

Keratin Immunoreactivity in the Benign and Neoplastic Human Prostate

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ABSTRACT

Keratin immunoreactivity in the benign and neoplastic human prostate was examined immunohistochemically using two monoclonal antibodies with differing specificities. One of these antibodies stained only the basal cells of the normal and hyperplastic prostatic epithelium, with no reactivity in tumor cells of prostatic adenocarcinoma. The other monoclonal antibody recognized a keratin protein present in all normal and hyperplastic columnar (secretory) epithelial cells, as well as in all cancer cells regardless of degree of tumor differentiation. In addition, the second antibody stained acinar and ductal epithelial cells exhibiting premalignant changes. Our findings indicate that keratin immunoreactivity differs among the epithelial cell populations of the human prostate, probably reflecting expression of different keratin proteins. The distinctive patterns of staining obtained with these two antibodies may assist in distinguishing hyperplastic from neoplastic prostatic epithelium, as well as in the recognition of basal cell hyperplasia, transitional cell metaplasia, and premalignant changes.

INTRODUCTION

Intermediate filaments comprise one subset of the eukaryotic cytoskeletal system. Five classes of intermediate filaments have been recognized, and their distinctive pattern of distribution has proven useful in classifying benign human tissues and tumors based on the cell of origin (1). The keratin proteins are one class of intermediate filaments which are unique to epithelial cells, and at least 19 keratins have been documented based on molecular weights and biochemical analyses (2). Examination of keratin proteins in numerous benign and malignant human tissues has shown that keratin proteins are uniformly present in epithelial cells and persist following malignant transformation. (1-28).

There have been few detailed reports on the pattern of keratin immunoreactivity in the human prostate. Utilizing polyclonal antisera raised against human stratum corneum, Schlegel *et al.* (22, 23) and Barwick and Mardi (27) reported immunoreactivity of keratin in basal cells of normal and hyperplastic prostatic epithelium, with no staining of adenocarcinoma. Conversely, Gabbiani *et al.* (13) identified keratin in one case of prostatic adenocarcinoma using polyclonal antiserum raised against bovine muzzle prekeratin.

Van Muijen *et al.* (28) reported the first studies utilizing anti-keratin monoclonal antibodies in human prostatic tissues. All 3 of their antibodies, directed against human callus cyokeratin, stained normal ductal epithelial cells, but only one antibody identified acinar cells. Details of the number of cases examined

and the specific staining pattern were not provided. In a comprehensive review of keratin immunoreactivity in normal human tissues, Gown and Vogel (15) noted that one of their anti-keratin monoclonal antibodies labeled only the prostatic basal cells, while another antibody with different specificity stained the columnar secretory cells in the single case of normal human prostate examined.

Utilizing the 2 well-characterized monoclonal antibodies of Gown and Vogel (14) which exhibit different specificities against keratin, we have examined keratin immunoreactivity in benign and neoplastic human prostatic epithelium. Our results show distinctive patterns of keratin immunoreactivity which may be diagnostically useful in distinguishing different types of epithelial cells in the prostate.

MATERIALS AND METHODS

Tissues. Human prostatic tissues were obtained from the files of the laboratory of Surgical Pathology, Stanford University Medical Center. Surgical procedures used to obtain these tissues included perineal needle biopsy, transurethral resection, radical cystectomy, and radical prostatectomy. All tissues had been fixed in 10% neutral buffered formalin for 12 to 24 h, embedded in paraffin, and serially sectioned at 5 μ m.

Antibodies. Two monoclonal antibodies with differing keratin immunoreactivity were purchased from Enzo Biochemicals, New York, NY. Antibody EAB 902 was raised against cytoskeleton derived from human hepatoma cells and recognizes a *M*, 54,000 keratin protein; antibody EAB 903 was raised against human stratum corneum and recognizes *M*, 49,000, *M*, 51,000, *M*, 57,000, and *M*, 66,000 keratins (14).

Immunohistochemistry. All reactions were carried out at room temperature. Tissue sections were deparaffinized with xylene followed by absolute ethanol. Endogenous peroxidase activity was blocked by immersing the slides in 0.3% hydrogen peroxide in absolute methanol for 30 min. Subsequent immunoreactivity was found to be considerably enhanced if the slides were exposed to a 0.1% solution of Pronase for 10 min before proceeding with antibody labeling (29).

Immunohistochemical staining was performed using the avidin-biotin-peroxidase complex (ABC) technique of Hsu *et al.* (30) (Vector Laboratories, Burlingame, CA). Normal horse serum was applied as a blocking agent to reduce nonspecific binding. Monoclonal antibodies against keratin were diluted 1:2000 and were applied overnight. Sections were developed with the chromagen 3,3'-diaminobenzidine (PolySciences, Warrington, PA) and lightly counterstained with Mayer's hematoxylin.

For each case, an adjacent section was stained with hematoxylin and eosin to confirm the histological findings and, in cases of prostatic adenocarcinoma, to evaluate the degree of tumor differentiation according to the Gleason grading system (31).

RESULTS

Tissue sections from 19 benign and 32 malignant human prostates were examined (Table 1). The results of immunohistochemistry are shown in Table 2. All grades of adenocarcinoma were represented. Premalignant change (intraductal dysplasia) was identified in 9 cases, each of which also exhibited invasive carcinoma in other areas of the same section. Basal cell hyper-

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Table 1
Keratin immunoreactivity in the human prostate: histology of cases examined

Histology	No. of cases examined		
	Anti-keratin antibody 902 (panepithelial)	Anti-keratin antibody 903 (basal cell-specific)	Both antibodies
Normal prostate	1	4	0
Nodular hyperplasia	14	15	11
Adenocarcinoma			
Gleason 1	3	3	3
Gleason 2	4	6	4
Gleason 3	11	10	10
Gleason 4	5	6	5
Gleason 5	5	6	4
Intraductal dysplasia	9	9	8
Basal cell hyperplasia	8	8	7
Transitional metaplasia	5	5	5

Table 2
Keratin immunoreactivity in the human prostate: immunohistological findings

	Anti-keratin antibody 902 (panepithelial)	Anti-keratin antibody 903 (basal cell-specific)
Normal secretory cells	+	-
Normal basal cells	+, -	+
Normal stroma	-	-
Secretory cells in nodular hyperplasia	+	-
Basal cells in nodular hyperplasia	+, -	+
Stromal cells in nodular hyperplasia	-	-
Adenocarcinoma (all grades)	+	-
Intraductal dysplasia	+	-
Basal cell hyperplasia	-	+
Transitional metaplasia	+	+

plasia was seen in 8 cases, and transitional metaplasia was present in 5 cases.

Antibody EAB 902 ("panepithelial" antibody) labeled all columnar secretory cells of normal and hyperplastic prostatic epithelium, as well as neoplastic epithelial cells (Fig. 1). The cytoplasmic staining was uniform and intense, and the degree of differentiation of neoplastic glands did not influence the staining pattern or the intensity of labeling. Basal cells in benign glands stained much less intensely than did the secretory cells of the same glands and in many cases were nonreactive. Foci of basal cell hyperplasia consistently failed to stain with antibody EAB 902, in contrast to transitional metaplasia, which was always positive. All acini and ducts exhibiting intraductal dysplasia also stained with this antibody.

The second anti-keratin monoclonal antibody, EAB 903 ("basal cell-specific" antibody), resulted in a different immunoreactive pattern (Table 2). Only the basal cells were labeled, while the secretory cells remained unstained (Fig. 2). All malignant glands failed to react with this antibody, including well-differentiated cancers (Gleason Grade I). All of the cells in foci of basal cell hyperplasia stained intensely. Intraductal dysplasia failed to stain, although in most ducts and acini exhibiting this lesion there was a basal layer which was decorated by this antibody. In many cases of intraductal dysplasia, focal disruption of the keratin-immunoreactive basal layer was noted. Transitional metaplasia stained with antibody EAB 903 as well as with EAB 902.

DISCUSSION

The present study indicates that there are characteristic patterns of keratin immunoreactivity in the benign and neoplastic

human prostate. Gabbiani *et al.* (13) and Schlegel *et al.* (22, 23) had previously reported differing results of keratin immunoreactivity in prostatic adenocarcinoma (the former obtaining positive staining, the latter negative). However, those investigators utilized polyclonal antibodies which were directed against different antigens, probably accounting for the conflicting results (Gabbiani used bovine muscle prekeratin; Schlegel used human stratum corneum). Additionally, Gabbiani used cryostat sections, which Schlegel's work was performed on paraffin-embedded formalin-fixed tissue. In our study, enzymatic predigestion considerably improved the immunostaining, giving results with formalin-fixed paraffin-embedded material comparable to that seen with cryostat sections.³ Interestingly, Schlegel's antibody stained only the basal cell layer of normal prostate. This pattern was identical to that seen with our basal cell-specific antibody; conversely, Gabbiani's antibody gave a pattern like our panepithelial antibody. These results confirm the presence of differing keratin epitopes in the basal cells and secretory (columnar) cells. It is intriguing that both the polyclonal antibody of Schlegel and the polyclonal antibody of Barwick and Mardi (27) were made against human stratum corneum, similar to the basal cell-specific monoclonal antibody which we used in this study.

The absence of immunoreactivity with the basal cell-specific antibody in prostatic carcinoma supports an earlier observation suggesting that malignant transformation of the prostate is accompanied by loss of the basal cell (32). The function of these basal cells is currently not known. Unlike the secretory cells, the basal cells are located at the periphery of the gland and have a characteristic axial orientation parallel to the basement membrane. Ultrastructurally, the basal cells are distinguished by the presence of many free ribosomes, few polysomes, little rough endoplasmic reticulum, absence of secretory granules, and prominent nucleoli. Similar cells have been found in the mouse prostate, and these have been called "myoepithelial" based on their morphological similarity to myoepithelial cells in the breast (33). We observed no immunological staining of cells in adenocarcinoma with the basal cell-specific monoclonal antibody, suggesting an absence of basal cells in prostate cancer. The absence of these cells, as demonstrated with this antibody, may be useful diagnostically in distinguishing benign and malignant prostatic tissue.

Basal cell hyperplasia is a benign process frequently encountered in the prostate, usually in association with nodular hyperplasia (34). It can be histologically confused with carcinoma, transitional metaplasia, or intraductal dysplasia. We found intense staining of foci of basal cell hyperplasia with the basal cell-specific monoclonal antibody and an absence of immunoreactivity with the panepithelial antibody. Transitional metaplasia, characterized by the replacement of simple columnar epithelium in the peripheral zone of the prostate by stratified transitional epithelium (urothelium), could be distinguished from basal cell hyperplasia by immunoreactivity for both of the monoclonal antibodies used in this investigation.

Intraductal dysplasia, characterized by proliferation and anaplasia of cells lining prostatic ducts and acini, is thought by McNeal and Bostwick (35) to be a precursor of invasive carcinoma. In its most severe grade, this lesion is felt to represent carcinoma *in situ*. The pattern of keratin immunoreactivity in this

³ Unpublished observations.

lesion was unique. The dysplastic cells lining the ducts and acini stained only with the panepithelial antibody; there was, however, in most ducts and acini exhibiting this lesion a basal cell layer decorated by the basal cell-specific antibody. In many areas of intraductal dysplasia, there was focal disruption of this basal layer with concomitant loss of keratin labeling by the basal cell-specific antibody. This finding, coupled with the observation that invasive carcinoma did not demonstrate immunoreactive basal cells, indicates that prostatic carcinoma shares some biochemical properties with the secretory cells, including pattern of keratin immunoreactivity, and that carcinoma may arise in the secretory cells (prostatic intraepithelial neoplasia). It is possible that the basal layer is breached and eventually obliterated in invasive prostatic adenocarcinoma. Alternatively, if the cellular profile of keratin proteins or keratin antigenicity changes during malignant transformation in the prostate, then the observed results would suggest that prostatic adenocarcinoma could arise in the basal cells.

Our findings indicate that the keratin immunoreactivity differs in the 2 epithelial cells of the prostate, probably due to expression of different keratin proteins. The fact that the antibodies used in this study recognize keratin proteins of different molecular weights, as shown by 2 dimensional gel electrophoresis, (14) supports this hypothesis. It is also possible that there is "masking" of antigenicity in the prostatic cells. It is clear that the combination of these 2 antibodies can assist in the differentiation of normal and hyperplastic epithelium from carcinoma. Additionally, transitional metaplasia, basal cell hyperplasia, and intraductal dysplasia can be distinguished.

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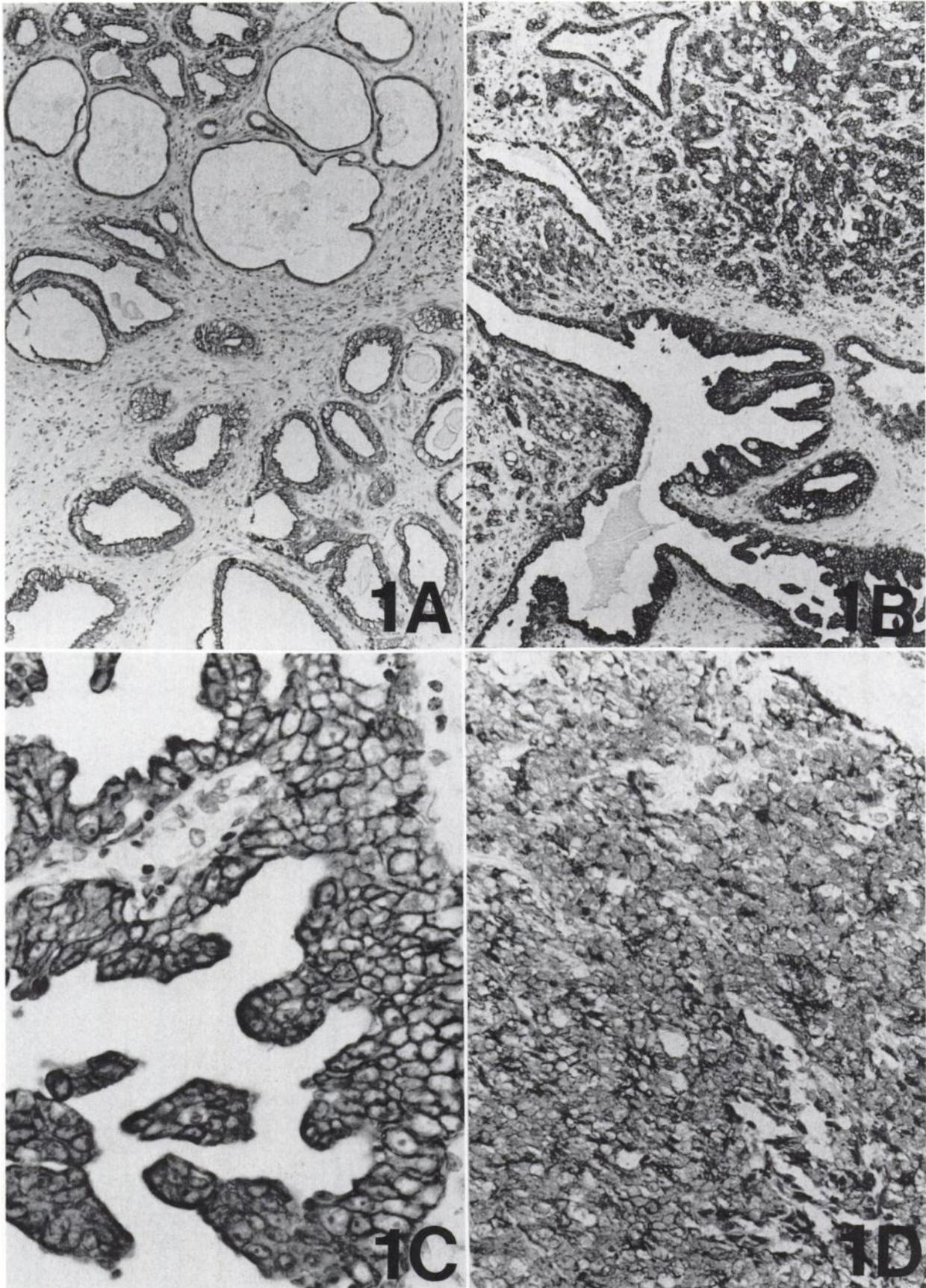


Fig. 1. Immunoperoxidase staining of human prostate with monoclonal anti-keratin antibody 902 (panepithelial antibody), showing intense cytoplasmic immunoreactivity in most normal and neoplastic epithelial cells. **A**, nodular hyperplasia. $\times 100$. **B**, moderately differentiated prostatic adenocarcinoma (Gleason Grade 4) and adjacent large duct exhibiting severe intraductal dysplasia. $\times 80$. Compare with **C**, showing higher magnification of keratin staining in intraductal dysplasia. Note prominent nucleoli in the dysplastic cells. $\times 450$. **D**, poorly differentiated prostatic adenocarcinoma (Gleason Grade 5) with keratin immunoreactivity in the majority of tumor cells. $\times 200$.

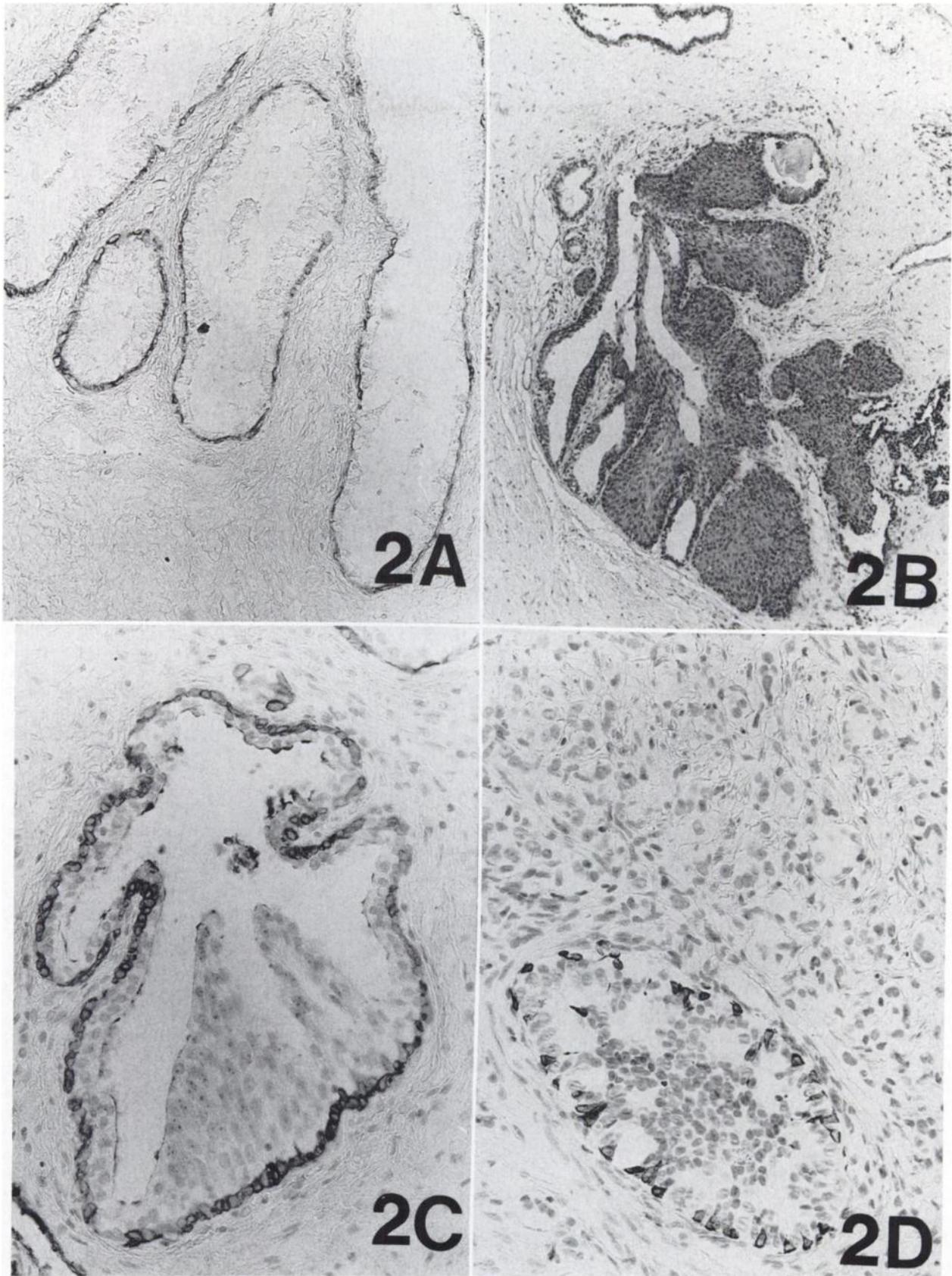


Fig. 2. Immunoperoxidase staining with monoclonal anti-keratin antibody 903 (basal cell-specific antibody), showing cytoplasmic immunoreactivity only within the basal cells. A, nodular hyperplasia. Note staining of the basal cell layer, with absence of staining in the secretory (luminal) cells. No counterstain. $\times 200$. B, basal cell hyperplasia. $\times 80$. C, severe intraductal dysplasia, with staining only within the basal cell layer (located peripherally). Note the focal piling up of the nonimmunoreactive dysplastic cells. $\times 250$. D, severe intraductal dysplasia and moderately differentiated prostatic adenocarcinoma (Gleason Grade 4) (same case as Fig. 1, B and C). A discontinuous immunoreactive basal cell layer outlines the duct which is filled with dysplasia; the duct is surrounded by nonimmunoreactive adenocarcinoma. $\times 250$.

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