

Injection of mice with antibody to interferon renders peritoneal macrophages permissive for vesicular stomatitis virus and encephalomyocarditis virus

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ABSTRACT Vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV) multiply in only a small percentage of peritoneal macrophages freshly explanted from 4- to 6-week-old male or female DBA/2, BALB/c, C3H, C57BL/6, or Swiss mice. However, when these mice were injected intraperitoneally with potent sheep (or goat) anti-mouse interferon α/β globulin 4 days prior to harvesting peritoneal macrophages, the viruses multiplied to high titers and most of the cells were infected, as determined by total virus yield (VSV and EMCV), percentage of VSV antigen-positive cells (immunofluorescence), and determination of VSV infectious centers. This effect was not observed when mice were inoculated with other sheep hyperimmune or normal serum globulins. Anti-interferon globulin appeared to act *in vivo* because incubation of this globulin with peritoneal macrophages during the period of cell attachment or during the 18 hr after virus absorption did not render these cells permissive for VSV. Injection of mice with anti-interferon globulin did not affect the binding and uptake of labeled VSV by peritoneal macrophages. Although the underlying mechanism of this phenomenon is unknown, the results suggest that there may be low levels of endogenous interferon that contribute to host defense by maintaining some cells in an antiviral state.

Macrophages are considered an important component in host defense against viral infections (1-5). Several animal viruses do not multiply in macrophages when these cells are first placed in culture (refs. 6-10; J. Brücher, I. Domke, C. H. Schröder, and H. Kirchner, personal communication), suggesting that macrophages may limit viral dissemination *in vivo* by restricting viral multiplication. Injection of silica, which is toxic for macrophages (11-13), markedly enhanced several virus infections in mice (14-17). Several factors that affect the resistance or susceptibility of an animal to a given virus [age of the host (18-21), virulence of the strain of virus (22), strain of the mouse (23-31)] have been correlated with the resistance or susceptibility of host macrophages to the given virus. The finding that inoculation of "resistant strains" of mice with anti-interferon globulin rendered them susceptible to virus infection (32, 33) suggested that in some instances resistance was due in part to interferon. Thus, A2G mice were resistant to influenza virus infection and this virus did not multiply in their peritoneal macrophages (33). However, influenza A virus did multiply to high titers in A2G mice inoculated with anti-mouse interferon globulin, and it also multiplied in the peritoneal macrophages taken from these mice (33). We show herein that vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV) do not multiply *in vitro* in the peritoneal macrophages from different strains of young mice but will multiply in these cells taken from mice previously injected with anti-interferon

globulin. These results suggest the possibility that endogenous interferon is present under some physiologic conditions and maintains some cells (peritoneal macrophages) in an antiviral state.

MATERIALS AND METHODS

Animals. Four to 6-week-old male and female DBA/2, BALB/c, C3H, C57BL/6, and Swiss mice were obtained from a pathogen-free colony at the Institut de Recherches Scientifiques sur le Cancer (Villejuif, France) and from the Comitato Nazionale Energia Nucleare (Casaccia, Italy).

Hyperimmune and Normal Serum Globulins. All sera were treated to remove complement and were extensively absorbed on murine cells (34). The immunoglobulin fractions were separated by precipitation with ammonium sulfate (protein content varied between 20 and 33 mg/ml) and shown to be devoid of any cytotoxicity (34). 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" (34).

The source and activities of the different immunoglobulin preparations are shown in Table 1.

Seeding of Peritoneal Macrophages in Culture Dishes. Mice were injected intraperitoneally (i.p.) with various globulins or test substances. At times thereafter, mice were killed and the peritoneal cavity was washed with 2.5 ml of nutrient medium (RPMI 1640 medium containing 10% fetal calf serum). Peritoneal cells from each mouse were seeded in 2 wells of a 24-well plastic plate (Nunc), each well containing approximately 0.5×10^6 cells in 1 ml. Cells were allowed to fix to the plastic culture dish at 37°C for 3½ hr, and nonadherent cells were discarded. Approximately 5×10^4 cells remained firmly adherent in each well after several washings. There was no significant difference in the number of cells recovered from the peritoneal cavities of mice injected with the different hyperimmune or normal serum globulins or in the number of cells adherent to the culture wells.

The experiments to be described were undertaken only with peritoneal cells firmly adherent to the culture wells after vigorous washing. The cells could be detached by trypsin only with some difficulty. Over 95% of the cells were stained for nonspecific esterase by using techniques previously described (36) and were positive in immunofluorescence studies using a rat monoclonal antibody (F4/80) specific for mouse macrophages (provided by S. Gordon and A. B. Ezekowitz). By electron microscopy these cells had a morphology characteristic of peritoneal macrophages.

Viruses. The origin, methods of preparation, and assay of VSV (Indiana strain) and EMCV have been described (37).

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Abbreviations: VSV, vesicular stomatitis virus; EMCV, encephalomyocarditis virus; i.p., intraperitoneally.

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Table 1. Sources and activities of immunoglobulins

Description	Source*	Neutralizing titer†	Reference
Anti-mouse interferon globulin			
Sheep no. 1-7	IRSC	1.6×10^{-6}	34
Sheep no. 5A	IRSC	2.5×10^{-5}	34
Sheep (NIH)	NIH	1.6×10^{-5}	Catalog no. G-024-501-568
Goat DM	E. De Maeyer	6.4×10^{-4}	35
Control hyperimmune globulins			
Sheep no. 11 anti-impurities	IRSC	$<1 \times 10^{-1}$	34
Sheep no. 4 partially immunized with mouse interferon	IRSC	2.5×10^{-1}	34
Normal serum globulins			
Sheep no. 2	IRSC	$<1 \times 10^{-1}$	34
Sheep (NIH)	NIH	$<1 \times 10^{-1}$	Catalog no. G-025-501-568
Goat	E. De Maeyer	$<1 \times 10^{-1}$	35

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†Titer measured in a test against 4–8 units of mouse α/β interferon.

Determination of Viral Multiplication in Peritoneal Macrophages: Virus Yield. In each well, 0.2 ml of a viral dilution was added to peritoneal macrophages. Because similar results were observed over a wide range of multiplicity of VSV infection of peritoneal macrophages, we used a multiplicity of infection of approximately 2 in most experiments. After 1 hr of incubation at 37°C the cell sheet was washed thoroughly, and 1 ml of nutrient medium containing 10% fetal calf serum was added. After incubation for 18 hr at 37°C in a 5% CO₂/air incubator, the cells were frozen and thawed three times, the cell extract was centrifuged, and the supernatant was titered on a monolayer of L 929 cells.

In a few experiments peritoneal macrophages were infected *in vivo*. Mice were injected i.p. with 10⁶ tissue culture infectious dose (TCID₅₀) of VSV. One hour later the peritoneal cells from individual mice were harvested and placed in culture. After 3 hr, the nonadherent cells were discarded and after several washings the adherent macrophages were left for an additional 17 hr at 37°C, at which time total virus yield was determined.

Immunofluorescence. Hyperimmune rabbit anti-VSV se-

rum (a gift of G. B. Rossi, Istituto Superiore di Sanità, Rome) was preabsorbed three times at 4°C for 1 hr with 1.8×10^7 peritoneal cells from DBA/2 mice. Peritoneal macrophages seeded on glass slides were infected with VSV at a multiplicity of infection of 1. After 1 hr at 37°C the cells were thoroughly washed and incubated with 100 μ l of a suitable dilution of anti-VSV serum. After 30 min at 4°C, the cells were washed and incubated with 100 μ l of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin (Nordic, Tilburg, Netherlands). After 30 min at 0°C, the cells were washed and 500 cells were examined to determine the percentage of fluorescent cells.

Infectious Centers. Peritoneal macrophages were infected with VSV at a multiplicity of infection of 10. After incubation for 1 hr, virus was removed, the cell sheet was washed, and anti-VSV serum was added to the nutrient medium for 20 min at 37°C and 10 min at 4°C. The medium was removed, the cell sheet was washed and treated with trypsin, and 100 μ l of a suspension containing different numbers of infected cells (10^2 to 10^3) was added to confluent monolayers of L cells in 35-mm Petri dishes (Falcon) (two dishes per dilution). The techniques of plaque formation in 1% Bacto agar have been described (38).

Preparation of ³⁵S-Labeled VSV. Confluent monolayers of L cells in roller bottles were maintained for 3 hr in methionine-free medium containing 0.5% dialyzed fetal calf serum. The cells were infected with VSV at a multiplicity of infection of 0.3. After 2 hr at 37°C, the virus was removed and medium containing [³⁵S]methionine (1,275 Ci/mmol, Amersham; 1 Ci = 37 GBq) at 37 μ Ci/ml was added. After 18 hr, total virus yield was collected and purified by sucrose gradient centrifugation as previously described for Sindbis virus (39). The virus titer after purification was 1.4×10^7 plaque-forming units/ml (1.3×10^7 cpm/ml). By polyacrylamide gel electrophoresis and fluorography, only viral proteins were detected in this preparation.

Statistical Analyses. All data were analyzed by Student's *t* test.

RESULTS

Effect of Injection of Mice with Anti-Interferon Globulin on the Multiplication of VSV and EMCV in Peritoneal Macrophages. Four- to 6-week-old male or female DBA/2 mice were inoculated i.p. with sheep anti-interferon globulin. Four days later, peritoneal cells were harvested and infected with VSV or EMCV. As can be seen from the results of three representative experiments (Table 2), minimal multiplication occurred in the peritoneal cells from untreated mice or mice treated with anti-impurities globulin. In contrast, a 100-fold or greater increase in virus yield was observed in peritoneal macrophages taken from mice that had been injected with anti-interferon globulin.

In accord with these results, only a small percentage (1.4–6%) of peritoneal macrophages from control mice were positive for VSV antigen at 12 hr, whereas most (75–81%) of the

Table 2. Effect of inoculation of mice with anti-mouse interferon globulin on the multiplication of VSV and EMCV in peritoneal macrophages *in vitro*

Globulin treatment	Mean virus yield/0.2 ml, log ₁₀			
	Exp. 1		Exp. 2	Exp. 3
	VSV	EMC	VSV	VSV
None	1.7 ± 0.1	2.4 ± 0.2	1.1 ± 0.1	2.5 ± 0.2
Sheep anti-impurities	1.7 ± 0.1	2.8 ± 0.3	1.3 ± 0.2	1.9 ± 0.3
Sheep no. 2 normal	NT	NT	1.6 ± 0.3	NT
Sheep anti-mouse interferon	4.3 ± 0.3	4.9 ± 0.7	4.1 ± 0.4	4.3 ± 0.2

Five- to 6-week-old male or female DBA/2 mice were injected i.p. with 0.2 ml of sheep no. 1-7 anti-mouse interferon globulin or anti-impurities globulin or normal sheep no. 2 globulin (diluted 1:10) 4 days before peritoneal cells were harvested. Virus yield per 0.2 ml was determined 18 hr after injection of peritoneal macrophages *in vitro*. Results are given \pm SEM. There were four mice per group in exps. 1 and 3 and five mice per group in exp. 2. NT, not tested; NS, not significant; *, *P* < 0.01; †, *P* < 0.001.

Table 3. Effect of inoculation of DBA/2 mice with anti-mouse interferon globulin on the multiplication of VSV in peritoneal macrophages as determined by immunofluorescence and by the number of infectious centers

Globulin treatment	Mouse no.	Fluorescence-positive cells after infection,* %			Infectious centers	
		3 hr	6 hr	12 hr	No. positive cells/ total no. cells plated	Positive cells, %
None	1	1.2	0.5	1.4	2/10 ⁵	0.002
	2	0.5	0.5	3.0	4/10 ⁵	0.004
	3	3.0	2.1	6.0	1.5/10 ⁴	0.015
Anti-impurities	1	0.5	2.5	5.0	2/10 ⁵	0.002
	2	4.0	5.0	3.5	2.5/10 ⁵ 1.5/10 ⁴	0.0025 0.015
Anti-mouse interferon	1	7.5	27.4	81.0	9/10 ²	9.0
	2	9.4	14.5	75.5	12.5/10 ²	12.5
	3	10.1	38.1	79.0	19.0/10 ²	19.0

*Six-week-old female DBA/2 mice were injected i.p. with 0.2 ml of a 1:10 dilution of either sheep anti-impurities globulin or sheep no. 1-7 anti-interferon globulin 4 days before peritoneal cells were harvested. Cells were seeded at 37°C for 4 hr in nutrient medium containing 10% fetal calf serum, washed twice with medium, and infected with VSV. VSV antigen expression was determined by indirect immunofluorescence techniques. Peritoneal cells were harvested from individual mice at the indicated times after infection and seeded on six slides (per mouse). Two slides were examined at each of the times. The numbers are the average of the two slides.

cells from anti-interferon globulin-treated mice were positive at this time (Table 3). Likewise, there was approximately a 1,000-fold difference between the number of infected cells taken from control mice and mice injected with anti-interferon globulin as determined by the infectious centers assay (Table 3).

Permissiveness of Peritoneal Macrophages for VSV Is Induced by Different Anti-Mouse Interferon Globulins but Not by Other Hyperimmune or Normal Serum Globulins. The results presented in Table 4 show that injection of anti-mouse interferon globulins from three sheep and one goat (from three laboratories) all rendered peritoneal macrophages permissive for VSV, whereas two hyperimmune globulins having slight or no anti-mouse interferon activity and two other normal serum globulins were ineffective.

We also undertook experiments in which mice in different groups were injected with thioglycolate, normal rabbit serum, rabbit anti-bovine serum albumin serum, rabbit anti-goat IgG serum, another normal sheep (no. 16) globulin, and sheep anti-human immunoglobulin. None of these rendered peritoneal macrophages permissive for VSV (data not shown). As a positive control, a group of mice was injected with sheep anti-mouse interferon globulin in each experiment.

Strain and Age of Mice. In addition to DBA/2 mice, injection of 4- to 6-week-old BALB/c, C3H, C57BL/6, and Swiss mice with anti-interferon globulin also abrogated the state of nonpermissiveness of peritoneal cells for VSV (data not shown). A similar effect was also observed in 5-week-old athymic nude C3H mice.

In the experiments described, we have used 4- to 6-week-old mice. In two experiments, we compared the response of peritoneal macrophages from 3-week-old and 5-month-old control and anti-interferon globulin-injected DBA/2 mice and 2- and 10-month-old C57BL/6 mice. VSV multiplication was significantly more pronounced in the peritoneal macrophages from older control mice than in the macrophages from younger control mice but was still further increased 12- to 100-fold by injection of mice with anti-interferon globulin (data not shown).

Kinetics of the Effect of Inoculation of Mice with Anti-Mouse Interferon Globulin on the Multiplication of VSV in Peritoneal Macrophages. The results of the experiments presented in Table 5 illustrate several points. Anti-interferon globulin rendered peritoneal macrophages permissive when injected 4 days (or 8 days; data not shown) prior to harvesting peritoneal cells. It was still effective when injected at -24 hr, albeit less effective than at -4 days. Anti-interferon

globulin was ineffective when injected 1 hr prior to harvesting peritoneal cells or when added to the nutrient medium either during the period of peritoneal cell attachment or during the period of viral multiplication.

Effect of Inoculation of Different Amounts of Anti-Mouse Interferon Globulin on the Multiplication of VSV in Peritoneal Macrophages. Inoculation of mice with a 1:10 or a 1:100 dilution of sheep no. 1-7 anti-interferon globulin (1.6×10^5 and 1.6×10^4 neutralizing units, respectively) exerted an effect, whereas no difference was observed when smaller amounts of globulin were injected.

Table 4. Effect of inoculation of DBA/2 mice with various anti-mouse interferon, control hyperimmune, and normal serum globulins on the multiplication of VSV in peritoneal macrophages

Globulin treatment	Mean VSV yield/0.2 ml, log ₁₀			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
None	2.3 ± 0.3	1.8 ± 0.2	≤0.7	1.9 ± 0.1
Anti-mouse interferon				
Sheep 1-7	4.5 ± 0.2	4.0 ± 0.2	4.4 ± 0.1	4.3 ± 0.4
Sheep 5A	4.5 ± 0.1			
Sheep NIH			4.6 ± 0.2	
Goat DM				4.0 ± 0.6
Control hyperimmune				
Sheep anti-mouse impurities		2.1 ± 0.3		
Sheep anti-mouse interferon (partially immunized)		1.8 ± 0.2		
Normal serum				
Sheep NIH			0.8 ± 0.2	
Goat				1.7 ± 0.1

Four- to 6-week-old male and female DBA/2 mice were injected i.p. with different globulin preparations 4 days before peritoneal cells were harvested. Virus yield per 0.2 ml was determined 18 hr after infection of peritoneal macrophages *in vitro*. There were five mice per group in each experiment. Results are given ±SEM. In expts. 1 and 2, mice were injected with immune globulins diluted 1:10. In exp. 3, the globulins of sheep 1-7 and sheep NIH were diluted 1:10 and 1:5, respectively. In exp. 4, the globulins of sheep 1-7 and goat DM were diluted 1:100 and 1:4, respectively. The difference between the mean VSV yield from peritoneal macrophages from mice injected with anti-interferon globulin and the virus yield from peritoneal macrophages from mice injected with control globulins was highly significant ($P < 0.001$). The significant values have been italicized.

Table 5. Kinetics of the effect of inoculation of anti-mouse interferon globulin in DBA/2 and C57BL/6 mice on the multiplication of VSV in peritoneal macrophages

Time of injection of anti-mouse interferon globulin	Virus yield/0.2 ml, log ₁₀		
	Exp. 1 (DBA/2)	Exp. 2 (DBA/2)	Exp. 3 (C57BL/6)
-4 days	4.2 ± 0.5	5.1 ± 0.4	3.7 ± 0.3
-1 day	NT	NT	2.3 ± 0.4
-1 hr	2.2 ± 0.1	2.2 ± 0.2	1.2 ± 0.2
<i>In vitro</i> treatment during cell attachment	2.5 ± 0.1	NT	NT
<i>In vitro</i> treatment after VSV infection	NT	2.4 ± 0.2	NT
No treatment	2.0 ± 0.2	2.2 ± 0.2	0.7 ± 0.3

Eight-week-old male DBA/2 or C57BL/6 mice were inoculated i.p. with 0.2 ml of sheep no. 1-7 anti-interferon globulin diluted 1:10. VSV yield per 0.2 ml was determined 18 hr after infection of peritoneal macrophages *in vitro*. Results are given ±SEM. For *in vitro* exposure of macrophages to anti-interferon globulin (sheep 1-7), the antiserum was diluted 1:100. There were four mice per group in expts. 1 and 2 and five mice per group in exp. 3. NT, not tested; NS, not significant; *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$.

Injection of Mice with Anti-Interferon Globulin Does Not Affect the Binding of Labeled VSV to Peritoneal Macrophages *in Vitro*. The experiments described above showed that injection of young DBA/2 mice with anti-interferon globulin rendered peritoneal macrophages permissive for VSV. The results of the experiment illustrated in Table 6 show that the increased yield of VSV from peritoneal macrophages from mice injected with anti-interferon globulin (compared to control mice) was not due to differences in virus absorption, because the binding and uptake of labeled VSV by peritoneal macrophages was similar for the different groups. Similar results were obtained with macrophages from 5-week-old male C57BL/6 mice.

Anti-Interferon Globulin Also Renders Peritoneal Macrophages Permissive for VSV When Infection Occurs *in Vivo*. In all the experiments described above, peritoneal macrophages were first placed in culture and then infected with virus. It was of interest to determine the effect of injection of anti-interferon globulin on the multiplication of VSV in peritoneal macrophages infected *in vivo*. Accordingly, 5-week-old male DBA/2 mice were injected i.p. with a 1:10 dilution of either sheep 1-7 anti-interferon globulin or normal serum globulin. Four days later mice were injected i.p. with 10⁶ TCID₅₀ of VSV. When the peritoneal macrophages were placed in culture, it was clear that VSV multiplied poorly in macrophages from mice preinjected with normal serum globulin (log₁₀ TCID₅₀ = 1.8 ± 0.4), whereas the virus did multiply in macrophages from mice injected with anti-interferon globulin (log₁₀ TCID₅₀ = 3.3 ± 0.6).

DISCUSSION

In the experiments described above, peritoneal cells from 4- to 6-week-old mice of different strains were placed in culture and the adherent esterase-positive cells (macrophages) were infected with VSV (most of the experiments) or EMCV. These viruses multiplied in only a few of these cells. In contrast, injection of mice with potent sheep or goat anti-mouse

interferon α/β globulin abrogated this state of nonpermissiveness, and the virus yield was 100- to 1,000-fold greater from macrophages from these mice than from mice injected with other hyperimmune or normal serum globulins. Similar results were obtained when peritoneal macrophages were infected *in vivo*, prior to being harvested. The results indicated that anti-mouse interferon globulin acted *in vivo*: (i) injection of mice with anti-interferon globulin 8 and 4 days prior to harvesting peritoneal cells was more effective than injection of mice with anti-interferon globulin 1 day before harvesting these cells; (ii) injection of mice with antibody 1 hr prior to harvesting macrophages or addition of anti-interferon globulin to macrophages *in vitro* did not render them permissive for VSV.

Only potent anti-mouse interferon globulins (prepared from four animals in three laboratories) and none of the other hyperimmune or normal serum globulins exerted this effect. How do the anti-interferon globulins render peritoneal macrophages permissive for VSV? It is clear that they do not alter the binding or uptake of radiolabeled VSV to peritoneal macrophages (Table 6). The simplest explanation would be that there are low levels of endogenous interferon in the peritoneum of young mice and that this interferon induces an antiviral state in some cells (i.e., peritoneal macrophages). Anti-interferon globulin would presumably neutralize this endogenous interferon. However, we have been unable to demonstrate any biologically active interferon in the peritoneal cavity, serum, or various tissues of young or old mice by using sensitive cell assay systems (40). There are, in fact, very few reports of the recovery of biologically active interferon in normal human or animal tissues. Cantell and Pyhälä (41) reported that sera from normal rabbits and especially pregnant rabbits exhibited anti-VSV activity in their routine assays for human interferon, but the inhibitory substance was not characterized. V. Bocci, M. Muscettola, L. Paulesu, and G. Grasso have recovered small amounts of an acid-labile interferon from the lymph of healthy rabbits (personal

Table 6. Lack of correlation between the binding and uptake of ³⁵S-labeled VSV and virus yield in peritoneal macrophages from mice injected with anti-interferon globulin

Globulin treatment	No. of mice	³⁵ S-labeled VSV bound,* cpm	³⁵ S-labeled VSV internalized,† cpm	VSV yield,‡ log ₁₀
None	5	3856 ± 158	460 ± 44	2.1 ± 0.2
Anti-impurities	4	4497 ± 255	471 ± 69	2.5 ± 0.2
Anti-mouse interferon	5	4003 ± 325	460 ± 29	4.1 ± 0.1

Seven-week-old DBA/2 mice were injected i.p. with 0.2 ml of a 1:10 dilution of either sheep anti-impurities globulin or sheep 1-7 anti-interferon globulin 4 days before peritoneal cells were harvested. Peritoneal cells were seeded and infected with 2.8 × 10⁴ plaque-forming units per well of ³⁵S-labeled VSV (27,136 cpm). Results are given as mean ± SEM. NS, not significant.

*After 1 hr at 37°C cells were washed three times with phosphate-buffered saline, solubilized in 150 μl of 1% sodium dodecyl sulfate, and added to 10 ml of scintillation liquid (Lumagel from Lumac). There were two wells for cells from each mouse.

†After 1 hr at 37°C, cells were washed twice with phosphate-buffered saline, treated with trypsin (1 mg/ml) and EDTA (1 mM), transferred to plastic tubes, and centrifuged. Cells were further washed with phosphate-buffered saline and pellets were solubilized in 150 μl of 1% sodium dodecyl sulfate and added to 10 ml of scintillation liquid.

‡Virus yield per 0.2 ml was determined 18 hr after infection of peritoneal macrophages *in vitro*.

communication). Lebon *et al.* (42) reported finding low levels of interferon in the amniotic fluid of 39 out of 40 pregnant women. Normal rabbit peritoneal macrophages (43) and human leukocytes (44) liberate an interferon-like substance after several hours in culture. It is possible, therefore, that biologically active interferon is present in very small amounts or that it is present in some combined form not detectable by our techniques.

One other possibility should be considered: i.e., that injection of anti-interferon globulin selected in some manner a macrophage population permissive for VSV. There was, however, no difference in the number of peritoneal cells recovered from mice in the different groups, in the number of cells adherent to the culture dishes, or in the percentage of esterase-positive cells.

Our previous work showed that injection of mice with antibody to interferon α/β markedly enhanced the severity of several virus infections (32–34, 45). The experimental results also showed that anti-interferon globulin neutralized the interferon produced shortly after viral infection, permitting viral multiplication to ensue unchecked in various organs. Large amounts of interferon were produced subsequently, but this interferon no longer appeared to benefit the host. It was thus the *early* production of interferon that appeared to be an important factor in determining the outcome of virus infection, a conclusion which has been supported by the recent work of Kirchner and co-workers (46, 47). We and others have stressed, therefore, the importance of interferon as an integral part of the host response to viral infection. The results presented herein suggest that low levels of endogenous interferon may also be present in mice *prior* to viral infection. This endogenous interferon would induce a constitutive antiviral state in some host cells (i.e., peritoneal macrophages in our experiments).

We postulate that, in some instances, the capacity of macrophages to restrict viral multiplication (see Introduction) is not necessarily an inherent property of these cells, as is usually thought, but stems from their interaction in the animal with an extrinsic factor—namely, interferon. Because these cells take up viruses but do not permit their multiplication, they would indeed serve as an efficient barrier to dissemination of virus in the organism. Last, in addition to its antiviral action, interferon can modulate cell function and division (48). It seems possible, therefore, that endogenous interferon may, in addition to inducing an antiviral state, also exhibit more general effects on normal cell physiology.

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1. Mims, C. A. (1964) *Bacteriol. Rev.* **28**, 30–71.
2. Gresser, I. & Lang, D. J. (1966) *Prog. Med. Virol.* **8**, 62–130.
3. Glasgow, L. A. (1970) *Arch. Intern. Med.* **126**, 125–134.
4. Mims, C. A. (1972) in *Microbial Pathogenicity in Man and Animals*, Symposia of the Society for General Microbiology, eds. Smith, H. & Pearce, J. H. (Cambridge University Press, Cambridge, England), No. 22, pp. 333–358.
5. Allison, A. C. (1974) *Prog. Med. Virol.* **18**, 15–31.
6. Dickinson, L. & Griffiths, A. J. (1966) *Br. J. Exp. Pathol.* **47**, 35–44.
7. Stevens, J. G. & Cook, M. L. (1971) *J. Exp. Med.* **133**, 19–38.

8. Mims, C. A. & Gould, J. (1978) *J. Gen. Virol.* **41**, 143–153.
9. Rodgers, B. & Mims, C. A. (1981) *Infect. Immun.* **31**, 751–757.
10. Stohlman, S. A., Woodward, J. G. & Frelinger, J. A. (1982) *Infect. Immun.* **36**, 672–677.
11. Kessel, R. W. I., Monaco, L. & Marchisio, M. A. (1963) *Br. J. Exp. Pathol.* **44**, 351–364.
12. Allison, A. C., Harington, J. S. & Birbeck, M. (1966) *J. Exp. Med.* **124**, 141–154.
13. Pearsall, N. N. & Weiser, R. S. (1968) *J. Reticuloendothel. Soc.* **5**, 107–120.
14. Zisman, B., Hirsch, M. S. & Allison, A. C. (1970) *J. Immunol.* **104**, 1155–1159.
15. Zisman, B., Wheelock, E. F. & Allison, A. C. (1971) *J. Immunol.* **107**, 236–243.
16. Selgrade, M. J. & Osborn, J. E. (1974) *Infect. Immun.* **10**, 1383–1390.
17. Mogensen, S. C. & Andersen, H. K. (1977) *Infect. Immun.* **17**, 274–277.
18. Johnson, R. T. (1964) *J. Exp. Med.* **120**, 359–374.
19. Hirsch, M. S., Zisman, B. & Allison, A. C. (1970) *J. Immunol.* **104**, 1160–1165.
20. Mogensen, S. C. (1978) *Infect. Immun.* **19**, 46–50.
21. Mintz, L., Drew, W. L., Hoo, R. & Finley, T. H. (1980) *Infect. Immun.* **28**, 417–420.
22. Roberts, J. A. (1964) *J. Immunol.* **92**, 837–842.
23. Bang, F. B. & Warwick, A. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 1065–1075.
24. Goodman, G. T. & Koprowski, H. (1962) *Proc. Natl. Acad. Sci. USA* **48**, 160–165.
25. Kantoch, M. & Bang, F. B. (1962) *Proc. Natl. Acad. Sci. USA* **48**, 1553–1559.
26. Kantoch, M., Warwick, A. & Bang, F. B. (1963) *J. Exp. Med.* **117**, 781–798.
27. Virelizier, J.-L. & Allison, A. C. (1976) *Arch. Virol.* **50**, 279–285.
28. Mogensen, S. C. (1977) *Infect. Immun.* **17**, 268–273.
29. Lindenmann, J., Deuel, E., Fanconi, S. & Haller, O. (1978) *J. Exp. Med.* **147**, 531–540.
30. Bang, F. B. (1981) *Adv. Exp. Biol.* **142**, 359–373.
31. Haller, O., Arnheiter, H. & Lindenmann, J. (1979) *J. Exp. Med.* **150**, 117–126.
32. Virelizier, J.-L. & Gresser, I. (1978) *J. Immunol.* **120**, 1616–1619.
33. Haller, O., Arnheiter, H., Gresser, I. & Lindenmann, J. (1979) *J. Exp. Med.* **149**, 601–612.
34. Gresser, I., Tovey, M. G., Bandu, M.-T., Maury, C. & Brouty-Boyé, D. (1976) *J. Exp. Med.* **144**, 1305–1315.
35. De Maeyer, E. & De Maeyer-Guignard, J. (1983) *J. Immunol.* **130**, 2392–2396.
36. Mueller, J., Brun del Re, G., Buerki, H., Keller, H. U., Hess, M. W. & Cottier, H. (1975) *Eur. J. Immunol.* **5**, 270–274.
37. Gresser, I., Bourali, C., Thomas, M.-T. & Falcoff, E. (1968) *Proc. Soc. Exp. Biol. Med.* **127**, 491–496.
38. Benedetto, A., Rossi, G. B., Amici, C., Belardelli, F., Cioè, L., Carruba, G. & Carrasco, L. (1980) *Virology* **106**, 126–132.
39. Smith, A. L. & Tignor, G. H. (1980) *Arch. Virol.* **66**, 11–26.
40. Brouty-Boyé, D. (1977) *Proc. Soc. Exp. Biol. Med.* **155**, 438–439.
41. Cantell, K. & Pyhälä, L. (1976) *J. Infect. Dis.* **133**, Suppl., A6–A12.
42. Lebon, P., Girard, S., Thépot, F. & Chany, C. (1981) *C.R. Hebd. Seances Acad. Sci. Ser. III* **293**, 69–71.
43. Wagner, R. R. & Smith, T. J. (1968) in *Interferon*, CIBA Foundation Symposium, eds. Wolstenholme, G. E. W. & O'Connor, M. (J. & A. Churchill, London), pp. 95–106.
44. Gresser, I. (1968) in *Interferon*, CIBA Foundation Symposium, eds. Wolstenholme, G. E. W. & O'Connor, M. (J. & A. Churchill, London), p. 107.
45. Gresser, I., Tovey, M. G., Maury, C. & Bandu, M.-T. (1976) *J. Exp. Med.* **144**, 1316–1323.
46. Zawatzky, R., Engler, H. & Kirchner, H. (1982) *J. Gen. Virol.* **60**, 25–29.
47. Kirchner, H., Engler, H., Schröder, C. H., Zawatzky, R. & Storch, E. (1983) *J. Gen. Virol.* **64**, 437–441.
48. Vilček, J., Gresser, I. & Merigan, T. C., eds. (1980) *Ann. N.Y. Acad. Sci.* **350**.