cursors in the absence of θ-carrying cells; or (iv) the low number of PFC represents the response of SRBC-stimulated precursors of PFC that differentiate in the absence of a proliferative stimulus afforded by θ-carrying cells.

In the reconstitution system, the precursors of the PFC were provided by spleen cells treated with anti-θ. Together with PFC themselves and hemopoietic stem cells, the precursors must therefore be θ-negative or θ-deficient cells and they most probably are not thymus-derived. The generation of direct and indirect PFC from their precursors requires the presence of θ-bearing cells which, in the system used here, can functionally be replaced by in vivo incubated thymus cells. It remains to be determined whether the population of θ-bearing cells has specific reactivity to SRBC and at what stage of the in vitro immune response these cells function to facilitate the generation of PFC (*16*).

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8. AKR/J mice were given six intraperitoneal injections of 2×10^7 C_3H/HeJ thymus cells weekly and bled 7 days after the last injection. Subsequent bleedings were made 7 and 14 days after single booster injections given at 3-week intervals. Although AKR/J and C3H/HeJ mice share the same H-2 alleles, they differ at the θ locus and probably also at the Ly-A locus (two above). Therefore it was possible for our AKR anti-θC3H to contain antibodies to Ly-A1 antigen. However, after absorption with BALB/c thymus cells which carry θC3H antigen but not Ly-A1 antigen, the antiserum had no detectable cytotoxic activity against thymus cells carrying θC3H and Ly-A1 antigens, indicating that there was little if any cytotoxic antibody against Ly-A1 antigen in our θ antiserum.
9. Three sources of guinea pig serum were used. One was a pool of serum from young guinea pigs, another a single batch of North American Biologicals frozen guinea pig serum, both known to have low cytotoxic activity for mouse thymus cells. The third was Hyland reconstituted guinea pig serum absorbed with an acetone powder of mouse liver and lymphoid cells.
10. Two methods of incubation have been used for thymus and marrow cells in cytotoxicity assays and for spleen cells in all other experiments. The one-step procedure consisted of treating cells at 1×10^7 cells/ml with antiserum at 1:40 and GPS at 1:10 for 45 minutes at 37°C. In the two-step procedure cells (5×10^7/ml) were incubated with antiserum (1:5 dilution) for 15 minutes at 4°C, washed, resuspended to the same concentration, and incubated at 37°C for 30 minutes with GPS (1:5 dilution). The medium in all cases was 5 percent fetal calf serum in Eagle's minimum essential medium. Similar results in the cytotoxic tests were obtained with both methods.
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16. *Note added in proof*: Since the completion of this manuscript, M. Schlesinger [*Nature* **226**, 1258 (1970)] has published data showing the resistance of PFC to anti-θ, and A. Schimpl and E. Wecker [*Nature* **226**, 1258 (1970)] have reported a decrease in the in vitro primary response of mouse spleen cells to SRBC after treatment of the spleen cells with anti-θ.
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Tumor Immunity Produced by the Intradermal Inoculation of Living Tumor Cells and Living Mycobacterium bovis (Strain BCG)

Abstract. *The intradermal inoculation of mixtures containing living tumor cells and living* Mycobacterium bovis *(strain BCG) into unimmunized syngeneic guinea pigs results in an inflammatory reaction to the BCG, and there is no progressive tumor growth. In the absence of BCG the tumor grows progressively, metastasizes, and kills the animal. By conventional methods, it has not been possible to immunize syngeneic guinea pigs to the tumor used. Guinea pigs that receive mixtures of BCG and tumor cells, however, develop specific systemic tumor immunity as measured by delayed cutaneous hypersensitivity and by suppression of tumor growth.*

Among the available methods for inducing tumor specific transplantation immunity to chemically induced syngeneic tumors are inoculation of (i) subthreshold doses of living tumor cells, (ii) irradiated tumor cells, (iii) living tumor cells intradermally (a site promoting growth and regression), (iv) living tumor cells followed by amputation of the growing tumor, and (v) cell-free extracts of tumor cells (*1*). Treatment of tumor cells by methods that cause impairment of their ability to multiply often leads to loss of immunogenicity. We now describe a method for inducing specific tumor immunity which permits the use of tumor cells that are capable of dividing but which avoids the danger of progressive tumor growth. Tumor cells are mixed with living *Mycobacterium bovis* (strain BCG) and the mixture is injected intradermally into unimmunized guinea pigs. An inflammatory response to the

Table 1. Specificity of immunity induced by intradermal injection of mixtures of BCG and line 10 tumor cells. Each animal received two intradermal injections: an injection of line 1 tumor cells and an injection of line 10 tumor cells. Because the antigen used in this experiment was living tumor cells, the delayed cutaneous hypersensitivity reactions to a cell inoculum was measured at 24 hours, and tumor growth of that same cell inoculum was measured at 8 days; *r*, radius.

Immunized with:	Animals (No.)	Skin reactions at 24 hours, $\langle r^2 \rangle$ (mm²) challenged with		Tumor nodule size at 8 days, $\langle r^2 \rangle$ (mm²) challenged with	
		Line 1*	Line 10†	Line 1*	Line 10†
BCG + line 10	4	1 ± 1	15 ± 5	25 ± 0	0
Line 1	3	20 ± 5	2 ± 0	0	23 ± 0
Nothing	4	1 ± 1	1 ± 1	21 ± 2	20 ± 0

* Number of line 1 cells, 3×10^6. † Number of line 10 cells, 10^6.

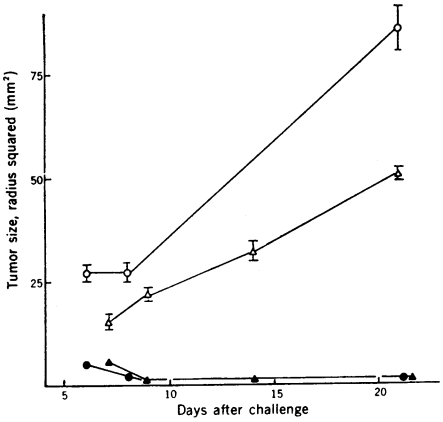

Fig. 1. Intradermal growth of 10^6 line 10 tumor cells in guinea pigs immunized to living BCG alone (△), to living line 1 tumor cells alone (○), or to mixtures of living BCG and living line 10 tumor cells (●, ▲). The results of two experiments are shown. In experiment 1, five guinea pigs were immunized intradermally with BCG alone, and three guinea pigs were immunized with a mixture of BCG and line 10. Each of these eight animals was challenged with line 10 tumor cells 35 days later. In experiment 2, three guinea pigs were immunized intradermally with line 1 tumor cells alone and five guinea pigs were immunized with a mixture of BCG and line 10. Each of these was challenged with line 10 tumor cells 31 days after immunization. The difference at day 21 between tumor nodule size in animals immunized with BCG alone, or immunized with line 1 alone compared to animals immunized with a mixture of BCG and line 10 is significant, at $P = .01$, as determined by t-test.

BCG occurs, and there is no progressive tumor growth. Animals immunized in this way are capable of suppressing the intradermal growth of tumor cells in a challenge inoculum containing ten times the lethal dose.

Strain-2 male guinea pigs (Sewell-Wright, inbred) were obtained from the breeding colony at the National Institutes of Health. The induction of primary hepatomas in the guinea pig by feeding the water-soluble carcinogen, diethylnitrosamine (*2*), and the antigenic and biologic characteristics of the transplantable hepatomas derived from the primary tumors have been described (*3*). We used ascites tumor line 10. This tumor, a poorly differentiated hepatocarcinoma, grows progressively after inoculation of 10^5 tumor cells intradermally. The tumor regularly metastasizes to lymph nodes draining the site of tumor injection. Animals injected intradermally with cells of this tumor line usually die about 60 days after injection. The Phipps strain of BCG was obtained from the Trudeau Mycobacterial Collection at a concentration of 1.2×10^8 bacteria/ml in Middlebrook 7H9 broth with Tween (*4*). The guinea pigs were immunized by intradermal inoculation of 6×10^6 bacteria mixed with 1.5×10^6 tumor cells. From 31 to 35 days after immunization, animals were challenged intradermally with 10^6 tumor cells.

The results of representative experiments presented in Fig. 1 and Table 1 can be summarized as follows. (i) All animals immunized with BCG and line 10 tumor cells completely suppressed the growth of line 10 cells. Protected animals have remained tumor free from the initiation of the experiment to the present, a period of 3 months. (ii) Previous immunization with BCG alone did not impair the growth of line 10 cells. (iii) Animals immunized with ascites line 1 did not suppress the growth of line 10 cells. (iv) Animals immunized with BCG and line 10 showed delayed cutaneous hypersensitivity reactions to line 10 cells alone and suppressed line 10 tumor growth. (v) Animals immunized with BCG and line 10 did not show delayed cutaneous hypersensitivity reactions to line 1 cells or suppress line 1 tumor growth. Line 1 is a transplantable ascites tumor induced by diethylnitrosamine in strain-2 guinea pigs. We have been unable to immunize against line 10 cell growth by conventional methods. Our results suggest that it may be possible to produce strong tumor immunity to weakly antigenic tumors by injecting the living tumor cells together with living BCG.

The paucity of reports of the use of complete Freund's adjuvant to induce specific cell-mediated tumor immunity suggests a lack of success with this method. Perhaps the labile tumor specific transplantation antigens are destroyed during emulsification. The procedure outlined does not require emulsification and evidently preserves antigens necessary for induction of specific tumor immunity.

There is already clinical evidence that BCG may be of value in the control of human cancer (*5*, *6*). One approach has been to inject BCG directly into intradermal metastases (*6*). Another approach would be to attempt the induction or augmentation of systemic tumor immunity by the inoculation of autologous living tumor cells mixed with BCG. Our work indicates that for the development of optimal systemic tumor immunity, direct contact between tumor cells and BCG is required (*7*).

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Hydroxy-L-proline- and 2,2′-Dipyridyl-Induced Phenovariations in the Liverwort Nowellia curvifolia

Abstract. *Two antagonists of "normal" proline-hydroxyproline-protein synthesis, 2,2′-dipyridyl and hydroxy-L-proline, induced the same kind of phenovariation in* Nowellia curvifolia *(Dicks) Mitt. (Cephaloziaceae) as they do in* Scapania nemorosa *(Scapaniaceae). This finding supports a hypothesized cardinal role for proline-hydroxyproline-protein in modulating aspects of morphogenesis and phylogeny in the leafy liverworts.*

Hydroxyproline and 2,2′-dipyridyl, compounds that interfere with the "normal" synthesis of hydroxyproline-protein, can induce phylogenetically and systematically significant changes in symmetry, branching pattern, and leaf morphology in the liverwort *Scapania nemorosa* (*1*, *2*). Because the pheno-

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