Virchows Arch A (1986) 410:221–229

Virchows Archiv A

Biological tissue markers in benign and malignant disease of the human prostate

Philippe Chastonay, Jean Hurlimann, and Daniel Gardiol
Department of Pathology, University of Lausanne, Medical School, CH-1011 Lausanne, Switzerland

Summary. Immunoperoxidase techniques were used to study, the distribution of peanut agglutinin receptors, blood group isoantigens and several epithelial antigens in hyperplasia, adenosis, microcarcinoma and well differentiated adenocarcinoma of the prostate. Intraluminal and luminal surface PNA receptors were seen in all well differentiated carcinomas, 53% of microcarcinomas and 50% of adenosis, while no such sites could be demonstrated in benign hyperplasia. The expected blood group isoantigen was expressed in 75% of benign hyperplasias. When compared to the hyperplastic epithelium nearby, appropriate ABH expression was seen in 60% of adenosis, 47% of microcarcinomas and 25% of well differentiated carcinomas. A keratin antibody specifically labelling the basal cells in the normal prostate identified a subset of well differentiated carcinomas with preferential staining of the apical cytoplasm while microcarcinomas and adenosis were consistently negative. Our study establishes a highly ordered PNA receptor distribution in prostatic epithelia; it confirms early changes in the expression of ABH isoantigens in epithelial proliferative disorders of the prostate; it identifies a subset of keratin-positive well differentiated carcinomas, possibly of different ontogeny.

Key words: Prostate – Keratin – ABH-isoantigens – Peanut-agglutinin

Introduction

The biological behaviour of prostatic carcinoma varies widely among individuals for a given histological grade (Whitmore 1973; Kern 1978). Its precise distinction from benign proliferative disorders can be a challenge (Tannenbaum 1974; Dhom 1979). It would be of use to have methods allowing either the subdivision of existing histological grades into subgroups better related to prognosis, or to establish a more accurate diagnosis of borderline lesions. Although the value of immunoperoxidase staining techniques, as an useful adjunct to diagnostic pathology is well established, few immunohistochemical studies on prostate tissue sections have been reported. The present study was designed to investigate benign, borderline and malignant prostatic disorders for the distribution of epithelial antigens, blood group isoantigens and peanut agglutinin receptors. These markers were chosen since they were shown to be of interest in a preliminary study including 23 markers and because quantitative and qualitative changes in their tissue expression are known to occur in various tumours (Schlegel et al. 1980; Franklin 1983; Ucci et al. 1984; Overbeck v. et al. 1985). A study of several markers, rather than one, was chosen since it might enable us to establish the phenotype of diseased prostatic epithelia.

Offprint requests to: P. Chastonay, at the above address

Material and methods

Prostatic biopsy slides from the 1982 and 1983 surgical pathology files of our institute were reviewed: 10 adenosis, 15 microcarcinomas, 25 well differentiated adenocarcinomas and 20 benign hyperplasias were randomly selected. Adenosis was defined according to the criteria first described by Brawn (1982): ie well circumscribed lobules of atypical prostate glands lined by epithelial cells containing no to moderate nuclear pleomorphism including occasional nucleoli and possibly small areas of an infiltrating pattern made of prostate glands lined by columnar cells with cytologically benign nuclei. Microcarcinoma was defined according to criteria selected by Akazaki and Stemmermann (1973) in their study of latent prostatic carcinoma: ie atypical glands and acini (occasionally solid cords) with back-to-back arrangements lined by anaplastic epithelial cells of cuboidal or polygonal shape with frequently present, often nucleo-

lated, pleomorphic and basophilic nuclei; invasive growth always striking with destruction of surrounding muscle fibres. The group of well differentiated carcinomas was made of grade I adenocarcinomas according to the MDAH grading system (Brawn et al. 1982): ie 75–100% of the tumour forms glands, predominantly cribriform-papillary tumours excluded; all cases were stage C or D carcinomas. Benign hyperplasia refers to the nodular modification of the prostate due to fibrous and muscular proliferation of the stroma as well as to the glandular proliferation, glands being lined by a double layered epithelium, inner columnar and outer cuboidal, thrown up into papillary buds or infoldings (Moore 1943). Normal prostatic tissue specimens were also included in the study. Snap-frozen carcinomatous, hyperplastic and normal prostatic tissue specimens were also tested in order to establish the reproductibility of staining on formalin-fixed paraffin-embedded material.

6 µm thick sections of formalin-fixed and paraffin-embedded tissues were obtained for immunoperoxidase staining. The initial histological diagnosis was verified on a further step section. Frozen tissues 6 µm thick were cut in a cryostat. The sections were air-dried for 2 h, fixed in acetone for 10 min and kept at −20° C until immunlogical processing. After blocking the endogenous peroxidase with 1% hydrogen peroxide in absolute methanol, sections tested with conventional antisera were immunostained according to the PAP method of Sternberger (DeLellis et al. 1979), while sections incubated with lectins and monoclonal antisera were immunostained according to the biotin-avidin technique (Hsu and Raine 1982).

To asses the value of immunostaining without enzymatic digestion prior to the incubation with the specific antisera we treated snap-frozen and formalin-fixed paraffin-embedded specimens with protease (protease VII, Sigma, 8 mg/loml PBS, 10 min at 37° C) and with pepsin (pepsin from hog stomach, Fluka, 20 mg/10 ml 0.01 N HCL, 60 min at 37° C). Modification of PNA receptor disponibility was investigated through neuraminidase tissue digestion prior to incubation with PNA: neuraminidase (neuraminidase V, Sigma) was used at optimal conditions previously established on normal kidney, i.e. neuraminidase 0.01% in acetate sodium buffer 0.05 M pH 5.0 and 0.4 M $CaCl_2$ for 30 min at 37° C.

A preliminary study limited to 5 cases of adenosis, 5 microcarcinomas, 5 well differentiated carcinomas and 5 hyperplasias was performed in order to detect the most promising markers which in turn were tested on the larger number of cases.

Antisera specific for keratin were obtained in rabbits with injection of keratin isolated from stratum corneum of human callus according to Sun and Green (1978). These antisera showed diverse specificities and labelled differently the epithelial cells from various tissues. We used diluted 1/80 a keratin antibody specifically staining the basal cells of normal prostatic epithelium and of normal endocervical glands (Rabbit 673). This antiserum revealed 8 bands of 48 to 68 K_d on immunoblot of human plantar keratins. A monoclonal anti-epithelial antibody (mAB lu-5) used 1/4 was a gift from Dr. C. Stähli, Central Research Department, F. Hoffmann-La Roche Co, Basel. Anti-smooth muscle myosin used 1/70 was a gift from Dr. J. Costa, Institut de Pathologie, Lausanne. Monoclonal antibodies specific for keratin were obtained from Enzo Biochemicals, New York, NY, USA. One, EAB 902, was raised against cytoskeleton from human hepatoma cells; the second, EAB 903, was raised against stratum corneum: they both were used 1/500. EAB 902 could be used on formalin-fixed paraffin-embedded tissue without preliminary proteolytic digestion whereas with EAB 903 digestion was mandatory. Two antikeratin antisera (Rabbits Z622 and A575), used 1/80, monoclonal anti-isoantigens A, B, H, used diluted 1/60, 1/60, 1/120 respectively, swine anti-rabbit antiserum and rabbit peroxidase antiperoxidase conjugate were obtained from Dakopatts A/S, Copenhagen, Denmark and biotinylated serum specific for mouse IgG from Vector Lab., Burlingame, USA.

In the preliminary study antisera against fat globule membranes, secretory component, lactoferrin, prostate specific antigen, prostate specific acid phosphatase, Oxford CAl antigen and CEA were tested: these various antisera did not show particular patterns of interest for our study.

Lectins. Peanut agglutinin (PNA) specific for β-D-gal (1–3)N-acetyl-D-galactosamine, was used in optimal concentration giving maximal contrast: i.e. 40 µg/ml without previous digestion. Negative controls included lectin incubation with the specific blocking sugar according to established techniques (Leathem and Atkins 1983). In the preliminary study we also tested UEA, WGA, SBA, RCA, DBA and CONA. These lectins did not show interpretable differences between the four groups of lesions and were not used thereafter. Digestion with neuraminidase was also tried; it gave the expected effect with more sites stained, but the various binding patterns in the four groups of lesions became identical: therefore this digestion was discontinued. Biotinylated lectins were purchased from Vector Lab., Burlingame, USA, as was the avidin-biotin complex system. PNA inhibiting sugars were obtained from Fluka, Buchs, Switzerland.

Results

PNA. Four glandular binding patterns, quite often concurrently present, were observed: diffuse and patchy cytoplasmic binding, luminal surface binding and intraluminal secretion binding (Fig. 1). Little or no binding to collagen nor to smooth muscle fibres occurred. Binding was evident in as high as 35% of the glands (Fig. 2). Results are summarized in Table 1. While all well differentiated carcinomas showed some positive staining the percentage of negative cases was highest in benign hyperplasia while adenosis and microcarcinoma lay in between in this respect. No luminal surface nor intraluminal distribution of PNA receptors could be demonstrated in benign hyperplasia whereas such a distribution was seen in all well differentiated carcinomas. In the normal prostate no luminal nor intraluminal binding occurred.

Epithelial antigens. In benign hyperplasia the superficial columnar cells were consistently negative with the antikeratin antibody raised in our laboratory (Rabbit 673) whereas more than 80% of the basal cells were positive (Fig. 3). Similar binding was seen in normal prostate tissue. The basal cells failed to stain when incubated with an antimyosin antibody. No positivity could be demonstrated neither in adenosis nor in microcarcinoma. 4/25 well differentiated carcinomas showed a preferential apical cytoplasmic staining with a strong submembraneous rim in over 90% of their glands (Fig. 4) (Table 2). The results were similar with 2 commer-

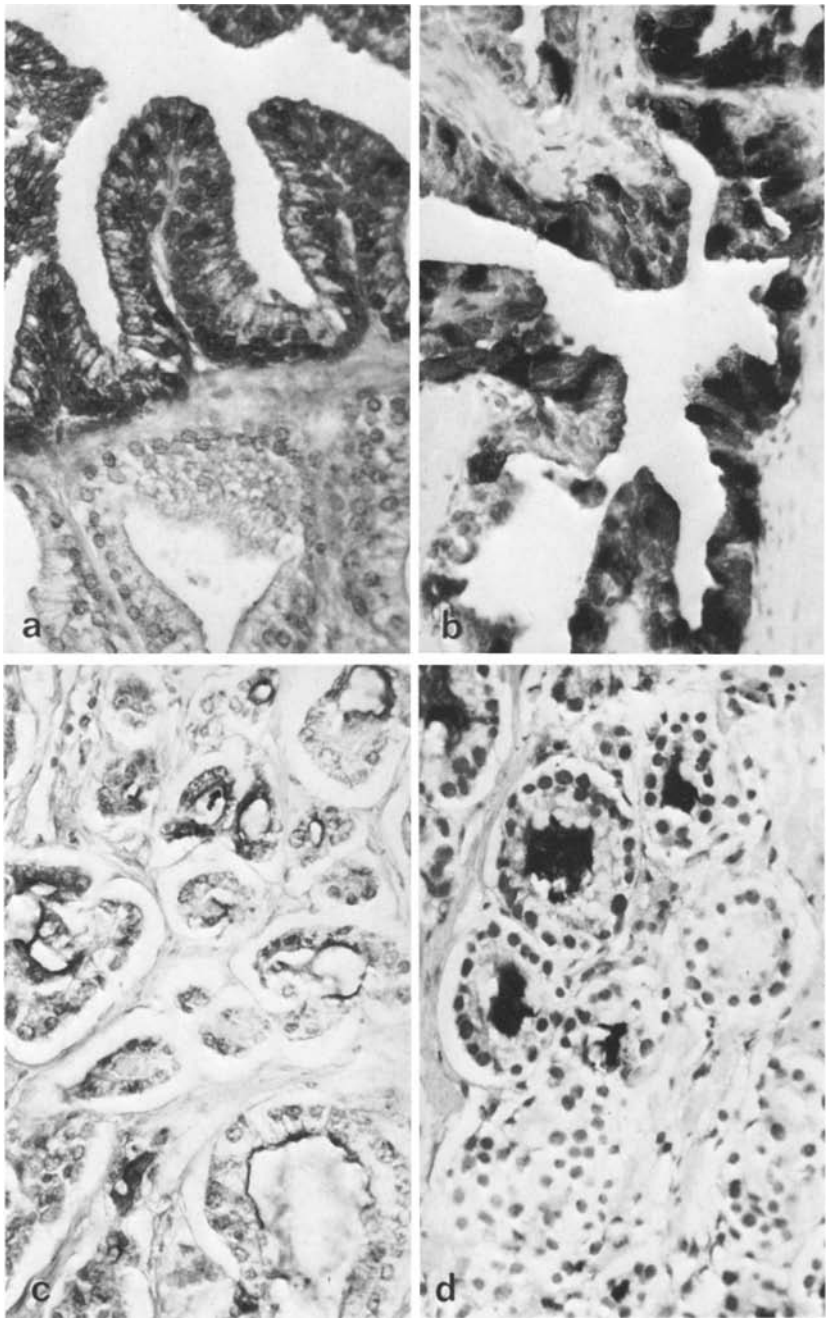

Fig. 1. Main immunoperoxidase binding pattern of PNA lectin in prostatic epithelial proliferative disorders: (**a**) diffuse cytoplasmic in hyperplasia (×320); (**b**) patchy cytoplasmic in hyperplasia (×320); (**c**) luminal surface membrane in carcinoma (×320); (**d**) intraluminal secretion in carcinoma (×320)

Table 1. Immunohistochemical distribution of PNA receptors (ABC technique) in epithelial proliferative prostatic disorders on paraffin sections

	Benign hyperplasia	Adenosis	Microcarcinoma	Well differentiated carcinoma
PNA −	7/20 (35%)	3/10 (30%)	5/15 (33%)	0/25 (0%)
PNA + luminal surface and intra-luminal secretion*	0/20 (0%)	5/10 (50%)	8/15 (53%)	25/25 (100%)
Diffuse and/or patchy cytoplasmic*	13/20 (65%)	7/10 (70%)	10/15 (67%)	11/25 (44%)

* percentage overlap due to concurrent staining

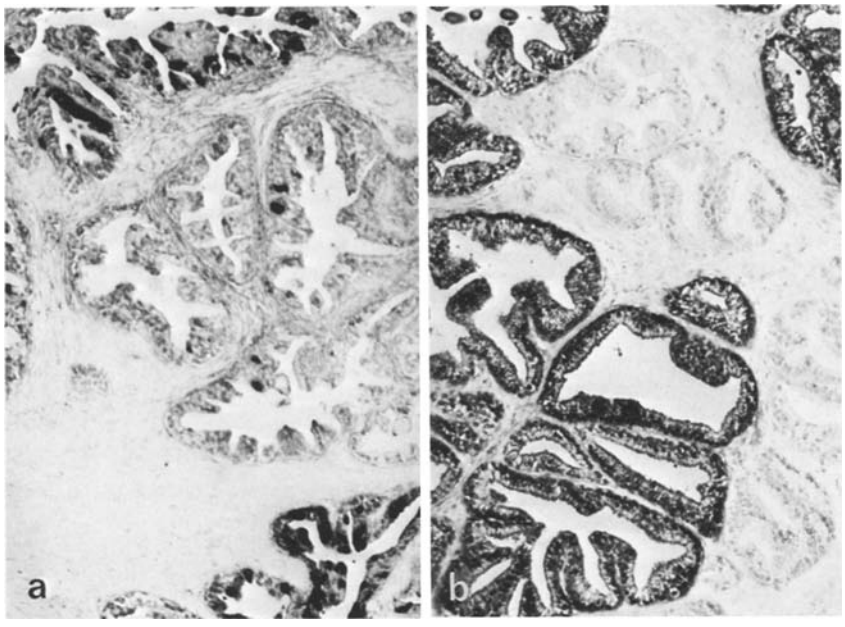

Fig. 2. Immunoperoxidase demonstration of PNA receptor distribution: cell heterogeneity outlined by variable staining: (**a**) patchy cytoplasmic staining (×80); (**b**) diffuse cytoplasmic staining (×80)

Table 2. Immunohistochemical distribution of keratin proteins (PAP technique) in epithelial proliferative prostatic disorders on paraffin sections

	Benign hyperplasia	Adenosis	Microcarcinoma	Well differentiated carcinoma
Keratin −	0/20 (0%)	10/10 (100%)	15/15 (100%)	21/25 (84%)
Keratin + basal cell	20/20	–	–	–
Cytoplasmic apex with strong submembraneous rim	0/20 (0%)	0/10 (0%)	0/15 (0%)	4/25 (16%)

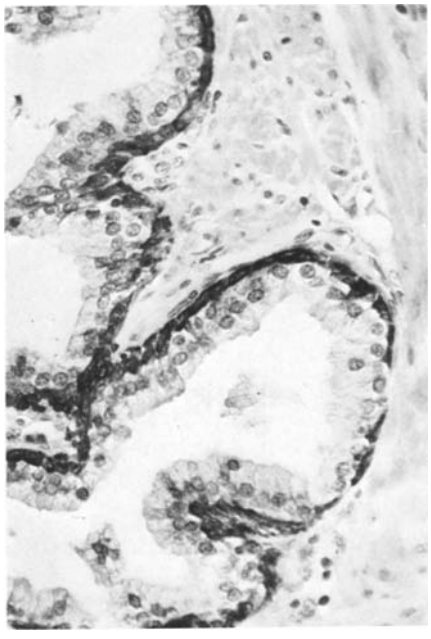

Fig. 3. Immunohistochemical localizaiton of keratin in benign hyperplasia with an antibody (Rabbit 673) to cytokeratins: basal cells are positive; columnar cells are negative (×320)

cially available antikeratin antisera (Dako Z622 and A575). EAB 903 revealed the basal cells of normal and hyperplastic prostatic epithelia as our polyclonal rabbit antiserum. When incubated after enzymatic digestion with EAB 903 2 of the 4 carcinomas that reacted with the polyclonal keratin antibodies showed similar positivity. EAB 902 revealed all the epithelial cells (basal and secretory) of the normal and hyperplastic prostate. It also revealed the carcinoma cells with a positivity of the whole cytoplasm.

Positive staining was seen in all cases incubated with the pan-epithelial antibody lu-5: diffuse cytoplasmic staining occurred as well in the basal as in the columnar cells which showed a predominant apical cytoplasmic positivty. Staining was seen in more than 75% of the glands without lesion-specific distribution.

Blood group ABH isoantigens. ABO blood group was identified by positive vascular endothelium staining on histological slides. It also served as a built in positive control. Glandular staining was

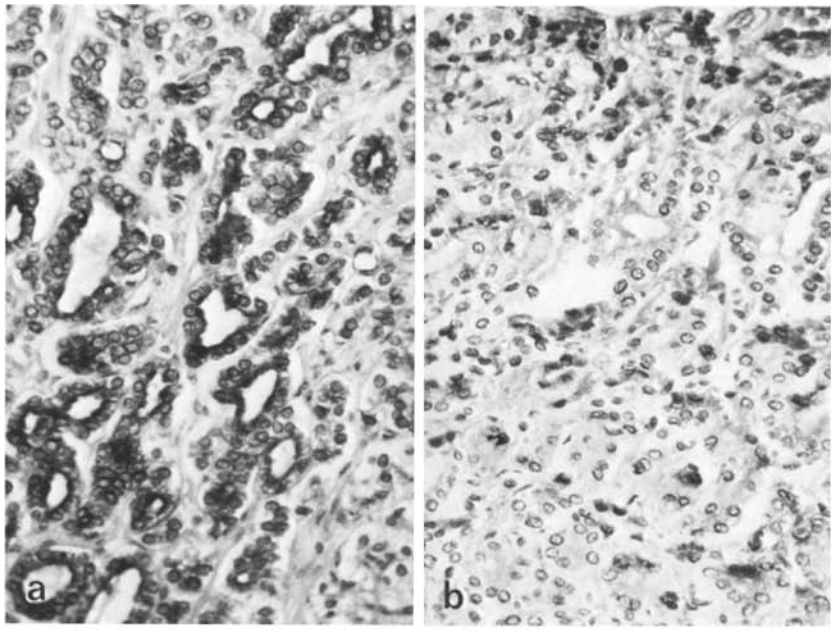

Fig. 4. Immunohistochemical localization of keratin in a subset of well differentiated carcinomas: **(a)** positive reaction with an antibody (Rabbit 673) to cytokeratins (×240); **(b)** negative control (×240)

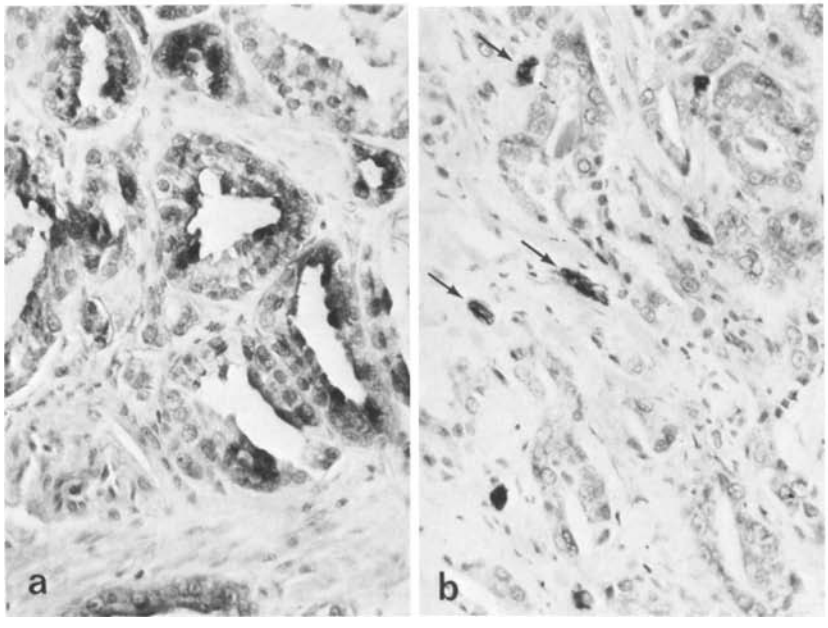

Fig. 5. Immunoperoxidase demonstration of ABH isoantigen expression: **(a)** inappropriate ABH expression in well differentiated carcinoma: vascular endothelium is negative, malignant epithelium is positive (×320); **(b)** loss of ABH expression in well differentiated carcinoma: vascular endothelium is positive (*arrows*), malignant epithelium is negative (×320)

intracytoplasmic as well as intraluminal. In most positive cases there were negative glands scattered among the positive ones (Fig. 5). In hyperplasia (n=20) the epithelial cells expressed the expected isoantigens except in 5 cases: i.e. 2 cases with blood group A expressed the isoantigen H; 1 case with blood group H expressed the isoantigen B; 1 case with blood group A and one with B expressed no isoantigen. In adenosis (n=10) 2 cases expressed the expected iso-antigen, 8 did not: 2 cases with blood group A, 1 with H and 1 with B expressed none; 2 cases with blood group A expressed 2 isoantigens, once H and A, once H and B; one case with blood group A expressed H and one with H expressed A. Comparing the lesion to the secretory status of the nearby glands rather than to the vascular endothelium yielded the following results: 6 cases expressed the same isoantigen; 1 case with an A secretory status expressed isoantigens A and H, 1 expressed H and B; 1 case with A secretory status and 1 with H expressed no isoantigens.

In microcarcinoma ($n=15$) only 3 cases expressed the expected blood group; 3 cases with

Table 3. Immunohistochemical ABH isoantigen expression (ABC technique) in epithelial proliferative prostatic disorders on paraffin sections as compared to ABH expression of the vascular endothelium or of the hyperplastic epithelium nearby

ABH expression of lesion	Benign hyperplasia	Adenosis	Microcarcinoma	Well differentiated carcinoma
Identical to vascular endothelium	15/20 (75%)	2/10 (20%)	3/15 (20%)	3/20 (15%)
Identical to hyperplastic epithelium nearby	–	6/10 (60%)	7/15 (47%)	5/20 (25%)

blood group H, 4 with A and 1 with B expressed no isoantigen; 1 with blood group A expressed H and B and 1 expressed all 3 isoantigens; 1 case with blood group H expressed A and 1 with B expressed H. When the secretory status of the nearby glands served as comparison 7 cases showed similar ABH status; 8 were different: 1 case with H secretory status, 1 with B and 2 with A expressed no isoantigen; 1 with A expressed H and B, 1 expressed all 3 isoantigens; 2 cases were nonsecretory: 1 expressed H and 1 expressed A.

In well differentiated carcinomas ($n=20$) 3 cases expressed the expected blood group, 17 did not: 6 cases with blood group H and 2 with B expressed no isoantigen; 5 cases with blood group A expressed H; 4 cases expressed more than one isoantigen, 1 H and 1 B expressed H and B, 1 A expressed A and H and 1 expressed all 3 isoantigens. Comparing the lesion to the secretory status of the nearby glands showed: 4 cases with H secretory status and 1 with A expressed no isoantigen; 3 cases with A secretory status expressed H and 1 with H expressed A; 3 cases expressed more than one isoantigen, 1 H expressed H and B, 1 A expressed A and H and 1 expressed all 3 isoantigens; 2 cases had similar ABH status.

Table 3 sums up the results of ABH expression of the various lesions studied compared to the vascular endothelial staining and to the secretory status of the nearby epithelial cells.

Discussion

In our preliminary study the results on formalin-fixed and paraffin-embedded tissues with and without protease digestion were similar; they were also identical with those obtained on frozen sections. Therefore we think that data presented here, though obtained without protease digestion, are valid.

PNA staining occurred in a minority of epithelial cells. An exclusive cytoplasmic PNA binding pattern was observed in benign hyperplasia as opposed to prominent luminal surface and intraluminal secretion PNA receptor distribution in well differentiated carcinoma. In this respect adenosis and microcarcinoma assumed an intermediate position.

Though lectin binding is known to be sugar specific most lectins do not detect glycoconjugates with terminal sialic acids without prior enzymatic tissue digestion (Brown and Hunt 1978): this could account for our observation of PNA staining being limited to a minority of glands in benign as well as in malignant epithelia. Such limited PNA staining has been reported in canine prostatic epithelium (Orgad et al. 1984) as well as in other epithelia (Franklin 1983). Reports of exclusively cytoplasmic PNA receptor distribution in the normal human prostatic epithelium (Bischof and Aumüller 1982) are confirmed and extended by our observations of similar distribution in benign hyperplasia, while such a distribution also occurs in carcinomas. In the breast such cytoplasmic PNA binding has been correlated with malignant transformation (Leathem et al. 1983; Lloyd et al. 1984). Our findings of a predominant luminal PNA binding in prostatic carcinoma differ from those of Orgad et al. (1984) and are opposed to reports of PNA receptor distribution in breast disease where luminal surface lectin binding is a hallmark of benign disease (Leathem et al. 1983; Lloyd et al. 1984).

Our study established a highly ordered distribution of PNA receptors in prostatic epithelia, a clear-cut difference appearing between benign and malignant epithelia. Though differences between the PNA binding patterns of malignant and non malignant epithelial cells have been reported (Louis et al. 1981 and 1983), it must be kept in mind that such variations might reflect functional or hormonal changes rather than malignant transformation: indeed according to some authors luminal PNA binding was exclusively limited to oestrogen modified cells (Orgad et al. 1984). Yet it might represent a way of differentiating borderline lesions from true carcinomas; if so the diagnosis of some cases of adenosis and microcarcinomas of the present study would have to be reconsidered.

Keratin protein localization was investigated with a heterologous antibody to total keratin which in the normal prostate specifically stained

the basal cells. With this antiserum (Rabbit 673) keratin proteins could be detected in the basal cells of all benign hyperplasias and at an apical cytoplasmic localization of a few well differentiated carcinomas. Keratin filaments located in the basal cells, as observed by us in benign hyperplasia and in the normal prostate, have previously been reported in the normal prostatec Schlegel et al. 1980b) and in other organs (Sun et al. 1979; Hurlimann and Gloor 1984). Our findings of keratin positive carcinomas differ from those of Schlegel et al. (1980a) who using a similar antibody could not demonstrate positive staining in prostatic carcinomas. The small number of carcinomas studied by these authors might account for the difference in results. Interestingly a recent survey (Achtstätter et al. 1985) using murine monoclonal antibodies (CK1 and Ks1818 directed against human cytokeratin polypeptide n° 18), as well as guinea pig antiserum (KV1 raised against bovine prekeratin VI and cross reacting with human keratin polypeptides n° 11 and n° 10) demonstrated differences in keratin expression of columnar and basal cells: indeed KV1 preferentially stained the basal cells while CK1 brightly stained the columnar cells but not the basal cells; Ks1818 stained both. Prostatic carcinoma and borderline lesions were unfortunately not investigated by these authors.

Used as a marker in prostatic disease, our antiserum to total keratin identifies benign hyperplasia but seems of no value in differentiating atypical hyperplasia from invasive carcinoma, though elsewhere it has been shown to be an indicator of cellular dysfunction (Hurlimann and Gloor 1984). However it identifies a well differentiated carcinoma subset, possibly of different metastatic potential and/or of different therapeutical response as has been suggested for various carcinomas of the same histological grade but harbouring a different immunological phenotype (Rognum et al. 1980; Klein et al. 1981; Wiley et al. 1981). Further it might identify a carcinoma subgroup of different ontogeny: i.e. malignant transformation of keratin positive basal cells would give rise to keratin positive carcinomas as has been speculated by some authors (Schlegel et al. 1980a), which seems all the more possible since the proliferative potential of the prostatic basal cells has been well documented (Kastendieck and Altenähr 1975).

Two commercially (Dako) available anti-keratin antisera gave the same results as the antikeratin raised in our laboratory. As to antikeratin EAB 902 it revealed cytokeratin in benign, borderline and malignant prostatic epithelia in accordance with data reported by others (Brawer et al. 1985). However none of the 28 prostatic carcinomas studied by these authors reacted positively with EAB 903 while 2 did in our series; their series included only 3 well differentiated carcinomas while ours was exclusively constituted of that kind of carcinomas. The lu-5 antibody, made against lung carcinoma cells, identified a cytoskeleton associated antigen which in our study showed a similar distribution as the cytokeratins identified by EAB 902. The lu-5 antibody therefore seems of no use as a diagnostic tool in our series though its value has been outlined in other circumstances (Overbeck v. et al. 1985).

Blood group ABH isoantigens. Frequent loss of the appropriate blood group isoantigen was noticed in diseased tissue other than benign hyperplasia. Non secretory status might partly account for this observation as it is well known that blood group isoantigen expression in body secretions is not universally present (Szulman 1966). Indeed when ABH isoantigen expression in the diseased epithelium was compared with that in the hyperplastic epithelium nearby, as has been suggested elsewhere (Kapadia et al. 1981), a far greater overlap was noticed. In this study using monoclonal antibodies no clear-cut difference between malignant and benign disorders could be established. This is in contrast with reports of various authors using the specific red cell adherence technique (Davidsohn 1972) who claim a 100% loss of ABH expression in prostatic carcinomas (Gupta et al. 1973a, Walker et al. 1984) as well as in other carcinomas (Gupta et al. 1973b). Our observations are in agreement with reports of variable degrees of loss of the blood group expression when immunoperoxidase techniques are used (Lee et al. 1984; Ernst et al. 1984). An inappropriate epxression as shown in the present study has been reported in gastric carcinomas and related to somatic mutation (Häkkinen 1970). We have observed the simultaneous expression of group H and another group or the loss of group A or B with the expression of group H; this has been demonstrated in gastric carcinomas (Slocombe et al. 1980) and seems due to the disturbance of the step-by-step elongation of the carbohydrate portion of blood group substances. Our data suggest that as well adenosis as microcarcinoma express the expected blood group more frequently than well differentiated carcinoma but quite less than benign hyperplasia. Quite similar progressive inappropriate ABH expression has been reported in borderline lesions of the oral mucosa (George et al. 1979).

Early change of the genetically predicted ABH

isoantigens in epithelial proliferative disorders of the prostate identifies a subpopulation of well differentiated carcinomas, possibly of different biological behaviour as reported in bladder carcinomas (Lange et al. 1978). Further investigations are required to determine to what extent our observations actually correlate with tumour prognosis and to establish their value in differentiating borderline lesions from malignant ones.

There is disagreement on the precise nature of adenosis: some authors consider it as precancerous (Helpap 1980; Kastendieck 1980) while others do not (Brawn 1982). The present study might contribute in settling the dispute should the PNA luminal surface binding pattern prove to be an early marker of malignant transformation; further investigations are certainly required. It remains to be seen whether carcinomas reacting with antibodies 673 to cytokeratins actually represent a distinctive subgroup of prostatic carcinomas. Some insight into this might hopefully be gained using new monoclonal antibodies specific to certain cytokeratin polypeptides. Our observations of prostatic tissue ABH expression, though of little value in differentiating borderline lesions, should contribute to the better understanding of tumour heterogeneity which might prove valuable with regard to the therapeutical considerations.

Acknowledgements. The authors wish to thank Mrs. J. Brautigam, M.M. Bertholet, C. Cattin and S. Burki for their technical assistance, Dr. M. Campiche for valuable suggestions during the preparation of the manuscript and Dr. C. Stähli and Dr. J. Costa for providing antisera.

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Accepted July 30, 1986