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Immunolocalization of Prostasomes in the Human Prostate

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ABSTRACT

Prostasomes are prostate-derived organelles, which can be isolated from seminal plasma. We have produced a panel of monoclonal antibodies against purified human prostasomes by intrasplenic immunization. Among the prostasome-positive mAbs obtained, one antibody (mAb 78) was selected for further characterization. SDS-PAGE and Western blots demonstrated that mAb 78 recognized a band of about 35 kDa from purified prostasomes, seminal plasma and extracts of prostatic gland tissues. Immunostaining with mAb 78 resulted in positive reactions in the apical parts of the secretory cells of the prostate epithelium and in the secretions of the gland lumen. The nuclei were not stained. The mAb 78 has the potentials of a prostasome marker.

INTRODUCTION

The prostate secretion in the ducts of the prostate gland is expelled at ejaculation and contributes to the seminal plasma. One of the components derived from the prostate secretion is the prostasomes. They appear as small vesicles or granules in the seminal plasma and have a diameter in a range of 50-800 nm (15).

Electron microscopy of human prostate glands demonstrated that the apical parts of the epithelium contained vesicles filled with smaller vesicles and granules, which had sizes similar to those of the prostasomes (2). Therefore it was suggested that the prostasomes were enclosed in storage vesicles which, when they fused with the apical cell membrane, released prostasomes into the gland ducts by exocytosis. When the prostate secretion is discharged at ejaculation, the prostasomes thus will be a component of the seminal plasma. In addition, the prostasomes will

coat the whole sperm cells (16).

The distribution of the prostasomes in the prostate gland and the changes in prostasome number caused by, for instance, hormonal changes and pathological conditions, are not known. Although immunohistochemical markers for the prostate epithelium, such as prostate-specific antigen (PSA) (5, 11), prostatic acid phosphatase (PAP) (1, 5, 14), and prostasin (25), have been reported, there is no specific immunomarker for the prostasomes available. Therefore we have raised a series of monoclonal antibodies against prostasomes in order to find immunohistochemical markers, which could be used for structural and functional studies of the prostasomes (12).

One of the antibodies obtained (mAb 78) detected a substance in the prostate epithelium which was distinct from the immunomarkers mentioned (6). The present contribution reports on the production and analyses of this antibody and on the distribution of the prostasomes in normal prostate glands as observed by immunostaining with the mAb 78.

MATERIALS AND METHODS

Anti-prostasome mAbs

The immunogen used was prostasomes, purified according to (4). In short, liquefied ejaculates were centrifuged for 20 min at 1000 g to remove spermatozoa and cell debris from the seminal plasma. The supernatant was subsequently subjected to preparative ultracentrifugation for 2 h at 105,000 g to pellet the prostasomes. The pellets were resuspended in 0.5 mL of 30 mM Tris-HCl buffer, pH 7.6, containing 130 mM NaCl and chromatographed on a Sephadex G 200 column (75 mL; Pharmacia AB, Uppsala, Sweden). Elution was performed with the Tris-HCl buffer at a flow rate of 6 mL/h. Fractions with a high protein concentration were pooled, centrifuged and adjusted to 2 mg protein/mL Tris-HCl buffer. Approximately 0.5 µl of this suspension was placed on a 5x5 mm^2 piece of nitrocellulose (NC) membrane (Schleicher & Schuell, Dassel, Germany). These prostasome blots were used for immunization.

Immunization was made intrasplenically by depositing the prostasome blots in the spleen tissue (13). Three female mice (NMRI) were taken as recipients. They were anaesthetized by an intraperitoneal injection of 2.5% Avertin in saline (0.02 mL/g body weight). The spleen was

exposed, and an NC membrane with prostasomes was introduced under the splenic capsule through a small incision. Each mouse received one prostasome blot at each immunization on 4 different occasions. Each blot contained prostasomes corresponding to approximately 1 μg of protein. Each mouse thus received a total of 4 μg of prostasomal protein. The interval between each deposition was 1-2 months.

The cell fusion between plasma cells in the spleen and SP2/0 mouse plasmacytoma cells was PEG mediated. The fused cells were distributed at a density of 1 x 10^5 viable hybrids per well in 96-well sterile culture trays. For screening antibodies of the supernatants obtained, approximately 0.5 μL of the prostasome suspension was dotted onto 5x5 mm^2 pieces of NC membranes, which then were processed by immunostaining. Among the supernatants containing mAbs against human prostasomes (12), one of these (mAb 78) was used in the present study.

SDS-PAGE

SDS-PAGE was carried out in mini-slab gels. Purified human prostasomes, seminal plasma and prostate extracts were each suspended in sample buffer. The prostate extract was prepared by mincing human prostate tissue for incubation in a solution of 10% SDS in water overnight. After centrifugation at 1000 g for 15 min, the supernatant was taken for electrophoresis. The proteins were separated under reducing conditions on 7.5% and 12% slab SDS-PAGE minigels (Mini-Protein II, Bio-Rad, Bedford, USA).

The separated proteins on gel slabs were visualized by Coomassie Blue or blotted onto a NC membrane. The blots were blocked over-night in Tris-HCl (pH 7.6) containing 10% fat-free milk powder and 0.01% Tween 20 and labeled with mAb 78 diluted 1:2 with Tris-HCl (pH 7.6) for 60 min at room temperature. The blots were then incubated in biotin-conjugated GAM-IgG followed by the ABC-HRP complex (Dacopatts A/S, Glostrup, Denmark). The corresponding antigens were visualized by an enhanced chemiluminescence kit (Amersham Int., Amersham, UK), and the results were recorded on photographic films. As a control, mouse IgG (Sigma), diluted to 10 μg/mL in Tris-HCl, was used as primary antibody.

Immmunohistochemical detection of prostasomes

Prostate glands were obtained from two patients subjected to total prostatectomy due to cancer of the bladder. Pieces of gland tissue were fixed in 4 % paraformaldehyde in Millonig buffer,

embedded in paraffin and sectioned at 2 μm with a rotary microtome (MICROM HM 360, Laborgeräte GmbH, Walldorf, Germany). Afterwards, the sections were placed in Coplin jars with citrate buffer (pH 6.0) and treated in a microwave-oven (Philips WhirlpoolJet 900 W) at 700W for 4 min (20). After cooling, the slides were rinsed and stained by the Vectastain ABC-AP protocol using supernatant with 0.01% sodium azide as primary antibody. As negative control, the primary antibody was omitted or an irrelevant antibody was used.

RESULTS

SDS-PAGE

The mAb 78 recognized protein bands corresponding to a molecular weight of about 35 kDa in Western blots from SDS-PAGE of purified prostasomes, seminal plasma and prostate tissues.

Immunohistochemical detection of prostasomes

The human prostate is an aggregate of small compound tubulo-alveolar glands embedded in a dense stroma which contains numerous strands of smooth muscle fibers. The secretory alveoli and tubules are irregular and vary in size and form due to the amount of secretion in the lumen. The gland epithelium contains cuboidal to columnar secretory cells lying on a thin layer of basal cells.

Immunohistochemistry using mAb 78 demonstrated a positive staining of all secretory cells in the epithelium (Figs. 1-2). The staining was most intense in the apical area of the cells, while it disappeared when the nuclei were approached (Fig. 3). The nuclei were not stained. In the stroma, some scattered cells were positive. The corpora amylacea of the gland ducts did not contain any component which was detected by mAb 78.

DISCUSSION

The prostate epithelium is folded, and the epithelial cells are cuboidal to columnar in type depending on their secretory activity. Among the epithelial cells, neuroendocrine cells are scattered (19). These are pleomorphic in shape with irregular dendritic processes that extend under and between adjacent epithelial cells and contain neurosecretory granules.

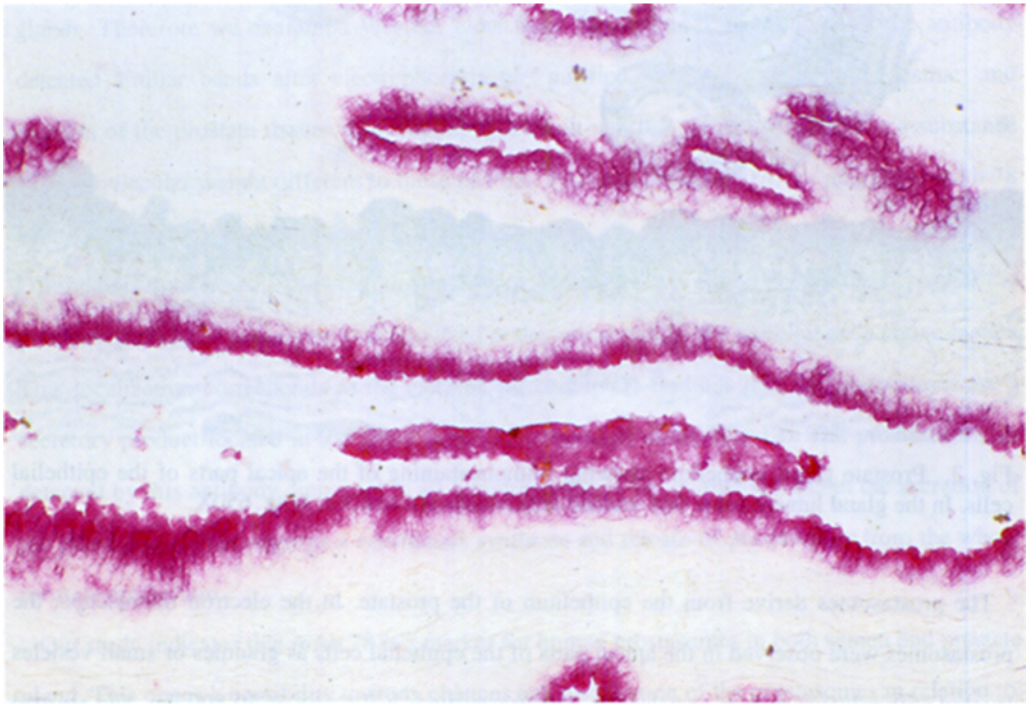

Fig. 1. A section of the prostate gland showing immunostained luminal secretion and apical parts of the epithelium. Mag. 250X.

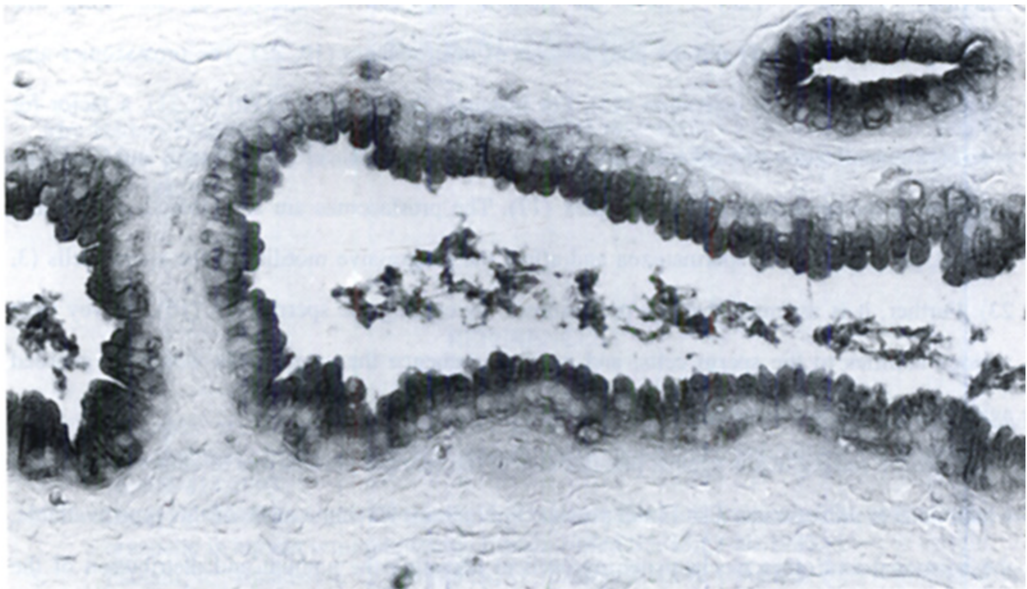

Fig. 2. A section of the prostate gland showing immunostained luminal secretion and apical parts of the epithelium. Mag. 400X.

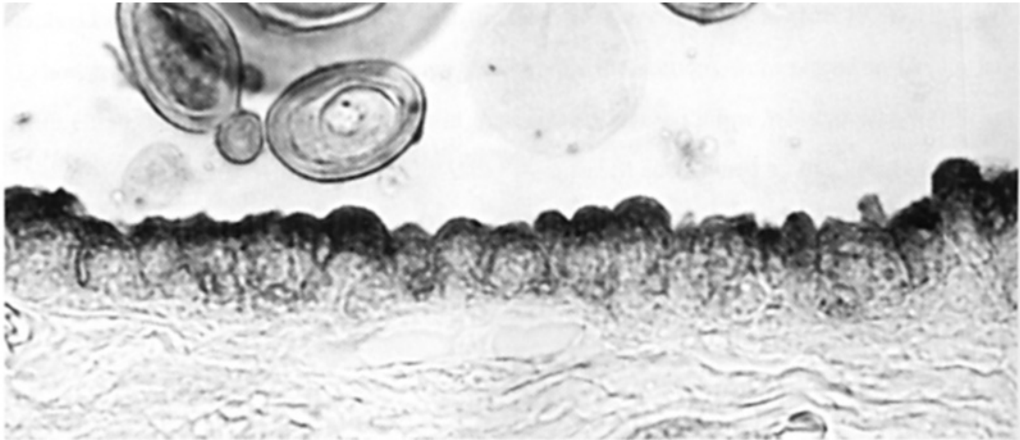

Fig. 3. Prostate gland epithelium showing immunostaining of the apical parts of the epithelial cells. In the gland lumen, some non-stained concretions are noticed. Mag. 650X.

The prostasomes derive from the epithelium of the prostate. In the electron microscope, the prostasomes were observed in the apical parts of the epithelial cells as granules or small vesicles located within larger storage vesicles (2). The prostasomes had a size of 50-800 nm, and seemed to be expelled by exocytosis when the storage vesicles fused with the apical cell membranes.

The prostasomes, which are assumed to derive from the prostate epithelium, are ascribed many functional effects. They have an immuno-suppressive capacity by inhibiting the lymphoproliferation (7, 8) and the phagocytosis of macrophages (10, 22). The prostasomes also regulate the complement activation (18). For instance, they contain CD46 (9, 21), a factor for proteolytic inactivation of C3b and C4b, CD55 (24), the decay accelerating factor, and CD59, an inhibitor of the membrane attack complex (17). The prostasomes are also able to attach onto washed, prostasome-free spermatozoa and affect the progressive motility of the sperm cells (3, 23). Further, it is assumed that the prostasomes, by coating the sperm cells (16), convey their various abilities to the sperm cells, and as a consequence the sperm cells would be guarded against attacks from the female immune system (8).

Our goal in raising mAbs against prostasomes of the seminal plasma was to obtain an antibody which detects prostasomes also in the prostate epithelium. Having a mAb of this type available, the hormonal and other mechanisms involved in adjusting the amount and distribution of the prostasomes in the epithelium could be analyzed. A prerequisite for this analysis is that the mAb detects the prostasomes, whether these are located in the seminal plasma or in the prostate

glands. Therefore we examined Western blots from these sources and found that the antibody detected similar bands after electrophoresis of purified prostasomes, seminal plasma and extracts of the prostate tissues. Considering this result and that the mAb 78 detects a substance with a molecular weight different to those of PSA, PAP and prostasin (6), we judge that mAb 78 is an immunomarker for prostasomes.

Immunohistochemical study demonstrated that mAb 78 binds to the supranuclear part of the prostate epithelia. The staining was localized at the apical part of the epithlial cells above nuclei. This localization corresponds to the electron microscopical findings that the prostasomes are a secretory product located in the apical parts of the prostate epithelium (2). The prostasomes, as detected by this antibody, appeared in all secretory cells of the epithelium and in the secretion of the gland ducts. This suggests a continuous synthesis and release of prostasomes from the whole prostate epithelium.

Our study indicates that mAb 78 is a marker for human prostasomes in both semen and prostate gland. This opens a possibility to study changes and distribution of the prostasomes in relation to, for instance, hormonal conditions or malignant transformation.

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