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Antibody Stimulation of Tumor Cells (39820)

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With a few exceptions, transplantable mouse tumors grow only in a syngeneic host. When transplanted to an allogeneic host the tumor is rejected because of the antigenic differences associated with histoincompatibility. However, if the allogeneic host is first immunized with either tumor tissue or normal tissue from the donor, the tumor may grow in the allogeneic host. This phenomenon is referred to as "immunological enhancement" (1–6). The enhancement has been demonstrated to be due to humoral antibody (7–11). Since many normal tissues, as well as tumor tissues, can serve to induce the enhancing antibody, it is apparent that histocompatibility antigens as well as tumor-specific antigens are responsible for the enhancing antibodies.

The question we wished to answer is whether it is possible to demonstrate immunological enhancement *in vitro*. That is, do antibodies to normal cells stimulate tumor cells to grow more rapidly in cell culture? To examine this possibility an antibody to primary dog kidney cells was prepared and tested on the growth of a transformed dog kidney cell line.

Materials and methods. Primary dog kidney cells (PDK) prepared by trypsinization were obtained from Armour–Baldwin Laboratories at passage 2 to 5. The transformed dog kidney cell line (TDK) arose spontaneously following extended passage of dog kidney cells. TDK cells grow in soft agar, show an infinite life (122 passages), pile up in random patterns, and have a shorter doubling time than do primary dog kidney cells. TDK and PDK cells were cultured in Dulbecco's modified Eagle medium with glutamine containing 10% (vol/vol) fetal calf serum (Rehatuin). Additions to the medium were 13.5 g/liter of sodium bicarbonate, 2 mg/liter of Amphotericin B, and 50 μg/liter of Gentamicin. Cells were incubated in an atmosphere of 95% air and 5% CO_2. Cells were counted with a model Fn Coulter counter which was calibrated weekly.

Antisera to the PDK cells were obtained by intramuscular injection of 20–25 $\times$ 10^6 cells into two Hereford cows weighing 500–650 lb. Four injections of cells were given at weekly intervals followed by a 2-week rest, one more injection was given, and the serum was collected 1 week later. After sterile filtration the serum was heated at 56° for 30 min to remove the heat-labile components of complement.

γ-Globulin was prepared from the bovine serum by ammonium sulfate precipitation as described by Campbell *et al.* (12).

The antiserum to PDK cells was assayed for cytotoxicity against PDK and TDK cells using the method of Gorer and O'Gorman as modified by Zimple *et al.* (13). The cytotoxicity titer of the bovine antiserum to PDK cells was 1:160 and 1:320. When tested against TDK cells, no cytotoxicity could be detected at any level in either antiserum. Normal calf serum was obtained from both animals prior to the immunization, filtered, and assayed for cytotoxicity. All dilutions of preimmune serum gave 95 to 100% viability in the cytotoxicity test.

Results. The antiserum to primary dog kidney cells (PDKS-1 and PDKS-2) was added to culture medium at 10% (vol/vol) containing PDK cells. At this concentration, the antiserum causes agglutination of the cells and, in the presence of added guinea pig complement, the cells rapidly lose viability as indicated by trypan blue staining. However, when the PDKS-1 and PDKS-2 were added to the TDK cells no agglutination occurred within 2 hr after mixing and there was no loss in viability in the presence of complement. The effect of the PDKS-1 and PDKS-2 on growth of TDK cells is shown in Table I. The greatest percentage increase in the cell number is seen at 96 hr. (127 vs 196% increase). Dilution of the antiserum with normal calf serum decreases the stimulatory effect so that, at a 1:19 dilu-

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tion of antiserum, stimulation of cell division is no longer apparent (Table II).

The γ-globulin fraction was removed from PDKS-1 by ammonium sulfate precipitation and used to determine whether the stimulation of cell division was associated with the γ-globulin fraction (Table III). The PDKS-1 antibody was added at different levels to normal calf serum and γ-globulin from normal calf serum was used as a control for the addition of γ-globulin from the PDKS-1. There is some suggestion of stimulation of cell division when normal calf serum γ-globulin is added to the cells. The increase in cell number in those groups receiving PDKS-1 γ-globulin is quite apparent particularly at 72 and 96 hr of incubation. No stimulation occurs when less than 0.15 mg/ml of PDKS-1 γ-globulin is added to the culture medium. The greater increase in cell number in the TDK cells treated with the PDKS-1 over the previous experiments is a result of counting both the cells in the supernatant and those that are attached to the dish. The antiserum-treated cells are more easily detached and float as small clumps in the medium. Counting the cells in the supernatant and those attached to the dish gives the higher counts, as seen in this experiment. The viability of the floating cells was determined to be 95% by dye exclusion. If the factor which stimulates growth is specific antibody it should be possible to absorb the antibody with PDK cells and remove the stimulatory effect. Absorption of PDKS-1 with PDK and TDK cells removes the stimulatory effect (Table IV). PDKS-1 adsorbed with HeLa, WI-38, or strain L cells was not diminished in its ability to stimulate TDK cells. Absorption of normal calf serum with either cell had no effect on the growth of TDK cells.

TABLE I. ANTISERUM TO PRIMARY DOG KIDNEY CELLS ON TRANSFORMED DOG KIDNEY CELLS.[a]

Antiserum	Cells × 10^3/cm²	
	48 hr	96 hr
NCS-1	12.7	97.3
NCS-2	13.0	94.2
PDKS-1	16.2	199.5
PDKS-2	16.4	176.0

[a] TDK seeding density was 4.1 × 10^3/cm²; 28-cm² dish contained 5.0 ml of Dulbecco's modified Eagle medium with 10% (vol/vol) of the designated serum. NCS-1 and NCS-2 are preimmunized normal calf serum and PDKS-1 and PDKS-2 are the postimmunized antiserum. $P < 0.05$ for comparison of PDK-S with NCS by Mann-Whitney U Test.

TABLE II. DILUTION OF PDKS-1 ON THE GROWTH OF TRANSFORMED DOG KIDNEY CELLS.[a]

Dilution	Cells × 10^3/cm²	
	48 hr	96 hr
NCS-1	16.4	90.9
PDKS-1 (undiluted)	18.6	186.0
PDKS (1:10)	17.0	119.6
PDKS (1:20)	15.9	89.5
PDKS (1:50)	16.5	79.6
PDKS (1:100)	15.5	80.3

[a] Seeding density was 6.0 × 10^3/cm². Final concentration of serum was 10% (vol/vol); PDKS-1 was diluted with NCS-1 to obtain indicated dilutions.

TABLE III. γ-GLOBULIN FROM THE ANTISERUM TO PRIMARY DOG KIDNEY CELLS ON THE GROWTH OF TRANSFORMED DOG KIDNEY CELLS.[a]

Antiserum	Cells × 10^3/cm²			
	24 hr	48 hr	72 hr	96 hr
NCS-1	6.7	15.0	48.5	156.0
PDKS-1	11.5	45.3	157.1	474.0
NCS-1 + NCS-1 γ-globulin (mg/ml)				
3.6	5.7	11.1	53.8	161.7
1.8	5.9	14.7	46.8	121.0
0.9	6.2	14.9	60.0	209.0
NCS-1 + PDKS-1 γ-globulin (mg/ml)				
3.6	11.4	40.0	149.0	463.0
1.8	10.1	32.2	138.7	449.2
0.9	10.4	33.5	135.8	406.8

[a] Seeding density was 6.4 × 10^3/cm². Normal calf serum (NCS-1) was added to Dulbecco's modified Eagle medium at 10% (vol/vol). γ-Globulin from PDKS-1 and NCS-1 was added at the indicated concentration to the final medium. For all values for NCS-1 γ-globulin compared with PDKS-1 γ-globulin at 96 hr, $P < 0.01$ by Mann-Whitney U Test.

TABLE IV. GROWTH OF TDK CELLS IN PDKS-1 AFTER ABSORPTION.[a]

Serum	Cells × 10^3/cm² (96 hr)
NCS-1	103.3
NCS-1 absorbed PDK	100.5
NCS-1 absorbed TDK	100.0
PDKS-1	179.0
PDKS-1 absorbed PDK	95.5
PDKS-1 absorbed TDK	116.0

[a] Seeding density was 4.5 × 10^3 cells/cm². All medium contained the indicated serum at 10% (vol/vol). Serum (25 ml) was absorbed with 200 × 10^6 cells. Cells were removed by centrifugation and the serum was filtered through a 0.45-μm filter. Each value is the average of three determinations. All determinations are in triplicate. PDKS-1 absorbed PDK compared to PDKS-1, $P < 0.01$ by Mann-Whitney U Test; PDKS-1 absorbed TDK, $P < 0.01$ by Mann-Whitney U Test.

TABLE V. MULTIPLE PASSAGE IN PDKS-1 ON THE GROWTH STIMULATION OF TDK CELLS.[a]

	Cells × 10^3/cm²			
	24 hr	48 hr	72 hr	96 hr
	No prior passage in PDKS-1			
NCS-1	7.5	16.1	42.0	132.0
PDKS-1	9.6	24.2	67.8	220.0
	After seven passages in PDKS-1			
NCS-1	7.1	13.6	42.8	114.0
PDKS-1	8.2	21.0	64.1	191.0

[a] Seeding density 3.2 × 10^3 cells/cm². The cells were grown for seven passages in 10% (vol/vol) PDKS-1 in Dulbecco's modified Eagle medium. NCS-1 and PDKS-1 differ $P < 0.05$ by Mann-Whitney U Test.

Stimulation of the cells is apparently not due to selection of a more rapidly growing cell type, since even after seven passages of TDK in antiserum the addition of the antiserum resulted in a similar increase in cell number (Table V). To determine the specificity of the antiserum to stimulate cell division, the following cell lines were investigated with medium containing 10% (vol/vol) PDKS-1: HeLa, strain L, WI-38, normal mouse fibroblasts, and F111 and F2412 rat embryo fibroblasts. None of these cell lines were stimulated or inhibited by the antiserum.

Discussion. "Immunological enhancement" was originally described as the successful establishment of a tumor allograft by immunization of the host with heat-killed tumor or normal tissues from the tumor donor (1-6). The establishment of the tumor allograft is known to be due to the development of antibody. Immunological enhancement may also be achieved by passive transfer of immunity (7-11). Since the antigen used may be tumor or normal tissue, the antibody required for immunological enhancement is probably directed toward normal tissue (histocompatibility) antigens and not tumor-specific antigens. An *in vitro* model of immunological enhancement would be stimulation of the cell division of transformed cells by antibody prepared to normal cellular antigens.

Our investigation with the transformed dog kidney cell and antibody to primary dog kidney cells is an approximation of an *in vitro* model of immunological enhancement. With this model, stimulation of a transformed cell by antibody to normal cells is attained. The antibody to PDK cells does not cause cellular injury to the TDK cells in the presence of complement, indicating an inability to fix complement in the presence of TDK cells. The stimulatory effect on cell division is apparently due to γ-globulin since the activity is present in an ammonium sulfate preparation of γ-globulin. Further, the activity of the antiserum may be removed by absorption with either PDK or TDK cells. It is particularly interesting that the activity is removed by the TDK cells in spite of the fact that these cells are not agglutinated by the antibody. This removal also indicates binding of the γ-globulin by the transformed cells.

Shearer *et al.* (14) demonstrated the humoral *in vitro* stimulation of tumor cell lines with antibody to tumor cells. The antiserum was cytotoxic at high concentrations and stimulated cell division at low concentrations. This differs from our observations with antibody to normal cells in that we observed no cytotoxicity, however, our antibody titer is much lower than that obtained by these authors. The antisera they prepared to HeLa, HEp-2, and strain L cells showed complete cross-reactivity with respect to stimulation, whereas the antiserum to normal dog kidney cells stimulated only the transformed dog kidney cell line. Only after a more extensive investigation of normal tissue antiserum will it be possible to accurately contrast the effects reported here

with those of antiserum to tumor cells on tumor cells (14–19).

One of the implications of the stimulation of tumor cell division by antibody to normal tissue antigens is that, in the very early stages of tumor development, tissue injury could enhance cancer cell division since tissue injury has been found to induce antibodies to normal tissue antigens (20) and is also known to promote tumors in carcinogen-treated animals (21, 22).

Summary. Antiserum to normal dog kidney cells was found to stimulate cell division in a transformed dog kidney cell line. The stimulation is obtained with the γ-globulin fraction of the antiserum and the effect is removed by absorption of the antiserum with either the transformed dog kidney cell or with primary dog kidney cells. No adaptation to the antiserum occurred after seven passages in antiserum; the cells were still stimulated by the antiserum as compared to normal serum. Because antibody to normal tissues occurs following injury, it is proposed that this may explain the promotion of tumors by tissue injury.

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