Effects of Lymphoid Cells and Serum on Target Cells in Xenogeneic Cell-Culture Systems[1] (34874)

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Several investigators, employing a variety of techniques, have demonstrated cell-killing effects of allogeneic lymphocytes (1–5). The cells serving as targets for the action of lymphocytes have been normal cells carrying different histocompatibility antigens, as well as transformed cells bearing new antigens, the so-called tumor transplantation antigens. Newly acquired antigens have also been detected on autochtonous cells (6). Lymphocytes from immunized animals were used in most of the studies, but evidence has also been presented demonstrating that lymphocytes from nonimmunized animals can also exert an anticellular effect under certain conditions (7). Considerably less is known about the xenogeneic system although antibodies raised in one species were found to sensitize cells of another donor species to the action of complement (8).

This report describes the *in vitro* reactions in a xenogeneic system consisting of spleen cells from rabbits immunized with KB cells (human carcinoma line) and KB cells used as targets. The immune sera from these rabbits were also tested against KB cells, normal human cells, and hamster cells from an adenovirus-induced tumor.

Materials and Methods. Immunizations. Rabbits were hyperimmunized with seven injections of KB cells in suspension (1×10^7 cells/ml) utilizing several routes. The first three immunizations consisted of injections of 0.2 ml of cells incorporated in complete Freund's adjuvant in each footpad, 1 ml of the same antigen subcutaneously and 1 ml of KB cells without Freund's adjuvant intravenously. In the four subsequent immunizations antigen was administered by the subcutaneous and intravenous routes only.

Spleen cell suspensions. Rabbit spleen lymphocytes were harvested by means of a fine mincing of the tissue. The cells were washed two or three times and resuspended in Eagle's Minimal Essential Medium (EMEM) supplemented with 30% inactivated fetal bovine serum (FBS). The suspension was adjusted to 5×10^6 viable cells/ml. The number of viable cells usually varied between 85 and 95% of the cell population.

Target cells. KB cells were grown in EMEM suspension with 10% nucleated inactivated FBS and antibiotics (100 units of penicillin and 100 μg of streptomycin). The cells employed as targets for the comparative study, human fetal skin, primary human amnion, and hamster cells transformed by adenovirus type 12, were also grown in the same medium.

Assays. Tests for lymphocyte-associated immunity were performed in stationary tubes in which a monolayer of KB cells was established after a 3-day growth period. The growth medium was discarded and the cells covered with 1 ml of lymphocytes from the spleen of an immunized rabbit or the spleen of a control rabbit. The monolayer of KB cells at this stage contained approximately 1×10^5 to 4×10^5 cells/ml. The cells were

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TABLE I. Results Obtained with Serum and Spleen Cells from Rabbits Inoculated with KB Cells.

Rabbit no.	Test	Days after 1st injection (no. of injections)					
		6(1)	16(2)	23(3)	53(4)	91(5)	140(7)
1	SCF[a]	<4	4	64	256	256	256
	SGI[b]	—	—	—	180	270	—
	A-CPE[c]	—	—	—	—	—	4+(48 hr)
	CT[d]	—	—	—	0.56	—	—
2	SCF[a]	<4	4	128	256	256	—
	SGI[b]	—	—	—	130	150	—
	A-CPE[c]	—	—	—	—	4+(48 hr)	—
	CT[d]	—	—	—	0.60	—	—

[a] Serum complement-fixation titer (expressed as reciprocal of serum dilution).

[b] Serum growth-inhibition (ED_{50} expressed as reciprocal of serum dilution).

[c] Adherence or cytopathogenic effect (expressed as degree of cell destruction in the presence of lymphocytes).

[d] Cytotoxic index (expressed as fraction of cells surviving the action of antibody + complement).

— = not done.

returned to the incubator and thereafter observed daily for adherence of lymphocytes to the target cells and for destruction of the target cells (A-CPE). The serum complement-fixation test (SCF) was performed in microtiter plates. The serum growth-inhibition (SGI) activity against KB cells and the other cells used for the comparative study was performed according to our modification of Eagle and Foley's technique (9) which was originally devised for screening of cytotoxic drugs. In this method, the extent of cell growth is measured by comparing the amount of total cellular protein on the third day of cultivation in treated and control tubes with the amount of protein at the start of the culture and prior to the treatment. Serum titers are based on 50% end points of activity. The cytotoxic index (CT) was performed according to Gorer and O'Gorman's (10) dye-exclusion technique by counting the number of viable cells before and after the addition of serum and complement to suspensions of KB cells. Normal sera were used in the various serological procedures as controls.

Results. As shown in Tables I and II, all rabbits developed CF antibodies to KB cells. The earliest antibodies were detected at 23 days. Maximum titers were attained between 36 and 67 days. Thereafter, they persisted at an unchanged level. The SGI was first measured at 53 days at which time significant activity was observed. Normal rabbit sera did not inhibit the growth of KB cells. Cytotoxic effects were also first measured at this time and found to be present. Normal rabbit sera were not cytotoxic when tested in the presence of complement. The rabbits were killed 80–140 days after the start of immunization and their spleen cells exerted destructive effects on KB monolayers. The lymphocytes first adhered to the target cells and subsequently within 48–72 hr caused cell rounding and detachment from the glass. Although normal lymphocytes showed some adherence to the target cells they did not bring about their destruction.

In order to test the specificity of the serum reaction, SGI experiments were performed with fetal human skin, human amnion, and hamster cells transformed by adenovirus type 12. Table III shows that the anti-KB serum inhibited the growth of the fetal cells but not of the amnion or of the hamster transformed cells.

Discussion. Immunization of rabbits with intact KB cells elicited both humoral and cell-associated immunity. The former was

TABLE II: Results Obtained with Serum and Spleen Cells from Rabbits Inoculated with KB Cells.

Rabbit no.	Test	Days after 1st injection (no. of injections)					
		36(3)	51(3)	67(4)	80(5)	94(6)	108(7)
3	SCF[a]	512	512	512	512	—	—
	SGI[b]	—	—	—	—	—	—
	A-CPE[c]	—	—	—	4+(72 hr)	—	—
	CT[d]	—	—	—	—	—	—
4	SCF[a]	512	512	1024	512	512	512
	SGI[b]	—	—	—	—	—	520
	A-CPE[c]	—	—	—	—	—	—
	CT[d]	—	—	0.64	—	—	0.52

[a] Serum complement-fixation titer (expressed as reciprocal of serum dilution).

[b] Serum growth-inhibition (ED_{50} expressed as reciprocal of serum dilution).

[c] Adherence or cytopathogenic effect (expressed as degree of cell destruction in the presence of lymphocytes).

[d] Cytotoxic index (expressed as fraction of cells surviving the action of antibody + complement).

— = not done.

amply manifested by the CF, SGI, and CT tests. The latter was evident from the ability of lymphocytes from immune spleens to kill KB cells. The results do not explain the mode of action of the lymphocytes as the killing action could have been either due to antibodies bound to the lymphocytes or generated by them, or related to other mechanisms which appear to be independent of classical antibody. Although complement was not added exogenously, the presence of endogenous complement was not ruled out.

That the killing action of the lymphocytes was the result of immunization was apparent from the absence of such action on the part of nonimmune lymphocytes. The specificity of this effect has not yet been tested. However, evidence was obtained for a relative specificity of the antibodies; thus, the immune sera had no effect against human amnion cells or hamster cells transformed by adenovirus type 12. The action of the rabbit anti-KB sera against human fetal skin cells is interesting because it is in line with the occurrence of carcino-embryonic antigens (11), but no conclusion is justified at this time because the findings may simply represent an instance of one fetus sharing antigens with the KB cells. Cells from other fetuses will have to be tested.

TABLE III. Specificity of Reaction in Serum Growth-Inhibition (SGI) Test with Serum from Rabbits Inoculated with KB Cells.

Rabbit no.	SGI (ED_{50}) of cell monolayer			
	KB	FS[a]	HuAm[b]	AV12[c]
1	270	65	0	0
2	150	50	0	0

[a] Fetal skin cells.

[b] Human amnion cells.

[c] Adenovirus 12 transformed cells.

Summary. Spleen lymphocytes of rabbits immunized with KB cells brought about destruction of these cells in tissue culture. No such effect was seen with normal lymphocytes. Immune sera from these rabbits reacted with KB cells in CF, CT, and SGI tests. The reaction was found to be specific as these sera did not react with cells of human amnion or of hamster tumors induced by adenovirus type 12. Anti-KB serum did react, however, with cells derived from fetal human skin.

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