cells (6).

The expression of the prepubertal Sertoli cell marker CK-18 in the affected testis of an adult raises the question of whether these Sertoli cells represent prepubertal cells that have been primarily arrested in their normal maturation, or, alternatively, if they are dedifferentiated Sertoli cells. In our case, the mixed histologic pattern, including tubules with arrested spermatogenesis and those with normal spermatogenic progression in the assessed biopsy samples, are consistent with a pattern of acquired azoospermia and not with that of a primary congenital defect (7). This secondary, or mixed form, of azoospermia is generally associated with the presence of Sertoli cells that appear to be immature and morphologically and functionally damaged (7).

The causal factor underlying the azoospermia in our patient was undergoing CCT in a pubertal period. At this time, Sertoli cells are normally in a fully differentiated state and have attained a mature phenotype. Accordingly, the observed positive immunoreactivity for CK-18 is likely a manifestation of a pathologic change; that is, a dedifferentiation of Sertoli cells is a consequence of CCT-induced testicular damage. This is consistent with the observed loss of D2-40 expression (an irreversible step in the transition of prepubertal Sertoli cells to an adult phenotype).

Dividing germ cells undergoing mitosis and meiosis are exquisitely sensitive to cytotoxic damage to the testis, including damage caused by CCT. In contrast, Sertoli cells that are fully differentiated and do not divide after puberty are relatively resistant. In fact, morphologically normal appearing Sertoli cells have been observed by light-microscopy after disruption of spermatogenesis or elimination of germ cells by a variety of treatments (8). This was initially interpreted to indicate that Sertoli cells were resistant to these treatments. Later studies using more sensitive techniques, including ultrastructural analysis, indicated that Sertoli cells are, in fact, also damaged by a variety of treatments that are cytotoxic to the testis and germ cells (8).

In the present study, using quantitative immunohistomorphometry, we determined that Sertoli cells that are immunopositive for CK-18 represented approximately 13% of Sertoli cells in tubules with impaired spermatogenesis, and 87% of Sertoli cells in these tubules were negative for CK-18. Interestingly, Sertoli cells positive for CK-18 were never observed in tubules with qualitatively normal spermatogenesis. These observations suggest that Sertoli cells are relatively resistant to CCT, and also that inactivation of only a fraction of Sertoli cells in a given tubule may have a disproportionately larger effect on spermatogenesis. This is not surprising, because Sertoli cells are involved in maintaining the blood–testis barrier that provides the appropriate physiologic environment for spermatogenic progression. Thus, inactivation of even a small number of Sertoli cells, like weak links in a chain-link fence, could compromise the integrity of the blood–testis barrier and disrupt spermatogenesis.

We conclude that, in cases of infertility following cancer chemotherapy, there is a primary cytotoxic effect of the drugs on both Sertoli cells and germ cells. The consequent Sertoli cell inactivation contributes to the impairment in spermatogenic progression, resulting in male factor infertility.

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References

