IMMUNOGENIC VARIANTS OBTAINED BY MUTAGENESIS OF MOUSE MASTOCYTOMA P815

II. T Lymphocyte-mediated Cytolysis

By THIERRY BOON, JACQUES VAN SNICK,* ALINE VAN PEL, CATHERINE UYTTENHOVE,* AND MARIE MARCHAND

From the Cellular Genetics Unit, Ludwig Institute for Cancer Research, UCL7459, B1200 Brussels, Belgium

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Various cytotoxic processes have been obtained in vitro in relation to the immune rejection of syngeneic tumor cells. Particular attention has been devoted to cytotoxicity mediated by T lymphocytes (1–4), macrophages (5, 6), and natural killer cells (7–9). Nevertheless, it has proved difficult to establish definitely to what extent these processes contribute in vivo to the rejection of the various tumors that have been analyzed. On this issue, one criterion of relevance lies in the degree of matching of the specificity observed in vitro with that observed in vivo. This requires a comparison of the effects of cytotoxic and rejection processes on a number of different tumors. Unfortunately, this comparison is often complicated because tumors differ not only for their tumor-associated antigens but also for many other cellular properties that influence the outcome of the immune processes both in vitro and in vivo.

We have developed a system of tumor variants that appears to be particularly suitable for the study of immunological reactions directed against a variety of surface antigens obtained on the same background cell. We reported that, by treating a teratocarcinoma and a Lewis lung carcinoma cell line with a mutagen, we obtained a number of cell variants that were rejected by syngeneic mice (tum^-)[1] (10, 11). Cross-immunization experiments performed in vivo indicated the presence of new antigens specific for each of these variants (12). However, neither with teratocarcinoma nor with Lewis lung carcinoma were we able to obtain immune cytotoxic spleen cells that showed any specificity for the immunizing tum^- variants.

In the search for the effector that causes the rejection of tum^- variants, we thought that it would be useful to obtain such variants from an ascitic tumor so as to be able to collect effector cells *in situ*. We show in the accompanying report (13) that we were able to obtain tum^- variants by mutagen treatment of a malignant cell line isolated from the ascitic mastocytoma P815. The cross-immunization pattern observed in vivo with five variants demonstrated the presence of singular antigens on three of them (Table I). We report here the existence of a syngeneic T lymphocyte-mediated cytolysis directed against P815 tum^- variants and we compare the results obtained in vitro to those obtained in vivo.

* Fonds National de la Recherche Scientifique, Brussels, Belgium.

[1] *Abbreviations used in this paper:* FCS, fetal calf serum; HAT, 10^{-4} M hypoxanthine, 3.8×10^{-7} M aminopterin, and 1.6×10^{-5} M 2-deoxythymidine; tum^+, variant cells that were capable of forming tumors in syngeneic mice; tum^-, variant cells that were incapable of forming tumors in syngeneic mice.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/80/11/1184/10 $1.00
Volume 152 November 1980 1184–1193

Materials and Methods

Cells. All the P815 cell clones were cultured in Petri dishes (Falcon 1001; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum (FCS). They were incubated at 37°C in an 8% CO_2 atmosphere. L1210 cells (a DBA/2 leukemia) were grown under the same conditions.

Azaguanine-resistant Mutants. Azaguanine-resistant mutants were selected in limiting-dilution conditions in culture medium that contained 10 or 30 μg/ml of azaguanine (ICN Nutritional Biochemicals, Cleveland, Ohio). Only those mutants were used that died in a culture medium supplemented with 10^{-4} M hypoxanthine (Merck AG, Darmstadt, Federal Republic of Germany), 3.8×10^{-7} M aminopterin (ICN Nutritional Biochemicals), and 1.6×10^{-5} M 2-deoxythymidine (Merck AG) (HAT).

Spleen and Peritoneal Cells. Immune spleen cells were taken from DBA/2 mice injected intraperitoneally ≥3 wk earlier with living tum⁻ P815 cells in culture medium without FCS. Control mice received the same volume of injection medium. The spleen cells were teased in Hanks' balanced salts solution supplemented with 5% FCS. Cell suspensions were filtered through a nylon-mesh gauze (80 μm; Nylon Swiss; Staniar, Manchester, England). Peritoneal cells were obtained by rinsing the peritoneal cavity with 10 ml of Hanks' balanced salts solution that contained 20 U/ml heparin (Roche, Basel, Switzerland). The cells were centrifuged for 10 min at 200 *g* and resuspended in RPMI-1640 medium (Grand Island Biological Co.) supplemented with 10% FCS and 10^{-2} M Hepes (Sigma Chemical Co., St. Louis, Mo.).

Mixed Lymphocyte-Tumor Cell Culture

STIMULATION WITH IRRADIATED CELLS. 3×10^7 immune spleen cells were mixed with P815 cells killed by gamma irradiation (5,000 rad, Cs source). The cells were incubated in 20 ml of RPMI-1640 medium that contained 10% FCS, 10^{-2} M Hepes, and 5×10^{-5} M 2-mercaptoethanol (Merck AG) in 50-ml Falcon flasks standing upright (Falcon 3013). After 4 d of incubation at 37°C in an 8% CO_2 atmosphere, the stimulated lymphocytes were washed in Hanks' balanced salts solution supplemented with 5% FCS and resuspended in RPMI-1640 medium that contained 10% FCS and 10^{-2} M Hepes before assay in the ^{51}Cr-release test.

STIMULATION WITH HAT-SENSITIVE CELLS. The stimulator cells were HAT-sensitive, azaguanine-resistant mutants. The stimulation conditions were the same as above, except that the medium was supplemented with HAT as described above. The stimulator cells were killed in <2 d, whereas the survival of the lymphocytes was not affected.

Cr-release Assays. Target cells grown in vitro were resuspended at 10^7 cells/ml of Hanks balanced salts solution medium supplemented with 5% FCS and incubated at 37°C with 100 μCi/ml of Na[^{51}Cr]O_4 (Institut des Radioéléments, Fleurus, Belgium). 1 h later, the labeled cells were washed first with 50 ml of Hanks' balanced salts solution medium supplemented with 5% FCS, then with 50 ml of RPMI-1640 medium that contained 10% FCS and 10^{-2} M Hepes. They were resuspended in the same medium, and 50-μl aliquots that contained 10^4 cells were distributed into microplates (96 wells; Linbro Chemical Co., Hamden, Conn.). Stimulated lymphocytes were added in 100 μl of the same medium. Assays were made in triplicate. The plates were centrifuged 5 min at 100 *g* and incubated 4 h at 37°C in an 8% CO_2 atmosphere. The plates were then centrifuged again and 75-μl aliquots of supernate were collected and counted. The percentage of ^{51}Cr-specific release was calculated as follows: the percent specific release = (ER − SR) × 100/(MR − SR); where ER was the observed experimental ^{51}Cr release, SR the spontaneous release measured by incubation of 10^4 labeled cells in 150 μl of RPMI-1640 medium alone, and MR the maximum release obtained by adding 50 μl 0.3% Triton X-100 (Sigma) to the target cells. The spontaneous release was ≃10% of the total label incorporated into the cells.

Lytic Units. 1 lytic unit is defined here as the number of spleen or peritoneal cells that lyse 50% of 10^4 target cells in 4 h (9). This number was estimated from the specific release obtained at various effector to target ratios by means of the regression $(1 - e^{-kx})$. The results are expressed in number of lytic units/10^6 cells.

Normalized Number of Lytic Units/10^6 Cells. To compare the lytic activities of an effector cell on the immunizing clone (self-lysis) and on other clones (cross-lysis), we corrected for differences

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in the general sensitivity of the targets. This was done by dividing the number of lytic units obtained with a given effector on a given target by the average number of lytic units obtained on the same target with all the effectors. This average was calculated after replacement of the self-lysis value by the average number of lytic units of the relevant effector on all the other targets. The corrected values were normalized by dividing them by the average of the corrected cross-lysis values of the relevant effector.

Results

When adult DBA/2 mice were injected intraperitoneally with 2×10^5 living cells of a number of tum^- variants, they completely rejected these cells in <20 d (Table I). After that period, these mice showed a significant protection against the immunizing variants, against other P815 tum^- variants, and against the original P1 cells that were capable of forming tumors in syngeneic mice (tum^+) (13). We tested spleen and peritoneal exudate cells of immune animals for their cytolytic activity.

Cytolytic T Lymphocytes in the Peritoneal Cavity. Mice that rejected a first challenge of living P815 tum^- cells received a secondary intraperitoneal injection of 2×10^5 cells of the same tum^- variant. After 4 d, all the mastocytoma cells had been eliminated from the peritoneal cavity as determined by an agar colony test as described in the

TABLE I

Tumorigenicity and Specificity of P815 Clones

	No. of mice with tumors/No. mice injected*	Presence of a specific antigen detected in vivo‡
Control clone:§		
tum^+: P1	15/15	
Mutagenized clones:‖		
tum^-: P21	0/20	+
P32	2/20	−
P35	0/20	+
P60	2/20	Not tested
P76	6/20	Not tested
P89	1/20	−
P91	0/20	+
tum^+: P22	6/6	
P26	6/6	
P30	5/6	
P33	3/3	
P38	6/6	
P40	6/6	
P53	6/6	
P58	6/6	
P87	5/6	
P93	6/6	

* DBA/2 mice were injected intraperitoneally with 2×10^5 living cells. Mice without visible tumors 3 mo after injection were considered negative.

‡ As demonstrated in the accompanying report (13) with immune protection experiments performed either directly or by adoptive transfer.

§ Clone isolated from the initial P815-X2 population.

‖ Clones isolated from a P1 population treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (13).

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accompanying report (13). At this time, the peritoneal cells were collected and their cytolytic activity was tested immediately on a number of P815 tum$^-$ variants. A significant cytolysis was obtained, as shown in Table II. A fractionation by nylon column indicated that the effector cells were T lymphocytes.

As seen in Table II, the peritoneal cells immunized against variants P21 and P32 preferentially lysed the immunizing variant. A similar specificity was obtained with variants P35 and P91. None was observed with peritoneal cells immunized against P89.

Cytolysis by Immune Spleen Cells Stimulated In Vitro. Spleen cells of animals that had been immunized and boosted so as to contain active peritoneal cells had no direct cytolytic activity. However, we observed a strong and specific cytolytic response after in vitro stimulation of immune spleen cells with the immunizing tum$^-$ clone (Table III). Similar results were obtained by stimulating either with tum$^-$ cells killed by irradiation or with their azaguanine-resistant, HAT-sensitive homologues in medium supplemented with HAT. The splenic effector cells were T lymphocytes, as shown by their presence in nylon-filtered population and by their inactivation with anti-Thy-1.2 serum (Table III).

Little or no specific cytolytic activity was obtained after stimulation of spleen cells from naive animals (Table III). Spleen cells from animals immunized 5 d earlier with living tum$^-$ cells already showed a significant specific cytolysis upon restimulation in vitro. The capacity of spleen cells to form cytolytic effector cells upon restimulation remained high between 10 d and 6 mo after immunization. A specific, but weaker, cytolysis could also be obtained with spleen cells from animals immunized with irradiated tum$^-$ variants (data not shown).

TABLE II

Cytolysis of tum$^-$ Variants by Peritoneal Cells

Experiment	Peritoneal effector cells*	Effector: target ratio	Percent specific ^{51}Cr release from target cells					
			P21	P32	P35	P89	P91	L1210
1	Anti-P21	40:1	91	13	14			<1
		20:1	75	5.7	4.7			
		10:1	34	2.0				
	Anti-P21, T cell‡	10:1	50					
		5:1	35					
	Anti-P32	20:1	6.6	42	12			<1
		10:1	3.0	23	6.7			
	Anti-P32, T cells‡	20:1	13	68	22			—
		10:1	7.4	53	—			—
		5:1	3	34	—			—
	Normal	40:1	<1	<1	<1			<1
2	Anti-P35	30:1	0.8	2.9	30	4.8	3.0	
	Anti-P89	30:1	1.8	3.5	8.9	8.4	8.6	
	Anti-P91	30:1	0.7	4.4	5	5.4	35.4	

* In experiment 1, DBA/2 mice were injected with 6×10^4 living P21 or P32 cells. 20 d later, they received an intraperitoneal boost with 2×10^5 living cells of the same clone. Control animals were injected and boosted with medium alone. The peritoneal cells were collected 3 d later and assayed immediately in a 4-h ^{51}Cr-release test. In experiment 2, the mice were injected with 2×10^5 cells and boosted 60 d later. The peritoneal cells were collected 4 d after the boost.

‡ Filtered once on nylon wool (14).

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TABLE III

Cytolysis by Spleen Cells Stimulated In Vitro

Experiment	Spleen effector cells*	Stimulator cells	Effector:target ratio	Percent specific ^{51}Cr release from target cells			
				P1	P21	P32	P91
1	Anti-P21	P21 irradiated	10:1	53	75	54	48
			3:1	30	61	30	27
			1:1	15	36	14	13
				(13)‡	(31)	(13)	(11)
	Anti-P32	P32 irradiated	10:1	54	47	86	64
			3:1	33	22	68	36
			1:1	18	8	48	18
				(14)	(10)	(68)	(18)
	Anti-P91	P91 irradiated	10:1	29	14	29	76
			3:1	9.2	3.8	11	70
			1:1	3.1	0.3	2.4	43
				(3.9)	(1.7)	(3.0)	(67)
	Normal	P21 irradiated	20:1	17	11	22	
			10:1	8.6	5.9	13	
				(1.1)	(0.8)	(1.6)	
	Normal	P32 irradiated	20:1	29	19	40	
			10:1	15	9.4	24	
				(2.5)	(1.4)	(3.3)	
2	Anti-P21	P21 azaguanine resistant	10:1	28	78	27	
			5:1	16	64	15	
			2.5:1	10	53	9.3	
				(4.8)	(29)	(3.9)	
	Anti-P21, T cells§	P21 azaguanine resistant	10:1	26	76	26	
			5:1	14	59	12	
			2.5:1	9	47	8.3	
				(4.3)	(28)	(4.3)	
	Anti-P21, T depleted‖	P21 azaguanine resistant	20:1	<1	<1	2.5	
				(<0.07)	(<0.07)	(0.18)	

* DBA/2 mice were immunized with 2×10^5 living P21, P32, or P91 cells. In experiment 1, the spleen cells were collected after 47 d. 3×10^7 spleen cells were incubated for 4 d with 6×10^5 irradiated stimulator cells. In experiment 2, 3×10^7 spleen cells collected after 26 d were incubated for 5 d with 2×10^5 living P21 azaguanine-resistant cells in HAT medium.

‡ Lytic units/10^6 cells are shown in parentheses.

§ Filtered once on nylon wool (14).

‖ Depleted from T lymphocytes by treatment with anti-Thy-1.2 (AKR anti-C3H) serum and complement.

To examine the possibility that the lytic response was directed against FCS components bound on P815 cells, we immunized mice with tum$^-$ variants grown in mouse serum for >7 d. The results were the same as those obtained after immunization with cells grown in FCS (data not shown).

Analysis of the tum$^-$ Antigenic Specificities. To examine the pattern of singular and common antigens found on tum$^-$ variants, seven independently isolated clones were used to immunize mice and stimulate their spleen cells in vitro. The cytolytic activities were tested against these seven tum$^-$ variants and the tum$^+$ clone P1 (Table IV). Spleen cells immunized and stimulated with P21, P32, P35, and P91 showed a markedly preferential cytolysis for the immunizing variant, indicating the presence of

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TABLE IV

Specificities Defined by Immune Spleen Cell Cytolysis

Spleen effector cells*	Lysis of target cells							
	P1	P21	P32	P35	P60	P76	P89	P91
	lytic units/10^4 cells							
Anti-P21	3.9	11‡	3.4	5.3	4.0	4.1	3.0	3.3
	(0.81)§	(6.44)	(0.97)	(1.20)	(0.95)	(1.17)	(0.90)	(0.93)
Anti-P32	27	6.8	75	22	15	16	16	18
	(1.20)	(0.88)	(4.55)	(1.07)	(0.76)	(0.96)	(1.04)	(1.05)
Anti-P35	21.7	5.0	11	93	16	11	16	12
	(1.20)	(0.81)	(0.86)	(5.57)	(1.05)	(0.85)	(1.32)	(0.93)
Anti-P60	5.6	2.7	5.2	5	8.6	4.9	3.8	5.5
	(0.85)	(1.19)	(1.07)	(0.82)	(1.50)	(1.00)	(0.83)	(1.12)
Anti-P76	6.32	2.0	5.1	6.7	8.3	11	2.5	5.6
	(0.91)	(0.84)	(1.01)	(1.05)	(1.39)	(2.18)	(0.52)	(1.09)
Anti-P89	15.8	6.0	16	18	18	11	11	13
	(0.88)	(0.97)	(1.22)	(1.10)	(1.16)	(0.85)	(0.86)	(0.96)
Anti-P91	3.26	1.1	2.7	3.5	3.4	3.7	2.8	66
	(0.85)	(0.88)	(0.98)	(0.99)	(1.03)	(1.31)	(1.05)	(23.5)

* DBA/2 mice were immunized with 2×10^5 living tum$^-$ variants. 21 d later, these spleen cells were restimulated in vitro with the irradiated immunizing variant and assayed in a 4-h ^{51}Cr-release test on various tum$^-$ variants.

‡ Boxed values were significantly different from the mean of the other cross-lysis values at a $P = 0.02$ level (Student's t test).

§ Normalized lytic index (corrects the differences in general sensitivity of the various targets) is shown in parentheses.

a singular antigenic specificity on each of these variants. No singular specificity was observed on P89. Variants P60 and P76 displayed a weak, but significant, singular specificity which was confirmed in a duplicate experiment. The effectors immunized against every tum$^-$ variant cross-reacted with all the variants and with the tum$^+$ cells. Not unexpectedly, we found that lymphocytes immunized in vivo with irradiated tum$^+$ cells had a uniform cytolytic activity against the tum$^-$ variants (data not shown).

To estimate more precisely the degree of cross-reaction between the different target cells, we normalized the lysis values, taking into account the general sensitivity of each target (Materials and Methods). With this normalized lytic index, shown in Table IV, values significantly >1 indicate that the relevant target cell and the immunizing variant share an antigenic specificity that is not found on the other targets. This procedure confirmed that six out of the seven tum$^-$ variants carried a singular antigenic specificity. Furthermore, it indicated that these six antigens were all different, and that at least those strong singular antigens carried by P21, P32, P35, and P91 were completely unrelated: no significant cross-reaction above that caused by the common antigen found on the tum$^+$ cell was observed with any pair of tum$^-$ variants.

This analysis has been extended to other tum$^-$ variants. From a total of 21 variants analyzed so far, we detected no individual antigen on 6, weak individual antigens on 4, and strong individual antigens (normalized lytic index >2) on 11. These tum$^-$ antigens appeared to be completely unrelated to each other.

The number of stimulator cells added to the responder spleen cells affected not only the intensity of the cytolytic response, but also its specificity (Table V). The highest and most specific cytolytic activities were observed with doses of 10^5–10^6 stimulator cells. We checked that our failure to observe a specific antigen on P89 was not a result of the use of an improper dose of stimulator cells: no specificity was

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TABLE V

Effect of the Number of Stimulator Cells on the Specificity of the Lysis by Spleen Cells

Number of P21 stimulator cells*	Lysis of target cells		
	P1	P21	P32
	lytic units/10^6 cells		
6×10^6	0.1	0.1	0.1
2×10^6	7.6	14.5	7.5
6×10^5	10.4	53	17
2×10^5	5.4	44	9.3

* 3×10^7 spleen cells from mice immunized 120 d earlier with 2×10^5 living P21 cells were incubated for 4 d with the number of irradiated P21 indicated.

TABLE VI

Absence of Specific Antigens on Mutagenized Cells That Have Retained Their Tumorigenicity

Effector cells*	Lysis of target cells			
	self‡	P1	P35	P91
		lytic units/10^6 cells		
Anti-P1 (control)		3.2	4.2	4.6
Anti-P22	6.9	5.0	4.1	5.3
Anti-P26	1.4	1.5	1.8	1.2
Anti-P30	9.4	11.6	8.9	9.5
Anti-P33	1.3	1.6	1.7	1.3
Anti-P38	1.9	1.6	2.6	2.2
Anti-P40	3.4	2.1	3.1	3.1
Anti-P53	10.6	6.4	8.4	11.5
Anti-P58	2.2	1.6	2.8	3.3
Anti-P87	1.8	1.4	2.3	1.3
Anti-P93	1.2	1.1	2.1	1.1

* Spleen cells from DBA/2 mice immunized with 6×10^6 irradiated cells 50 d earlier, stimulated with irradiated cells of the same variant for 4 d.
‡ Target cells were the immunizing variant.

observed after stimulation at doses ranging from 3×10^3 to 10^7 P89 cells/3×10^7 spleen cells (data not shown).

Absence of New Antigens on Mutagenized Cells That Have Remained Tumorigenic. We selected 10 P815 clones whose ability to form tumors was unaffected by the mutagenic treatment (Table I). These tum^+ clones were isolated from the mutagenized population that contained tum^- clones P21, P32, P35, and P91. DBA/2 mice were immunized with irradiated cells and their spleen cells were restimulated in vitro. No evidence for a singular antigen was obtained with any of these clones (Table VI).

tum^- and the tum^+ Antigens Are Recognized by Distinct Populations of Lymphocytes. Spleen cells recovered from mice immunized with P32, were stimulated in vitro with the same variant and assayed for their cytolytic activity against different ^{51}Cr-labeled P815 targets in the presence of various cold competitor cells (Fig. 1). When the target cell was the immunizing variant P32, we found that this variant was a more effective competitor than P21 or P1. When the target was another tum^- or the tum^+ cell, we

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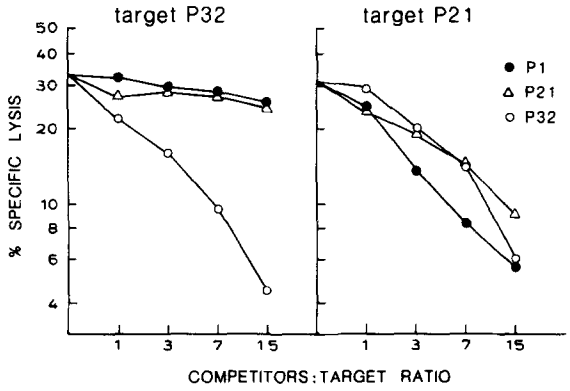

FIG. 1. Spleen cells from DBA/2 mice immunized with 2×10^5 living P32 cells were stimulated in vitro with P32 and tested against ^{51}Cr-labeled P32 targets (2.5:1 effector:target ratio) or P21 targets (20:1 effector:target ratio). Cold competitor cells were added: P1 (●), P21 (△), and P32 (○).

did not observe this preferential competition by P32. If there was only one population of lymphocytes with higher affinity for P32, this variant would compete better than the others against any P815 target. Therefore, immunization of DBA/2 mice with P32 generated two distinct populations of immune lymphocytes: one directed against the individual specificity of P32 and another that recognized a common antigen present on all the tum$^-$ variants and on the tum$^+$ cells. Similar results were obtained with lymphocytes immunized against P21.

Discussion

We have examined the cytolytic reaction of immune syngeneic T lymphocytes against a number of independently isolated P815 tum$^-$ variants. From the 21 variants analyzed so far, 15 were found to carry new antigens that are not present on the original tum$^+$ cells. The presence of a strong new antigenic specificity detected by T lymphocyte-mediated cytolysis appears to be diagnostic for the tum$^-$ character: none was found on 10 clones that had remained tum$^+$ after the mutagenesis. The converse is clearly not true: some tum$^-$ variants like P89 fail to show any new antigen detectable by T cell cytolysis. The cause of the rejection of this class of tum$^-$ variants remains to be determined. An obvious possibility is an increased expression of an antigen that is already present on the P815 tum$^+$ cell.

The lymphocytes immunized against each tum$^-$ variant exerted a significant cytolysis against the other variants and against the original tum$^+$ clone, indicating the persistence on the tum$^-$ variants of an antigen present on the tum$^+$ cell. This is consistent with previous reports which demonstrate that P815 is capable of inducing a syngeneic cytolysis (15, 16). Moreover, we found no instance of cross-reaction between any pair of tum$^-$ clones that could not be accounted for by this common tum$^+$ antigen. The 15 tum$^-$ variants that carry new antigens all carry different specificities. Therefore, it is probable that the number of completely independent tum$^-$ antigens that can be obtained on P815 is >30. This diversity makes it unlikely that the tum$^-$ antigens appear as a consequence of the induction of viral components by the mutagen.

For five P815 tum$^-$ variants, it was possible to compare the pattern of specificity

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observed in vitro to that obtained in immune protection experiments described in the accompanying report. For variants P21, P35, and P91, specific antigens were detected both in vivo and in T cell-mediated lysis. For variant P89, no specific antigen was found either in vivo or in vitro. However, variant P32 showed no specificity in vivo, whereas it showed a definite specificity in T cell cytolysis. Thus the pattern of the specificity obtained in T cell cytolysis matches that observed in vivo very significantly, but not completely. Although this correlation favors a major role of T cytolytic cells in the rejection of most P815 tum$^-$ variants, it does not exclude other effector mechanisms. In this respect, it should be mentioned that even though teratocarcinoma and Lewis lung carcinoma tum$^-$ variants generate a very specific immune protection in vivo, we have consistently failed to obtain specific cytolytic spleen cells in mice that were immunized with these variants. (11, 12; and T. Boon, O. Kellermann, A. Van Pel, J. C. Leclerc, and J. Van Snick. Unpublished observations.). It has also been reported that, for the male-specific antigen of the mouse, the rejection of male grafts does not correlate well with the presence of cytolytic T cells (17).

The results described here suggest that the tum$^-$ variants obtained in the P815 mastocytoma system may serve as a convenient system for the study of T lymphocyte-mediated cytolysis directed against a variety of antigens on a uniform background. It will be of interest to examine the relation of the various tum$^-$ antigens with the products of the major histocompatibility locus.

Summary

Tumor cell variants that were rejected by syngeneic mice (tum$^-$) were obtained from mastocytoma P815 by mutagenesis (as described in the accompanying report (13). A considerable T lymphocyte-mediated lysis was observed upon incubation of these tum$^-$ variants with peritoneal exudate cells collected a few days after an intraperitoneal challenge of immune animals. Spleen cells from these animals were cytolytic after stimulation in vitro with the immunizing variant.

New antigens, absent from the original P815 tum$^+$ cells, were detected on 15 of the 21 tum$^-$ variants that were tested. All these antigens appeared to be different. No new antigen was detected on any of 10 mutagenized P815 clones that had retained their ability to form tumors.

We compared the evidence obtained in vivo and in vitro for the presence of specific antigens on five tum$^-$ variants. Three variants were shown both in vivo and in vitro to carry an individual antigen. One showed no specificity either in vivo or in vitro. However, for one variant, no specificity was observed in vivo, although cytolysis tests demonstrated the existence of a singular antigenic specificity.

We thank Myriam Georlette, Martine Marchand, and Anne-Marie Lebacq-Verheyden for their contribution to the experiments. We also thank Jean-Claude Leclerc, Elisabeth Simpson, Jean-Charles Cerottini, H. Robson Macdonald, and Pierre Masson for stimulating discussions and advice. The excellent technical assistance of Jean-Claude Gaudin and Huguette Delhez is acknowledged.

Received for publication 2 June 1980.

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