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9. Supported by NIH grant FR-05399. We thank Drs. George Todaro and Howard Green for their advice on tissue culture methods and Mrs. Merle Eichner and Miss Dora Brenholz for technical assistance.

22 April 1969

Tumor-Specific Antigens Detected by Inhibition of Macrophage Migration

Abstract. *Tumor-specific antigens of a guinea pig hepatoma induced by diethylnitrosamine were detected by the inhibition of migration of specifically sensitized macrophages from capillary tubes, and by the local passive transfer of delayed skin hypersensitivity and the suppression of growth of intradermally injected tumor.*

Tumor-specific transplantation antigens of a hepatoma induced in guinea pigs (strain 2) by diethylnitrosamine were detected by methods for measuring cellular immunity to tumors. These methods were: (i) inhibition of migration of macrophages from capillary tubes and (ii) local passive transfer of both delayed skin reactivity and the ability to suppress the growth of intradermally injected tumor cells. All three methods were studied simultaneously with peritoneal exudate cells from the same animals.

Age-matched pairs of male guinea pigs (Sewell-Wright NIH strain 2) were immunized to line 1 tumor by three courses of injections. Each course consisted of intradermal injections of 3.0×10^6 live tumor cells once a week for 3 weeks. There was a 2- or 3-week pause between courses. Peritoneal exudate cells were obtained from these animals in the following manner. Thirty milliliters of a light mineral oil (Drakeol 6 VR, Butler Refining Co., Gettysburg, Pa.) was sterilized and injected into the peritoneal cavity. The animal was killed 4 days after injection of Drakeol, and the peritoneal cavity was washed once with 80-ml, and three times with 40-ml portions of medium 199. The exudate and washings were placed in a separatory funnel at 4°C for 45 minutes, and the cell-rich fraction was then withdrawn from the bottom. Cells were washed three times with medium 199, and a sample was tested for viability with trypan blue (GIBCO, 0.4 percent). Differential cell counts of the peritoneal exudate (PE) usually showed about 50 percent macrophages, 40 percent lymphocytes, and 10 percent granulocytes.

Ascites-variant forms of two separate transplantable hepatomas, lines 1 and 7, were harvested (*1*). These lines have different tumor-specific transplantation antigens as shown by cross-protection studies (*2*). Cells were washed three times with medium 199 and then tested for viability with trypan blue. The tumor cells in these suspensions consisted mostly of single cells; only a few small clumps were present.

Tumor-cell suspensions (line 1 or line 7) were mixed with equal volumes of peritoneal exudate cells from animals immunized to line 1. For macrophage-migration tests, the following mixtures were made—60×10^6 PE cells per milliliter with 60×10^6 tumor cells per milliliter, and 60×10^6 PE cells per milliliter with 6×10^6 tumor cells per milliliter. These mixtures were incubated for 10 minutes, with shaking, at 37°C.

Cell mixtures were placed in capillary tubes and sealed in pairs into tissue chambers, after the method of David (*3*). The chambers were filled with McCoy's 5a medium (modified) with 30 percent fetal bovine serum (GIBCO) and incubated for 5 days at 37°C. The areas covered by the migrating cells in 24 hours were measured with a planimeter (Table 1).

Migration of PE cells from animals immunized to line 1 tumor is inhibited by an equal number of line 1 tumor cells, but not by an equal number of line 7 tumor cells. Mixtures of PE and tumor cells (10 : 1) showed no inhibition of migration when tumor cells of either line 1 or line 7 were used. There was no inhibition of migration of PE cells from nonimmunized animals when the cells were mixed with line 1 or line 7 tumor cells (Table 2).

Cell mixtures were also prepared for use in local passive transfer of delayed skin sensitivity. One-tenth of a milliliter of these mixtures contained 3×10^6 PE cells and 1.5×10^6 tumor cells of either line 1 or line 7. Age-matched pairs of male guinea pigs (strain 2) were injected intradermally with 0.1 ml of each of these mixtures and at a separate site with 1.5×10^6 cells from each tumor line. Delayed skin reactions were measured with a caliper at 24 hours (Table 3). Because each intradermal injection contained live tumor cells, it was possible to mea-

Table 1. Areas of migration (measured by planimetry from a projected image and expressed in square millimeters) of peritoneal exudate (PE) cells from animals immunized with line 1 tumor. Line 7 shares no tumor-specific transplantation antigen with line 1.

Cells (No./10^6) per mixture			Migration area* (mm^2)
PE	Line 7	Line 1	
60	60		21.51 ± 0.42
60		60	10.15 ± 0.68
60	6		19.92 ± 0.55
60		6	20.25 ± 0.67
60			18.47 ± 0.51

* Mean area ± standard error of the mean for eight capillary tubes.

Table 2. Areas of migration (measured by planimetry from a projected image and expressed in square millimeters) of peritoneal exudate (PE) cells from nonimmunized animals.

Cells (No./10^6) per mixture			Migration area* (mm^2)
PE	Line 7	Line 1	
60	60		15.47 ± 0.27
60		60	15.68 ± 0.34
60			14.40 ± 0.17
	60		0.99 ± 0.05
		60	0.91 ± 0.08

* Mean area ± standard error of the mean for six capillary tubes.

Table 3. Local passive transfer of delayed cutaneous hypersensitivity to tumor-specific transplantation antigens measured as the skin reaction at 24 hours. Size of intradermal papules at 5 days is a measure of the ability of the tumor cells in the injected mixtures to grow. Peritoneal exudate (PE) cells were from animals immunized with line 1; r, radius.

Cell mixture (No./10^6) injected in 0.1 ml			Skin reaction at 24 hours ($< r^2 >$, mm^2)	Intradermal papule size at 5 days ($< r^2 >$, mm^2)
PE	Line 7	Line 1		
3	1.5		1.2	5.9
3		1.5	8.5	0
	1.5		0	5.8
		1.5	1.2	7.6

sure growth or suppression of growth (Table 3).

Local passive transfer of delayed hypersensitivity could be accomplished with PE cells from these same animals. Skin reactions caused by mixtures of PE cells with line 1 tumor cells were significantly larger than were reactions caused by mixtures of PE cells with line 7 tumor cells. Reactions of PE cells alone, line 1 alone, line 7 alone, and medium 199 alone had a radius squared less than 1.3 mm^2.

Five days after injection intradermal growth of line 7 tumor cells alone was the same as that of mixtures of PE cells and line 7 tumor cells (Table 3). Peritoneal exudate cells from animals immunized to line 1 caused suppression of growth of line 1 tumor compared to the growth of an equal number of line 1 cells not mixed with PE cells. Additional experiments showed that the growth of tumor cells from either line 1 or line 7 was not suppressed by PE cells from nonimmunized animals.

Tumor-specific transplantation antigens can be detected by these three methods. We do not know, however, whether these three methods detect the same antigens.

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18 February 1969

Chloramphenicol: Effects on Mouse Myeloma Cells in Tissue Culture

Abstract. *Within 36 hours of being administered, chloramphenicol (50 micrograms per milliliter) inhibits by 50 percent the rate of protein synthesis in mouse myeloma cells grown in suspension culture. Although there is a decrease in the amount of globulin synthesized, the rate of synthesis per cell is unchanged; the observed decrease is traced to the inhibition of cell proliferation caused by chloramphenicol.*

The cells of the hematopoietic and immune systems are most susceptible to the deleterious effects of chloramphenicol (CM), hematopoietic depression and toxicity being the most common and serious clinical side effects of treatment with this agent (*1*). Chloramphenicol inhibits induced antibody synthesis both in vivo and in vitro (*2*), presumably by inhibiting protein synthesis. However, attempts to demonstrate protein inhibition directly in mammalian systems have led to widely variable results (*3*).

Mouse myeloma cells have been adapted to tissue culture and have been grown continuously in suspension for several years (*4*). These cells offer several advantages. (i) They are susceptible to CM; (ii) they secrete measurable amounts of gamma globulin; and (iii) their growth pattern is highly reproducible. Cells which die remain identifiable and can be counted with no more than 5 percent disintegration loss for 4 to 5 days. This means that cumulative cell counts plotted against time are significant, and rates of division and death per viable cell per hour can be computed from the first derivative of these curves.

Myeloma cells were suspended in fresh growth medium (400×10^3 viable cells per milliliter), and the pooled cells were divided into two equal portions; CM was added to one [50 μg/ml ($1.5 \times 10^{-4} M$)]. Samples (6 ml) were then placed in enough flasks to provide four for each time point (two control, two with CM). Portions (0.5 ml) were then removed in duplicate, and the cells were counted with a Coulter counter to assure that each flask contained the proper number. All flasks were incubated at 37°C. One hour before each interval, four flasks were removed, and 5 μc of C^{14}- labeled amino acids (reconstituted protein hydrolyzate) were added to each. After incubation for 1 hour a portion (0.2 ml) was stained with trypan blue, and the cells were counted in a hemocytometer. Viable cells were defined as those not staining with trypan blue. Duplicate samples (0.5 ml each) were removed, and the cells were counted in the Coulter counter. Total numbers of viable cells were calculated by multiplying the cell number determined with the Coulter counter by the percentages of viable cells as determined by trypan blue exclusion; total numbers of dead cells were determined similarly. Protein synthesis, expressed as disintegrations per minute (dpm), in the fraction precipitable by trichloroacetic acid (TCA) (*5*) and globulin synthesis, measured by the method of radial diffusion (*6*), were determined on the remainder of the culture.

The number of cells in the control cultures increased threefold during 60 hours, whereas in cultures treated with CM the cell number reached a maximum at 36 hours. At this time the treated cultures contained about 60 percent of the number of cells in the control cultures. The total number of dead cells per milliliter was essentially the same in all cultures during 60 hours.

The total number of viable cells can be thought of as the result of cell division and cell death. Because division adds one cell to the total population, the curve of the increase in the total number of cells (viable and dead) as a function of time is also an expression of the total number of divisions. Similarly, a graph of the cumulative increase in the number of dead cells expresses the total number of deaths as a function of time. Comparison of these data shows that the entire difference between the number of viable cells in the control and the treated cultures can be attributed to the far greater number of cell divisions in the former. Cell death contributed nothing, for there was always