Murine Lymphoma-Induced Immunosuppression: Requirement for Direct Tumor Cell Contact

Abstract. *The FBL-3 lymphoma cell line caused impaired antibody formation in vivo when injected into mice intraperitoneally, and in vitro when added to normal syngeneic spleen cells immunized in vitro with sheep erythrocytes. Immunosuppression occurred only when intact viable tumor cells were cocultivated with the normal spleen cells. As few as 10^5 FBL-3 cells, when added to 5×10^6 normal cells, impaired antibody formation. However, cell-free extracts or filtrates from even much larger numbers of tumor cells did not affect antibody formation, either in vitro or in vivo. Heating the tumor cells at 56°C or irradiation with as little as 1000 rads completely abolished immunosuppressive activity, both in vitro and in vivo. Separation of viable tumor cells from target antibody-forming cells by cell-impermeable membranes prevented immunosuppression, showing that direct cell-to-cell contact is required for immunosuppression.*

Many tumors and tumor-inducing substances adversely affect the immune response mechanism. Cell-free extracts derived from virus-induced tumors, as well as oncornaviruses per se, suppress humoral or cell-mediated (or both) immunity (*1*). For example, incubation of normal spleen cells in vitro with cell-free extracts from leukemia virus-infected lymphoid cells resulted in impaired immune responses. Suppression of antibody formation also occurred when normal spleen cells were cultured in chambers containing tumor cells separated by a cell-impermeable membrane, suggesting that immunosuppression was mediated by a relatively small-molecular-weight substance or possibly the virus shed by the leukemic cells (*2, 3*).

Although many of the widely studied transplantable lymphomas were initially derived from tumor virus–injected donor animals, little attention has been given to the effects of virus-free lymphoma cells on immune competence. In this regard, the FBL-3 lymphoma cell line, derived originally from leukemia virus–resistant C57B1/6 mice injected at birth with Friend leukemia virus (FLV), has been studied with respect to antitumor immunity (*4*). The virus responsible for this lymphoma is markedly immunosuppressive when injected into susceptible mouse strains (*5*). Nevertheless, FBL-3 cells, which do not normally shed FLV, have not been examined for their effects on the immune response system. However, as was shown in our study, tumor cells of this type were immunosuppressive for normal syngeneic mouse spleen cells. Unlike the situation with FLV-infected leukemic cells per se, direct cell-to-cell interaction was necessary for immunosuppression by FBL-3 cells. Neither cell-free extracts nor nonviable tumor cells suppressed antibody formation.

For our study the FBL-3 lymphoma (*6*) was passaged by intraperitoneal injection of 10^5 cells into adult C57B1/6 mice. Inoculation of as few as ten tumor cells intraperitoneally into this mouse strain resulted in a rapidly fatal lymphoma, with death of most animals occurring by 30 days. Depression of antibody formation to challenge immunization with sheep red blood cells (SRBC) occurred after tumor cell inoculation (Table 1). Mice were injected intravenously with 4×10^8 SRBC, and their splenic antibody plaque-forming cell (PFC) responses were determined 4 days later (*7*). Whereas control mice developed relatively large numbers of PFC's, tumor-bearing mice showed a progressive decline of antibody formation as a function of time after FBL-3 injection.

In order to determine whether immunosuppression was due to a direct effect of tumor cells on immunocytes per se, we used a completely in vitro system. For this purpose 5×10^6 spleen cells from normal C57B1/6 mice were cultured in Marbrook vessels (Bioresearch Glass) in 1.0 ml of Eagle's minimal essential medium (MEM) containing 10 percent fetal calf serum (Flow Laboratories) in the inner chamber and 11 ml of medium in the outer reservoir chamber (*8*). Immunization of the spleen cells with 0.1 ml of a 0.1 percent suspension of SRBC (2×10^6 erythrocytes) resulted in the rapid appearance of hemolytic PFC's, with peak responses 4 to 5 days after culture initiation. Cocultivation of the normal splenocytes with graded numbers of FBL-3 cells obtained from the peritoneum of syngeneic mice resulted in a marked diminution of the expected antibody response (Table 2). Addition of 10^6 tumor cells to five times as many normal splenocytes completely suppressed antibody response. Five hundred thousand tumor cells resulted in a 95 percent suppression; reducing the number of tumor

Table 1. Hemolytic antibody response by spleen cells from FBL-3 tumor-bearing mice immunized with sheep erythrocytes

Time in days after FBL-3 injection*	PFC response† (number of cells)			
	Per spleen	Percent of control	Per 10^6 spleen cells	Percent of control
None‡	29,090 ± 2,700		233 ± 41	
+7 days	17,100 ± 1,950	59	112 ± 16	48
+10 days	8,900 ± 650	31	92 ± 16	39
+14 days	9,745 ± 738	33	63 ± 16	27

*Groups of C57B1 mice injected intraperitoneally with 10^5 FBL-3 tumor cells on indicated day prior to challenge immunization by intravenous injection of 4×10^8 SRBC. †Average PFC response for spleen cells from five to six mice per group 4 days after immunization with SRBC. ‡Controls.

Table 2. Effect of graded numbers of FBL-3 tumor cells on the in vitro antibody response of normal spleen cells immunized with SRBC; N.S., not significant.

FBL-3 cells added to normal spleen cell cultures* (No.)	PFC per 10^6 spleen cells†	Percent of control	P‡
None (control)	1472 ± 137		
FBL-3 cells			
5×10^4	1620 ± 185	97	
1×10^5	991 ± 72	67	< .01
2×10^5	644 ± 51	44	< .01
5×10^5	50 ± 7	3	< .01
1×10^6	3 ± 1	1	< .01
Normal spleen cells			
1×10^6	1426 ± 43	97.3	N.S.

*5×10^6 normal C57B1 spleen cells cocultivated in vitro with indicated number of FBL-3 cells and immunized with 2×10^6 SRBC. †Average number [± standard error (S.E.)] of hemolytic PFC per 10^6 viable spleen cells for 9 to 12 Marbrook culture chambers 5 days after immunization in vitro. ‡Calculated according to Student's t-test.

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cells to 2×10^5 or 1×10^5 per culture reduced the suppressive effect, but immunologic impairment was still significant. Fifty thousand tumor cells had no effect on antibody formation.

Suppression of the hemolytic antibody response by FBL-3 appeared to be due to a direct interaction of tumor cells with normal spleen cells. Prior treatment of intact tumor cells with 1000 rads or more as well as heating for 20 to 30 minutes at 56°C completely abolished immunosuppressive activity, both in vivo and in vitro (Table 3). Furthermore, homogenates prepared from 10×10^6 tumor cells with a Sorvall Omnimixer had little if any immunosuppressive activity. The slight to moderate immunosuppression appeared to be due to small numbers of intact tumor cells (approximately 1 percent) in the homogenates. This number of tumor cells appeared sufficient to induce a partial suppression of the in vitro immune response. However, homogenates from 10^6 or 5×10^6 tumor cells had no suppressive effect in vitro. None of the same concentrations of the homogenates had any effect on the in vivo response. Furthermore, cell-free filtrates derived from the homogenates by passage through a 0.45-μm Millipore filter showed no suppressive effect, either in vivo or in vitro.

In an additional series of experiments with double-chambered periscopic Marbrook culture vessels (*3*), immunosuppression was only evident in those chambers in which tumor cells were added directly to normal spleen cells immunized with SRBC. For these experiments culture chambers contained either normal spleen cells or spleen cells plus FBL-3 tumor cells. Normal spleen cells in the outer chamber immunized with SRBC developed large numbers of PFC 5 days after in vitro immunization (Fig. 1). Cultures containing FBL-3 cells in a second inner chamber separated by either a Nuclepore membrane (0.45-μm pore size) or a dialysis membrane had no effect on antibody formation by spleen cells in the outer chamber. Only the responses of cultures with FBL-3 cells in the outer chamber containing the spleen cells being immunized with SRBC were suppressed.

Our results show that FBL-3 lymphoma cells are markedly immunosuppressive in vivo and in vitro. However, immunologic impairment occurred only with intact, viable tumor cells. This contrasts with the effects of oncornavirus-induced leukemia. Extracts of virus-induced leukemic cells, as well as virus per se, readily impaired antibody formation, both in vivo and in vitro (*3*). Our findings also contrast with a number of reports indicating that cell-free extracts or culture supernatants derived from a variety of tumor cell lines, regardless of origin, are immunosuppressive (*9*). Various soluble products derived from tumor cells or tumor-bearing individuals, including various serum proteins, ascites fluids, and polypeptide antigens (including tumor-associated antigens), have been implicated in immunosuppression associated with malignancy (*10*).

Table 3. Effect of FBL-3 cells, homogenates, or filtrates on in vivo and in vitro antibody responses of normal mouse spleen cells to SRBC.

FBL-3 preparation*	PFC response per 10^6 spleen cells†	
	In vivo‡	In vitro§
None (controls)	677 ± 51	1630 ± 213
Intact tumor cells (untreated)	95 ± 63 (14%)	4 ± 2 (1%)
Inactivated (1000 rads)	657 ± 72 (97%)	1467 ± 158 (90%)
Heated (56°C, 20 minutes)	732 ± 60 (103%)	1826 ± 207 (112%)
Tumor cell homogenate	780 ± 54 (115%)	986 ± 112 (61%)
Tumor cell filtrate	677 ± 103 (100%)	1387 ± 196 (83%)

*10^6 FBL-3 cells, untreated, irradiated, or heated, or homogenates or filtrate of untreated cells, injected intraperitoneally into normal mice or added to cultures of normal spleen cells in vitro. †Average PFC response for 10^6 spleen cells from five to six mice or cultures per group 4 to 5 days after immunization with SRBC. ‡Mice injected intravenously with 4×10^8 SRBC on day of injection with indicated FBL-3 preparation. §5×10^6 normal spleen cells incubated with 0.1 ml of FBL-3 preparation on day of culture initiation and in vitro immunization with 2×10^6 SRBC.

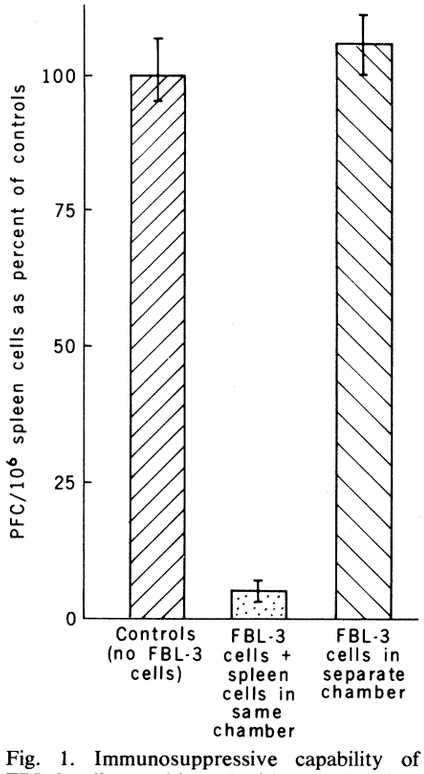

Fig. 1. Immunosuppressive capability of FBL-3 cells cocultivated with normal spleen cells in same chamber or separated by a cell-impermeable membrane in a double-chambered culture vessel. All cultures contained 20×10^6 normal spleen cells immunized in vitro with 4×10^6 SRBC. The bars represent the average PFC response (± S.E.) for 9 to 12 cultures 5 days after in vitro immunization.

The results of our study with FBL-3 cells point to the complexity of immunosuppression mediated by tumors and show that in at least one system direct cell-to-cell contact is necessary for tumor-induced immunologic impairment. Whether the immunosuppression induced in this system is due to "antigenic competition" by tumor cells per se, which carry a virus-associated or tumor-specific transplantation antigen on their surface, or to other mechanisms is not yet clear. Nevertheless, this system provides a model for examining the effects of tumors per se on immunity without the complication of soluble cell-free factors or viruses which also influence immunity.

ROBERTA S. CIMPRICH
STEVEN SPECTER
HERMAN FRIEDMAN
Department of Microbiology and Immunology, Albert Einstein Medical Center, Philadelphia, Pennsylvania 19141

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