*SERUM-MEDIATED PROTECTION OF NEOPLASTIC CELLS FROM INHIBITION BY LYMPHOCYTES IMMUNE TO THEIR TUMOR-SPECIFIC ANTIGENS**

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Abstract and Summary.—The combined effect of immune lymphocytes (lymph-node cells or blood lymphocytes) and serum from tumor-bearing donors was assessed in four tumor systems with the use of the colony inhibition assay: (*a*) Moloney virus-induced sarcomas in mice, (*b*) Shope papillomas in rabbits, (*c*) spontaneous mammary carcinomas in mice, and (*d*) two adenocarcinomas of the colon and two adenocarcinomas of the lung in humans. The neoplasms studied had previously been shown to possess tumor-specific antigens, against which cellular immunity could be detected *in vitro*. In all four systems, it was found that sera from hosts with progressively growing neoplasms could abrogate the inhibitory effect of lymphocytes which were immune to the specific antigens of the corresponding tumor type. Studies with Moloney sarcomas, in particular, showed that the serum effect had at least some degree of specificity.

Lymphocytes from tumor-bearing animals and patients can inhibit or destroy cells of their autochthonous neoplasms *in vitro*. The immunological reaction is directed against tumor-specific transplantation antigens (TSTA).[1-4] The demonstration of tumor-cell inhibition by immune lymphocytes, which are incapable of eradicating the neoplasms *in vivo*, suggested[1] that the ability of the lymphocytes to destroy their targets may be diminished *in vivo* by serum "factors" that protect the neoplastic cells specifically (akin to enhancing antibodies)[5-7] or nonspecifically.[8]

Möller[9] and Brunner *et al.*[10] have shown that mouse tumor cells can be protected from destruction by immune H-2 incompatible lymphocytes by being incubated with antisera against those of their H-2 antigens, which are foreign to the immune cells. The present report shows that serum from tumor-bearing hosts can abrogate the inhibitory effect of lymphocytes immune to the TSTA of the target cells.

Experimental.—The tumors investigated were of four types: (*a*) Moloney virus-induced sarcomas in mice; (*b*) Shope virus-induced papillomas in rabbits; (*c*) "spontaneous" mammary carcinomas in mice, which carried the mammary tumor virus; and (*d*) two adenocarcinomas of the colon and two adenocarcinomas of the lung in humans. The colony inhibition (CI) test,[11] which had been previously employed for the detection of cellular immunity to TSTA of a variety of neoplasms, was used.[1-3] It was carried out[11] by measuring the reduction in the plating efficiency of target tumor cells subsequent to incubation with lymph-node cells (in experiments with animal tumors) or blood lymphocytes (in experiments with human cells) from donors immune to the TSTA of the target tumors; both the lymph-node cells and the blood lymphocytes are referred to as lymphocytes, although it is realized that other cell types may also be involved. Our procedures for preparation of lymphoid cell suspensions have been described.[1-2] Lymphocytes from

nonimmune donors were used in the controls, since previous CI tests had shown that such lymphocytes behaved similarly to lymphocytes immune to antigens lacking in the target cells.[1-3] The target cells were exposed to the serum to be tested before the lymphocytes were added (see footnotes to the tables for details). In some of the tests, the lymphocytes were added immediately after the serum; in others the target cells were exposed for 30 min to the serum, which was then removed before the lymphocytes were added.

Results.—Moloney sarcomas: Sarcomas induced by the Moloney virus possess a common TSTA.[12] Tumors induced in immunologically competent hosts regress, while tumors induced in immunologically hyporesponsive hosts (such as in BALB/c or A/Sn mice less than 20 days old) grow progressively until death of the host.[13] Regression of Moloney tumors is mediated by an immunological reaction against TSTA,[13] and lymphocytes from regressor mice inhibit colony formation of syngeneic Moloney sarcoma cells.[14]

Moloney sarcoma cells of A/Sn or BALB/c origin were exposed to mouse serum of four types, followed by syngeneic lymphocytes from mice in which Moloney sarcomas had regressed 7–14 days previously. The sera studied were derived from (*a*) untreated A/Sn or BALB/c mice; (*b*) A/Sn or BALB/c mice of 25–30 days of age which had been inoculated when 14–20 days old with the Moloney sarcoma virus and which carried progressively growing tumors; (*c*) mice which carried primary methylcholanthrene (MCA)-induced sarcomas or primary mammary carcinomas; and (*d*) mice in which Moloney sarcomas had regressed spontaneously.

As shown in Table 1, lymphocytes from mice in which Moloney sarcomas had undergone spontaneous regression (called regressors) reduced colony formation by syngeneic Moloney sarcoma cells, as compared to lymphocytes from untreated syngeneic mice, if serum from untreated controls was added (column I). This agrees with data from previous experiments with regressor lymphocytes in which no serum was added.[14] However, the reduction was abrogated when serum from mice with progressively growing Moloney sarcomas was added to the target cells before the lymphocytes (column III, expts. 1–14). Sera from mice with spontaneous mammary carcinomas (column II, expts. 1–9) did not abrogate the inhibitory effect of immune lymphocytes in eight of nine experiments but, instead, behaved as serum from untreated mice. Five experiments in which sera were added from mice with progressively growing MCA sarcomas were performed (expts. 10–14). Only one of these sera protected the target cells. Regressor sera gave no such protection (column III, expts. 15–16).

Table 1 also shows that the protective property of serum from mice with progressively growing Moloney sarcomas was abolished by absorption with suspended Moloney sarcoma cells (expts. 17–19, column III). Treatment with mammary tumor cells was ineffective.

Shope papillomas: Shope virus in rabbits induces papillomas that either regress spontaneously or persist and may develop into carcinomas. Rabbits in which papillomas have regressed (regressors) are resistant to tumor induction by DNA prepared from the Shope virus; rabbits with persistent papillomas (persistors) are susceptible to tumor induction by DNA.[15] Nevertheless, lymphocytes from both regressors and persistors reduce colony formation of plated Shope papilloma cells by reacting with TSTA common to such tumors[16]—which

TABLE 1. *Abrogation of the inhibitory effect of regressor lymphocytes on Moloney sarcoma cells by exposure of the target cells to serum from mice which have progressively growing Moloney sarcomas.*

Expt.	Target cells strain of origin	Reduction (%) of Colony Counts of Target Cells Exposed to Regressor Lymphocytes after Incubation with Serum from: I. Untreated syngeneic mice	II. Mice with mammary carcinomas (expts. 1–9) or MCA sarcomas (expts. 10–14)	III. Syngeneic mice with Moloney sarcomas
A. Pretreatment of target cells with unabsorbed serum				
1	BALB/c	48.8‡	34.7‡	0
2	A/Sn	52.9*	58.7*	17.1
3	A/Sn	44.3‡	56.1‡	0
4	A/Sn	42.5‡	38.3†	1.1
5	A/Sn	43.6‡	11.1	6.3
6	A/Sn	92.5‡	100.0‡	24.8
7	A/Sn	36.7‡	46.0‡	12.4
8	BALB/c	36.3†	36.4‡	13.7
9	BALB/c	33.9‡	31.8‡	6.1
10	A/Sn	36.0‡	18.3†	5.4
11	A/Sn	50.2†	36.3†	19.8
12	A/Sn	31.8‡	34.7†	0
13	BALB/c	56.1‡	55.0†	10.5
14	BALB/c	56.0*	44.4	6.7,
15	BALB/c	47.4†		53.1‡ (tumor regressed)
16	A/Sn	31.8†		40.4† (tumor regressed)
B. Pretreatment of target cells with serum absorbed with mammary carcinoma or Moloney sarcoma cells				
17	BALB/c	Nonabsorbed	47.3†	Nonabsorbed 13.7
		Absorbed mammary ca.	51.8†	Absorbed mammary ca. 7.5
		Absorbed Moloney sa.	47.7*	Absorbed Moloney sa. 49.0†
18	BALB/c	Nonabsorbed	38.4*	Nonabsorbed 15.9
		Absorbed mammary ca.	30.4	Absorbed mammary ca. 1.9
		Absorbed Moloney sa.	36.7*	Absorbed Moloney sa. 40.7*
19	A/Sn	Nonabsorbed	44.3*	Nonabsorbed 0
		Absorbed mammary ca.	30.0*	Absorbed mammary ca. 0
		Absorbed Moloney sa.	36.8†	Absorbed Moloney sa. 32.7†

Sera were harvested from untreated syngeneic mice; mice that were 25–30 days old with progressively growing sarcomas which had been induced by inoculation with Moloney sarcoma virus at days 14–20 (expts. 1–7); mice in which Moloney sarcomas had regressed (expts. 15–16); and mice with progressively growing mammary carcinomas (expts. 1–9) or MCA-induced primary sarcomas (expts. 10–14), which were either allogeneic (expts. 1–5) or syngeneic (expts. 6–14). Different pools of serum were used for each experiment. In expt. 1, the target cells were incubated with the sera during 30 min at 37°C before they were exposed to lymphocyte (lymph node cell) suspensions, with 0.5 ml serum diluted 1:7 in Waymouth's medium being added to each Petri dish. In expts. 2–19, lymphocytes were added immediately following 0.5 ml serum, diluted 1:7. All sera were heat-inactivated during 30 min at 56°C. The absorptions carried out in expts. 17–19 were performed by incubating serum diluted 1:7 with 5×10^6 syngeneic Moloney sarcoma or mammary carcinoma cells/ml serum during 30 min at 37°C.

The percentage reduction of colony counts was calculated by comparing groups receiving 5×10^6 lymphocytes per Petri dish from mice in which Moloney sarcomas had regressed 7–14 days previously with groups given 5×10^6 lymphocytes from untreated mice of the same age; both groups were exposed to serum of the same type. The probabilities that the differences between groups with regressor and control lymphocytes (and the same serum) were due to chance are indicated: * <0.05, † <0.01, ‡ <0.001.

Ca., carcinoma; sa., sarcoma.

implies that the differences in the immune status between regressors and persistors evident *in vivo* cannot be ascribed to differences between the lymphocytes of the animals.

Table 2 presents experiments in which Shope papilloma cells were pretreated with serum from rabbits of three types: (*a*) persistors, (*b*) regressors, and (*c*) rabbits that had not been in contact with the Shope virus. The findings show that sera from rabbits with persistent Shope papillomas strongly reduced the inhibitory effect on Shope papilloma cells exhibited by lymphocytes from regressors. Sera from regressors had no such inhibitory effect.

Mammary carcinomas: Lymph-node cells from mice with autochthonous or transplanted mammary carcinomas caused by the mammary tumor virus reduce colony formation of plated mammary tumor cells.[17] Based on this experience, tests were made with mammary carcinomas that had appeared spontaneously in A.CA, C3H, A × CBA F_1, A/Sn, and BALB/c f C3H mice. They showed that the serum of mice with primary mammary carcinomas could decrease the inhibitory effect of their immune lymphocytes on cultured cells of their respective tumors. The colony counts were compared with counts obtained in controls with serum from normal females of the same age. For example, in one experiment with an A.CA carcinoma as target, control Petri dishes receiving normal lymphocytes developed 28.5 ± 0.5 and 30.7 ± 0.7 colonies when incubated with normal serum and serum from mice with a mammary tumor, respectively. With immune lymphocytes, the corresponding figures were 13 and 22.7 ± 1.7 colonies. The specificity of the serum effect was indicated by the observation that sera that protected mammary carcinoma cells from the effect of immune lymphocytes did not protect Moloney sarcoma cells (Table 1).

Human neoplasms: Previous studies have shown that neuroblastomas[2] and certain other human neoplasms, such as adenocarcinomas of the colon and of the lung, have TSTA,[3] against which cellular immunity can be detected with the CI assay. Both lymphocytes from patients with tumors and from patients who were clinically free of detectable tumor after therapy were found to inhibit the patient's own tumor cells *in vitro*. Tests have now been made with serum from patients with progressively growing neoplasms. In cases of adenocarcinomas of the colon, which possess cross-reacting TSTA,[3] additional tests were conducted with allogeneic lymphocytes and sera from patients with colon cancer.

The data are presented in Table 3. Although the experimental material is limited, the findings indicate that sera from patients with adenocarcinoma of the colon can protect cells of the same tumor type against the inhibitory effect of lymphocytes from these patients. Sera from two patients with adenocarcinomas of the lung protected the target cells from inhibition by autochthonous lymphocytes. The serum effect appeared again to be specific. However, more tests are needed before firm conclusions can be drawn.

Discussion.—The results of the present experiments, performed with Moloney sarcomas in mice, Shope papillomas in rabbits, spontaneous mammary carcinomas in mice, and carcinomas of the colon and the lung in man, indicate that sera of animals and patients with progressively growing neoplasms often are capable of abrogating the colony-inhibitory effect of specifically immune lymphocytes.

TABLE 2. *Abrogation of the inhibitory effect of regressor lymphocytes on Shope papilloma cells by exposure of the target cells to serum from rabbits which have persistent Shope papillomas.*

Expt.	Designation of regressor from which immune lymphocytes were derived§	Reduction (%) of Colony Counts of Target Cells exposed to Regressor Lymphocytes after Incubation with Serum from:** Untreated rabbits	Regressor rabbits	Persistor rabbits
1	U 65	39.2†	...	0
	U 66	22.2	...	0
	T 94	35.4†	...	0
	T 79	56.2‡	...	12.4
2	U 65	54.5	...	14.2
	U 66	63.9	...	29.1
	T 78	65.9*	...	44.7
	T 94	70.7†	...	37.2
3	T 897	...	41.7*	0
	T 895	...	50.0	0
	T 894	...	15.4	0
	T 892	...	42.5	0
4	U 56	38.2†	...	0
	U 57	22.2*	...	4.6
	S 606	32.4	...	0
	T 144	22.8*	...	0
	T 897	37.1†	...	15.8
	T 893	24.7*	...	0
5	T 892	39.1*	...	17.2
	T 895	45.6†	...	8.5
	T 896	34.8*	...	0
	T 898	26.0	...	0

The percentage reduction of colony counts was calculated by comparing groups that received lymphocytes from rabbits in which Shope papillomas had regressed with groups given lymphocytes from untreated rabbits; both groups were exposed to serum of the same type. Each group consisted of three to six Petri dishes and each dish received 5×10^6 lymphocytes. The probabilities that differences between groups were due to chance are indicated: * <0.05, † <0.01, ‡ <0.001.

§ Papillomas were induced with Shope virus on the back of St. Juan and Chinchilla rabbits. In rabbits in which the papillomas regressed spontaneously (called regressors), axillary lymph nodes were harvested within 1 month after regression and used as a source of immune lymphocytes. Control lymphocytes were derived from untreated rabbits of the same strain and age.

** Serum was harvested from regressor rabbits, from untreated rabbits, and from rabbits in which the Shope papillomas did not regress and would probably have later developed into carcinomas (called persistors). Different pools of serum were studied in each of the five experiments. In expts. 3–5, the target cells were incubated with serum during 30 min at 37 °C before lymphocyte suspensions were added; each Petri dish received 0.5 ml serum, diluted 1:7. In expts. 1–2, lymphocytes were added to the target cells immediately after incubation with sera. All sera were heat-inactivated during 30 min at 56 °C.

Since the serum effect appeared to be specific, it is suspected that the effect is, at least partly, due to the presence of "enhancing" antibodies which bind to the antigens of the target cells and protect them from attack by immune lymphocytes. Immunological enhancement of transplanted cells with TSTA has been repeatedly demonstrated *in vivo*,[6, 18–20] but the role of enhancement for the development of autochthonous neoplasms has so far been unclear. Alternatively, the antibodies could have induced antigenic modulation,[21] so that the TSTA of the target cells would not have been expressed in their presence.

The present findings offer one way to explain the seemingly paradoxical situation in which tumors grow progressively *in vivo* despite the fact that their cells

TABLE 3. *Abrogation of the inhibitory effect of immune lymphocytes on human tumor cells after exposure of the target cells to serum from patients with progressively growing neoplasms of the same type as the target cells.*

Expt.	Donor of target cells	Lymphocyte donor	Serum donor	Number of colonies with 5×10^6 lymphocytes/dish (mean ± SE)	Reduction (%) with immune as compared to control lymphocytes
1	Ca.colon-J.B.	Contr.-L.H.	Contr.	43.7 ± 1.7	...
			Sq.cell ca.-H.L.	52.3 ± 2.0	...
			Ca.colon-L.C.	57.8 ± 2.4	...
		Ca.colon-C.G.	Contr.	29.0 ± 2.1	33.6†
			Sq.cell ca.-H.L.	29.0 ± 2.6	44.6‡
			Ca.colon-L.C.	55.5 ± 2.2	4.0
2	Ca.colon-J.B.	Contr.-B.D.	Contr.	80.5 ± 4.1	...
			Ca.lung-A.M.	85.5 ± 4.1	...
			Ca.colon-J.B.	84.5 ± 4.8	...
		Ca.colon-L.C.	Contr.	24.3 ± 1.3	69.8‡
			Ca.lung-A.M.	40.0 ± 3.2	53.2‡
			Ca.colon-J.B.	68.3 ± 3.7	19.2
3	Ca.colon-J.B.	Contr.-R.J.	Contr.	47.3 ± 5.5	...
			Ca.colon-J.B.	44.5 ± 5.3	...
		Ca.colon-W.P.	Contr.	24.3 ± 4.9	48.5*
			Ca.colon-J.B.	41.5 ± 2.1	6.7
4	Ca.colon-L.C.	Contr.-F.H.	Contr.	39.3 ± 1.8	...
			Ca.prost.-J.H.	32.0 ± 1.2	...
			Ca.colon-L.C.	44.0 ± 2.1	...
		Ca.colon-L.C.	Contr.	16.0 ± 1.0	59.3‡
			Ca.prost.-J.H.	12.2 ± 0.5	61.9‡
			Ca.colon-L.C.	38.8 ± 1.0	11.8
5	Ca.colon-L.C.	Contr.-B.M.	Ca.lung-G.M.	112.5 ± 12.5	...
			Ca.colon-T.C.	126.3 ± 6.3	...
		Ca.colon-L.C.	Ca.lung-G.M.	73.3 ± 8.1	34.8*
			Ca.colon-T.C.	124.4 ± 8.0	1.5
6	Ca.lung-A.B.	Contr.-G.L.	Ca.colon-T.C.	130.5 ± 5.3	...
			Ca.lung-A.B.	132.6 ± 6.5	...
		Ca.lung-A.B.	Ca.colon-T.C.	95.7 ± 3.5	26.7†
			Ca.lung-A.B.	134.7 ± 8.2	0
7	Ca.lung-B.D.	Contr.-S.W.	Contr.	60.3 ± 9.1	...
			Ca.prost.-J.H.	60.0 ± 16.5	...
			Ca.lung-B.D.	57.3 ± 1.9	...
		Ca.lung-B.D.	Contr.	30.5 ± 0.5	49.4*
			Ca.prost.-J.H.	28.7 ± 3.2	52.1
			Ca.lung-B.D.	59.7 ± 1.2	0

Serum was harvested from a normal control subject (contr.), from a patient with a progressively growing tumor of the same type as the target tumor (or from the donor of the target tumor cells in some tests), and from a patient with a progressively growing neoplasm of another histological type. The sera were heat-inactivated during 30 min at 56°C and were diluted 1:7 in Waymouth's medium. Lymphocytes were harvested by separating leukocytes from peripheral blood with plasma gel and sedimenting the granulocytes in glass bottles with added glass beads during 60 min, a procedure which yielded lymphocyte suspensions that were 70–80% pure. The lymphocytes were added to the target cells immediately after sera.

The percentage reduction of colony formation was calculated by comparing groups receiving lymphocytes from patients with the same diagnosis as the donor of the target cells (or from the autochthonous patients), with groups receiving lymphocytes from patients who did not have tumors of the same type as the target cells; both groups were exposed to serum of the same type. Each group consisted of three to six Petri dishes. The probabilities that differences between groups were due to chance are indicated: * <0.05, † <0.01, ‡ <0.001.

Ca., carcinoma.

are inhibited by autochthonous lymphocytes *in vitro:* presumably the neoplastic cells are protected from destruction by serum factors. Attempts to reduce or eliminate such factors should be made, since they might provide information of value in tumor therapy. It is of interest that serum from mice in which Moloney sarcomas or Shope papillomas had spontaneously regressed did not interfere with the action of immune lymphocytes but, instead, often contained antibodies that were capable of inhibiting colony formation of the respective target cells.[14-16]

The suggested mechanism for tumor cell protection by serum factors *in vivo* from inhibition by immune lymphocytes does not exclude the presence of other mechanisms, which probably also operate *in vivo.*[1, 20]

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Abbreviations used: TSTA, tumor-specific transplantation antigens; CI, colony inhibition.

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