CELLULAR IMMUNOABSORBENTS IN TRANSPLANTATION IMMUNITY

Specific In Vitro Deletion and Recovery of Mouse Lymphoid Cells Sensitized against Allogeneic Tumors*

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Specifically sensitized lymphoid cells and, in certain cases, circulating antibodies have been shown to play a major role in the immunological rejection of homografts (1, 2). However, the mechanisms by which sensitized lymphoid cells reject foreign grafted tissue is still largely unknown. The introduction of tissue culture systems (3–8) has allowed a better understanding and characterization of the rejection and destruction of allografts. It has been demonstrated that lymphoid cells from an animal that had undergone graft rejection were subsequently capable in vitro of specifically destroying target cells of the same strain as the graft donor. Brondz and his colleagues (9, 10) have demonstrated the possibility of depleting a lymphoid cell population of cells that are active in the in vitro lysis of target cells by incubating sensitized lymphoid cells on specific macrophage monolayers.

This report presents the results of a series of experiments which demonstrate that in vitro absorption of mouse spleen cells sensitized against allogeneic tumors onto fibroblast monolayers specifically depletes the immunoreactive cell population. Furthermore, the immunoreactive cells can subsequently be recovered from the fibroblast monolayers and are reactive in in vitro lysis of tumor cells and in vivo retardation of tumor growth.

The results indicate that the population of lymphoid cells reactive in the rejection of tumor allografts in vivo is the same as that involved in the destruction of tumor cells in vitro.

Materials and Methods

Animals.—Mice of the following strains were used: DBA/2J ($H\text{-}2^d$) females, A/J ($H\text{-}2^a$) females, BALB/cJ ($H\text{-}2^d$) males, C57BL/6J ($H\text{-}2^b$) males and females, and (C57BL/6J ×

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A/J)F_1 hybrid females ($B6AF_1$).[1] Animals were 3–6 months old when used. Sources of embryos for preparation of fibroblast monolayers were pregnant mice on days 14–17 of gestation. All animals were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Tumors.—Spindle cell sarcoma (SaI) of A/J was obtained from the Jackson Laboratory as a subcutaneous tumor and was carried in the ascites form by weekly transfer of 5×10^6 tumor cells into A/J females. EL4 leukosis of C57BL/6 (EL4) was obtained from Dr. John Wunderlich, National Cancer Institute, Bethesda, Md., and was carried as an ascites tumor by weekly transfer of 3×10^7 tumor cells in C57BL/6J males. P815 mastocytoma of DBA/2 (P815) was obtained from Dr. M. Potter, National Cancer Institute, and was carried in an ascites form by weekly injection of 1×10^6 tumor cells in DBA/2J females.

Immunization of Mice against Tumors.—Animals were immunized against allogeneic tumors by intraperitoneal injection of 3×10^7 tumor cells which had been washed in phosphate-buffered saline (PBS).

Preparation of Spleen Cell Suspensions.—Animals were killed 10–11 days after immunization and the spleens were removed under sterile conditions and placed in cold PBS containing 10% heat-inactivated fetal calf serum (PBS-FCS). The spleens were minced, pressed through fine nylon mesh, filtered through gauze, and centrifuged in the cold at 250 *g* for 10 min. The pellet was resuspended in PBS-FCS and the cells were centrifuged again. Nucleated cells were counted and the number of dead cells was determined by eosin dye exclusion. Spleen cells prepared as above and kept on ice did not lose reactivity for at least 4 hr when tested in the systems described below.

In Vitro Assessment of Lysis of Tumor Cells.—The assessment of lysis of tumor cells was done as previously described (4, 11, 12), with the following modifications. Tumor cells were collected in PBS-FCS via syringe from the peritoneal cavity, and 3×10^7 washed cells were labeled with 100 μCi (^{51}Cr) Na_2CrO_4 (Amersham/Searle Corp., Arlington Heights, Ill.) in 1 ml of PBS-FCS for 45 min at 37°C with occasional shaking. At the end of the labeling period, cells were washed twice with 40 ml of PBS-FCS and then were recounted. Usually, 80–90% of the labeled cells were recovered. 5×10^4 ^{51}Cr-labeled tumor cells and 5×10^6 spleen cells in MEM-FCS (Eagle's minimal essential medium containing 100 units of penicillin and 100 μg of streptomycin/ml, supplemented with 10% heat-inactivated fetal calf serum)[2] were plated in 1 ml volumes in 35-mm plastic Petri dishes (Falcon Plastics, Division B-D Laboratories, Inc., Los Angeles, Calif.). The plates were rocked on a rocker platform (Bellco Glass, Inc., Vineland, N. J.) at 5 rocks/min at 37°C in a humidified incubator gassed with 10% CO_2 in air. To determine the amount of released radioactivity, 1 ml of cold PBS-FCS was added to the plates and the suspensions were transferred to small plastic tubes. The tubes were centrifuged in the cold at 1000 *g* for 10 min, and the radioactivity in the supernatant was assayed using a well-type gamma scintillation counter. To assay the maximum amounts of releasable radioactivity, labeled tumor cells were frozen and thawed three times, centrifuged, and the radioactivity of the supernatant assayed. This represented 90–95% of the radioactivity that was incorporated into the labeled cells.

Four, and in a few cases two, replicate cultures were used for the assessment of the lysis of tumor cells; the relative standard deviation from the mean of the amount of released radioactivity was below 3%.

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[1] *Abbreviations used in this paper*: $B6AF_1$, (C57BL/6J × A/J)F_1; BSA, bovine serum albumin; EL4, EL4 leukosis of C57BL/6J; MEM-FCS, Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum; P815, P815 mastocytoma of DBA/2J; PBS, phosphate-buffered saline; PBS-FCS, phosphate-buffered saline supplemented with 10% heat-inactivated fetal calf serum; SaI, spindle cell sarcoma of A/J mice.

[2] Tissue culture media and supplements were obtained from Grand Island Biological Company, Grand Island, N. Y.

Results were expressed as per cent lysis calculated with the following equation (11, 12):

$$\text{Per cent lysis} = \frac{(\text{cpm released by immune spleen cells}) - (\text{cpm released by normal spleen cells})}{\text{cpm released by freeze-thawed tumor cells}} \times 100.$$

Embryo Fibroblast Monolayers.—Mouse primary fibroblast cultures were prepared using 14–17-day-old embryos by a technique which has been described in detail elsewhere (13). In brief, primary fibroblast monolayers were cultured in MEM-FCS; these were trypsinized and transferred to secondary cultures every 7–12 days. Only confluent secondary culture were used for the absorption experiments. Human fibroblasts that were cultured from fetal tissue were also used.

Absorption of Spleen Cells on Fibroblast Monolayers.—Confluent fibroblast monolayers in 100-mm plastic Petri dishes (Falcon Plastics) containing 7–12 million fibroblasts per dish were used for absorption of the spleen cells. 60×10^6 spleen cells in 5 ml of MEM-FCS were plated per dish and the cultures were incubated at 37°C for 3–4 hr with occasional shaking. At the end of this absorption period, the plates were rotated for 3 min at 50 rotations/min on a mechanical rotator (Arthur H. Thomas Co., Philadelphia, Pa.) and the fluid phase was removed and diluted with cold PBS-FCS. The number of nucleated cells was redetermined; usually, 50–70% of the nucleated cells was recovered; the cells were assayed in an in vitro tumor cytotoxicity assay or in an in vivo tumor neutralization test.

The calculation of per cent decrease in lysis of target cells obtained in the absorption experiments was done as follows:

$$\text{Per cent decrease in lysis} = \frac{\left(\begin{matrix}\text{Per cent lysis with spleen cells}\\ \text{incubated in empty plates}\end{matrix}\right) - \left(\begin{matrix}\text{Per cent lysis with spleen cells incubated}\\ \text{on fibroblast monolayers}\end{matrix}\right)}{\text{per cent lysis with spleen cells incubated in empty plates}} \times 100.$$

Fractionation of Cells on Albumin Density Gradient (14).—Cells were suspended in 17% bovine serum albumin (BSA) and placed on top of a discontinuous BSA gradient and separated by centrifugation at 1000 *g* for 30 min at 4°C. BSA fraction V (Sigma Chemical Co., St. Louis, Mo.), 35% (refractive index determination) and 320 mOsM (by freezing point depression), was used for fractionation. After dilution with PBS, 1 ml of 32% albumin solution was placed at the bottom of plastic tubes; on top of this was layered sequentially 1 ml of 25% and 1 ml of 20% BSA. Cells were recovered from the gradient interfaces with a Pasteur pipette, washed twice in an excess of MEM-FCS, counted differentially, and assayed for reactivity.

Tumor Neutralization Assay.—Winn's assay (15) with some modifications was used. A mixture of tumor cells and spleen cells was prepared in cold PBS, and 0.05 ml of this suspension was injected subcutaneously into the shaved abdominal wall of $(C57BL/6J \times A/J)F_1$ female mice.

The growth of the tumors was measured with calipers at intervals of 1–5 days. Two different diameters were recorded for each tumor at the time of observation. The average geometric mean was calculated by division of the sum of the mean tumor diameters by the number of animals measured. Mortality of animals was also recorded.

RESULTS

Cytolytic Effects of Immune and Normal Spleen Cells on Tumor Cells In Vitro.—The cytolytic effects on target tumor cells of spleen cells sensitized by tumor homografts and of normal spleen cells were studied by incubating the spleen cells in vitro with ^{51}Cr-labeled tumor cells and measuring the amount of released radioactivity. The results (Table I) demonstrate that ^{51}Cr was eluted into the medium during the incubation period and that the extent of the release of ^{51}Cr was specific for the tumor used for immunization. When labeled tumor

TABLE I

Cytotoxic Effects of Spleen Cells on Tumor Cells In Vitro

Spleen cells*	Extent of ^{51}Cr release from labeled tumor cells‡		
	EL4	P815	SaI
	%	%	%
C57BL/6 ♀ anti-P815	19.5 ± 0.4§	40.5 ± 2.3	NT‖
C57BL/6 ♀ anti-SaI	15.4 ± 1.2	NT	35.7 ± 0.3
BALB/c ♂ anti-EL4	63.2 ± 1.8	19.6 ± 1.0	7.5 ± 0.8
Normal C57BL/6 ♀	9.3 ± 0.7	15.3 ± 1.8	6.7 ± 1.6
Normal BALB/c ♂	11.6 ± 1.4	16.8 ± 2.8	8.1 ± 2.1
None	12.1 ± 2.7	16.2 ± 1.2	10.9 ± 0.9

* Immune spleen cells: BALB/c anti-EL4, C57BL/6 ♀ anti-SaI, and C57BL/6 ♀ anti-P815 cells were prepared from mice that had been injected 11 days previously with 3×10^7 cells of the appropriate tumor. Normal spleen cells were from noninjected animals.

‡ Extent of ^{51}Cr released from the labeled tumor cells was determined after 4 hr of incubation with the spleen cells by division of the amount of the radioactivity that was released into the medium by the total radioactivity.

§ All figures correspond to the mean of four replicates ± relative standard deviation from the mean.

‖ NT, not tested.

cells were incubated with normal spleen cells, the amount of ^{51}Cr released was very similar to that obtained when no spleen cells were added. It can also be seen that the relative standard deviation from the mean between replicate cultures was small (below 3%).

The release of ^{51}Cr from labeled cells has been considered as an expression of cellular damage (4, 13, 16). Hence, hereafter, the expression per cent lysis (see the Materials and Methods section) rather than per cent ^{51}Cr released is used to describe the extent of cytotoxicity for labeled tumor cells.

Effects of Absorption of Sensitized Spleen Cells with Fibroblast Monolayers on the Subsequent Lysis of Tumor Cells.—The lytic capacity of sensitized spleen cells previously incubated on fibroblast monolayers and subsequently harvested from the medium (nonadherent cells) has been studied utilizing the above three allograft systems.

Spleen cells of BALB/c ♂ anti-EL4, C57BL/6 ♀ anti-P815, and C57BL/6 ♀ anti-SaI were prepared. 60×10^6 sensitized spleen cells were incubated in vitro on fibroblast monolayers of various strains of mice. At the end of the incubation period, the nonadherent spleen cells were harvested and assayed in vitro for their lytic reactivity against labeled tumor cells. The results obtained are shown in Tables II and III and in Fig. 1. From Table II, it is evident that the non-

TABLE II

Lysis of EL4 Cells by Nonadherent BALB/c Anti-EL4 Spleen Cells

Exp. No.	Absorption with	Lysis‖	Decrease‖
		%	%
1*	C57BL/6J	12.9	67.7
	BALB/cJ	36.2	9.5
	DBA/2J	36.0	10.0
	Empty plates	40.0	0
	No absorption	41.9	−4.7
2‡	C57BL/6J	8.3	81.7
	BALB/cJ	42.7	5.9
	DBA/2J	40.3	11.2
	Empty plates	45.4	0
3§	C57BL/6J	5.4	89.4
	A/J	58.3	−14.0
	CBA/J	44.7	12.5
	Human	59.0	−15.4
	BALB/cJ	47.9	6.2
	DBA/2J	50.1	1.9
	Empty plates	51.1	0

* Spleen cells were absorbed for 3 hr with fibroblast monolayers. Then the nonadherent cells were harvested and mixed with ^{51}Cr-labeled EL4 cells and the extent of lysis was determined after 4 hr of incubation.

‡ Absorption for 3.5 hr, lysis for 4 hr.

§ Absorption for 3.75 hr, lysis for 5 hr.

‖ See Materials and Methods for assessment and calculations.

adherent spleen cells of Balb/c ♂ anti-EL4 were only slightly cytotoxic to EL4 cells when they were harvested from C57BL/6 fibroblast monolayers (syngeneic with EL4), whereas, BALB/c anti-EL4 spleen cells, harvested after incubation on third-party monolayers or empty plates, were fully cytotoxic to the tumor cells. Similar results were obtained with C57BL/6 anti-P815 spleen cells (Table III) which, after absorption with DBA/2 or BALB/c fibroblasts (both *H-2*^{d}), were less cytotoxic than those spleen cells that had been absorbed with C57BL/6 (*H-2*^{b}) fibroblast monolayers. Nevertheless, absorption with C57BL/6 fibroblasts produced a partial decrease in the lytic effect when compared with spleen cells absorbed on empty plates. A very efficient diminution of the cytolytic

effect was obtained also with the third tumor, SaI (Fig. 1), in which absorption of C57BL/6 ♀ anti-SaI spleen cells for 3 hr with A/J fibroblasts (syngeneic with SaI) specifically diminished the subsequent killing of the tumor cells. On the other hand, absorption of the sensitized spleen cells with CBA or C57BL/6 fibroblast monolayers or with empty plates left a population of cells capable of lysing SaI target cells.

Basis for the In Vitro Elimination of Specifically Sensitized Spleen Cells.—Two

TABLE III

Lysis of P815 Cells by Nonadherent C57BL/6 Anti-P815 Spleen Cells

Exp. No.	Absorption by	Lysis‖	Decrease‖
		%	%
1*	DBA/2J	4.2	79.6
	BALB/cJ	2.0	90.3
	C57BL/6J	13.0	36.9
	Empty plates	20.6	0
	No absorption	19.9	3.3
2‡	DBA/2J	4.4	85.5
	BALB/cJ	2.4	92.9
	C57BL/6J	19.6	35.5
	Empty plates	30.4	0
3§	DBA/2J	9.1	62.3
	C57BL/6J	15.6	35.5
	Empty plates	24.2	0

* Spleen cells were absorbed for 3 hr with fibroblast monolayers, then the nonadherent cells were mixed with ^{51}Cr-labeled P815 cells and the extent of lysis determined after 4 hr of incubation.

‡ Absorption for 3.5 hr, lysis for 5 hr.

§ Absorption for 4 hr, lysis for 4 hr.

‖ See Materials and Methods for the assessment and calculations.

possibilities were considered to explain the diminution of the observed extent of lysis obtained with the nonadherent spleen cells. The first and most obvious possibility was that specifically sensitized killing cells were selectively adsorbed onto the monolayer fibroblasts and remained adherent. The second possibility was that sensitized spleen cells were selectively destroyed during the incubation period. To support the first hypothesis, it was necessary to recover the adherent cells and to demonstrate their capacity to lyse target cells. Recovery of adherent cells was accomplished by trypsinization of monolayers, together with the adsorbed cells, followed by the separation of the resultant cell suspension on a discontinuous albumin gradient.

BALB/c ♂ anti-EL4 spleen cells were preincubated on C57BL/6 and on A/J fibroblast monolayers and in empty plates. After 3 hr of incubation, the non-

adherent cells were removed and the remaining adherent lymphoid cells, together with the monolayer fibroblasts, were trypsinized (trypsin 0.25%) for 10 min to obtain a single cell suspension. The three types of cell suspensions, those

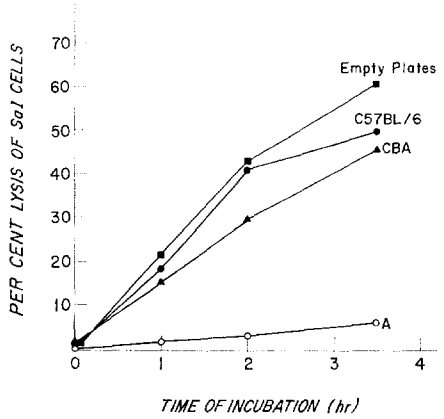

FIG. 1. Time course of the lysis of SaI cells by nonadherent C57BL/6 ♀ anti-SaI spleen cells. ^{51}Cr-labeled SaI cells were incubated with C57BL/6 ♀ anti-SaI spleen cells previously absorbed with A (○—○), CBA (▲—▲), or C57BL/6 (●—●) fibroblast monolayers or on empty plates (■—■). Each point is the mean of three replicates.

TABLE IV

Recovery of BALB/c Anti-EL4 Spleen Cells on Albumin Gradients

Cells for separation	Extent of lysis of EL4 cells by cells harvested from albumin density interfaces (in per cent BSA)		
	17/20	20/25	25/32
	%‡	%	%
Adherent* (to C57BL/6 fibroblasts)	14.6§	44.3‖	36.1¶
Adherent* (to A fibroblasts)	7.3	14.8	17.1
Nonadherent (to C57BL/6 fibroblasts)	2.1	13.8	16.8
Nonadherent (to A fibroblasts)	2.7	63.4	54.3
Nonadherent (to empty plates)	0.7	85.3	70.9

* Adherent cells (lymphoid cells and fibroblasts) were harvested by trypsinization for 10 min (trypsin 0.25%).

‡ Per cent lysis of ^{51}Cr-labeled EL4 cells was determined after 5 hr of incubation with the fractionated spleen cells.

§ 35% fibroblasts.

‖ 5% fibroblasts.

¶ <1% fibroblasts.

nonadherent to empty plates, those nonadherent to C57BL/6 and A/J monolayers, and the cells adherent to the fibroblasts (with the latter included), were layered on individual discontinuous albumin gradients and fractionated. The

cells from the various fractions were collected, counted, and assayed for their cytolytic activity against ^{51}Cr-labeled EL4 cells. The results (Table IV) show that it was possible both to recover the reactive cells from the syngeneic fibroblasts (C57BL/6) and to demonstrate their cytolytic capacity. On the other hand, cells recovered from the allogeneic strain A/J fibroblasts did not show significant reactivity.

It is clear from Table IV that the procedure employed for the fractionation of the cells did not confer lytic reactivity on the adherent lymphoid cells since the

TABLE V

*Effect of Nonadherent BALB/c Anti-EL4 Spleen Cells on the Growth of EL4 Tumor in B6AF$_1$ ♀ Mice**

Fibroblast for absorption	No. of spleen cells injected	Average tumor diameter at time in days‡								
		5	7	9	11	14	16	18	20	23
	(× 10^{-6})	*mm*								
C57BL/6	6.65	1.0§	3.8	5.3	8.7	11.8	14.8	16.9	19.4	22.4
	3.32	1.7	5.7	8.0	10.3	14.5	16.0	19.2	22.8	24.4
	1.61	1.6	5.1	7.6	10.7	14.0	18.3	20.0	23.3	24.6
A	6.65	— ‖	—	—	—	—	—	—	—	—
	3.32	—	—	1.1	4.0	5.8	6.8	8.7	9.9	15.9
	1.61	—	1.7	4.1	7.9	11.0	14.4	17.8	20.8	23.2
CBA	6.65	—	—	—	—	2.6	4.3	6.4	8.4	10.9
	3.32	—	—	4.2	4.8	7.4	10.8	14.1	15.4	17.2
	1.61	1.8	6.5	6.7	9.6	14.3	17.8	20.5	22.6	23.7
—	—	3.1	7.8	10.4	13.4	16.7	20.3	21.8	23.2	24.5

* Mixtures of nonadherent spleen cells together with 665 × 10^3 EL4 cells were injected into B6AF$_1$ ♀ mice.

‡ See Materials and Methods for determination.

§ Each figure is the mean of tumor measurements for four animals.

‖ No measureable tumor.

cells which were nonadherent to C57BL/6 fibroblasts remained nonreactive after the fractionation. As can also be seen, the fractionation procedure was not damaging because the cells that were nonadherent to A/J fibroblasts apparently retained reactivity after fractionation.

Effects of Absorption of Sensitized Spleen Cells on Fibroblast Monolayers on the Growth of Tumor Cells In Vivo.—As has been described above, sensitized spleen cells were specifically depleted of their in vitro cytolytic reactivity against tumor cells by preincubation on fibroblast monolayers syngeneic with the tumor donor. To demonstrate in vivo the efficacy of the selective elimination of the reactive cells in vitro, a modified in vivo tumor neutralization assay was utilized.

BALB/c ♂ anti-EL4 spleen cells were preincubated for 3 hr on fibroblast monolayers of C57BL/6, A/J, and CBA. The nonadherent cells were then harvested, mixed with EL4 cells, injected into $B6AF_1$ mice, and the growth of the tumors was followed. The data (Table V) show that the spleen cells that had been absorbed with C57BL/6 fibroblasts (syngeneic with EL4) failed to neutralize the tumor; whereas, the same cells adsorbed with A/J or CBA fibroblast monolayers were more efficient in the inhibition of tumor growth in vivo.

The same type of approach also was utilized for the SaI system. C57BL/6 ♀ anti-SaI spleen cells were preincubated in vitro for 3 hr on C57BL/6 and A/J

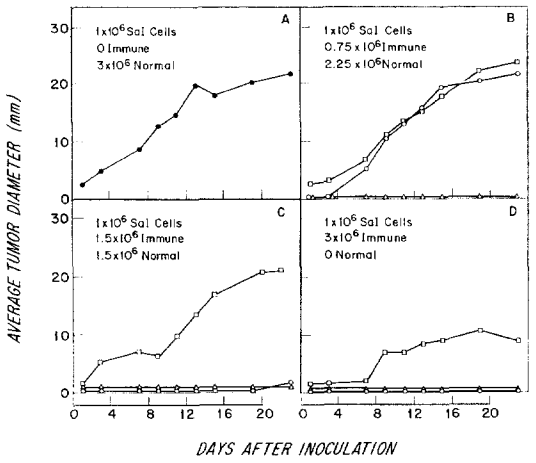

FIG. 2. Effects of nonadherent C57BL/6 ♀ anti-SaI spleen cells on the growth of SaI tumor cells in $B6AF_1$ ♀ mice. C57BL/6 ♀ anti-SaI spleen cells were preincubated on fibroblast monolayers of C57BL/6 (○—○), A (□—□), or on empty plates (△—△). The nonadherent cells were harvested and mixed with normal C57BL/6 spleen cells in the various proportions shown, and 3×10^6 immune and nonimmune spleen cells, together with 1×10^6 SaI cells, were injected. Each point is the average tumor measurement for four animals.

fibroblast monolayers, and in empty dishes. The nonadherent spleen cells were then harvested and mixed with SaI tumor cells together with normal C57BL/6 spleen cells, and the mixture was injected subcutaneously into susceptible $B6AF_1$ mice. The growth of the tumor was followed with time. The results (Fig. 2) demonstrate that C57BL/6 ♀ anti-SaI spleen cells which were absorbed with A/J fibroblasts failed to neutralize the tumor at all immune spleen cell doses tested, although a certain degree of suppression was obtained when 3×10^6 cells were injected. On the other hand, 1.5×10^6 or 3×10^6 nonadherent C57BL/6 ♀ anti-SaI spleen cells harvested from C57BL/6 fibroblast monolayers neutralized the SaI tumor (Figs. 2 C and D); however, the tumor did grow when only 0.75×10^6 nonadherent spleen cells were injected (Fig. 2 B). C57BL/6 ♀ anti-SaI spleen cells that did not adhere to empty plastic Petri

dishes were capable of neutralizing the tumor in the three different concentrations used. From Fig. 2 it is also clear that normal C57BL/6 spleen cells were ineffective in retarding tumor growth.

In Vivo Effects of Recovered Adherent Cells.—It has been demonstrated (Table IV) that the specifically adherent cells recovered from the fibroblast monolayers retained their cytotoxic potential against tumor cells in vitro. Table VI extends these observations to an in vitro–in vivo system. C57BL/6 ♀ anti-SaI spleen cells were absorbed on fibroblast monolayers of A/J and CBA and in empty

TABLE VI

*SaI Tumor Growth in B6AF$_1$ ♀ Mice: Effect of Recovered Adherent Cells**

Spleen cells	Average tumor diameter at time in days‡									
	4	8	11	13	16	18	22	24	27	29
					mm					
Nonadherent to A fibroblasts	7.7 (6/6)§	13.9 (6/6)	16.4 (6/6)	18.8 (6/6)	18.9 (6/6)	19.1 (6/6)	22.2 (4/4)	23.2 (3/3)	28.4 (2/2)	28.7 (1/1)
Recovered from A fibroblasts	—‖	—	—	7.2 (1/6)	9.0 (1/6)	8.7 (1/6)	10.0 (1/6)	8.4 (1/6)	8.2 (1/6)	7.7 (2/6)
Nonadherent to CBA fibroblasts	6.3 (1/6)	9.0 (1/6)	10.0 (2/6)	10.4 (2/6)	10.9 (2/6)	10.3 (2/6)	13.5 (2/6)	15.7 (2/6)	16.5 (2/6)	17.5 (2/6)
Recovered from CBA fibroblasts	—	11.2 (3/6)	13.8 (4/6)	14.9 (4/6)	16.0 (4/6)	16.1 (4/6)	17.8 (5/6)	17.9 (5/6)	21.2 (5/6)	23.7 (5/6)
Nonadherent to plastic dishes	—	—	—	7.2 (1/6)	9.2 (1/6)	10.9 (2/6)	14.4 (2/6)	16.0 (2/6)	13.1 (2/6)	18.0 (2/6)

* 1.5×10^6 C57BL/6 anti-SaI spleen cells were injected together with 1×10^6 SaI cells, subcutaneously into B6AF$_1$ ♀ mice.

‡ See Materials and Methods for determination.

§ Number of mice having tumors per number of mice observed.

‖ No measurable tumor.

plates for 3 hr. The nonadherent cells were harvested and kept at 4°C. The adherent cells together with the fibroblasts were trypsinized to form a monodispersed cell suspension and fractionated on a discontinuous albumin gradient, as described above. The cells from the lower two gradient fractions were combined, and these and the nonadherent cells were then separately mixed with 1×10^6 SaI tumor cells and injected into six panels of mice. The tumor growth and mortality were observed. The data (Table VI) show that cells nonadherent to a monolayer syngeneic with the tumor cells (A/J strain) were less effective in retarding tumor growth than those absorbed on an allogeneic (CBA) monolayer or in empty plates. Conversely, recovery of specifically adherent cells yielded those that were efficient in the inhibition of the growth of the tumor. In the group of mice treated with cells recovered from the A/J monolayer, there was no mortality at 31 days.

DISCUSSION

In this paper experiments are presented which show that the preincubation of mouse spleen cells sensitized against tumor allografts on mouse fibroblasts, syngeneic with the tumor donor, depletes the immunoreactive cell population. Two different procedures were used to demonstrate that the nonadherent cells were not only less effective in retarding tumor growth in vivo, but also markedly inefficient in lysing tumor cells in vitro. Furthermore, the adsorbed reactive cells that were recovered were specifically efficient in inhibiting tumor growth in vivo and in killing target tumor cells in vitro.

The results which indicate that preincubated cells were depleted of their cytolytic effects against tumor cells in vitro are in good agreement with findings reported recently by Brondz and associates (9, 10), although in a few cases presented here, "all or none" effects for the diminution of the in vitro lytic reactivity of preincubated sensitized spleen cells have not been achieved. However, it should be noted that these experiments employed different tumors, different cells and methods for the absorption, and most important, a different method for the assessment of the cytolytic effect. On the other hand, the findings in those experiments which demonstrate a diminution in the capacity of preincubated sensitized spleen cells to neutralize the growth of tumor cells in vivo appear to be unique.

Recently (4–7, 11), it has been shown that sensitized lymphoid cells from animals which had undergone graft rejection were capable of in vitro destruction of cells from the same strain as the graft donor. Those findings have substantiated, at least circumstantially, the concept that the lymphoid cell (and other cells as well) are actually capable of exerting a cytotoxic effect against grafted cells in vivo. The experiments that have been reported in this paper might supply further evidence for the suggestion that the destruction of target cells in vitro by sensitized lymphoid cells is truly representative of the mode of destruction of grafted cells in vivo, for the data represent parallel in vitro and in vivo evidence for the diminution of the capacity of preincubated cells to destroy target cells in vitro and to neutralize tumor cells in vivo. However, this also is only circumstantial evidence and final proof will rest on the demonstration of the performance of these two effects by the same cell rather than by populations of cells. The recovery of the specifically adherent lymphoid cells by albumin density gradient separations, together with the subsequent demonstration that the recovered cells were reactive in the destruction of the tumor cells in vitro and in the neutralization of tumor cells in vivo, provides a rationale for the assumption that the same lymphoid populations mediate the two effects.

Several reports have recently described the specific elimination of immunoreactive lymphoid cell populations. Zoschke and Bach (17) reported such a deletion by antigen–bromodeoxyuridine–light manipulations. Rich et al.[3] have

[3] Rich, R. R. Personal communication.

successfully used this procedure in a mixed lymphocyte reaction and in the graft-vs.-host reaction in rats. Salmon et al. (18) were able to accomplish the deletion of lymphoid cell populations that are otherwise capable of responding to human transplantation antigens by selective cell killing with tritiated thymidine. The specific absorption of antigen-reactive cells by antigen-coated inert material has been successfully used by Wigzel and Andersson (19) for the production of lymphoid cell populations specifically deprived of immunological reactivity and has allowed a detailed analysis of antigen-reactive cell populations.

The data recorded here and elsewhere (9) indicate that another general method may be available in transplantation biology for the removal and recovery of sensitized lymphoid cell populations. Furthermore, these experiments show that lymphoid cell populations sensitized against tumor allografts are subsequently able to recognize antigenic determinants present on embryo fibroblasts. Therefore, absorption of these lymphoid cell populations may yield a useful method to recover cells active in tumor immunity.

These data and that of other experiments[4] justify the expectation that under appropriate conditions it may be possible to selectively delete in vitro unwanted lymphoid cells from lymphoid and bone marrow grafts in vivo.

SUMMARY

Mouse lymphoid cells, sensitized against tumor allografts, can be deprived of the immunoreactive cells by in vitro absorption with specific fibroblast monolayers. Populations of lymphocytes so depleted are less effective in retarding tumor growth in vivo and in lysing tumor cells in vitro. Moreover, the adsorbed immunoreactive cells can be recovered specifically and are subsequently efficient in inhibiting tumor growth in vivo and in killing tumor cells in vitro.

Further evidence is presented for the suggestion that the destruction of target cells in vitro by sensitized lymphoid cells is truly representative of the mode of destruction of grafted cells in vivo.

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