Androgen receptor expression of proliferating basal and luminal cells in adult murine ventral prostate

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Abstract

Maintenance of the size and differentiated function of the adult prostate is dependent on testicular androgens. In this study, simultaneous androgen receptor (AR) immunohistochemistry and [³H]thymidine labelling was used to characterise the proliferating epithelial cells of the murine ventral prostate. Proliferation in the adult prostate was more prevalent in the basal cell population with 1.8% AR-negative cells labelled with [³H]thymidine as compared with 0.7% AR-expressing luminal cells. Three weeks following castration of mice, the atrophied prostate contained rudimentary glands composed of both luminal and basal cells with the proportion of AR-expressing basal cells reduced from 50 to 25%. Administration of testoster-

one enanthate to castrated mice induced a recapitulation of the prostate gland that was preceded by up-regulation of AR expression in basal cells to normal adult levels (50% AR-positive cells) by 12 h following testosterone injection. Proliferation of AR-positive luminal cells peaked at 48 h (22.8%) while proliferation of AR-negative basal cells peaked at 96 h (6.1%) following testosterone administration. These results suggest that distinct populations of luminal and basal cells are resistant to castration-induced involution of the prostate but remain responsive to direct or indirect testosterone effects and recapitulate the gland following administration of testosterone.

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Introduction

Development of both the human and rodent prostate gland is completed during a pubertal growth phase that occurs in response to increasing production of testosterone by the Leydig cells of the testes (Cunha *et al.* 1987). In order for adult maturation of the prostate gland and development of secondary male characteristics to occur, testosterone must be converted to 5 α -dihydrotestosterone by the enzyme 5 α -reductase (Bruchovsky & Wilson 1968, Wilson 1984), and the androgen receptor (AR), which mediates androgen activity in androgen target tissues such as prostate, must contribute a minimum level of ligand binding and transcriptional activity (Quigley *et al.* 1995).

Throughout adult life the prostate retains its dependence on androgens. Under normal physiological levels of testosterone the adult prostate gland remains at a relatively constant size via a steady but slow turnover of epithelial and stromal cells (Berry & Isaacs 1984, Isaacs 1984). Withdrawal of testicular androgens (e.g. by castration) induces epithelial cell apoptosis and the prostate atrophies to a rudimentary gland. The rapid induction of proliferation and recapitulation of the adult secretory gland that is observed when testosterone is re-administered, and the ability of the prostate to undergo multiple cycles of

involution and recapitulation in response to withdrawal and re-administration of testosterone, have indicated the presence of a single or multiple populations of androgen-responsive progenitor cells within the prostatic epithelium.

The glandular epithelium of the prostate is separated by basal lamina from an inter-glandular stroma that consists of smooth muscle cells and fibroblasts (McNeal *et al.* 1990). In the adult prostate, the glands and ducts are lined by a dual layer of luminal and basal epithelial cells. Basal cells, which can be distinguished morphologically and by their unique profile of cytokeratin expression (Verhagen *et al.* 1992), form close contact with the underlying basement membrane but not the glandular lumen (Macklin & Macklin 1963). The basal cell layer has been proposed to contain the prostatic stem cells that give rise to both basal and luminal epithelial cells of the secretory prostate gland, and to recapitulate the adult differentiated prostate gland following testosterone treatment of castrated mice (Isaacs 1987). Basal cells in the rat and human prostate have been reported to be negative for AR expression (Wernert *et al.* 1988, Masai *et al.* 1990, Mobbs & Lin 1990, Ruizeveld de Winter *et al.* 1990, Zegers *et al.* 1991) although a single study has identified positive AR immunohistochemical staining in a proportion of basal cells in normal and

hyperplastic human prostate specimens (Bonkhoff & Remberger 1993).

The luminal cells of the prostate produce the prostatic secretions including prostate specific antigen in humans and other kallikreins and additional enzymes in rodents and humans (Verhagen *et al.* 1992, Partin & Coffey 1998). Luminal cells express AR and extensive apoptosis of these cells is observed following androgen withdrawal (Isaacs *et al.* 1992). The luminal cells have been reported as the differentiated progeny of basal progenitor cells of the prostate (Isaacs 1987). However, the findings that not all luminal cells die during prostatic involution induced by androgen withdrawal (Sinha *et al.* 1981, Sinha & Bentley 1984, English *et al.* 1987, Evans & Chandler 1987*a*) and that luminal cells proliferate rapidly following re-administration of testosterone (Sinha & Bentley 1984, English *et al.* 1987, Evans & Chandler 1987*a*), have led to an alternative proposal of cellular hierarchies within the prostate. This model ascribes two separate cell lineages within the prostatic epithelium, a basal cell and luminal cell lineage, each with its own subpopulation of stem cells (Evans & Chandler 1987*a,b*).

In this study the androgen responsiveness of the ventral prostatic epithelium was evaluated by determination of AR distribution and expression in proliferating cells of the adult differentiated gland, in the rudimentary prostate in castrated mice and in the recapitulating prostate gland in testosterone-treated castrated mice. AR-expressing luminal cells and AR-negative, but not AR-expressing basal cells were observed to proliferate in the ventral prostate of intact and testosterone-treated castrated mice. Although the basal cell compartment was the most highly proliferative in intact mice, luminal cells exhibited a higher proliferation rate in testosterone-treated castrated mice. These findings have demonstrated both direct and indirect activity of testosterone in the adult prostate and a proliferative potential of both luminal and basal cells.

Materials and Methods

Animals

Nine-week-old male BALB/c mice weighing 25–35 g (Animal Resource Centre, Perth, Western Australia) were used in this study. Mice were housed under regulated lighting conditions (12 h light:12 h darkness). Food and water were freely available. All animal procedures were carried out according to protocols approved by the University of Western Australia Animal Ethics Committee. Castration of mice was carried out as follows. Mice were anaesthetised by a single i.p. injection of Avertin according to Hogan *et al.* (1994). The abdominal fat pads with testes were removed via an abdominal incision and the testicular artery and veins sealed by electro-cautery prior to excision of the testes. Twenty-one days following surgery, testosterone was administered to

castrated mice as an s.c. depot of testosterone enanthate (3.6 µg/g body weight; Schering, Alexandria, Australia) diluted in sesame oil. One hour prior to killing, mice were given a single i.p. injection of [³H]thymidine (specific activity 24 Ci/mmol, Amersham International, Amersham, Bucks, UK) diluted in PBS (pH 7.4) (final dose 1 µCi/g body weight). At the time of killing, mice were anaesthetised as described above, blood was removed by heart puncture, they were killed by cervical dislocation and abdominal organs were immediately removed.

Serum testosterone levels

Blood samples were incubated at 4 °C for 30 min, centrifuged at 5000 *g* for 5 min and serum removed and stored at –80 °C. Serum testosterone levels were quantitated using an ACS II Automated Testosterone Analyser (Chiron Diagnostic, Scoresby, Australia).

Preparation of tissues

Ventral, dorsal and lateral prostates, seminal vesicles, coagulating glands and bladder and a portion of small intestine (to confirm [³H]thymidine incorporation) collected at necropsy were washed at room temperature in PBS (pH 7.4) for 5 min, fixed in 4% paraformaldehyde (w/v in PBS pH 7.4) for 1 h and washed in PBS for 20 min. Specimens were dehydrated through 70, 95 and 100% ethanol, cleared in chloroform and embedded in paraffin (Tissue-Tek VIP tissue processor, Miles Scientific, Elkhart, IN, USA). Five-micron serial sections were mounted on glass microscope slides coated with 3-aminopropyltriethoxysilane (Sigma Chemical Co., St Louis, MO, USA).

AR immunohistochemistry

Sections were dewaxed in xylene, rehydrated through alcohol and washed in distilled water. Antigen retrieval was performed in 5 mM EGTA pH 8.0 (Sigma) by microwaving slides for 13.5 min (9 × 1.5 min). The sections were incubated in 5 mM EGTA buffer for 5 min then washed in distilled H₂O (2 × 5 min). Sections were blocked with 1% H₂O₂ in methanol for 15 min, then blocked in 20% normal horse serum in Tris-buffered saline (TBS) pH 7.4 for 30 min. Sections were incubated overnight at 4 °C in a humidified chamber in rabbit polyclonal AR (N-20) primary antibody (Santa Cruz Biotechnology, CA, USA) diluted 1:100 in TBS containing 1% BSA. Sections were then incubated for 40 min with biotinylated swine anti-rabbit secondary antibody (Dako, Sydney, Australia) diluted 1:200 in TBS containing 1% BSA, followed by streptavidin-peroxidase (Silenus Labs, Melbourne, Australia) diluted 1:200 in TBS for 40 min. Between incubations, sections were washed 3 × 5 min in PBS containing 0.2% Tween-20. All incubations were

carried out at room temperature unless stated otherwise. Visualisation of immunoreactivity was achieved using metal enhanced 3,3'-diaminobenzidine tetrahydrochloride (Pierce, Rockford, IL, USA) for 1·5 min. The sections were washed in warm distilled H₂O (2 × 5 min) then transferred to 70% ethanol overnight before being dipped in autoradiographic emulsion. For negative control sections, TBS containing 1% BSA replaced the primary antibody. No immunoreactivity was observed in control sections.

Autoradiography

Ventral prostate sections and control small intestine sections were coated in Kodak NTB-2 liquid emulsion (Ajax Chemicals, Melbourne, Victoria, Australia) diluted 1:1 with distilled H₂O and placed in light-proof boxes for 10 days at 4 °C. The slides were developed in Kodak D-19 developer (Ajax) diluted 1:1 with distilled H₂O, for 2·5 min, stopped in 1% acetic acid for 40 s, fixed in Hypam fixer containing Hypam hardener (40:1) (Ilford, Mt Waverley, Australia) for 4·5 min, and washed in distilled H₂O for 1 h. All slides were counterstained lightly with haematoxylin, dehydrated through ethanol, cleared in xylene and mounted in Depex (BDH, Kilsyth, Australia).

Western blotting

Three mice per group were used in this experiment. Ventral prostates removed at necropsy were stored in liquid nitrogen. The human prostate cancer cell lines LNCaP and DU145 were used as positive and negative controls respectively. Tissue samples were powdered in a liquid-nitrogen-chilled Mikro Dismembrator-U (B Braun, Melsungen, Germany), dissolved in sample buffer (0·05 M Tris pH 6·8, 10% sucrose, 2% SDS and 4% (v/v) β-mercaptoethanol and 1 µg/ml (each) protease inhibitors (chymostatin, leupeptin, antipain, pepstatin and aprotinin) (Sigma)), heated at 95 °C for 5 min and stored at -20 °C. Samples were electrophoresed in 12% SDS polyacrylamide gels, then transferred onto polyvinylidene difluoride membranes (Amersham). For AR Western blotting, membranes were blocked with 3% skim milk powder in TBS for 90 min, rinsed in TBS and incubated for 90 min in rabbit polyclonal AR (N-20) primary antibody (Santa Cruz Biotechnology) diluted 1:1000 in TBS containing 1% skim milk powder. Membranes were then incubated for 90 min with sheep anti-rabbit horseradish peroxidase-conjugated secondary antibody (Silenus Labs) diluted 1:4000 in TBS containing 1% skim milk powder. Between incubations, sections were washed 3 × 10 min in TBS containing 0·2% Tween-20. All incubations were carried out at room temperature. Visualisation of immunoreactivity was achieved using enhanced chemiluminescence according to the manufacturer's instructions

(Amersham). A densitometer (Molecular Dynamics, Sunnyvale, CA, USA) was used to scan the developed Western blots. The results were standardised for protein loading against Coomassie Blue-stained gels using Image Quant Software (Molecular Dynamics). Specificity of the AR primary antibody was confirmed by abolition of immunoreactivity of AR antibody that had been pre-incubated with the peptide used to generate it (Santa Cruz Biotechnology).

Data collection and analysis

Epithelial cells counts were performed on sections selected by systematic random sampling from the entire ventral prostate of each mouse. These representative sections were counted, moving from top left to bottom right, at × 1000 magnification (oil immersion) using an Olympus BX-40 light microscope. At least 2000 epithelial cells from each animal were counted encompassing the entire cross-section of ducts and glands within ventral prostate sections. Basal and luminal cells were identified histomorphologically using criteria described previously for rodent prostate (English *et al.* 1987). Immunostaining of ventral prostate sections for high molecular weight cytokeratin (Dako-34βE12), which labels basal cells (O'Malley *et al.* 1990), indicated >95% concordance in identification of basal cells by histological and high molecular weight-immunohistochemical criteria. Basal and luminal cells were scored according to both their AR expression (positive or negative) and thymidine labelling. Autoradiographic background was low in all slides (<four grains per field) and cells with five or more silver grains over the nucleus were scored as thymidine labelled. All data are expressed as the mean ± s.e.m. of three or four animals. Group differences were statistically analysed using a Welch modified two-sample *t*-test (to account for unequal variances) and were considered significant at *P* < 0·05.

Results

Serum testosterone levels

In intact adult mice, mean (± s.e.m.) serum testosterone levels were 8·8 ± 1·6 nmol/l (Fig. 1). Three weeks following castration, the concentration of testosterone was significantly reduced to 3·6 ± 0·1 nmol/l (*P* < 0·05). Administration of testosterone to castrated mice increased serum testosterone levels to a peak of 48·6 ± 4·9 nmol/l at 12 h (Fig. 1). Serum testosterone levels decreased steadily after 12 h and were 9·2 ± 1·9 nmol/l at 144 h.

Prostatic histological analysis

Haematoxylin and eosin staining of paraffin-embedded prostate tissue sections indicated that the ventral prostates

Figure 1 Serum testosterone levels in intact mice ($n=4$), 3-week castrated mice ($n=6$) and castrated mice following testosterone administration ($n=4$ per timepoint). Results are expressed as means $\pm$ S.E.M. * = Significantly different from intact animals, $P < 0.05$; + = significantly different from 3-week castrated mice, $P < 0.05$.

of intact adult mice consisted of ducts and highly infolded glands separated by stroma (Fig. 2A). Stromal cells were usually spindle shaped and loosely organised around glands and ducts. Glands and ducts were surrounded by basal lamina, which appeared as an eosinophilic membrane separating the epithelial cells from the stroma (Fig. 2A inset). In the ventral prostate, glands contained a single layer of tall columnar luminal cells while ducts were lined with cuboidal luminal cells. Underlying triangular-shaped basal cells which contained very small amounts of cytoplasm were observed adjacent and parallel to the basal lamina (Fig. 2A inset).

Three weeks following castration, ventral prostates consisted of rudimentary glandular structures containing both luminal and basal cells (Fig. 2B). Luminal cells were cuboidal in shape with less cytoplasm than that evident in intact animals, while the basal cells appeared morphologically similar under light microscopy to prostatic basal cells of intact mice (Fig. 2B inset). In comparison with the normal ventral prostates of intact mice, a greater density of stromal cells was evident between rudimentary ventral prostatic glands of castrated animals (Fig. 2B).

Thirty-six hours following administration of testosterone to castrated mice, the cuboidal luminal cells had become tall and regained a columnar epithelial appearance (results not shown). By 48 h the basal lamina had thinned and appeared similar to that in the ventral prostates of intact mice. At this time, glands began to form epithelial infolds as in intact mice. By 96 h after testosterone

administration, the stroma appeared less dense and more closely resembled intact mouse prostate (results not shown).

AR immunohistochemical analysis

In intact mice, AR staining was localised exclusively in the nucleus of epithelial and stromal cells (Fig. 2C). Within the ventral prostate, no differences in staining patterns were observed between luminal cells of glands and the ducts. All luminal cells stained positively for AR, while $49.2 \pm 0.6\%$ of the basal cells were also positive for AR in both glands and ducts (Fig. 3). Generally, luminal cells showed more intense staining for AR compared with basal cells. AR staining appeared heterogeneous between cells within the same gland or duct with the staining intensities in luminal cells ranging from very strong to moderate and basal cells containing moderate to very weak staining. Nuclear AR staining was also observed in a proportion of stromal cells (results not shown). However, these cells stained more weakly for AR, suggesting that epithelial cells expressed higher levels of AR than stromal cells.

Three weeks following castration, the rudimentary glandular structures contained luminal cells that were all strongly AR positive (Fig. 2D). However, the proportion of AR-positive basal cells had reduced significantly to only $24.5 \pm 0.9\%$ ($P < 0.001$) (Fig. 3).

In ventral prostates of castrated mice treated with testosterone, AR expression in luminal cells remained at 100%. Administration of testosterone to castrated mice resulted in an increase in the number of AR-positive basal cells. The proportion of basal cells staining positively for AR reached normal levels, that is approximately 50% AR-positive staining at 12 h (Fig. 3).

Cell counts

In the ventral prostates of intact mice, the ratio of luminal to basal cells in glands and ducts was approximately 13:1 (Fig. 4). Low levels of proliferation were seen in this group with approximately $0.80 \pm 0.03\%$ of epithelial cells in the ventral prostates labelled with [^3H]thymidine (Fig. 4). Proliferation was only observed in AR-negative basal or AR-positive luminal cells (Fig. 2C). In intact mice, $1.8 \pm 0.2\%$ of basal cells were proliferating. This was approximately two and a half times greater than that observed in the luminal cell layer where $0.70 \pm 0.02\%$ of cells were proliferating (Fig. 5).

Three weeks following castration, the ratio of luminal to basal cells in ventral prostates had been reduced to approximately 3:1 (Fig. 4). Castration also resulted in decreased thymidine labelling such that very low levels of proliferation were seen in epithelial cells of these mice $0.34 \pm 0.03\%$ (Fig. 4). Only AR-positive luminal cells were labelled (results not shown).

Figure 2 Light microscopy (haematoxylin and eosin staining) of ventral prostates in (A) intact adult mice showing glandular structures (G) surrounded by stroma (S). Bar=54 μ m. Inset: the glandular epithelium consisted of tall columnar luminal cells (arrows) and triangular flattened basal cells (arrowhead) lying parallel to the eosinophilic basal lamina (Δ). Bar=20 μ m. (B) Ventral prostates from castrated mice were composed of rudimentary glandular structures (G) and dense stroma (S). Bar=54 μ m. Inset: luminal cells (arrows) were more cuboidal in appearance. The light microscopic appearance of basal cells (arrowhead) remained similar to that in intact mice. Bar=20 μ m. (C) In ventral prostate from an intact mouse, nuclear AR staining was observed in all luminal cells and a proportion of basal and stromal cells. [3 H]Thymidine labelling (silver grains) was observed in AR-negative basal cells (arrowhead) and AR-positive luminal cells (arrow). Bar=13 μ m. (D) Three weeks following castration, luminal epithelial cells remained strongly positively stained for AR. Bar=33 μ m. (E) Forty-eight hours following administration of testosterone numerous AR-positive luminal (arrows) and AR-negative basal (arrowhead) epithelial cells were labelled with [3 H]thymidine. Bar=13 μ m.

Figure 3 AR expression in basal and luminal cells of the mouse ventral prostate. The proportions of basal and luminal cells expressing AR in ventral prostates of intact mice ($n=3$), 3-week castrated mice ($n=3$) and testosterone-treated castrated mice ($n=4$ per timepoint) were calculated as a percentage of total basal or luminal cells respectively. Results are expressed as percentage mean $\pm$ S.E.M. * = Significantly different from intact animals, $P < 0.05$; + = significantly different from 3-week castrated mice, $P < 0.05$.

Following treatment of castrated mice with testosterone, the ratio of luminal to basal cells increased to 13:1 by 144 h (Fig. 4). Very few ($0.32 \pm 0.01\%$) labelled cells

Figure 4 Epithelial cell proliferation (bars), as determined by [3 H]thymidine autoradiography, and expressed as a percentage of total cells in ventral prostates of intact mice ($n=3$), 3-week castrated mice ($n=3$) and castrated mice following administration of testosterone ($n=4$ per timepoint). Results are expressed as means $\pm$ S.E.M. * = Significantly different from intact animals, $P < 0.05$; + = significantly different from 3-week castrated mice, $P < 0.05$. The luminal to basal cell ratio (●) decreased significantly ($P=0$) in castrated mice and increased to levels similar to intact mice by 144 h following testosterone treatment.

Figure 5 Proliferation of basal and luminal cells of the mouse ventral prostate in intact mice ($n=3$), 3-week castrated mice ($n=3$) and castrated mice following administration of testosterone ($n=4$ per timepoint). AR-negative basal cells and AR-positive luminal cells were the only two cell types that were observed to be labelled with [3 H]thymidine. Results are expressed as means $\pm$ S.E.M.

were detected 12 h after administration of testosterone. Epithelial proliferation began to increase in mice 24 h following testosterone treatment ($1.10 \pm 0.15\%$) and continued increasing to $21.0 \pm 1.7\%$ at 48 h. Thereafter, labelling of epithelial cells declined to $0.37 \pm 0.16\%$ at 144 h. Similar to intact mice, thymidine labelling was only observed in the AR-negative basal and AR-positive luminal cells (Fig. 2E). In mice treated with testosterone, luminal cell proliferation commenced 24 h following administration of testosterone (Fig. 5). Proliferation of luminal cells peaked at $22.8 \pm 1.8\%$ at 48 h after testosterone treatment. By 144 h after testosterone administration, proliferation of luminal cells had reduced to $0.30 \pm 0.14\%$. A similar pattern of proliferation was observed in basal cells. Basal cell proliferation commenced 36 h after administration of testosterone and peaked at $6.1 \pm 0.9\%$ at 96 h after testosterone treatment. Subsequently, basal cell proliferation had decreased to $1.4 \pm 0.8\%$ 144 h after administration of testosterone.

Western analysis

A 110 kDa band corresponding to the AR protein was evident in all ventral prostate tissue samples and in the LNCaP cells. No AR protein bands were detected in extracts of DU145 cells or on membranes incubated with peptide-absorbed AR antibody (results not shown). The linear range of AR detection by Western blotting was determined using increasing amounts of LNCaP cell extracts (Fig. 6). In prostate tissue samples, densitometric

Figure 6 Quantitation of AR protein levels by Western blotting. (A) Western blot analysis for AR protein in total protein extracted from LNCaP cells. Lanes: 1, 0 µl; 2, 1 µl; 3, 2.5 µl; 4, 7.5 µl; 5, 10 µl; 6, 12.5 µl LNCaP cell extract. (B) Quantitation of AR protein relative to total protein, as determined by comparative densitometric analysis of AR Western blot and Coomassie Blue-stained polyacrylamide gels respectively.

analysis indicated a 3.5-fold reduction of AR protein levels in ventral prostates from 3-week castrated mice compared with intact mice. Twelve hours following administration of testosterone to castrated mice, ventral prostate AR protein levels increased approximately 17-fold compared with castrated mice (Fig. 7). AR protein levels decreased after 12 h and by 96 h were similar to levels observed in intact mice.

Discussion

The present studies have demonstrated that castration of male adult mice leading to atrophy of the prostate results in decreased AR protein expression in the whole gland, a reduction in the proportion of basal cells expressing AR and no alteration in the proportion of surviving luminal epithelial cells that express AR protein. Following administration of testosterone to the castrated mice, the androgen responsiveness of the prostatic epithelium is characterised by the up-regulation of AR expression in prostatic basal cells and the rapid induction of proliferation of AR-expressing luminal cells and AR-negative basal cells. These findings indicate that populations of cells with progenitor-like potential may reside in both the luminal

Figure 7 AR protein levels as determined by Western blot analysis. (A) Western blot of AR protein levels in ventral prostates of intact mice, 3-week castrated mice and castrated mice following administration of testosterone. (B) Densitometric quantitation of relative AR protein levels. Results are expressed as means $\pm$ S.E.M. of three animals per group.

and basal cell compartments of the prostatic glands and ducts. While androgen-responsive for proliferation and differentiated (secretory) functions, these cells appear to be androgen-independent for survival.

Involution of the prostate gland following castration is associated with increased programmed cell death of luminal cells resulting in an approximately 80% loss of prostatic epithelial cells (Coffey *et al.* 1968, Isaacs *et al.* 1992). The glandular atrophy is incomplete, however, and histological findings in this and previous studies indicate that rudimentary glandular structures remain even after prolonged periods of castration (Sinha *et al.* 1981, Evans & Chandler 1987a). These glandular rudiments consist of both luminal and basal cells, with a decreased relative proportion of luminal:basal cells (3:1 as compared with 13:1 in the intact animal). Although castration results in a significant reduction of serum testosterone levels, a low but detectable level of serum testosterone remains. It is feasible therefore that the rudimentary glandular structures are maintained by residual testosterone levels or that all or a proportion of these cells are androgen-independent for survival.

The direct actions of androgens in target cells are mediated by the AR (Lubahn *et al.* 1988). In the prostate, androgens appear to act as an apoptosis inhibitor since androgen withdrawal (i.e. castration) results in rapid induction of apoptosis that leads to atrophy of the gland (Kyprianou & Isaacs 1988, Isaacs *et al.* 1992). In the present and in previous studies (Wernert *et al.* 1988, Masai *et al.* 1990, Mobbs & Lin 1990, Ruizeveld de Winter *et al.* 1990, Prins *et al.* 1991, Zegers *et al.* 1991, Bonkhoff & Remberger 1993), prominent AR expression was evident in the luminal cells of the prostatic glands and it is these cells that undergo apoptosis following castration of rodents and other species (Price & Williams-Ashman 1961, Brandes 1966, Lesser *et al.* 1973, Sinha *et al.* 1981, Isaacs 1984, English *et al.* 1987, Kyprianou & Isaacs 1988, Isaacs *et al.* 1992). Although at 3 weeks following castration of mice, histological evidence of cell death (apoptosis) or cell proliferation was virtually absent in the prostate, almost all of the surviving luminal cells expressed AR at similar immunohistochemical levels to prostatic luminal cells of intact mice. The mechanisms responsible for the apparent apoptosis resistance of these residual AR-expressing luminal cells is currently unknown. Whether these cells represent a single population or contain progenitor cells of the differentiated prostatic secretory cells of the recapitulated adult gland is also unresolved.

In contrast to the luminal cells, immunohistochemical methods detected low levels of AR in 50% of prostatic basal cells in the intact animal. Previous studies of rat and canine prostate have identified basal cells as AR negative (Prins *et al.* 1991), findings that suggest species-specific differences in AR distribution in cell types of the prostate or increased sensitivity of AR detection resulting from utilisation of different AR antibodies. Although several previous studies using human prostate tissues have reported AR expression exclusively in the prostatic luminal cells (Wernert *et al.* 1988, Masai *et al.* 1990, Mobbs & Lin 1990, Ruizeveld de Winter *et al.* 1990, Zegers *et al.* 1991), AR expression in a proportion of basal cells in normal and hyperplastic human prostate has recently been described in a single report (Bonkhoff & Remberger 1993). The relationship between AR-expressing and AR-negative basal cells and the functional role of the basal cell population in the adult prostate gland is at present unclear.

In the adult prostate, the basal cell population has been suggested to contain stem cells of the luminal epithelium, to function as a regulator of secretory cell numbers (Isaacs 1987, Bonkhoff & Remberger 1996) or as a cellular mediator of stromal cell modulation of the luminal cell population (Cunha *et al.* 1987). Studies in both rat and human prostate have demonstrated that the basal cells do not have a myoepithelial or contractile role in the prostate (Srigley *et al.* 1990, Prins *et al.* 1991). Basal cells are not as responsive as luminal cells to androgen withdrawal and their numbers are not believed to alter dramatically

following castration (English *et al.* 1987). In this study, the proportion of AR-expressing basal cells was found to be reduced 3 weeks following castration and to increase to normal levels by 12 h following testosterone administration. Since this up-regulation of AR expression occurred prior to the testosterone-induced initiation of basal cell proliferation, a common lineage of at least a proportion of the AR-expressing and AR-non-expressing basal cells is suggested. AR-expressing basal cells labelled with [³H]thymidine were never observed in intact, castrated or testosterone-treated mice, suggesting that proliferation of these cells does not contribute directly to maintenance or testosterone-induced recapitulation of the gland. Preservation of a consistent proportion of AR-expressing cells in intact mice and the rapid restoration of this ratio following testosterone administration to castrated mice indicate an important role for these cells, potentially in directing the secretory function of the adjacent luminal cells of the gland or as precursors of this luminal cell population.

In addition to maintenance of prostatic glandular size, testicular androgens are required for maintenance of the differentiated function of the prostate gland. In the rodent, these include androgen-induced expression of prostatic enzymes such as the glandular kallikreins and production of prostatic secretions (Cunha *et al.* 1987, Clements 1994, Partin & Coffey 1998). Histological features of the prostate in castrated mice, including the more flattened appearance of luminal epithelial cells and the increased density of the periglandular stroma, are similar to those observed in pre-pubescent male mice (Sugimura *et al.* 1986). These findings, and the previously described absence of expression of prostatic secretory proteins in castrated mice, suggest a loss of differentiation in cells of the prostate following androgen withdrawal. The thickened basement membrane surrounding glands and ducts of castrated mice has been attributed to infolding of the basal lamina around glands that have rapidly decreased in size following castration (Decarvalho & Line 1996). It is feasible that the basement membrane would equilibrate to a more normal thickness following longer time periods post-castration.

Re-administration of testosterone to castrated mice results in recapitulation of the prostate to its original size and differentiated function. Following injection of testosterone, the histology of the prostate alters and the luminal cells become tall and columnar as in intact mice, and stromal cell density and the thickness of the basement membrane are reduced as the rudimentary glands enlarge. These histological alterations are correlated with the up-regulation of expression of prostatic secretory proteins (Cunha *et al.* 1987, Clements 1994, Partin & Coffey 1998) and are similar to the growth and initiation of adult secretory function of the prostate that is observed during pubertal development. Androgen-induced resurrection of the differentiated appearance and function of the prostate in castrated mice occurs concurrently with the

up-regulation of prostatic AR and widespread induction of cellular proliferation.

Proliferation of cells of the prostatic epithelium in adult mice was only observed in AR-negative basal cells and AR-expressing luminal cells. These findings suggest direct and indirect effects of androgens on prostatic epithelial cells both during steady-state maintenance of the gland and mediating testosterone-induced recapitulation of the prostate in castrated mice. A low but reproducible level of proliferation was evident in the basal and luminal compartments of intact mice, findings that were consistent with previous reports (Sinha *et al.* 1981, Sinha & Bentley 1984). Of interest was the observation that basal cell proliferation was more than double luminal cell proliferation in intact mice. Such findings indicate that a subpopulation of basal cells exhibits a high turnover rate as compared with luminal cells. Alternatively, if as suggested by Isaacs (1987) the basal and luminal cell populations are representative of a single cell lineage with the progenitor cells residing in the basal cell population, a proportion of the intermediate or amplifying cells may be located in the basal cell layer in the adult steady-state gland.

The steady-state proliferation rate in the prostatic epithelium was reduced to virtually undetectable levels at 3 weeks following castration. Although following testosterone administration serum testosterone levels had peaked by 12 h, peak proliferation of prostatic epithelial cells was detected at 48 h, the lag time consistent with previous reports (Lesser & Bruchovsky 1973) and potentially due to induction of expression of accessory proteins required for S-phase progression. Synthesis of RNA (Butler & Schade 1958, Liao *et al.* 1965, Coffey *et al.* 1968, Katz *et al.* 1989) and protein (Coffey *et al.* 1968, Chung & Coffey 1971*a,b*), but not DNA, has been demonstrated during this lag phase. While proliferation of luminal cells peaked at 48 h post testosterone administration, basal cell proliferation peaked at 96 h after testosterone injection. The longer lag time prior to S-phase progression in basal cells may be due to androgen effects being indirect in this AR-negative cell type and requiring protein synthesis in other cells (luminal and stromal cells) to initiate cell cycle progression.

This study has demonstrated the existence of androgen-regulated homeostasis within the adult prostate gland controlling total AR content of the gland, relative proliferation rates of basal and luminal epithelial cells, the relative proportions of AR-expressing and AR-negative basal cells and the basal:luminal cell ratio. These effects are the result of both direct and indirect androgen activity. Populations of AR-positive luminal and AR-positive and AR-negative basal cells are resistant to the androgen depletion caused by castration of mice and are activated in a coordinated manner to recapitulate the gland following androgen administration. Further evaluation of the functional activity of epithelial cell types described in this study will identify cell lineages within the adult prostate gland, their role in differentiated secretory prostatic function and

potentially the cell of origin and associated biological processes that lead to dysplasia and formation of prostate cancer.

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