The Antitumor Effect of Interferon in Lymphocyte- and Macrophage-Depressed Mice (37706)

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It seems likely that the antitumor effect of interferon observed in mice results in part from a direct inhibitory action of interferon on the multiplication of tumor cells (1–4). However, the finding that interferon treatment also increased the survival of DBA/2 mice inoculated with interferon-resistant L 1210 tumor cells (4) suggested that, under some conditions, inhibition of tumor growth was due to stimulation by interferon of host-mediated rejection mechanisms, since multiplication of these resistant cells was not inhibited by interferon. Since lymphocytes (5–12) and macrophages (13–20) are considered important in host rejection of transplantable tumors, it was of interest to determine whether the antitumor effect of interferon might be mediated by lymphocytes or macrophages. We present herein the results of experiments on the efficacy of interferon treatment in tumor inoculated mice pretreated either with antilymphocytic sera (ALS) or X-irradiation to depress lymphocytes (21–28), or with silica to depress macrophages (29–32).

Materials and Methods. Mice. Balb/c and DBA/2 mice were obtained from the breeding colonies of the Institut de Recherches Scientifiques sur le Cancer.

Tumor cells. The origin of lymphoid leukemia L 1210 cells and the Ehrlich ascites (EA) cells has been previously described, as well as the techniques used in determining the number of cells inoculated (2). The origin and characteristics of a subline of interferon-resistant cells have also been previously described (33), but may be summarized here as follows: cultivation of L 1210 cells in the presence of interferon resulted in the selection of a subline of L 1210 cells resistant to the inhibitory effect of interferon on cell division (33). These cells were also resistant to the antiviral effect of interferon. The character of resistance to interferon did not change after passage of these cells in the mouse (4). Interferon-resistant L 1210 cells have the same generation time in suspension cultures as the parental interferon-sensitive L 1210 cells, the same karyotype, the same colony-forming efficiency in agarose, and the same tumorigenicity for DBA/2 mice.

Interferon. The preparation and assay of concentrated mouse brain interferon and control mouse brain extract have been described in detail elsewhere (1, 2, 33). One unit of interferon, as expressed in the text, corresponds to 4 mouse reference units.

Pretreatment of mice with ALS, X-ray or silica. Rabbit anti-mouse lymphocytic serum (purchased from Microbiological Associates) titered 1:5,120, as tested in our laboratory by complement-dependent lysis of C57Bl/6 thymocytes labeled with radioactive chrome; it decreased the number of circulating lymphocytes and prolonged the survival of allogeneic skin grafts. (Mean survival of C57Bl/6 skin grafts on untreated DBA/2 mice was 16 days and $>$ 34 days in ALS-treated DBA/2 mice.) X-irradiated mice received 500 rad total body irradiation from a cobalt source. Silica was kindly given by Dr. A. C. Allison. It was Dörentrup quartz No. 12, $<$ 5 μm, provided through the generosity of Dr. K. Röbock (Steinkohlenbergbauverein, Federal Republic of Germany). Peritoneal cells harvested from mice inoculated ip with 25 mg silica per mouse failed to adhere to glass or plastic, whereas peritoneal cells from uninoculated mice adhered and spread on

glass or plastic.

Tumor systems. (i) DBA/2 mice were inoculated ip with cells of cloned lines of either interferon-sensitive (S) or interferon-resistant (R) L 1210 cells. The efficacy of interferon treatment was determined by comparing the mean survival times of tumor-inoculated mice (2).

(ii) Balb/c mice were inoculated ip with Ehrlich ascites (EA) cells, and the efficacy of interferon treatment was determined by quantitative estimates of the number of EA cells recovered from the peritoneal cavity (2).

Results. Effect of pretreatment of DBA/2 mice with ALS or silica on the efficacy of interferon in increasing survival after inoculation of L 1210 S or L 1210 R cells. ALS. Pretreatment with ALS decreased the survival time of DBA/2 mice inoculated with L 1210 S or L 1210 R cells compared to tumor-inoculated mice pretreated with normal rabbit serum or not pretreated (Table I, experiments 1 and 3). Thus, the mean survival time of ALS-pretreated tumor-inoculated mice was 23 and 21 days (experiments 1 and 3) compared to 29 and 26 days for mice not receiving ALS ($p < 0.001$).

In experiment 1 (Table I) DBA/2 mice were inoculated with L 1210 S cells, and in experiment 3 mice were inoculated with L 1210 R cells. In both experiments, interferon treatment was associated with an increased survival time compared to untreated tumor-inoculated mice. Furthermore, the antitumor effect of interferon was not affected by pretreatment of mice with ALS. (Thus, no significant difference was noted when the difference

TABLE I. Effect of Antilymphocytic Serum or Silica on the Efficacy of Interferon in the Treatment of DBA/2 Mice Inoculated with Interferon-Sensitive L 1210 S or Interferon-Resistant L 1210 R Cells.

Expt no.[a]	Cells inoculated (no. cells/mouse)	Pretreatment	Treatment	No. of mice surviving >90 days / No. of mice injected	Mean harmonic survival (days)	Confidence interval (0.95)
1	L 1210 S (3.3×10^3 cells)	Normal serum	None	0/15	29]*	27–30
			Interferon	5/15	67]*	42–104
		ALS	None	0/15	23]*	22–25
			Interferon	3/15	56]*	44–77
2	L 1210 S (1×10^3 cells)	None	None	0/14	24]*	22–25
			Interferon	0/15	38]*	35–40
		Silica	None	0/15	22]*	21–23
			Interferon	0/12	29]*	25–32
3	L 1210 R (6×10^3 cells)	None	None	0/16	26]*	24–28
			Interferon	0/16	40]*	37–44
		ALS	None	0/15	21]*	20–23
			Interferon	0/15	33]*	31–35
		Silica	None	0/16	28]*	26–30
			Interferon	0/15	36]*	32–40

* $p < 0.001$.

[a] In all experiments tumor cells were inoculated ip on Day 0.

Experiment 1. Two-month-old female DBA/2 mice inoculated sc with 0.1 ml ALS on Day −1, Day +1, Day +2, and twice weekly for 2 weeks thereafter. Mouse brain interferon (32,000 units) inoculated ip on Day +1 and daily for 1 month.

Experiment 2. Six-week-old female DBA/2 mice inoculated ip with 25 mg silica on Day −1. Mouse brain interferon (8,000 units) inoculated ip on Day +1 and daily for 1 month.

Experiment 3. Two-month-old female DBA/2 mice inoculated with ALS and silica as described for experiments 1 and 2. Mouse brain interferon (16,000 units) inoculated ip on Day +1 and daily for 1 month.

between control and interferon-treated mice not receiving ALS was compared to the difference between the control and interferon treated mice pretreated with ALS, experiments 1 and 3).

Silica. In contrast to the effect of ALS in decreasing the survival time of L 1210 inoculated mice, intraperitoneal inoculation of silica was not associated with a significant reduction in survival time of tumor-inoculated DBA/2 mice (experiments 2 and 3, Table I).

Interferon treatment was associated with an increased survival in DBA/2 mice preinjected with silica and inoculated with either L 1210 S or L 1210 R cells. However, the efficacy of interferon in silica-treated mice was less than in interferon-treated mice not receiving silica. ($p < 0.01$ in experiment 2 and $p < 0.05$ in experiment 3, when the difference between control and interferon-treated mice was compared to the difference between control silica-pretreated mice and interferon-treated silica-pretreated mice).

Effect of pretreatment of Balb/c mice with ALS, X-ray, or silica on the efficacy of interferon in inhibiting the multiplication of Ehrlich Ascites cells. ALS or X-ray. Pretreatment of Balb/c mice with ALS or X-ray did not appear to modify the multiplication of EA cells (open symbols, Fig. 1). Thus, 5 days after inoculation of 10^5 EA cells 4×10^6 to 10^8 cells were recovered from the peritoneum of each mouse. Daily interferon treatment (closed symbols, Fig. 1) proved as effective in inhibiting the multiplication of EA cells in ALS-pretreated or X-irradiated mice, as in control interferon-treated mice.

Silica. Interferon treatment was also associated with an inhibition of the multiplication of EA cells in mice pretreated with silica, although it appeared to be somewhat less effective than in the control interferon-treated mice not pretreated with silica.

Discussion. To determine whether the anti-

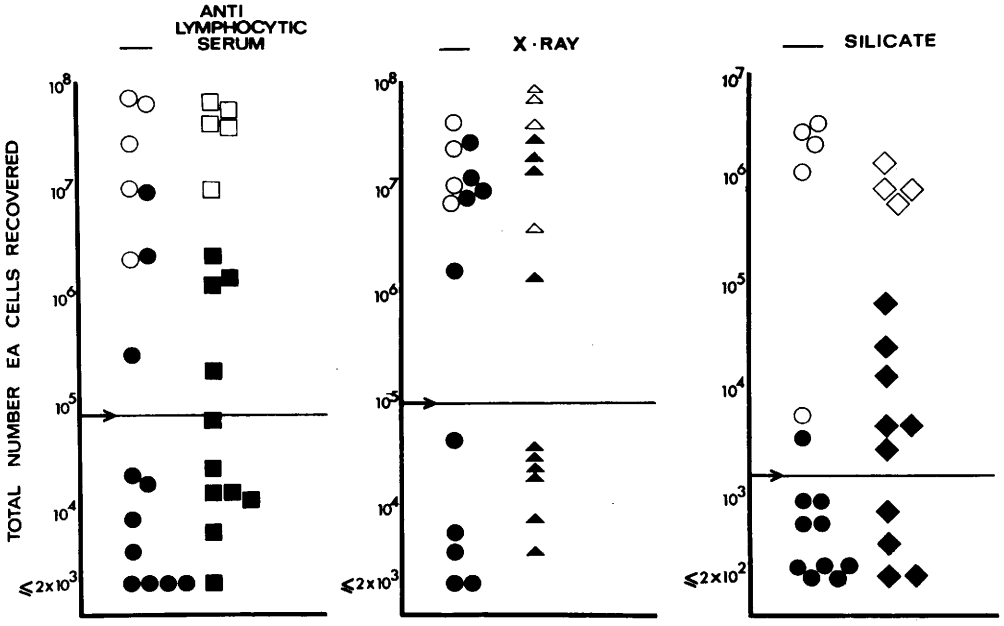

FIG. 1. ALS (0.25 ml) was given ip on Day −4, and sc Day −3, Day −2 and Day −1. *X-ray:* 500 rad given on Day 0; 25 mg *silica* inoculated ip on Day 0. The number of EA cells inoculated on Day 0 per mouse is indicated by the horizontal arrow. Mouse brain interferon (16,000–32,000 units) inoculated ip on Day +1, and daily thereafter for 4 days. On Day 5, mice were sacrificed, and the total number of EA cells recovered from the peritoneum of each mouse was determined by techniques previously described (2). Open figures (○, □, △, ◇) indicate *no* interferon treatment. Closed figures (●, ■, ▲, ◆) indicate interferon treatment.

tumor effect of interferon was mediated in part by lymphocytes or macrophages, we have attempted to depress the overall lymphocyte activity of the host by treatment with antilymphocytic sera (21–28) (or X-ray) and to depress peritoneal macrophage function by the inoculation of silica (29–32). The efficacy of interferon treatment was determined in 2 experimental systems: (a) intraperitoneal inoculation of DBA/2 mice with interferon-sensitive (S) or interferon-resistant (R) L 1210 cells and determination of the mean survival time of tumor inoculated mice; (b) intraperitoneal inoculation of Balb/c mice with Ehrlich ascites (EA) cells and quantitative estimation of the multiplication of tumor cells in the peritoneal cavity.

Although depression of lymphocyte function by ALS resulted in a decreased survival time of L 1210 S or L 1210 R tumor-inoculated mice, interferon treatment proved as effective in increasing survival in these mice as in mice not receiving ALS. Likewise, interferon proved as effective in inhibiting EA cell multiplication in lymphocyte-depressed mice as in the control interferon-treated mice. Thus, under the experimental conditions employed (*i.e.*, intraperitoneal inoculation of tumor cells and interferon), treatment of mice with ALS (or X-ray in the experiments with EA cells) did not modify the antitumor effect of interferon.

Interferon treatment also proved effective in tumor-inoculated mice injected with silica, although somewhat less effective than in interferon-treated tumor-inoculated mice not receiving silica. Thus, although the antitumor effect of interferon in mice inoculated intraperitoneally with tumor cells was not abolished by destruction of peritoneal macrophages, the results suggest that the overall antitumor effect was more pronounced when the function of peritoneal macrophages was unimpaired.

It seems likely that under some experimental conditions interferon can inhibit directly the multiplication of tumor cells, for example, when interferon is administered intraperitoneally in mice inoculated with Ehrlich ascites cells and the antitumor effect is determined after a few days of treatment. Analysis of the antitumor effect appears more complicated when the criterion is one of survival time. The finding that interferon preparations increased the survival of DBA/2 mice inoculated with interferon resistant L 1210 cells as reported previously (4) and repeated herein (see experiment 3, Table I) suggests that the antitumor effect in these mice did not result from a direct inhibitory effect on tumor cell multiplication, but was host mediated. However, our experimental results do not permit us to implicate either lymphocytes or macrophages as mediators of the antitumor effect of interferon. In this regard, it is of interest that the antitumor effect of the interferon-inducer polyinosinic–polycytidylic acid (poly I·poly C) (34–35) [which is known to enhance the immune response (36–38)] may also not be mediated by lymphocytes (39–40). Thus, immunosuppression of mice with X-ray or ALS did not eliminate the antitumor effect of poly I·poly C. (40). These considerations raise the possibility that the antitumor effect of interferon (and possibly poly I·poly C) is mediated in part by the host, but not necessarily by those cellular elements considered important in allograft rejection.

Summary. The antitumor effect of interferon was assayed in mice pretreated with ALS to depress lymphocytes or with silica to depress macrophages. Pretreated DBA/2 mice were inoculated intraperitoneally with interferon-sensitive or interferon-resistant L 1210 cells, and Balb/c mice were inoculated intraperitoneally with Ehrlich ascites cells. The efficacy of interferon was determined by its effect either on survival time in L 1210 tumor-inoculated mice, or on the multiplication of EA cells. Interferon proved as effective in ALS-pretreated tumor-inoculated mice as in mice not receiving ALS. Pretreatment of mice with silica diminished somewhat, but did not abolish the antitumor effect. It is concluded that interferon can exert an antitumor effect in lymphocyte- and macrophage-depressed mice.

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