Urol Res (1988) 16:407–414

Urological Research

Stability of Lectin Binding Properties Expressed by Human Bladder Carcinoma Cell Lines Passaged in Vitro or in Nude Mice

P. J. Russell[1], J. Wotherspoon[2], M. Jelbart[1], J. Philips[3], and D. Raghavan[1]

[1] Urological Cancer Research Unit, Royal Prince Alfred Hospital, Sydney, and Department of Surgery, University of Sydney, Australia
[2] Clinical Immunology Research Centre University of Sydney, Australia
[3] Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia

Accepted: May 10, 1988

Summary. Binding of a panel of lectins by sublines of two human bladder carcinoma cell lines, UCRU-BL-17 CL and UCRU-BL-13 CL, assessed flow cytometrically following passage of the cells in vitro and in nude mice, was compared with that of human leukaemic cell lines, K562 and HL60, and found to be different. Marked glycosylation of sublines of both bladder cancer cell lines was found compared with normal human bladder transitional epithelium (assessed cytochemically). Neuraminidase pretreatment increased the binding of some lectins indicating that some galactose and N-acetylgalactosamine residues were sialylated. Lectin binding by UCRU-BL-17 CL sublines was remarkably constant on prolonged passage in vitro even though the lines underwent changes in physical characteristics and ploidy when grown in nude mice. This suggests that glycosylation of the tumour cell surface may represent an intrinsic feature of this bladder tumour.

Key words: Lectin binding – Human bladder TCC cell lines – Flow cytometry

Introduction

Changes in the expression of cell surface glycoconjugates accompany both normal tissue differentiation and malignant transformation (see [14] review). Studies using animal models have revealed that patterns of lectin binding appear to correlate with indices of tumour function including tumour enzyme activity [9], the ability to metastasize [10], cell adhesiveness [9] and inter-actions with the cell matrix [13]. The lectin binding properties of a tumour may predict its natural history or likely pattern of spread, analogous to the extensive literature on the prognostic significance of expression of ABO blood group antigens by bladder tumours [8].

Two human bladder cancer cell lines, UCRU-BL-13 and -17 have been established and characterized in our laboratory [11–13]. UCRU-BL-13 was derived from an invasive (stage T3) grade II transitional cell carcinoma; UCRU-BL-17 originated from an invasive (stage T4) grade III transitional cell carcinoma with an admixture of squamous and adenocarcinomatous features. Sublines, which have been maintained over several passages in vitro and in nude mice, have been used to determine whether lectin binding patterns alter with tumour evolution or with the differentiation of the tumour.

We have also compared these lectin binding profiles with those of normal tissue and with unrelated tumours in the hope of defining lectin binding properties specific to urothelial malignancy.

Materials and Methods

Cell Lines

Continuous cell lines of human transitional cell carcinoma of the bladder, UCRU-BL-13 CL and UCRU-BL-17 CL, were established and characterised in our laboratory from xenografts of human tumour biopsies grown in nude mice [11–13]. For simplicity the tumours are referred to as 13 and 17, xenografts derived from them are called 13/(X), and cultured cell lines are termed 13/CL or 17/CL. The number after the slash refers to the number of serial passages of the xenograft in vivo, and for cell lines, the xenograft passage from which the cell line was established. Different in vitro sublines of 17 CL were established from either the initial xenograft or from later passages in vivo (see Fig. 1). Serial xenografting of the cells of the line, 17/0 CL, was accompanied at passage 2 by a change in presentation from solid tumours in early passage to the formation of fluid filled sacs. Cultured sublines established from these cysts include 17A CL derived from the fluid cells, and 17S CL, from the surrounding tissue. Two sublines were derived from 13: 13/0 CL, from the first xenograft in nude mice, and 13/0/X0 CL (abbreviated 13/X CL), following passage of 13/0 CL cultured cells through nude mice. All of the bladder carcinoma cell lines were maintained in RPMI medium 1640, (Flow, North Ryde, NSW, Australia) with 10% fetal calf serum (FCS, Commonwealth Serum Laboratories, Melbourne, Australia) in 5% oxygen, 7.5% carbon dioxide, 87.5% nitrogen, at 37 °C. Other lines

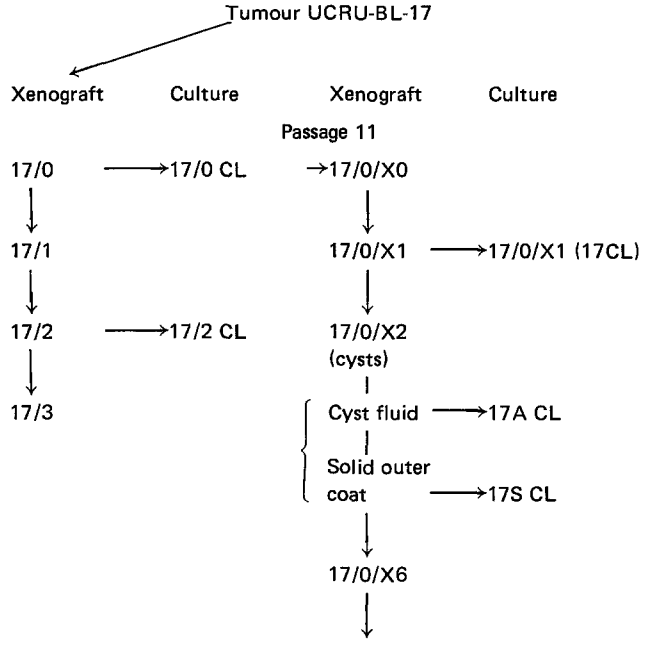


Fig. 1. Sublines of UCRU-BL-17 established in culture

used as controls included HL60, a promyelocytic leukemia line, and K562, an erythroleukemic line. At the time of assessing lectin binding, all cell lines were free of Mycoplasma as judged by staining with the fluorochrome, Hoechst 33258 (Flow) [2].

Assessment of Differentiation of Xenografts from Which Cell Lines were Derived

Xenografted tissue was fixed in buffered formalin, paraffin embedded and stained with haematoxylin and eosin, or with special stains for mucin, including Periodic Acid Schiff plus diastase (PAS + D), mucicarmine and alcian blue. Immunoperoxidase staining for carcinoembryonic antigen and keratin were carried out on deparaffinised sections as previously described [13]. For electron microscopy, small fragments of tissue were fixed in cacodylate buffered glutaraldehyde and processed as previously described [12]. Xenografted tumours were graded histologically, and were assessed for adenocarcinomatous features by mucin staining and electron microscopic evidence of intracytoplasmic lumina, or for features of squamous carcinoma by the presence of keratin ringing, and ultrastructurally, by the presence of tonofibrils. Such features have previously been described in the 17 lines [11, 13].

Assessment of Lectin Binding by Fluorescence

Nonadherent or adherent cultured cells were harvested by centrifugation or by treatment with 1 mM sodium ethylene diamine tetraacetic acid (EDTA, Flow) in HBSS respectively. Cells of the 17 CL sublines detached after 1–2 h; 13 CL cells required up to 8 h before harvest. For specific experiments, cells were harvested with 0.25% trypsin (Commonwealth Serum Laboratories) whose activity was stopped by adding FCS. Harvested cells were washed twice in Dulbecco's phosphate buffered saline (PBS, Oxoid, UK). Before staining, some cells were treated with neuraminidase (0.2 U/ml, ex *Clostridium perfringens*, Sigma per 10^8 cells) at 37 °C for 45 min, and washed twice. Untreated and treated cells were suspended in PBS plus 2% FCS plus 0.1% sodium azide, or for incubation with fluoresceinated Concanavalin A, in Tris (hydroxymethyl amino methane) (Sigma) buffered saline (TBS, 0.005 M, with 0.001 M calcium chloride, 0.015 M sodium chloride, pH 7) with FCS and azide, aliquoted ($0.3–0.5 \times 10^6$ viable cells/tube, judged by trypan blue exclusion) and incubated on ice with 1 μg fluorescein-conjugated (FITC-) lectin plus or minus the appropriate inhibiting sugar at a final concentration of 0.2 M, for 30–45 min. The lectins and inhibiting sugars used were obtained from E-Y Laboratories (San Mateo, California), and included: Fluorescein conjugated: – peanut agglutinin (Arachis hypogaea), PNA-FITC: (D-galactose); Horse gram (Dolichus bifloris), DBA-FITC: (D-galactose); Osage Orange seed (Maclura pomifera), MPA-FITC: (D-galactose); soy bean agglutinin (Glycine max), SBA-FITC: (D-galactose); Bandeiraea (Griffonia simplicifolia), GS-I-FITC: (D-galactose); Gorse agglutinin (Ulex europaeus), UEA-FITC: (fucose); wheat germ agglutinin (Triticum vulgaris), WGA-FITC: (N.N'N''-triacetyl chitotriose); and Jack Bean meal (Concanavalin enisformis), Con A-FITC: (mannose). All cells were washed twice in appropriate buffer with FCS and azide, then fixed in 1% paraformaldehyde in PBS (pH 7.4). Green fluorescence was assessed by flow cytometric analysis on a FACS 440 (Becton Dickinson, Sunnyvale, CA) using the 488 nm line of an argon ion laser (200 mW). Fluorescent emission was selectively collected using 535/15 nm band pass filters and using logarithmic scaling. Daily system alignment and calibration was carried out using fluorescent "Calebrite" beads (Becton Dickinson) to standardise the fluorescent intensity scale so that analyses could be compared between experiments. The lectin binding is expressed as the difference in peak channel fluorescence (measured on a log scale) in the absence or presence of the specific inhibiting sugar at a final concentration of 0.2 M (except for N.N'N'' triacetylchitotriose, which was at 0.2 mM). Differences were arbitrarily scaled as follows: difference of 0–10 channels, –; 10–20 channels, +; 21–40 channels, ++; 41–60 channels, +++; 60 channels, ++++. A difference of 20 channels represents an approximate two fold increase in the intensity of fluorescent signal.

Fixation of Cells

Cells were harvested by treatment with 1 mM EDTA, washed and fixed in 50% ethanol in PBS on ice for 5 min [16].

Immunoperoxidase Staining of Normal Bladder Specimens

Staining was carried out on:

(a) 3 bladder cytoscopy specimens without neoplastic change, derived from patients with mycobacterial cystitis, urinary tract obstruction and inflammatory changes respectively. These were fixed in 10% buffered formalin and paraffin embedded;

(b) 2 snap frozen bladder specimens from normal transplant donors cut as frozen sections after storage in liquid nitrogen, and fixed in cold acetone overnight at –20 °C;

(c) 2 normal bladder specimens obtained post mortem, formalin fixed and paraffin embedded.

Prior to staining, paraffin embedded sections were deparaffinised and selected sections were pretreated with neuraminidase (0.4 U/ml, ex *Clostridium perfringens*, Sigma) [6] and washed twice. Treatment with pronase E (Sigma, 1 mg/ml) for 10 min at 37 °C failed to change any of the staining patterns obtained. All sections were pretreated with 0.3% hydrogen peroxide (H_2O_2) in methanol for

Table 1. Histological features of xenografts from which cell lines were derived

UCRU-xenograft (ploidy)[a]	UCRU-BL cell line (ploidy)	Morphology	Differentation features[b]		Mucin stains			CEA
			Squamous	Glandular	Alcian Blue	PAS + D	Mucicarmine	
BL-17/0 (2N, 4N)	17/0 CL (P8: 2N, 4N)	TCC grade II–III	+	+	+++	+++	+++	+++
BL-17/2 (2N, 4N)	BL-17/2 CL (P4: 2.2N, 4.4N)	TCC grade II–III	+	+	+++	+++	+++	+++
BL-17/0/X1 (2N, 3.5N)	17/0/X1 CL (P25: 3N)	TCC grade III less diff	±	+	+	–	+	+++
BL-17/0/X2								
Cysts:								
fluid (2N, 3.5N)	17A CL (P2: 2.9N, 3.5N)	TCC grade II–III	+	+	++	++	++	+
cyst (2N, 3.6N)	17S CL (P2: 2.6N, 5N)	less diff						
BL-13/0 (2N, 2.5N, 2.9N)	13/0 CL (P26: 3.5N)	TCC grade II–III	±	–	–	–	–	++
BL-13/0/X0 (2.3N, 4.4N)	13/X CL (P40: 3.5N, 3.7N, 4.2N)	TCC grade III	+	+	+ focal	+ focal	+ focal	+++

BL = bladder; CEA = carcinoembryonic antigen; TCC = transitional cell carcinoma; PAS + D = periodic acid Schiff plus diastase; ND = not done; less diff = less differentiated; P = passage number in vitro

[a] Ploidy was assessed by DNA flow cytometry using propidium iodide as previously described (11–13)

[b] Differentiation features were assessed by special stains (keratin, mucin stains) and by electron microscopy (see methods)

5 min to block endogenous peroxidases and incubated in 20% horse serum in TBS with 0.01% azide to reduce non-specific staining. Lectin binding was studied using biotinylated lectins (E-Y Laboratories, San Mateo, CA.) (B-lectin): B-DBA, B-SBA, B-GS I, B-UEA, B-WGA, and B-Con A. Each section was stained with approximately 40 μl of a 1/40 dilution of lectin at 1 mg/ml. For PNA, the peroxidase anti-peroxidase (PAP) method was used. Sections were sequentially incubated with appropriate dilutions of PNA (E-Y Laboratories), rabbit anti-PNA (Sigma) in TBS, affinity purified swine anti-rabbit IgG (Dako) in TBS, rabbit PAP complexes (DAKO), and finally with diaminobenzidine tetrahydrochloride (DAB) and 0.03% H_2O_2 to demonstrate peroxidase. Sections were counterstained in Harris' haematoxylin, dehydrated, cleared and mounted. For avidin biotin labelling, sections were sequentially incubated with biotinylated lectins for 60 min, washed with TBS, then with streptavidin peroxidase (Amersham, 1:300) for 60 min, followed by further washing and then DAB solution, counterstaining and mounting as above. Positive controls were carried out on formalin-fixed tissue from the xenografts, UCRU-BL-17/0/X3 or UCRU-BL-17/2. Negative controls were performed on each tissue by omitting the lectin step.

Results

Flow cytometric assessment of lectin binding by tumour cell lines: The histological features of the xenografts from which the 13 CL and 17 CL sublines were derived is summarized in Table 1 and Fig. 2. These sublines were used to study the changes in lectin binding with time in culture, or following passage in and out of nude mice. The results are compared with lectin binding by two cultured leukaemic cell lines, HL60 and K562 in Table 2.

The panel of lectins tested on sublines of 13 CL and 17 CL failed to discriminate multiple populations of cells. Exceptions were seen for 17 CL sublines (Table 2, Fig. 3) in the case of DBA, GS I, SBA and WGA, and for 13 CL sublines (Table 2) for PNA and MPA, where two distinct populations were observed in either untreated or neuraminidase treated cells in some but not all passages.

The 17 CL sublines showed major differences in the intensity of binding of lectins with specificity for galactose (Table 2). PNA was more strongly bound by each subline than MPA and SBA, whereas DBA was bound only by the line, 17/0 CL, but not by the other sublines. There was very little binding of GS I by any 17 subline. In contrast, SBA and PNA were bound to a similar extent by cells of 13 CL sublines. Pretreatment with neuraminidase increased the intensity of binding to a variable extent by both PNA and SBA in each case, but except for 17/0/X1 CL cells (passage 17), had little effect on the binding of MPA (Fig. 3).

The lectin binding profiles of each of both the 17 and 13 sublines were similar for PNA, GS I, SBA, MPA, WGA

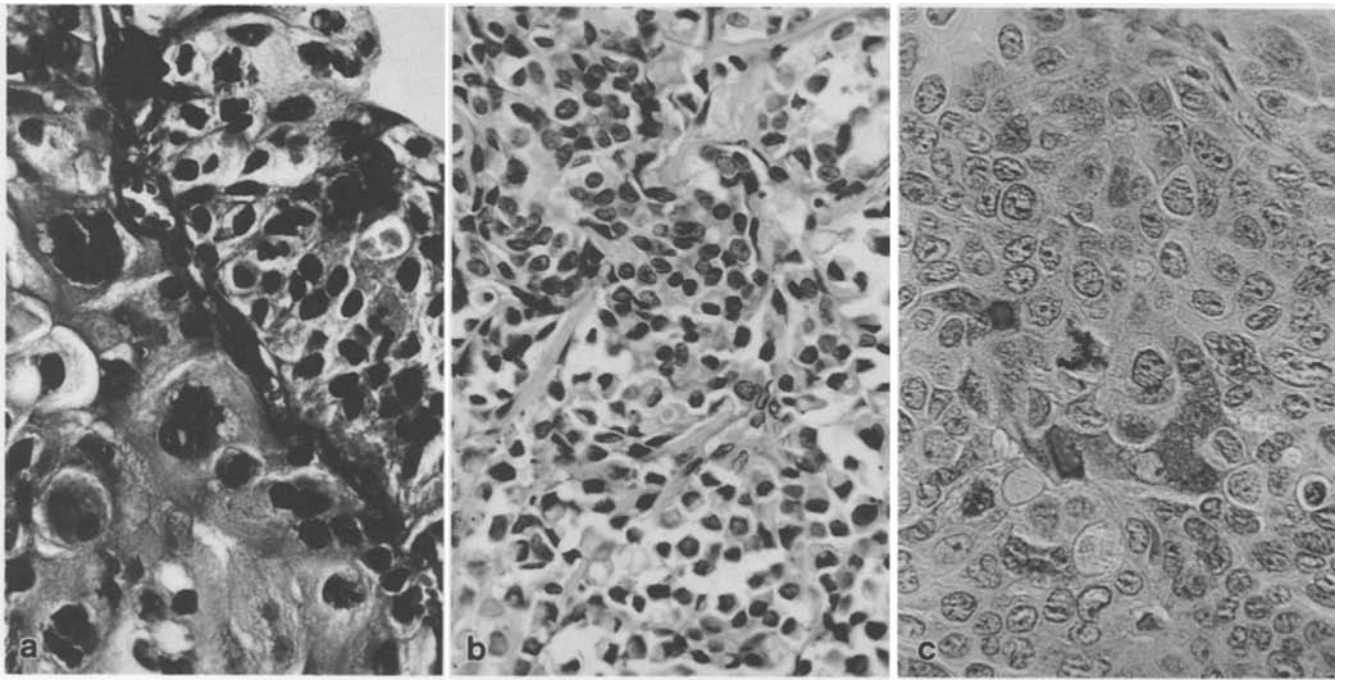
Table 2. Lectin binding by tumour lines assessed by flow cytometry

UCRU-BL	UCRU-BL-17 Sublines								UCRU-BL-13 Sublines			Leukaemic cell lines	
Line	17/0		17/0/X1		17/0/X2		17/2		13/0		13/0/X0	K562	HL60
Passage	P_8	P_{36}	P_{17}	P_{45}	17A P_{19}	17S P_{33}	P_{11}	P_{18}	P_{13}	P_{25}	P_{25}		
Lectin													
PNA	+++	++++	+++	++++	++++	+++	++	++++	+, +++	++	+++	+	++
PNA + N	++++	++++	++++	++++	++++	+++	+++	++++	++	ND	ND	+++	++
DBA	++++	–, +++	–	–	–	–	–	–	ND	–	–	–	–
DBA + N	ND	ND	–	–	–	–	–	–	ND	ND	ND	–	–
GS-1	–	+	–	+	–	–	+	+	ND	–	ND	–	–
GS-1 + N	+	–	–	+	+	–, +	–	–	ND	ND	–	–	–
SBA	ND	++	ND	+++	++	+	ND	–, +++	ND	ND	+++	–	–
SBA + N	ND	++	ND	ND	ND	+++	ND	+++	ND	ND	ND	+	+++
MPA	++	+++	+	++	++	++	++	++	ND	++	+, –	–	+++
MPA + N	++	+++	++	++	++	ND	+, +++	++	ND	ND	ND	+++	++
WGA	++++	ND	++++	++++	++++	ND	++, +++	++++	ND	ND	+++	++++	++++
WGA + N	+++	ND	+++	+++	++	ND	+++	+	ND	ND	ND	+++	++++
ConA	+++	+	+++	+	+	–	+++	+	ND	++	++	+	+++
ConA + N	+++	+	+++	ND	+	–	+++	+	ND	ND	ND	+	+++
UEA	++	+++	+++	++	+++	+	++	++	+++	+++	ND	++	–
UEA + N	++	+++	+++	++	+++	+++	++	++	++	ND	ND	++	–

FITC-lectin binding is expressed as the difference in peak channel fluorescence (log scale) in the absence or presence of the specific inhibiting sugar, arbitrarily scaled as: 0–10 channels, –; 10–20 channels, +; 21–40 channels, ++; 41–60 channels, +++; 160 channels, ++++. A difference of 20 channels represents an approximate two fold increase in the intensity of fluorescent signal.

Lectin abbreviations (inhibiting sugar used at 0.2 M final concentration): PNA: peanut agglutinin (D-galactose); DBA: Dolichus bifloris (D-galactose); MPA: Maclura pomifera (D-galactose); SBA: soybean agglutinin (D-galactose); GS 1: Griffonia simplicifolia (D-galactose); UEA: Ulex europaeus agglutinin (fucose); WGA: wheat germ agglutinin (N.N′N″-triacetylchitotriose, used at 0.2 mM final concentration); Con A: Concanavalin A (mannose)

+N: pretreated with neuraminidase; ND: not done



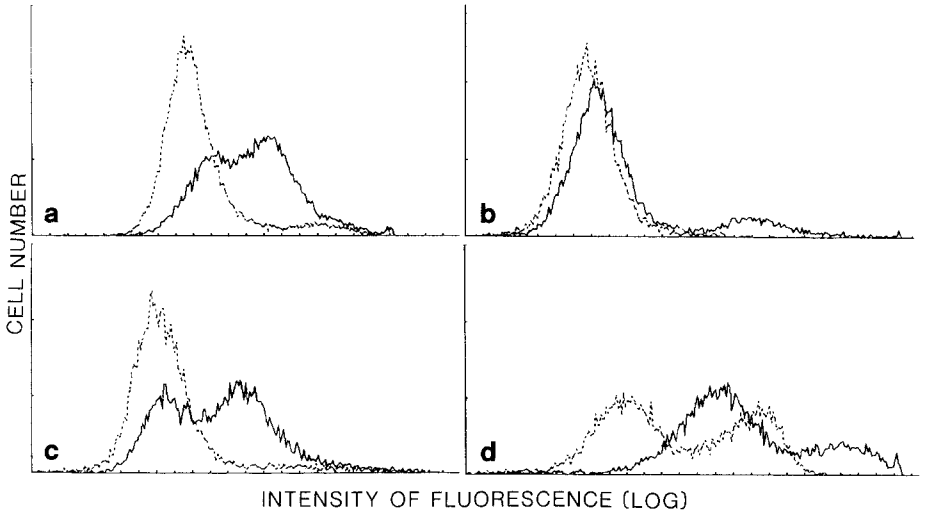


Fig. 3a–d. Subpopulations of UCRU-BL-17CL sublines distinguished by lectin binding **a** MPA binding by neuraminidase treated UCRU-BL-17/2 cells, passage II **b** GS I binding of neuraminidase treated UCRU-BL-17S cells, passage 33 **c** SBA binding by untreated UCRU-BL-17/2 cells, passage 18 **d** WGA binding by neuraminidase treated UCRU-BL-17/2 cells, passage II. Solid line: binding of lectin, Dotted line: binding of lectin in presence of inhibitory sugar – a–c 0.2M galactose; d 0.2 mM N.N′N″-triacetylchitotriose

and UEA. Differences were observed with DBA, which was only bound by the 17/0 CL subline, and with Con A, which bound with decreased intensity as the time of culture of the 17 CL sublines was increased (Table 2). Binding of WGA (which was very intense) decreased after pretreatment of the cells with neuraminidase, whereas binding of UEA and Con A was unaffected by this treatment.

By comparison with the bladder cancer lines, the leukaemic lines showed weaker binding of PNA, no binding of SBA, (except after neuraminidase pretreatment), and contrasting patterns of binding of UEA and MPA. The intensity of binding of WGA was similar in both the bladder and the leukaemic cell lines.

Effects of Fixation and Trypsinization on the Lectin Binding by 17 CL Sublines

The effect of fixation in 50% ethanol in PBS on ice for 5 mins [16] was tested to provide preliminary data for experiments on the effects of cell cycle on lectin binding properties. Fixation of the cells did not alter the binding of PNA or Con A, but increased the intensity of binding of UEA and WGA (by approximately 15 channels) and of SBA (by 30 channels).

Since some cell lines appear to be refractory to removal with EDTA, the effect of trypsin harvest on lectin binding was examined. Trypsin harvested 17/0/X1 CL cells showed less intense binding of all lectins tested, except WGA, which was unaltered. However, when the cells were pretreated with neuraminidase prior to lectin binding, the same results were obtained whether the cells had been harvested with EDTA or with trypsin.

Lectin Binding by Normal Bladder Transitional Epithelium

Short term cultures were established from 2 normal bladder specimens obtained from transplant donors. However, only small pockets of epithelial cells grew, insufficient for staining with a large panel of lectins. Fibroblasts were obtained in large quantities, but these were not considered relevant for comparison with cultured tumour epithelial cells. For this reason, our results on lectin binding by cancer cells in vitro were compared with lectin binding by non-neoplastic transitional epithelium of bladder in vivo. Parallel staining of xenografts of UCRU-BL-17/0/X3 (derived from inoculation and passage of the 17/0 CL subline in nude mice) or UCRU-BL-17/2 (derived directly from passage of the patient's tumour in nude mice – this line had never been cultured in vitro) was carried out as a positive control. The results are shown in Table 3. PNA binding was seen in normal bladder specimens following pre-treatment with neuraminidase only. The two post mortem specimens showed nonspecific binding of B-WGA, B-Con A and following neuraminidase treatment, of B-SBA. Apart from the specimen from the patient with cystitis, which bound B-WGA, and following neuraminidase treatment, B-UEA, all specimens failed to stain with the other lectins tested. In contrast, xenograft specimens of UCRU-BL-17 showed strong binding of a few cells with B-UEA and B-SBA, of more cells with PNA, of approximately half the cells with B-DBA and of most cells with B-WGA. Mouse host stromal cells present in xenograft sections failed to stain with any lectins tested.

Fig. 2. **a** Transitional cell carcinoma with squamous and adenocarcinomatous differentiation (original tumor, UCRU-BL-17). Light micrograph, haematoxylin and eosin, x280. **b** area of predominantly adenocarcinomatous differentiation (Xenograft 17/0 established from original tumor). Light micrograph, haematoxylin and eosin, x560. **c** poorly differentiated cells with increased nuclear pleomorphism (Xenograft, passage 2, established from tissue cultured line, UCRU-BL-17 CL). Light micrograph, haematoxylin and eosin, x560

Table 3. Lectin binding by normal/non-neoplastic bladder transitional epithelium (Number of specimens showing positive binding by transitional cells)

Lectin[a]	Pretreatment with Neuraminidase	Normal bladder[b] $n = 4$	Bladders with normal transitional epithelium[c] $n = 3$
–	–	0	0
	+	0	0
PNA	–	0	0
	+	4	3
B-ConA	–	0[d]	0
	+	0[d]	0
B-WGA	–	0[d]	1
	+	0[d]	0
B-DBA	–	0	0
	+	0	0
B-UEA	–	0	0
	+	0	1
B-SBA	–	0	0
	+	0[d]	0
B-GS-1	–	0	0
	+	0	0

[a] Sections were stained with PNA using the PAP method, or with biotinylated lectins (B-lectin) plus streptavidin

[b] 2 specimens were obtained post-mortem, and were formalin fixed; 2 specimens were snap frozen from transplant donors, and frozen sections were acetone fixed

[c] Cystoscopy specimens were obtained from patients with non-neoplastic bladder changes; I: nonspecific cystitis, I: urinary tract obstruction, I: inflammatory change

[d] In the 2 post mortem specimens, high background staining was observed and the interpretation of the results was difficult

Discussion

The results described in this paper show clearly that there is active glycosylation of the cell surface of urothelial tumours (Table 2) compared with that in normal tissue (Table 3). It is possible that the glycosylation of the bladder cancer cells occurred as a result of some artefact of tissue culture. However, we believe that this is unlikely, since staining of the xenograft, UCRU-BL-17/2, which had never been in culture, gave similar lectin binding profiles to those obtained in culture in lines derived from this xenograft (Table 2). Furthermore, PNA binding was demonstrated in the donor tumours, comparable to that seen in the xenografts [12, 13]. For this reason, we believe that it is valid to compare the results obtained on cancer cell lines with those of staining of normal human bladder specimens.

Confirmation of these results has been obtained from a study of lectin probing of Western blots derived from plasma membrane components of normal and carcinomatous urothelial tissues to be reported elsewhere. The increased binding of some of the lectins (PNA, SBA, Table 2) following treatment with neuraminidase indicates that some of the cell surface galactose and N-acetyl galactosamine residues on the tumour cells are sialylated. These findings are complementary to studies indicating sialylation of exposed glycoproteins of bladder cancer cell lines [15].

Previous studies have compared lectin binding by bladder tumours [7] or cell lines [5] of differing grade or invasiveness. This is the first description of serial studies carried out on cell lines during their evolution as xenografts or on long term passage in vitro. Reports of lectin binding by 8 human bladder carcinoma cell lines showed that lines from higher grade or more invasive tumours showed a higher intensity of binding of WGA, PNA and Con A [5], correlating with the presence of more highly branched tri- and tetraantennary-N-acetyllactosamine glycans and fewer biantennary glycans in the more invasive, tumorigenic lines [4]. In contrast, in the study described here, lectin binding by the sublines of the same bladder tumours appeared to be stable, as exemplified by comparing the patterns of lectin binding by various sublines of 13 CL or 17 CL (Table 2) following passage through nude mice (see Fig. 1), or in culture. The only exceptions to this were the changes in binding of DBA and Con A by the 17 CL sublines (Table 2).

We previously observed that there was a significant increase in the proportion of bizarre, pleomorphic cells in the xenografts derived from injection of cultured cells (17/0/X0 (X), see Fig. 1, Fig. 2) compared with the xenograft line established directly from the patient tumour (17/0 (X), 17/2 (X), see Fig. 1, Table 1); This cellular pleomorphism was accompanied by the emergence of a near triploid DNA component (Table 1), a faster growth rate in vivo, and reduced staining for CEA [13]. The apparent overall stability of the lectin binding patterns suggests that the glycoprotein profile intrinsic to each particular tumour line is independent of the state of differentiation. These results compare with those described by Debray et al. [4] as an exception to their general findings of increased branching of glycans with increasing pathological grade of a bladder tumour line. The two sublines derived from the same tumour, Hu609 (non-tumorigenic, non-invasive) and Hu609T (tumorigenic and invasive) showed the same pattern of multi-branched carbohydrates.

The tumours we studied differed from those reported by Dus et al. [5] in that we observed PNA binding in the absence of neuraminidase pretreatment in both 13 CL and 17 CL sublines. We have previously described PNA binding by both the untreated xenografts from which the cell lines were derived, and by the tumours of the donor patients [11, 13]. Others have also reported binding of PNA to untreated bladder [7] and colon [17] tumours and have correlated exposure of the T-antigen with tumorigenicity.

The results of lectin binding by normal bladders (Table 3) contrast with those described by Alroy et al. [2]; these

workers studied lectin binding by bladder fragments from 16 patients with non-neoplastic urologic disorders and found binding of Con A and WGA (as well as Ricinus communis agglutinin I, RCA-I, and Limulus polyhedra, LPA) in all 16, UEA binding by blood group 0 and DBA binding by blood group A1 positive patients. The discrepancy between the results may reflect differences in fixation, methodology, or lectin concentration used, or may reflect the state of "normality" of the tissues examined. For this study, tissues were fixed in either 10% buffered formalin or in cold acetone at −20 °C (Alroy and colleagues used 2% cold paraformaldehyde), and biotin-labelled lectins were used (not fluorescein-conjugated) at a concentration of approximately 1 μg lectin/section (as compared with 10 μg/tissue block used by Alroy and colleagues). This concentration was selected since it was used in flow cytometric analysis of bladder cell lines (Tables 2). The patterns of lectin binding by xenografted tissues of UCRU-BL-17 reflected those obtained by flow cytometric analysis of the corresponding cell lines. Of the 7 "normal" bladders examined in this study, the post mortem specimens showed nonspecific binding of WGA and Con A, but the only other staining by WGA was of a specimen from a patient with cystitis. Healthy bladder tissues from transplant donors showed no staining (Table 3). Blood types were not assessed in patients from whom bladder specimens were obtained.

The absence of binding of DBA by some of the 17 CL sublines (Table 1) is of particular interest, since these tumour lines were established from a patient who was blood group A positive, and DBA binds to A1 positive red blood cells. Previously, the 17/0/X0 CL (17 CL) subline had been shown to bind antibodies to both blood group A and B antigens [11], yet this line bound only trace levels of DBA (Table 2). However, binding of B-DBA to xenografted tissue of 17/0/X3 (X), derived from 17/0/X0 CL, showed heterogeneous staining, with approximately equal proportions of DBA positive and DBA negative cells. Flow cytometric profiles of DBA also indicated two populations of cells in the UCRU-BL-17/0 line studied at passage 36. Similarly, two populations of cells were differentially stained by SBA, WGA, or following neuraminidase treatment, with MPA on only one occasion (Table 2). Possible explanations for such lectin binding patterns are that one of the sub-populations subsequently became dominant in culture, or that the expression of particular sugar configurations at the cell surface may be altered by a factor such as the phase of the cell cycle. We are currently studying lectin binding profiles in a series of clones established by limit dilution from UCRU-BL-17/2 in order to answer these questions.

The two bladder cell lines studied showed similar lectin binding profiles (Table 2), which differed from those of the leukaemia cell lines, with respect to the intensity of binding of PNA (greater in bladder cancer cells) and UEA (absent in leukaemic lines).

The observed stability of the expression of carbohydrate moieties at the cell surface of two human bladder carcinoma cell lines following passage in vivo and in vitro is important since it will allow the use of lectin markers as diagnostic and prognostic determinants, e.g., binding of WGA is increased in bladder tumours showing poorer prognoses [5]. The finding that lectin binding profiles of the bladder cell lines were not grossly affected following mild fixation in 50% ethanol in ice should enable other immunohistological staining techniques to be developed and used in conjunction with lectin binding. To this end, we are preparing a panel of bladder tumour specific monoclonal antibodies, many of which appear to be directed against carbohydrates. The stability of expression of these carbohydrate moieties at the cell surface will determine future usefulness of the antibodies.

Acknowledgements. We are grateful to staff of the Kanematsu Laboratories, Sydney: Miss G. Croaker and Mr. T. Radloff, for providing us with HL60 and K562 cells, to Miss Jane Wass for DNA flow cytometry, and to Dr. P. C. Vincent, for the use of facilities. This study was supported by a grant from the National Health and Medical Research Council, Australia. Miss M. Jelbart was supported by the New South Wales Women's Bowl for Others Club.

References

1. Alroy J, Szoka FC, Heaney JA, Ucci AA (1982) Lectins as a probe for carbohydrates in non-neoplastic urothelium of human urinary bladder. J Urol 128:189–193
2. Chen TR (1977) In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 104:255–262
3. Coombe DR, Parish CR, Ramshaw IA, Snowden JM (1987) Analysis of the inhibition of tumor metastasis by sulphated polysaccharides. Int J Cancer 39:82–88
4. Debray H, Qin Z, Delannoy P, Montreuil J, Dus D, Radzikowski C, Christensen B, Kieler J (1986) Altered glycosylation of membrane glycoproteins in human uroepithelial cell lines. Int J Cancer 37:607–611
5. Dus D, Radzikowski C, Debray H, Montreuil J, Christensen B, Kieler J (1985) Lectin binding to nonmalignant and malignant human uroepithelial cells in vitro. In: Lectins, vol IV. de Gruyter, Berlin New York, pp 65–74
6. Leathem AJC, Atkins NJ (1983) In: Bullock GR, Petrusz P (eds) Techniques in immuno-cytochemistry, vol 2. Academic Press, New York, pp 40–70
7. Lehman TP, Cooper HS, Mulholland SG (1984) Peanut lectin binding sites in transitional cell carcinoma of the urinary bladder. Cancer 53:272–277
8. Limas C, Lange PH (1985) Lewis antigens in normal and neoplastic urothelium. Am J Pathol 121:176–183
9. Mullins DE, Rohrlich ST (1983) The role of proteinases in cellular invasiveness. Biochim Biophys Acta 695:177–214
10. Nicolson GL (1982) Cancer metastatis: organ colonization and the cell-surface properties of malignant cells. Biochim Biophys Acta 695:113–176
11. Russell PJ, Jelbart ME, Wills E, Singh S, Wass J, Wotherspoon J, Raghavan D (1988) Establishment and characterization of a new human bladder cancer cell line showing features of squamous and glandular differentiation. Int J Cancer 42:74–82

12. Russell PJ, Raghavan D, Gregory P, Philips J, Wills EJ, Jelbart ME, Wass J, Zbroja RA, Vincent PC (1986) Bladder cancer xenografts: a model of tumor cell heterogeneity. Cancer Res 46:2035–2040
13. Russell PJ, Wills EJ, Philips J, Wass J, Jelbart M, Gregory P, Raghavan D (1988) Features of squamous and adeno-carcinoma in the same cell in a xenografted human transitional cell carcinoma: evidence of a common histogenesis? Urol Res 16: 79–85
14. Smets LA, van Beek WP (1984) Carbohydrate of the tumour cell surface. Biochim Biophys Acta 738:237–249
15. Steele JG, Rowlett C, Sandall JK, Franks LM (1983) Identification of exposed surface glycoproteins of four human bladder cancer cell lines. Biochim Biophys Acta 732:219–228
16. Walker KZ, Hayden GE, Goodnow CC, Adams E, Basten A, Raison RL (1985) Coexpression of an epitope on human free k light chains and on a cytoplasmic component in activated T cells. J Immunol 134:1059–1064
17. Yuan M, Itzkowitz SH, Boland CR, Kim YD, Tomita JT, Palekar A, Bennington JL, Trump BF, Kim YS (1986) Comparison of T antigen expression in normal, premalignant and malignant colonic tissue using lectin and antibody immunohistochemistry. Cancer Res 46:4841–4847

Pamela J. Russell, MD
Urological Cancer Research Unit
Blackburn Building
D06
University of Sydney
Sydney, NSW
Australia 2006