Urol Res (1986) 14:145–148

Urological Research

Detection of Transitional Cell Carcinoma in Bladder by Intravesical Injection of Monoclonal Antibodies*

D. K. Chopin** and J. B. deKernion

Department of Surgery, Division of Urology, the Jonsson Cancer Center and the Center for Interdisciplinary Research in Immunologic Diseases, UCLA School of Medicine, Los Angeles, California, USA

Accepted: August 30, 1985

Summary. Monoclonal antibodies produced against tumor associated antigens of human bladder transitional cell carcinoma have proved to be useful in detecting malignant cells in tumor sections and bladder washings. The present study evaluated the capacity of one such antibody G4 to identify sites of malignancy when introduced into the lumen of intact bladders immediately after cystectomy. An ex vivo immunoperoxidase staining (IPS) method was applied to four cystectomy specimens – three with solitary invasive transitional cell carcinomas (TCC) and one with carcinoma in situ (CIS) using monoclonal antibody G4 injected intravesically immediately after cystectomy. The tumor sites, normal appearing mucosa and other exposed and non exposed tissues from the same patient were examined for G4 binding. In all cases preferential intravesical binding of G4 antibody to the tumor size was demonstrated without binding to the normal mucosa except in one case. This study showed that monoclonal antibody injected intravesically can be focused on the tumor site and indicates that G4 and other antibodies may be useful intravesically for selectively assessing field changes associated with malignancies or as specific therapeutic agents.

Key words: Anti-TCC, Monoclonal Antibodies, Bladder mapping, In vivo TCC diagnosis.

Introduction

The significance of urothelial histologic alteration in normal appearing mucosa distant from a primary bladder tumor is now well established. Field changes in the surrounding non-papillary urothelium are important prognostic factors to determine the likelihood of development of invasive bladder tumor in patients with non-invasive papillary lesions [12–14, 18]. Invasive TCC is frequently associated with carcinoma in situ (CIS) [3, 6, 8, 15]. Although variability in the biologic activity of CIS is acknowledged [16] careful retrospective pathologic observations suggest that cases of invasive bladder cancer represent a progression of the in situ stage [15]. Therefore, in vivo detection of malignant and premalignant bladder urothelium has become a major issue in diagnosis and treatment of bladder tumors.

This study was designed to determine if differential in vivo staining can be achieved using monoclonal antibodies directed against TCC-tumor associated antigens. Prior to any attempt to utilize monoclonal antibodies intravesically for diagnostic or therapeutic purposes, their ability to bind tumor in vivo should be demonstrated. Monoclonal antibody G4 was injected intravesically in four patients with TCC. Binding of monoclonal antibody to bladder tumor or normal appearing mucosa was determined with immunoperoxidase staining (IPS) of fresh frozen sections from several sites of each specimen.

Material and methods

Monoclonal antibody G4 was used in this study and has been previously described [1]. This monoclonal antibody reacts with high grade TCC, carcinoma in situ and some low grade TCC and is of the IgM subclass. Tissue culture supernatant from 3–4 day old flasks containing 2 x 10^6 hybridoma cell/ml were pooled and kept at 4 °C until use. Bladder specimens from 4 male patients undergoing cystectomy were utilized for ex vivo immunoperoxidase staining using a procedure previously described [9]. Three patients had solitary non-papillary invasive TCC and one patient had diffuse carcinoma in situ without invasive disease. The bladder was filled with 300 cc of hybridoma supernatant G4 in the operating room immediately after removal.

After 1 h incubation time, the bladder was opened in the presence of the pathologist and selective biopsies of the tumor site and "normal appearing" mucosa were immediately processed for frozen section. Frozen sections of other tissues from the same patient's

* Work supported by National Bladder Cancer Project CA 3271-01, the Blalock Foundation, the Concern Foundation and l'ARC

** *Present address:* Service d'Urologie, Hôpital Henri Mondor, F-94000 Creteil, France, to whom requests for reprints should be directed

Table 1. Ex vivo immunoperoxidase (IPS) after intravesical injection of monoclonal antibody (MoAb) G4

	Pt1[a]	Pt2[a]	Pt3[b]	Pt4[a]
Tumor site(s)[c]	+ (1)[e]	+ (1)[e]	+ (1)[e]	+ (2)[e]
Trigone[c]	–	–	–	+
Bladder dome[c]	–	–	some area +	–
Ureter[d]	–	–	–	–

[a] Invasive TCC Grade III/IV
[b] Carcinoma in situ
[c] Tissues exposed to G4
[d] Tissues non-exposed to G4
[e] Number of biopsies; + positive for IPS; – negative for IPS

Table 2. Immunoperoxidase staining (IPS) of cryostat sections of exposed (a) and non-exposed (b) tissues to G4 ex vivo with monoclonal antibodies G4, 3C12 (positive control), Ig(1b) (negative control)

	No. of patients	G4	3C12	Ig(1b)
Tumor sites[a]	4	4 (+)		
Trigone[a]	4	$1^+/3^-$		
Bladder dome[a]	4	$1^+/3^-$		
Ureter[b]	4	4–	4 (+)	4–
Skin[b]	1	1–	1+	1–
Ileum[b]	3	3–	3+	3–
Prostate[b]	4	4–	4+	4–

ureter, prostate, skin or ileum were prepared at the same time. The cryostat sections were stained using two different immunoperoxidase staining (IPS) techniques. The first IPS (experimental IPS) was performed as follows: Cryostat sections were air dried for 20 min fixed in acetone at 4 °C for 10 min and washed in phosphate buffer saline (PBS; 136 mM NaCl; 2.7 mM KCl; 8 mM Na_2HPO_4; 1.5 mM KH_2PO_4 pH 7.4). The slides were incubated with normal goat serum (dilution 1/10 in PBS) for 30 min which was blotted before application of a goat antimouse antibody fragment $F(ab)'_2$ conjugated with peroxidase (Boehringer Mannheim, Indianapolis, IN) diluted 1/20 in PBS. After washing in PBS, peroxidase was revealed with 0.02% 3 amino 9 ethylcarbazole 0.03% H_2O_2, 5% dimethylformamide in 0.02 M acetate buffer pH 5.4 (AEC) (SIGMA, St. Louis, MI). Slides were washed in tap water counterstained in hematoxylin, and mounted with gelatin.

The second IPS, (control IPS), included the addition of an incubation step with monoclonal antibody G4 just after the acetone fixation and before the incubation with the goat antimouse antibody as previously described [9].

Using these two IPS techniques allowed comparisons of the staining of monoclonal antibody G4 injected intraavesically with the staining of the same antibody on cryostat sections. The first staining (experimental IPS) detected the fixation of an immunoglobulin to the surface of the urothelium of the cystectomy specimen. The second staining (control IPS) detected the binding of the antibody to a cell surface antigen on a cryostat section and was used to confirm the staining pattern defined by the experimental IPS. In this procedure 3 monoclonal antibodies were used; G4, 3C12 (a non-specific mouse antihuman monoclonal antibody used as a positive control also of the IgM subclass, established at the same time as G4), and Ig (1b) [10] an antimouse antibody of the IgG subclass used as a negative control.

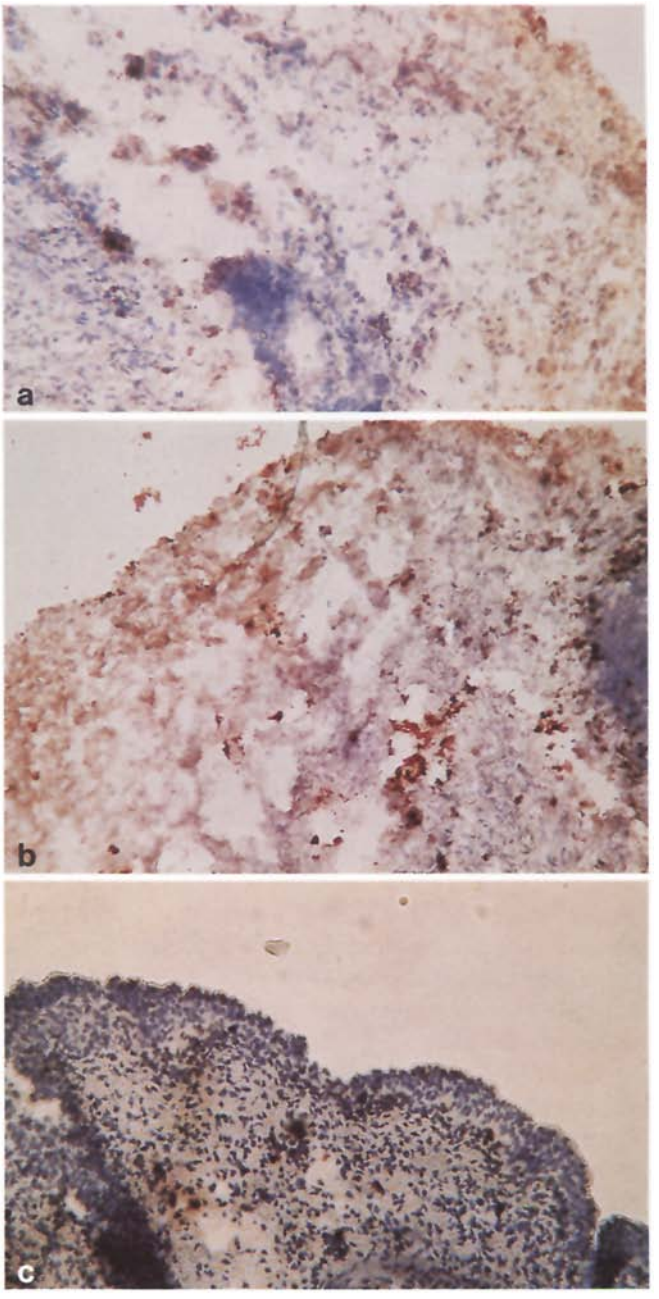

Fig. 1a–c. Immunoperoxidase staining (IPS) from a tumor site of an invasive TCC (a) obtained by intravesical injection of monoclonal antibody (Mab) G4 (experimental IPS). (b) Same section after incubation with MabG4 (control IPS). (c) IPS from a biopsy of a normal appearing mucosal site after intravesical injection of MabG4 (experimental IPS)

Results

In the three patients with invasive TCC, a definite IPS was observed on sections obtained from the tumor site (Table 1). The ex vivo (experimental) staining was limited to the luminal surface of the tumor. This staining extended to the entire tumor section when the frozen section was stained using the control IPS, i.e. using hybridoma G4 as a primary antibody, followed by the goat antimouse antibody conjugat-

ed with peroxidase as secondary antibody (Fig. 1). In two cases, histologically normal urothelium was available on the same section as the tumor site and was not stained. Biopsies from other exposed tissues, normal appearing mucosa from the trigone and the bladder dome and the prostatic urethra, were negative for the experimental IPS except one section from the bladder dome of one patient which demonstrated some focal staining. The same set of slides of exposed tissues was submitted to the control IPS (Table 2). The same patient showed focal staining of normal appearing mucosa from two biopsy sites, trigone and bladder dome, and all other samples were negative.

Non-exposed tissues, ureter, skin or ileum were negative for both experimental and control IPS. The third patient had an extensive CIS with a very roughened mucosal surface above the bladder neck. In this patient two areas of CIS were stained with both IPS (ex vivo experimental and control) and two histologically normal urothelial areas did not stain.

Discussion

This study demonstrated that monoclonal antibodies injected intravesically can bind to abnormal bladder urothelium. Monoclonal antibody G4 has been found to react preferentially with high grade TCC and carcinoma in situ [1]. These results indicate that G4 is capable of binding to living TCC. G4 antibody showed a clear preferential staining for TCC when compared to histologically normal urothelium in samples studied. For one case, however, staining was found in normal appearing bladder mucosa. It is not clear if this staining represents a non-specific binding of G4 to normal urothelium or binding to dysplastic urothelium since these cryostat sections do not allow precise definition of atypia. This study demonstrated that the use of a specific antibody probe to map malignant urothelial changes in the whole bladder is possible. Antibody G4 can easily recognize only high grade TCC and CIS. This limits its usefulness for detecting low grade malignancies and field changes. Use of G4 together with other anti-TCC antibodies should broaden the capability of immunohistological methods to detect a spectrum of urothelial changes in the human bladder. These studies did not provide adequate information regarding premalignant field changes because a single probe was used and because cryostat sections do not provide adequate morphologic details to grade urothelial dysplasia. This problem is being explored using cold cup biopsies and a panel of monoclonal antibodies.

Other in vivo staining tests such as methylene blue bladder staining and microscopic chemocystoscopy or fluorescein ultraviolet cystoscopy have been described for bladder mapping [2, 7, 17]. These techniques are based on a differential uptake of a dye related to modification of the vascularization or cell junctions in malignant urothelium which are not specific and can also occur in inflammatory lesions. Use of monoclonal antibodies which can be conjugated to fluoroscein or dye may provide more specific information, on tumor presence and location.

The first step in therapeutic application of monoclonal antibody for direct tumor cell killing or for focusing cytotoxic agents in the area of the tumor requires preferential binding to tumor in vivo [4].

Here we have demonstrated that G4 injected intravesically can bind preferentially to living TCC, and that monoclonal antibodies may be useful intravesically for understanding and treating urothelial changes associated with malignancy. Further studies of this monoclonal antibody and of conjugated, tumor toxic derivates are planned.

References

1. Chopin DK, Bubbers JE, deKernion JB, Fahey JL (1984) Monoclonal antibodies against tumor associated antigens (TAA) on human transitional cell carcinoma of the bladder. J Urol 131:107A
2. Gill WB, Huffman JL, Lyon ES, Bagley DH (1983) In vivo urothelial surface histology by microscopic chromocystoscopy. J Urol 130:669
3. Kakizoe T, Matsumoto K, Nishio Y, Kishi K (1984) Analysis of 90 step-sectioned cystectomized specimens of bladder cancer. J Urol 131:467
4. Koprowski H (1984) Human tumor antigens in "Cancer cell." In: The transformed phenotype vol. 1. Levine AJ, Vandewoude GF, Topp WC, Watson JD (eds) Cold Spring Harbor Laboratory Press, New York, pp 293–296
5. Brawn PN (1984) The relationship between non-invasive papillary lesions and invasive bladder carcinoma. Cancer 54:620
6. Farrow GM, Utz DC, Rife CC (1976) Morphological and clinical observations of patients with early bladder cancer treated with total cystectomy. Cancer Res 34:2495
7. Fukui I, Yokokawa M, Mitani G, Ohwada F, Wakui M, Washizuka M, Tohma T, Igarashi K, Yamada T (1983) In vivo staining test with methylene blue for bladder cancer. J Urol 130:252
8. Melicow MM (1952) Histological study of vesical urothelium intervening between gross neoplasm in total cystectomy. J Urol 68:261
9. Messing EM, Bubbers JE, Whitmore K, deKernion JB, Nestor MS, Fahey JL (1984) Murine hybridoma antibodies against human transitional carcinoma-associated antigens. J Urol 132: 167–172
10. Oi VT, Herzenberger LA (1979) Localization of murine Ig-1b and Ig-1a (IgG_{2a}) allotypic determinants detected with monoclonal antibodies. Mol Immunol 16:1005–1007
11. Reisfeld RA, Varki NM, Walker LE (1984) Drug monoclonal antibody conjugates for cancer therapy. Proc. 75th Ann Meeting Am Assoc Cancer Res, May 9–12, Toronto, Canada, p 410
12. Schade ROK, Swinney J (1973) The association of urothelial atypism with neoplasia: its importance in treatment and prognosis. J Urol 109:619
13. Schade ROK, Swinney J (1983) The association of urothelial abnormalities with neoplasia: a 10 year follow-up. J Urol 129:1125

14. Soloway MS, Murphy W, Rao MK, Cox CE (1978) Serial multiple biopsies in patients with bladder cancer. J Urol 120: 57
15. Soto EA, Friedell GH, Tiltman AJ (1977) Bladder cancer as seen in giant histologic sections. Cancer 39:447
16. Weinstein RS, Miller AW, Pauli BU (1980) Carcinoma in situ: Comments on the pathobiology of a paradox. Urol Clin North AM 7:3–523
17. Whitmore WF, Bush IM (1966) Ultraviolet cystoscopy in patient with bladder cancer. J Urol 95:201
18. Wolf M, Hojgaard K (1983) Urothelial dysplasia concomittant with bladder tumors as a determinant factor for future new occurrences. Lancet II:134

D. Chopin
Service d'Urologie
Hôpital Henri Mondor
F-94000 Creteil
France