Reversion of the differentiated phenotype and maturation block in Sertoli cells in pathological human testis

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To study the relationship between abnormal Sertoli cell differentiation and spermatogenic impairment, we examined the expression of Sertoli cell markers normally lost at puberty, cytokeratin 18 (CK18), anti-Müllerian hormone (AMH) and M2A antigen, in three children (aged 1–2 years), 50 adults (aged 19–45 years) with obstructive or non-obstructive azoospermia or oligozoospermia, and six patients (aged 1–18 years) with 5α-reductase deficiency. There was CK18 and/or AMH expression, but never M2A antigen expression, associated with spermatogonial arrest or Sertoli cell-only (SCO) syndrome in infertile men. Loss of M2A antigen suggests the transition of Sertoli cells to an adult phenotype, while CK18 and/or AMH expression may be a manifestation of de-differentiation of Sertoli cells. In 5α-reductase deficiency, there was a sequential loss of CK18, M2A antigen and AMH around puberty, associated with partial spermatogenesis. The persistence of immature Sertoli cells expressing M2A antigen was associated with prepubertal seminiferous cords and SCO syndrome. Therefore, 5α-reductase deficiency may prevent the maturation of Sertoli cells, resulting in impairment of spermatogenesis, and loss of M2A antigen expression coincides with a critical step in the Sertoli cell maturation. High follicle stimulating hormone concentrations due to failure of normal Sertoli cell differentiation indicate a normal development pattern of the hypothalamic–pituitary–gonadal axis.

Keywords: anti-Müllerian hormone/cytokeratin/male infertility/M2A antigen/5α-reductase deficiency

Introduction

The maturation of Sertoli cells at puberty is critical for the initiation and maintenance of spermatogenesis. Intriguingly, Sertoli cells with immature characteristics are found in adult testis in some human pathological conditions usually associated with impaired spermatogenesis. These characteristics include alterations in Sertoli cell nuclear shape and a supposed re-expression of differentiation markers normally lost at puberty. First, a reduction in nuclear infoldings is seen in spermatogenic arrest and Sertoli cell-only (SCO) syndrome (Terada and Hatekayama, 1991; Bruning et al., 1993). Second, while the intermediate filaments of immature Sertoli cells contain both cytokeratin and vimentin, only vimentin persists in adult testis (Franke et al., 1979; Paranko et al., 1986; Aümüller et al., 1988, 1992; Stosiek et al., 1990; Dinges et al., 1991; Rogatsch et al., 1996). However, cytokeratin expression has been observed in adult Sertoli cells in testis showing mixed atrophy associated with spermatogenic arrest at the level of spermato- gonia or SCO syndrome (Miettinen et al., 1985; Stosiek et al., 1990; Aümüller et al., 1992; Bergmann and Kliesch, 1994; Steger et al., 1996), as well as in the vicinity of carcinoma in-situ (CIS) cells (Miettinen et al., 1985; Stosiek et al., 1990; Soosay et al., 1991; Aümüller et al., 1992; Rogatsch et al., 1996; Kliesch et al., 1997). Third, anti-Müllerian hormone (AMH), a glycoprotein dimer of the transforming growth factor-β (TGF-β) family (Cate et al., 1986; Josso et al., 1993a), is also expressed in prepubertal, but not adult, Sertoli cells (Tran et al., 1987; Josso et al., 1993b; Rey et al., 1996; Steger et al., 1996). Although AMH plays a key role in the development of internal genitalia by inducing regression of the Mullerian ducts in the male fetus (Josso et al., 1993b), serum concentrations remain high throughout childhood and wane at puberty in response to rising androgen concentrations (Rey et al., 1993). AMH expression is downregulated in seminiferous tubules when the androgen receptor becomes functionally expressed in Sertoli cells and germ cells enter meiosis (Al-Attar et al., 1997).

In pathological conditions, we have recently reported variable patterns of expression of CK18 and AMH in Sertoli cells in adult men exhibiting various degrees of spermatogenic dysfunction (Steger et al., 1996). In separate biopsies of individual testes with mixed tubular atrophy, we found either apparently normal adult-type Sertoli cells that did not express CK18 or AMH, those that expressed both markers, or those that expressed one of the two markers. It is not known to what extent abnormal Sertoli cells associated with defective spermatogenesis are the consequence of the underlying pathological process or, because of their malfunction, a contributing factor to the spermatogenic dys- function. In addition, the above results raise the question whether these Sertoli cells represent prepubertal Sertoli cells arrested in their normal maturation, or alternatively, adult-type
Sertoli cells that have reverted to a less differentiated state. In order to address these issues, we have now examined testicular biopsies from a larger cohort of patients with defective spermatogenesis, including the testes of six patients with 5α-reductase deficiency, aged 1–18 years, which are known to lack normal pubertal testicular development (Damjanov and Drobnjak, 1974; Müller, 1984; Aumüller and Peter, 1986; Johnson et al., 1986). We have also included an additional immunological marker of Sertoli cell maturation, the M2A antigen, defined by reactivity with the monoclonal antibodies M2A and D2-40. This antigen is expressed in prepubertal, but not adult, Sertoli cells (Bailey et al., 1986; Baumal et al., 1989).

Our results indicate that there is a reversion of the Sertoli cell phenotype to a less differentiated state as a heterogeneous process in which the expression of CK18, AMH and M2A antigen is not coordinately regulated. Furthermore, we provide evidence that, in adult subjects with 5α-reductase deficiency, aged 16 years, and two adults (aged 18 years each), with 5α-reductase deficiency. Diagnosis was confirmed by clinical examination (female phenotype with clitoromegaly, blind-ending vagina and missing derivatives of the Müllerian ducts, uterus and tubes, and primary amenorrhoea in adults) and chromosome analysis (46, XY; mutation in the androgen receptor gene). We examined a total of 79 biopsies from 59 patients (see Tables I and II).

The testicular biopsy specimens, each about the size of a grain of rice, were fixed by immersion in Bouin’s fixative and embedded in paraffin.

**Serum follicle stimulating hormone**

Serum follicle stimulating hormone (FSH) concentrations were measured by fluoroimmunoassay on at least two occasions for each patient (Jockenhövel et al., 1989). Serum concentrations of >7 IU/l were regarded as elevated compared with normal men of proven fertility (Cooper et al., 1991).

**Immunohistochemistry**

For cytokeratin 18 (CK 18) immunostaining, 5-μm sections were stained with monoclonal antibody to human CK 18 (Coulter Immunotech, Hamburg, Germany) using the avidin–biotin–peroxidase complex (ABC) method (Vectastain Elite ABC Kit, Vector, Burlingame, CA, USA), as follows. After deparaffinization and rehydration, sections were digested with 1 mg pronase/ml Tris-buffered saline pH 7.4 (TBS) for 8 min, and treated with 3% H₂O₂ for 30 min, followed by 3% bovine serum albumin in TBS (TBS-3% BSA) for 30 min. Sections were then incubated with the primary antibody (1:50 in TBS-3% BSA) overnight, followed by the biotinylated secondary antibody for 30 min, and ABC also for 30 min. Following each incubation, sections were washed thoroughly with TBS. For colour development, the sections were incubated with DAB/H₂O₂ (Research Genetics, Huntsville, AL, USA). Finally, sections were mounted in DePeX for microscopic examination.

For CK18 and vimentin double-immunostaining, sections were first stained with the anti-CK 18 antibody, as described above, followed by the anti-vimentin antibody (Coulter Immunotech) using the alkaline

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### Table I. Immunohistochemical detection of vimentin (VIM), cytokeratin 18 (CK 18), anti-Müllerian hormone (AMH) and M2A antigen (recognized by the monoclonal antibodies M2A and D2-40) in Sertoli cells in histologically normal and pathological seminiferous tubules.

<table>
<thead>
<tr>
<th>Total patients</th>
<th>Total biopsies</th>
<th>Age range years (mean)</th>
<th>Serum FSH mean ± SD (IU/l)</th>
<th>Clinical diagnosis</th>
<th>Histology</th>
<th>Vim (%)</th>
<th>CK18 (%)</th>
<th>AMH (%)</th>
<th>M2A D2-40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
<td>1–2 (1.3)</td>
<td>0.8 ± 0.6</td>
<td>Ectopic testis</td>
<td>Prepubertal NSPG (100%)</td>
<td>100</td>
<td>85</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>30–44 (35.4)</td>
<td>4.9 ± 2.4</td>
<td>OAZ</td>
<td>Adult NSPG (100%)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>19–45 (31.4)</td>
<td>19.8 ± 11.4</td>
<td>NOAZ</td>
<td>SCO (5%)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>19–43 (31.4)</td>
<td>11.2 ± 7.0</td>
<td>OAT</td>
<td>SCO (25%)</td>
<td>100</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (55%)</td>
<td>100</td>
<td>55</td>
<td>50</td>
<td>0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (14%)</td>
<td>100</td>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (13%)</td>
<td>100</td>
<td>75</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (5%)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (10%)</td>
<td>100</td>
<td>0</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (12%)</td>
<td>100</td>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCO (3%)</td>
<td>100</td>
<td>55</td>
<td>100</td>
<td>0</td>
</tr>
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</table>

OAZ = obstructive azoospermia; NOAZ = non-obstructive azoospermia; OAT = oligoasthenoteratozoospermia; SCO = Sertoli cell-only syndrome; NSPG: qualitatively normal spermatogenic progression; SDA = arrest at the level of round spermatids; SCA = arrest at the level of spermatocytes; SGA = arrest at the level of spermatogonia. The percentage of immunopositive Sertoli cells is a semiquantitative estimate based on examination of 10 microscopic fields.
phosphatase–anti-alkaline phosphatase (APAAP) technique according to Cordell et al. (1984).

AMH immunohistochemistry was performed as previously described (Rey et al., 1996). Briefly, 5-µm sections were dehydrated, microwaved and incubated with rabbit anti-recombinant human AMH antibody followed by an alkaline phosphatase-conjugated goat anti-rabbit Ig antibody. For colour development, the sections were incubated with a solution of nitroblue-tetrazolium and 5-bromo-4-chloro-3-indolylphosphate.

Monoclonal antibodies M2A and D2-40 were produced by immunizing mice with the human ovarian adenocarcinoma cell line HEY (Bailey et al., 1986) and a dysgerminom tumour (A.Marks, unpublished results), respectively. Both antibodies are directed to the same antigen and react with tissues fixed in Bouin’s fixative. In addition, D2-40 (but not M2A) reacts with tissues fixed in formalin. The antibodies were purified from ascitic fluid of mice by chromatography on a protein A–Sepharose (Pharmacia, Uppsala, Sweden) column and concentrated to 1.6 mg/ml in phosphate-buffered saline (PBS; 140 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄ pH 7.2).

For immunostaining, the sections were incubated with M2A (2 µg/ml) or D2-40 (0.1 µg/ml) for 1 h at room temperature. The sections were then incubated with biotinylated goat anti-mouse IgG antibody (DAKO, Carpinteria, CA, USA), at a 1:400 dilution, followed by a horseradish peroxidase–avidin conjugate (Zymed, San Francisco, CA, USA) at a 1:600 dilution. For colour development, the sections were incubated with 0.05% diaminobenzidine and 0.03% H₂O₂ for 1 min, rinsed in water, and counterstained with haematoxylin.

For each immunoreaction, control incubations were performed by substituting buffer for the primary antibody. These sections were completely immunonegative.

Results

The results of analysis of four ectopic testes from children, aged 1–2 years, and a total of 65 testicular biopsies from 50 adults, aged 19–45 years, with spermatogenic dysfunction associated with obstructive azoospermia (OAZ), non-obstructive azoospermia (NOAZ) and oligoasthenoteratozoospermia (OAT) are presented in Table I.

Serum FSH concentrations were <1 IU/l in the three children and <7 IU/l in the five adults with OAZ. Patients with NOAZ and OAT exhibited elevated serum FSH concentrations (NOAZ, 19.8 ± 11.4 IU/l; OAT, 11.2 ± 7.0 IU/l).

The histological appearance of the seminiferous tubules varied from qualitatively normal prepubertal seminiferous epithelium in ectopic testes (Figures 1–3) and normal adult spermatogenic progression (NSPG) in OAZ, to SCO in NOAZ. Biopsies from patients with OAT exhibited a range of histological appearances, including NSPG, arrest at various stages of spermatogenic development, and SCO (Figures 4–6).

Vimentin was a ubiquitous immunohistochemical marker present in testicular biopsies of all ages, associated with both normal and impaired spermatogenesis. The three markers of immature Sertoli cells, CK18, AMH and M2A antigen, were uniformly expressed in prepubertal Sertoli cells found in ectopic testes (Figures 1–3) and were absent from adult testicular tubules with NSPG. Of these three markers, CK18 and AMH were variably expressed in Sertoli cells in adult testis exhibiting various stages of arrest in spermatogenic progression. All possible combinations of expression of these two markers were observed, including absence of CK18 and AMH expression, expression of either one of the markers, or expression of both markers. Remarkably, the M2A antigen was always absent in Sertoli cells of adults, irrespective of any histological evidence of spermatogenic dysfunction.

The results of analysis of a total of 10 testicular biopsies from six patients with 5α-reductase deficiency are shown in Table II.

Serum FSH concentrations were <1 IU/l in prepubertal patients, aged 1, 2 and 10 years (mean 0.8 ± 0.1 IU/l). The 16-year-old patient, and the two 18-year-old patients exhibited elevated serum FSH concentrations (20.3 ± 12.8 IU/l).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Serum FSH (IU/l)</th>
<th>Biopsy no.</th>
<th>Histology</th>
<th>Vim (%)</th>
<th>CK18 (%)</th>
<th>AMH (%)</th>
<th>M2A D2-40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>Prepub. sem. cords with presg. (100%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
<td>Prepub. sem. cords with presg. (100%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.1</td>
<td>5</td>
<td>Prepub. sem. cords with presg. and sg. (100%)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean</td>
<td>0.8 ± 0.1</td>
<td></td>
<td>6</td>
<td>Prepub. sem. cords (1%)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>38.27</td>
<td>6</td>
<td>Prepub. sem. cords (60%)</td>
<td>100</td>
<td>40</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>9.0</td>
<td>7</td>
<td>Prepub. sem. cords (8%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>13.5</td>
<td>9</td>
<td>Prepub. sem. cords (4%)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean</td>
<td>20.3 ± 12.8</td>
<td></td>
<td>10</td>
<td>Prepub. sem. cords (4%)</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Prepub. sem. cords = prepubertal seminiferous cords; presg. = prespermatogonia; sg. = spermatogonia; SCO = Sertoli cell-only syndrome; SCA = arrest at the level of spermatocytes; SGA = arrest at the level of spermatogonia. The percentage of immunopositive Sertoli cells is a semiquantitative estimate based on examination of 10 microscopic fields.
Figures 1–6. Immunohistochemical detection of cytokeratin 18 (Figures 1 and 4), anti-Müllerian hormone (Figures 2 and 5) and M2A antigen (Figures 3 and 6, recognized by the monoclonal antibody D2–40) in a 2-year-old boy (Figures 1–3) and in a 33-year-old infertile man with oligozoospermia (Figures 4–6). (Figures 1–3) The seminiferous cords of the 2-year-old boy contained prepubertal Sertoli cells and prespermatogonia (arrowheads). Prepubertal Sertoli cells showed strong immunoreactivity for CK18, AMH and M2A antigen. Scale bar = 515 μm. (Figures 4–6) CK18 and AMH were variably expressed in Sertoli cells in adult testis exhibiting Sertoli cell-only syndrome or spermatogenic arrest at the level of spermatogonia (arrowheads). M2A antigen was absent. Scale bar = 10 μm.

Biopsies from all of these patients were examined (Figures 7–12). The histological examination of the prepubertal patients revealed the presence of prepubertal seminiferous cords with Sertoli cells and prespermatogonia in two patients, aged 1 and 2 years, and in addition to the former cell types, spermatogonia were also seen in the patient aged 10 years. Testicular tissue of the 16-year-old patient (Figures 7–9) exhibited a wide range of histological appearances, with a small fraction of prepubertal seminiferous cords, a majority of tubules with SCO, and some tubules showing arrest in spermatogenic progression at either the level of spermatogonia or spermatocytes. A small proportion of the tubules of the two adult patients (Figures 10–12) had evidence of remnants of prepubertal seminiferous cords. However, the predominant histology of the tubules in these patients was SCO.

Again, vimentin was found to be a ubiquitous immunohisto-
Figures 7–12. Consecutive sections showing the immunohistochemical detection of cytokeratin 18 (Figures 7 and 10), anti-Müllerian hormone (Figures 8 and 11) and M2A antigen (Figures 9 and 12, recognized by the monoclonal antibody M2A) in a 16-year-old patient (Figures 7–9; patient 4) and in an 18-year-old patient (Figures 10–12; patient 5), both with 5α-reductase deficiency. (Figures 7–9) The seminiferous tubules of the 16-year-old patient exhibited a wide range of histological appearance, including a small fraction of prepubertal seminiferous cords (arrowheads), which were immunopositive for CK18 and AMH. M2A antigen was absent. Scale bar = 15 µm. (Figures 10–12) The predominant histology of the tubules in the 18-year-old patient was Sertoli cell-only syndrome. Sertoli cells expressed AMH and M2A antigen. Note that some Sertoli cells were immunonegative for M2A antigen (arrowhead). The expression of CK18 was variable. Scale bar = 15 µm.

Discussion

It is known that infertile men with non-obstructive azoospermia or severe oligozoospermia exhibit elevated serum FSH concentrations (Bergmann et al., 1994, Martin-du-Pan and Bischof, 1995), and that Sertoli cells are the only cells in the testis that express FSH receptors (Böckers et al., 1994; Kliesch et al., 1997). CK18 and/or AMH expression in Sertoli cells of SCO tubules or tubules with spermatogonial arrest (Bergmann and Kliesch, 1994; Steger et al., 1996; this paper) indicate that the increase of FSH in such patients is due to the immature characteristics of part of the Sertoli cell population, showing normal functioning of the hypothalamic–pituitary–testicular axis. Elevated serum FSH concentrations are due to insufficient inhibin B secretion (Anawalt et al., 1996). It is known from the male rat that pituitary–gonadal negative feedback regulation starts during the last days of fetal life (Huhtaniemi, 1995). In the human, prepubertal serum FSH concentrations are low (<1 IU/l) and increase to 2–7 IU/l in adult men with normal spermatogenesis (Andersson et al., 1998). Our patients with 5α-reductase deficiency showed either normal prepubertal FSH concentrations (ages 1–10 years) together with normal testicular developmental pattern, or elevated FSH (ages 16–18 years) together with maturation arrest or SCO comparable with that of infertile men, confirming the well-known lack of normal pubertal testicular development (Damjanov and...
The high FSH concentrations due to the failure of normal Sertoli cell differentiation indicate a normal development pattern of the hypothalamic–pituitary–gonadal axis in 5α-reductase deficiency. It is still a matter of debate whether spermatogenic impairment is a direct effect of the mutation or a secondary consequence of incomplete testicular descent (Wilson et al., 1993).

At puberty, Sertoli cells undergo marked morphological and physiological changes in response to hormonal and paracrine effectors (for review see Gondos and Berndston, 1996). This maturation of Sertoli cells is essential for the normal development of prepubertal seminiferous cords to functional adult tubules exhibiting normal spermatogenic progression. Neither the mechanisms through which Sertoli cells influence this process, nor the reciprocal effects of spermatogenesis on the maintenance of Sertoli cells are understood. However, it is certain that the definition of sequential differentiation markers to follow the timely maturation of Sertoli cells would lead to important insights into these questions. Our results have a direct bearing on this issue.

First, we confirm the validity of the three previously reported markers (Steger et al., 1996) of immature Sertoli cells, CK18, AMH and M2A antigen. These three markers were coordinately expressed in normal prepubertal testes, but were absent in normal adult testes. Second, we found that in various pathological disorders of the adult testis associated with infertility and histological abnormalities in spermatogenic progression, CK18 and/or AMH were expressed sporadically. In keeping with previous reports (Rey et al., 1996; Al-Attar et al., 1997), AMH was expressed only in premeiotic seminiferous tubules. Remarkably, M2A antigen was never expressed in this large cohort of patients, including 65 testicular biopsies from 50 adults.

The expression of cytokeratin (Miettinen et al., 1985; Stosiek et al., 1990; Soosay et al., 1991; Aumüller et al., 1992; Bergmann and Kliesch, 1994; Rogatsch et al., 1996; Steger et al., 1996, deMiguel et al., 1997) and AMH (Steger et al., 1996) in postpubertal Sertoli cells in pathological conditions associated with infertility has already been reported. The expression of these markers may indicate a de-differentiation of adult-type Sertoli cells in the context of testicular tubular pathology. This suggests that normal spermatogenic progression may be required to maintain Sertoli cells in their fully differentiated adult stage. In contrast, the absence of expression of M2A antigen in Sertoli cells in these pathological tubules is a novel finding, suggesting that loss of expression of M2A antigen is an irreversible marker of transition of prepubertal Sertoli cells to a differentiated adult state. Therefore, it is likely that the observed abnormalities in Sertoli cells in adult infertility associated with tubular dysfunction are not a primary defect, but may be interpreted as a partial de-differentiation as a consequence of the underlying derangement in spermatogenesis resulting in the re-expression of CK18 and AMH.

With the above inferences in mind, the interpretation of our results in patients with 5α-reductase deficiency provides an additional insight into the relationship between Sertoli cell maturation and spermatogenesis. Again, the three markers of immature Sertoli cells, CK18, AMH and M2A antigen, were uniformly expressed in prepubertal spermatogenic cords in testis from young children with this genetic defect. Remarkably, Sertoli cells in the tubules of the prepubertal child, aged 10 years, the 16-year-old patient and the two 18-year-old patients displayed a gradation of expression of these markers, suggesting that Sertoli cells in this disease are arrested at various stages of transition between the immature and adult state. Furthermore, since these stages of transition of Sertoli cells were associated with various degrees of aborted spermatogenic progression, it is possible that 5α-reductase deficiency interferes with the maturation of Sertoli cells, as a primary defect. In turn, these Sertoli cells at various stages of functional maturation would allow only partial progression of spermatogenesis to sequential end-points.

The loss of expression of M2A antigen appears to coincide with a mandatory step in the functional maturation of Sertoli cells. In the 16-year-old patient, loss of expression of M2A antigen in Sertoli cells coincided with the presence of tubules exhibiting spermatogenic progression to the level of spermatagonia and spermatocytes. In contrast, in the two 18-year-old patients, whose Sertoli cells expressed M2A antigen, indicating that they were functionally immature, there was no histological evidence of spermatogenesis, only the presence of prepubertal seminiferous cords and SCO.

The loss of expression of the other two markers of Sertoli cell maturation, CK18 and AMH, appear to bracket temporally the loss of expression of M2A antigen. Loss of CK18 expression occurs very early during maturation of Sertoli cells. The loss of this marker may signal the earliest transition of these cells to a functional state which is, therefore, manifested inconsistently. Thus, in the 10-year-old prepubertal patient whose Sertoli cells showed loss of CK18 expression, prepubertal cords contained spermatogenesis in addition to spermatagonia. However, in one 18-year-old adult whose Sertoli cells also did not express CK18, there was no evidence of spermatogenic progression.

In contrast, loss of AMH expression appears to be a late maturation marker. It never preceded the loss of expression of M2A antigen. In fact, in the 16-year-old patient, AMH continued to be expressed in Sertoli cells even after the loss of expression of M2A antigen. Only in those tubules in which spermatogenesis progressed to the stage of spermatocytes was there loss of AMH expression. This suggests that loss of AMH expression coincides with a further functional maturation of Sertoli cells, allowing the progression of spermatogenesis to a more advanced stage. This is in keeping with results derived from mice (Al-Attar et al., 1997).

The notion (Damjanov and Drobnjak, 1974; Müller, 1984; Aumüller and Peter, 1986; Johnson et al., 1986) that 5α-reductase deficiency affects Sertoli cell maturation as a primary defect with resulting effects on spermatogenesis is consistent with our data. However, this does not exclude the possibility of a more complex phenotype with spermatogenic impairment as a direct consequence of this genetic defect and, in addition, secondary effects of impaired spermatogenesis on Sertoli cell differentiation. For example, the observed expression of CK18 in Sertoli cells that no longer expressed M2A antigen (patient...
4) may be an indication of de-differentiation of these cells, following an earlier loss of CK18 expression during early maturation. It is also likely that the penetration of the genetic defect varies among individual patients restricting the maturation of Sertoli cells to different degrees, and allowing partial spermatogenic progression in some patients, but not others. Our combined results provide evidence for a reciprocal regulation between Sertoli cells and spermatogenesis. Functional Sertoli cells are required for normal spermatogenic progression, which in turn is necessary to maintain Sertoli cells in their fully differentiated state. We suggest that Sertoli cells with partially reverted maturation markers in adult testis, associated with some forms of spermatogenic dysfunction, acquire this phenotype through de-differentiation. Conversely, in 5α-reductase deficiency, the failure of Sertoli cell to progress fully to a mature functional state at puberty is a primary defect which, in turn, does not allow normal spermatogenic progression. In this respect, our description of immature Sertoli cells in two adults with this genetic defect represents the first report of the persistence of immature, rather than de-differentiated Sertoli cells in adult testis.

We also suggest a temporal sequence for the three markers of Sertoli cell maturation, with the loss of CK18 expression being the earliest, followed by the loss of M2A antigen expression, and finally the loss of AMH expression. Each of these maturation steps is associated with an expanded capability of Sertoli cells to allow spermatogenesis to progress to a further end-point. The loss of expression of M2A antigen appears to coincide with the most important transition of Sertoli cells to a functional state. Furthermore, the loss of expression of this antigen may be irreversible, in the sense that it does not revert in de-differentiated Sertoli cells associated with impaired spermatogenesis. The elucidation of the molecular mechanisms which control the sequential loss of expression of CK18, AMH and M2A antigen, and the definition of the effector functions which are acquired by Sertoli cells at these maturation steps will contribute to a further understanding of the relationship between Sertoli cells and spermatogenesis.

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