American Journal of Pathology, Vol. 131, No. 3, June 1988

Deletion of Antigens of the Lewis a/b Blood Group Family in Human Prostatic Carcinoma

WILLIAM W. YOUNG, Jr., PhD,
STACEY E. MILLS, MD,
MARGUERITE C. LIPPERT, MD,
PARVEZ AHMED, BA, and S. K. LAU, DVM

From the Departments of Pathology and Urology and the Cancer Center, University of Virginia Medical Center, Charlottesville, Virginia

The expression of antigens of the blood group Lewis a/b family were studied in a series of 42 prostatectomy specimens from patients with adenocarcinoma clinically confined to the prostate; 19 of these were later reclassified as pathologic Stage C. Staining of normal or hyperplastic *versus* neoplastic epithelium was assessed in routinely processed, paraffin-embedded tissue using murine monoclonal antibodies and an avidin–biotin immunoperoxidase technique. Antigens screened and the antibodies used to recognize them were Lewis a (CF4C4), Lewis b and Type 1 H (NS10), monosialosyl Lewis a I (19.9), and disialosyl Lewis a and monosialosyl Lewis a II (FH7). FH7 strongly stained the benign epithelium of all 39 Lewis positive cases, suggesting that the sialyltransferase responsible for synthesis of FH7-reactive determinants is highly active in benign prostatic tissue. When compared to the reactivity of benign epithelium in Lewis positive cases, the staining of the carcinomas was markedly reduced in 18 cases (46%) and absent in 16 cases (41%). This reduction or loss of staining of the malignant epithelium was observed for all antibodies that stained the corresponding benign epithelium of each case. In only five of the cases (13%) was the intensity of staining in the carcinoma equal to that of the surrounding benign epithelium. No cases in this latter group had recurrence of disease, whereas in the other staining groups 25–33% of the cases had recurrences; median follow-up for the entire group was 78 months. No correlation was apparent between Gleason score and the staining pattern with these antigens. In summary, antigens of the Lewis a/b family are deleted in a high percentage of cases of prostatic adenocarcinoma. (Am J Pathol 1988, 131: 578–586)

PROSTATIC CARCINOMA is the second most common tumor in men in the United States and the second leading cause of male cancer deaths.[1] Since the discovery of acid phosphatase as a tumor marker for prostate cancer in 1938[2] and the finding of androgen sensitivity of prostate cancer in 1941,[3] little progress has been made in the diagnosis or treatment of the disease. Expression of carbohydrate antigens has been found to be of use in the management of several types of human cancers. A correlation between deletion of blood group ABH antigens in transitional cell carcinomas of the urinary bladder and subsequent deep muscle invasion has been well established.[4] Recently, a similar correlation was proposed for deletion of antigens of the Lewis a/b blood group system in these tumors.[5,6] In tumors arising at other sites, monoclonal antibodies specific for carbohydrate epitopes have shown considerable promise, as illustrated by the successful treatment of several melanoma patients with monoclonal antibodies specific for the ganglioside GD3[7] and GD2.[8] Few studies have reported on carbohydrate antigens in prostate cancer. The only studies to date have found deletion of compatible blood group A and B antigens in the majority of prostate cancers.[9–12] In the current study normal/hyperplastic prostatic tissues and prostatic carcinomas were screened for expression of antigens of the Lewis a/b blood group family.

Materials and Methods

Patients

Specimens were obtained from a series of patients with nonfocal carcinoma confined to the prostate on

Supported by Grant No. PDT-335 from the American Cancer Society and a grant from the Research and Development Committee, University of Virginia School of Medicine.

Accepted for publication February 3, 1988.

Address reprint requests to William W. Young, Jr., PhD, Department of Pathology, Box 214, University of Virginia Medical Center, Charlottesville, VA 22908.

clinical examination who underwent radical prostatectomy at our institution between 1967 and 1983. The clinical status and pathologic features of this group were described previously.[13] Of the 68 cases described at that time, we have complete staining data and follow-up on 42; two patients were lost to follow-up, in three cases no tumor tissue was present on the available slides, and the remaining 21 cases did not have paraffin-embedded material available for study. The prostatic carcinomas in the available 42 cases previously had been graded histologically using the Gleason system[14] (Table 1). Thirty-one cases had capsular involvement, of which 19 were defined as pathologic Stage C due to capsular penetration or seminal vesicle involvement (Table 1).

Mouse Monoclonal Antibodies

Antibody CF4C4 specific for the Lewis a antigen was described previously.[15] Antibody FH7 specific for disialyl Lewis a[16] was kindly donated by Dr. S. Hakomori, Fred Hutchinson Cancer Center (Seattle, Wash). Antibodies 19.9 specific for sialyl-Lewis a[17] and NS10 which recognizes Lewis b[18] were kindly donated by Dr. T. L. Klug, Centocor, Corp. (Malvern, Pa). Ascites fluids containing antibodies CF4C4 and FH7 were used in the staining method at dilutions of 1:15,000 and 1:9000, respectively. Purified antibodies 19.9 (stock concentration 4.3 mg/ml) and NS10 (1.5 mg/ml stock) were used at dilutions of 1:8000 and 1:1000, respectively.

Glycolipid Plate Binding Assay

The specificity of antibody NS10 was evaluated with a plate binding assay performed as previously described[15] using Immulon 1 removawells (Dynatech, Alexandria, Va). The following glycolipids were kindly provided by Dr. K-A. Karlsson, University of Göteborg (Göteborg, Sweden): Lewis a glycolipid (Galβ1 → 3[Fucα1 → 4]GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer), Lewis b glycolipid (Fucα1 → 2Galβ1 → 3[Fucα1 → 4]GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer), lacto-N-fucopentaosyl (I) ceramide (Type 1 H; Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer), and lactotetraosylceramide (Galβ1 → 3GlcNAcβ1 → 3-Galβ1 → 4Glcβ1 → 1Cer).

Staining Method

Tissue sections, obtained from the files of the Division of Surgical Pathology of this institution, were fixed in 10% buffered formalin and embedded in paraffin. All tissue specimens were placed in fixative within minutes of their removal from the patients. Total prostatectomy specimens were routinely sliced in parallel sections at 2–3 mm intervals to aid in fixation. After fixation for 4–6 hours or occasionally overnight, 2–3 mm thick tissue blocks were trimmed for processing. Three specimens were biopsies of subsequently resected carcinomas, and the remainder were tissue blocks from total prostatectomy specimens. Sections were cut at 4–6 μ thickness and stained by the avidin–biotin–peroxidase complex method of Hsu et al.[19] Briefly, sections were deparaffinized and rehydrated through xylene and graded alcohols, after which endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol. Following blocking of nonspecific antibody binding with normal horse serum, sections were incubated with the mouse monoclonal antibodies for 30 minutes at room temperature. Serial sections were used when comparing the reactivities of different antibodies. The reaction was visualized by means of the avidin–biotin–peroxidase ABC kit (Vector, Burlingame, Calif) using 3,3′-diaminobenzidine (Polysciences, Warrington, Pa) as the substrate. Sections were counterstained with Harris hematoxylin and dehydrated prior to microscopic examination. Antibody staining of benign and malignant glandular epithelium was graded as positive if numerous glands, usually a majority of the total, showed prominent cellular staining. Weak staining corresponded to only rare cells in scattered glands. Negative staining implied a total absence of staining. In practice the cases segregated easily into these three groups.

To determine the antibody staining patterns of benign prostatic tissue from patients of known Lewis phenotype, Lewis blood group typing of erythrocytes from 10 patients was performed in the blood bank at the University of Virginia. The benign prostatic epithelium from all five Lewis a^-b^+ patients was strongly stained by NS10 and FH7 and weakly to moderately by 19.9; CF4C4 reacted moderately to strongly with three cases but weakly and in a scattered pattern in the other two cases. This pattern of Lewis a reactivity in patients with the Lewis a^-b^+ erythrocyte phenotype has been reported previously in gastric mucosa[20,21] and urothelium.[22] The benign epithelium from four Lewis a^+b^- patients was stained strongly with antibodies CF4C4 and FH7 and weakly to moderately with antibody 19.9. Two of these cases were unreactive with antibody NS10 in agreement with the erythrocyte phenotype, whereas two exhibited reactivity with NS10. These cases may be examples of the anomalous Lewis b expression in Lewis a^+ cases as described recently by Limas[22] in human urothelium and by Bjork et al[23] in human small intestine. The

Table 1—Clinical Follow-up and Pathologic Data of Prostatectomy Patients Grouped According to Lewis a/b Staining Patterns

Patient no.	Lewis a/b phenotype	Gleason score	Pathologic stage	Total survival (months)	Disease-free survival (months)	Status
Benign$^+$ tumor^{+*}						
1	a^+	7	B	69	69	DNED
2	b^+	7	B	104	104	ANED
3	b^+	9	B	78	78	ANED
4	b^+	7	B	82	82	ANED
5	a^+	7	NA	46	46	ANED
Benign$^+$ tumor^{weak}†						
6	b^+	7	B	98	95	DWD
7	b^+	7	B	68	68	DNED
8	b^+	7	B	131	131	ANED
9	‡	7	C	40	18	DWD
10	a^+	6	B	129	129	ANED
11	b^+	9	C	129	12	AWD
12	b^+	5	B	99	99	ANED
13	a^+	10	C	24	8	DWD
14	b^+	4	B	94	94	ANED
15	b^+	9	C	89	89	ANED
16	b^+	9	C	58	57	DWD
17	b^+	6	C	86	86	ANED
18	b^+	8	C	78	78	ANED
19	a^+	5	B	75	75	ANED
20	a^+	4	C	79	79	ANED
21	b^+	4	B	66	66	ANED
22	b^+	6	B	39	36	AWD
23	b^+	8	B	19	19	ANED
Benign$^+$ tumor$^-$§						
24	a^+	7	C	133	133	ANED
25	b^+	7	B	132	132	ANED
26	b^+	5	C	116	116	DNED
27	a^+	9	B	105	47	AWD
28	b^+	7	B	82	82	ANED
29	b^+	9	B	90	90	ANED
30	b^+	5	B	78	78	ANED
31	b^+	9	C	64	64	ANED
32	a^+	7	C	67	67	ANED
33	b^+	7	C	66	6	AWD
34	b^+	6	B	65	65	ANED
35	b^+	7	C	62	47	AWD
36	a^+	7	B	60	60	DNED
37	b^+	7	B	56	56	ANED
38	b^+	9	C	51	49	AWD
39	a^+	8	C	50	50	ANED
Benign$^-$ tumor$^-$‖						
40	–	7	C	130	74	AWD
41	–	8	C	82	82	ANED
42	–	8	C	39	39	ANED

DNED: died, no evidence of disease; DWD: died with disease; ANED: alive, no evidence of disease; AWD: alive with disease; NA: not available.

* Median total survival: 78 months; median disease-free survival: 78 months.

† Median total survival: 78.5 months; median disease-free survival: 76.5 months.

‡ Stained with antibody FH7 only.

§ Median total survival: 66.5 months; median disease-free survival: 64.5 months.

‖ Median total survival: 82 months; median disease-free survival: 74 months.

benign epithelium from a Lewis a^-b^- patient was unreactive with all four antibodies used in this study.

Results

Antibody Specificities

The antigens recognized by the panel of mouse monoclonal antibodies used in this study are shown in Table 2. Because publications of the specificity of antibody NS10 had indicated some reactivity with the H Type 1 structure,[18,24] we tested this antibody in a glycolipid plate binding assay. Figure 1 indicates that NS10 reacted nearly as strongly with the H Type 1 structure as with the Lewis b glycolipid. In addition, there was detectable cross-reaction with the Type 1 chain precursor structure found in lactotetraosylceramide, but there was no reaction with the Lewis a glycolipid. Thus, a broad spectrum of fucosylated Type 1 structures was tested with this panel of antibodies.

Benign Tissue Staining Patterns

In the prostatectomy series of 42 cases being evaluated in this retrospective study, three of the specimens were unreactive with all four antibodies and were therefore determined to be of the Lewis negative phenotype. In five of the remaining cases antibody CF4C4 strongly stained the benign epithelium (Figure 2) whereas NS10 staining was very weak or negative; these cases were classified as Le a^+b^-. The benign epithelium in the remaining 34 cases reacted strongly with NS10, whereas the staining with CF4C4 varied from weak to strongly positive; these were categorized as Lewis a^-b^+. In all of the Lewis positive cases, antibody FH7 strongly stained the benign epithelium (Figures 3 and 4). In contrast, the staining produced by antibody 19.9 was the weakest of the four antibodies tested.

The staining pattern produced by these antibodies in benign epithelium generally consisted of a homogeneous staining of the cytoplasm (Figures 2–4). In many cases secretions were also stained (Figures 2 and 4); the epitope recognized by antibody 19.9 was previously found on seminal plasma glycoproteins.[25] As described previously for the staining of prostatic tissue with blood group ABH monoclonal antibodies,[12] there was considerable variation in staining intensity between histologically similar areas within the same specimen. This variability was also seen in sections of fresh frozen tissue (data not shown), indicating that it was not due to fixation and/or embedding procedures. Similarly, in other tissues the Lewis a/b antigens, which are carried on both glycoproteins and glycolipids, have been found to be equally expressed in fixed and fresh frozen sections.[26]

In several cases the superficial cell layer of benign glands stained more heavily than the basal cell layer

Table 2—Determinants Recognized by Monoclonal Antibodies

Antibody	Determinant	Structure
CF4C4	Lewis a	Galβ1 → 3[Fucα1 → 4]GlcNAc–
19.9	Sialosyl Lewis a I	SAα2 → 3Galβ1 → 3[Fucα1 → 4]GlcNAc–
FH7	Disialosyl Lewis a	SAα2 → 3Galβ1 → 3[Fucα1 → 4, SAα2 → 6]GlcNAc–
	Sialosyl Lewis a II	Galβ1 → 3[Fucα1 → 4, SAα2 → 6]GlcNAc–
NS10	Lewis b and Type 1 H	Fucα1 → 2Galβ1 → 3[Fucα1 → 4]GlcNAc–
		Fucα1 → 2Galβ1 → 3GlcNAc–

Gal: galactose; Fuc: fucose; GlcNAc: N-acetylglucosamine; SA: sialic acid.

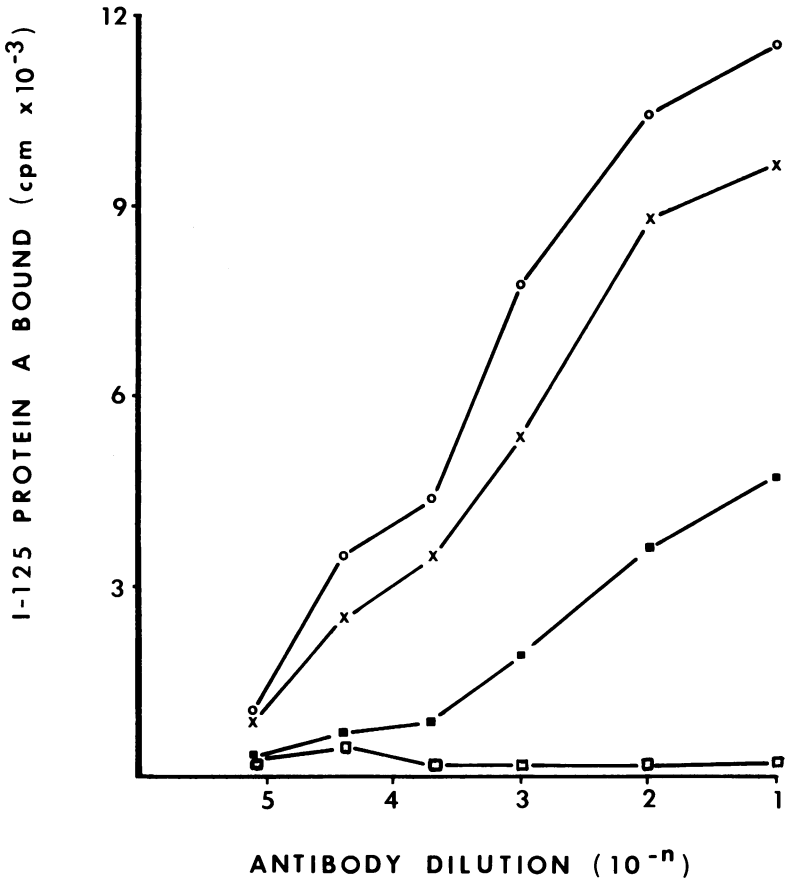

Figure 1—^{125}I-protein A plate binding assay. Wells of plastic microtiter plates were precoated with glycolipid, treated with bovine serum albumin to block nonspecific protein adsorption, and then incubated in succession with the indicated dilution of purified antibody NS10 (stock concentration 1.5 mg/ml), rabbit antimouse immunoglobulin, and ^{125}I-protein A. Individual wells were counted for ^{125}I activity in a gamma counter. Glycolipids: o, Lewis b active ceramide hexasaccharide; ×, Type 1 chain H active glycolipid (lacto-N-fucopentaosyl[I]ceramide); ■, lactotetraosylceramide; and □, Lewis a active ceramide pentasaccharide.

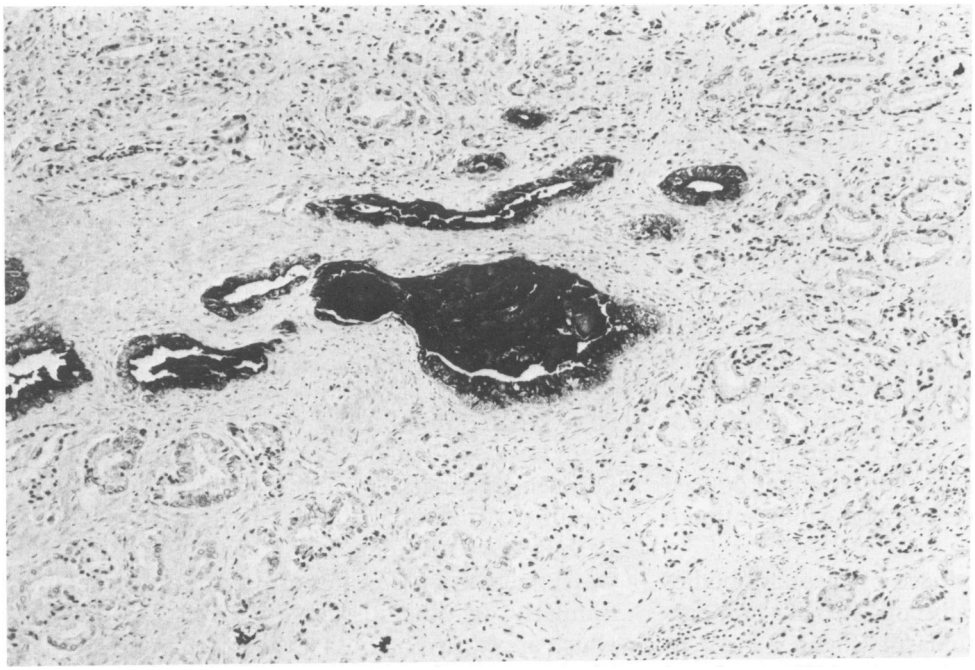

Figure 2—Prostate section stained with the Lewis a specific monoclonal antibody CF4C4 using the avidin–biotin–immunoperoxidase technique. Cells and secretions of the benign epithelial glands stain intensely while the surrounding tumor cells are unreactive. The surface layer of the benign epithelium stains more intensely than the basal layer. (×100)

(Figure 2). However, in other cases staining of many benign glands was restricted to the basal cell layer (Figure 3), whereas in other glands of the same sections both cell layers were stained. Such differences in staining between layers of the epithelium were generally found for all antibodies that stained that particular case. Ernst et al[27] observed in some colon carcinomas a differential staining of Lewis a/b such that areas stained for Lewis a were negative for Lewis b. We did not observe such differential expression of Lewis a and Lewis b in the two cell layers of benign prostatic epithelium. In most cases the staining of glands included the majority if not all of the cells in the gland (Figures 2 and 4). However, in several cases only a few scattered cells in each gland stained, suggesting reactivity with a subset of cells.

Staining of Prostatic Carcinoma

When compared to the reactivity of the benign epithelium, the staining of the carcinomas was markedly reduced in 18 cases (46% of the Lewis positive cases; Figure 4, Table 1) and absent in 15 cases (41%; Figure 2). This reduction or loss of staining of the malignant epithelium was observed for all of the antibodies that stained the corresponding benign epithelium of each case; ie, there was an apparent global deletion of all Lewis a/b antigens detected by the four antibodies tested. In only five of the cases (13%) was the intensity of staining in the carcinoma equivalent to that of the surrounding benign epithelium (Figures 5 and 6). The common finding of variability of staining intensity just described for the benign epithelium was also apparent in the malignant tissue. No case contained $CF4C4^+$, $NS10^-$ benign epithelium, and $NS10^+$ malignant glands, which would have indicated incompatible Lewis b antigen expression in the tumor tissue.

Clinical Follow-up

The median disease-free interval of these 42 patients was 71.5 months (range, 6–133 months) and the median total survival was 78 months (range, 19–133 months) postoperatively (Table 1). Thirty-one patients (74%) were clinically free of tumor at the end of the observation period. Four died with disease, two died of other causes, and five are alive with disease. Of the five patients in which Lewis antigen intensity was equivalent for both the benign and malignant tissue, none had a recurrence of disease during the follow-up period (Table 1). One patient in this group died from a second primary tumor. None of the cases were pathologic Stage C. In the group of Lewis antigen negative patients, one of three developed a recurrence of disease 74 months after surgery. All three were reclassified as pathologic Stage C due to capsular invasion or seminal vesicle involvement. Of the 18 patients in whom the benign epithelium was Lewis antigen positive and the malignant tissue was markedly reduced in staining, six suffered recurrence of disease and four died with disease. Eight patients in this group were reclassified as pathologic Stage C. Of the eight, three died with disease, one was alive with disease, and four were alive and disease free. In the 16 patients in which

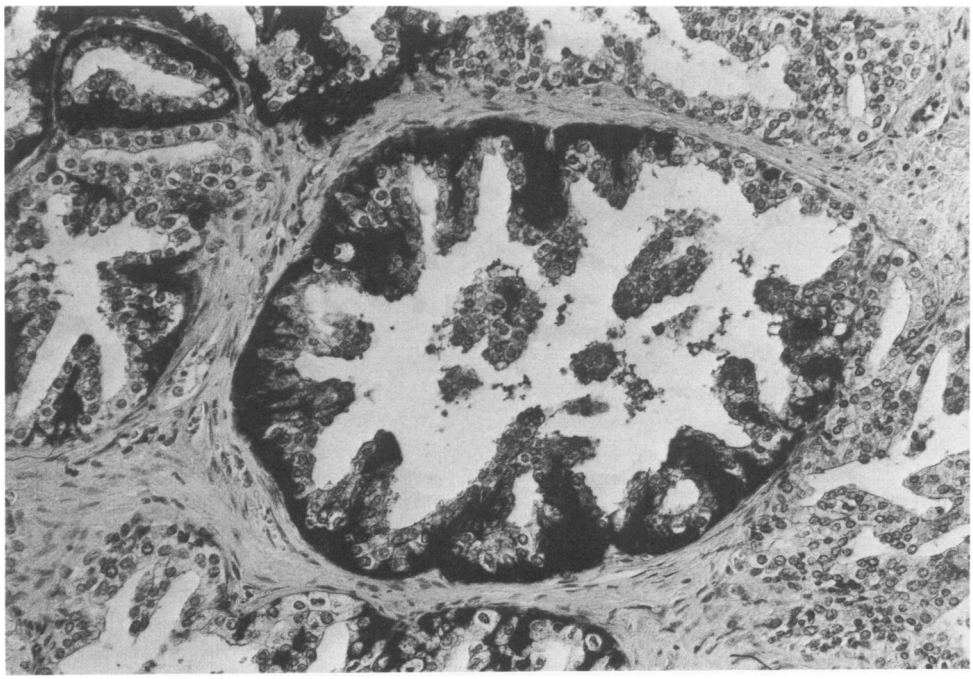

Figure 3—Benign prostate glands stained with anti-disialyl Lewis a monoclonal antibody FH7. Staining is restricted to the basal layer of cells. (×175)

the benign epithelium was Lewis antigen positive and the malignant tissue was negative, four had recurrence of disease. None of these died due to recurrence of disease, and one died of other causes.

Discussion

Studies on carbohydrate antigens in human prostate tissue have focused on blood group ABH expression. Using a red cell adherence assay, Gupta et al[9] reported negative staining of all carcinomas for the compatible ABH antigens, whereas areas of benign prostatic hyperplasia stained positive in 60% of the cases. Ghazizadeh et al[10] used the same assay and found 46% of benign hyperplastic samples to be antigen negative, whereas all adenocarcinomas were negative, regardless of the stage or degree of differentiation of the tumor. They concluded that, unlike the situation with bladder cancer,[4] ABH antigen expression

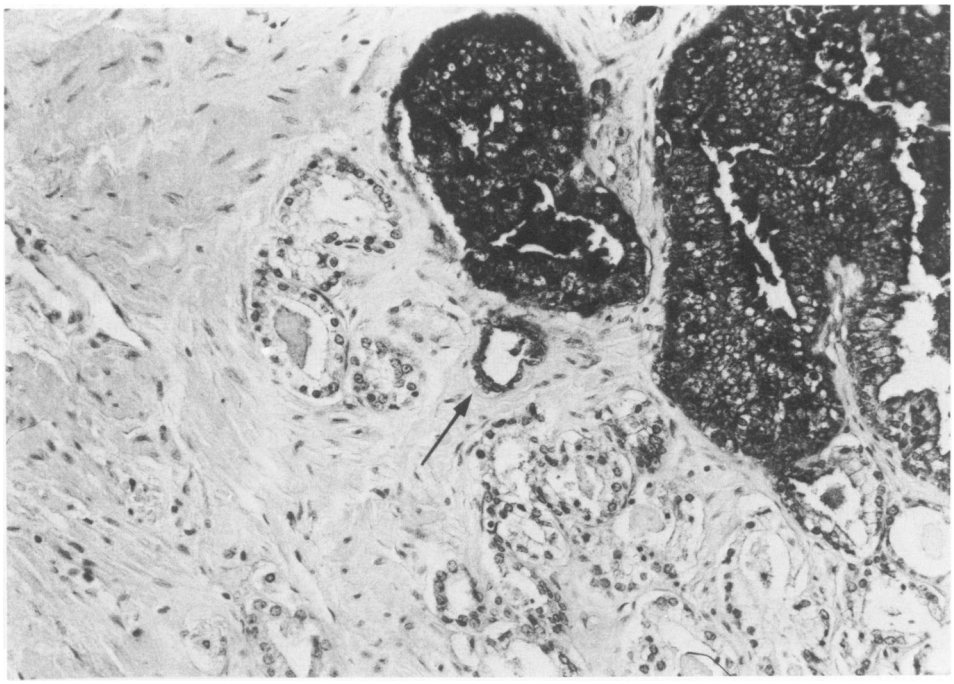

Figure 4—An example in which the benign epithelium expresses Lewis antigens while expression in the malignant glands is weak and focal. The benign glands and their secretions in the upper right portion of the field stain intensely with monoclonal antibody FH7. A few of the malignant glands in the lower half of the field show weak staining (arrow). (×175)

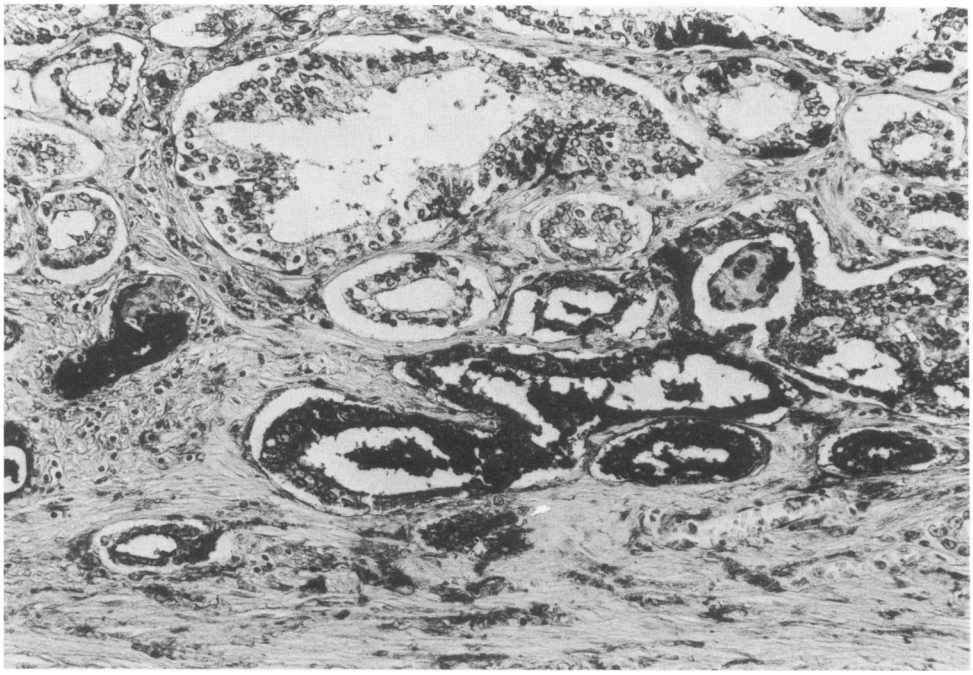

Figure 5—An example in which the benign and malignant glands express Lewis antigens with equivalent intensity. This field shows only malignant glands, some of which stain intensely in both the cells and secretions with antibody FH7. In other fields benign glands stained positive with this antibody. (×175)

was of no predictive value in prostate cancer. Walker et al[11] reached the same conclusion using a similar method. Using monoclonal antibodies and an immunoperoxidase staining technique, Vowden et al[12] found deletion of compatible A and B antigens in all prostatic tumors tested but preservation of the Type 2 chain H antigen and Y antigen in 10 of 12 tumors.

The results of the present study indicate the deletion of Type 1 chain fucosylated structures including the Lewis a/b antigens in a high percentage of cases of prostatic adenocarcinomas. In other naturally occurring, as well as experimentally induced malignancies, deletion of complex carbohydrate determinants has been found to be a common feature of the malignant phenotype.[26] In some cases this deletion has been accompanied by accumulation of precursor structures;[26] we have utilized such an accumulated precursor as a target for immunotherapy.[28] Future studies

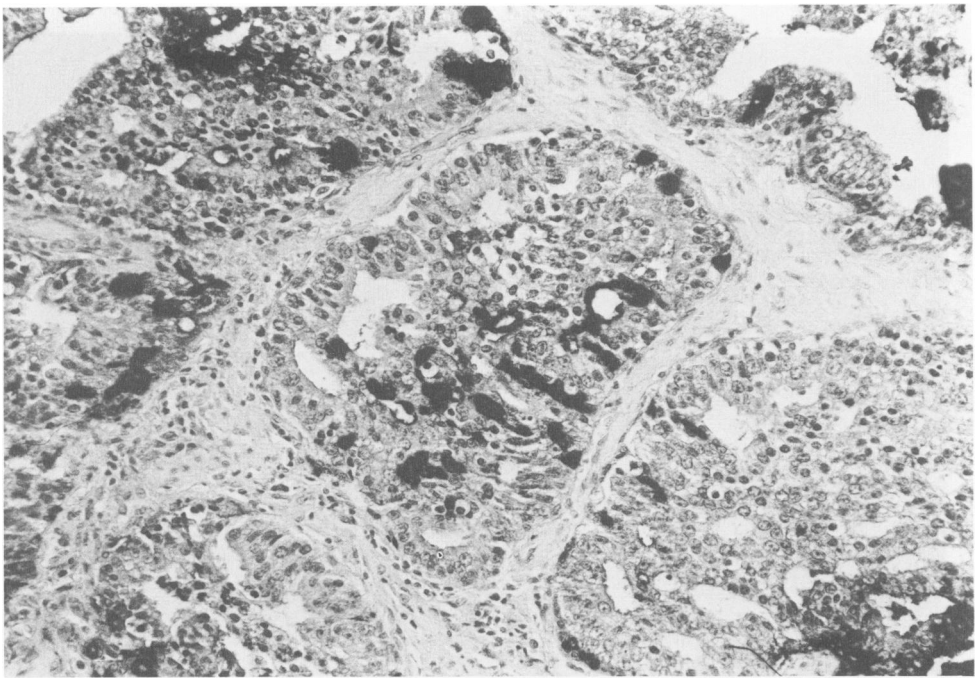

Figure 6—Staining of prostatic carcinoma, cribriform pattern with anti-Lewis b antibody NS10. (×175)

will attempt to detect such precursor determinants on prostatic carcinoma cells.

In all of the Lewis antigen positive cases, antibody FH7 stained the benign epithelium intensely, whereas antibody 19.9 was generally the weakest of the four antibodies tested. This staining pattern suggests that the sialyltransferase responsible for attachment of sialic acid to the N-acetyl glucosamine residue (structures in Table 2) is highly active in benign prostatic epithelium, converting potential 19.9 epitopes to FH7 reactive sites. Future studies will be required to determine the pathway of synthesis of the FH7 determinant, as has been accomplished for the epitope recognized by antibody 19.9.[29]

In the current study no correlation was apparent between deletion of the Lewis a/b family of antigens in carcinomas and their Gleason score. However, none of the five cases that retained strong expression of these antigens in the tumor cells had evidence of recurrence of the tumor following prostatectomy, whereas between 25% and 33% of the remaining cases suffered recurrences. Ten- to 15-year follow-up is frequently necessary to adequately evaluate the benefits of therapeutic changes in Stage B prostate cancer. Therefore, longer follow-up of these 42 cases may further demarcate the differences in total and disease-free survival in these four patient groups defined by staining patterns. In addition, study of a larger population will be required to determine if there is a significant correlation between deletion of Lewis antigen expression, pathologic stage, and tendency of the tumor to metastasize. If such a correlation does exist, it may be independent of conventional histologic grading systems such as the Gleason score.

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Acknowledgments

The authors thank Drs. S. Hakomori and T. Klug for kindly donating monoclonal antibodies and Dr. K-A. Karlsson for kind gifts of glycolipids.