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ABSTRACT

Seven mouse monoclonal antibodies and the lectin from Ulex europaeus, detecting blood group specificities of the ABH and Lewis systems, have been used to define the expression and/or modulation of these antigenic structures in human normal urothelium and tumors of the urinary bladder. The reagents employed recognize the following blood group related antigens: A, B, H, Lewis* (Le°), Lewis* (Le^), Lewis* (Le^), Lewis* (Le^), Lewis* (Le^), and type 1 precursor chain.

Immunohistochemical studies have demonstrated that these antigenic systems are differentially expressed in the urothelium of secretor and nonsecretor individuals. The normal urothelium of secretors is particularly rich in ABH blood group antigens as well as Le° and Le^ specificities. Nonsecretors, however, either lack or show decreased and patchy expression of H, Le°, and Le^ antigens. In general, areas affected by carcinoma in situ showed deletion of ABH, as did invasive carcinomas, as demonstrated by other investigators. This was not a universal observation, however, as variable expression of ABH antigens occurred in a few invasive tumors. Le° antigen was not expressed in normal urothelium except for occasional umbrella cells, but was demonstrated in the majority of invasive tumors, regardless of blood type and secretor status of the individuals studied. Le^ determinant, which was poorly expressed in the normal urothelium of nonsecretor individuals, was found in all tumors analyzed. An accumulation of non-fucosylated precursor structure was also a feature of invasive carcinoma, particularly in secretor individuals.

A panel of anti-blood group antibodies, encompassing A, B, Le°, Le^ and type 1 precursor chain specificities for use in patients of known secretor status, may provide useful early markers of malignant change in the urothelium.

INTRODUCTION

Determinants of the A, B, H, and Lewis blood group-related antigens represent an assortment of carbohydrate structures detected on erythrocytes and certain epithelial cells, and in body fluids and secretions (1, 2). The expression of blood group antigens in secretions and in tissue cells (other than erythrocytes) are under separate control (3, 4). Their presence in epithelial cells and secretions depends on the secretor status of the individual (4, 5).

Blood group antigen expression in normal and neoplastic urothelium has been a major focus of interest for years, at least in part because of the potential clinical relevance of several observations: (a) loss of ABH antigens in transitional cell carcinoma (6–9); (b) predictive value with respect to invasion by urinary bladder tumors after ABH deletion (10–14); (c) correlation of abnormal patterns of Lewis^ and Lewis^ with histological grade of transitional cell carcinomas (15); (d) possible prognostic value of Lewis^ expression in Stage P3 transitional cell carcinomas (16). However, most of the previous studies concerning blood group-related antigens and their expression in normal and neoplastic urothelium have been performed with restricted panels of antibodies, mainly those detecting ABH and/or Le° antigens. Moreover, these studies usually lack information on secretor status and in general they do not include comparative studies of normal and neoplastic tissue samples from the same individual.

This report makes use of a wide panel of well-characterized mouse mAb2 and Ulex europaeus lectin with specificities for A, B, H, Lewis^, Lewis^, Lewis^, Lewis^, and precursor type 1 chain antigens to characterize their immunopathological expression and modulation in urinary bladder tumors, and to further define the anatomic distribution of these antigens in normal urothelium (17). Furthermore, the present analysis correlates the antigenic profiles of normal and neoplastic urothelium from each case with blood type and secretor status of the individuals under study.

MATERIALS AND METHODS

Tissues

Normal and neoplastic tissues were obtained from surgical pathology specimens within 1–2 h of resection. Fresh tissues were fixed in 10% formaldehyde in PBS, pH 7.5, and embedded in paraffin.

Cystectomy specimens from 19 patients with urinary bladder tumors were obtained from the present study. Medical histories of these patients were reviewed and Table 2 summarizes patient’s information, including therapy prior to cystectomy. In the majority of the cases we were able to examine normal urothelial mucosa adjacent to the tumor, urothelial mucosa distant from the tumor, carcinoma in situ and invasive urothelial carcinoma. Selection of cases was based on excellence of tissue preservation, availability of tissue blocks of normal and neoplastic urothelium, and knowledge of secretor status of the individuals under study. From two to nine tissue blocks were available on each case, and sections from every block were studied with the entire panel of antibodies. All tumors were classified by stage and histological grade and pattern. The patients included eight blood group O, six group A, three group B, and two group AB individuals. Secretor status was determined in all cases either by presence of Lewis antigens in saliva and/or in samples of peripheral blood red blood cells drawn from these patients (18).

Reagents

Purified agglutinin I from Ulex europaeus at 4 µg/ml (Vector Laboratories, Burlingame, CA) served to identify the H antigen. Mouse mAb HIT 29–36 (T-36) recognizing A antigen (all variants) (19), mAb S8 detecting B antigen (20), mAbs T-174, T-218, P-12, and F-3 with specificities for Le°, Le°, Le°, and Le^ antigens, respectively, were also used (19, 21, 22). Finally, precursor type 1 chain antigen was detected by mAb K-21 (22). The antibodies were used as undiluted culture supernatants, or purified immunoglobulin preparation at an approximate concentration of 25–40 µg/ml (Cambridge Research Laboratories, Cambridge, MA).

Immunohistochemistry

The method chosen for the present analysis was the avidin-biotin complex immunoperoxidase technique (23, 24). Formalin-fixed and
paraffin-embedded tissue sections were deparaffinized, then treated for 30 min in 1% hydrogen peroxide in PBS to remove endogenous peroxidase activity (no staining was observed when 1% periodic acid was used instead of 1% hydrogen peroxide). Tissue sections were washed in PBS and then incubated with 10% blocking normal serum (heat inactivated) and 1% hydrogen peroxide in PBS for 20 min. Blocking normal serum was drained off and sections were incubated with mAb overnight at 4°C. The secondary antibodies were biotinylated horse anti-mouse IgG or goat anti-mouse IgM (Vector Laboratories, Burlingame, CA). They were incubated on sections for 1 h, the sections were then washed and incubated with the avidin-biotin complex for 30 min. The peroxidase reaction was performed by incubating tissue sections for 6–12 min with 5 mg of diaminobenzidine tetrahydrochloride (Sigma Chemicals, St. Louis, MO) in 100 ml of Tris buffer containing 100 μl of 0.3% hydrogen peroxide. Sections were washed with distilled water, counterstained with hematoxylin, and mounted with permount.

Immunoperoxidase Analysis Using a Lectin. The lectin **Ulex europaeus** was incubated on paraffin-embedded tissue sections for 2 h at room temperature. Sections were washed with PBS and incubated with goat anti-**Ulex** lectin antibody (1:1000 dilution) (Vector) overnight at 4°C. The immunoperoxidase method was performed as described above using biotinylated rabbit anti-goat immunoglobulins as secondary reagent.

Controls. Paraffin-embedded tissues expressing the appropriate blood group antigen were used for titration of the reagents as well as positive and negative controls. Negative controls included substitution of the mAb by another mAb of the same species and subtype, or incubation with PBS alone.

RESULTS

Table 1 summarizes the derivation of the panel of mAbs, their immunoglobulin subtype, and their specificity for blood group antigens. Tables 2 through 5 summarize the clinical information and immunoreactivities of these antibodies on sections of normal urothelium, carcinoma *in situ*, and invasive urothelial carcinoma of the urinary bladder; correlating blood type and secretor status of the individuals. Figs. 1–3 illustrate the immunohistological staining patterns of these mAbs with normal urothelium, carcinoma *in situ* of the urinary bladder, and invasive urothelial carcinomas.

**Blood Group Expression in Normal Adult Urothelium**

As previously reported, antibodies detecting blood group antigens were found to be differentially expressed in normal urothelium of secretor and nonsecretor individuals (15). Tables 3–5 include the reactivities observed in tissue sections of histologically normal human urothelium from all individuals analyzed in the present study: Le~"~b~"~ (secretors), Le~""~b~ (nonsecretors), and Le~"~b~"~ (secretors or nonsecretors) individuals.

*Anti-A (T36) and anti-B (S8) antibodies reacted only with tissue specimens from blood group A-positive and B-positive individuals, respectively. They were also expressed by the two AB individuals tested. In each case, endothelial cells and erythrocytes were found to stain with the corresponding antibody or antibodies. Urothelium of secretors was immunoreactive throughout (Fig. 1A), with some variation in staining intensity and usually greater reactivity in basal cells. Urothelium of the one evaluable nonsecretor (Case 15) was group AB and unreactive for both A and B antigens (Table 4). Urothelium of Le~"~b~"~ individuals (Table 5) showed strong A and weak B antigenic profiles.*

Expression of H antigen was observed in endothelial cells and erythrocytes of all specimens studied, regardless of secretor status, as previously described (17, 25). Histologically normal urothelium of type O secretors expressed H antigen either throughout the mucosa (Fig. 1D), or with intense immunostaining of the basal layers. H antigen was undetected in normal urothelium of the four type A secretors and there was patchy staining in one of two type B secretors (Table 3). In the nonsecretors, there was staining of basal epithelium in two of four type O cases (Fig. 1G), and no staining in the AB case.

**Lewis antigens were expressed on normal urothelium with distinct patterns of reactivities. Lewis* (T174) in secretors was found to be positive in the superficial epithelial cell layers and weak or absent in deeper cell layers in eight patients (Fig. 2A), while in two samples, both from type A patients, there was no staining of the urothelium. In nonsecretors, Lewis* showed at least patchy reactivity in three of four group O cases (Fig. 3A) and was negative in one group O and one group AB case; while in the Le~"~b~"~ individuals staining was observed only in one group B case (Table 5). Lewis* (T218) was expressed in the urothelium of all secretors with intense immunoreactivity (Fig. 2D). Three of five nonsecretors lacked expression of Lewis* in their normal urothelium; the remaining specimens showed patchy staining, mainly found in basal and suprabasal cell layers (Fig. 3D). Expression of Le* paralleled that of Le~"~b~"~ individuals, being only partially immunoreactive in two cases and negative in two cases (Table 5). The reactivity of anti-Le* (P12) was absent or weak except for an occasional umbrella cell, regardless of secretor status (Figs. 2G and 3G). Le* determinant (F3) was detected in endothelial cells and erythrocytes in O individuals. The reactivity with normal urothelium of secretors was intense and homogeneous (Fig. 2J), with the
exception of one case in which only patchy staining was observed (A specimen) (Table 3). Nonsecretor individuals either lacked expression of Le' determinant (four of five cases) (Fig. 3J) or showed patchy basal staining in the urothelium. Of the four Le'~b~ individuals, two showed luminal immunoreactivities and the other two were unreactive.

Finally, the precursor type 1 chain (K21) was not detected in any normal urothelium of secretor individuals (Table 3). However, immunoreactivity was observed in one of five nonsecretors and one of four Le'~b~ individuals, mainly in basal and suprabasal cell layers (Table 4).

Blood Group Expression in Areas of Neoplastic Urothelium

Table 2 summarizes patient's age, sex, blood group type, secretor status, treatment prior to this study, and number of blocks analyzed. Tables 3–5 summarize immunoreactivities observed in the normal urothelium compared with in situ and invasive carcinoma from the same individual.

Blood Group Antigens in Flat in Situ and Noninvasive Papillary Carcinoma. Areas of CIS were identified in 14 of 19 cases; the appropriate blood group antigen was expressed in normal epithelium of 11 of the 14 cases. Deletion of ABH antigens was noted in the neoplastic epithelium of seven of these 11 cases (Fig. 1B). The remaining four cases showed variable and patchy patterns of reactivities (Fig. 1E). In one case (type A specimen, Le'~b~ individual) (Case 17) H antigen was expressed homogeneously throughout the in situ and invasive tumor but only in luminal epithelium in benign areas. It should be emphasized that some nonsecretor individuals lacked expression of these antigens in their normal urothelium, leaving only two evaluable cases with areas of CIS to study in individuals expressing the antigen in normal epithelium. Two type O nonsecretors were negative for H antigen in areas of CIS, as well as in normal mucosa (Cases 11 and 14) (Fig. 1H). An AB nonsecretor was unreactive for H, A, and B antigens in normal and all neoplastic urothelium. Specimens from Le'~b~ individuals showed variable patterns of immunoreactivities (Table 5).

Lewis' antigen in areas of CIS did not differ appreciably from normal urothelium in the majority of the cases. It was expressed in seven of nine secretors, one of three nonsecretors (Fig. 3B), and one of two Le'~b~ individuals. The pattern of reactivity was luminal, as in the normal urothelium, or patchy. The remaining cases, two secretors (Fig. 2B), two nonsecretors, and one Le'~b~ individual, were unreactive for the Le' determinant.

Lewis' antigen was homogeneously stained in areas of CIS in three of nine secretors (Fig. 2E), while in the remaining cases there was heterogeneous reactivity. Similarly, in nonsecretors and Le'~b~ individuals Le' expression mimicked that of normal urothelium, being positive in two of five cases (Fig. 3E).

Lewis' determinant expression appeared in areas of CIS in seven of nine secretor individuals, showing a strong and patchy distribution (Fig. 2H). Two of three nonsecretors and the two Le'~b~ individuals who were tested were immunoreactive with

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**Table 3** Secretor individuals: Antibodies and lectins detecting blood group antigens

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* BG, blood group.
* Analysis of formalin-fixed paraffin-embedded tissue sections using immunoperoxidase techniques (avidin-biotin). N, normal urinary bladder; C, carcinoma in situ; T, primary urinary bladder invasive tumor. PS, blood group precursor substance.
* H, homogeneously stained; B, basal immunostaining; L, luminal immunostaining; D, heterogeneous stained; O, undetectable immunoreactivity.

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**Table 4** Nonsecretor individuals: Antibodies and lectins detecting blood group antigens

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* Abbreviations as in Table 3.
* Symbols as in Table 3.
* Immunoreactivity was observed only in occasional umbrella cells.

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**Table 5** Lewis'~b~ individuals: Antibodies and lectins detecting blood group antigens

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* Abbreviations same as in Table 3.
* Symbols same as in Table 3.
* Immunoreactivity was observed only in occasional umbrella cells.
an heterogeneous pattern of staining (Fig. 3H).

Lewis\(^*\) determinant was universally expressed in all areas where CIS was identified, regardless of blood type and secretor status; uniform immunoreactivity was observed in six of 14 patients and variable staining occurred in the remaining eight (Figs. 2K and 3K). Thus, expression was similar to that seen in normal urothelium. However, in two nonsecretors and one Le\(^*\)\(^{-}\)\(^{-}\) patient areas of CIS were positive whereas the normal tissue was negative.

Precursor type 1 chain determinant was absent in areas of CIS in 13 of 14 cases in which it could be evaluated; one O-type nonsecretor showed patchy immunoreactivity (Table 4).

Blood Group Antigens in Invasive Bladder Tumors. A antigen of tumor cells was deleted in three of four A-type secretors (Fig. 1C), and diminished in the other one, which showed heterogeneous staining. It was similarly diminished on invasive tumor cells in the two A-type Le\(^*\)\(^{-}\)\(^{-}\) individuals. A antigen was undetected in the AB-type nonsecretor, and lost on invasive tumor cells in the AB-type Le\(^*\)\(^{-}\)\(^{-}\) patient.

B antigen was deleted in tumor cells of the two B-type secretor cases and a B-type Le\(^*\)\(^{-}\)\(^{-}\) individual. The AB-type nonsecretor was unreactive for B antigen; however, the AB-type Le\(^*\)\(^{-}\)\(^{-}\) individual showed patchy expression of B antigen in a subpopulation of tumor cells.

H antigen was homogeneously expressed in invasive urothelial carcinoma in three of 19 cases (Fig. 1F), while patchy immunoreactivity was observed in 10 cases. Three O-type, one A-type, one B-type, and one type-AB cases lacked H antigen reactivity in invasive tumor (Fig. 1F), but in five of these six cases the normal urothelium was also nonreactive so this could not be considered an antigenic deletion.

Lewis\(^*\) antigen was expressed by tumor cells in seven of 10 secretors and three of nine cases of nonsecretor and Le\(^*\)\(^{-}\)\(^{-}\) individuals. In five cases Le\(^*\) antigen was present in normal urothelium and deleted in invasive tumor cells (Fig. 2C); in three cases it was expressed in tumor but not in normal epithelium. The immunoreactivities observed were heterogeneous in most cases, but diffuse immunostaining was found in two B and one A type specimens (Fig. 3C).

Lewis\(^*\) antigen was expressed by invasive tumor cells in all secretor cases (Fig. 2F), with various patterns of reactivities; six of 10 cases were homogeneously stained and the rest showed
BLOOD ANTIGENS IN URINARY BLADDER TUMORS

Fig. 2. Localization of Lewis blood group-related antigens in normal urothelium, CIS, and invasive urothelial carcinoma of the urinary bladder of a secretor (Table 3: Case 4). A, Lewis~ antigen heterogeneously expressed in normal urothelium; B, deletion of Lewis~ antigen in CIS; C, Lewis~ antigen was not detected in invasive tumor cells in this case; D, Lewis~ antigen homogeneously expressed in normal urothelium; E, localization of Lewis~ antigen in invasive tumor cells; F, localization of Lewis~ antigen in normal urothelium; G, Lewis~ antigen was not detected in normal urothelium, with the exception of occasional umbrella cells; H, neosynthesis of Le~ antigen in CIS; I, enhanced expression of Le~ antigen in invasive tumor cells; J, localization of Le~ antigen in invasive tumor cells; K, detection of Le~ antigen in CIS; L, expression of Le~ antigen in invasive tumor cells. Fig. 2A to L, x 400.

patchy staining. There was staining in three of nine nonsecretor and Le~b~ cases, the same cases that stained in areas of CIS and/or in normal urothelium (Fig. 3F).

Lewis~ determinant was expressed by tumor cells in all secretors, nonsecretors and Le~b~ individuals, showing variable heterogeneity in their immunophenotypic patterns (Fig. 2I and 3J). Staining was homogeneously positive in four cases, and heterogeneous in the others; in the former there was an enhanced antigenic expression in the invasive tumor as compared to the corresponding CIS. In all these cases normal urothelium was Le~ antigen negative or showed only staining of occasional surface umbrella cells (see above).

Lewis~ determinant was detected in the invasive tumor of all cases under study, as it was in CIS, regardless of blood type and secretor status. Secretor individuals usually showed diffuse immunoreactivity, that is staining in 80–100% of invasive tumor cells (Fig. 2L). Nonsecretors and Le~b~ individuals had either enhanced expression of Le~ antigen in invasive tumor cells when compared to their normal urothelium (three of nine cases), or neosynthesis of Le~ antigen in the invasive tumor when the Le~ determinant was undetectable in their normal urothelium (six of nine cases) (Fig. 3L).

Precursor type I chain determinant showed patchy expression by invasive tumor cells in five of 10 secretors, one of five nonsecretors, and three of four Le~b~ individuals. In nonsecretors and Le~b~ individuals, two of the positive cases for anti-precursor antibody showed no reactivity for anti-H antibodies; while three of five negative cases for precursor type I determinant stained for H antigen.

DISCUSSION

In this report we describe a detailed immunohistological analysis of normal human urothelium and transitional cell carcinomas using an extensive panel of reagents detecting precursor, A, B, H, Le~, Le~, Le~, and Le~ blood group antigens. The reactions with each reagent were compared in consecutive tissue sections of normal and tumor samples of individuals with known blood type and secretor status (saliva and/or Lewis typing studies). This analysis of blood group antigen phenotypes in human urothelium is an extension of our earlier studies on the human nephron (17).

Changes in blood group-related antigen expression in the urogenital system have been extensively discussed in the literature (6, 7, 17, 26–29). Pioneering studies were based on an RCA test to detect such antigens in normal and neoplastic urothelium (30, 31). With this method neoplastic urothelium was reported to show deletion of A, B, and/or H antigens (6, 7, 10), and antigen deletion was said to precede or be predictive of tumor invasion (11–13). With the recent technical improvements in immunohistochemistry and the availability of antibodies detecting ABH antigens, these early observations were re-examined (28, 32, 33). Immunohistochemical methods proved to be more sensitive than the red cell adherence test, and

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Fig. 3. Localization of Lewis blood group-related antigens in normal urothelium, CIS, and invasive urothelial carcinoma of the urinary bladder of a nonsecretor (Table 4: Case 14). A, Lewis' antigen homogeneously expressed in normal urothelium; B, Lewis' antigen patchy expressed in CIS (note punctate cytoplasmic immunostaining); C, localization of Lewis' in invasive tumor cells; D, Lewis' antigen heterogeneously expressed in normal urothelium (note reactivity in basal and supra-basal(s) cell layers); E, Lewis' antigen with patchy expression in CIS; F, localization of Lewis' in invasive tumor cells; G, Lewis' antigen was not detected in normal urothelium, with the exception of occasional umbrella cells; H, neosynthesis of Le' antigen in CIS; I, enhanced expression of Le' antigen in invasive tumor cells; J, Le' antigen was not detected in normal urothelium in this case; K, neosynthesis of Le' antigen in CIS; L, enhanced expression of Le' antigen in invasive tumor cells. A, B, D, E, G, H, J, K, and L, × 400; C, F, and I, × 200.

minimized the false-negative results that characterized the latter (26, 34).

In the present study we have observed either complete or partial deletion of ABH antigens in areas of CIS, independently of blood type and secretor status. However, since a subgroup of nonsecretors lack expression of H antigen in normal urothelium, the lack of expression in areas of CIS in these individuals should not be considered a deletion phenomenon. Incompatible blood group expression (e.g., A blood group antigen in O or B individuals) was not observed in CIS. Le' and Le'' immunoreactivity in CIS paralleled that in normal urothelium; only in a few cases was Le' detected in CIS of individuals with undetectable levels in the normal urothelium. Le' and Le'' determinants usually showed either increased expression or neosynthesis in CIS. In the case of Le' antigen, these phenomena were mainly observed in nonsecretor and Le''−− individuals. Expression of Le' and Le'' antigens was considered a neosynthesis phenomenon when the normal mucosa showed undetectable levels of these antigens. Precursor type I structure was expressed in only one case with CIS.

Invasive bladder tumor cells usually showed deletion of A and B antigens. However, neither all tumors nor all cells within a given tumor showed complete deletion of ABH determinants. Absence of A and/or B antigens could be due to lack of the corresponding glycosyltransferases, which in turn could result in an accumulation of both H and precursor molecules. Using the red cell adherence test, Limas and Lange reported increased reactivity for H antigen in patients of blood group A or B, whereas A or B isoantigens were reduced or absent (35). In the present study, 68% of invasive tumors expressed H antigen, and 47% showed heterogeneous immunoreactivities for the precursor type 1 structure. It should be noted that, in general, cases in which invasive tumor cells were strongly reactive for anti-H did not react intensely for anti-precursor antibody, and vice versa. Again, enhanced fucosylation of the precursor could explain the increased immunoreactivity of the H antigen seen in some cases, while lack of fucosylation could explain absence of H antigen and expression of precursor type I molecule in other cases.

Lewis' and Lewis'' determinants in general are expressed in invasive tumor cells and CIS, as in the normal urothelium. However, in five cases, Le' showed down-regulation, being detected on normal urothelium but not on invasive tumor cells. Expression on tumor cells of Le' and/or Le'' structures in Le''−− and Le''++ RBC-typed individuals was not totally unexpected and has been reported in gastrointestinal and normal urothelium (19, 36). The Lewis' determinant was expressed with variable heterogeneity by invasive tumor cells in all individuals studied regardless of secretor status, but not in normal urothelium except for occasional umbrella cells. The Le' antigen showed increased expression or neosynthesis in invasive tumor cells of nonsecretors. In secretors, Le' antigen was expressed similarly in the invasive tumor cells but found in the normal urothelium also. Our studies are performed on formalin-fixed paraffin-embedded tissues, but as deparaffinization has been reported to alter...
antigenic expression (37–39), we have reexamined this question in comparative analyses of frozen and deparaffinized tissue sections of normal kidney/urothelium (17) and normal colonic mucosa/colonic carcinoma (19, 40). We did not observe a loss of antigen expression in formalin-fixed paraffin-embedded sections in comparison with frozen sections. In the present study we also compared the immunophenotypes of bladder tumor and normal urothelium of five cases in frozen and paraffin-embedded tissue sections side by side, and found no differences. Thus, it is likely that the determinants recognized by the present panel of antibodies reside on glycoproteins. Moreover, since the present study analyzes normal adjacent versus tumor cells in the same specimens from the same individuals processed in the same way, we can also correlate antibody reaction in tumor cells with the normal epithelial counterpart of the specimen in each case. The known normal phenotypes of RBC and epithelial cells serve as internal controls for our analysis.

It has also been reported that patients treated with radiotherapy had strong RCA-positive tests for ABH isoantigens, while untreated patients showed no reaction with the RCA test (41). However, four patients who were studied by us had received radiotherapy (Table 2), and all retained distinct phenotypes as described (Table 2) when normal urothelium, CIS, and invasive carcinoma were compared using the immunoperoxidase technique. Endothelial cells, erythrocytes, and normal urothelium served as internal controls for our analysis.

Previous investigators have emphasized that loss of ABH antigen expression is correlated with malignant change (6–14). We show that this is not a uniform feature of urothelial carcinomas, depending on the blood type and secretor status of the patient. It appears that there is a combination of changes in the various blood group specificities rather than a single change during malignant transformation, and that these vary depending on normal phenotype of the individual. Loss of A or B antigens and gain of H and Le" specificities was noted in A and B secretor individuals. In O secretors, there was loss of H antigen and gain in Le" determinant expression. Down-regulation of Le" was observed on tumor cells of O individuals. Expression of Le" on tumor cells of a Le"+ RBC-type individual was also noted. In nonsecretors there was gain or enhancement in Le" expression. In all individuals, uniform or heterogeneous expression of Le" antigen, not evident in normal urothelium, was highly characteristic of tumor cells. Also, expression of type I precursor determinant was preferentially seen in invasive bladder tumor cells and not in areas of CIS. Thus, it seems possible that enhanced expression of Le" antigen, accompanied by one or more changes in other blood group antigens, outlined above, could be reliable indicators of malignant transformation in bladder urothelium. A panel of well-characterized reagents to blood group-related antigens could be extremely useful in the immunopathological analysis of bladder tumors, particularly when the secretor status of the patient is known.

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REFERENCES

Blood Group-related Antigens in Human Urothelium: Enhanced Expression of Precursor, Le\(^{x}\), and Le\(^{y}\) Determinants in Urothelial Carcinoma


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