INJECTION OF MICE WITH ANTIBODY TO INTERFERON ENHANCES THE GROWTH OF TRANSPLANTABLE MURINE TUMORS*

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Antibody to mouse interferon has proven useful in demonstrating the role of endogenous interferon in the pathogenesis of different viral diseases (1–7) and in determining to what extent some of the biologic effects of interferon inducers are mediated by endogenous interferon (8). Recently, it has been shown that injection of athymic nude mice with antibody to mouse interferon enhanced the growth of several xenogeneic transformed cells as well as human prostatic tumor cells (9) and hepatoma cells.[1] We show herein that antibody to mouse interferon markedly enhances the transplantability of a variety of murine tumors in immunocompetent mice. Furthermore, we have analyzed some of the experimental conditions relative to this phenomenon using two lines of Friend erythroleukemia cells (FLC),[2] one characterized as interferon-sensitive and the other as interferon-resistant (10, 11). The results suggest that there may be low levels of endogenous interferon in mice that contribute to host defense against transplantable tumors.

Materials and Methods

Mice. 5–8 wk-old male and female DBA/2, BALB/c and C57Bl/6 mice were obtained from the pathogen-free breeding colonies of the Institut de Recherches Scientifiques sur le Cancer (Villejuif).

Tumor Cells. The following tumor cells were used:

Cell line	Characteristics	Received from	References
FLC-745	Friend virus erythroleukemia; interferon-sensitive line	Dr. E. Affabris Rome, Italy	(10, 11)
FLC-3Cl-8	Friend virus erythroleukemia; interferon-resistant line	Dr. E. Affabris Rome, Italy	(10, 11)

* Supported by grants from the Richard Lounsbery Foundation and from the Institut National de la Santé et de la Recherche Médicale (CRL 81-20-08) and Centre National de la Recherche Scientifique (ATP ETATS-UNIS).

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[1] Shouval, D., B. Rager-Zisman, P. C. Quan, B. R. Bloom, D. A. Shafritz, and L. M. Reid. 1983. Restriction of tumorigenicity in nude mice of a human hepatoma cell line persistently infected with hepatitis B virus. Submitted for publication.

[2] *Abbreviations used in this paper:* BSA, bovine serum albumin; FCS, fetal calf serum; FLC, Friend erythroleukemia cells; FLV, Friend leukemia virus; Ig, immunoglobulin; i.p., intraperitoneal; NK, natural killer; PBS, phosphate-buffered saline; s.c., subcutaneous; SE, standard error.

Cell line	Characteristics	Received from	References
L1210R	Methylcholanthrene-induced tumor cell line; interferon-resistant	Our laboratory	(12, 13)
RBL5	Rauscher virus lymphoma line	Dr. J.-P. Lévy, Paris France	
EL4	7,12-Dimethylbenz(a)anthracene-induced tumor cell line	Our laboratory	(12)
J558	Myeloma IgA, λ1 cell line	Dr. M. Stanislawsky IRSC, Villejuif	
MOPC 104	Myeloma IgM, λ1 cell line	Dr. M. Stanislawsky IRSC, Villejuif	
CGV	Gross virus tumor cell line	Dr. J.-P. Lévy Paris, France	
NS 1	Myeloma cell line	Dr. J.-P. Lévy Paris, France	
HFL/B	Friend virus erythroleukemia line	Dr. J.-P. Lévy Paris, France	
Ehrlich ascites	Spontaneous	Our laboratory	(12)

Tumor cells were either passaged in culture in RPMI medium (Flow, Irvine, Scotland) supplemented with 10% fetal calf serum (FCS), or by weekly intraperitoneal (i.p.) injections in syngeneic mice. The different mouse strains are indicated in Table I.

Quantitative Estimation of the Number of FLC in the Peritoneal Cavity by Colony Formation in Agarose. Mice were sacrificed and the peritoneal cavity was washed with 3 ml of RPMI medium containing 10% FCS. The total number of viable cells recovered from each mouse was determined by trypan blue dye exclusion. The techniques of preparation of agarose and plating of cells have been described (10). After 4 d of incubation at 37°C (in a humidified air-5% CO_2 mixture) colonies of FLC were counted using an inverted light microscope. The total number of colonies per dish was calculated as previously described (14). Cells recovered from the peritoneal cavity of normal mice do not form colonies in agarose (14).

Hyperimmune and Normal Serum Globulins. The source and activities of the different Ig preparations were as follows:

Description	Source	Neutralizing titer against 4–8 U of mouse α/β interferon	Reference
Anti-mouse interferon globulins:			
Sheep no. 1–7	Our laboratory	1.6×10^6	(2)
Sheep no. 5A	Our laboratory	2.5×10^5	(2)
Sheep (NIH)	Research Resources Branch, NIH, Bethesda, MD	1.6×10^5	Catalog no. G-024-501-568
Goat D.M.	Dr. E. De Maeyer, Orsay, France	6.4×10^4	(15)
Rabbit serum	Our laboratory	3.2×10^3	
Control hyperimmune globulins:			
Sheep no. 11 anti-impurities	Our laboratory	<10	(2)
Rabbit-anti-L1210 cells	Our laboratory	<10	
Normal serum globulins:			
Sheep no. 2	Our laboratory	<10	(2)
Sheep (NIH)	Research Resources Branch, NIH, Bethesda, MD	<10	Catalog no. G-025-501-568
Goat	Dr. E. De Maeyer, Orsay, France	<10	(15)

TABLE I

Effect of Anti-mouse Interferon Globulin on the Survival of Mice Injected with Different Murine Tumors

Tumor cell, Conditions of passage	Strain of mouse, No. of cells injected (i.p./mouse)	Treatment				
		Anti-mouse interferon globulin	Anti-impurities globulin	Normal serum globulin	None	p
	DBA/2					
FLC 745,	10^5	8/8*	8/8		8/8	
In vivo		22.1‡	24.5		25.7	§
		(21.3–23.0)	(23.9–25.1)		(25.3–26.1)	
	10^2	10/10		10/10		
		27.4		33.2		¶
		(26.9–27.9)		(32.6–33.8)		
	10^1	10/10		6/10		
		31.0		53.5		¶
		(29.8–32.3)		(46.5–62.9)		
	DBA/2					
FLC 745,	10^5	6/6	3/5		4/6	
In vitro		25.6	47.6		50.1	¶
		(24.4–26.9)	(40.6–57.6)		(45.0–56.5)	
FLC-3CL-8,	10^5	8/8			6/8	
In vitro		21.3			74.6	¶
		(20.5–22.2)			(61.7–90.9)	
	C57BL/6					
RBL 5,	10^6	9/10		2/10		
In vitro		25.5		89.3		¶
		(23.0–28.5)		(77.2–106.0)		
	10^5	5/9		0/9		
		41.8		—		‖
		(34.7–52.6)				
	DBA/2					
L1210R,	10^5	10/10		10/10	10/10	
In vitro		21.5		26.0	27.0	¶
		(20.7–22.3)		(25.1–27.0)	(25.5–28.7)	
	10^2	9/9		9/9	8/9	
		26.1		30.8	32.2	‖
		(25.6–26.7)		(29.7–31.9)	(30.1–34.7)	
	BALB/c					
MOPC 104,	10^5	9/9			3/9	
In vivo		31.8			72.3	¶
		(30.9–32.7)			(62.5–85.8)	
	BALB/c					
J 558,	2×10^3	11/12			8/12	
In vivo		28.0			41.6	‖
		(26.2–30.1)			(36.8–47.8)	
	C57BL/6					
EL 4,	10^3	12/12		12/12	12/12	
In vivo		23.3		26.0	28.7	§
		(22.5–24.2)		(24.9–27.1)	(26.5–30.9)	

In these experiments 5–8-wk old male or female mice were used. Mice were injected i.p. with 0.2 ml of 1:10 dilutions of sheep no. 1–7 anti-mouse interferon globulin; sheep no. 11 anti-impurities globulin; sheep no. 2 normal serum globulin; or left untreated. Mice were injected −2, 0, +7, and +14 days (tumor cells were inoculated on day 0). Experiments usually were terminated between 3 and 4 months, at which time surviving mice were autopsied. Significant differences in these experiments were observed only between the group of mice injected with anti-interferon globulin (underlined) and the control groups. The p values refer to this difference. No significant difference was observed between the control groups.

* Number of mice dead with tumor/Total no. of mice inoculated.

‡ Harmonic mean day of death ± SE.

§ $p < 0.05$.

‖ $p < 0.01$.

¶ $p < 0.001$.

All sera were decomplemented and extensively absorbed on C-243 cells and on Swiss mouse thymocytes and spleen cells as previously described (2). For some experiments sera were further absorbed on DBA/2 mouse spleen cells and on a pool of FLC taken from mice with ascites. The Ig fractions were separated by ammonium sulfate precipitation (protein content varied between 20 and 33 mg/ml) and were shown to be devoid of any cytotoxicity (2). The anti-mouse interferon α/β globulins did not neutralize interferon γ. A sheep was immunized against the contaminating proteins in the partially purified interferon preparations. This serum globulin is referred to as "anti-impurities" (2). The hyperimmune rabbit sera were absorbed on murine cells but the Ig fractions were not prepared.

Determination of Interferon in Various Tissues; and Spleen NK Cell Activity. The techniques used to determine the presence of interferon in various tissues have been described (2) as well as the techniques used to determine NK cell activity using YAC target cells (16).

Statistical Analyses. All data were analyzed by Student's *t*-test (comparison of harmonic mean and slopes of regression lines).

Results

Injection of Mice with Anti-interferon Globulins Enhances the Transplantability of Several Murine Tumors and Shortens the Survival Time of Tumor-inoculated Mice

The results illustrated in Table I may be summarized as follows:

Injection of mice with anti-interferon globulin enhanced the transplantability of murine tumor cells injected i.p. (compared with mice injected with anti-impurities globulin, normal serum globulin or left untreated) as manifested by a marked decrease in survival time and in some instances an increased percentage of tumor-bearing mice. (*a*) The effect was observed using 6 murine tumors (8 cell lines) of different origin and in 3 strains of mice. (*b*) The effect was observed in mice injected with a large or small number of tumor cells. (*c*) The effect was observed in mice injected with tumor cells that had been passaged in vitro or in vivo, although the difference was much more pronounced when in vitro passaged cells were injected.

In some tumor systems, however, injection of mice with anti-interferon globulin did not affect tumor transplantability under the experimental conditions used (data not shown). Thus, it failed to affect: (*a*) the transplantability of in vitro passaged 745 FLC injected into allogeneic C57Bl/6 mice or BALB/c mice (which like DBA/2 mice are H-2^d), (*b*) the growth of three tumor cell lines (CVG, NSI, and HFL/B) that had been passaged in vitro and were of very low tumorigenicity in syngeneic mice, or (*c*) the growth of a highly tumorigenic in vivo passaged Ehrlich ascites cell line.

Analysis of Various Experimental Conditions Relative to the Effect of Anti-mouse Interferon Globulin in DBA/2 Mice Injected i.p. With FLC

Both 745 and 3Cl-8 FLC have been transplanted in mice and can then be passaged by further i.p. inoculation. Tumor cells multiply rapidly and form solid tumor masses in the mesentery and induce a tumor ascites (14). After several i.p. passages, 1–10 FLC constitute 1 LD_{50} (14). In contrast, in vitro passaged FLC are considerably less tumorigenic when first inoculated i.p. than in vivo passaged FLC. Thus, when 10^5 to 10^6 in vitro cultivated FLC were injected i.p., few mice developed an intra-abdominal tumor, although approximately half of the mice developed a subcutaneous tumor (along the needle track).

However, mice injected with anti-interferon globulin and inoculated with in vitro cultivated FLC developed large intra-abdominal tumor masses with ascites similar to those seen in DBA/2 mice injected with in vivo passaged FLC. Furthermore, anti-interferon globulin–treated mice injected with 10^5 in vitro passaged FLC died at approximately the same time as control mice injected with 10^5 in vivo passaged cells. It should be stressed that the effect of anti-interferon globulin on the transplantability of in vitro passaged FLC was the same for both interferon-sensitive (745) and interferon-resistant (3Cl8) FLC (Table I).

The effect of anti-interferon globulin in altering the usual behavior of in vitro passaged FLC injected i.p. was so pronounced that we undertook a series of experiments using these cells to define some of the experimental conditions and to determine whether antibody to mouse interferon was indeed the responsible factor.

Injection of Various Numbers of FLC: Constant Amount of Anti-interferon Globulin. Table II shows that anti-interferon globulin shortened the survival time of mice injected with various numbers of in vitro passaged 745 FLC compared with mice injected with anti-impurities globulin or left untreated. The comparison of the slopes of the regression lines between mice injected with anti-interferon globulin and anti-impurities globulin was highly significant ($t_{54} = 9.459$, $p < 0.001$).

Injection of Various Dilutions of Anti-Interferon Globulin: Constant Number of FLC. Fig. 1 shows the relationship between the amount of anti-interferon globulin injected and the survival time of DBA/2 mice injected with in vitro passaged 3Cl-8 FLC. The dose-response regression line was highly significant: $p < 0.001$ (coefficient of $r_{50} = -0.852$; $t = -11.54$). Moreover, 18/18 mice injected with $10^{5.7}$ or $10^{4.7}$ interferon neutralizing units of antibody died (all 18 had intra-abdominal tumors) compared with 4/25 mice injected with $10^{1.7}$ or $10^{0.7}$ neutralizing units or PBS with BSA (3 had subcutaneous (s.c.) tumors only and 1 had

TABLE II

Effect of Anti-interferon Globulin on Mice Injected with Various Numbers of 745 FLC

Treatment	No. of FLC injected i.p./mouse			
	10^5	10^4	10^3	10^2
Anti-interferon globulin	6/7*	6/7	5/7	6/7
	33.9‡	45.1	59.8	60.3
	(29.4–40.0)	(39.3–52.9)	(50.3–73.7)	(54.7–67.2)
Anti-impurities globulin	5/7	6/7	4/7	2/7
	63.7	70.6	86.9	85.1
	(56.4–73.2)	(64.1–78.6)	(75.7–102.1)	(71.9–104.1)
None	5/7	NT	NT	NT
	69.9			
	(61.5–80.8)			

5-wk old female DBA/2 mice were injected with 0.2 ml of a 1:10 dilution of anti-interferon globulin (sheep 1–7) or anti-impurities globulin −2, 0, +7, +14 days (tumor cells were injected on day 0). The experiment was terminated at 118 d.

* $\frac{\text{No. of mice dead with tumor}}{\text{Total no. of mice inoculated}}$.

‡ Harmonic mean day of death ± SE ( ).

NT, not tested.

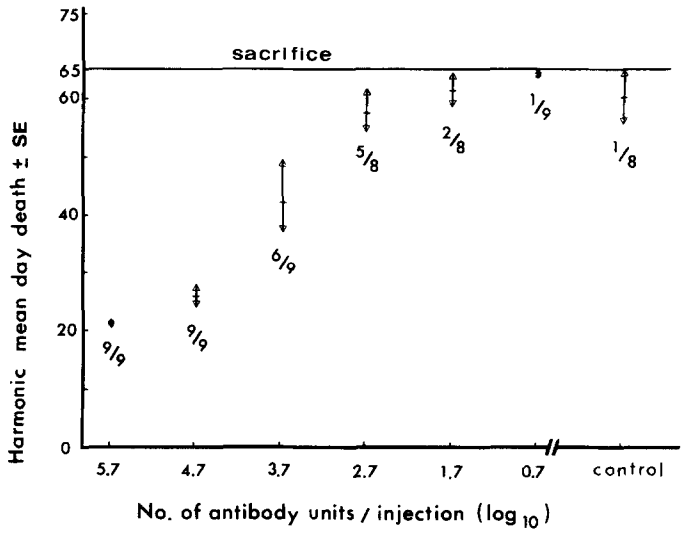

FIGURE 1. Effect of injection of varying amounts of anti-interferon globulin on the survival of DBA/2 mice injected i.p. with 3Cl-8 FLC. 5-wk-old male DBA/2 mice were injected i.p. with 10^5 3Cl-8 cells on day 0. They received injections of 0.2 ml of anti-interferon globulin (sheep 1–7) i.p. on days −2, 0, +7, +14. Anti-interferon globulin was diluted in PBS containing crystalline (RIA grade) BSA (Sigma) 50 μg/ml. The control group was injected on the same days with the same concentration of BSA in PBS. Numbers indicate the number of mice dead with tumor/total no. of mice inoculated. Arrows indicate SE.

TABLE III

Effect on Survival of the Time of Injection of Anti-interferon Globulin Relative to the Time of Tumor Cell Injection

Treatment	No. of mice dead with tumor / Total no. of mice inoculated	Harmonic mean day of death ± SE	
None	5/12	62.5 (58.8–66.7)	¶
Anti-interferon (−2, 0, +7, +14 days)	12/12	24.4 (23.8–25.0)	NS ¶
Anti-interferon (−2 days only)	11/12	27.8 (25.6–30.3)	
Anti-interferon (+7, +14 days)	12/12	40.0 (37.0–43.5)	

5–6-wk old male DBA/2 mice were injected i.p. on day 0 with 10^5 745 cells cultivated in vitro. 0.2 ml of a 1:10 dilution of anti-interferon globulin (sheep 1–7) was injected on the days as indicated. The experiment was terminated at 74 d.

¶ $p < 0.001$.

NS, not significant.

an intra-abdominal tumor). A significant difference in the survival time was still obtained in mice injected with $10^{3.7}$ interferon neutralizing units, compared with control tumor-inoculated mice.

Time of Injection of Anti-interferon Globulin Relative to the Time of Tumor Cell Injection. In the preceding experiments, anti-interferon globulin was injected at −2, 0, +7, and +14 days (tumor cells injected on day 0). As can be seen in Table III, the effect on survival was most marked when antibody was inoculated i.p.

TABLE IV

Effect of Different Preparations of Antibody to Interferon or Control Preparations on the Survival of DBA/2 Mice Inoculated i.p. With FLC

Experiment no.	FLC line injected	Treatment	No. mice dead with tumor / Total no. mice inoculated	Harmonic mean day of death ± SE	
1	745	Anti-interferon globulin (sheep no. 5)	9/9	27.1 (25.9–28.4)	¶
		Normal serum globulin (sheep no. 2)	5/9	70.6 (62.6–80.8)	
		None	5/8	64.9 (57.5–74.5)	NS
2	3Cl-8	Anti-interferon globulin (sheep no. 1–7)	12/12	28.4 (27.1–29.9)	NS
		Anti-interferon globulin (goat DM)	11/11	26.3 (25.7–27.0)	¶
		Normal serum globulin (goat DM)	10/12	57.7 (55.5–60.0)	NS
		None	9/12	50.8 (46.7–55.7)	
3	745	Anti-interferon globulin (sheep no. 1–7)	4/6	33.8 (30.1–38.6)	NS
		Anti-interferon globulin (sheep NIH)	0/8	34.5 (31.1–38.8)	¶
		Normal serum globulin (sheep NIH)	5/8	>64	NS
		None	0/8	>64	
4	3Cl-8	Rabbit anti-interferon serum	10/10	22.7 (21.7–23.7)	¶
		Rabbit anti-L1210 cell serum	5/8	42.6 (37.7–48.8)	NS
		None	6/8	45.0 (41.7–49.0)	

5-wk old male or female DBA/2 mice were injected i.p. with 0.5 ml containing 10^5 in vitro passaged FLC. Mice were injected with 0.2 ml of the various preparations on days −2, 0, +7, and +14 (tumor cells were inoculated on day 0).

In experiment 1 the globulins were diluted 1:5.

In experiment 2 the sheep 1–7 and goat globulins were diluted 1:25 and 1:2, respectively.

In experiment 3, sheep 1–7 and NIH globulins were diluted 1:8 and 1:3, respectively.

In experiment 4, the rabbit sera were injected undiluted.

¶ $p < 0.001$.

before injection of 745 FLC, but a significant effect was still observed when mice were first inoculated with antibody 7 and 14 d after tumor cell injection.

Specificity of the Effect of Anti-mouse Interferon Globulin. In the experiments described above only antibody to mouse interferon enhanced the transplantability of in vitro passaged FLC. No effect had been observed in mice injected with anti-impurities globulin or normal sheep serum globulin (Tables I and II). It was considered important to determine the effect of other anti-interferon and normal serum globulins on the survival time of DBA/2 mice injected i.p. with FLC. As shown in Table IV, inoculation of anti-interferon globulins from two other sheep (No. 5 and NIH), one goat (DM), and anti-serum from one rabbit all increased

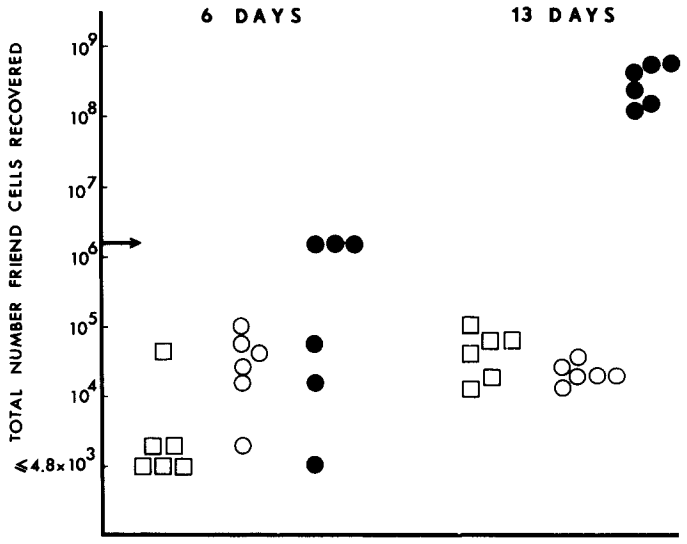

FIGURE 2. Kinetics of growth of 3Cl-8 FLC tumors in the peritoneum of mice injected with anti-interferon globulin. 5-wk-old DBA/2 mice were injected with 0.2 ml of a 1:6 dilution of anti-interferon globulin (●) or anti-impurities globulin (○) at −1, 0, and +7 days or left untreated (□). On day 0 mice were injected i.p. with 2×10^6 FLC as indicated by arrow. At 6 and 13 days, 6 mice in each group were sacrificed, and the total number of FLC recovered from the peritoneum of each mouse was determined by colony formation in agarose.

the percentage of tumor-bearing mice and decreased the survival time in mice injected with in vitro passaged FLC. In contrast, none of the control preparations exerted a similar effect, and there was no difference in survival time or incidence of transplantability of FLC between mice injected with control preparations and untreated mice.

Growth of FLC Tumors in the Peritoneum of Mice Injected with Anti-interferon Globulin. In the experiments described above injection of anti-interferon globulin increased the percentage of mice dying with tumor and decreased the survival time. It was of interest to determine the kinetics of growth of FLC tumors in mice injected with anti-interferon globulin.

As can be seen in Fig. 2, 6 d after inoculation of 3CL-8 FLC, 3/6 mice injected with anti-interferon globulin had 2×10^6 FLC in the peritoneum and by 13 d, 6/6 mice in this group had 10^8 to 10^9 FLC in the peritoneum compared with 6 mice injected with anti-impurities globulin and 6 untreated mice who had only 10^4 to 10^5 FLC in their peritoneal cavities.

The livers and spleens of all mice were examined microscopically for tumor metastases. None were seen, although in 2/6 mice treated with anti-interferon globulin and sacrificed at 13 d, tumor cells extended from the surface of the liver into the parenchyma. This was not observed in control mice.

Effect of Splenectomy on the Action of Anti-interferon Globulin. FLC harbor Friend leukemia virus (FLV), but the FLV present in the 745 and 3Cl-8 cell lines is only weakly leukemogenic as determined by the development of splenomegaly in DBA/2 mice injected with cell-free extracts of 10^8 FLC (data not shown). Nevertheless, one might suggest on theoretical grounds that after injection of anti-interferon globulin, the FLV might become leukemogenic and the tumor

TABLE V

Effect of Anti-interferon Globulin on the Growth of FLC Tumors in Splenectomized Mice

Splenectomy	Treatment	No. of mice dead with tumor / Total no. of mice inoculated	Harmonic mean day of death ± SE	
NO	Anti-interferon globulin	7/8	27.0 (24.7–29.8)	¶
NO	Normal sheep globulin	0/8	>52	
YES	Anti-interferon globulin	8/8	22.4 (22.0–22.9)	¶
YES	Normal sheep globulin	5/8	35.2 (35.1–35.3)	

7-wk old male DBA/2 mice were splenectomized or left intact. 1. wk later, all mice were injected with 0.2 ml of a 1:6 dilution of anti-interferon globulin (sheep 1–7) or normal sheep (no. 2) serum globulin at −1, 0, +7, +14 days. They were injected i.p. on day 0 with 10^5 745 FLC cultivated in vitro. The experiment was terminated at 52 d. None of the surviving mice had any gross evidence of tumor.

¶ $p < 0.001$.

TABLE VI

Effect of Anti-interferon Globulin on the Growth of 745 FLC Tumors Implanted Subcutaneously

Treatment	Harmonic mean day of death ± SE	
Anti-interferon globulin	30.9 (29.5–32.5)	¶
Anti-impurities globulin	47.3 (43.4–51.9)	NS
None	46.5 (43.5–49.9)	

5-wk old female DBA/2 mice were injected s.c. in the dorsal area with 0.2 ml of sheep 1–7 anti-interferon globulin diluted 1:10 or anti-impurities globulin at −2, 0, +6, +13 days. On day 0, 10^5 745 FLC cultivated in vitro were injected s.c. at the site of globulin injections. There were 8 mice in each of the different groups. All mice in the different groups died.

NS, Not significant.

¶ $p < 0.001$.

cells observed in the peritoneum of anti-interferon globulin treated mice might be derived from FLV-transformed spleen cells. Thus, we determined the effect of anti-interferon globulin on the transplantability of in vitro passaged FLC in splenectomized DBA/2 mice.

As can be seen from Table V, splenectomy did not abrogate the effect of anti-interferon globulin on the survival time of mice injected i.p. with in vitro passaged 745 FLC, even though splenectomy decreased the survival time of control mice injected with 745 FLC.

Effect of Anti-interferon Globulin on the Growth of FLC Tumors Implanted s.c.

Mice were injected s.c. with in vitro passaged 745 FLC and also injected at the same time with either anti-interferon globulin or anti-impurities, or left untreated. As can be seen from Table VI, a significant difference in the survival time was observed between mice injected with anti-interferon globulin and the two other groups when 10^5 FLC were injected.

Attempts to Demonstrate the Presence of Interferon in DBA/2 Mice Before or After Injection of FLC

The presence of interferon could not be demonstrated in peritoneal washings, splenic extracts, or sera of normal DBA/2 mice. In several experiments DBA/2 were injected i.p. with varying numbers of 745 or 3Cl-8 FLC passaged in vitro or in vivo, and peritoneal washings, splenic extracts, and sera tested at 2, 6, 24, 48, 72, 96 hours, 1 and 2 weeks thereafter. Interferon was not detected in any of the samples. As injection of interferon enhances splenic NK cell cytotoxicity (16), we injected varying numbers of FLC i.p. and determined splenic NK cell cytotoxicity at intervals thereafter. Injection of FLC i.p. did not increase splenic NK cell cytotoxicity.

Cultivation of FLC in the Presence of Anti-interferon Globulin Does Not Alter Their Transplantability

To determine whether anti-interferon globulin acted directly on the tumor cells, 745 FLC cultivated in vitro were passaged three times in the presence of a 1:100 dilution of sheep 1–7 anti-interferon globulin or anti-impurities globulin. DBA/2 mice were then injected i.p. with 10^5 cells from each group. There was no significant difference in the mean day of death (or the percentage of mice dying) between the two groups (data not shown).

Discussion

The results presented herein show that injection of male and female DBA/2, C57Bl/6, or BALB/c mice with antibody to mouse interferon enhanced the i.p. transplantability of six different murine tumors (eight tumor cell lines). This effect was manifested by a pronounced decrease in survival time and in some instances by an increase in the percentage of tumor-bearing mice. The enhancement in transplantability was most marked when tumor cells had been previously passaged in vitro and were of low tumorigenicity or when only a few in vivo passaged tumor cells were injected (Table I). Thus, in vitro cultured FLC induce subcutaneous tumors but do not readily form tumors in the peritoneum. However, in mice injected with anti-interferon globulin, in vitro passaged FLC grew rapidly i.p. and formed extensive intra-abdominal tumors similar to those observed in mice injected with in vivo passaged FLC.

Analysis of some of the experimental conditions using FLC, showed that the enhancing effect was observed over a wide range of tumor cell inocula (Table II); that the effect was directly related to the amount of antibody to interferon injected (Fig. 1); and was most pronounced when antibody was administered at the time of tumor cell injection (Table III). An enhancing effect was also observed when FLC were injected s.c. Antibody to interferon did not act directly on the tumor cells as cultivation of FLC for three passages in the continued presence of anti-interferon globulin or anti-impurities globulin did not affect their tumorigenicity.

This ensemble of results raises two main questions. Is antibody to mouse interferon responsible for the effects observed? And if so, how does it act?

Concerning the specificity, we have shown that hyperimmune anti-mouse interferon α/β globulins from three sheep and one goat (produced in three

laboratories) exerted an enhancing effect on the growth of in vitro passaged FLC injected i.p. The main control preparation consisted of the Ig from a sheep immunized with "impurities" present in the partially purified interferon. Neither this Ig nor normal sheep and goat serum globulins affected the growth of FLC. Crude rabbit anti-mouse interferon serum exerted an effect, whereas crude serum from a rabbit immunized against mouse lymphoma L1210 cells was ineffective. We conclude therefore, that antibody to mouse interferon is indeed responsible for the enhancement of transplantability of these tumors in mice.

We have previously shown that administration of interferon to mice results in the inhibition of growth of most of the tumor cells used in this study (12) including the interferon-sensitive and interferon-resistant L1210 cells (17) and FLC (14, 18, 19). These previous results suggested the possibility that the anti-tumor effect of interferon was at least in part host-mediated (17–20). Thus, if endogenous interferon inhibits the transplantability of in vitro cultivated FLC, as our experiments with anti-interferon globulin suggest, it is not surprising that the anti-interferon globulin was equally effective in mice inoculated with interferon-sensitive and interferon-resistant tumor cell lines (Table I).

The most likely explanation for the results observed would be that antibody against mouse interferon α/β increased transplantability of tumor cells because it neutralized endogenous mouse interferon that was either present *prior* to inoculation of tumor cells or was induced by inoculation of tumor cells. However, despite a variety of experimental protocols, we have been unable to demonstrate any biologically active interferon in the peritoneum, sera, or splenic extracts of mice before or after tumor cell inoculation. It is possible, nevertheless that biologically active interferon is present in very small amounts or that it is present in some combined form not detectable by our techniques. In previous work, interferon had also not been detected in the organs of polyoma virus–infected mice and yet, anti-interferon globulin markedly enhanced the disease (4). Likewise, Reid et al. (9), demonstrated the marked effects of sheep no. 1 anti-interferon globulin on the growth and metastases of xenogeneic tumor cells in nude mice but failed to detect interferon in the mice. The authors were, however, able to show that injection of tumor cells resulted in an increase in NK cell activity, which was presumed to be due to endogenous interferon as inoculation of mice with anti-interferon globulin abrogated the increased NK cell activity (9). However, we were unable to show any increase in NK cell activity in mice injected with FLC.

It is possible that the anti-interferon globulin acts because of its anti-interferon activity, but in some manner other than by neutralizing biologically active free interferon. For example, if interferon were bound to certain specialized cells, macrophages or lymphocytes, anti-interferon globulin might affect the function of these cells.

We failed to demonstrate an effect of anti-interferon globulin in some tumor systems. For example, anti-interferon globulin enhanced the transplantability of in vitro passaged FLC in syngeneic but not in allogeneic mice. In some instances, the tumor cells were of very low tumorigenicity even in syngeneic mice (CVG and NS1). Possibly, the low tumorigenicity was an inherent characteristic of these cells independent of the host response and therefore these cells might not be

affected by any interferon-induced effect. On the other hand Ehrlich ascites cells multiply rapidly in several mouse strains and these cells may be uninfluenced even by interferon-enhanced host defense mechanisms.

The point we should like to stress from these studies and those of Reid et al. (9) and Shouval et al.[1] is that endogenous interferon appears to be an important factor in inhibiting the transplantability in mice of some tumors, regardless of their origin. These results raise the possibility that endogenous interferon may play some role in controlling the growth and possibly the metastases of autochthonous tumors in man.

Summary

Injection of DBA/2, C57Bl/6, or BALB/c mice with antibody to mouse interferon α/β enhanced the i.p. transplantability of six different murine tumors, as manifested by an increase in the percentage of tumor-bearing mice and a decrease in survival time. The effect was observed in mice injected with antibody to interferon raised in three sheep, a goat, and a rabbit, but not with sheep antibody to "impurities" present in the mouse interferon preparations or with normal sheep or goat globulins. The enhancement in transplantability was most marked when tumor cells had been previously passaged in vitro and were of low tumorigenicity. Analysis of some of the experimental conditions using interferon-sensitive and interferon-resistant lines of Friend erythroleukemia cells (FLC) showed that the enhancing effect was observed over a wide range of tumor cell inocula, was directly related to the amount of antibody to interferon injected and was most pronounced when antibody was administered at the time of tumor cell injection. Enhancement was also observed when FLC were injected subcutaneously (s.c.). Antibody did not act directly on the tumor cells in vitro. Although we were unable to demonstrate any biologically active interferon in mice before or after tumor cell inoculation, the results suggest that endogenous interferon is present and plays a role in inhibiting the transplantability of some murine tumors in immunocompetent mice.

We are indebted to Dr. E. De Maeyer (Orsay, France) and Dr. M. W. Myers of the National Institute of Allergy and Infectious Diseases (NIH) Bethesda, MD, for gifts of goat and sheep anti-mouse interferon serum and globulin, respectively, and to Drs. S. Gisselbrecht and J.-P. Lévy for four tumor cell lines.

Received for publication 18 July 1983 and in revised form 30 August 1983.

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