Detection of A, B and H(O) Antigens in Normal and Neoplastic Epithelium of the Urinary Bladder by the Specific Red Cell Adherence Test (SRCA)

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KATO, T. *Detection of A, B and H(O) Antigens in Normal and Neoplastic Epithelium of the Urinary Bladder by the Specific Red Cell Adherence Test (SRCA)*. Tohoku J. exp. Med., 1977, 121 (3), 239-246 — A, B and H(O) blood group antigens in some tissues are readily demonstrable by the specific red cell adherence test (SRCA) which is a modification of Coombs' mixed cell adherence technique. Paraffin-embedded tissue sections obtained from tumors of the urinary bladder were submitted to SRCA. Normal bladder epithelium adjacent to neoplastic lesions and the leukoplakia were found to contain the three antigens. The antigens were completely absent in 18 cases and partially present in one of the bladder carcinoma. It was also suggested that the prognosis of the tumor correlates with the antigen loss. SRCA is sensitive, reproducible and technically easy for detection of tissue antigens, and it would be expected to play a crucial role for demonstration of immunological dedifferentiation in tissues which normally keep the antigens. ——— ABH(O) antigens; normal and neoplastic urinary bladder; immunological dedifferentiation; specific red cell adherence test (SRCA)

Some tissues and body fluids contain antigens identical to A, B and H(O) blood group antigens which form one of the most thoroughly explored systems in immunology (Landsteiner 1901; Shirai 1924; Hartmann 1941; Davidsohn 1972). Many investigators have suggested that, with highly sensitive and specific technique, these substances can serve as tracer antigens for the study of cancerous transformations (Masamune et al. 1958; Kay and Wallace 1961; Davidsohn et al. 1966; Davidsohn 1972). The earliest technique for the demonstration and identification of the three antigens in tissue was the agglutination inhibition test (Kabat 1956), and more recently the immunofluorescence technique was introduced (Coons et al. 1950). Although the latter permits the location of the group antigens in tissues and has been widely used, Davidsohn et al. (1968) reported that the specific red cell adherence test (SRCA), which was a modified Coombs' mixed cell agglutination technique, was much sensitive and technically easier for detection of tissue antigens.

In the present study, formalin-fixed paraffin-embedded tissue sections obtained from 20 cases of urinary bladder tumors were submitted to SRCA for determination of ABH antigens in the urinary transitional epithelium with special reference to cancerous transformation.

MATERIALS AND METHODS

The cases submitted to the present study are shown in Table 1.

TABLE 1. *Pertinent data of the cases of urothelial tumors submitted to SRCA*

Patient	Age	Blood type	Histology	SRCA		Remarks	
				Tumor lesion	Normal epith.		
1	K.T.	31	A	Pap. Ca., G 1, St O	±	+	
2	E.T.	53	B	Pap. Ca., G 2, St A	—	+	
3	M.I.	67	O	Pap. Ca., G 2, St A	—	+	Tumor recurrence
4	Y.K.	62	O	Pap. Ca., G 2, St O	—	+	Tumor recurrence
5	S.K.	55	B	Pap. Ca., G 2, St A	—	+	
6	K.I.	74	B	Pap. Ca., G 2, St A	—	+	
7	K.T.	69	A	Trs. Ca., G 3, St B2	—	+	Cancer death
8	T.K.	84	B	Trs. Ca., G 2, St B1	—	+	
9	K.Y.	78	O	Pap. Ca., G 1, St A	—	+	Tumor recurrence
10	J.H.	78	A	Trs. Ca., G 3, St C	—	+	
11	K.T.	57	AB	Pap. Ca., G 2, St O	—	+	
12	S.S.	67	A	Sqm. Ca., G 3, St C	—	± *	
13	T.S.	68	B	Trs. Ca., G 3, St C	—	+	
14	T.O.	63	B	Pap. Ca., G 2, St B1	—	+	
15	M.S.	56	B	Trs. Ca., G 3, St B2	—	+	
16	T.O.	71	A	Pap. Ca., G 1, St A	—	+	
17	G.K.	39	A	Pap. Ca., G 2, St A	—	+	Tumor recurrence
18	Y.F.	82	O	Trs. Ca., G 3, St B2	—	+	
19	K.T.	53	B	Trs. Ca., G 3, St A	—	+	
20	K.S.	30	A	Leukoplakia	+	+	

* Squamous metaplasia.

The tissue specimens of the urinary bladder were obtained by means of biopsy, trans-urethral resection or open surgery from 19 cases of bladder carcinoma and one case of leukoplakia of the bladder. The specimens were then foramlin-fixed and paraffin-embedded according to the usual histological procedure. Approximately 4 μ m thick sections of paraffin-embedded tissues were mounted on standard glass slides. Both the neoplastic and nonneoplastic normal tissues were examined in all of the cases studied.

Antisera. Commercially prepared human anti-A and anti-B sera with titers of about 500 were used for detection of antigens A and B, and extracts of *Ulex europaeus* with titers ranging from 64 to 128 were prepared for identification of antigen H in group O (Kovarík et al. 1968).

Indicator erythrocytes. Erythrocytes of groups A, B and O washed in phosphate buffer solution (PBS) were used as a 5% suspension in the same buffer. The reaction with *ulex* extract and O erythrocytes was enhanced by treatment of O erythrocytes with a 0.01% trypsin solution for 60 min at 37°C.

SRCA. (1) Tissue sections were deparaffinized in the usual manner and washed briefly in PBS. (2) Each slides were placed in Petri dishes lined with moist filter paper. (3) The sections were covered with antisera for 15 min at room temperature. It was necessary to keep the *ulex* extract on the sections for 4 hr. (4) The slides were returned to the slide holder and uncombined antiserum on the surface was washed off with three changes of PBS. The slides should remain in each bath for at least 10 min with occasional stirring. (5) Excess buffer was drained off and the sections were covered with AB serum for 5 min to prevent nonspecific adherence of erythrocytes to slide glass. (6) Each section was then covered with the indicator erythrocyte suspension. (7) After 15 min the erythrocytes

sedimented and came into contact with the tissue section. For detection of H antigen, O erythrocytes should remain on the section for 30 min. (8) Another set of Petri dishes was then filled with minimal amounts of buffer. The slides with the sedimented erythrocytes were turned upside down and placed immediately on two supporting pieces of wooden applicators so that they just touched the buffer solution. (9). The indicator erythrocytes which did not react specifically with the homologous immune serum became detached and sank to the bottom of the Petri dish. After 15 min each slide was moved aside on the supporting applicators to an area of clear buffer. (10) Each slide in the Petri dish was ready for microscopic observation.

RESULTS

Reagent controls were performed in 10 cases selected at random, with reactions combining non-isologous antiserum and isologous indicator erythrocytes, as well as isologous antiserum and non-isologous indicator erythrocytes. In all of the cases

Fig. 1. Biopsy specimen from normal bladder showing that the indicator erythrocytes react specifically to the transitional epithelium, erythrocytes and endothelium. H & E stained section (a) and SRCA section (b).

Fig. 2. Leukoplakia (Case 20). SRCA is positive, but the basal layer cells do not react to the indicator cells. H & E stained section (a) and SRCA section (b).

the indicator erythrocytes did not react to the tissues. The antigens were clearly outlined by the indicator erythrocytes reacting with erythrocytes present in/or extravasated from the lumina of blood vessels and with the endothelial cells lining blood vessels in the tissues subjected to the SRCA (Fig. 1). On the other hand, the stromal components, namely muscle fibers and connective fibers, were proved not to have the isoantigens (Fig. 1). In agreement with the results reported previously by others (Davidsohn et al. 1972; Kovarik et al. 1968), these findings permitted to adopt the former as a built-in positive control and the latter as built-in negative control.

The normal transitional epithelial cells of the bladder reacted to the indicator erythrocytes (SRCA+), indicating the presence of the antigens in the bladder

Fig. 3. Papillary carcinoma, stage B1, grade 2 (Case 14). The antigen is absent in neoplastic cells, and the indicator cells attached to the endothelium and erythrocytes in the tissue alone. H & E stained section (a) and SRCA section (b).

Fig. 4. Transitional cell carcinoma, stage B2, grade 3 (Case 7). SRCA is negative. H & E stained section (a) and SRCA section (b).

epithelium (Fig. 1).

The pertinent data of the present study are summarized in the table. The non-neoplastic epithelium adjacent to the neoplastic lesions was SRCA-positive in all of the cases except for one in which the bladder epithelium showed diffuse squamous metaplasia (Case 12).

The SRCA was positive in leukoplakia, but the cells of the basal layer were not coated with indicator erythrocytes (Fig. 2). This result is in agreement with that of the stratified squamous epithelium of the skin and the cervix reported previously (Davidsohn et al. 1969).

With the SRCA the antigens could not be detected in 18 cases of bladder

Fig. 5. Anaplastic transitional cell carcinoma, stage C, grade 3 (Case 10). SRCA is negative. H & E stained section (a) and SRCA section (b).

Fig. 6. Papillary carcinoma, stage O, grade 1 (Case 1). The neoplastic epithelium is coated with the indicator cells in part as shown in this picture. H & E stained section (a) and SRCA section (b).

carcinoma (Figs. 3-5), in which one died of advanced tumor growth 6 months after radical cystectomy (Case 7) and four suffered from recurrences after resection of the tumors (Cases 3, 4, 9 and 17). In one case (Case 1) the isoantigen was partially present (Fig. 6) and partially absent, so the reaction was estimated as SRCA $\pm$. No recurrence has been found in this case for 5 years since partial cystectomy.

The follow-up periods of the investigated cases ranged from 6 months to 5 years.

DISCUSSION

Coombs et al. (1956) demonstrated the A and B antigens in epidermal cells by the mixed agglutination technique, which was shortly applied to detection of these antigens in isolated urinary bladder epithelial cells (Kay 1957). Davidsohn and associates (1968) improved Coombs' technique so that paraffin-embedded tissue sections as well as frozen sections were able to be employed, and designated this immunohistological technique as the specific red cell adherence test (SRCA). As Gupta et al. (1973) stated, the water soluble and alcohol soluble antigens are readily demonstrable by SRCA and remain in a well preserved state even after many years of fixation. Distribution of the antigens in tissues can be so clearly identified by this technique as to make a histological diagnosis possible. The present study shows that SRCA on paraffin embedded tissue sections is sensitive, specific and reproducible, and moreover it can be performed without any special apparatus nor highly specific technique.

Normal transitional epithelium of the bladder was proved to have the three antigens by SRCA in the present study, and the result was in agreement with previous reports (Kay and Wallace 1961; Kovarik et al. 1968).

Kay and Wallace (1961) studied the A and B antigens on fresh cells scraped from tumors arising out of the urinary epithelium by Coombs' agglutination technique. They observed that complete or partial loss of the antigens was frequent and that frequency of antigen loss increased particularly among the histologically malignant and rapidly fatal tumors. It was found, however, that some degree of antigenicity remained in some tumors which were actually invasive at the time of testing. Some of these reactions could be falsely positive. Such false positives are eliminated by SRCA which uses sections through the tumors itself in which blood vessels and normal tissues can be identified.

Decenzo et al. (1975) studied SRCA on 22 patients with low staged bladder tumors and found that the absence of the antigens in the original lesions correlated with the development of invasive carcinomas in 9 cases and that in the presence of the antigens invasive condition failed to develop within 5 to 14 years of follow-up. Davidsohn (1972) summarized his extensive work on SRCA with 933 carcinomas of the lung, cervix, pancreas and stomach. He stated that no antigens were detected in 88%, partial loss was present in 7% and no demonstrable loss also in 4%. The present results also revealed that the bladder epithelial cells in malignant lesions lose the antigens which are present in non-malignant epithelium and that

the antigen loss correlates with the prognosis of the tumors in general. From the above-mentioned results, the loss of the three antigens could be interpreted as the evidence of immunological dedifferentiation analogous to morphological dedifferentiation in anaplasia. SRCA affords valuable clues in the diagnosis of early carcinoma in tissues that normally contain the antigens as well as in the prognosis of manifest carcinoma.

It is well known that the ABH(O) blood group substances show ontogenic development, which can be contrasted with those of carcinoembryonic antigen (Gold et al. 1965), and α -fetoprotein (Abelev et al. 1963). Furuhashi et al. (1954) stated that the blood group substances in fetal period had considerably lower antigenicity than in adult. The antigenicity of these substances rapidly develops after birth through 5 or 7 years of age.

Recently Simmons and Perlmann (1973) reported that carcinoembryonic antigens were incomplete ABH blood group substances which were cryptic in normal situation, and concluded that the genetic changes in oncogenesis included deletion of the genes controlling the transferases for the terminal sequences of ABH-active glycoproteins.

Thus, further investigation of the three antigens in tissues would be expected to play a crucial role for cancer research.

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