Eur J Cancer Clin Oncol, Vol. 17, No. 10, pp. 1083–1087, 1981.
Printed in Great Britain.

0277-5379/81/101083-05$02.00/0

Immunogenic Cell Variants of a Mouse Teratocarcinoma Confer a Protection Against the Original Non-immunogenic Transplantable Tumor

MYRIAM GEORLETTE and THIERRY BOON

Ludwig Institute for Cancer Research, Brussels branch, UCL 74.59, 74 avenue Hippocrate, B-1200 Bruxelles, Belgium and Cellular Genetics Unit, Université de Louvain, Bruxelles, Belgium

Abstract—*We reported previously that, by mutagenesis of teratocarcinoma cell line PCC4. aza1, it is possible to obtain immunogenic cell variants [tum⁻ (incapable of forming progressive tumors)], that are rejected by syngeneic mice. These tum⁻ variants confer an immune protection against PCC4.aza1, even though this line does not elicit any rejection response in syngeneic mice.*

We show here that these variants also confer an immune protection against other malignant cell lines derived from the same transplantable teratocarcinoma as PCC4.aza1 and also against this transplantable tumor itself. These results rule out the possibility that the protection against PCC4.aza1 is caused by an artefactual antigen acquired in vitro. *They suggest that it may be possible to use tum⁻ variants to elicit a rejection response against non-immunogenic tumors in the primary host.*

INTRODUCTION

THE RATIONALE of a large part of cancer immunotherapy is based on the presence of specific "tumor-associated" transplantation antigens on cancer cells. While a large body of experimental evidence suggests that a variety of human tumors carry specific antigens [1–3], there subsists a considerable doubt regarding the presence of specific transplantation antigens on all tumors. In the mouse it has been shown that tumors induced either with oncogenic viruses or with carcinogens like methylcholanthrene usually elicit an immune rejection process in syngeneic hosts [4–6]. On the contrary, little or no transplantation immunogenicity was observed with tumors that arose spontaneously [7]. It is, therefore, not surprising that a number of attempts have been made to increase the immunogenicity of tumor cells. Increased immunogenicity has been reported for tumor cells infected with viruses [8–10], for tumor cells whose surface was modified with chemicals [11] and for tumor cells treated *in vivo* with antineoplastic drugs [12].

We reported previously that upon treatment of the non-immunogenic malignant mouse teratocarcinoma cell line PCC4.aza1 with the mutagenic compound, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, we obtained immunogenic variant clones which failed to form tumors in syngeneic 129/Sv mice. These stable variants, which we called "tum⁻", undergo a process of immune rejection [13]. Most of these variants carry a new singular transplantation antigen. Moreover, mice that have rejected these tum⁻ variants are partially resistant to a challenge with the original teratocarcinoma PCC4.aza1 cell line (tum⁺). This is remarkable because no protection can be induced either with irradiated tum⁺ cells or with living tum⁺ cells subsequently removed by surgery [14, 15]. This protection conferred by tum⁻ variants does not appear to be caused by a non-specific stimulation of the immune system as it extends over many months and is largely radiation resistant [14, 15].

We have observed that tum⁻ variants can also be obtained by mutagenesis in two other mouse tumor systems, namely Lewis lung carcinoma [16] and mouse mastocytoma P815 [17]. In both systems the tum⁻ variants confer a significant protection against the tum⁺ cells. This is, however, less remarkable than the results

Accepted 5 May 1981.

obtained with teratocarcinoma, as both Lewis lung carcinoma and P815 mastocytoma show a weak, but definite, immunogenicity.

If it proves possible to make use of immunogenic variants to induce a protection against non-immunogenic tumor cells in the original host, this will widen considerably the class of tumors that may be amenable to immunotherapy. It is, therefore, of importance to find out whether the latent transplantation immunogen present on the teratocarcinoma cell line PCC4.aza1 is present not only on this cultured cell line but also on the original transplantable tumor and, therefore, possibly on the primary tumor.

We present here evidence that tum$^-$ variants obtained from cell line PCC4.aza1, which was derived from the transplantable teratocarcinoma OTT6050, induce a significant protection against other cell lines independently isolated from the same transplantable tumor and against tumor OTT6050 itself.

MATERIALS AND METHODS

Mice

Mice from the inbred 129/Sv line, originally obtained from J. L. Guénet (Institut Pasteur, Paris), were raised in "specific pathogen-free" conditions in our animal house. The mice were approximately 12 weeks old at the time of immunization. Steel/+ and +/+ mice were used indifferently.

Tumor and cell lines

The transplantable ascitic teratocarcinoma OTT6050 [18] was maintained in 129/Sv mice by serial i.p. passage every three weeks. A number of teratocarcinoma cell lines were isolated independently from OTT6050: PCC4 [19], PCC3/A/1 [19, 20] and F-9 (isolated by Mrs Grandchamps in the laboratory of Dr Ephrussi). These lines are clonal and malignant. PCC4.aza1 is a clone of PCC4, resistant to a concentration of 15 μg of azaguanine per ml [19]. Tum$^-$ clones 20 and 25 were isolated from a culture of PCC4.aza1 treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [13]. The doses of injected cells that produce tumors in approximately 50% of the animals (TD_{50}) with OTT6050, PCC4.aza1, PCC3/A/1 and F-9 are 10^4, 3×10^5, 2×10^4 and 2×10^3 respectively.

Tum$^-$ clones L20 and P21 were isolated after treatment with the same mutagen of cell lines derived from Lewis lung carcinoma (C57B1/6) and mastocytoma P815 (DBA/2) respectively [16, 17]. The TD_{50} for L20 and P21 in irradiated syngeneic animals are 10^4 and 10 cells respectively.

Culture conditions

The teratocarcinoma cell lines were cultured in Falcon "tissue culture" dishes with the Dulbecco modification of Eagle's medium supplemented with 10% fetal calf serum (FCS) (Gibco) at 37°C in an atmosphere of 12% CO_2, as previously described [19]. For F-9, the dishes were coated with gelatin [19]. The Lewis lung carcinoma cells and P815 cells were grown in the same culture medium in an atmosphere of 8% CO_2.

Immunization and challenge

For immunization and challenge, mice were injected with cells re-suspended in culture medium containing 1% FCS. For the teratocarcinoma permanent lines, one-third of the cells were injected subcutaneously (s.c.) and the remainder intraperitoneally (i.p.). We have observed that this procedure gives an optimal yield of tumors. OTT6050 tumor cells were injected i.p. with cells from the ascitic fluid. Lewis lung carcinoma cells were injected s.c. and P815 i.p. Unless otherwise mentioned, the mice were challenged between 22 and 30 days after immunization.

RESULTS

Mice of the 129/Sv strain formed progressive tumors when they were inoculated with 2×10^6 cells of the syngeneic permanent teratocarcinoma line PCC4.aza1. On the other hand, less than 5% of these mice formed tumors when they were injected with an equivalent number of cells from tum$^-$ clones 20 or 25, two clones isolated after a mutagenic treatment of PCC4.aza1 [13]. Mice that had rejected tum$^-$ clones 20 or 25 showed significant protection against a challenge with PCC4.aza1 (Table 1). These results confirmed those reported previously [13, 14].

In order to find out whether the common antigen shared by PCC4.aza1 and its tum$^-$ derivatives is restricted to this permanent culture line or whether it may be present on the original tumor, we examined the protection conferred by tum$^-$ clones 20 or 25 against permanent malignant lines F-9 and PCC3, which were independently isolated from the same transplantable teratocarcinoma OTT6050. As shown in Table 1, we observed a protection comparable to that obtained against PCC4.aza1.

In addition, we examined the protection obtained against transplantable teratocarcinoma OTT6050, which was never passed *in vitro* and was always maintained in the syngeneic 129/Sv mice. This tumor grows in the peritoneal cavity in the form of free floating

Table 1. *Protection by tum⁻ variants* 20 *and* 25 *against cell lines isolated from the transplantable tumor OTT*6050

Immunizing clone†	Challenging clone‡	% Mice with tumors (no. with tumors/no. inj.) d50§	d70	d90
20	PCC4.azal	32	42	42 (25/60)**
25	PCC4.azal	33	39	48 (22/46)**
—	PCC4.azal	80	81	88 (52/59)
20	F-9	17	28	28 (5/18)**
—	F-9	72	72	72 (13/18)
20	PCC3/A/1	22	33	33 (6/18)**
25	PCC3/A/1	24	47	47 (8/17)*
—	PCC3/A/1	72	78	78 (14/18)

†Mice were immunized with 2×10^6 living cells.
‡Mice challenged with PCC4.azal, F-9 and PCC3/A/1 received 2×10^6, 6.5×10^5 and 6.5×10^4 cells respectively.
§Number of days after the challenge.
**$P < 0.01$ (chi-square test).
*$P < 0.05$.

aggregates of 10–100 cells. The cell content of these aggregates could be evaluated approximately and they were used as challenge. As shown in Fig. 1, mice immunized with teratoma variant 20 showed a definite protection against OTT6050. The specific nature of the immune protection induced by tum⁻ variant 20 was indicated by the lack of protection observed with mice that were immunized with tum⁻ cells derived from Lewis lung carcinoma. These results were confirmed in two independent experiments reported in Table 2. Mice immunized with tum⁻ variants 20 or 25 were significantly protected against OTT6050. No protection was observed in mice that were previously injected with irradiated OTT6050 or with tum⁻ cells derived from allogeneic mastocytoma P815.

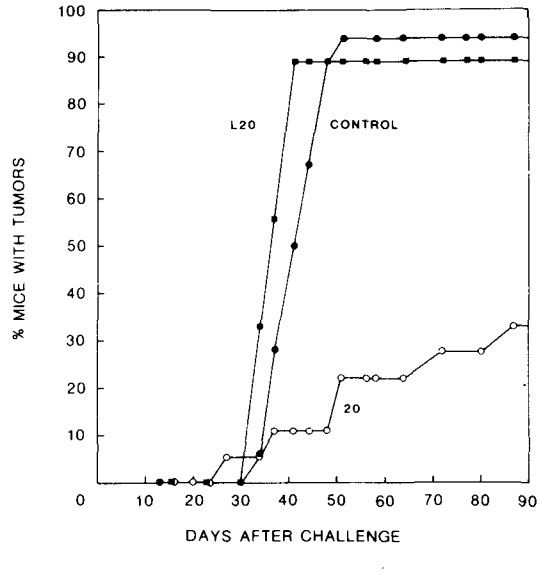

Fig. 1. *129/Sv mice were injected either with* 2×10^6 *living cells of teratoma tum⁻ clone* 20 [20] *or with* 2×10^5 *living cells of Lewis lung carcinoma tum⁻ clone L*20 (*L*20). *The control group received the same amount of injection medium. The mice were challenged* 25 *days later with* 4×10^4 *cells of teratocarcinoma tumor OTT*6050.

DISCUSSION

We have confirmed our previous observations that teratocarcinoma tum⁻ variants isolated from PCC4.azal confer a protection against this non-immunogenic line. This protection has been found to be long lasting and largely radiation resistant [14, 15]. Moreover, it was not observed after immunization with tum⁻ variants obtained from two other tumors. We therefore believe that this is a specific immune protection and that PCC4.azal and the tum⁻ variants carry a common transplantation antigen, which is immunogenic only when located on the tum⁻ variants. We have shown that this common antigen is also present on two other permanent cell lines that were independently isolated from the same transplantable tumor as PCC4.azal. Finally, we have demonstrated its presence on this transplantable tumor itself. This excludes the possibility that this antigen was acquired in the course of the adaptation of the teratocarcinoma cells to *in vitro* growth or during the culture passages.

However, these results do not prove rigorously that this antigen was present on the primary teratocarcinoma and could have been recognized by the original tumor-bearing mouse. The primary tumor was obtained more

Table 2. Protection by tum⁻ variants against the transplantable teratocarcinoma OTT6050

Experiment	Immunizing clone	% Mice with tumors† (no. with tumors/no. inj.) d50‡	d70	d90
I	tum⁻ clone 20§	44	55	55 (10/18)**
	tum⁻ clone 25§	47	53	53 (9/17)**
	—	72	94	94 (17/18)
II	tum⁻ clone 20§	30	45	50 (50 (10/20)*
	tum⁻ clone 20 (2 times)‖	20	30	35 (7/20)**
	OTT6050 irr.¶	100	100	100 (20/20)
	P815 tum⁻ clone P21††	77	82	86 (19/22)
	—	70	80	80 (16/20)

†Mice were challenged with approximately 4×10^4 cells of the ascitic OTT6050 tumor at d42 (exp. I) and d23 (exp. II).
‡Number of days after the challenge.
§Mice were immunized with 2×10^6 living cells.
‖These mice received a first injection of 2×10^6 living cells and, 21 days later, a second injection of 10^7 living cells.
¶Mice immunized with OTT6050 irradiated cells (6000 rads of gamma radiation) received 3.6×10^6 cells.
††Mice were given 2×10^5 living cells.
**$P < 0.01$ (chi-square test).
*$P < 0.05$.

than ten years ago by intratesticular grafting of a syngeneic 6-day-old embryo in a 129/Sv mouse [18]. In the course of its numerous transplantations in 129 mice it may have acquired a new antigen, for instance a viral determinant. Moreover, we cannot exclude the possibility that our 129 mice differ from the primary mouse by having lost or altered a weak histocompatibility antigen which they now recognize as a foreign determinant on the tumor.

Nevertheless, we consider it likely that this antigen was present on the primary teratocarcinoma. Whether it proves to be specific for this individual tumor or for all mouse teratocarcinomas could be answered by examining the protection against other teratocarcinomas obtained independently in the 129 mice. If a protection against recently isolated teratocarcinomas is observed, this will eliminate the possibility that our results are due to minor histocompatibility changes of the 129/Sv mice.

Our observations suggest that it may be possible to induce transplantation immunity against non-immunogenic primary tumors with tum⁻ variants derived from them *in vitro*. A large number of tumors will have to be tested to ascertain the generality of our results and reduce the possibilities of spurious antigenicity mentioned above. Spontaneous mouse tumors, which have been transplanted for only a brief period in a colony of mice carefully maintained in inbred conditions, ought to prove adequate for this purpose.

Acknowledgements—We thank J. Van Snick and A. Van Pel for stimulating discussions and G. Warnier for the expert production of mice. We are grateful for the excellent technical and secretarial assistance of D. Donckers, J. C. Gaudin and G. Schoonheydt. We thank J. Maryanski for her critical reading of the manuscript.

REFERENCES

1. Carey TE, Lloyd KO, Takahashi T, Travassos LR, Old LJ. AU cell-surface antigen of human melanoma: solubilization and partial characterization. *Proc Natl Acad Sci USA* 1979; **76**, 2898–2902.
2. Carrel S, Theilkaes L. Evidence for a tumour-associated antigen in human malignant melanoma. *Nature (Lond)* 1973; **242**. 609–610.
3. Herlyn M, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. *Proc Natl Acad Sci USA* 1979; **76**: 1438–1442.
4. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. *J Natl Cancer Inst* 1957; **18**: 769–779.
5. Klein E, Klein G. Antigenic properties of lymphomas induced by the Moloney agent. *J Natl Cancer Inst* 1964; **32**: 547–568.

6. LECLERC JC, GOMARD E, LEVY JP. Cell-mediated reaction against tumors induced by oncornaviruses. I. Kinetics and specificity of the immune response in murine sarcoma virus (MSV)-induced tumors and transplanted lymphomas. *Int J Cancer* 1972; **10**: 589–601.
7. HEWITT HB, BLAKE ER, WALDER AS. A critique of the evidence for active host defense against cancer, based on personal studies of 27 murine tumors of spontaneous origin. *Br J Cancer* 1976; **33**: 241–259.
8. LINDENMANN J, KLEIN A. Viral oncolysis: increased immunogenicity of host cell antigen associated with influenza virus. *J Exp Med* 1967; **126**: 93–108.
9. KOBAYASHI H, SENDO F, SHIRAI T, KAJI H, KODAMA T. Modification in growth of transplantable rat tumors exposed to friend virus. *J Natl Cancer Inst* 1969; **42**: 413–418.
10. KOBAYASHI H, SENDO F, KAJI H, SHIRAI T, SAITO H, TAKEICHI N, HOSOKAWA M, KODAMA T. Inhibition of transplanted rat rumors by immunization with identical tumor cells infected with friend virus. *J Natl Cancer Inst* 1970; **44**: 11–19.
11. GALILI N, NAOR D, ASJO B, KLEIN G. Induction of immune responsiveness in a genetically low responsive tumor-host combination by chemical modification of the immunogen. *Eur J Immunol* 1976; **6**: 473–476.
12. BONMASSAR E, BONMASSAR A, VADLAMUDI S, GOLDIN A. Immunological alteration of leukemic cells *in vivo* after treatment with an antitumor drug. *Proc Natl Acad Sci USA* 1970; **66**: 1089–1095.
13. BOON T, KELLERMAN O. Rejection by syngeneic mice of cell variants obtained by mutagenesis of a malignant teratocarcinoma cell line. *Proc Natl Acad Sci USA* 1977; **74**: 272–274.
14. BOON T, VAN PEL A. Teratocarcinoma cell variants rejected by syngeneic mice: protection of mice immunized with these variants against other variants and against the original malignant cell line. *Proc Natl Acad Sci USA* 1978; **75**: 1519–1523.
15. BOON T, VAN PEL A, WARNIER G. Mouse teratocarcinoma cell variants obtained by mutagenesis: rejection by syngeneic mice and immunization against the original tumor cell line. In: *Protides of biological fluids, 27th Colloquium.* Oxford: Pergamon Press, 1979: 173–177.
16. VAN PEL A, GEORLETTE M, BOON T. Tumor cell variants obtained by mutagenesis of a Lewis lung carcinoma cell line: immune rejection by syngeneic mice. *Proc Natl Acad Sci USA* 1979; **76**: 5282–5285.
17. UYTTENHOVE C, VAN SNICK J, BOON T. Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815: rejection by syngeneic mice. *J. Exp Med* 1980; **152**: 1175–1183.
18. STEVENS LC. The development of transplantable teratocarcinomas from intratesticular graft of pre-and post-implantation mouse embryos. *Dev Biol* 1970; **21**: 364–382.
19. JAKOB H, BOON T, GAILLARD J, NICOLAS JF, JACOB F. Tératocarcinome de la souris: isolement, culture et propriétés de cellules à potentialités multiples. *Ann Microbiol (Paris)* 1973; **124B**: 269–282.
20. NICOLAS JF, AVNER P, GAILLARD J, GUENET JL, JAKOB H, JACOB F. Cell lines derived from teratocarcinomas. *Cancer Res* 1976; **36**: 4224–4231.