the intact cell. Although further characterization of this enzyme is necessary, these observations are consistent with the interpretation that this phosphatase is a part of a specific enzyme system that mediates the energy-dependent translocation of calcium across the plasma membrane at the serosal surface of the mucosal cell. Therefore, the role of sodium in intestinal calcium transport may be the activation of this enzyme system.

STANLEY J. BIRGE, JR.
HELEN R. GILBERT
LOUIS V. AVIOLI

Department of Medicine,
Jewish Hospital of St. Louis,
St. Louis, Missouri 63110

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19. Supported by NIH grants AM 14571 and 11674.

4 October 1971; revised 19 January 1972

The Immune Reaction as a Stimulator of Tumor Growth

Abstract. *Various numbers of spleen cells from specifically immunized mice were mixed with constant numbers of target tumor cells, and were inoculated subcutaneously into thymectomized, x-irradiated recipients. Small numbers of admixed immune spleen cells produced a statistically significant, and reproducible, acceleration of tumor growth in the inoculum as compared with controls of either nonimmune spleen cells or spleen cells from animals immune to a different, noncross-reacting, tumor. Larger numbers of specifically immune spleen cells, however, produced inhibition of tumor growth. These data imply that the normal immune reaction may have a dual function in relation to neoplasia: (i) stimulation of tumor growth, early in the course of the disease, or whenever the immune reaction is minimal; (ii) inhibition of tumor growth at other times.*

I recently advanced the theory that the effect of immunity on target tumor cells might be biphasic, that is, a mild reaction might stimulate tumor growth although a strong one is cytotoxic (*1*, *2*). This theory is based primarily on the considerable data suggesting that, under some circumstances, a slight degree of immune reactivity may benefit fetal growth and survival (*2*). Since a fetus and a tumor share the quality of being antigenically foreign to their hosts, tumor growth might likewise be stimulated by a mild immune reaction. The mechanisms by which the immune reaction might actually stimulate tumor growth are open to speculation, but a clue is provided by the report that small concentrations of lymphotoxin are stimulatory, rather than cytotoxic, to target cells (*3*).

The theory was tested with Winn procedures (*4*), that is, varying numbers of immune spleen cells were mixed with tumor cells and the effect on the tumor was assayed by inoculating the mixtures subcutaneously into test mice. The tumors were sarcomas that had been induced by treatment of inbred DBA/2 or F_1 hybrid mice [(C_{57}BL × BALB/c) and (C_{57}BL × C_3H)] with 3-methylcholanthrene. The tumor cells were suspended in modified Eagle tissue culture medium by the action of Pronase and deoxyribonuclease. The live cells were counted after they were stained with trypan blue, and a small number (usually 10^4) were mixed with graded numbers of immune or nonimmune, syngeneic, spleen cells. The tumors were in the first to fifth transplant generation when they were used. Because the different tumors varied in their growth rates, the time selected for analysis of the data varied slightly among experiments but it was always when the diameter of the largest tumor most nearly approximated 10 mm.

The syngeneic recipients had been previously thymectomized as adults and then, 24 hours before inoculation of the mixtures of tumor and spleen cells, had been given 450 roentgens of total body x-irradiation. This regimen of thymectomy and x-irradiation crippled the capacity of the mouse to reject a primary skin allograft. Therefore, the effects of the admixed spleen cells on the growth of the inoculated tumors were probably not complicated by host immunity.

The donors of immune spleen cells were syngeneic mice that had grown the particular tumor for 10 to 20 days. Usually the tumors were excised and the spleens were harvested 7 to 12 days after excision. In one experiment, however, spleens were harvested without prior excision of the immunizing tumors.

Initially, there were five experiments with three tumors that were induced separately. Experimental and control recipients were paired for inoculation. Control spleen cells were obtained from nonimmunized donors in four of the five experiments. In the other experiment, the control spleen cells were obtained from mice that had been immunized in the standard manner against a different, and noncross-reacting, tumor.

The results of this first series of experiments are presented in Fig. 1. It became apparent that tumor growth was accelerated when normal syngeneic spleen cells were mixed with the tumor cells as compared with that when no spleen cells were present. A similar finding has been reported by Deckers *et al.* (*5*). In addition, in my experiments, the immune spleen cells produced an even greater acceleration than did the control spleen cells when, and only when, these immune cells were added in amounts of less than 10^5, that is,

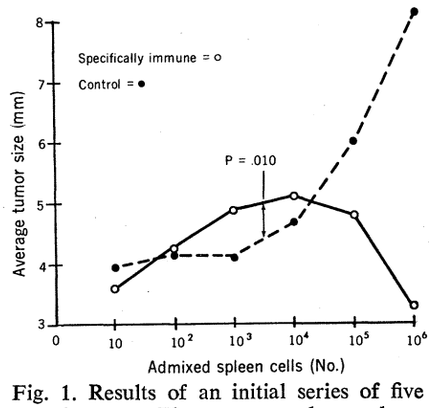

Fig. 1. Results of an initial series of five experiments. The curves have been smoothed by averaging: that is, the plot for ten spleen cells is actually the average of the values for 0, 10, and 10^2; that for 10^2 is the average of 10, 10^2, and 10^3, and so forth. There were 13 to 37 observations per point.

when they were approximately equal to, or less than, the number of tumor cells. At higher proportions of spleen cells, those from the specifically immune donors produced an inhibition of tumor growth.

The acceleration of tumor growth in these experiments (as measured by greater tumor size) with 10^3 or 10^4 specifically immune spleen cells was statistically significant (the paired sign test). Thirty-six of the 54 unequal pairs available had the largest tumor with the admixed immune spleen cells [$P =$ .010 (one-tail distribution)]. Only one of the individual experiments was large enough to yield statistically significant results, although the data of each, with one exception, pointed in the same direction. The one experiment in which the data did not show this trend was the experiment in which the "immune" spleen cells had been obtained from donors bearing unexcised tumors. There are reports that such cells may lack cytotoxic activity in vitro in the colony inhibition assay. However, the possible influence of this factor in the present type of experiment is yet to be determined (*6–8*).

These initial results were then confirmed in an experiment with one of the tumors used in the previous series. The experimental method was substantially the same as before except that only three amounts of spleen cells were used: 0, 5×10^4, and 10^7. The groups were somewhat larger in an attempt to achieve a statistically significant result within the data of the single experiment. Also, a second control group was added in which the spleen cells were obtained from animals that had been immunized against a different, and noncross-reacting, tumor.

The results of the confirmatory experiment are presented in Fig. 2. With 5×10^4 spleen cells, there was better tumor growth in the specifically immune spleen cell group. There was no apparent difference between the groups receiving the nonspecifically immune cells and those that received the normal spleen cells. A statistical analysis of the specifically immune as compared to the nonspecifically immune spleen cell group, by the paired sign test, gave $P =$ .002 (one-tailed test). When compared to the groups that received nonimmune spleen cells, the value was $P =$ .035. The combined value was $P < .001$. The results of this experiment thus confirmed the relative stimulation of tumor growth by a low dosage of immune spleen cells.

Prior immunization can lead to accelerated growth of subsequent tumor implants, the phenomenon commonly called "tumor enhancement." This effect can be transferred by passage of immune lymphoid cells (*9*); a biphasic reaction has also been reported (*10*). However, this acceleration has only been described in comparison with growth in nonimmunized, but immunologically competent, controls and is generally ascribed to the formation of blocking antibodies or immune complexes. In contrast with the usual type of enhancement, the tumor acceleration that I have reported here involved recipient animals that had been thymectomized and then exposed to 450 roentgens of x-irradiation 24 hours prior to tumor implantation. Thus, the accelerated tumor growth observed in my experiment is probably not explainable on the basis of a blockage of recipient immunity. However, there is a possibility that the sensitized spleen cells of the inoculum produced blocking factors that interfered with further stimulation of the inoculated immune spleen cells themselves, or with the sensitization of previously nonsensitized portions of the "immune" inoculum.

Whatever the mechanism may be, my experiments show that normal syngeneic spleen cells in contact with a tumor can stimulate tumor growth and that, if these spleen cells were specifically sensitized, they stimulated growth even more provided that a critical dosage level is not exceeded.

There may be a time, in the early evolution of tumors, when the immune

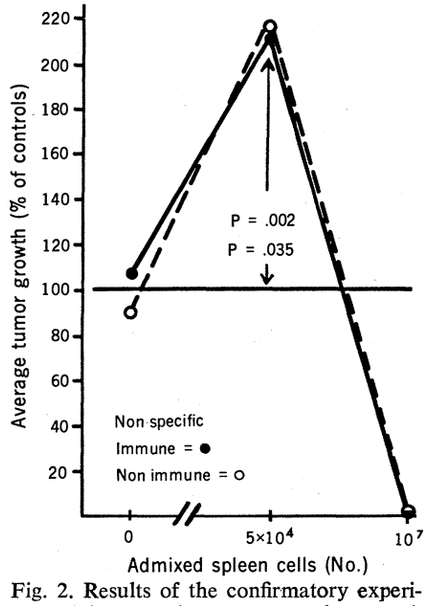

Fig. 2. Results of the confirmatory experiment. The two plots represent the growth of the same series of tumors as compared with two different types of control: controls with normal spleen cells and controls with nonspecifically immune spleen cells, that is, spleen cells from animals immune to a different, and noncross-reactive, tumor. There were 5 to 13 observations per point.

response is incipient and therefore weak. If this situation is comparable to a low dosage of immune spleen cells, the normal immune reaction may actually assist the growth of nascent tumors. Furthermore, if a weak immunity stimulates tumor growth, immunoselection by the growth-stimulatory immune reaction would provide an explanation of the fact that most, and perhaps all, tumors are antigenic.

RICHMOND T. PREHN
Institute for Cancer Research,
7701 Burholme Avenue,
Philadelphia, Pennsylvania 19111

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26 October 1971; revised 24 January 1972