Ductal epithelial cells cultured from human foetal epididymis and vas deferens: relevance to sterility in cystic fibrosis

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Summary
A tissue culture system for epithelial cells derived from male human foetal genital ducts has been established. The cells show morphological and biochemical characteristics of ductal epithelial cells, and can be passaged and maintained in culture for considerable periods of time. These cells will provide a suitable system for investigating, by electrophysiological, biochemical and molecular biological methods, the cause of sterility in cystic fibrosis.

Key words: vas deferens, epididymis, epithelial cells, cystic fibrosis, sterility.

Introduction
The majority (more than 97%) of males with cystic fibrosis (CF), the most common autosomal recessive disease in Caucasian races, are sterile. This sterility is due to an abnormality of the epididymis and vas deferens, which, instead of forming a duct system, along which mature spermatozoa pass from the testis to the urethra, are blind-ended. It is thought that abnormal epididymes and absence of vasa deferentia are already evident at birth (Kaplan et al. 1968; Gracey et al. 1969; Holsclaw, 1969).

It is known that the cystic fibrosis gene is already functional in some tissues of the mid-trimester foetus. Post-mortem examination of foetuses diagnosed as having CF on the basis of abnormal levels of microvillar enzymes and alkaline phosphatase have shown periodic acid-Schiff positive material in the pancreas (Boué et al. 1986). Further, in some cases foetuses already show extensive fibrosis of the epididymis with only a few ducts present (A. Ornoy, personal communication).

The components of the male reproductive system are anatomically well developed by 18 weeks gestation, with clear testes, epididymes and vasa deferentia. It seems likely that between the mid-trimester and birth the vas deferens in CF foetuses becomes gradually blocked by deposits of secreted materials. These blockages then cause destruction of the vas deferens and the associated regression of the epididymis. How and why these secreted deposits occur in the vas deferens is unknown. However, it seems probable that the situation is similar to that seen in the pancreas in CF. Here blockage of pancreatic ducts with inspissated secretions causes gradual autolysis of the pancreas and acini become replaced by cystic spaces.

The basic defect in CF is thought to be one of regulation of chloride ion transport. This defect is seen in the sweat gland duct epithelium and certain airway epithelia (Quinton, 1983; Sato & Sato, 1984; Widdicombe et al. 1985; Knowles et al. 1986; Welsh & Liedtke, 1986; Frizzell et al. 1986). By analogy with these specialized epithelia, it is likely that the pancreatic duct epithelium is expressing similar abnormalities in regulation of anion channels. Though the pancreatic duct epithelium secretes bicarbonate ions primarily rather than chloride ions the movements of these two ions across the apical cell membrane are closely inter-related (Gray et al. 1988). Similarly, it is probable that the ductal epithelium lining the vas deferens and/or epididymis shows defective regulation of chloride ion transport in CF. Resulting abnormalities in the ionic microenvironment of the vas lumen could in turn result in deposition of secreted materials, either directly or by the reactions of cells lining the lumen.

We have recently established a tissue culture system for ductal epithelial cells derived from human foetal pancreas (Harris & Coleman, 1987, 1988). This system has now been adapted to culture ductal epithelial cells from the lining of the epididymis and vas deferens of mid-trimester human foetuses.

Materials and methods

Materials and cell culture
Vasa deferentia and epididymes were obtained within 48 h from mid-trimester prostaglandin-induced terminations or spontaneous abortions. These included eight normal foetuses, and four with other known abnormalities. Male genital ducts were
removed while still attached to the testes, and washed in tissue culture medium. They were then dissected from surrounding mesentery and chopped into 1-2 mm diameter pieces in a solution of collagenase (Sigma type 1A at 0.5 mg ml\(^{-1}\)) at 37°C. After 1-5 min in collagenase, duct pieces were washed in tissue culture medium and plated out onto Primaria flasks (Falcon, Becton Dickinson) in CMRL 1066 medium, containing 20% foetal calf serum (FCS) (Gibco, UK); penicillin (100 units ml\(^{-1}\)); streptomycin (100 \mu g ml\(^{-1}\)); l-glutamine (4 mM); insulin (0.2 unit ml\(^{-1}\)); cholera toxin (10\(^{-6}\) M) and hydrocortisone (1 \mu g ml\(^{-1}\)), all from Sigma. Cultures were routinely maintained at 37°C in a humidified 5% CO\(_2\) incubator. Cells were seen to migrate from duct explants after 5-10 days. Fibroblasts, which occasionally contaminated primary epithelial cell cultures, were eliminated by physical removal with a rubber policeman. Cells were routinely passaged with trypsin (0.25%) and EDTA (0.02%).

**Histology**
Genital ducts from 18- to 20-week male foetuses were divided into epididymis and vas deferens, fixed in methanol: chloroform:acetic acid (6:3:1, by vol.) for 1 h and then embedded in paraffin wax using standard procedures. Sections of 3 \mu m were cut and processed for immunocytochemistry.

**Immunocytochemistry**
Immunocytochemistry was done by standard procedures. LE 61 (Lane, 1982), a monoclonal antibody that reacts with a range of cytokeratins, characteristic of simple epithelial cells (mainly with cytokeratin 18), was donated by Dr Birgitte Lane. Monoclonal antibodies to cytokeratins 8 and 19 were purchased from Amersham. Monoclonal antibody Ca2 (Bramwell et al. 1983), which reacts with a mucous glycoprotein, known to be expressed on certain ductal epithelia (Harris, 1987) was donated by Professor Harris. 19-9 (Magnani et al. 1983) is a monoclonal antibody that detects a mucin found in the sera of many patients with gastrointestinal and pancreatic cancer. DU-PAN-2 (Lan et al. 1985) detects another mucin associated with human pancreatic adenocarcinomas. The three mucins detected by Ca2, 19-9 and DU-PAN-2 are distinct, unrelated molecules.

Peroxidase-conjugated rabbit anti-mouse immunoglobulins were purchased from Dako-Patts.

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**Fig. 1.** Vas deferens (A, B) and epididymis (C, D) epithelial cells. A, C, \(\times 180\); B, D, \(\times 90\).
Fig. 2. Cytokeratin expression in vas deferens (A, E, I) and epididymis (B, F, J) epithelial cell cultures and in sections through foetal vas deferens (C, G, K) and epididymis (D, H, L). Monoclonal antibodies LE 61 (A, B, C, D); anti-cytokeratin 8 (E, F, G, H); anti-cytokeratin 19 (I, J, K, L). Cell cultures, ×220; sections, ×110.
Fig. 3. Mucin production in vas deferens (A, E, F) and epididymis (B) epithelial cell cultures and in sections through foetal vas deferens (C) and epididymis (D). Monoclonal antibodies Ca2 (A, B, C, D); 9-19 (E) and DU-PAN-2 (F). Cell cultures, ×220; sections, ×110.
Results

The cells that migrate from primary explants of vas deferens and epididymis are morphologically of two main types (Fig. 1). The percentage of the two cell types in any culture varies and seems to depend on the particular set of male reproductive ducts from which it is derived. The first is a large angular cell type that does not appear tightly packed even when confluent (Fig. 1B,D). The second is a relatively small, 'cobblestone' cell type that always appears in tightly packed colonies (Fig. 1A,C). These two cell types bear marked similarity to the secretory and resorptive cells of the sweat gland duct described by Lee et al. (1986). In primary cell cultures the larger cell type usually appears first and always represents the major cell type in a confluent culture. The smaller type appears 24–48 h later. Both cell types can be successfully passaged at least five times with trypsin (0-25 %) and EDTA (0-02 %). However, the larger cell type leaves the substratum first. This ability to withstand trypsin/EDTA is in contrast to the pancreatic duct epithelial cells described previously (Harris & Coleman, 1987, 1988), which can only be passaged with dispase. Cells can be passaged at least 10 times before showing signs of senescence.

Cell cultures can be frozen in liquid nitrogen using standard methods. However, the small cell type seems less able to withstand this than the larger cells, as judged by the percentage of the two cell types in one tissue culture flask prior to freezing and post recovery of the same vial.

Culture substrata

A range of different culture substrata were tested for their effect on vas deferens and epididymis epithelial cell growth. These included (1) the following plastic tissue culture flasks: Sterilin, Nunclon (Gibco), Falcon (Becton Dickinson) and Primaria, Falcon (Becton Dickinson); and (2) glass coverslips with or without a coating of collagen (Sigma type VI from human placenta at 1 mg ml⁻¹). Primaria flasks or collagen-coated glass were the preferred substratum for both cell types, although Sterilin flasks also provide an adequate growth support.

Culture media

A range of culture media were tested including (1) CMRL 1066 (Gibco UK) with 20 % foetal calf serum (FCS) and with or without the following supplements: insulin (0-2 unit ml⁻¹); cholera toxin (10⁻¹⁰ M) and hydrocortisone (1 μg ml⁻¹), all from Sigma; (2) the same combination of CMRL 1066 with or without supplements as described in (1) but with only 10 % FCS; (3) Dulbecco's MEM (Gibco) with 10 % FCS; RPMI 1640 (Flow) with 10 % FCS; Ham's F10 (Gibco) with 10 % FCS. CMRL 1066 with 10 or 20 % FCS and supplements achieved the best results. The presence of insulin, cholera toxin and hydrocortisone in the culture medium was essential for maintenance of the distinctive morphology of the two cell types shown in Fig. 1.

Immunoctytochemistry

For all antibodies used expression of the specific antigen was monitored simultaneously in wax sections through vas deferens and epididymis and in cultured cells (derived from the other male genital duct from the same foetus) to establish the distribution of particular cell types in vitro.

Cytokeratins

The mixture of cytokeratin intermediate filament proteins characteristic of simple epithelia (mainly cytokeratin 18), that are detected by the monoclonal antibody LE 61 (Lane, 1982) are expressed abundantly in both cell types seen in vas deferens and epididymis epithelial cultures (Fig. 2A and B). The physical distribution of cytokeratin 18 in the two cell types is rather different. The smaller cells show more uniform staining with LE 61 throughout their cytoplasm than the larger, loosely packed, cells in which clearly staining fibres can be seen in perinuclear bundles as well as dispersed through the cytoplasm. A very similar pattern of expression of cytokeratin 8 is also seen in both vas deferens and epididymis cell cultures (Fig. 2E and F). However, in sections through foetal vas deferens and epididymis, little, if any expression of either cytokeratin 18 (Fig. 2C and D) or 8 (Fig. 2G and H) is seen. A similar lack of in vitro expression of cytokeratins 8, despite substantial levels being seen in cultured cells derived from the same tissue, has been previously reported in foetal pancreatic duct (Harris & Coleman, 1988). The reason for this is unclear but may relate to culture conditions, which are known to affect cytokeratin expression in vitro. It is of course possible that antigen masking may be occurring in the sections. However, protease treatment of sections prior to immunocytochemistry did not reveal in vitro expression of these cytokeratins. The pattern of cytokeratin expression in the cell cultures remains stable from early primary culture for at least 8 weeks (the latest time at which it has been analysed).

Cytokeratin 19 is expressed in both cell types in vas deferens and epididymis cultures, again with slightly different distribution in the two cell types (Fig. 2I and J). In sections through foetal vas deferens (Fig. 2K) and epididymis (Fig. 2L) strong expression of cytokeratin 19 is only seen in cells lining the ducts themselves.

Mucins

The Ca antigen that is detected by the antibody Ca2 is known to be present on the luminal surfaces of certain ductal epithelia (Harris, 1987), including the adult epididymis. The Ca antigen is expressed in the vas deferens and epididymis cells we are culturing (Fig. 3A and B). Low levels only are seen in the loosely packed cells, but substantially more is produced in the tightly packed cells. It is possible that these cells have a secretory role in vitro. In sections through foetal vas deferens (Fig. 3C) and epididymis (Fig. 3D), Ca2 binding only to the epithelial cells lining the ducts is clear.

The mucous glycoprotein detected by antibody 19-9 (Magnani et al. 1983) (and found in the sera of many patients with gastrointestinal and pancreatic cancer) is
expressed in all the vas deferens and epididymis-derived cells in culture, though its level of production is variable in different cells (Fig. 3E). In sections through these ducts, there is no substantial binding of 19–9 (data not shown). The mucin DU-PAN-2 (Lan et al. 1985) (that is associated with human pancreatic adenocarcinomas) is also expressed in all the cultured cells though at variable levels, with the smaller cell type binding more DU-PAN-2 (Fig. 3F). In sections through foetal vas deferens and epididymis DU-PAN-2 appears to bind non-specifically to all parts of the ductal tree (data not shown). This is likely to be a property of the DU-PAN-2 antibody rather than reflecting true expression of the antigen.

Discussion

A tissue culture system for epithelial cells derived from mid-trimester human foetal vas deferens and epididymis has been established. Two main cell types predominate in the cultures (Fig. 1). To date, genital ducts from eight normal male foetuses and four with other known abnormalities (one each with trisomy 21, two with Noonan's syndrome and amniogenesis malformation, respectively) have been cultured.

Characterization of the cultured epithelial cells with monoclonal antibodies specific for cytokeratins 8, 18 and 19 has confirmed that they are indeed epithelial cells (Fig. 2). Further, from the level and patterns of expression of cytokeratin 19 in sections through 18-week foetal male genital ducts and in the cultured cells it seems likely that the latter are indeed derived from the epithelium lining the ducts themselves. On the basis of morphology and patterns of cytokeratin expression, the cultured cells can be divided into two main populations. These populations are apparently the same in cultures derived from the vas deferens and the epididymis, though the proportion of the two may vary.

Further evidence in support of a ductal origin for the cultured cells is provided by their expression of the mucin detected by the monoclonal antibody Ca2. This mucin is known to be present on the luminal surface of adult epididymis (Harris, 1987) and has been shown here to be produced by the epithelial cells lining the foetal vas deferens and epididymis ducts (Fig. 3). Again, a marked difference in the amount of Ca antigen produced by the two cultured cell types may reflect clear functional differences between them. By analogy with the morphology of cell types grown from sweat gland duct epithelium, it is possible that the smaller cells are secretory and the larger ones resorptive cells.

The different physiological functions of the two cell types being cultured will be elucidated by analysis of ion channels and their regulation. These cells will provide a valuable additional tool, at both molecular and cellular levels, in understanding how the cystic fibrosis gene defect causes dysfunction of specialized epithelial cells in vivo.

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References


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