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Androgen Receptor Expression in Sertoli Cells as a Function of Seminiferous Tubule Maturation in the Human Cryptorchid Testis*

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ABSTRACT

Androgen receptor (AR) immunohistochemistry was performed in an archival collection of adult human cryptorchid testes to determine whether AR cellular distribution and intensity of immunostaining were functions of the severity of cellular dysgenesis. The seminiferous tubule histology of cryptorchid testes collected from adults is marked by three specific patterns. 1) Seminiferous tubules are characterized as maintaining focal areas of germinal cell differentiation (albeit incomplete) that are interspersed with 2) tubules composed of Sertoli cells only, these latter cells being principally of the adult type, although dysgenetic and immature Sertoli cells may also be detected. 3) In contrast, there is a class of tubule that is characterized as being composed exclusively of Sertoli cells that are extremely dysgenetic in appearance. The majority of adult-type Sertoli cells found in the first types of tubules exhibited either robust or moderate AR staining intensity. Peritubular cells of these tubules also expressed a similar AR staining intensity. In contrast, in the more dysgenetic and immature type Sertoli cells found in the second type of tubules, the intensity of AR staining was significantly less, if not missing altogether. Finally, in the most dysgenetic tubules, Sertoli cell AR staining was never detected. To our knowledge, this is the first report in the literature that addresses the intensity of AR immunostaining in Sertoli cells of cryptorchid testes. The results presented herein are consistent with the interpretation that the intensity of AR staining in Sertoli cells diminishes as a function of the severity to which the cells are afflicted within a cryptorchid testis and that focal absence of AR expression in Sertoli cells correlates with a lack of local spermatogenesis in the tubules. (*J Clin Endocrinol Metab* **86:** 413–421, 2001)

CRYPTORCHIDISM in the human male may be caused by a variety of anomalies, all of which alter the normal descent of the testis into the scrotum. The undescended testis may be found retained at several sites, including within the abdominal cavity proper, along the inguinal canal, or in the higher portions of the scrotal sac. In cryptorchid testes of patients having experienced puberty, lesions in the seminiferous tubules and interstitium are readily apparent and highly dependent on the location of the testis and the age of the individual at the time of orchidopexy in infant patients or orchiectomy in adult patients. The most remarkable lesions are found in those testis located within the abdominal cavity or in the superior-most region of the inguinal canal. In addition, the lesions are more intense in extirpated testes from patients older than 25–30 yr of age (reviewed in Ref. 1). In testes from these patients it is possible to detect distinct histological patterns in the lesions of the seminiferous tubules and to group these into three types. In the first type, tubules are relatively large, exhibit evidence of pubertal growth, are composed principally of adult-type Sertoli cells, and may contain a focal differentiation (albeit incomplete) of the germ cell line. Variations in these types of tubules include those that present as Sertoli cell only tubules (SCO), although the tubules are also relatively large and are composed of the mature-type Sertoli cell. A second type of tubule is defined as being relatively large and expressing some evidence of pubertal growth, but in focal regions, most frequently all along their length, there is a significant absence of pubertal maturation; and the Sertoli cells are often of the immature type. In the third class, the tubules are characterized by a small diameter and significant dysgenesis without any evidence of germ cell line components, the Sertoli cells are either round or fusiform, and the tubules are most often grouped into almost complete sclerotic areas of the tubular-interstitial zones (2). Of importance to the present study, all of these types of tubules may be found within the same testis.

That naturally occurring cryptorchid testes are frequent in the human population and express distinct seminiferous tubule histology led several investigators to develop experimental models to possibly identify hormonal environments that may be responsible for these remarkably distinct phenotypes (reviewed in Refs. 3 and 4). In these models, normally descended testes from pre- and postpubertal animals were surgically transposed to the abdominal cavity, and the

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inguinal ring was sutured shut to prevent testicular prolapse back into the scrotal sac (3, 4). Unfortunately, one limitation of using these experimental models to study testicular physiology is that the cryptorchidism is most usually performed on well developed adult testes that have undergone normal descent. These experimental models, then, more correctly address the changes in the testes produced by the abdominal environment, especially the increase in temperature (5), and are unlikely to fully correspond to true human cryptorchidism (1). More recently, animal models with congenital prenatal cryptorchidism have been developed (6, 7), but these also have limitations as a model for human cryptorchidism. In the latter, for example, detectable genomic alterations are infrequent, and when they do occur are more commonly associated with other complex genito-urinary malformations or with polymalformed syndromes (4). Therefore, to our knowledge, human cryptorchidism is one of the few naturally occurring biological models in which paracrine regulation phenomenology within the testis can be best understood. In particular, as discussed above, this is because after puberty distinct lesions develop in a cryptorchid testis, even within the length of the same seminiferous tubules, although presumably all seminiferous tubules within the same testis were subjected to identical, global conditions of hormones and growth factors.

In the mammalian testis, including that of humans, it is now clear that one means by which paracrine regulation between spermatogenesis and steroidogenesis occurs is via the androgen receptor (AR) located in the nuclei of Sertoli cells (8–18), and as we recently demonstrated, immunopositive AR levels vary as a function of the cycle of the seminiferous epithelium in humans (19). Therefore, in the present study we evaluated the level of AR immunoexpression in Sertoli cell nuclei of cryptorchid testes removed from adult patients as a means to further our understanding of AR expression *vis-à-vis* its potential association with the regulation of spermatogenesis. In addition, we relate this expression to the presence or absence of germinal cells as well as the grade of Sertoli cell dysgenesis found within hypoplastic seminiferous tubules of cryptorchid testes removed from adult patients. To our knowledge, a detailed examination of AR immunostaining in the cryptorchid testis has not been reported.

Subjects and Methods

Patient population

All testes (n = 37) used in this study were collected from postpubertal patients who presented at various hospitals in Spain with unilateral cryptorchidism and who were counseled to undergo surgical intervention by the urological services of the respective hospitals. Consent forms were obtained from each patient, and in patients younger than 18 yr of age parents were asked to sign the consent forms. The indication for surgical intervention of a cryptorchid testis in postpubertal patients is universally accepted to avoid future development of testicular malignancy (germ cell tumors) that is more frequent in the cryptorchid testis relative to the general population, and this risk increases with age.

All testes were located in the highest portion of the inguinal canal, or in the abdominal cavity proper in this select patient population, making it impossible to try and lower the testes into the scrotum. In all cases, the cryptorchid testis was removed after puberty (mean age, 24.05 ± 7.05; range, 15–44 yr) at Hospital La Paz of Madrid (University Autonoma, Madrid, Spain) or University Hospital of Guadalajara (University of Alcala, Alcala de Henares, Spain). Control testes were obtained from the right testes of 20 patients, older than 40 yr of age (mean age, 56 ± 6.98; range, 43–65 yr), who had been autopsied (performed between 6–12 h after death) at the Department of Pathology of La Paz Hospital who died from various causes. However, control testes were selected from patients known to be free of liver and renal disease as well as endocrinological and metabolic conditions that could adversely affect testicular function. A detailed histological study did not reveal lesions in either the testes or spermatic duct, and the general autopsy study also did not reveal endocrine, renal, or hepatic pathologies known to produce morphological and functional changes in the testis. Although it is recognized that the mean ages of the cryptorchid and control testes differ by approximately 32 yr, possibly affecting the comparison between control and deceased testes, we wish to emphasize that normal testes from a younger population is difficult, if not impossible, to obtain.

Tissue collection

The cryptorchid testes were removed by abdominal laparotomy (if located intraabdominally) or were excised from an opening of the inguinal canal (if located in the inguinal canal), and the control testes were removed at autopsy along with the epididymis and the spermatic cord. Both groups of testes were fixed by immersion in buffered 10% formalin for 2–3 days after first nicking the tunica albugenea to permit diffusion of the fixative into the tissue. On day 2, 2- to 3-mm transverse slices were made of the testes, and testes were cut into 0.25-cm^2 pieces. All of the tissues were embedded in paraffin using conventional methods, and 6-μm sections were cut and stained with hematoxylin-eosin to assess tissue morphology.

Morphometry

Measurements were performed on the transverse sections of the cryptorchid testes using a semiautomatic analyzer (Videoplan-Kontron Instruments Ltd., Oberkochen, Germany). The point-counting method reported by Weibel (20) was used. This method superimposes Weibel's B36 test lattice with its 144 intersections over each microscopic field, and each intersection is recorded. At least 2000 intersections were counted for each cryptorchid testis, and the data are reported as a percentage of the surface area.

Three distinct histological patterns were measured for each testis: tubules containing germ cells, Sertoli cell-only tubules, and Sertoli cell-only tubules exclusively exhibiting dysgenetic Sertoli cells (hypoplastic tubules). The presence of hypoplastic tubules in a testis is considered significant when they make up more than 3% of the total area of the testis, given that in the normal, adult fertile testis hypoplastic tubules may also be present. Germ cell-containing tubules were characterized as those that exhibited basally localized spermatogonia associated with or without more mature spermatocytes. Again, the 3% rule of total surface area was used to distinguish a testis as exhibiting germ cell-containing or Sertoli cell-only. A testis was considered to be composed exclusively of Sertoli cell-only tubules when, by definition, germ cells were absent from the testis.

Immunohistochemistry

New 6-μm sections were cut, and AR were immunolocalized using prediluted, commercial antibody (BioGenex Laboratories, Inc., Dublin, Ireland) and employing a biotin-streptavidin-immunoperoxidase method as described previously, including epitope retrieval (19). At the completion of the epitope retrieval, the sections were allowed to cool for approximately 20 min. Primary antibody was applied to the sections and allowed to incubate overnight at 4 C. At this point the sections were treated exactly as described in the instruction manual supplied with the manufacturer's immunostaining kit for 3′,5′-diaminobenzidine as the chromogen (Zymed Laboratories, Inc., South San Franscisco, CA). Hematoxylin counterstaining of the sections was performed briefly for 30–60 s, and the sections were mounted with DPX (Probus, Badalona, Spain). As controls, additional sections were treated as follows: 1) primary antibody was omitted; 2) normal rabbit sera was used instead of primary antibody; 3) dilutions of primary antisera were performed to quench positive staining as a function of specific antibody concentration; and 4) sections from adult human prostate were immunostained in

parallel as a positive verification of the antibody potential. The sections were photographed using a Leitz Labor Luxe D microscope (Rockleigh, NJ) fitted with an 80A color filter, and images were recorded on Kodak Elite Chrome 100 ASA film (Eastman Kodak Co., Rochester, NY) set at an ASA sensitivity of 50. For publication, photographs were scanned at 300 dpi using a Hewlett-Packard scanner (Palo Alto, CA), and the final prints were made using Adobe Photoshop imaging software (Abacus Concepts, Inc., Berkeley, CA) and a Fuji Photo Film Co., Ltd. 3000 pictography color printer (Tokyo, Japan).

Results

Morphometry

The results of the morphometric analysis of the cryptorchid testes are presented in Tables 1 and 2 and Fig. 1. Three distinct patterns histological patterns were measured in seminiferous tubules: 1) tubules containing germ cells, 2) tubules that were Sertoli cell only, and 3) tubules that were Sertoli cell only and the cells were dysgenetic. In 21 of the 37 testes examined 21 testes exhibited seminiferous tubules with some form of germ cell development, whereas 16 exhibited seminiferous tubules of the Sertoli cell type only. There did not appear to be a correlation between the duration of cryptorchidism and the incidence of SCO or between the location of the testes and the incidence of SCO (data not shown). Importantly, all three histological patters could be observed within the same testis, as is evident by examining the results in Table 2.

AR immunoexpression in normal adult human testis

To establish baseline levels of AR immunostaining intensity with the commercial antisera, AR immunoexpression was first evaluated in seminiferous tubules expressing complete spermatogenesis that were collected from normal adult human testis. Using the commercial prediluted anti-AR antibody, robust immunostaining was detected in all testes examined (Fig. 2A). Although slight variations in the AR immunostaining intensity between different testes were sometimes discerned, these differences were interpreted as resulting from varying the length of exposure to the color reaction or counterstaining with hematoxylin, differences to be expected when the immunostaining protocols were performed on different days. Specific immunostaining was detected exclusively in the nuclei of Sertoli cells, Leydig cells, and peritubular myoid cells (Fig. 2A). Cytoplasmic AR immunostaining was never observed in Sertoli cells, nor was staining ever detected in the germ cells (Fig. 2A). The intensity of the AR immunostaining was more robust in the Sertoli cell nuclei than in the peritubular cell nuclei, although in the latter cell types positive immunostaining appeared to be present more frequently than in the Sertoli cell. The apparent absence of AR immunostaining intensity in a few of peritubular cell nuclei photographically captured in our data appeared to be a function of the length of time that the sections were subjected to hematoxylin counterstaining. This inter-

TABLE 1. Tubule type in cryptorchid testes

	n (37 cases) (%)
Germ cell-containing tubules	21 (56.8)
With dysgenesis	13 (61.9)
Without dysgenesis	8 (38.0)
Sertoli cell only tubules	16 (43.2)
With dysgenesis	15 (93.2)
Without dysgenesis	1 (6.2)

TABLE 2. Tubule histology in cryptorchid testes

	No. of testes (n = 37)	% of testicular surface area
Testes with tubules exhibiting germ cells	21	18.90 ± 8.21
Testes with Sertoli cell only tubules	16	100
Testes exhibiting Sertoli cell only (dysgenetic) tubules	28	9.71 ± 3.58

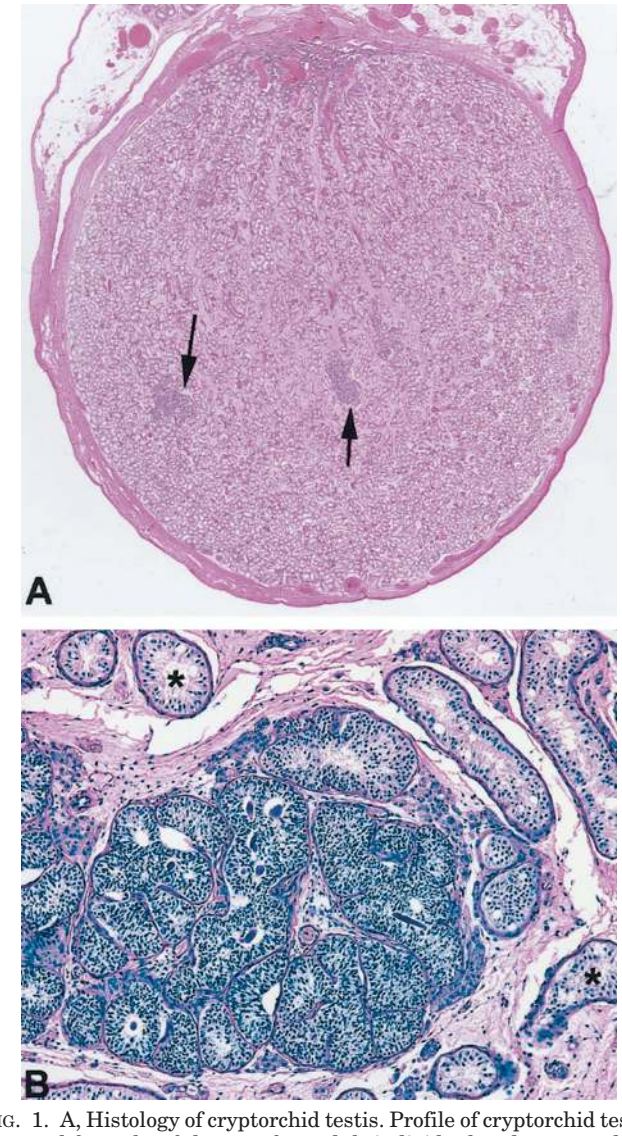

FIG. 1. A, Histology of cryptorchid testis. Profile of cryptorchid testis removed from the abdomen of an adult individual and processed for routine hematoxylin-eosin staining. Although not evident at this magnification, all seminiferous tubules of this testis were of the Sertoli cell-only type. In addition, *arrows* point to foci of hypoplastic seminiferous tubules (composed exclusively of dysgenetic Sertoli cells) that are present throughout the testis. B, Higher magnification view of hypoplastic tubule foci interspersed between SCO tubules (labeled with *asterisks*). Even at this magnification it is possible to distinguish the distinct histology between a SCO tubule without hypoplasia and a SCO with hypoplasia.

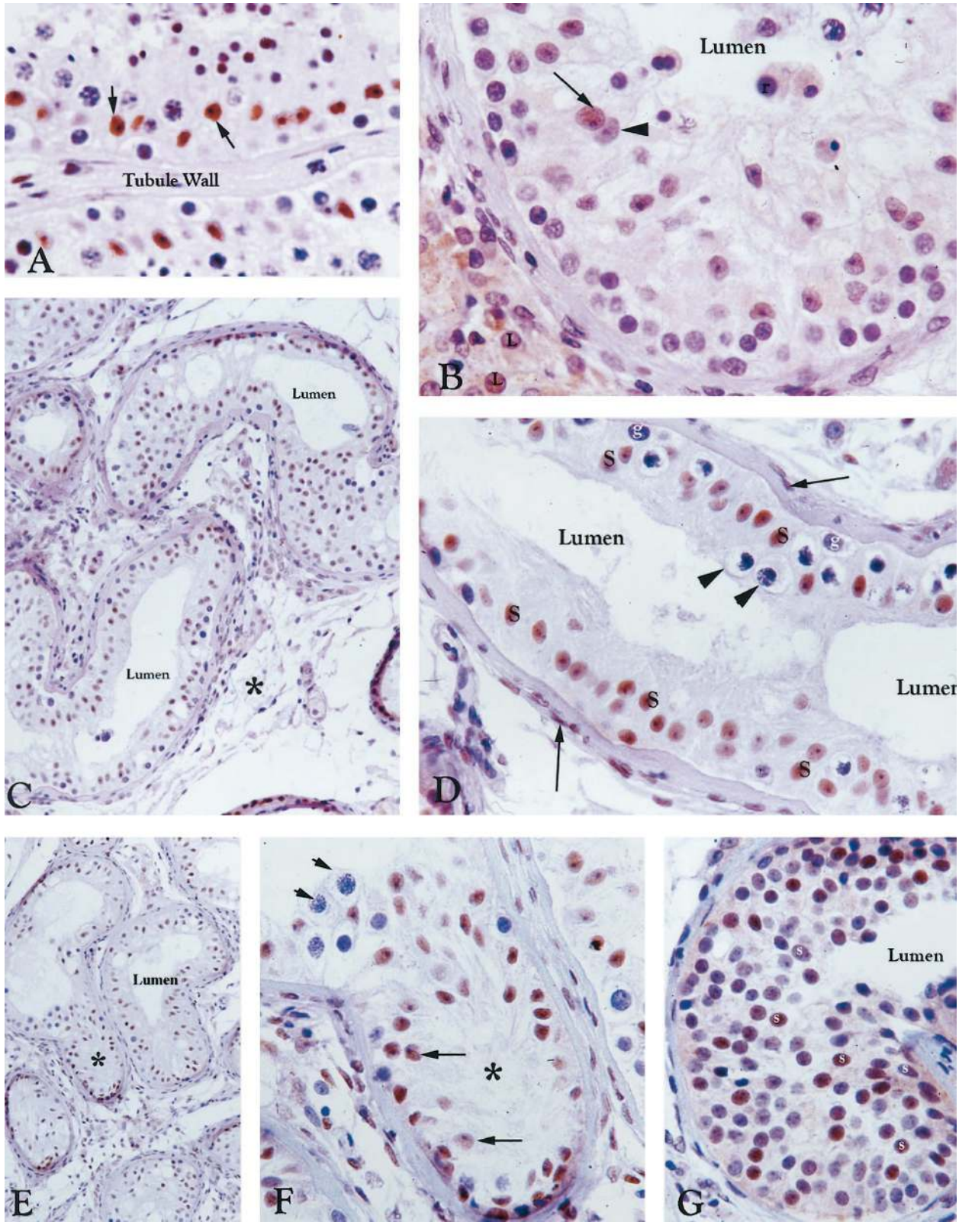

FIG. 2. AR localization in normal and cryptorchid testes. A, AR immunostaining in the nuclei of Sertoli cells (*arrows*) located within the seminiferous epithelium and peritubular myoid cells contained within the tubule wall is illustrated in adult normal testis. B, Differential AR immunostaining intensity in Sertoli cell nuclei of normal adult males is shown. The *arrow* points to intense nuclear staining that is absent from Sertoli cell nuclei exhibiting only the blue color of the hematoxylin counterstain (*arrowhead*). Leydig cells (L) in the interstitium also display differences in AR staining intensity. C, AR immunostaining of cryptorchid testis found within the proximal inguinal canal. All seminiferous tubules have undergone pubertal growth, although the tubule diameters are less than in normal testes. Some tubules exhibit dilated lumens

pretation is based on the fact that when the immunostained tissue sections were observed before counterstaining, all of the peritubular cell nuclei appeared AR positive, albeit some were quite faint (data not shown). In contrast, Leydig cells exhibited only tenuous immunostaining intensity, and an appreciable number appeared to be AR negative (Fig. 2B). Within the same seminiferous tubule cross-section it was possible to detect a marked variation in the AR immunostaining intensity between different Sertoli cell nuclei, whereas some cells exhibited robust immunostaining homogeneously distributed throughout the nucleus, others expressed less intense immunostaining more focally localized, still other nuclei were evidently weakly AR immunopositive, and finally some were completely devoid of any detectable AR-positive staining (Fig. 2B). These results were consistent with our previous studies in the normal adult human testis using a different antibody, PG-21, in which we observed that the intensity of AR staining varied as a function of the cycle of the seminiferous epithelium (19). As in the previous study, the intensity of AR staining of Sertoli cell nuclei appeared to be highest in those stages associated with spermiation, stage 3, although no quantitation was performed with the commercial antibody because of cost limitations. In general, we wish to emphasize that these observations also coincide with prior reports in the rat (11, 13, 16).

AR immunoexpression in Sertoli cells from cryptorchid tubules exhibiting pubertal progression

Seminiferous tubules from cryptorchid testes that have experienced pubertal growth with moderately decreased mean tubular diameter, dilation of the tubule lumen, atrophy of the epithelium, and thickening of the peritubular wall (Fig. 2C). In these tubules it is possible to detect cellular components of the germ cell line, especially spermatogonia and primary spermatocytes, but more developed spermatids are rarely, if ever, observed (Fig. 2C). Epithelial areas containing germ cells are frequently interspersed between tubular areas composed exclusively of Sertoli cells only (SCO) of the adult type. The Sertoli cells are characterized by their ample fibrillar and/or microvacuolated cytoplasm, and with either an overall round or triangular-shaped nucleus, exhibiting an irregular surface and occasional deep fissures (Fig. 2D). In addition, nucleoli are always evident in the Sertoli cells.

The majority of Sertoli cells found in seminiferous tubules from cryptorchid testes that experienced some pubertal growth exhibited moderate, AR immunostaining intensity homogeneously distributed throughout the nucleoplasm, although in a few nuclei the staining was weak or completely absent (Fig. 2D). In the tubule walls from these regions it was possible to observe positive AR immunostaining in the nuclei of the peritubular myoid cells, although this staining often appeared less strong than in the Sertoli cell nuclei (Fig. 2D). In tubules from these testes, expressing evidence of postpubertal growth, tubule segments with germ cell differentiation were interspersed between segments exhibiting a lesser mean tubular diameter and a SCO pattern (Fig. 2, E and F); in all regions of these tubules a highly collagenized tubule wall persisted, often with two or three layers of myoid cells that were AR positive.

In those tubules from cryptorchid testes exhibiting evidence of postpubertal development in addition to the adult-type Sertoli cells, it was also possible to find the immature-type Sertoli cell. These immature type cells were characterized by a round nucleus, similar to that of the prepubertal infantile cell. In addition, although less frequently, it was possible to discern even more immature-type Sertoli cells that exhibited an elongated nucleus, and these corresponded to the dysgenetic Sertoli cell. AR immunostaining in these more immature (dysgenetic) Sertoli cells was highly variable, although a clear trend was detected. The intensity of AR staining was greater in the nuclei of the more mature cells and in those exhibiting a round nucleus and a prominent nucleolus than in the Sertoli cells scored as dysgenetic characterized by an elongated nucleus (Fig. 2G).

The cryptorchid testis in the adult contained abundant numbers of Leydig cells that were grouped together in small nests within the interstitial compartment, although at times the Leydig cells formed large cell aggregates or nodules. These Leydig cells exhibited a polygonal or round contour, and their cytoplasm was either homogeneous or microvacuolated. AR immunostaining of these cells ranged from weak but homogeneously distributed in the nucleoplasm to completely absent (Fig. 2F).

AR immunoexpression in dysgenetic Sertoli cells from hypoplastic tubules

In long-term cryptorchid testes obtained from adults, it was possible to frequently observe various nodules of small diameter, quite hypoplastic seminiferous tubules made up of extremely immature, dysgenetic Sertoli cells (Fig. 3, A and B). These nodules were found well delimited from the pubertal type tubules by connective tissue, were well collagenized

and atrophic epithelium. A moderate amount of interstitial fibrosis is present (*asterisk*). Sertoli cell nuclei in these types of tubules exhibit robust AR immunostaining intensity, as do peritubular myoid cells. D, Longitudinal section of a seminiferous tubule exhibiting focal differentiation of primary spermatocytes (*arrowheads*). However, the majority of the epithelium of this tubule demonstrates the characteristics of the Sertoli cell-only pattern, and all of the Sertoli cell nuclei exhibit positive AR immunostaining. E, Seminiferous tubules from an adult cryptorchid testis. Note the presence of dilated tubule lumens, atrophic epithelium consisting principally of Sertoli cells (*asterisk*), and only limited sites of focal germ cell differentiation. F, Higher magnification view of the area demarcated by the *asterisk* in E. Sertoli cells (*arrows*), having undergone pubertal maturation, and a limited number of spermatocytes (*arrowheads*) are evident in these types of tubules. The hypoplastic tubule segment (*asterisk*) is composed solely of Sertoli cells. The nuclei from these cells express highly variable AR immunostaining intensity, including cells that appear to express no detectable AR immunostaining. In contrast, all peritubular cells from these regions were AR positive. G, AR immunostaining in dysgeneic Sertoli cell nuclei from a cryptorchid testis. In these postpubertal seminiferous tubules a characteristic area of Sertoli cell hyperplasia is evident. An appreciable number of Sertoli cell nuclei express robust AR immunostaining; however, immature-type Sertoli cells, lacking nucleoli, are AR negative. Germ cells are not detected in these precise areas of Sertoli cell hyperplasia. Magnification: A, ×500; B, D, F, and G, ×600; C and E, ×125.

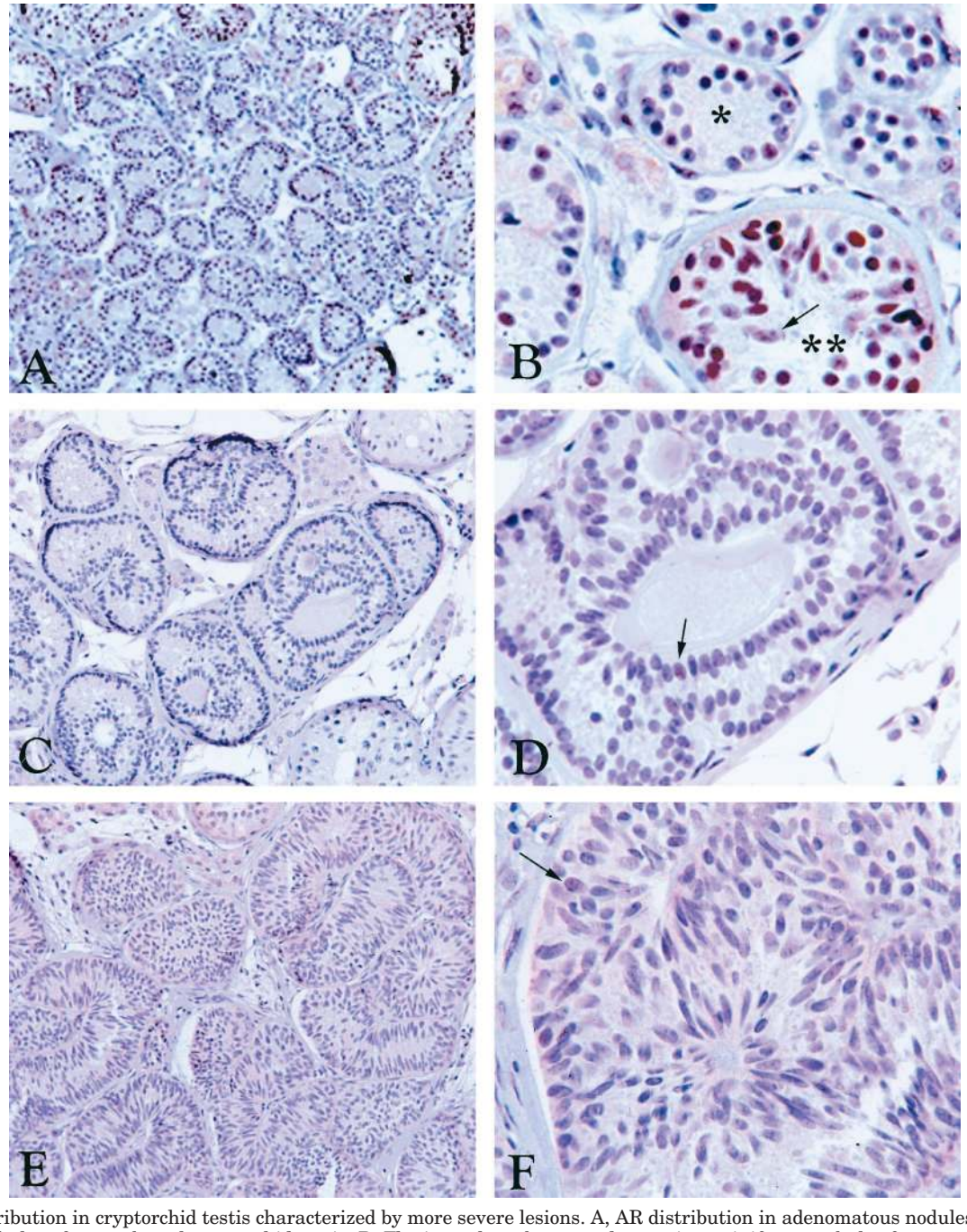

FIG. 3. AR distribution in cryptorchid testis characterized by more severe lesions. A, AR distribution in adenomatous nodules from dysgeneic seminiferous tubules of postpubertal cryptorchid testis. B, The interphase between dysgeneic seminiferous tubules from a tubule not having undergone pubertal differentiation (*single asterisk*) and one having gone through pubertal differentiation (*double asterisks*). The prepubertal tubules are characterized by immature Sertoli cells exhibiting round nuclei that express either limited or no AR immunostaining. In contrast, in the tubules exhibiting postpubertal maturation, Sertoli cell nuclei are AR positive, although occasional fusiform-type nuclei (*arrow*) that are AR negative can be discerned within these tubules. C, Dysgeneic Sertoli cells in adenomas from annulated tubules. These are AR negative for the most part. D, Higher magnification view of a pleated seminiferous tubule composed exclusively of Sertoli cells. The Sertoli cells are arranged concentrically around homogeneous, hyalinized areas. All Sertoli cells are of the prepubertal type, appear quite dysgeneic, and lack AR immunostaining. Occasionally, deposition of chromogen (*arrow*) could be discerned in a few Sertoli cells, but the rarity of this event raises the possibility that it is a staining artifact. E, Sertoli cell adenoma from immature type tubules. These tubules exhibit a highly irregular tubule wall. No interstitial cells were evident in these testes. F, Higher magnification view of the fusiform-type Sertoli cells, exhibiting significant dysgenesis and lacking AR immunostaining. Only the rare Sertoli cell nuclei whose morphology is round (*arrow*) exhibit any evidence of AR staining, albeit an insignificant amount. Magnification: A, C, and E, ×125; B, D, and F, ×600.

and disorganized, and did not present with a true perinodular capsule (Fig. 3, C and D). Each nodule of hypoplastic tubules, regardless of its size, was made up exclusively of the identical type of immature type Sertoli cell. Whereas some nodules contained the infantile-type Sertoli cell with round nuclei (Fig. 3B), other nodules were made up of fusiform-

type Sertoli cells that were quite dysgenetic (Fig. 3, C and D). A few of the round-type Sertoli cells expressed minimal AR staining, but the majority of these cells lacked any form of AR immunostaining. Further, the fusiform-type Sertoli cells were even more dedifferentiated and exhibited very limited cytoplasm, and only rarely (if at all) was AR immunostaining detected in the nuclei (Fig. 3F). In these nodules of hypoplastic tubules the interstitial tissue was minimal; the tubules were practically in contact with one another. In addition, the tubule walls were very fine and were composed of a single, discontinuous layer of myoid cells whose nuclei expressed constant AR immunostaining, although the intensity of the staining was minimal (Fig. 3B).

One variety of nodule of hypoplastic tubules, present in a few cases and only in cryptorchid testes found with the abdominal cavity proper, was that constituted by annular, seminiferous tubules composed of a SCO pattern of the infantile type (Fig. 3, C and D). These tubules were extremely pleated, such that the Sertoli cells were arranged as concentric circles surrounding areas of hyaline material (Fig. 3, C and D). These pseudoadenomas with the annular tubular pattern were exclusively made up of very immature-type Sertoli cells with round nuclei, the majority of which were AR negative except for the exceptional AR-positive nucleus (Fig. 3D). Even in these latter positive cases, however, the AR immunostaining intensity was less than the intensity of staining in nuclei of Sertoli cells in seminiferous tubules that experienced some pubertal growth and surrounded the nodules of annular tubules.

Discussion

In the present study the expression of AR in cryptorchid testes was examined, but only in testes removed from patients who suffered from unilateral cryptorchidism. In addition, testes were selected whose locations were either within the abdominal cavity proper or high up in the inguinal canal, frequent locations when removal of the cryptorchid testes are performed in adult men (1). We specifically excluded those cases of ectopic testes (or of cryptorchid testes found lower down in the inguinal canal) that may best be considered retractile testes whose pathological involvements most likely were either inconsequential or still poorly resolved (21–24).

It is now well established that normal gonadal differentiation and development of secondary male characteristics are processes determined by androgenic stimulation, in which a direct interaction between androgens and tissue AR must occur (25–28). In addition, it is clear that to maintain normal spermatogenesis it is necessary to deliver adequate levels of testosterone to the seminiferous tubules (29, 30) and that testosterone acts directly via the AR (31). Given that the cryptorchid testes examined in these studies were removed from men exhibiting normal plasma hormone levels and that the contralateral testes were normal, it is safe to assume that the pathological alteration exhibited in these cryptorchid testes were manifestations of more local phenomena. Thus, they serve as ideal models to study local paracrine interactions entailing the AR-androgen system, a system vital to the normal maintenance of spermatogenesis (30).

In cryptorchid testes of adult men, the seminiferous tubules with pubertal growth may have partial differentiation of the germ cell line, *e.g.* spermatogonia and primary spermatocytes that are focally localized along the length of the tubule and alternate with other tubule segments that have an exclusive SCO pattern of the adult type. In both types of tubule segments in cryptorchid testes the majority of the Sertoli cells expressed a moderate to intense AR immunostaining, although in those segments that were of the SCO type it was possible to detect Sertoli cells that were AR negative or at best expressed only slight AR immunostaining. Our findings of AR immunostaining in Sertoli cells in cryptorchid testes coincide in part with the only two studies in the literature addressing AR disposition in pathological testes (14, 15). In these two reports AR distribution was examined in biopsy material from subfertile patients presenting with hypospermatogenesis and/or SCO tubules, but without alteration in testicular descent. Interestingly, these researchers did not report any change in AR immunoexpression as a function of the severity or grade of hypospermatogenesis or the grade of differentiation of the Sertoli cell (14, 15). Close examination of the published data, however, revealed that the AR-positive Sertoli cells from these two studies exhibit morphological features of postpubertal maturation, precisely what was observed in the present investigation. That is, even in the most disorganized and seriously affected SCO tubules, if the Sertoli cell nuclei were of the adult type, the majority of these were AR positive.

The mechanisms by which the formation of these postpubertal, SCO tubules occurs in the seminiferous tubules of cryptorchid testes is not known, but it is reasonable to speculate on two plausible alternatives. First, the germinal cells were initially present during the period of prenatal development, but then disappeared during either infancy or puberty as a result of metabolic changes. Second, the germinal cells never differentiated in these segments from the cryptorchid testes. In this regard it is interesting to note the following. First, in approximately 30% of infantile patients the cryptorchid testes are found in the inguinal canal, and in these children it is possible to find spermatogonia in numbers not dissimilar from normal levels (1, 24). Second, in biopsies obtained before and after puberty from the same patient suffering from androgen insensitivity syndrome, it is possible to observe spermatogonia in an appreciable number of tubules during infancy, but these are then conspicuously absent after puberty (32). These findings would suggest that in cryptorchidism the SCO segments of tubules with adult-type Sertoli cells expressing positive AR immunostaining corresponded to zones of germ cell development but experienced progressive germ cell atrophy, culminating in their complete extinction. That a diminution in AR immunostaining was observed in a few Sertoli cells of this region varies slightly from the aforementioned prior studies of AR distribution in pathological testes (14, 15). We suggest, however, that this discrepancy is of limited importance and may be irrelevant, perhaps resulting from methodological differences in the three laboratories, including use of different antibodies, varied staining protocols, or different tissue processing. Furthermore, we had access to archival material that could be repeatedly examined for AR staining, whereas in

the initial two reports the investigators only had access to limited biopsy material (14, 15).

In the nodules of the hypoplastic tubules, the majority of the Sertoli cells were dysgenetic, containing only a limited cytoplasm, and their nuclei were round or fusiform without showing evidence of differentiation. These nuclei were AR negative except for the few exhibiting a vestigial level of AR staining. In contrast, the peritubular myoid cells of these tubules were AR positive. These observations lead us to suggest that a primary lesion exists in the Sertoli cells of these most dysgenetic tubules and that the lack of AR immunostaining is not due to destruction of the epitope during tissue fixation or the lack of epitope unmasking by microwave treatment during the staining process (9, 10). Indeed, we wish to emphasize that the present results of AR localization in excised cryptorchid testes and in normal testes obtained at autopsy do not differ from our previous investigation of AR immunolocalization in biopsied, testes of adult normal that were quickly fixed. Significantly, the results were identical when two different primary antibodies were employed, first the PG21 (19) and now a commercial preparation. Therefore, we suggest that the focal absence of AR staining in the dysgenetic Sertoli cells of the most hypoplastic tubules provides evidence of a specific alteration in the expression of AR in these cells.

In reality, we do not know the mechanism by which lack of AR immunostaining in the dysgenetic Sertoli cells occurs. As stated above, it is possible to speculate that an alteration occurs in the AR gene in the dysgenetic tubules from cryptorchid testes and that the antibody ceases to recognize the AR epitope. That is, in addition to the physical blockage of testicular descent, in tubules from cryptorchid testes there is perhaps the development of a specific lesion in the expression of AR in a certain number of seminiferous tubules. More likely, however, we favor the interpretation that the dysgenetic Sertoli cells of the hypoplastic tubules have undergone a variety of alterations, one of which we now report is lack of normal AR expression. Indeed, in support of this interpretation is the complex set of parameters known to regulate the expression of AR in the normal testis. First, AR immunostaining in the rat correlates with normal levels of androgens (11), and the AR concentration is 4 times greater in Sertoli cells treated with FSH and testosterone or dihydrotestosterone than in control cells (33). Second, Sertoli cells of neonate rodents, up to 1 week before the onset of puberty, are AR negative, and the AR immunoexpression increases gradually during prepubertal development (15–17). Third, AR gene transcript and receptor numbers increase (34) after treatment of Sertoli cells with FSH, and the number of FSH receptors in the rat testis also increases during development and maturation of Sertoli cells (35). Whether this AR maturation is secondary to initial priming with FSH as a prerequisite of fetal maturation (36) as well as subsequent maturation during infancy and puberty of the seminiferous tubules (37) remains to be determined. What is clear, however, is that blockage of any one of these steps may lead to the absence of AR expression in the Sertoli cells of hypoplastic seminiferous tubules of cryptorchid testes.

In the present study using cryptorchid testes removed from adult men, we observed that germ cell development and maintenance of spermatogenesis correlated with the local expression of AR in Sertoli cells. Conversely, the absence of AR immunoexpression in dysgenetic Sertoli cells precisely correlated with hypoplasia of the seminiferous tubules and the absence of germ cell differentiation. Significantly, in these hypoplastic tubules even spermatogonia were rare, if not completely absent. One plausible interpretation of these findings is that congenital focal lesions may be present at the level of the somatic cells in cryptorchid testes. The presence of such lesions in a majority of seminiferous tubules may help explain why surgical intervention to descend the testis into the scrotum, even when performed early in life, fails to restore the normal onset of spermatogenesis and eventual fertility in some cases of unilateral cryptorchid testes (38–40).

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