Effect of Cyclophosphamide on the *in Vitro* Production of Immune Globulins by Lymphoid Tissue in Allograft Rejection[1] (34410)

MILDRED E. PHILLIPS[2]

New York University Medical Center, New York, New York 10016

It was previously reported (1) that following the application of a subcutaneous graft of sarcoma I (a transplantable tumor derived from the inbred A-strain mice) in $C_{57}Bl/6$ mice, the initial synthesis of immunoglobulins by the regional lymph nodes consisted of the high molecular weight 19S γ-globulin accompanied by a significant 7*S* γ_2. It was also found that as the level of 7*S* γ_2 rose, IgM production disappeared and at that time a striking 7*S* γ_1 synthesis, that reached its peak 10 days after antigenic stimulation (the peak of graft rejection in this particular donor–host combination) was noted. The sequence of immunoglobulin formation after the application of a tumor graft was found to be comparable with that following the use of conventional antigens (2). The technique used was that of radioimmunoelectrophoresis.

Several studies have shown that cyclophosphamide (CY) significantly prolongs survival of homografts of skin in several species (3–5) and that it markedly inhibits the ability of rats and mice to produce measurable antibody to inoculation of sheep red blood cells (6–8). The present experiments were undertaken to study the effect of CY on the synthesis of immunoglobulins by sensitized lymph nodes from murine hosts following the application of a tumor graft.

Materials and Methods. Male mice of the inbred strains A/JAX were used for the passage of sarcoma I and male $C_{57}Bl/6$ mice were used as recipients of the tumor grafts. All mice, weighing between 20 and 25 g each, were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The sarcoma I was obtained in the ascites form (from Dr. George Snell, Jackson Memorial Laboratories, Bar Harbor, Maine) and maintained by transfer (every 4–6 days) of 3 $\times$ 10^6 cells in a volume of 0.5 ml of physiologic saline solution containing 2 units of heparin/ml given intraperitoneally to A/JAX mice. Subcutaneous tumors for immunization were produced by inoculation of 10^6 cells in a volume of 0.05 ml into shaved sites on the right flank of $C_{57}Bl/6$ mice. The hosts were given a single ip injection of 300 mg/kg of CY within 1 hr of tumor grafting.

On days 4, 5, 7, 10, and 12 after transplantation, the ipsilateral lymph nodes were aseptically removed, and the contralateral nodes were used as controls. The tissues were minced, and approximately 40–50 mg of lymphoid tissue were cultured for 24 to 48 hr at 37° in roller tubes with 1 ml of the appropriate media to which C^{14}-lysine and isoleucine had previously been added. It was necessary to pool the minced tissues from the lymph nodes from two animals in order to obtain a uniform weight. After the culture period, the culture fluids were dialyzed against 0.015 *M* phosphate buffer at pH 7.2 for 48 hr, lyophilized, and redissolved with 0.1 or 0.15 ml of distilled water, after which they were subjected to microimmunoelectrophoresis. A nonlabled mouse carrier serum was added to the antigen well before the addition of the culture fluids to provide enough mouse proteins to make immunoelectrophoretic patterns. The patterns were developed with a rabbit anti-mouse serum (Hyland Laboratories, Los Angeles, Calif.). A few slides were developed

[1] This investigation was supported by Grant T 404 from the American Cancer Society, and by Grant 5 R01 CA-07863 from the United States Public Health Service.

[2] Faculty Research Associate, American Cancer Society.

TABLE I. Types of Immune Globulins Present in Regional Lymph Nodes at Various Intervals after Tumor Graft.

Day	IgG_1		IgG_2		IgM	
	Experimental	Control	Experimental	Control	Experimental	Control
4	—	—	—	—	—	—
5	—	—	—	—	—	—
7	—	—	VW[a]	—	—	—
10	—	—	4+[b]	1+	3+	1+
	—	—	3+	1+	3+	1+
12	—	—	3+	1+	2+	1+
	—	—	3+	1+	1+	1+

[a] VW, very weak.

[b] Numbers 1+ to 4+ represent the intensity of the radioactive lines on the autoradiographs.

with a goat immune serum, specific for individual mouse immune globulins (kindly supplied by Dr. Richard Asofsky, National Institutes of Health, Bethesda, Maryland). This antiserum was used to identify the 7*S* γ_1.

Results. Although no attempt was made to study the effect of CY on graft survival, it was apparent that on the 10th day after the application of the tumor graft, a time when rejection of sarcoma I could be expected in $C_{57}Bl/6$ mice, many of the grafts were still viable. This finding is compatible with the observations that single or multiple doses of CY (4, 5) prolonged the survival of allogeneic skin grafts in rats and mice.

The results concerning the synthesis of immune globulins are summarized in Table I. A total of 10 culture fluids from sensitized lymph nodes and an equal number of control cultures were analyzed by radioimmunoelectrophoresis. Significant immunoglobulin labeling did not appear until day 10 at which time very strong labeling of 7*S* γ_2, accompanied by labeling of IgM appeared (Fig. 1). The labeling of 7*S* γ_2 was somewhat stronger than the labeling of IgM. The intensity of labeling of neither 7*S* γ_2 nor IgM was significantly altered by day 12. Culture fluids, from lymph nodes obtained 10 and 12 days after tumor grafting, when developed with an antiserum used to identify 7*S* γ_1 failed to show labeling of a precipitation arc.

Discussion. Previous studies from this laboratory have shown (1) that following the application of a subcutaneous graft of sarcoma I, IgM synthesis by the regional lymph nodes appeared on day 4 and was negligible by day 10 whereas 7*S* γ_2 synthesis appeared at the same time, reached its peak on day 7 and diminished slightly thereafter. Labeling of 7*S* γ_1 did not appear until day 7 and reached its peak on day 10.

Several interesting observations emerged from the present study. The most significant findings were the complete suppression of labeling of 7*S* γ_1 and virtually complete suppression of immunoglobulin synthesis during the first 7 days after grafting. The labeling of 7*S* γ_2 on days 10 and 12 was significantly stronger than that obtained with culture fluids of lymph nodes from hosts that were not treated with CY. Similarly, labeling of IgM, although occurring later, was generally stronger than in nontreated tumor grafted hosts (1).

The marked suppression of immunoglobulin synthesis in the present study is in accord with observations of others (8) that CY suppressed the primary antibody response of CY-treated mice to sheep RBC over a 28-day period of observation, irrespective of the intervals of CY inoculations in relation to immunization. The differences between the findings in the present study and those of others (7) in regard to the types (19 *S* or 7

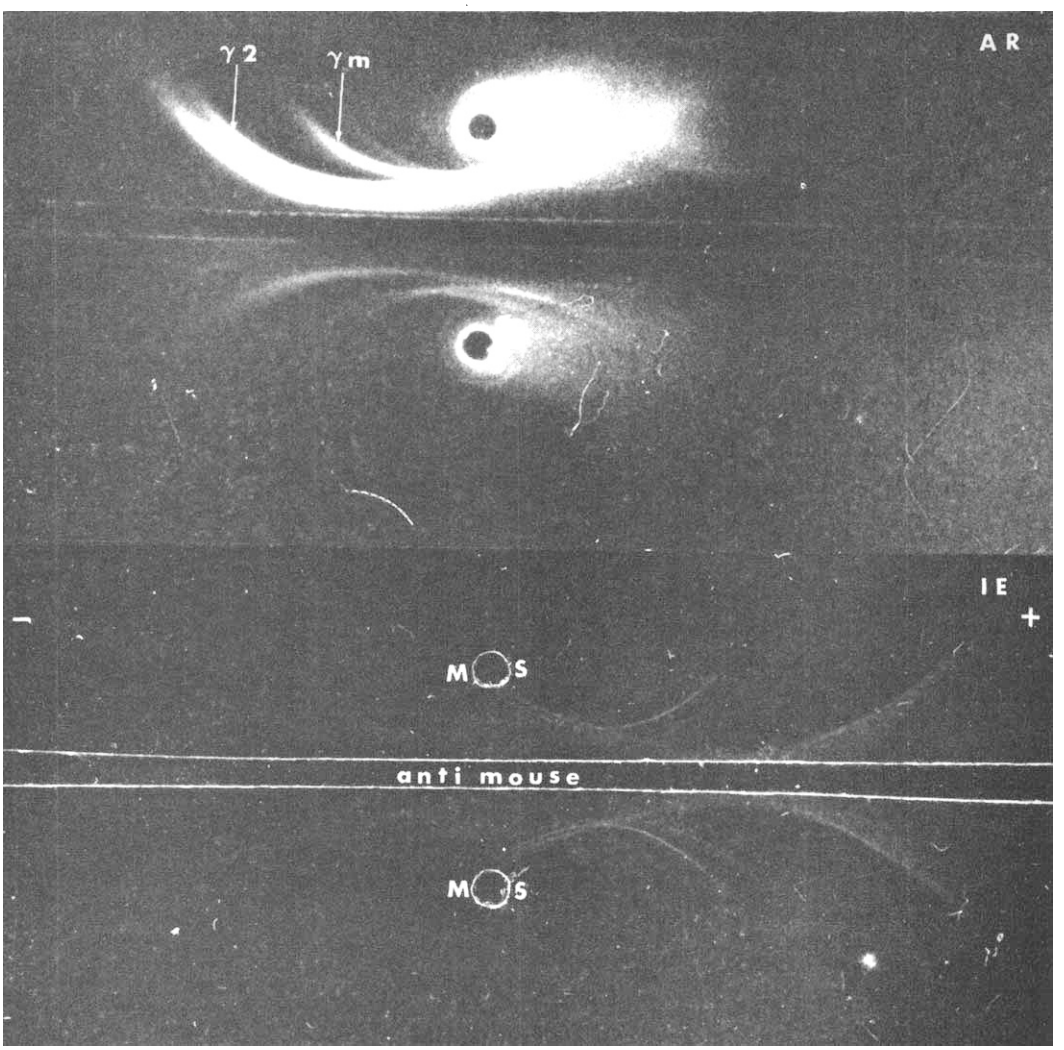

FIG. 1. Autoradiograph (AR) of immunoelectrophoretic (IE) patterns and IE pattern of culture fluids from sensitized and control lymph nodes 10 days after tumor grafting. The pattern was developed with mouse serum (MS) as a carrier and antimouse serum. The culture fluid from the sensitized nodes is in the top well. Labeling of IgG_2 (γ_2) and IgM (γ_m) is present.

S) of measurable antibody response may be due to such variables as species, type of antigenic stimulation, the dosage and schedule of CY in relation to antigenic stimulation as well as to the technique used to detect the antibody response.

Summary. Immune globulin synthesis by sensitized lymph nodes, obtained from mice treated with cyclophosphamide, following the application of subcutaneous grafts of sarcoma I was studied by the technique of radioimmunoelectrophoresis. There was complete suppression of labeling of *7S* γ_1 during the homograft reaction as well as almost complete suppression of immunoglobulin synthesis during the first 7 days after grafting. Labeling of *7S* γ_2 and IgM appeared on the 10th day after the application of the graft. These findings differed from those obtained with untreated tumor-bearing murine hosts.

1. Phillips, M. E., Lab. Invest. **19**, 539 (1968).

2. Coe, J. E., J. Immunol. **96**, 744 (1966).

3. Jones, J. W., Brody, G. L., O'Neal, R. M., and Haines, R. F., J. Surg. Res. **3**, 189 (1963).

4. Berenbaum, M. C., Nature **200**, 84 (1963).

5. Santos, G. W. and Owens, A. H., Jr., Bull. Johns Hopkins Hosp. **114**, 384 (1964).

6. Santos, G. W. and Owens, A. H., Jr., Bull. Johns Hopkins Hosp. **116**, 327 (1965).

7. Santos, G. W. and Owens, A. H., Jr., Nature **209**, 622 (1966).

8. Ùy, Q. L., Srinivasan, T., Santos, G. W., and Owens, A. H., Jr., Exptl. Hematol. **10**, 4 (1966).

Received Sept. 5, 1969. P.S.E.B.M., 1970, Vol. 133.