Eur J Cancer Clin Oncol, Vol. 20, No. 12, pp. 1557–1560, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Letter to the Editor

Polyoma Virus-induced Tumor-specific Transplantation Antigen (TSTA) is a Mouse and Rat Cross-species-reacting Antigen*

†TORBJÖRN RAMQVIST and TINA DALIANIS
Department of Tumor Biology, Karolinska Institute, Box 60400, 104 01 *Stockholm, Sweden*

POLYOMA virus-infected normal mice have a virtually watertight immune surveillance that protects them against the outgrowth of polyoma virus-transformed cells [1]. Furthermore, mice or rats immunized with polyoma virus or polyoma virus-induced tumor cells of the same species can reject an inoculum of living polyoma tumor cells, which otherwise grows and kills the animals [2–5].

The target of the host response, operationally defined as the tumor-specific transplantation antigen (TSTA) [4, 5], must be a direct or indirect product of the early region, since only this region of the viral genome is active in transformed cells. It may be expected to correspond to one or more of the three products, small, middle or large T-antigen, coded for by the early region [6] or to a so far unidentified cellular membrane component.

We have previously shown the ability of various deletion [7, 8] and host range (hr-t) [9] mutants to induce a TSTA-type immunity against wild-type polyoma virus-induced tumor cells [10]. The finding that an hr-t mutant, expressing a complete large T-antigen and nonfunctional fragments of middle and small T-antigens, could induce tumor rejection suggested that full-length middle and small T-antigens were not necessary for this response. Moreover, since intact middle T-antigen was the only virus-coded protein known to be associated with the plasma membrane [6], the possibility that the polyoma TSTA was a cellular component had to be considered. Although we cannot exclude the possibility that the N-terminal part common to all T-antigens could be a TSTA candidate, recent studies indicate that middle T-antigen is not exposed on the outside surface of the plasma membrane [11] and thus support the notion of TSTA being a cellular component. If this indeed is the case, one would like to know whether polyoma TSTA is species-specific or not. Karl Habel showed that it was not possible to immunize mice against polyoma TSTA with one polyoma-induced hamster tumor [12]. Although the data were limited, no cross-reaction was demonstrated and a cell-derived TSTA was discussed.

In this study we show that it is possible to immunize mice against polyoma TSTA with polyoma virus-induced rat tumors.

The mouse and rat cell lines used in these experiments are described in Table 1. Three wild-type polyoma virus-induced mouse tumors, SEBA, SEBB and SECA, were used both as immunogens and for living challenge in rejection tests. Mice were also immunized with wild-type polyoma virus (a plaque-purified derivative of the A-2 Pasadena large plaque strain [13, 14]) and three rat cell lines. The Rat-1 line is a polyoma virus-negative fibroblast line while wtRat-1z is a wild-type polyoma virus-transformed derivative of the Rat-1 line and SEBDAS is a polyoma virus-induced renal tumor.

Mice were immunized weekly × 5 either with six hemagglutination units of virus [10] or with approximately 1–2 × 10^5 cells (irradiated with 10,000 rad). The total number of cells given during each immunization was between 0.5 and 1 × 10^6 cells per mouse. The hemagglutination inhibition (HI) titers of controls and immunized mice were checked regularly as described

Accepted 25 June 1984.
*This work was supported by the Swedish Cancer Society.
†To whom requests for reprints should be addressed.

previously [10] after the last immunization. Except for mice immunized with wild-type polyoma virus, which showed titers up to 2560, none of the other groups, i.e. controls, or mouse or rat cell line-immunized animals, showed HI titers corresponding to any significant polyoma virus release. Before living tumor challenge all mice received 400 rad of whole-body irradiation. This proved necessary to distinguish between unspecific and anti-polyoma TSTA-specific immune responses [10, 15].

Rejection tests described in Tables 2–4 show that all three tumors are rejected in mice immunized with either wild-type virus (Tables 2 and 4) or by mice immunized with autologous tumor (Tables 2–4). This is not unexpected since

Table 1. Cell lines used

Designation	Origin	Polyoma TSTA	Large T	Middle T	Small T	Relative polyoma DNA content§	References
SEBA*	CBA mouse	+	+	+	+	30	[10]
SEBB*	CBA mouse	+	+‡	+	+	30	[10]
SECA*	ACA mouse	+	+‡	+	+	NT	[10]
SEBDAS*	BDX rat	+	+‡	+	+	2	[17]
wt Rat-1 z*	polyoma virus-transformed Rat-1 line	+	+	+	+	4	[18]
Rat-1†	Fischer rat	-	-	-	-	0	[19]

*Polyoma virus tumor.
†Nonpolyoma line.
‡Truncated form of large T-antigen.
§Tested by dot blot hybridization: cells were treated with 10 mM Tris (pH 7.4), 10 mM EDTA, 10 mM NaCl, 0.5% SDS containing proteinase K 50 ml/10^6 cells) for 5 hr at 37°C. The DNA was extracted using phenol separation and ethanol precipitaion followed by RNAs treatment [50 μg/ml in 10 mM Tris (pH 7.4), 1 mM EDTA and 10 mM NaCl] and a new phenol separation and ethanol precipitation. The purified DNA was spotted on a nitrocellulose filter in series from 10 to 0.3 μg and hybridized with a ^{32}P-labeled wild type polyoma DNA probe as described by Maniatis *et al.* [20].
NT = not tested.

Table 2. Tumor takes of SEBA in CBA mice

Exp. No.	Challenge No. of cells*	Control	wt-virus	Mice immunized with: SEBA	wtRat-1z	Rat-1	SEBA+ wtRat-1z
1	1×10^5	5/5	2/5	0/5			
1	1×10^4	5/5	0/5	0/5			
2	1×10^4	6/6		0/8	4/9		0/10
3	1×10^4	9/9		2/9	5/9	7/7	0/8
4	1×10^3	5/5		1/5	0/5	2/4	
Total takes*		35/35	2/10	3/32	9/23	9/11	0/18
% takes		100	20§	9§	39§	82	0§

*No significant differences were observed for the different cell doses and therefore the data were pooled (10).
§$P < 0.001$ compared to controls.

Table 3. Tumor takes of SECA in ACA mice

Exp. No.	Challenge No. of cells*	Control	Mice immunized with: SECA	wtRat-1z	Rat-1
1	5×10^3	5/5	0/3		
	1×10^4	5/5	1/5		
	1×10^5	5/5	0/3		
2	1×10^3	5/5	2/5	0/5	3/3
3	1×10^3	5/5	0/4	3/4	4/5
Total takes*		25/25	3/20	3/9	7/8
% takes		100	15§	33§	87

*No significant differences were observed for the different cell doses and therefore the data were pooled (10).
§$P < 0.001$ compared to controls.

Table 4. Tumor takes of SEBB in CBA mice

Exp. No.	Challenge No. of cells*	Mice immunized with: Control	wt-virus	SEBB	SEBDAS	Rat-1	SEBB+ SEBDAS	SEBB+ Rat-1
1	1×10^4	5/5	3/5	2/5	3/5			
2	1×10^4	6/11	1/9	4/8		5/7		0/7
3	1×10^4	6/6		2/4	3/7		1/7	
4	1×10^5	5/5		2/5	4/5		2/5	
Total takes*		22/27	4/14	10/22	10/17	5/7	3/12	0/7
% takes		81	29‡	45†	59	71	25‡	0§

*No significant differences were observed for the different cell doses and therefore the data were pooled (10).
†$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$ compared to controls.

it has previously been shown that all three tumors express TSTA [10]. It is, however, somewhat remarkable that SEBB immunizes poorly against itself, as seen in Table 4.

The rat polyoma line wtRat-1z immunizes less efficiently against SEBA (39% tumor take) and SECA (33% tumor take) than the two mouse tumors do against themselves (i.e. for SEBA 9% and for SECA 15% tumor take). The take incidence in controls was 100% (Tables 2 and 3). The SEBDAS polyoma tumor shows a rather poor immunization against the SEBB tumor (Table 4). However, the same immunization pattern is observed in Table 4 as in Tables 2 and 3, i.e. mouse polyoma tumors are better immunogens in mice than rat polyoma tumors.

The Rat-1 nonpolyoma control line does not show a significant immunizing effect against any of the mouse polyoma tumors, as expected (Tables 2–4).

Since rat cells express antigens foreign to mice, we were interested in studying if the presence of such antigens would inhibit the response against polyoma TSTA when mouse polyoma tumors were used as immunogens in parallel. This was not the case, as demonstrated in Tables 2 and 4. On the contrary, if anything, a better immunizing effect was observed.

In summary, we have shown that it is possible to immunize mice with polyoma virus-induced rat tumors against polyoma TSTA, although in general they are less efficient than mouse polyoma tumors as immunogens in the mouse.

Quantitative differences in the expression of TSTA on the mouse and rat polyoma cells used could exist, especially since one could find quantitative differences in the amount of polyoma DNA in the different lines, as shown in Table 1. However, both SEBDAS and wtRat-1z immunize efficiently against polyoma TSTA in rats [21].

It is also possible that the inefficiency of rat polyoma tumors to immunize mice is due to the presentation of the antigen in the context of xenogeneic major histocompatibility antigens [16]. The presence of rat antigens *per se*, however, together with, for example, polyoma TSTA of the mouse, as shown in Tables 2 and 4, does not inhibit the anti-TSTA response of the mouse.

An alternative explanation for the differences between mouse and rat polyoma tumors as immunogens in the mouse could be that polyoma TSTA is a virus-induced cellular antigen, which is cross-reacting between mouse and rat, but is not completely identical.

REFERENCES

1. Allison AC. Immune responses to polyoma virus induced tumors. In: Klein G, ed. *Viral Oncology*. New York, Raven Press 1980, 481–488.
2. Sjögren HO, Hellström I, Klein G. Resistance of polyoma virus immunized mice to transplantation of established polyoma tumors. *Exp Cell Res* 1961, **23**, 204–208.
3. Vandeputte M, De Somer P. Transplantation of polyoma tumors in rats. *Nature* 1963, 199, 391–392.
4. Sjögren HO. Studies on specific transplantation resistance to polyoma virus induced tumors: III, Transplantation resistance to genetically compatible polyoma tumors induced by polyoma tumor homografts. *JNCI* 1964, **32**, 645–659.
5. Sjögren HO, Hellström I, Klein G. Transplantation of polyoma virus induced tumors in mice. *Cancer Res* 1961, **21**, 329–337.
6. Ito Y. Organization and expression of the genome of polyoma virus. In: Klein G, ed. *Viral Oncology*. New York, Raven Press 1980, 447–480.

7. Griffin BE, Ito Y, Novak U *et al.* Early mutants of polyoma virus (dl 8 and dl 23) with altered transforming properties: is polyoma virus middle T-antigen a transforming gene product? *Cold Spring Harbor Symp Quant Biol* 1979, **44**, 271–283.
8. Magnusson G, Berg P. Construction and analysis of viable deletion mutants of polyoma virus. *J Virol* 1979, **32**, 523–529.
9. Benjamin TL. Host range mutants of polyoma virus. *Proc Natl Acad Sci USA* 1970, **67**, 394–399.
10. Dalianis T, Magnusson G, Ito Y, Klein G. Immunization against the polyoma virus-induced tumor-specific transplantation antigen by early region mutants of the virus. *J Virol* 1982, **43**, 772–777.
11. Schaffhausen BS, Dorai H, Arakere G, Benjamin TL. Middle T-antigen relationship to cell membranes and apparent lack of ATP-binding activity. *Mol Cell Biol* 1982, **2**, 1187–1198.
12. Habel K. Antigenic properties of cells transformed by polyoma virus. *Cold Spring Harbor Symp Quant Biol* 1962, **17**, 433–439.
13. Fried M, Griffin RE, Lund E, Robberson DL. A study of wild type mutant and defective DNA's. *Cold Spring Harbor Symp Quant Biol* 1974, **39**, 45–52.
14. Griffin BE, Fried M, Cowie A. Polyoma DNA a physical map. *Proc Natl Acad Sci USA* 1974, **71**, 2077–2081.
15. Klein G, Klein E. Antigenic properties of other experimental tumors. *Cold Spring Harbor Symp Quant Biol* 1963, **27**, 463–470.
16. Matzinger P, Bevan M. Induction of H-2 restricted cytotoxic T-cells: *in vivo* induction has the appearance of being unrestricted. *Cell Immunol* 1977, **33**, 92–100.
17. Szigeti R, Dalianis T, Klein G, Ito Y. Detection of polyoma virus-induced tumor antigen(s) by macrophage migration inhibition. *Int J Cancer* 1982, **30**, 69–74.
18. Ito Y, Spurr N. Polyoma virus T-antigens expressed in transformed cells: significance of middle T-antigen in transformation. *Cold Spring Harbor Symp Quant Biol* 1980, **44**, 149–157.
19. Freeman AE, Gilden RV, Vernon ML, Wolford RG, Hugunin PE, Huebner RJ. 5-Bromo-2-deoxyuridine potentiation of transformation of rat leukemia gs antigen in transformed cells. *Proc Natl Acad Sci USA* 1973, **70**, 2415–2419.
20. Maniatis T, Fritsch EF, Sambrook J. *Molecular Cloning*. Cold Spring Harbor, Cold Spring Harbor Laboratory, 1982, 387–389.
21. Dalianis T, Ramqvist T, Klein G. Studies on the polyoma virus induced tumor specific transplantation antigen (TSTA)—does middle or large T-antigen play a role? *Int J Cancer* In press.