ANTIBODY TO MOUSE INTERFERON α/β ABROGATES RESISTANCE TO THE MULTIPLICATION OF FRIEND ERYTHROLEUKEMIA CELLS IN THE LIVERS OF ALLOGENEIC MICE

By I. GRESSER,* C. MAURY,* F. VIGNAUX,* O. HALLER,‡
F. BELARDELLI,§ and M. G. TOVEY*

*From the *Laboratory of Viral Oncology, Groupe de Laboratoires de l'Institut de Recherches
Scientifiques sur le Cancer, BP 8, 94802 Villejuif, France; the ‡Institut für
Immunologie und Virologie der Universität Zürich, Zürich, Switzerland; and the
§Istituto Superiore di Sanità, 00161 Rome, Italy*

We have previously noted that when Friend erythroleukemia cells (FLC)[1] (H-2^d) passaged intraperitoneally in DBA/2 mice were injected subcutaneously or intraperitoneally into adult allogeneic immunocompetent mice, they multiplied rapidly in the first few days before their further growth was abruptly arrested. In contrast, FLC injected intravenously into allogeneic adult C57Bl/6 mice, implanted in the liver and spleen, but did not multiply to any significant degree; whereas in syngeneic DBA/2 mice, they multiplied rapidly and preferentially in these organs and killed all the mice between 7 and 12 d (1). These observations suggested that when FLC were injected subcutaneously or intraperitoneally the growing tumors were rejected after several days in allogeneic mice, whereas when the same cells were injected intravenously and lodged in the liver and spleen they simply could not multiply in these organs in adult allogeneic mice. It seems plausible that the mechanisms underlying the host response to these tumor cells in these different tissues are different.

Our previous work has suggested that IFN-α/β may constitute an important host defense against the growth of transplantable tumors: (a) treatment of athymic nude mice with anti–mouse IFN-α/β globulin markedly enhanced the subcutaneous growth and metastatic capacity of several xenogeneic cell lines (2); (b) in adult immunocompetent mice, administration of anti–mouse IFN-α/β globulin enhanced the growth of a variety of transplantable murine tumors injected subcutaneously or intraperitoneally in syngeneic mice but did not abrogate the capacity of allogeneic mice to reject the growing tumors (3).

In the studies reported herein, we show that administration of antibodies to IFN-α/β completely abrogates the resistance of adult C57Bl/6 (H-2^b) and C3H (H-2^k)

[1] *Abbreviations used in this paper:* FLC, Friend erythroleukemia cells; VSV, vesicular stomatitis virus.

mice to the rapid multiplication of FLC in the liver. Our results suggest that the endogenous IFN-α/β induced by intravenous injection of FLC is a crucial component in the resistance of the liver to the multiplication of these allogeneic tumor cells.

Materials and Methods

Mice

Adult DBA/2, C57Bl/6, C3H, BALB/c mice were obtained from the pathogen-free breeding colonies of the Institut de Recherches Scientifiques sur le Cancer (Villejuif). F1 hybrid mice were obtained by mating male or female C57Bl/6 with male or female BALB/c. In two experiments, BALB/cBy, C57Bl/6By, and congenic B6.C-H-2^d (HW19) mice (4) were a generous gift of Dr. Edward De Maeyer (Institut Curie, Orsay, France). In all experiments (unless otherwise specified) adult mice >6 wk old were used.

Mx$^+$-congenic Mice

The congenic B.6A2G-Mx (N20,F4) mouse strain (5) was produced at the Institute for Immunology and Virology of the University of Zürich by introducing the influenza virus resistance allele Mx$^+$ of A2G mice (6) into virus-susceptible C57Bl/6 mice.

Tumor Cells

The IFN-α/β resistant clone, 3Cl8, of FLC passaged in vitro was obtained from Dr. Affabris, Istituto Superiore di Sanità, Rome, Italy (7). These cells were subsequently passaged in our laboratory by weekly intraperitoneal injection of DBA/2 mice (H-2^d) and were highly metastatic for the liver and spleen (1, 8, 9). In the experiments to be described, tumor cells were harvested while in the exponential growth phase in the peritoneum.

Quantitative Estimation of the Number of FLC in Liver and Spleen

Colony Formation in Agarose.   The liver or spleen was cut into small pieces with a scalpel and suspended in 20 ml of a prewarmed (37°C) solution of collagenase 100 U/ml (crystalline collagenase 156 U/mg CLSII; Worthington Biochemical Corp., Freehold, NJ), in RPMI 1640 nutrient medium (Flow Laboratories, Irvine, Scotland) without serum (1). After gentle mixing on a magnetic stirrer at 37°C for 45 min, the cell suspension was centrifuged, and the cell pellet was resuspended in 4 ml (liver) or 2 ml (spleen) of RPMI 1640 medium supplemented with 10% FCS (Flow Laboratories). The total number of FLC recovered was calculated by the number of FLC colonies in agarose according to techniques previously described (10). Cells from the liver of normal adult mice do not form colonies in agarose, and only very few colonies were obtained from the spleen of normal mice (<10^3/spleen) (1). These colonies could easily be distinguished morphologically from typical FLC colonies in agarose.

Irradiation of FLC

FLC in a petri dish were irradiated with 50 Gy (5,000 rad): (cesium γ rays: 200 rad/min) by Dr. M. Guichard, Hôpital Gustave Roussy, Villejuif, France.

Hyperimmune Anti-IFN Globulins and Normal Serum Globulins

The sources and activities of the different Ig preparations are shown in Table I.

All sera were decomplemented and extensively absorbed on murine cells (11). 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 (11). The anti–mouse IFN-α/β globulins did not neutralize IFN-γ.

Sheep Nos. 1 and 5A were immunized with partially purified IFN-α/β (11), whereas sheep No. 18-2 was immunized only with highly purified mouse IFN-α/β (12). Sheep (Mona) polyclonal antibody to IFN-α/β$^{(K)}$ (prepared in mouse L cells) serves as the National Institutes of Health (Bethesda, MD) reference antibody (13). Anti–mouse IFN-β mAb was produced and partially purified in our laboratory (F. Belardelli) from the hybridoma cell line 7F-D3 (14); a generous gift of Dr. Y. Kawade, Institute for Virus Research, Kyoto, Japan. The R4-6A2

TABLE I

Sources and Activities of the Different Ig Preparations

Description	Source	Polyclonal (P) or monoclonal (M)	IFN specificity	Neutralizing titer against 4–8 U of mouse IFN-α/β or -γ	Reference
Anti–mouse IFN globulins					
Sheep No. 1	IRSC*	P	α/β	6.4×10^6	11
Sheep No. 5A	IRSC	P	α/β	2.5×10^5	11
Sheep No. 18-2	IRSC	P	α/β	4.0×10^4	—
Sheep (MONA)	Research Resources Branch (NIH)‡	P	α/β	5.0×10^4	13
Rat:mouse hybridoma 7F-D3	Kawade	M	β	4.0×10^5	14
Rat:mouse hybridoma R4-6A2	Havell (A) IRSC (B) (C)	M	γ	6.4×10^4 1.3×10^5 1.5×10^5	15
Control hyperimmune globulins					
Sheep No. 11 antiimpurities	IRSC	—	—	$<1 \times 10^1$	11
Normal serum globulins					
Sheep No. 2	IRSC	—	—	$<1 \times 10^1$	
Sheep No. 18-1 preimmunization	IRSC	—	—	$<1 \times 10^1$	

* IRSC, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.
‡ National Institutes of Health, Bethesda, MD. NIH catalog no. G-024-501-568.

rat:mouse hybridoma cell line was kindly provided by Dr. E. Havell (Trudeau Institute, Saranac Lake, NY) (15). The crude tumor ascites was partially purified by Dr. R. Marcowitz in our laboratory by precipitation with 45% ammonium sulfate. The anti–mouse IFN-γ showed no neutralizing activity at a 1:40 dilution against 8 U of mouse IFN-α/β.

The polyclonal sheep anti–mouse IFN-α/β globulins were shown to be completely devoid of any anti-H-2^d, anti-H-2^b, or H-2^k activity at a 1:2 dilution (these tests were kindly performed by Dr. M. Pla, Laboratory of Mouse Immunogenetics, Hôpital Saint-Louis, Paris, France).

IFN Neutralization Assay

Serial twofold dilutions of Ig were incubated for 1 h at 37°C and 2 h at 4°C with 4–8 U of mouse IFN-α/β (C-243 cell IFN) or mouse IFN-γ. Mixtures were then incubated with monolayer cultures of L cells for 18 h before challenge with ∼100 tissue culture infective doses (TCID$_{50}$) of vesicular stomatitis virus (VSV). In each instance a simultaneous titration of the IFN used was included in the test. The neutralizing titer was taken as the highest dilution of antibody that inhibited by 50% the protective activity of 4–8 U of IFN. A PHA-induced spleen cell IFN preparation partially purified on Con A-Sepharose columns was provided by Dr. E. A. Havell (Trudeau Institute, Saranac Lake, NY).

Anti-Mx Antibody and Immunofluorescence Assay for the Mx Protein

AP5 is a monospecific anti-Mx antibody produced by immunizing rabbits with a synthetic peptide corresponding to the COOH terminus of murine Mx protein (position 616–631) (16). It has the same specificity in immunoprecipitation and immunofluorescence assays as the

polyclonal and monoclonal murine anti-Mx antibodies described previously (17–19) and was provided by Dr. P. Staeheli (Research Institute of Scripps Clinic, La Jolla, CA).

Frozen 6–8-μm cryostat sections of liver and spleen were fixed and permeabilized in 3% paraformaldehyde and 0.5% Triton X-100 as described (18) and were then incubated for 15 min with appropriate dilutions of rabbit antibody AP5. Bound antibody was revealed with rhodamin (TRITC)-conjugated goat anti–rabbit IgG (Cappel Laboratories, Cochranville, PA). A Reichert-Jung Polyvar microscope was used for UV incident light fluorescence microscopy.

Detection of mRNA for Murine Interferons in Organs of Mice

Extraction of RNA from Liver and Spleen.   Quick frozen liver or spleen was homogenized in lysis buffer (6 M urea/3 M LiCl/0.1% NaDodSO4/heparin 20 U/ml/10 mM sodium acetate, pH 5.0) and the RNA was precipitated at 0°C, extracted twice with phenol/chloroform, and chromatographed twice on oligo(dT)-cellulose. Poly(A)$^+$ RNA was recovered by precipitating the RNA with 2.5 vol of ethanol at −20°C overnight and ultracentrifuging the RNA precipitate (100,000 g for 1 h).

RNA Blot Hybridization.   RNA was electrophoresed in 1.5% agarose gels in the presence of formaldehyde, transferred to nylon filters (Hybond-N; Amersham Corp., Arlington Heights, IL) in the presence of 20× SSC (1× SSC: 0.15 M NaCl/7.5 mM sodium citrate), and irradiated for 4 min at 312 nm (180 W). The RNA blots were prehybridized in 5 × SSC/1× Denhardt's reagent (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% BSA/100 mM sodium phosphate buffer, pH 6.5/0.1% NaDodSO4/denatured salmon sperm DNA (100 μg/ml) at 68°C for 4 h.

RNA was then hybridized to a 600-bp Bam HI–Hinc II fragment of the mouse IFN-α2 gene (20) cloned into the vector pSP64 (21), a cDNA clone of the mouse IFN-β gene (22), and a 330-bp Hind III–Cla I fragment containing the first exon of the mouse IFN-γ gene (23) cloned into the vector pBR322 labeled by nick translation to a specific radioactivity of 0.5–4 × 10^9 cpm/μg of DNA using α-[^{32}P]dATP and α-[^{32}P]dCTP (6,000 Ci/mmol; Amersham Corp.). The blots were hybridized at 68°C for 20 h in a fresh aliquot of the above solution. The filters were then washed at 42°C for 2 h in a buffer containing 50% deionized formamide/5× SSC/100 mM sodium phosphate and then were repeatedly washed in 0.1× SSPE/0.1% NaDodSO4 at room temperature. The filters were then exposed to Fuji RX film with a Cronex intensifying screen (DuPont Co., Wilmington, DE) at −80°C.

Results

Role of the MHC in Determining the Multiplication of FLC in the Liver after Intravenous Inoculation

FLC Injected Intravenously in BALB/c (H-2^d) and C57Bl/6 Mice (H-2^b), in F1 Hybrid Mice, and in C57Bl/6 Mice Congenic with BALB/c Mice at the MHC Locus.   FLC passaged in syngeneic DBA/2 mice (H-2^d) are highly tumorigenic (10) and when injected intravenously they rapidly multiply in the liver and spleen (1); previous work has shown that intravenous inoculation of approximately four FLC constitutes 1 LD$_{50}$ for adult DBA/2 mice (1). FLC also multiplied extensively in the livers of BALB/c (H-2^d) mice but not at all in the livers of adult C57Bl/6 (H-2^b) mice. We wanted to determine whether FLC would also multiply in the livers of C57Bl/6 mice congenic with BALB/c mice at the MHC locus. It is clear from the results presented in Fig. 1 *A* that fewer FLC were recovered from the livers of congenic mice than from the livers of BALB/c mice. Likewise, 6 d after intravenous inoculation fewer FLC were recovered from the livers of F$_1$ C57Bl/6 × BALB/c hybrid mice (Fig. 1 *B*) than from the livers of BALB/c mice. These results indicated that permissivity for the multiplication of FLC in the liver after intravenous inoculation was not determined solely by the MHC, and that other factors might be involved.
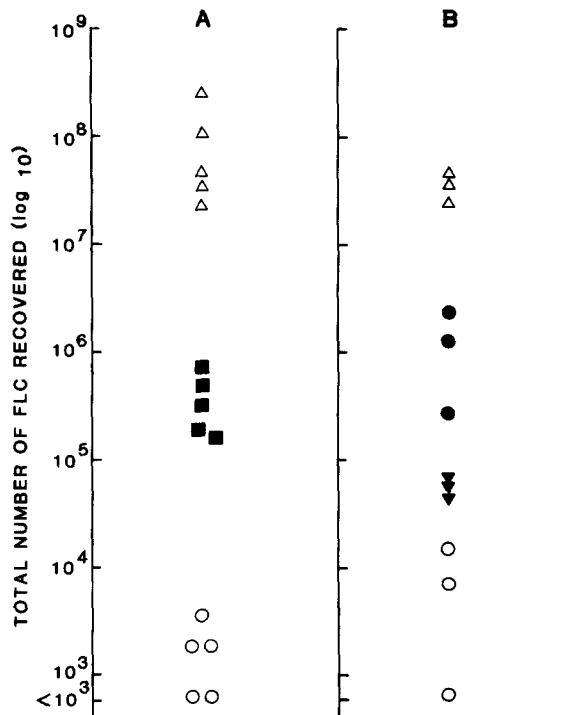
FIGURE 1. Recovery of FLC from the livers of BALB/c mice (H-2^d), C57Bl/6 (H-2^b), C57Bl/6 mice (B$_6$-C-H-2^d) congenic with BALB/c mice at the MHC locus and F1 hybrid mice (C57Bl/6 × BALB/c). (*A*) 12-wk-old male BALB/c (By) mice (△), C57Bl/6 (By) mice (○), or C57Bl/6 mice B6.C-H-2^d (HW19) congenic with BALB/c mice at the MHC locus (■) were injected intravenously with 2 × 10^6 3C18 FLC. 6 d later, five mice in each group were killed and the total number of FLC in the liver of each mouse was estimated by colony assay in agarose. (*B*) 6-wk-old male BALB/c (△), C57Bl/6 (○), or F1 hybrid mice (●) (C57Bl/6♀ × BALB/c□) or (▼) (BALB/c♀ × C57Bl/6□) were injected with 2 × 10^6 3C18 FLC. 6 d later, three mice in each group were killed and the total number of FLC in the liver of each mouse was estimated by colony assay in agarose.

Role of Endogenous IFN in Determining Whether FLC Multiply in the Liver of Allogeneic C57Bl/6 or C3H Mice after Intravenous Inoculation

FLC Multiply in the Livers of C57Bl/6 Mice Treated with Antibody to Mouse IFN-α/β. Although FLC do not multiply in the livers of adult C57Bl/6 mice (Fig. 2, ○), they did multiply rapidly in the livers of all C57Bl/6 mice injected with antibody to mouse IFN-α/β (Figs. 2 and 3, ●). FLC do not multiply in the livers of most BALB/c mice and all BALB/c mice treated with antibody to IFN-α/β (Fig. 2, △, ▲). Between the 9th and 12th day after inoculation of tumor cells, two of two other antibody-treated C57Bl/6 mice and five of five other antibody-treated BALB/c mice died with extensive tumor in the liver: whereas three of six BALB/c and zero of two C57Bl/6 mice treated with normal sheep globulin died.

The number of tumor cells recovered from the liver 3 h after intravenous inoculation of FLC was the same for mice treated with antibody to IFN-α/β, or control Ig, or for mice left untreated. Thus, injection of antibody did not affect the homing to the liver of FLC injected intravenously (data not shown).

Influence of Various Experimental Conditions Relative to the Multiplication of FLC in the Livers of C57Bl/6 Mice Treated with Antibody to Mouse IFN-α/β.

AMOUNT OF ANTIBODY INJECTED. FLC multiplied in the livers of C57Bl/6 mice pretreated with 2 × 10^6, 2 × 10^5, and 2 × 10^4 neutralizing units of antibody to IFN-α/β but not in mice injected with 2 × 10^3, 2 × 10^2 neutralizing units (Fig. 4).

NUMBER OF FLC INJECTED. Although in the experiments described above mice were injected intravenously with 2 × 10^6 FLC, it can be seen from Fig. 5 that the
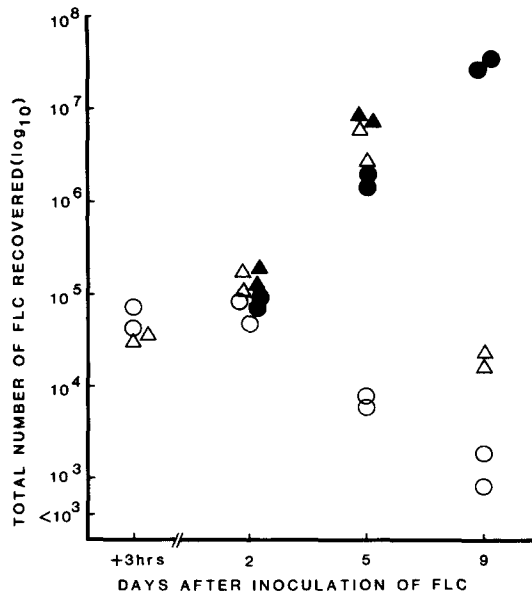
FIGURE 2.   Multiplication of FLC in the livers of C57Bl/6 and BALB/c mice injected with antibody to mouse IFN-α/β. 10-wk-old male C57Bl/6 (O, ●) or BALB/c (△, ▲) mice were injected (0.2 ml i.v.) with 10⁶ neutralizing units of sheep 1 anti-IFN-α/β globulin (●, ▲) or normal sheep globulin (O, △) 2 h before an intravenous injection of 2 × 10⁶ 3Cl8 FLC. At the times indicated, individual mice were killed and the number of FLC in each liver was determined by colony formation in agarose. Each symbol represents the number of FLC per mouse.

phenomenon of abrogation by anti-IFN globulin of the resistance of C57Bl/6 to the multiplication of FLC in the liver was observed for all groups regardless of the number of FLC injected at the dose of antibody injected.

AGE OF MICE.   Although FLC injected intravenously do not multiply in the livers of 6 wk and older C57Bl/6 mice, FLC do multiply in the livers of some 4-wk-old mice (Fig. 6). FLC did multiply in the livers of all 4- and 12-wk-old mice treated with anti-IFN-α/β globulin (Fig. 6).

TIME OF INJECTION OF ANTI-IFN GLOBULIN.   Injection of anti-IFN-α/β globulin 3 h before intravenous injection of FLC was more effective in abrogating the resistance of C57Bl/6 mice to multiplication of FLC in the liver than injection of the same amount of globulin 2 or 3 d before tumor cell inoculation (however, this treatment was also effective) (data not shown). The somewhat lower efficacy of anti-IFN-α/β globulin injected 2-3 d before FLC injection was probably due to the progressive decrease in the circulating serum titer of the anti-IFN globulin in the days after injection·(11).

RELATIVE NUMBER OF FLC IN LIVER AND SPLEEN.   After intravenous inoculation of syngeneic DBA/2 mice, FLC multiply extensively and equally in both the liver and spleen (1). In C57Bl/6 mice treated with anti-IFN-α/β globulin, 1,000-fold more FLC were recovered from the liver than from the spleen (Fig. 7). There was, nevertheless, a difference between the number of FLC recovered from the spleen of antibody treated mice compared with control-treated C57Bl/6 mice (Fig. 7).

Effect of Different Anti-IFN Globulin Preparations on the Multiplication of FLC in the Livers of C57Bl/6 and C3H Mice.   In all the above experiments we used potent polyclonal sheep No. 1 anti-IFN-α/β globulin to abrogate the allogeneic resistance of C57Bl/6 mice (H-2^b) to the multiplication of FLC (H-2^d) in the liver. The evidence that
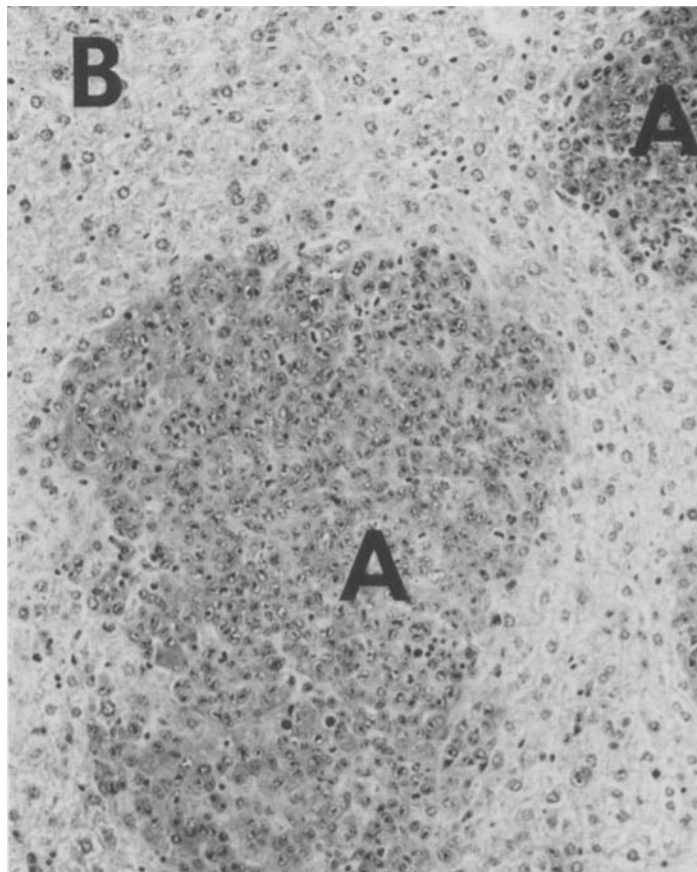
FIGURE 3. Foci of FLC in the liver of a C57Bl/6 mouse treated with anti-IFN-α/β globulin. Experimental conditions were those described in legend to Fig. 2. C57Bl/6 mouse was killed on day 6. Note foci of tumor (A) surrounded by normal hepatic parenchyma (B) × 150. FLC were not seen in the livers of C57Bl/6 mice treated with normal sheep globulin.

abrogation of resistance did not stem from a peculiarity of this particular sheep Ig may be summarized as follows.

Polyclonal anti-IFN-α/β globulin from another source (Dr. Y. Kawade, Institute for Virus Research, Kyoto, Japan) also abrogated the resistance of C57Bl/6 mice to the multiplication of FLC in the liver (Fig. 8 A). Likewise, polyclonal anti-IFN-α/β globulin from two other sheep immunized in our laboratory (one with partially purified interferon [5A] [11] and the other with highly purified interferon [18-2]) was also equally effective in abrogating resistance in C57Bl/6 mice (data not shown). In contrast, normal sheep serum globulins (the preimmunization serum globulins of sheep No. 18; and sheep No. 2, and the serum globulin from sheep No. 11 immunized with the impurities in the IFN preparations) did not abrogate the resistance of C57Bl/6 mice, and FLC did not multiply in the livers of these mice (data not shown).
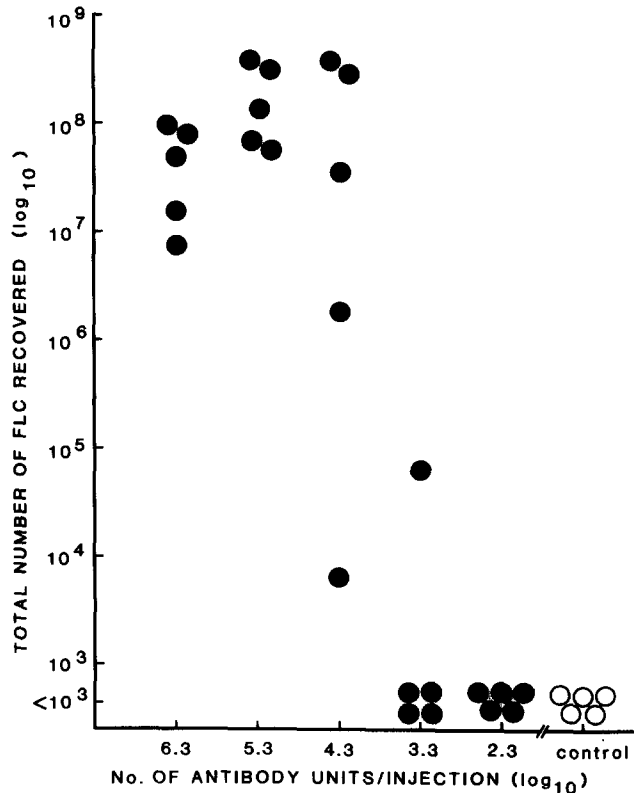
FIGURE 4. Relationship between the amount of anti-IFN-α/β globulin injected and the multiplication of FLC in the livers of C57Bl/6 mice. 8-wk-old male C57Bl/6 mice were injected with various amounts of sheep 1 anti-IFN-α/β globulin (0.15 ml i.v. and 0.15 ml i.p.) (●) or left untreated (O) 3 h before injection of 2 × 10^6 3Cl8 FLC. 7 d later five mice in each group were killed and the number of FLC per liver was determined. Each symbol represents the number of FLC/mouse.

Administration of monoclonal anti–mouse IFN-β Ig was ineffective in C57Bl/6 mice (Fig. 8 *A*), and monoclonal anti-IFN-γ Ig was ineffective in C57Bl/6 and C3H mice (Fig. 8, *B* and *C*).

Antibody to IFN-α/β also Abrogates the Resistance of C3H Mice (H-2^k) to the Multiplication of FLC in the Liver.   The experiment illustrated in Fig. 8 (C) shows that polyclonal antibody to IFN α/β also abrogates the resistance of C3H mice (H-2^k) to the multiplication of FLC in the liver.

Reproducibility of the Effect of Polyclonal Antibody to IFN-α/β on the Multiplication of FLC in the Liver of Adult C57Bl/6 Mice.   As can be seen in Table II, treatment of adult C57Bl/6 mice with polyclonal antibody to IFN-α/β consistently abrogated the resistance of the liver to multiplication of FLC, whereas FLC did not multiply in the livers of adult C57Bl/6 mice treated with control globulins or left untreated.

Injection of Anti–Mouse IFN-α/β Globulin Results in Death of C57Bl/6 and C3H Mice Injected Intravenously with 3Cl8 FLC.   In six experiments some C57Bl/6 or C3H mice treated with anti-IFN-α/β globulins or control globulins were kept for at least 2 mo to determine the effect of antibody treatment on long-term survival. As can be seen from Table III, 15 of 18 (83%) C57Bl/6 and C3H mice treated with antibody to IFN-α/β died with massive tumor involvement of the liver 8–13 d after inoculation of FLC. Syngeneic DBA/2 mice (H-2^d) die at this time after intravenous inoculation of FLC (1). In contrast, none of 32 mice treated with control globulins died.
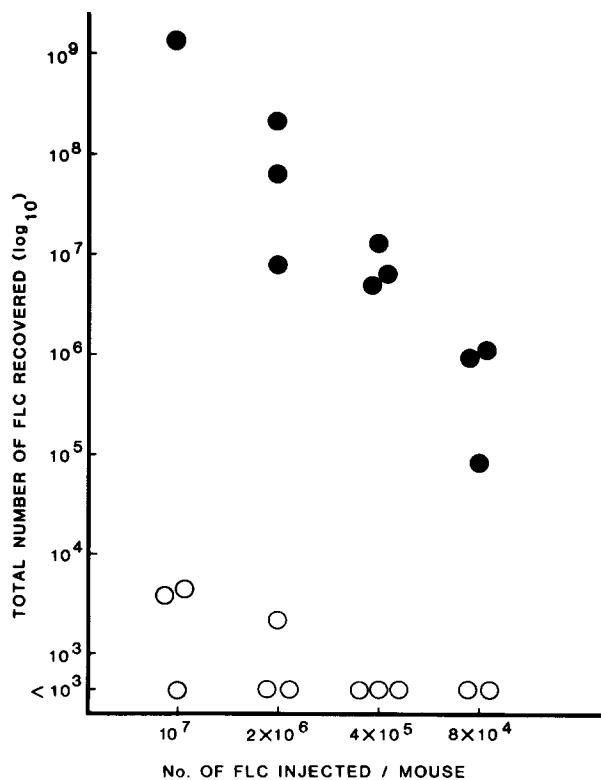
FIGURE 5. Effect of treatment of C57Bl/6 mice with anti-IFN-α/β globulin on the multiplication of FLC in the liver after intravenous injection of varying numbers of FLC. 8-wk-old male C57Bl/6 mice were injected with 2 × 10⁶ neutralizing units of sheep 1 anti-IFN-α/β globulin (0.15 ml i.v. and 0.15 ml i.p.) 3 h before the injection of varying numbers of 3Cl8 FLC. There were three antibody-treated mice (●) and three control mice (O) per group. 7 d later mice were killed and the number of FLC in the liver was determined by colony formation in agarose. Each symbol indicates the number of FLC per mouse. Two antibody-treated mice injected with 10⁷ FLC were dead on the seventh day. Both had massive tumor replacement of the liver.

Demonstration of mRNA in the Livers of C57Bl/6 Mice Injected with 3Cl8 FLC Which Hybridizes with Mouse IFN-α and -β DNA Probes. In three experiments using cell culture IFN assays, we could demonstrate an antiviral inhibitor (at serum dilutions of 1:10 to 1:40) in most pools of sera from C57Bl/6 mice 24 h (but not at 48 h) after inoculation of 2 × 10⁶ 3Cl8 FLC. As the activity of this inhibitor was low it was difficult to characterize it, so we determined whether injection of FLC induced the appearance of mRNA hybridizable with specific mouse IFN-α or -β cDNA probes.

In four experiments, poly(A)⁺ RNA harvested from the liver of C57Bl/6 mice 8 h after inoculation of FLC hybridized with the mouse IFN-α2 probe. No hybridization was detected with RNA from the livers of mice taken 24 h after injection of FLC or with RNA prepared from uninjected mice (Fig. 9 A). A lower signal of hybridization was observed with the IFN-α2 probe for poly(A)⁺ RNA extracted from the spleen 24 h after injection of FLC (data not shown). Newcastle Disease virus (NDV)-induced IFN-α mRNA in the spleen had an approximate size of 1.2 kb (Fig. 9 A). In each experiment the size of the hybridizable RNA in the liver (8 h) or spleen (24 h) after inoculation of FLC was ~1.5 kb.

In two experiments, poly(A)⁺ RNA harvested from the liver of mice 8 h after inoculation of FLC (but not after 24 h nor from uninoculated mice) hybridized with the mouse IFN-β probe (Fig. 9 B). NDV-induced IFN-β mRNA in the spleen had an approximate size of 0.9 kb, whereas the RNA in the liver of FLC-injected mice had an approximate size of 1.2 kb.
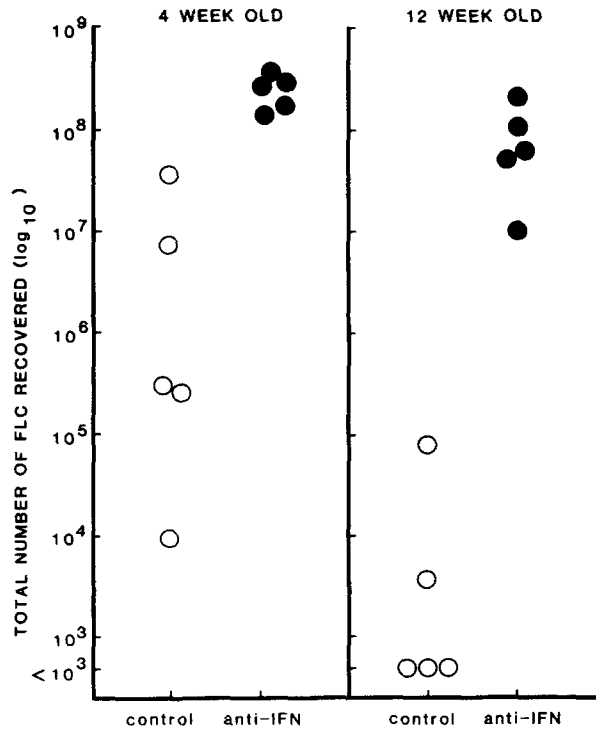
FIGURE 6. Recovery of FLC from the livers of 4- and 12-wk-old C57Bl/6 mice injected with anti-IFN-α/β globulin. Male C57Bl/6 mice were injected with 2 × 10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin (0.25 ml i.p.) (●) or left untreated (O) 5 h before intravenous injection of 2 × 10^6 3Cl8 FLC. 7 d later mice were killed and the number of FLC in the liver was determined by colony formation in agarose. Each symbol indicates the number of FLC/mouse.
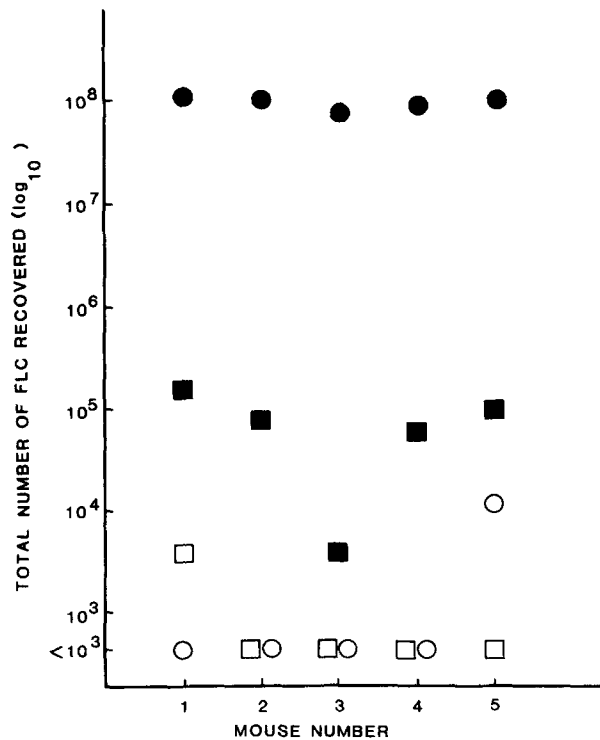

FIGURE 7. Effects of anti-IFN-α/β globulin on the recovery of FLC from the liver and spleen of C57Bl/6 mice. 9-wk-old male C57Bl/6 mice were injected with 2 × 10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin (solid symbols) or with normal sheep globulin (open symbols) (0.15 ml i.v. and 0.1 ml i.p.) 3 h later mice were injected intravenously with 2 × 10^6 3Cl8 FLC. 7 d later mice were killed and the number of FLC in the liver (●, O) or spleen (■, □) was determined. Each symbol indicates the number of FLC/organ.
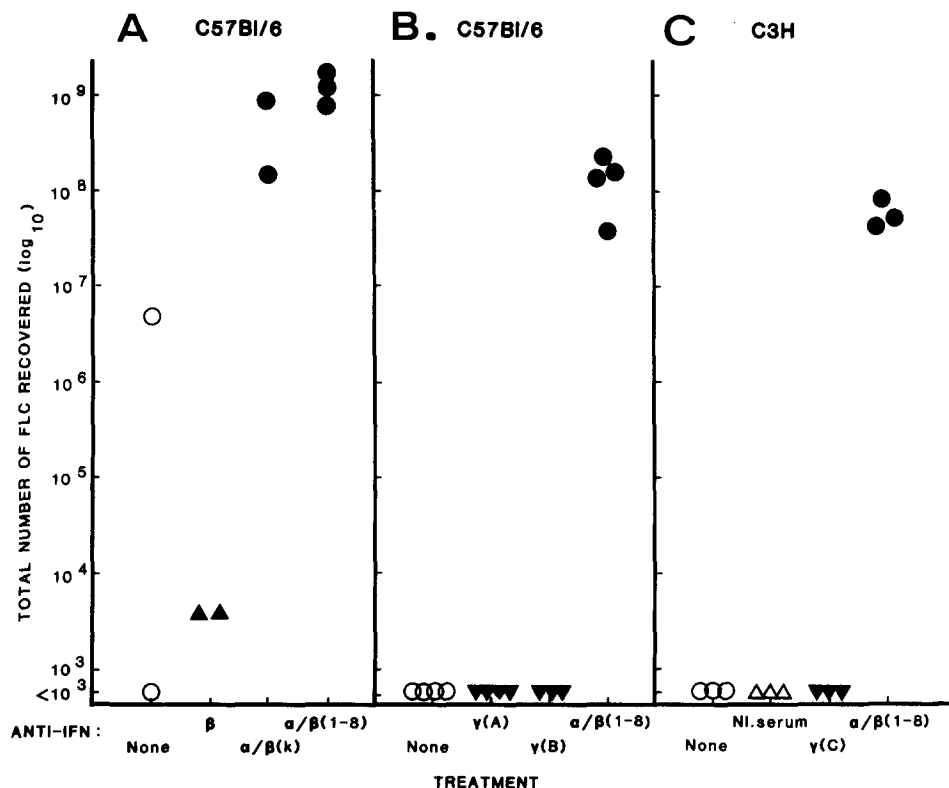
FIGURE 8.   Effect of different anti-IFN globulin preparations on the multiplication of 3Cl8 FLC in the livers of C57Bl/6 and C3H mice (A) 10-wk-old male C57Bl/6 mice were injected with 2×10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin (●); or 5×10^4 neutralizing units of sheep Mona (K) anti-IFN-α/β globulin (●); or 4×10^5 neutralizing units of anti-IFN-β globulin (▲) or left untreated (○). Globulins were injected (0.15 ml i.v. 0.1 ml i.p.) 3 h before intravenous inoculation of 2×10^6 3Cl8 FLC. 7 days later the mice were killed and the number of FLC in the liver of each mouse was determined. In this experiment, FLC multiplied in the liver of one untreated C57Bl/6 mouse. This was the only instance of 107 untreated C57Bl/6 mice (>6 wk old) in 22 experiments (Table II). (B) 8-wk-old male C57Bl/6 mice were injected with 2×10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin (●); or two different batches of anti-mouse IFN gamma globulin: 6.4×10^4 neutralizing units (A) or 1.28×10^5 neutralizing units (B) (▼) (0.15 ml i.v., 0.1 ml i.p.) or left untreated (○). 3 h later mice were injected intravenously with 2×10^6 3Cl8 FLC. 7 d later the mice were killed and the number of FLC in the liver of each mouse was determined. (C) 9-wk-old male C3H mice were injected intraperitoneally with 2×10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin (●), 1.5×10^5 neutralizing units of rat (R4-6A2) anti-IFN-γ (▼), normal sheep 2 serum globulin (△) or were left untreated (○). Three hours later, mice were injected intravenously with 2×10^6 3Cl8 FLC. 6 d later the mice were killed and the number of FLC in the liver of each mouse was determined.

Hybridizable RNA was not detected in extracts of kidney, lung, thymus, or mesenteric ganglion using the mouse IFN-α2 or -β probes. No hybridizable RNA was detected in organ extracts using a mouse IFN-γ probe (data not shown).

Induction of Mx Protein Expression in C57Bl/6 Mice Congenic with A2G Mice at the Mx Locus.   The preceding experiments showed that after intravenous injection of FLC, mRNA hybridizing with cDNA probes for mouse IFN-α and -β was present in the

TABLE II

Reproducibility of the Effect of Treatment with Polyclonal Antibody to IFN-α/β
on the Multiplication of FLC in the Liver of Adult C57Bl/6 Mice

Treatment	Number of experiments	Number of mice in which FLC multiplied in the liver/ total number of mice injected	Percentage
None	22	1/107	1
Control globulins	7	0/36	—
Polyclonal antibody to IFN α/β	23	100/102	98

In all these experiments adult C57Bl/6 mice (>6 wk old) were injected intravenously with >2 × 10^6 3C18 FLC. To determine FLC multiplication, the number of FLC in the liver was determined between the fifth and ninth day after intravenous inoculation.

liver at 8 h, and a viral inhibitor was present in very low titer of the serum at 24 h. As mouse IFN-α/β (but not IFN-γ) induces the expression of the Mx protein in Mx$^+$ mice (24, 25) we determined whether inoculation of FLC into C57Bl/6 mice congenic with A2G mice at the Mx locus would result in the expression of the Mx protein in the liver and spleen, thus constituting further evidence that injection of FLC results in the production of IFN-α/β.

Accordingly, congenic B6.A2G-Mx mice were injected intravenously with 2 × 10^6 3C18 FLC and organs were harvested 3, 5, and 7 d thereafter. In uninoculated Mx$^+$ mice there is a background level of Mx protein expressed in a subset of cells in the liver and spleen (probably in macrophages and endothelial cells) (18, 24) (Fig. 10, A and D), but it is less prominent in other organs. In Mx$^+$ mice injected intravenously with FLC, there was an induction of Mx expression in most hepatocytes (Fig. 10 E), and cells of the white pulp of the spleen (Fig. 10 B), kidney, and lung (data not shown). Administration of anti-IFN-α/β globulin markedly inhibited this induced expression of the Mx protein in both the liver and spleen of FLC-injected mice (Fig. 10, C and F), although it did not appear to affect the low background level of spontaneous Mx expression (Fig. 10, compare A and D with C and F).

TABLE III

Mortality in Mice Treated with Anti-IFN-α/β Globulin and Injected Intravenously with FLC

Mouse strain	Number of experiments	Treatment*	Number of mice dead[t]/ total number	Mean day of death ± SE
C57Bl/6	6	Anti-IFN α/β	13/15	9.7 ± 0.3
		Control Ig	0/25	—
C3H	1	Anti-IFN α/β	2/3	11.5 ± 1.5
		Control Ig	0/7	—

All mice were injected intravenously with 2 × 10^6 3C18 FLC.

* Mice were injected with 2 × 10^6 neutralizing units of sheep 1 anti-IFN-α/β globulin. Control Ig consisted of normal sheep globulin from sheep 11 immunized against impurities in the IFN preparations.

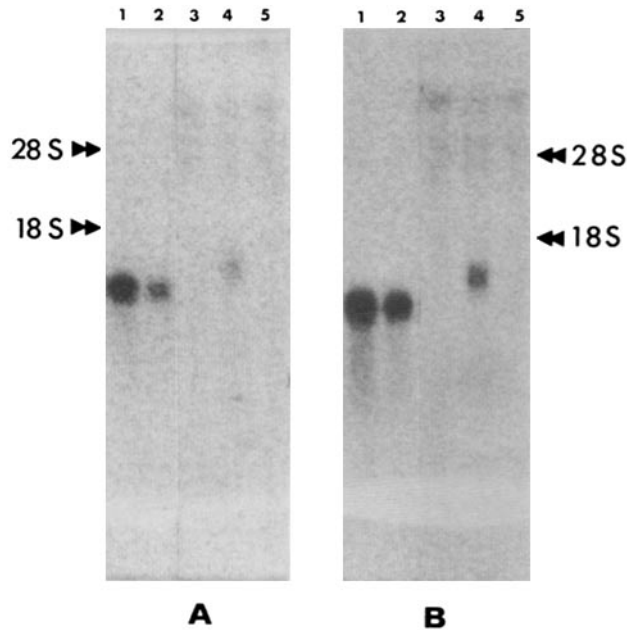[t] All dead mice had extensive gross tumor invasion of the liver.

FIGURE 9. Northern blot hybridization analysis of IFN-α and -β mRNA in the livers of 8-wk-old male C57Bl/6 mice injected intravenously with 3Cl8 FLC. (*A*) Hybridization with cloned murine IFN-α2 DNA; (*B*) hybridization with cloned murine IFN-β cDNA. The film was exposed for 4 d at −80°C. Lanes *1* and *2* are the poly(A)⁺ RNA from the spleens of C57Bl/6 mice injected intravenously with Newcastle disease virus as a positive control for the induction of mRNA for IFN-α and -β. *A*: 0.5 and 0.2 µg/*B*: 1.0 and 0.5 µg). Lane *3*, RNA from liver from uninjected C57Bl/6 mice; lanes *4* and *5*; RNA from livers of C57Bl/6 mice killed 8 h (lane *4*) or 24 h (lane *5*) after intravenous injection of FLC. 20 µg of poly(A)⁺ RNA were loaded per sample in lanes *3-5*.

Serial Passage of FLC in C57Bl/6 Mice Treated with Anti-IFN-α/β Globulin Does Not Result in the Emergence of FLC Capable of Multiplying in Untreated C57Bl/6 Mice

The preceding results indicated that after intravenous inoculation, FLC multiplied in the livers of C57Bl/6 or C3H mice treated with antibody to mouse IFN-α/β. It might be hypothesized that injection of FLC from syngeneic DBA/2 mice into these anti-IFN globulin–treated allogeneic mice resulted in the selection of a population of tumor cells (perhaps with an altered H-2 expression) capable of multiplying in untreated C57Bl/6 mice. Likewise, it might be hypothesized that injection of FLC liberated a factor (virus ?) that induced a transformation of host cells in C57Bl/6 mice treated with anti-IFN-α/β globulin. We undertook two experiments to test these hypotheses.

First, FLC were serially passaged every 7 d for 10 passages in C57Bl/6 mice treated with 10⁶ neutralizing units of anti–mouse IFN-α/β globulin. At each passage, 2 × 10⁶ FLC from the livers of antibody treated mice were injected intravenously into three other antibody-treated or three untreated mice. At each passage level, FLC multiplied only in the livers of mice pretreated with antibody to IFN-α/β and never in the livers of untreated mice. After the second and tenth passage of FLC in the livers of antibody-treated mice, these FLC were injected both intravenously and subcutaneously into untreated allogeneic C57Bl/6 mice and also into syngeneic DBA/2 mice. FLC formed large subcutaneous tumors and multiplied extensively in the livers of DBA/2 mice (compared in the same experiment with FLC passaged intraperitoneally in DBA/2 mice) but did not multiply in untreated C57Bl/6 mice. These results indicated that passage of FLC in the livers of antibody-treated C57Bl/6 mice was not associated with acquisition of the capacity to multiply in the livers (or subcutane-
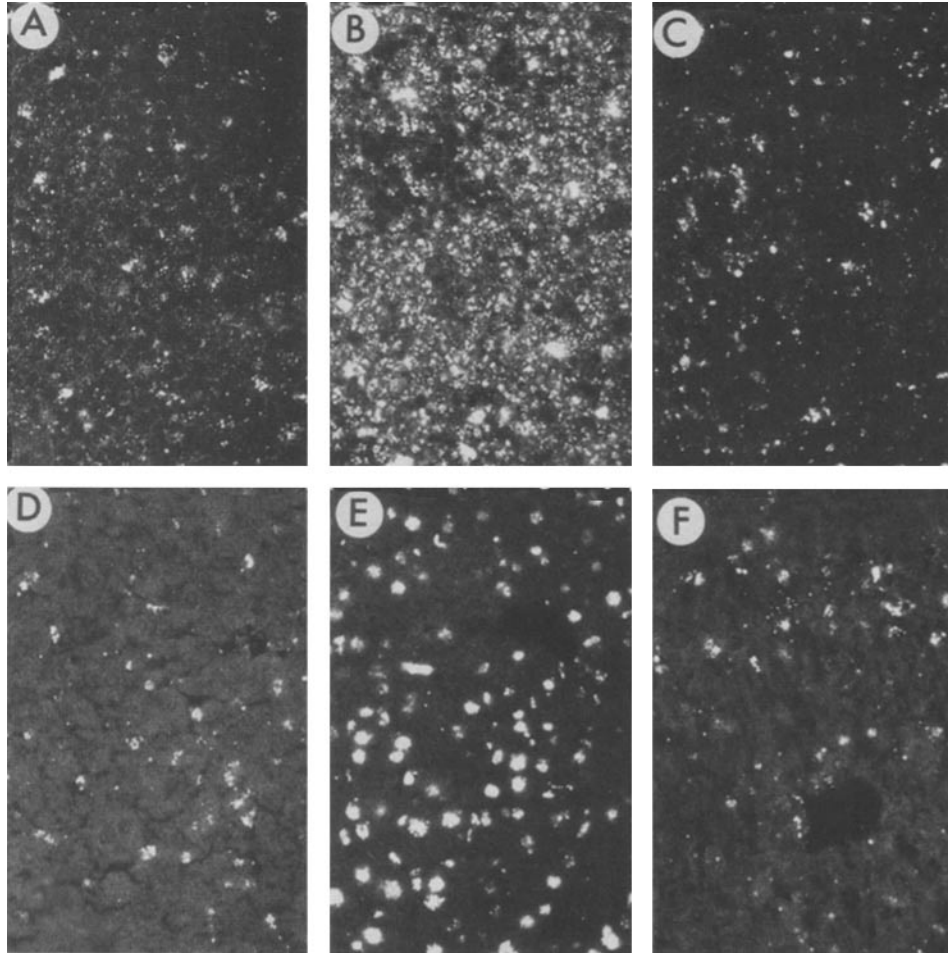
FIGURE 10.   Induction of Mx protein expression in Mx$^+$ C57Bl/6 mice injected intravenously with 3Cl8 FLC. 5-mo-old male congenic B6.A2G-Mx$^+$ mice were separated into three groups. One group was not injected (A, D); one group was injected (0.2 ml i.p.) with normal sheep 2 serum globulin (B, E); one group was injected with 2 × 10^6 i.p. neutralizing units of sheep 1 anti-IFN-α/β globulin C, F). 3 h later mice in groups B, C, E, and F were injected intravenously with 2 × 10^6 3Cl8 FLC. 3 d later frozen cryostat sections of spleen (A, B, C) and liver (D, E, F) were stained by indirect immunofluorescence with specific anti-Mx antibodies. Note that Mx$^+$ cells are present in a subset of cells of spleen and liver of uninjected Mx^+ mice (A, D) (24). Note marked increase in expression of Mx protein in cells (nuclei [18, 25]) of spleen and liver of tumor-injected, control serum–treated Mx$^+$ mice (B, E). Note relative decrease in expression of Mx protein in spleen and liver of tumor-injected Mx$^+$ mice preinjected with antibody to IFN-α/β (C, F) compared with B, E.

ously) of untreated allogeneic C57Bl/6 mice or in a decreased tumorigenicity for syngeneic DBA/2 mice.

In a second experiment, FLC were irradiated with 5,000 rad, which abolished their capacity to multiply in the liver of C57Bl/6 mice treated with anti-IFN-α/β globulin (or left untreated). These results indicated that the injected FLC must have

the capacity to multiply in order to induce tumors in the livers of C57Bl/6 mice treated with antibody to IFN-α/β. These results do not support, therefore, the hypothesis that virus released from FLC induced a transformation of host cells.

Discussion

The results presented herein suggest the possibility that the mechanisms operative in restricting transplantable tumor growth in allogeneic mice may be different depending on the site of tumor growth. For example, transplantable mouse tumors usually grow for several days when injected subcutaneously or intraperitoneally into allogeneic mice before rejection. Thus, in previous experiments FLC (H-2^d) injected intraperitoneally into allogeneic C57Bl/6, C3H, or Swiss mice multiplied 500-fold in the peritoneum by the fifth day (comparable numbers of FLC were present in the peritoneum of syngeneic mice at this time) before being eliminated in the ensuing few days (our unpublished observations). It is usually accepted that immune mechanisms are responsible for this tumor cell rejection across a strong MHC barrier (26).

In contrast, when FLC were injected intravenously they implanted in the liver of syngeneic DBA/2 and allogeneic C57Bl/6 mice to the same extent (see Fig. 2; 3 h after injection), but multiplied only in the livers of syngeneic mice and not to any significant degree in the livers of adult allogeneic mice. We are not aware of a distinction having been made, heretofore, between rejection of a growing tumor and failure of the tumor to grow at all in the adult allogeneic host. Furthermore, the behavior of tumor cells injected intravenously into hybrid or congenic mice suggested that factors in addition to the MHC may be important in restricting tumor growth in different organs after intravenous inoculation. Thus, several days after intravenous inoculation, 100-fold fewer FLC could be recovered from the livers of C57Bl/6 mice congenic with BALB/c mice at the MHC locus (or from F1 hybrid C57Bl/6 × BALB/c mice) than from the livers of BALB/c mice (H-2^d) (Fig. 1). Likewise, after intravenous inoculation of the B16 melanoma, ∼10-fold fewer tumor nodules were observed in the lungs of F1 hybrid mice compared with the lungs of syngeneic mice (27). In contrast, no difference was observed in mesenteric tumor formation between syngeneic and F1 hybrid mice when the tumor cells were injected intraperitoneally (27). Jack and McVeigh concluded that after intravenous injection, tumor growth in the lung was restricted by a "locus other than H-2" (27).

The results presented herein strongly suggest that IFN-α/β is one of the important factors in restricting FLC multiplication in the liver after intravenous inoculation of allogeneic mice. Whereas administration of antibody to IFN-α/β did not prevent tumor cell rejection when tumor cells were injected subcutaneously or intraperitoneally into allogeneic mice (3), this antibody completely overcame the barrier to FLC multiplication in the livers of adult allogeneic immunocompetent mice. Thus, in antibody-treated C57Bl/6 or C3H mice, FLC injected intravenously multiplied rapidly in the liver (as determined by quantitative estimations of the number of FLC [Figs. 2, 4–8] and histopathologic examination [Fig. 3]) and almost all the mice died with extensive tumor invasion of the liver (Table III). In fact, the kinetics of FLC muliplication in the liver of C57Bl/6 mice treated with anti-IFN-α/β globulin (Fig. 2) and the mean day of death of these mice resembled that observed in syngeneic DBA/2 mice. The effect of antibody treatment of allogeneic mice in abrogating

liver resistance was highly reproducible (Table II) under a variety of experimental conditions (i.e., varying numbers of FLC injected, age of mice, amount of antibody) as detailed in Figs. 4–6.

Several arguments attest to the specificity of the antibodies to IFN-α/β used: (a) Polyclonal antibodies raised in three sheep in our laboratory against either partially purified C243 cell IFN-α/β (sheep 1–8, and 5A), or highly purified mouse IFN-α/β (12) (sheep 18), or antibody from a sheep (Mona) prepared in another laboratory against mouse L cell IFN all proved effective in abrogating the resistance of C57Bl/6 or C3H mice to the multiplication of FLC in the liver (Table I, Fig. 8). (b) Neither antibody raised in sheep 11 against the impurities in the IFN-α/β preparations or the serum globulin from two normal sheep (one being the preimmunization serum of sheep 18) exerted any effect.

These considerations suggest that these anti-IFN-α/β globulins acted by neutralizing either "spontaneous" endogenous IFN-α/β present before injection of tumor cells or an endogenous IFN-α/β resulting from the intravenous injection of FLC. Although we have not detected IFN in normal mice, other investigators have demonstrated the presence of small amounts of IFN-α/β in the serum of a few normal mice (28) and our previous work using antibody to IFN-α/β has provided indirect evidence to suggest the presence of biologically active "spontaneous" endogenous IFN in mice (29–31). More recently we have shown that the mRNA for IFN-α1 and -α2 is present in various tissues of normal individuals in the apparent absence of an inducer (32).

To our knowledge there have been very few previous reports demonstrating the production of interferon in experimental animals after inoculation of tumor cells (33, 34), although tumor cells have been shown to induce interferon production by lymphocytes in vitro (35, 36). Several experimental results strongly indicated that in our experiments the intravenous inoculation of FLC does result in the induction of IFN: (a) 8 h after intravenous inoculation of FLC, poly(A)$^+$ RNA hybridizable with specific DNA probes for mouse IFN-α2 and -β was detectable in the liver (Fig. 9) (hybridizable poly(A)$^+$ RNA was also present in the spleen at 24 h).[2] (b) Using IFN cell culture assays a viral inhibitor of low activity was detected in the serum of C57Bl/6 mice 24 h after injection of 3Cl8 FLC. (c) To date only IFN-α/β has been shown to induce the expression of Mx protein in Mx$^+$ cells (24). Injection of C57Bl/6 mice with FLC resulted in the induced expression of the Mx protein in most cells of the liver, spleen (Fig. 10, B and E), lung, and kidney, and this enhancement was blocked by treatment of the mice with anti-IFN-α/β globulin (Fig. 10, C and F). The most likely explanation of our results appears to be that intravenous injection of 3Cl8 FLC results in the induction of IFN-α/β that was neutralized by potent polyclonal antibody to IFN-α/β.

We do not mean to imply, however, that IFN is the sole factor in determining the resistance of the liver to allogeneic tumor growth, but it does seem to be one of the important factors. IFN may also play a role in the resistance of the liver of syngeneic mice to FLC injected intravenously, but the effect is less obvious as the

---

[2] If both IFN-α and -β mRNAs are translated, this may explain the failure of a monoclonal anti-IFN-β globulin alone to abrogate resistance of C57Bl/6 mice (Fig. 8). Unfortunately a potent monoclonal anti-IFN-α globulin was not available to permit us to test the combined effect of both mAbs.

tumor cells multiply so rapidly. FLC injected intravenously into BALB/c mice (H-2^d) congenic with A2G mice at the Mx locus also induced an increase in the expression of the Mx protein in the liver, spleen, lung, and kidney, therefore indicating the production of IFN-α/β (data not shown). Injection of syngeneic DBA/2 mice (H-2^d) with antibody to IFN-α/β decreased the mean day of death from 9.0 ± 0.6 d for control FLC-injected mice to 7.0 ± 0 days for antibody-treated mice ($p < 0.01$) (data not shown). Lastly, FLC do multiply in the livers of allogeneic nu/nu mice (data not shown).

Landolfo and colleagues (37) showed that monoclonal antibodies to mouse IFN-γ abrogated alloantigen recognition and induction of CTL in mixed leukocyte reactions. When allogeneic mice were injected with a fibrosarcoma, and treated repeatedly with an mAb to mouse IFN-γ, tumor rejection did not occur. In our experiments, intravenous inoculation of FLC did not result in the appearance in any of the tissues tested of a poly(A)$^+$ RNA hybridizable with a mouse IFN-γ cDNA probe. Likewise, treatment of allogeneic C57Bl/6 or C3H mice with a potent mAb to mouse IFN-γ did not abrogate the resistance to multiplication of FLC in the liver (Fig. 8). Thus, in our experiments we found no evidence that IFN-γ played an important role, emphasizing once more the possibility that the site of tumor growth may elicit different host mechanisms and different inhibitory factors.

In our previous work (1) on the antitumor effects of IFN we have shown that administration of IFN markedly inhibits the multiplication of IFN-resistant 3Cl8 FLC in the liver and spleen of syngeneic DBA/2 mice, even after these cells were present in large numbers in these target organs. As we could show that these 3Cl8 FLC were resistant to several activities of IFN in vivo (as well in vitro) (38, 39), we postulated that the antitumor effect of IFN was not due to a direct effect of IFN on the tumor cells themselves, but was host mediated (1). The nature of the host mechanisms remains obscure, but we have found no evidence that humoral or cellular immune mechanisms played an important role (40). Likewise, in the experiments reported herein we have used the IFN-resistant 3Cl8 line of FLC. As we still have no clear idea how exogenous IFN acts in syngeneic tumor–injected mice, it is premature to speculate how endogenous IFN might act in restricting FLC muliplication in the liver of allogeneic mice. In allogeneic mice treated with antibody to IFN-α/β, FLC multiplied to a far greater extent in the liver than in the spleen (Fig. 7), whereas in syngeneic mice these FLC multiplied equally well in the liver and spleen. These results suggest that multiple factors are operative in determining the organ resistance to tumor cell multiplication and that IFN may be more important in the liver than in the spleen.

To our knowledge it is not known whether autochtonous primary or metastatic tumors in man elicit an IFN response. As tumor metastases in the liver do not always grow rapidly and exponentially, it seems reasonable to suggest that the rate of tumor growth in certain tissues may be determined not only by the inherent growth pattern of the individual tumor but also by the presence of soluble growth inhibitors such as IFN.

Likewise, it is unknown whether transplantation of normal liver induces an IFN response in the immunodepressed host, although it has been shown that IFN-α/β is induced in mice in the course of graft-vs.-host disease (41). The finding that in experimental animals "foreign liver is often better tolerated than other organ grafts

(skin, kidney, or heart) being rejected less aggressively or requiring less immunosuppression to prevent rejection" (42) may suggest that mechanisms responsible for liver rejection are qualitatively or quantitatively different from those observed in other grafts. In a recent review on the immunology of liver transplantation in the rat, Kamada summarized as follows: "It seems quite likely that rejection of liver grafts across a whole haplotype barrier is under multigenic control, with important roles both for classical MHC-linked Ir genes and for non-MHC-linked background genes" (42). If IFN production is important in liver transplantation it would be of interest to determine whether administration of antibody to IFN would facilitate liver (or other organ) transplantation in experimental animals (and possibly in man).

Summary

Friend erythroleukemia cells (FLC) (H-2^d) injected intravenously into adult syngeneic DBA/2 or allogeneic C57Bl/6 (H-2^b) or C3H (H-2^k) mice lodge in the liver but only multiply in the liver of syngeneic mice. Our results indicated that endogenous IFN-α/β was a crucial factor in preventing the multiplication of FLC in the liver of adult allogeneic mice. (a) Treatment of allogeneic adult C57Bl/6 or C3H mice with polyclonal antibody to mouse IFN-α/β (but not antibody to IFN-γ) completely abrogated the resistance to the multiplication of FLC in the liver and 87% of tumor-injected, antibody-treated C57Bl/6 mice died with extensive tumor involvement of the liver. In contrast, after intravenous inoculation FLC do not multiply at all (or very rarely) in the liver of adult C57Bl/6 mice left untreated or treated with a variety of control globulins, and no deaths occurred. (b) 8 h after intravenous inoculation of FLC, poly(A)$^+$ RNA hybridizable with specific DNA probes for mouse IFN-α or -β (but not -γ) was present in the liver of injected C57Bl/6 mice. Using the expression of the Mx protein as an indicator of the presence of IFN-α/β, we showed that Mx$^+$ congenic C57Bl/6 mice injected with FLC exhibited a marked increase in the expression of the Mx protein in the liver, spleen, kidney and lung, and this increase was blocked by treatment of mice with antibody to IFN-α/β. The possibility that different host mechanisms are elicited depending on the site of tumor growth in allogeneic mice is discussed. IFN-α/β appears to be of particular importance in determining the resistance of the liver to FLC in allogeneic mice.

References

1. Gresser, I., C. Maury, D. Woodrow, J. Moss, M. G. Grütter, F. Vignaux, F. Belardelli, and M.-T. Maunoury. 1988. Interferon treatment markedly inhibits the development of tumor metastases in the liver and spleen and increases survival time of mice after intravenous inoculation of Friend erythroleukemia cells. *Int. J. Cancer.* 41:135.
2. Reid, L. M., N. Minato, I. Gresser, J. Holland, A. Kadish, and B. R. Bloom. 1981. Influence of anti-mouse interferon serum on the growth and metastasis of tumor cells persistently infected with virus and of human prostatic tumors in athymic nude mice. *Proc. Natl. Acad. Sci. USA.* 78:1171.

3. Gresser, I., F. Belardelli, C. Maury, M.-T. Maunoury, and M. G. Tovey. 1983. Injection of mice with antibody to interferon enhances the growth of transplantable murine tumors. *J. Exp. Med.* 158:2095.

4. Bailey, D. W. 1981. Recombinant inbred strains and bilineal congenic strains. *In* The Mouse in Biomedical Research. Vol. 1. H. L. Foster, J. D. Small, and J. G. Fox, editors. Academic Press, New York. 223–239.

5. Haller, O., M. Prochazka, P. Stäeheli, and J. Lindenmann. 1984. Mx⁺ - congenic mouse strains. *Mouse News Lett.* 71:53.

6. Haller, O. 1981. Inborn resistance of mice to orthomyxoviruses. *Curr. Top. Microbiol. Immunol.* 92:25.

7. Affabris, E., C. Jemma, and G. B. Rossi. 1982. Isolation of interferon-resistant variants of Friend erythroleukemia cells: effect of interferon and ouabain. *Virology.* 120:441.

8. Belardelli, F., M. Ferrantini, C. Maury, L. Santurbano, and I. Gresser. 1984. On the biologic and biochemical differences between in vitro and in vivo passaged Friend erythroleukemia cells. I. Tumorigenicity and capacity to metastasize. *Int. J. Cancer.* 34:389.

9. Amici, C., M. Ferrantini, A. Benedetto, F. Belardelli, and I. Gresser. 1984. On the biologic and biochemical differences between in vitro and in vivo passaged Friend erythroleukemia cells. II. Changes in cell surface glycoproteins associated with a highly malignant phenotype. *Int. J. Cancer.* 34:397.

10. Belardelli, F., I. Gresser, C. Maury, and M.-T. Maunoury. 1982. Antitumor effects of interferon in mice injected with interferon-sensitive and interferon-resistant Friend leukemia cells. I. *Int. J. Cancer.* 30:813.

11. Gresser, I., M. G. Tovey, M.-T. Bandu, C. Maury, and D. Brouty-Boyé. 1976. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. I. Rapid evolution of encephalomyocarditis virus infection. *J. Exp. Med.* 144:1305.

12. De Maeyer-Guignard, J., M. G. Tovey, I. Gresser, and E. De Maeyer. 1978. Purification of mouse interferon by sequential affinity chromatography on poly(U)- and antibody-agarose columns. *Nature (Lond.).* 271:622.

13. Kawade, Y. 1980. An analysis of neutralization reaction of interferon by antibody: a proposal on the expression of neutralization titer. *J. Interferon Res.* 1:61.

14. Kawade, Y., and Y. Watanabe. 1986. Characterization of rat monoclonal antibodies to mouse interferon α and β. *Proc. TNO-ISIR Meet. Interferon System, Espoo, Helsinki, Finland.* 72.

15. Spitalny, G. L., and E. A. Havell. 1984. Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activities. *J. Exp. Med.* 159:1560.

16. Staeheli, P., O. Haller, W. Boll, J. Lindenmann, and C. Weissmann. 1986. Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. *Cell.* 44:147.

17. Staeheli, P., Ph. Dreiding, O. Haller, and J. Lindenmann. 1985. Polyclonal and monoclonal antibodies to the interferon-inducible protein Mx of influenza virus resistant mice. *J. Biol. Chem.* 260:1821.

18. Dreiding, Ph., P. Staeheli, and O. Haller. 1985. Interferon-induced Mx accumulates in nuclei of mouse cells expressing resistance to influenza viruses. *Virology.* 140:191.

19. Wüthrich, R., P. Staeheli, and O. Haller. 1985. Monoclonal antibodies detect protein Mx in lung cells of interferon-treated influenza virus resistant mice. *In* The Biology of the Interferon System 1984. H. Kirchner and H. Schellekens, editors. Elsevier Science Publishers B. V., Amsterdam. 317–323.

20. Shaw, G. D., W. Boll, H. Taira, N. Mantei, P. Lengyel, and C. Weissmann. 1983. Structure and expression of cloned murine IFN-α genes. *Nucleic Acids Res.* 11:555.

21. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.

1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035.

22. Higashi, Y., Y. Sokawa, Y. Watanabe, Y. Kawade, S. Ohno, C. Takaoka, and T. Taniguchi. 1983. Structure and expression of a cloned cDNA for mouse interferon-β. *J. Biol. Chem.* 258:9522.

23. Gray, P. W., and D. V. Goeddel. 1983. Cloning and expression of murine immune interferon cDNA. *Proc. Natl. Acad. Sci. USA.* 80:5842.

24. Staeheli, P., and O. Haller. 1987. Interferon-induced Mx protein: A mediator of cellular resistance to influenza virus. *In* Interferon 8, 1987. I. Gresser, editor. Academic Press, London. 1–23.

25. Horisberger, M. A., and H. K. Hochkeppel. 1985. An interferon-induced mouse protein involved in the mechanism of resistance to influenza viruses. *J. Biol. Chem.* 260:1730.

26. Bach, F. H., and D. H. Sachs. 1987. Current concepts: immunology. Transplantation immunology. *N. Engl. J. Med.* 317:489.

27. Jack, A. S., and K. L. McVeigh. 1987. A demonstration of a strain related restriction effect in the formation of experimental metastases. *J. Pathol.* 152:37.

28. Galabru, J., N. Robert, C. Buffet-Janvresse, Y. Rivière, and A. G. Hovanessian. 1985. Continuous production of interferon in normal mice: effect of anti-interferon globulin, sex, age, strain and environment on the levels of 2-5A synthetase and p67K kinase. *J. Gen. Virol.* 66:711.

29. Belardelli, F., F. Vignaux, E. Proietti, and I. Gresser. 1984. Injection of mice with antibody to interferon renders peritoneal macrophages permissive for vesicular stomatitis virus and encephalomyocarditis virus. *Proc. Natl. Acad. Sci. USA.* 81:602.

30. Gresser, I., F. Vignaux, F. Belardelli, M. G. Tovey, and M.-T. Maunoury. 1985. Injection of mice with antibody to mouse interferon α/β decreases the level of 2-5′-oligoadenylate synthetase in peritoneal macrophages. *J. Virol.* 53:221.

31. Haller, O., H. Arnheiter, I. Gresser, and J. Lindenmann. 1979. Genetically determined, interferon-dependent resistance to influenza virus in mice. *J. Exp. Med.* 149:601.

32. Tovey, M. G., M. Streuli, I. Gresser, J. Gugenheim, B. Blanchard, J. Guymarho, F. Vignaux, and M. Gigou. 1987. Interferon messenger RNA is produced constitutively in the organs of normal individuals. *Proc. Natl. Acad. Sci. USA.* 84:5038.

33. Svet-Moldavsky, G. J., E. G. Slavina, I. L. Leipunskaya, B. M. Nemirovskaya, S. N. Zinzar, and L. F. Morozova. 1975. Interferon, immunologic recognition and cytotoxicity of lymphoid cells. II. Specific inhibition with alloantiserum; interferon production after syngeneic tumor cells inoculation (Personal communication). *In* Interferon Scientific Memoranda. Memo I-A144.

34. Svet-Moldavsky, G. J., E. G. Slavina, I. L. Leipunskaya, N. V. Leneva, and B. M. Nemirovskaya. 1976. Interferon, immune recognition and cytotoxicity of lymphoid cells. III. Interferon production after inoculation of syngeneic hepatoma cells and alphafetoprotein. Interferon production at the interaction of lymphocytes and target-cells (Personal communication). *In* Interferon Scientific Memoranda. Memo I-A356/1.

35. Trinchieri, G., D. Santoli, B. B. Knowles. 1977. Tumour cell lines induce interferon in human lymphocytes. *Nature (Lond.).* 270:611.

36. Berger, R., W. Knapp, and H. Kirchner. 1984. Interferon induction in human mixed leukocyte-tumor-cell reactions: evidence for restriction to a certain lineage expressing glycophorin A. *Int. J. Cancer.* 34:39.

37. Landolfo, S., F. Cofano, M. Giovarelli, M. Prat, G. Cavallo, and G. Forni. 1985. Inhibition of interferon-gamma may suppress allograft reactivity by T lymphocytes in vitro and in vivo. *Science (Wash. DC).* 229:176.

38. Gresser, I., F. Belardelli, C. Maury, M. G. Tovey, and M.-T. Maunoury. 1986. Anti-

tumor effects of interferon in mice injected with interferon-sensitive and interferon-resistant Friend leukemia cells. IV. Definition of optimal treatment regimens. *Int. J. Cancer.* 38:251.

39. Locardi, C., F. Belardelli, M. Federico, G. Romeo, E. Affabris, and I. Gresser. 1987. Effect of mouse interferon α/β on the expression of H-2 (class I) antigens and on the levels of 2'-5'oligoadenylate synthetase activity in interferon-sensitive and interferon-resistant Friend leukemia cell tumors in mice. *J. Biol. Regulators Homeostatic Agents.* 1:189.

40. Gresser, I. 1985. How does interferon inhibit tumor growth? *In* Interferon 6. I. Gresser, editor. Academic Press, London. 93–126.

41. Reyes, V. E., and G. R. Klimpel. 1987. Interferon α/β synthesis during acute graft-versus-host disease. *Transplantation (Baltimore).* 43:412.

42. Kamada, N. 1985. The immunology of experimental liver transplantation in the rat. *Immunology.* 55:369.