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Blood Group Antigens in Normal and Neoplastic Urothelium

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Abstract The ABO and Lewis blood group antigens are cell surface carbohydrate determinants formed by the sequential addition of saccharides to precursor backbone structures of membrane lipids and proteins. Suppression of normally active glycosyltransferases results in the absence of antigens that are normally expressed. ABH antigen deletion in malignant and premalignant urothelium has been extensively evaluated; it appears to correlate with significantly higher rates of tumor recurrence and disease progression. However, we have recently shown that the ABH blood group system is differentially expressed in the normal urothelium of secretors in contrast to nonsecretor individuals. The normal urothelium of nonsecretors does not express A, B or H determinants; therefore, deletion of ABH antigens can only be ascertained in secretor individuals. Although nonsecretors only comprise 22–24% of the population, this observation mandates a re-evaluation of earlier studies. Deletion of A, B or H antigens is noted in carcinoma *in situ* (CIS), and in invasive and metastatic transitional cell carcinoma (TCC) of secretor individuals. Increased synthesis or activation of normally quiescent glycosyltransferases in bladder tumors can result in the expression of aberrant tumor-associated blood group antigens. Immunohistochemical analysis has demonstrated that Lewis X (Le^x) is not detected in normal adult urothelium except for occasional umbrella cells. However, papillomas, CIS and TCC expressed Le^x in 84% of cases, regardless of grade, stage, blood type or secretor status of the individual. The presence of Le^x-positive cells in bladder lavage specimens enhanced the detection of urothelial tumor cells, correctly identifying bladder tumors in 253/293 (86%) cases compared to a 63% sensitivity for cytology alone. The specificity of Le^x immunocytology was 87%; 57 of 65 control subjects were negative for the Le^x antigen. Furthermore, the detection of the Le^x antigen in exfoliated bladder cells is a useful marker for predicting bladder tumor relapse in high risk, disease-free patients. All 17 Le^x-positive patients recurred with clinical evidence of disease between 2 and 33 months (mean 8.4 months), while only 8 of 39 Le^x-negative patients recurred; 31 patients remain NED (2–40 months, mean 16.2 months). © 1992 Wiley-Liss, Inc.

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Blood group antigens are cell surface carbohydrate structures formed by the sequential addition of saccharides to precursor backbone structures of membrane lipids and proteins [1]. Although blood group antigens were originally detected on the surface of erythrocytes, they are found in many extra-erythroid tissues, including urothelium [1]. Blood group antigen expression in normal and malignant urothelium has been the focus of considerable interest for many years, primarily because early reports indicated that the absence of cell surface blood group antigens from low grade superficial bladder tumors heralded an unfavorable clinical course compared to tumors that retained their respective antigens [2–11].

Kay and Wallace first suggested that the absence of A and B antigens on the surface of transitional cell carcinomas (TCCs) was correlated with increased histologic dedifferentiation [2]. In 1973, Davidsohn and associates [3] confirmed this observation when they found a correlation between histologic grade and blood group antigen expression using the red cell adherence (RCA) technique. In their experience most high grade lesions were RCA negative.

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In a 1975 landmark study, DeCenzo *et al.* [4] studied the expression of ABH antigens in 22 patients with stage A TCC who were followed a minimum of 5 years. They used a mixed cell agglutination test with polyclonal antibodies on paraffin-embedded, formalin-fixed tissue sections. They noted that antigenic deletion of ABH antigens from the original tumor correlated with the subsequent development of muscle invasive cancer. Eight of nine patients without ABH antigen expression in the original superficial tumor progressed to muscle infiltration while none of the 13 patients whose initial tumors retained their ABH antigens suffered an invasive recurrence during a 5–14 year follow-up [4]. This landmark observation was corroborated by other investigators (Table I).

Newman *et al.* [5] studied ABH expression in 322 patients with superficial bladder tumors and noted that ABH antigens were deleted in 71 out of 80 (88%) patients who subsequently progressed to invasion. Furthermore, 127 of 146 (87%) patients who had no recurrence of superficial tumor for at least 5 years retained A, B or H antigens; and of 96 patients who had superficial recurrences, 86 (90%) retained antigens on the initial and subsequent tumors.

D'Elia [6] studied 40 patients with stage 0 papillary TCC of the bladder for the presence or absence of ABH antigens in the tumor. Eleven of 15 (73%) tumors without antigen expression progressed to stage B or greater in a mean time of 4.3 years while 21 of 25 (84%) patients whose tumors retained ABH antigens remained stage 0 with a mean follow-up of 13.3 years.

Limas [7] noted that of 60 patients who presented with noninvasive TCC and who were followed at least 5 years, 49 (81%) patients whose tumors were RCA positive did not develop invasive tumors and 16 (27%) had no recurrences, while all 34 patients (56%) whose tumors were ABH negative experienced recurrences, and 21 (62%) of those developed invasive lesions.

Despite the enthusiasm that resulted from the early promising data from multiple centers using ABH testing in bladder tumors, several problems precluded therapeutic decision making and widespread clinical applicability. First of all, the RCA technique had serious limitations with a significant incidence of false positives [12]. Second, heterogeneity of blood group antigen expression within tumors complicated interpretation of results in terms of quantitation and biologic significance [13]. Third, the clinical correlation between ABH expression and tumor aggressiveness was not precise enough and was never tested prospectively [13]. Lastly, the influence of additional factors such as secretor status on blood group antigen expression in normal urothelium was not appreciated [1].

Using a well-characterized panel of monoclonal antibodies and improved immunohistochemical methodology, Cordon-Cardo *et al.* [1] have recently shown that the ABH blood group antigen system is differentially expressed in the normal urothelium of secretor individuals as compared to nonsecretor individuals. The normal urothelium of nonsecretors does not express A, B or H determinants; therefore, deletion of ABH antigens can only be ascertained in secre-

TABLE I. Incidence of Stage Progression Related to ABH (O) Status of Initial Superficial Bladder Tumor

AUTHOR	NUMBER OF PATIENTS	PROGRESSION ABH POSITIVE (%)	PROGRESSION ABH NEGATIVE (%)
DeCenzo [4]	22	0/13 (0)	8/9 (98)
D'Elia [6]	49	4/25 (16)	11/15 (73)
Limas [7]	60	5/26 (19)	21/34 (62)
Young [8]	23	1/9 (11)	13/14 (93)
Johnson [10]	30	0/15 (0)	9/15 (60)
Newman [5]	322	9/223 (4)	71/99 (72)

tor individuals who do express their respective A, B or H antigens in normal urothelium. Although nonsecretors only comprise approximately 22% of the population [14], this observation mandates a critical re-evaluation of earlier studies and may explain some inconsistencies and false negatives in previous reports.

In addition to more sensitive and specific antibodies available since the advent of hybridoma technology, we now have a better understanding of the genetic and biosynthetic pathways of blood group antigens. The sequential addition of carbohydrate residues by gene-specific glycosyltransferases to precursor structures is shown in Figure 1. The Lewis antigens are genetically and biochemically related to the ABO blood group [15]. The ABO and Lewis antigenic profile of urothelial cells is the result of complex interaction between three structural gene loci: ABO, *Lele* and *Hh* [1]. A fourth regulatory gene, the dimorphic secretor gene (*Sese*), regulates the 2-fucosyltransferase encoded by the H gene [1,16]. The 4-fucosyltransferase controlled by the *Le* gene converts precursor type 1 chain to Le^a by addition of a fucose residue. However, in the presence of the H gene and at least one copy of the dominant allele of the *Se* gene, precursor type 1 and type 2 chains are preferentially converted to H substance by the addition of a fucose via the H controlled 2-fucosyltransferase. This critical step apparently does not take place in nonsecretors (*Sese*). H substance is an important intermediate structure which can then be converted to Le^b (type 1 chain) or Le^y (type 2 chain) by addition of a second fucose by the 4-fucosyltransferase controlled by the *Le* gene, or to A and B determinants in the presence of the appropriate gene and its specific glycosyltransferase (*N*-acetyl galactosamine transferase or D-galactose transferase, respectively [1,16]). Consequently, the normal urothelium of secretor individuals is very rich in A, B, H, Le^b and Le^y determinants, while that of nonsecretors has minimal or undetectable expression levels of these antigens due to their decreased ability to fucosylate precursor type 1 and 2 chains to H substance [1,16]. Expression of Le^x is not influenced by the secretor gene and is only seen on occasional umbrella cells in normal urothelium [1].

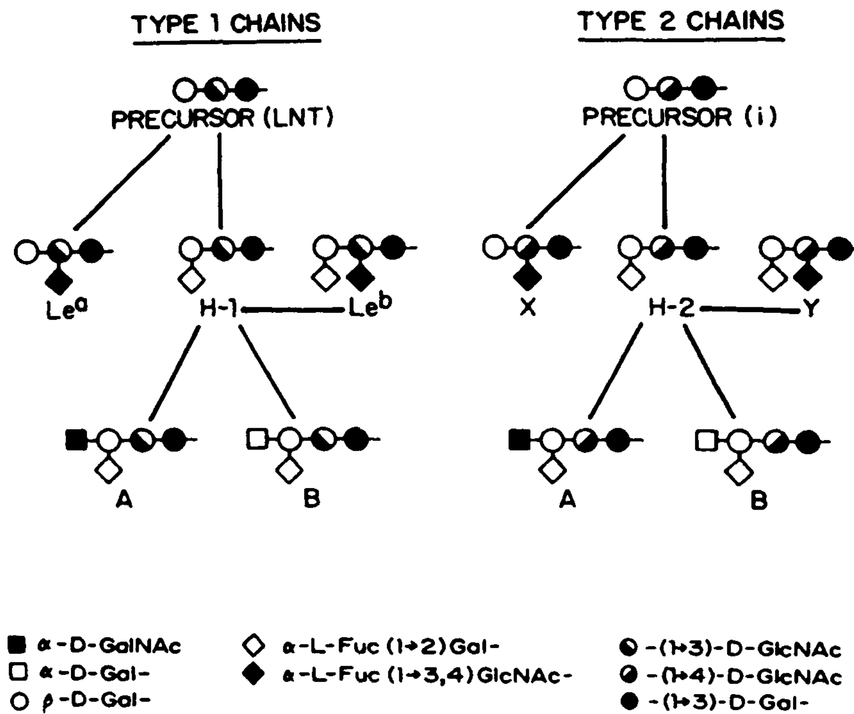

Fig. 1. *Biosynthetic pathways of type 1 and type 2 blood group antigens in humans.* Gal, galactose; Gal Nac, *N*-acetyl galactosamine; Glc, glucose; fuc, fucose; Glc NAC, *N*-acetylglucosamine.

In a detailed immunohistochemical analysis of 19 cystectomy specimens obtained from individuals of known blood type and secretor status where normal urothelium, carcinoma *in situ* (CIS) and invasive carcinoma were studied, Cordon-Cardo *et al.* [1] made several important observations. First of all, in secretor individuals, complete or partial deletion of ABH antigens in areas of CIS and invasive carcinoma was noted; deletion of A, B or H antigens cannot be determined in nonsecretors since they do not express these antigens in normal urothelium. Secondly, precursor type 1 structure was found to accumulate in approximately 50% of invasive TCCs, but was never detected in normal urothelium, and was seen in only one case of CIS. Third, neoplastic urothelium in nonsecretors demonstrates neoexpression of H substance, Le^b and Le^y, structures that are not detected in normal urothelium of nonsecretors. Secretor individuals show enhanced expression of Le^y in malignant urothelium [7]. Finally, histologically normal urothelium was invariably negative for the Le^x determinant except for occasional umbrella cells, while areas of CIS expressed it in 11 of 14 cases. All 19 cases of invasive TCC expressed Le^x regardless of blood type or secretor stature [17]. It appears that a combination of changes occurs in the blood group specificities during malignant transformation, and that these vary depending on the normal phenotype of the individual [17]. Suppression of normally active glycosyltransferases results in the reduction or absence of antigens that are normally expressed, as seen in A, B or H deletion in secretors [17] and the accumulation of precursor structures not normally present, such as precursor type 1 chain. Conversely, increased synthesis or activation of normally quiescent glycosyltransferases in bladder tumors can result in aberrant expression of blood group antigens not detected in normal urothelium. Examples of this phenomenon include the neosynthesis of Le^x in most tumors regardless of blood type or secretor status and the neoexpression of H, Le^b and Le^y in nonsecretors [17].

Furthermore, the antigenic modulation of cell surface structures that accompanies neoplastic transformation may precede overt histological changes [18]. Detection of such tumor-associated antigenic changes would enhance our ability to detect bladder tumors and potentially allow for the objective monitoring of an unstable urothelium and thus predict tumor recurrences.

As stated earlier, the Le^x determinant is a tumor-associated antigen in urothelium. It is formed by the 1-3 fucosylation of a type 2 blood group backbone chain. In normal urothelium it is only detected in occasional umbrella cells; however, extensive immunohistochemical studies of more than 250 specimens show that approximately 85% of all tumors express this antigen to some extent regardless of grade or stage of the tumor, blood type, or secretor status of the individual [17,19,20].

In a pilot study, Sheinfeld *et al.* [19] evaluated the Le^x expression by exfoliated bladder epithelial cells from 129 bladder lavage specimens using an anti-Le^x monoclonal antibody P-12, and the avidin-biotin immunoperoxidase method. The presence of Le^x-positive cells (exclusive of umbrella cells) identified tumors in 76 of 89 cases for a sensitivity of 85% compared to a 62% sensitivity for cytology alone. The combination of a positive Le^x and/or a positive cytology yielded a sensitivity of 93%. The specificity in the study of 40 controls was 85%. We have recently expanded this analysis to almost 300 cases with virtually identical results; *i.e.*, the specificity of Le^x immunocytology was 87%, while the sensitivity of cytology alone was 63%. These results are very similar to those obtained by Huland and associates [22] with monoclonal antibody 486 P3/12 on bladder lavage specimens. It appears that this antibody recognizes either the Le^x antigen or an immunodeterminant structure shared by Le^x [23]. Furthermore, we studied a group of high risk, disease-free patients and found that all Le^x-positive patients recurred with clinical evidence of disease while only 21% of Le^x-negative patients recurred, suggesting that the detection of the Le^x antigen in exfoliated bladder epithelial cells is a useful marker in predicting tumor relapse [24].

SUMMARY

Blood group antigens, complex carbohydrate structures, are found on the surface of urothelial cells. Recent technical improvements in immunohistochemistry and the availability of highly specific monoclonal antibodies have enhanced the detection of blood group antigens. Furthermore, recent knowledge regarding the

importance of the secretor status and the genetic and biochemical regulation of A, B, H and Lewis blood group antigen synthesis has provided us with a better understanding and a more precise definition of the non-neoplastic urothelium.

Malignant transformation of urothelial cells is associated with changes in the carbohydrate composition of membrane glycoproteins and glycolipids. Suppression of normally active glycosyltransferases results in the absence of antigens that normally are expressed (A, B, H in secretor individuals). Conversely, increased synthesis or activation of transferases with little or no activity in normal urothelium results in the expression of aberrant oligosaccharides on the surface of tumor cells (Le^x). The potential clinical applicability of this conceptually interesting phenomenon continues to evolve. Identifying specific markers of neoplastic transformation in exfoliated urothelial cells, such as the Le^x determinant, significantly improves the detection rate of urothelial tumor cells, and appears to predict bladder tumor recurrences. More reliable reagents, plus improved and more reproducible methodology, coupled with a knowledge of the patient's secretor status, will allow prospective evaluation of blood group antigens in the setting of disease recurrence and progression.

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