A functional model of adult human prostate epithelium

The role of androgens and stroma in architectural organisation and the maintenance of differentiated secretory function

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Summary

A functional model of adult human prostate epithelium is described. This model shows that stromal cells, but not an androgenic stimuli, are required for architectural organisation of prostate epithelium. Within an organised structure, androgenic stimulation is required for the establishment of secretory epithelial cell morphology and associated function. In the absence of stromal cells but in the presence of androgens architectural organisation and secretory function are lost. Epithelial parenchymal units (organoids) from human prostate tissue were isolated, cultured within a three-dimensional collagen matrix, and xenografted subcutaneously into athymic mouse hosts. The grafted gels were rapidly invaded by host fibroblasts. Epithelial organisation initially disappeared but was re-established concurrently with the stromal cell invasion. In intact male hosts, cuboidal and columnar cells that expressed human prostate-specific secretory markers were found. In castrated male and in female hosts epithelial structures were lined with flattened epithelium with no secretory function. This phenomenon could be reversibly replicated by treating intact male hosts with the anti-androgen Flutamide. Gels containing organoids grafted within 0.45 μm Millipore chambers were not invaded by stromal cells and rapidly lost all epithelial organisation and secretory function. When organoids cocultured with human foreskin fibroblasts were grafted within chambers, structural organisation of the epithelium was supported. These results indicate that both heterologous human fibroblasts and mouse stromal cells are capable of permissively supporting adult human prostate epithelial function.

Key words: epithelium, stroma, prostate, human, differentiated.

Introduction

One of the main problems in the investigation of prostatic disorders is the lack of easily established and manipulated models of the human prostate. Ideally these should be able to exhibit the normal secretory function of the gland, and respond to changes in hormonal status. A model should also allow for interactions between epithelial and stromal elements because of their great importance in the definition and regulation of tissue architecture and function (Haffen et al., 1987). The principal problem with in vivo models, based on laboratory animals, is that their prostatic structure and function are not exact replicas of the human situation. This is especially true with respect to the patterns of disease found in other mammalian prostates. Thus, results obtained with animal model systems are not necessarily applicable to human prostatic diseases such as benign prostatic hyperplasia (BPH) and carcinoma of the prostate (Coffey and Isaacs, 1980).

In vitro models have tended to rely on epithelial monolayer cell culture systems, either on tissue culture plastic or on a protein matrix. This form of culture seems to encourage the appearance of cells with a morphology that is distinctly different from that found in the tissue of origin. Care is therefore required in the application of these techniques to the study of higher cellular functions, such as cell-cell interactions and organ-specific secretions by defined epithelial cell subclasses (Sandberg and Kadomah, 1980; White et al., 1990). Recently there has been a move towards cell culture within biologically recognisable matrices and on released collagen gel matrices, and systems are now
available for the culture of functional mammary epithelium (Burwen and Pitelka, 1980; Hahm et al., 1990). Such a satisfactory in vitro system is, however, unavailable for human prostatic epithelium.

The prostate is completely dependent for the maintenance of its structural and functional integrity on testicular androgens. Castration of an adult male, in the absence of an external androgenic stimulus, gives rise to a rapid involution and regression of the prostate, involving specifically a loss of the epithelial component (de Voogt et al., 1988). It has been demonstrated in the mouse that this regression is not a uniform phenomenon (Cunha et al., 1985). Cells at the distal portion of the ductal-acinar network are lost before those in the more proximal portion of the gland. At a cellular level, investigations of the rat prostate have shown that luminal cells are lost before the basal cells (Verhagen et al., 1988). This implies that the tall columnar secretory epithelium is more dependant than the basal epithelium on the maintenance of an androgenic stimulus.

The ability of mesenchyme and stroma to affect the structure and function of epithelium is now well established. Two classes of epithelial-mesenchymal and epithelial-stromal interaction have been recognised. These are permissive inductive effects and instructive inductive effects (Haffen et al., 1987). A permissive effect triggers a previously determined developmental programme already specified by the epithelium, whilst an instructive effect elicits a new programme specified by the mesenchyme. The instructive role of urogenital sinus mesenchyme during the development of the prostate has been extensively studied and described (Cunha, 1985). However, the instructive or permissive role of the stroma in the maintenance of function of adult prostatic epithelium has not been thoroughly examined. The work described here addresses this problem. BPH-epithelium has not been thoroughly examined. The stroma in the maintenance of function of adult prostatic epithelium was considered to be a suitable system could be used to elucidate the nature of the epithelial-stromal interaction have been recognised.

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The instructive role of urogenital sinus mesenchyme during the development of the prostate has been extensively studied and described (Cunha, 1985). However, the instructive or permissive role of the stroma in the maintenance of function of adult prostatic epithelium has not been thoroughly examined. The work described here addresses this problem. BPH-derived epithelium was considered to be a suitable source material with which to establish this model, as this tissue is slow growing and maintains appropriate expression of the secretory epithelial markers, prostate-specific acid phosphatase (PSAP) and prostate-specific antigen (PSA). The aim of the work reported here was to establish a functional model of adult human prostatic epithelium in an in vivo environment. Such a model system could be used to elucidate the nature of the epithelial-stromal interactions involved in the permissive support of adult prostate function.

Materials and methods

Tissue collection

Human prostate tissue was obtained from 12 patients, age range 62 to 78, undergoing transurethral resection of the prostate for BPH, causing urinary blockage. The benign nature of the excised tissue was established by histological examination of representative fragments. Approximately 20-30 g of tissue was collected from each resection, transferred to a wide-mouthed bottle containing 80 ml of RPMI-1640 (without phenol red; Gibco, Paisley, Scotland) with 2.5% foetal calf serum (Gibco) and 100 μg/ml gentamycin (Sigma, Poole, Dorset) and transported under aseptic conditions to the laboratory. Patients who had undergone any hormonal therapy within the last six months or who were suffering from any malignant disease were excluded.

Tissue processing

The tissue was processed as previously described (Hayward et al., 1987; Deshpande et al., 1989). Briefly, it was extensively washed in calcium- and magnesium-free phosphate-buffered saline containing 100 μg/ml gentamycin, to remove any blood clots and debris. It was then cut into small pieces (1 mm × 1 mm × 3 mm) using a scalpel and digested in RPMI-1640 (without phenol red) containing 10% foetal calf serum with collagenase type I (Sigma, 225 units/ml) and hyaluronidase (Sigma, 125 units/ml) at 37°C in a conical flask on a magnetic stirrer for 16-18 hours. When the digestion was nearly complete the released epithelial structures were pelleted and thoroughly washed with RPMI-1640 to remove the proteolytic enzymes. They were then filtered using 1000 μm and 600 μm filters to remove undigested tissue fragments.

Epithelial organoids (acini and ducts) were separated from the stromal fraction of the digest by repeated unit gravity sedimentation and washing until as much stromal contamination as possible had been removed. The organoids were then counted and samples containing approximately 1200 were gently pelleted.

Type I collagen

Type I collagen was prepared from rat-tail tendons as previously described (Hallowes et al., 1980). Briefly, tails from mature rats were soaked in 70% ethanol, and then skinned. The proximal and distal portions of the tails were discarded and the four main tendons removed from the remaining part with a scalpel. The excised tendons were weighed and stirred gently at 4°C for seven days in a 1/500 solution of glacial acetic acid in water (100 ml acid per gram of tendon). The mixture was then centrifuged at both low and high speed to remove undissolved fragments and fungal spores. Collagen was stored at 4°C in acid solution and the pH was neutralised with 1 M sodium hydroxide immediately before use.

Human foreskin fibroblasts (HFF)

HFF cells were obtained from the ICRF Cell Production Unit. They were maintained in 775 tissue culture flasks in RPMI-1640 containing 2.5% foetal calf serum until use. Cells were released from the flasks with trypsin, washed with growth medium containing 5% FCS and pelleted. They were resuspended in 2 ml of cold neutralised type I collagen solution and mixed to ensure even distribution. Organoids were then added to the mixture as described below.

In vitro culture

Pellets containing 1200 organoids were suspended in 2 ml of neutralised type I collagen solution (or collagen solution containing suspended HFF cells, as described above) and plated onto 30 mm Petri dishes. The gels were allowed to set for 30 min at 37°C. When a solid gel had formed, 5 ml of phenol red-free RPMI-1640 containing 2.5% foetal calf serum and 3×10^-10 M testosterone (Steraloids, Sutton, Surrey) was added to each Petri dish. Dishes were incubated for three days at 37°C in a humidified atmosphere of 3% CO₂ in air. Medium was changed after 24 hours of incubation.

Xenografts

Epithelial organoids in a three-dimensional type I collagen gel were xenografted according to the technique of Del Buono et
Anti-androgen treatment were sealed into 0.45 μm Millipore chambers (Millipore, Watford, Herts.) prior to grafting. Cocultured organoids and HFF cells were sealed into chambers before grafting.

Animals were killed at days 7, 10, 14, 21, 28, 35, 42 and 56. The grafts were excised, fixed in neutral buffered formalin and processed to paraffin for histological examination.

Staining of graft sections

Sections were cut at 4 μm, dewaxed and stained with Mayer’s haematoxylin and cosin (H and E). Sections were also stained, using the avidin-biotin complex reaction (Dako, High Wycombe, Bucks.). Rabbit antisera raised against PSA or PSAP (Dako), monoclonal antibodies against desmin (Dako), vimentin (Dako) and smooth muscle α-actin (Sigma), the anti-cytokeratin CAM 5.2 (keratins 7 and 8) and 34βE12 (keratins 1, 5, 10 and 11-Enzo Diagnostics), and the anti-PCNA (proliferating cell nuclear antigen) antibody PC10 (Hall et al., 1990; gift from Dr D. Lane) were used as the first stages for this reaction. Sections of formalin-fixed prostate tissue were used as positive controls in all cases. Sections without a primary antibody were used as negative controls.

The degree of PSAP expression was determined by examination of all duct-like structures in graft sections. Expression was graded as: 0 (no staining), + (<50% of luminal cells in any structure stained), ++ (50-90% of luminal cells stained), +++ (equivalent to concurrently stained sections of prostate tissue, used as a positive control).

Sections were stained with the Hoechst 33258 dye according to the method of Chen (1977). Briefly, freshly cut sections were dewaxed and hydrated to isotonic phosphate-buffered saline (PBS). Incubated for one minute at room temperature in a 4 μg/ml solution of Hoechst 33258 stain in PBS. Washed gently for three minutes in running tap water, mounted in an aqueous mount and sealed. They were viewed using a Leitz Dialux 22 fluorescence microscope equipped with filter block A.

Anti-androgen treatment

Gels were grafted into intact male mice as described above and allowed 28 days to become established. Flutamide was dissolved in arachis oil and administered for fourteen days by daily subcutaneous injection, at a site distant from the graft, at a dose of 10 mg/kg per day. Animals were killed at various time points throughout the treatment period and the xenografts were examined at these points. After fourteen days of treatment, the remaining animals were left untreated. These animals were then killed at different times after treatment to determine whether, and how quickly, the effects of the drug were reversed.

Results

Investigation of the initial tissue digest revealed clean epithelial organoids with very little stromal contamination. These had the appearance of open spheres of basal epithelium lined with tall columnar epithelial cells that exhibit immunoreactivity to anti-PSA and anti-PSAP antisera. Gels fixed after three days in vitro revealed that the organoids were apparently unchanged from the original digest, although immunoreactivity to the secretory marker antisera was reduced.

Grafts have been performed on 70 animals using organoids derived from a total of 12 patients; 100% of these grafts have become established. Each graft initially contained around 300 epithelial structures of which between 35 and 80% are recognisable on sectioning. This difference appears to be patient-specific, with estimates of intergraft variations using organoids derived from the same patient being less than 10%. Once established, organoid remnant viability does not decrease through the time scale of these experiments.

In intact male mice, grafts were invaded from their margins by stromal cells. The progression with time of this invasion, and the morphology of representative epithelial structures is shown in Figs 1-4. After 10 days in the host the epithelial structures typically formed condensed cellular balls, without obvious architectural organisation (Fig. 1). Most of the organoids observed at this time point did not have a lumen. This may be due to the structures collapsing as a result of experimental manipulation or of host movement. Expression of the secretory markers PSA and PSAP was not detectable in these structures. There were no stromal cells in the immediate vicinity of the epithelial structures. However, stromal cells could be observed invading from the margins of the grafts. Proliferating cell nuclear antigen (PCNA) expression, as detected by the antibody PC10, showed that at the edges of the graft the invading stromal cells were dividing. Many of the epithelial cells also exhibited nuclear staining with this antibody, providing evidence of proliferative activity.

After 21 days, stromal cell invasion of the gel was much more extensive. A lumen had started to form in most of the epithelial structures, as shown in Fig. 2. Limited PSAP and PSA immunoreactivity was demonstrable in some epithelial cells at this stage (see Table 1); however, the majority of the lumina were lined with flattened or cuboidal cells that did not express the secretory markers. Staining with the antibody PC10 showed that PCNA is expressed in the nuclei of most of the cells, indicating that proliferative activity was occurring in both the stromal and epithelial components of the model at this stage.

At 28 days after grafting stromal invasion was still more extensive. The epithelial structures formed a well-defined bilayer with flat basal epithelium lined by cuboidal and columnar secretory epithelial cells (Fig. 3), many of which reacted to the anti-PSA and anti-PSAP antisera (see Table 1). There was a well-defined lumen often containing a mass of either dead cells or secreted material. PCNA expression in the nuclei of epithelial cells in both the luminal and basal compartments revealed that epithelial proliferative activity continued at this time point. By 35 days post-grafting the structures had essentially matured; experimental data in this model to 56 days show no significant changes beyond this point. The lumen was clearly defined and there was intense staining of the luminal cells by both PSA and PSAP antisera, as shown in Fig. 4. Table 1 documents the changes in immunoreactivity to anti-PSAP antisera over the first 35 days after grafting into an intact male host. At 35 days after
Fig. 1. An organoid within a collagen gel xenograft after 10 days in an intact male mouse. H&E stained. A single epithelial structure can be seen. There is no clear organisation of the structure. No stromal cells are associated with the structure. There is very little invasion of the graft by host stromal cells. x100.

Fig. 2. An organoid within a collagen gel xenograft after 21 days in an intact male mouse. H&E stained. Host stromal cell invasion of the graft is becoming apparent. A lumen (L) has started to form in the epithelial structure. x100.

Fig. 3. An organoid within a collagen gel xenograft after 28 days in an intact male mouse. H&E stained. There is extensive host stromal cell invasion of the graft. The epithelial structure shown has a clearly defined lumen surrounded by columnar luminal cells. x100.

Fig. 4. An organoid within a collagen gel xenograft after 35 days in an intact male mouse. Stained against PSAP. There is extensive host stromal cell invasion of the graft. The epithelial structure shown has a clearly defined lumen surrounded by cuboidal and columnar luminal cells. There is intense staining of the luminal cells, indicating expression of the secretory marker. x100.
Table 1. Expression of PSAP in duct-like structures

<table>
<thead>
<tr>
<th>Host type</th>
<th>Age of graft (days)</th>
<th>Assessment of PSAP expression (%)</th>
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<tr>
<td>Intact male</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Intact male</td>
<td>21</td>
<td>94 (85-100)</td>
</tr>
<tr>
<td>Intact male</td>
<td>28</td>
<td>34 (29-40)</td>
</tr>
<tr>
<td>Intact male</td>
<td>35</td>
<td>7 (6-11)</td>
</tr>
<tr>
<td>Castrated male</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Castrated male</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>100</td>
</tr>
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</table>

All structures in four sections of graft (separate grafts, using tissue from four different patients in the case of male hosts, and two patients in the case of female hosts) were assessed for their intensity of staining as: 0 (no staining), + (<50% of luminal cells in any structure stained), ++ (50-90% of luminal cells stained), +++ (equivalent to concurrently stained sections of prostate tissue, used as a positive control). Values are the mean and range of the assessed expression of the marker.

Fig. 5. An organoid within a collagen gel xenograft after 35 days in a female host. H&E stained. There is extensive host stromal cell invasion of the graft. The epithelial structure has a well-defined lumen lined with flattened cells. Note the lack of any columnar secretory epithelium. ×100.

Fig. 6. An organoid within a collagen gel xenograft after 35 days in a castrated male host. H&E stained. There is extensive host stromal cell invasion of the graft. The epithelial structure has a well-defined lumen lined with flattened cells. Note the lack of any columnar secretory epithelium. ×100.

grafting PCNA expression was found only occasionally in the epithelial cells although this marker was still found in the stromal cells.

In female and in castrated male hosts the organisational processes occurred in the same manner as in intact males. The structures initially collapsed and there was gradual formation of an organised structure with a well-defined lumen. This process was accompanied by the same extensive stromal cell invasion of the gel as occurred in the intact male animals. However, while epithelial structures with a well-defined lumen were clearly visible these were lined with a flattened epithelial cell type and displayed no cuboidal or columnar luminal phenotype, as shown in Figs 5 and 6. No immunoreactivity to either of the secretory markers could be observed at any stage in the castrated male or female animals.

Gels grafted within a 0.45 μm Millipore chamber illustrate the effects of androgen in the absence of stromal support. These chambers prevent stromal cell
invasion of the gel while not affecting the flow of soluble factors (Del Buono et al., 1991). After 28 days in such a chamber the epithelium had become disorganised (Fig. 7). Sheets and balls of cytokeratin positive (as determined by reactivity to the anti-cytokeratin antibodies 35/SE12 and CAM 5.2; data not shown) epithelial cells could be found but there were no organised structures with a defined lumen. No expression of either secretory marker was detected. There was no host stromal cell invasion of the gel and human stromal cells were not found, as all cells detected in the chambers showed reactivity to the anti-cytokeratin antibodies.

In order to assess further the role of stromal cells in epithelial architectural organisation, human foreskin fibroblasts were cocultured with the organoids and grafted within Millipore chambers. The results of this experiment showed that the structures retain an organised morphology under these conditions, indicating that vascularisation of the graft is not required for lumination of the structures, as shown in Fig. 8. Duct-like structures were found in all chambers in which fibroblasts were cocultured with the organoids. Their incidence (as a proportion of the total number of organoids grafted) was in the same range as was found in subcutaneous grafts of epithelium from the same patients.

Hoechst 33258-stained sections were examined from all of the grafts under investigation. These revealed that the epithelial structures always showed the diffusely stained nuclei characteristic of human origin while the stroma predominantly showed the speckled pattern indicative of rodent origin (Cunha and Vanderslice, 1984), as shown in Fig. 9. There were a small number of human stromal cells closely associated with some of the structures and comprising of the order of 1-5% of stromal cells found in the immediate vicinity of the epithelium. In the body of the gel, however, the stromal cells were exclusively of murine origin.

Examination of intermediate filament expression in the grafts showed that only the epithelial structures demonstrated reactivity to anti-cytokeratin antibodies (Fig. 10). In grafts established for 28 days or more these structures were surrounded by a narrow band of smooth muscle α-actin-expressing cells (Fig. 11). This layer lies outside the epithelial structures, indicating that it is stromal in origin. It should be noted that human prostate basal epithelial cells are not myoepithelial in nature and do not express smooth muscle α-actin (Srigley et al., 1990). The majority of the stromal cells showed reactivity to anti-vimentin antibodies (Fig. 12), but not to anti-desmin antibodies. This indicates that the principal invading stromal cell population is composed of fibroblasts.

The effects of the anti-androgen Flutamide on the morphology and secretory activity of the epithelial structures was investigated using grafts pre-established
Fig. 9. (A) An organoid within a collagen gel xenograft after 35 days in an intact male host. Hoechst 33258 stained. The epithelial structure encloses a lumen containing some brightly staining debris. The epithelial cells show the large diffusely stained nuclei characteristic of human cells. The stromal cell shows the smaller "speckled" nuclei characteristic of murine origin. ×200. (B) Diagrammatic summary of the Hoechst 33258 section shown in A.

Fig. 10. An organoid within a collagen gel xenograft after 35 days in an intact male host. Stained to show expression of cytokeratin (using the antibody 34βE12). The epithelial nature of the structure is confirmed by expression of this intermediate filament class. The surrounding stromal cells exhibit no such expression. ×100.

Fig. 11. An organoid within a collagen gel xenograft after 35 days in an intact male host. Stained to show expression of smooth muscle α-actin. A band of α-actin can be seen surrounding the structure. ×100.
Fig. 12. An organoid within a collagen gel xenograft after 35 days in an intact male host. Stained to show expression of vimentin. The vast majority of the stromal cell population express this marker. The epithelial structure by contrast does not. ×100.

Fig. 13. An organoid within a collagen gel xenograft after 28 days in an intact male host followed by 3 days of treatment with Flutamide (10 mg/kg per day). Stained to show expression of PSAP. The graft is extensively invaded by host stromal cells. The epithelial structure has a well-defined lumen lined with cuboidal and columnar cells. These show intense staining with anti-PSAP antisera indicating secretory activity. ×100.

Fig. 14. An organoid within a collagen gel xenograft after 28 days in an intact male host followed by 11 days of treatment with Flutamide (10 mg/kg per day). Stained to show expression of PSAP. The graft is extensively invaded by host stromal cells. The epithelial structure has a well-defined lumen lined with flattened cells. These show no staining with anti-PSAP antisera, indicating that there is no secretory activity. ×100.

for 28 days in intact male hosts. These experiments showed that this drug specifically affected the secretory epithelial cell structure and function in the grafts. After three days of treatment the grafts were little different from untreated controls. There was expression of both of the secretory markers, with columnar and cuboidal secretory cells in evidence (Fig. 13). After eight days of treatment, expression of the markers had declined markedly, and tall columnar cells could no longer be found. After 11 days there was no detectable expression of the secretory markers. The luminal epithelial cells had a flattened appearance, as shown in Fig. 14 and the structures closely resembled those found in grafts in female and castrated male hosts (shown in Figs 5 and 6). When intact male hosts were treated with Flutamide for fourteen days and were then allowed to recover for a further fourteen days the grafts showed recommenced expression of the secretory markers as shown in Fig. 15. The effects of Flutamide treatment on PSAP expression were assessed and the results are shown in Table 2.

Discussion

The model presented here is interesting in that human prostate epithelium establishes and maintains a structure and morphology similar to that found in vivo, with associated secretory activity. The data show that functionally active, adult human prostate epithelium
Fig. 15. Organoids within a collagen gel xenograft after 28 days in an intact male host followed by 14 days of treatment with Flutamide (10 mg/kg per day) and a further 14 days without treatment. Stained to show expression of PSAP. The graft is extensively invaded by host stromal cells. The epithelial structures have a well-defined lumen lined with cuboidal and columnar cells. These show intense staining with anti-PSAP antisera indicating secretory activity. x100.

Table 2. Effect of Flutamide on the expression of PSAP in duct-like structures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Assessment of PSAP expression (%)</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Flutamide, 3 days</td>
<td>19 (15-21)</td>
</tr>
<tr>
<td>Time-matched control</td>
<td>6</td>
</tr>
<tr>
<td>Flutamide, 8 days</td>
<td>57 (45-62)</td>
</tr>
<tr>
<td>Time-matched control</td>
<td>4</td>
</tr>
<tr>
<td>Flutamide, 11 days</td>
<td>100</td>
</tr>
<tr>
<td>Time-matched control</td>
<td>6</td>
</tr>
<tr>
<td>Flutamide, 14 days, followed by 14 days untreated</td>
<td>14</td>
</tr>
<tr>
<td>Time-matched control</td>
<td>5</td>
</tr>
</tbody>
</table>

Gels were grafted into intact male hosts and allowed to become established for 28 days. Animals were injected daily with Flutamide for 14 days (three samples per point) and were then left untreated for a further 14 days (single observation). All structures in sections from three grafts were assessed for their intensity of staining as: 0 (no staining), + (<50% of luminal cells in any structure stained), ++ (50-90% of luminal cells stained), +++ (equivalent to concurrently stained sections of prostate tissue, used as a positive control). Values are the mean and range of the assessed expression of the marker. Results for untreated time-matched control animals (one per time point) are also shown.

can be established in collagen gel xenografts in intact male athymic mice. They indicate that stromal cells are required for the maintenance of architecturally organised prostatic epithelial structures with a well-defined lumen. In addition to this organisational level, an androgenic stimulus appears to be required for the establishment of secretory epithelial cell morphology and function.

When grafted into an intact male host, the epithelial structures follow a clear sequence of events. This consists of an initial collapse followed by gradual re-establishment of structures with a well-defined lumen,
and finally the formation of secretory cell morphology and function. This process occurs concurrently with the invasion of the gel by murine stromal cells. Xenografts into castrated male and female hosts showed the same pattern of collapse followed by establishment of duct-like epithelial structures, again occurring concurrently with host stromal cell invasion. While the general structure of the organoids was maintained in these animals there was no establishment of columnar luminal epithelium and an associated lack of detectable prostate-specific secretory marker expression. The resulting structures are comparable in appearance to the prostate of recently castrated animals having lumina lined with flattened epithelium. Exclusion of host stromal cells, by grafting within Millipore chambers, showed that in the absence of stromal cell support the epithelial structures were unable to establish an organised morphology.

Examination of intermediate filament expression in the grafts allowed the stromal cells to be identified as fibroblasts, as they exhibited immunoreactivity to antivimentin antibodies but not to anti-desmin, or anticytokeratin, antibodies. Hoechst 33258 staining confirmed that the vast majority of these stromal cells were of rodent origin. However, a small number of human stromal cells were also found to be associated with some of the epithelial structures. This indicates that the initial enzymic digestion of the tissue was not complete. The role played by these human stromal cells in the establishment and maintenance of prostatic function in this model is unclear. In the Millipore chambers, in the absence of introduced fibroblasts, organisation and secretory function of the epithelium was not maintained, despite this small component of human stromal cells. This observation underlines the key role played by murine stromal cells in this model and the importance of relative cell numbers in instructive and permissive relationships (Cunha et al., 1983). This also shows that these murine cells may act permissively across species barriers.

Cocultured HFF cells within a Millipore chamber were capable of supporting organised epithelial structures, showing that heterologous human fibroblasts can also exhibit permissive support of secretory epithelium. This result is important as it shows that the chamber environment is not inherently incapable of allowing organisation to be maintained. This finding also suggests that the process of vascularisation that occurs in the grafted gels is peripheral to epithelial organisation and is not its direct cause.

Together these findings indicate that prostatic epithelial organisation and morphology, defined during organogenesis, can be maintained with the permissive support of heterologous, and heterospecific, stromal cells, with the stromal cells acting to support duct-like organisation. This suggests a generalised stromal function, which may relate to factors such as mechanical support or the production of locally diffusible factors. Alternatively, the presence and exact composition of the extracellular matrix is known to affect epithelial cell function (Hadley et al., 1985; Blum et al., 1987; Li et al., 1987). Therefore, the mechanism of the permissive support may be related to the deposition of a complete matrix or of specific matrix components by the stromal cells. Comparison of results in intact and castrated male, and female, hosts with results obtained in the absence of stromal cells demonstrates that stromal cells support a certain basic level of overall structural organisation. Beyond this organisational level an androgenic stimulus is additionally required for secretory epithelial activity.

The data obtained with Flutamide treatment lend further weight to the idea of androgens acting within a predetermined, stromally supported, organised structure. The drug specifically affects luminal cell morphology and thus secretory function while not destroying the organisation of the epithelial structures around a central lumen. Upon termination of the drug treatment, the secretory cell morphology and function are quickly re-established. This shows that even after a period of androgen deprivation the model retains an ability to respond to an androgenic stimulus by changing luminal epithelial morphology and function.

In the adult human, as in the adult mouse, androgen receptors and 5α-reductase are expressed in the prostatic epithelium as well as in the stroma (Chang et al., 1989; Cooke et al., 1991; Cowan et al., 1977; Habib et al., 1983). This strongly suggests a role for the adult epithelium, which exhibits a functional morphological programme determined by the mesenchyme during development, in the interpretation of androgenic stimulus. In an attempt to clarify the role of prostate epithelium in this process, the ability of the stromal and epithelial portions of this model system to respond directly to androgens is currently under investigation. The response to treatment and withdrawal of treatment with the anti-androgen Flutamide, considered in combination with the results obtained in castrated male and in female hosts shows that there is an ultimate response in the epithelial cells. However, this does not determine in which component this response is mediated.

Norman et al. (1986) reported that adult rat prostatic epithelium maintained the ability to produce new ductal growth in the presence of an embryonic inducer. The development of several hundred ducts from a single ductal tip in renal capsule implants of recombinant foetal urogenital sinus mesenchyme with adult prostate epithelium was described, representing a massive increase in prostatic epithelium. Expression of the proliferating cell marker PCNA has been noted in this model; however, this seems to be related to the rearrangement of epithelial structures to re-form functioning secretory units. In an intact male host very little PCNA expression is found in the epithelial portion of this model beyond 35 days. While we have not accurately quantified the epithelial content of our xenografts, it is apparent that an increase of the magnitude described by Norman et al. (1986) has not occurred. So, while it would appear that adult epithelium can maintain a morphogenetic competence, this model system does not exercise this potential. The permissive role of the heterologous and heterospecific
adult mouse stromal cells on adult human epithelium is clearly different from the effects induced by an instructive foetal urogenital sinus mesenchyme on adult rodent epithelium. Since, in the adult, the prostate does not normally undertake a rapid and massive increase in its epithelial component, the system reported here may be considered to represent a realistic model of the stable adult situation.

The finding that the majority of the stromal cells show reactivity to anti-vimentin antibodies but not to anticytokeratin or anti-desmin antibodies indicates that these are fibroblasts. However, the thin ring of smooth muscle actin seen around the epithelial structures does beg the question of whether a smooth muscle cell phenotype is induced in these stromal cells by the epithelium. As already noted, the basal epithelial cells of the human prostate are not myoepithelial in nature and do not express smooth muscle actin (Srigley et al., 1990), indicating that the epithelial cells are not the source of this material. The possibility of smooth muscle α-actin induction in murine fibroblasts by heterospecific epithelium is consistent with results reported from a similar model of differentiated gel (Del Buono et al., 1992). Also, staining shows that some human stromal cells are associated with the epithelial structures. However, the distribution of these is uneven, while the expression of actin is consistent and apparently unbroken around the structures. This may indicate an effect exerted by adult epithelial cells to induce formation of conditions permissive to epithelial function, representing the establishment of an interactive system between the human epithelial and murine stromal cells. Work is underway to determine the source of origin of the actin-expressing cells and to elucidate the mechanism of the induction of this expression.

The use of this simple technique, particularly xenografting within Millipore chambers, represents an alternative to some applications of the sub-renal capsule implant. The success rate is higher, and the technical difficulties much lower than the renal capsule implant. The results are consistent and reproducible. The chambers are a closed environment in which defined cell types, or combinations of cell types, can be cultivated without direct contact with host cells, while still retaining the biological support inherent in an in vivo model. The utilisation of the prostate model described here provides a system in which the effect of drug modalities on biologically active human prostatic epithelium can be tested in vivo, and in which mechanisms of epithelial-stromal interactions in prostate can be investigated.

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