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Injection of Mice with Antibody to Mouse Interferon α/β Decreases the Level of 2′-5′ Oligoadenylate Synthetase in Peritoneal Macrophages

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Injection of conventional or axenic weanling mice with potent sheep or goat antibody to mouse interferon α/β resulted in a decrease in the basal level of 2-5A synthetase in resting peritoneal macrophages and rendered these cells permissive for vesicular stomatitis virus. There was a good inverse correlation between the level of 2-5A synthetase in peritoneal macrophages and the permissivity of these cells for vesicular stomatitis virus. The peritoneal macrophages of 1- and 2-week-old mice had low levels of 2-5A synthetase and were permissive for vesicular stomatitis virus, whereas at 3 weeks (and after) there was a marked increase in the level of 2-5A synthetase in peritoneal macrophages, and these cells were no longer permissive for vesicular stomatitis virus. We suggest that low levels of interferon α or β or both are produced in normal mice, and that this interferon contributes to host defense by inducing and maintaining an antiviral state in some cells.

Several animal viruses do not multiply in macrophages freshly explanted from adult mice, suggesting that in vivo macrophages may limit virus spread by inhibiting virus multiplication. The resistance of resting peritoneal macrophages of adult mice to vesicular stomatitis virus (VSV) and encephalomyocarditis viruses can, however, be abrogated by injection of mice with antibody to interferon α/β (4). Although the simplest explanation for this phenomenon was that endogenous interferon had induced an antiviral state in peritoneal macrophages, we and others (36) have been unable to demonstrate any biologically active interferon in the peritoneum or in different tissues of adult mice by using sensitive cell assay systems. As an increase in the cellular level of 2′-5′ oligo-adenylate (2-5A) synthetase has been considered a reliable marker of interferon action in vitro (3, 6, 9, 12, 13, 15, 16, 29, 31, 41; reviewed in references 23 and 36) and in vivo (5, 14, 21, 22, 27, 28, 30, 33–35), it was of interest to determine whether injection of normal mice with antibody to interferon would alter the basal level of this enzyme. The results presented herein show that administration of antibody to interferon α/β decreases the level of 2-5A synthetase in peritoneal macrophages and renders these cells permissive for VSV, suggesting that low levels of interferon are present in the peritonea of normal mice.

MATERIALS AND METHODS

Mice. Male and female DBA/2 and Swiss mice were obtained from a pathogen-free colony at the Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.

Hyperimmune and normal serum globulins. All sera were decomplemented and extensively absorbed on murine cells (10). The immunoglobulin 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 (10). The anti-mouse interferon α/β globulins did not neutralize interferon γ.

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

In one experiment, we further purified the sheep no. 1-7 immunoglobulin. The immunoglobulin was delipidated according to the method of Harboe and Ingild (11). After centrifugation, the supernatant in an acetate buffer (0.05 M; pH 5.0) was passed on a carboxy methyl-Sepharose column. Analysis of the antibody and protein content of the "unabsorbed" and eluted fractions showed that the unabsorbed fraction (3B) had the highest specific activity (resulting in a ca. 10-fold purification). These results were in accord with the findings of Mogensen and Cantell (26) for the purification of sheep antibody to human interferon α (leukocyte).

Monoclonal rat anti-mouse interferon γ (and a suitable control ascitic preparation) was kindly provided by E. A. Havell (Trudeau Institute, Saranac, N.Y.) (38). The crude ascites were partially purified in our laboratory by precipitation with 45% ammonium sulfate. After purification, the anti-mouse interferon γ (rat immunoglobulin G [IgG] class 1) had a titer of 10^{-6} against 4 U of mouse interferon γ. Mouse interferon γ, a phytohemagglutinin-induced spleen cell interferon preparation partially purified on concanavalin A-Sepharose columns, was also kindly provided by E. A. Havell. The anti-mouse interferon γ showed no neutralizing activity at a 1:40 dilution against 8 U of mouse interferon α/β.

Preparation of interferon. Mouse interferon α/β was produced, partially purified (specific activity, 10^7 reference units per mg of protein), and assayed as previously described (39). One of the units as expressed in the text is the equivalent of 4 interferon reference units.

Seeding of peritoneal macrophages in culture dishes. Preliminary experiments showed that similar results were obtained when mice were injected intraperitoneally (i.p.) with antibody to mouse interferon 8, 4, or 1 day before peritoneal macrophages were harvested. In the experiments to be described below, mice were routinely injected with the

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TABLE 1. Sources and activities of the different immunoglobulin preparations

Description	Source[a]	Neutralizing titer against 4 to 8 U of mouse interferon α/β	Reference
Anti-mouse interferon globulin			
Sheep no. 1-7	IRSC	1.6×10^{-6}	10
Sheep no. 5A	IRSC	2.5×10^{-5}	10
Sheep (NIH)	Research Resources Branch, NIH[b]	1.6×10^{-5}	
Goat DM	E. De Maeyer (Orsay, France)	6.4×10^{-4}	7
Control hyperimmune globulins			
Sheep no. 11 anti-impurities	IRSC	$<1 \times 10^{-1}$	10
Sheep no. 4B partially immunized with mouse interferon	IRSC	2.5×10^{-1}	10
Normal serum globulins			
Sheep no. 2	IRSC	$<1 \times 10^{-1}$	10
Goat	E. De Maeyer (Orsay, France)	$<1 \times 10^{-1}$	7

[a] IRSC, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France; NIH, National Institutes of Health, Bethesda, Md.
[b] NIH catalog no. G-024-501-568.

different preparations 4 days before peritoneal cells were harvested.

The peritoneal cavity was washed with 3 ml of nutrient medium (RPMI 1640 medium containing 10% fetal calf serum. Peritoneal cells from each mouse were seeded in 3 wells of a 24-well plastic plate (Nunc), each well containing ca. 0.5×10^6 cells per ml. Cells were allowed to fix to the plastic culture dish at 37°C for 3 h, and nonadherent cells were discarded. One well was used to determine protein concentration, one well for determination of 2-5A synthetase, and one well for VSV yield.

The experiments to be described below 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, using techniques previously described (4), and were positive by immunofluorescence when a rat monoclonal antibody (F4/80) specific for mouse macrophages (provided by S. Gordon and A. B. Ezekowitz, Sir William Dunn School of Pathology, Oxford, England [1]) was used. By electron microscopy, these cells had a morphology characteristic of peritoneal macrophages.

Determination of protein. The protein content of peritoneal macrophages in each well was determined by the method of Lowry et al. (24). There was no significant difference in the protein content between wells containing macrophages from mice injected with the different hyperimmune or normal serum globulins.

Determination of 2-5A synthetase in peritoneal macrophages. The method described by Revel et al. (30) was used for determination of 2-5A synthetase. In brief, peritoneal macrophages seeded in one well were lysed in 100 μl of lysis buffer, and after centrifugation the supernatants were stored at −70°C before assay. In the assays, 20 μl of cell extract was mixed with beads of poly(rI):(rC)-agarose (P-L Biochemicals Inc.) (50 μl) which had been prewashed with a solution of 50 mM KCl, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.5) 20% glycerol, 5 mM $MgCl_2$, and 7 mM dithiothreitol (buffer C), and the tubes were incubated for 15 min at 30°C. The beads were washed with 1 ml of buffer C and incubated for 22 h at 30°C with a 10-μl reaction mixture containing buffer C, 3 mM ATP, and 1.6 μCi of [α-^{32}P]ATP (Amersham Corp.; ca. 400 Ci/mmol), 10 mM creatine phosphate, 3 mg of creatine kinase per ml, and 40 μg of poly (rI):(rC) per ml. Preliminary experiments showed that the kinetics of conversion of ATP into oligo-adenylate phosphatase-resistant cores increased linearly from 1 to 23 h for extracts of peritoneal macrophages from both control and anti-interferon immunoglobulin-treated mice. In the experiments described below, mixtures were incubated routinely for 22 h; 1 U of bacterial alkaline phosphatase (Sigma Chemical Co.) in 40 μl of 0.1 M Tris base was added to the tubes, which were then incubated for 2 h at 37°C. After centrifugation, 20 μl of the supernatant was added to acid alumina columns (Sigma). The oligo-adenylate phosphatase-resistant cores were recovered from the columns with 1 M glycine-hydrochloride buffer (pH 2) and counted by Cherenkov radiation in a scintillation counter. The results are expressed as picomoles of ATP incorporated per hour per microgram of protein.

Determination of the multiplication of VSV in peritoneal macrophages. The origin, methods of preparation, and assay of VSV (Indiana strain) have been previously described (4). 0.2 ml of a viral dilution (0.2 ml) (multiplicity of infection, ca. 2) was added to peritoneal macrophages in each well (after the 3-h period of cell attachment to the plastic plate and washing). After 1 h 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 h at 37°C in a 5% CO_2-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. The results are expressed as $\log_{10}$ 50% tissue culture infectious doses per 0.2 ml.

Preparation of thymocytes and mesenteric lymph node cells: determination of 2-5A synthetase and multiplication of VSV. Thymocytes and mesenteric lymph node cells were obtained by teasing and filtering the cells through a metallic grid. The level of 2-5A synthetase was determined for 2×10^7 thymocytes and 5×10^6 mesenteric lymph node cells, using the techique described for peritoneal macrophages. VSV multiplication was determined in suspensions of thymocytes (5×10^6 cells per ml) or mesenteric lymph node cells (2.5×10^6 cells per ml) after incubation for 18 h at 37°C in a 5% CO_2-air incubator.

Statistical analysis. Results were analyzed by using Student's *t* test and covariance analysis.

RESULTS

Effect of antibody to interferon α/β on the level of 2-5A synthetase in peritoneal macrophages and their permissivity for VSV. In a series of four experiments, 4- to 6-week-old male and female DBA/2 and Swiss mice were injected i.p. with 0.2 ml of sheep 1-7 antibody to mouse interferon α/β or with control immunoglobulins, or left untreated. Four days later, the peritoneal macrophages were harvested and the levels of 2-5A synthetase were determined, as well as the

capacity of these macrophages to support multiplication of VSV.

Lower levels of 2-5A synthetase were present in macrophages from mice injected with antibody to interferon compared with the levels in macrophages from control mice (Fig. 1). VSV multiplied in macrophages from mice injected with antibody to interferon, whereas VSV multiplied poorly or not at all in macrophages from control mice. (Statistical analysis of the results of 52 values plotted on a logarithmic scale showed a linear relationship between the level of 2-5A synthetase and the VSV yield [$y = -3.120 \times +4.514$; $r = -0.794$; $P < 0.001$]).

Relationship between the amount of antibody to interferon injected, the level of 2-5A synthetase, and the permissivity of peritoneal macrophages for VSV. A clear-cut effect on both VSV multiplication and the level of 2-5A synthetase was observed in mice injected with 10^3 U or more of neutralizing antibody to interferon α/β (Fig. 2). Again, there was an inverse correlation between the level of 2-5A synthetase activity and the permissivity of peritoneal macrophages for VSV.

Injection of different anti-mouse interferon globulins but not control globulins results in a decrease in the level of 2-5A synthetase in peritoneal macrophages and renders them permissive for VSV. In all of the experiments described above, we injected mice with sheep no. 1-7 antibody to mouse interferon. It was considered important to determine the effect of other anti-interferon and normal serum globulins on the level of 2-5A synthetase in peritoneal macrophages and on the permissivity of these cells for VSV.

Injection of DBA/2 mice with anti-mouse interferon globulins from three sheep and one goat (from three laboratories) rendered peritoneal macrophages permissive for VSV and resulted in a significant decrease in the level of 2-5A synthetase (Table 2). In contrast, injection of globulins from a normal sheep, a normal goat, and from a sheep (4B) partially immunized with mouse interferon (see above) were ineffective. Statistical analysis of the results of 45 determinations plotted on a log scale showed a linear relationship between the level of 2-5A synthetase and the VSV yield ($y = -2.673 \times +4.310$; $r = -0.841$; $P < 0.001$).

Injection of a monoclonal antibody to mouse interferon γ did not render peritoneal macrophages permissive for VSV or lower the basal level of 2-5A synthetase (data not shown).

Antibody to interferon α/β is also effective in axenic mice. The experiments described above were undertaken with pathogen-free mice. It was of interest to determine whether peritoneal macrophages from adult axenic mice were also nonpermissive for VSV and whether administration of antibody to interferon would exert any effect. For this experiment, 5-week-old female axenic Swiss mice were injected i.p. with 10^5 U of sheep 1-7 antibody to mouse interferon or a control immunoglobulin (sheep no. 11), and 4 days later the peritoneal macrophages were harvested. Injection of antibody to interferon resulted in a decrease in the level of 2-5A synthetase in peritoneal macrophages, and the VSV yield

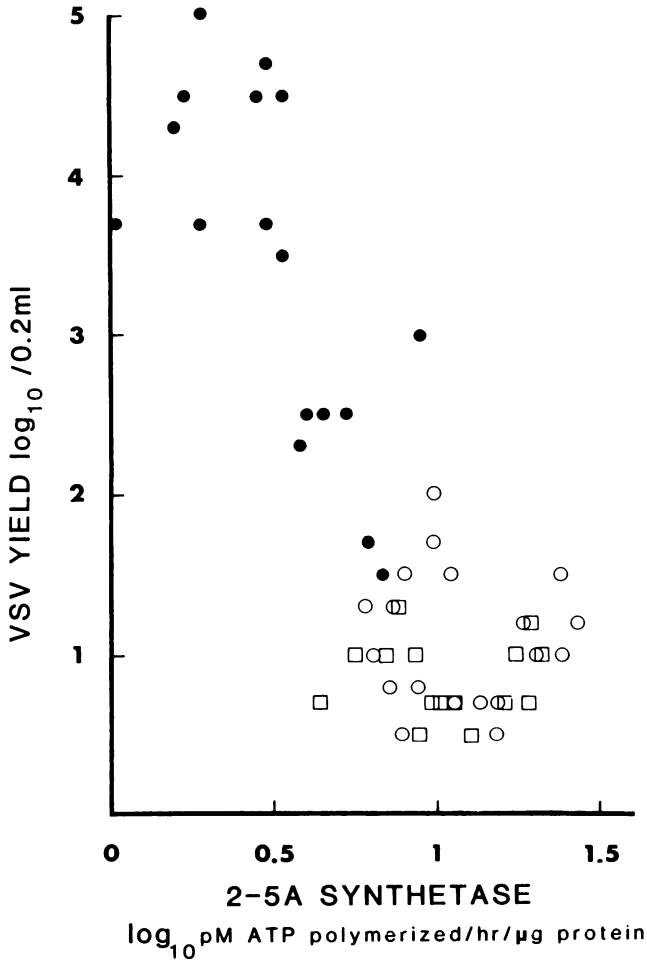

FIG. 1. Relationship between the level of 2-5A synthetase in peritoneal macrophages from mice injected with antibody to interferon α/β or control globulins and the permissivity of these macrophages for VSV. Four- to 5-week-old male and female DBA/2 mice and 5-week-old female Swiss mice were injected i.p. with 0.2 ml of sheep 1-7 antibody to mouse interferon α/β (10^5 neutralizing units) (●) or control immunoglobulin (○) 4 days before peritoneal macrophages were harvested. Other mice were left untreated (□). The VSV yield and the 2-5A synthetase level were determined as described in the text. Each point represents the value obtained with peritoneal macrophages from an individual mouse.

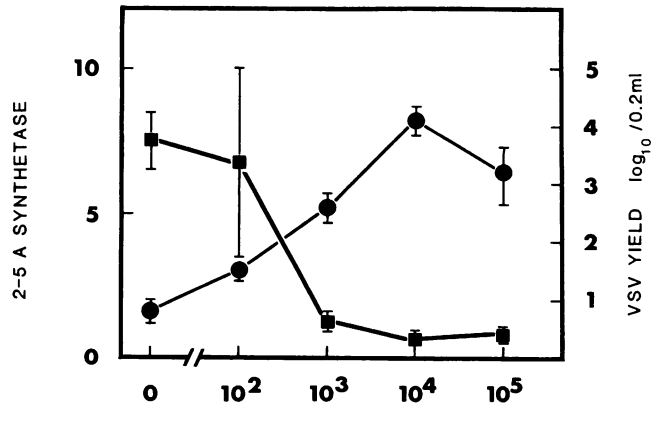

FIG. 2. Effect of injection of different dilutions of antibody on the level of 2-5A synthetase and the permissivity of peritoneal macrophages for VSV. Five-week-old male DBA/2 mice were injected i.p. with 0.2 ml of 10-fold dilutions of sheep (1-7) antibody to interferon α/β or were left untreated. Four days later, the peritoneal macrophages were harvested and the level of 2-5A synthetase (■) (picomoles of ATP polymerized per hour per microgram of protein) and the multiplication of VSV (●) were determined. The results are expressed as the mean ± standard error for five mice tested individually in each group.

TABLE 2. Injection of DBA/2 mice with anti-interferon globulin decreases the level of 2-5A synthetase in peritoneal macrophages and renders them permissive for VSV[a]

Treatment	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	2-5A synthetase	VSV yield	2-5A synthetase	VSV yield	2-5A synthetase	VSV yield	2-5A synthetase	VSV yield
None	8.2 ± 1.6	0.8 ± 0.1	12.8 ± 2.3	0.8 ± 0.0	14.7 ± 2.7	1.0 ± 0.1	4.3 ± 1.5	<0.5
Anti-mouse interferon α/β globulin								
Sheep no. 1-7	3.0 ± 0.4	4.1 ± 0.6			2.2 ± 0.6	3.7 ± 0.3	0.8 ± 0.1	2.1 ± 0.1
Sheep no. 1-7(3B)							0.3 ± 0.1	2.3 ± 0.6
Sheep no. 5A					2.5 ± 0.5	3.0 ± 0.4		
Sheep NIH					2.6 ± 0.4	3.7 ± 0.3		
Goat DM			5.3 ± 1.1	3.0 ± 0.5				
Control globulin								
Sheep no. 2	11.3 ± 0.3	1.1 ± 0.3			11.5 ± 3.2	1.5 ± 0.1		
Sheep no. 4B					10.6 ± 2.4	1.7 ± 0.2		
Goat			20.9 ± 2.2	1.0 ± 0.1				

[a] Four- to five-week-old male and female DBA/2 mice were injected i.p. with 0.2 ml of the different globulin preparations 4 days before peritoneal cells were harvested. The neutralizing titers of the anti-interferon globulin preparations injected were as follows: sheep 1-7, 1.6×10^5; sheep 1-7 (3B), 8×10^4; sheep 5A, 2.5×10^4; sheep NIH, 3×10^4; goat DM, 2×10^4. The neutralizing titers of all the control globulin preparations were less than 1:10. Macrophages were tested from each mouse individually, and the results are expressed as the mean ± standard error for five mice per group. 2-5A synthetase was determined on peritoneal macrophages lysed 3 h after plating, and is expressed as picomoles of ATP polymerized per hour per microgram of protein. Virus yield per 0.2 ml $\log_{10}$ was determined 18 h after infection of peritoneal macrophages in vitro. The difference between the mean level of 2-5A synthetase and VSV yield in peritoneal macrophages from mice injected with anti-mouse interferon globulins and the mean level of 2-5A synthetase and VSV yield in peritoneal macrophages from control mice (injected with control preparation or left untreated) was highly significant in all experiments. The significant values have been underlined.

was more than 100-fold greater in these macrophages than in macrophages from control mice (Table 3).

Administration of interferon increases the level of 2-5A synthetase in peritoneal macrophages. As injection of antibody to interferon resulted in a decrease in the level of 2-5A synthetase in peritoneal macrophages, it was of interest to determine the effect of administration of interferon α/β on the level of the enzyme in macrophages. Accordingly, 4-week-old female DBA/2 mice were injected i.p. daily for 4 days with 0.2 ml of a partially purified interferon preparation having a titer of 10^{-6}, or with a control preparation, or were left untreated (there were five mice per group). The level of 2-5A synthetase in peritoneal macrophages of untreated mice was 1.62 ± 1.1 pmol/h per μg of protein, 1.20 ± 0.26 for control-treated mice, and 9.82 ± 1.79 for interferon-treated mice. Thus, interferon administration clearly increased the level of 2-5A synthetase in peritoneal macrophages ($P < 0.01$).

In another series of experiments, we incubated freshly plated peritoneal macrophages from 4-week-old mice for 24 h with various amounts of interferon. A clear-cut increase (three- to fourfold) in the level of 2-5A synthetase was observed in macrophage cultures incubated with 100 or 1,000 U of partially purified mouse interferon α/β (data not shown).

TABLE 3. Injection of axenic Swiss mice with anti-interferon globulin decreases the level of 2-5A synthetase in peritoneal macrophages and renders them permissive for VSV[a]

Treatment	2-5A synthetase[b]	VSV yield[c]
Control hyperimmune globulin (no. 11)	3.2 ± 0.6	2.1 ± 0 4
Anti-mouse interferon globulin (no. 1–7)	1.8 ± 0.4	4.4 ± 0.1

[a] Five-week-old axenic Swiss mice were injected i.p. with 0.2 ml of the indicated globulin 4 days before peritoneal cells were harvested. The activity of 2-5A synthetase picomoles of ATP polymerized per hour per microgram of protein) and VSV yield ($\log_{10}$) were determined as described in the text. The results are expressed as the mean ± standard error for four mice per group.
[b] For these two values, $P < 0.05$.
[c] For these two values, $P < 0.01$.

Influence of mouse age on the level of 2-5A synthetase in peritoneal macrophages and their permissivity for VSV. As shown previously (4) and herein, macrophages from 4-week-old DBA/2 mice were nonpermissive for VSV. However, peritoneal macrophages from 1- and 2-week-old DBA/2 mice were permissive for VSV, and the cells had low levels of 2-5A synthetase (Fig. 3). In contrast, peritoneal macrophages from 3- and 4-week-old DBA/2 mice were no longer permissive for VSV, and levels of 2-5A synthetase were increased.

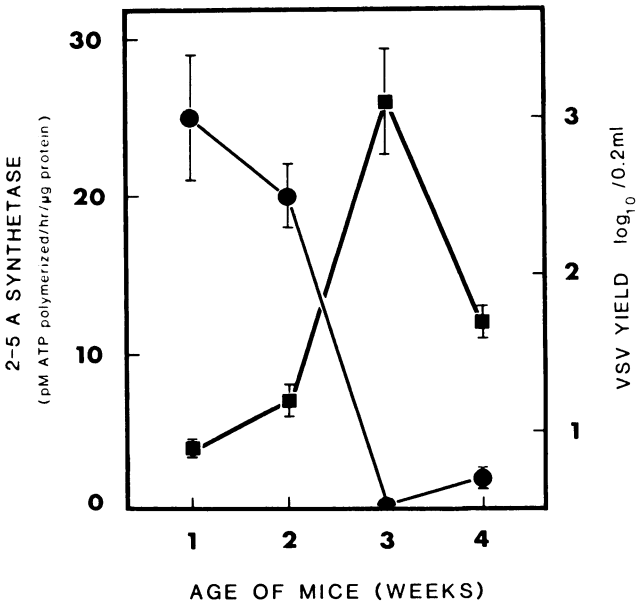

FIG. 3. Peritoneal macrophages were harvested from normal DBA/2 mice. Macrophages were taken at 1 and 2 weeks from male and female mice (five pools of two mice) and at 3 and 4 weeks from five male mice per group. The results presented are the mean ± standard error for the levels of 2-5A synthetase (■) and VSV yield (●) determined as previously described.

TABLE 4. Effect of injection of 1-week-old DBA/2 mice with interferon or anti-interferon globulin: level of 2-5A synthetase and the permissivity for VSV in peritoneal macrophages[a]

Treatment	2-5A synthetase	VSV yield
None	<0.5	3.6 ± 0.3
Anti-mouse interferon globulin	1.1 ± 0.6	3.6 ± 0.3
Interferon	9.6 ± 0.8	<0.5

[a] Newborn mice from three litters of DBA/2 mice were divided into three groups: (i) untreated; (ii) injected i.p. with 10^5 neutralizing units of anti-interferon globulin 1-7 four days before peritoneal cells were harvested; (iii) injected daily i.p. and subcutaneously with 0.1 ml of interferon α/β (titer, 6×10^5) on days 5 to 7. All mice were sacrificed on day 8. The activity of 2-5A synthetase (picomoles of ATP polymerized per hour per microgram of protein) and VSV yield ($\log_{10}$) were determined as described in the text. The results are expressed as the mean ± standard error for five suckling mice per group. Differences among values in each column were statistically significant ($P < 0.001$).

The finding that peritoneal macrophages from 1-week-old mice exhibited low levels of 2-5A synthetase activity and were permissive for VSV suggested that either endogenous biologically active interferon was not present in the peritonea of these young mice or that their peritoneal macrophages were resistant to interferon action. To test these possibilities, suckling mice were either injected with antibody to interferon α/β or treated with interferon. Peritoneal macrophages from untreated 1-week-old mice again exhibited low levels of 2-5A synthetase and were permissive for VSV (Table 4). Injection of these mice with antibody to interferon α/β did not significantly alter these values, whereas injection of interferon increased the level of 2-5A synthetase in the peritoneal macrophages and rendered them totally resistant to VSV.

In most of the experiments described herein, peritoneal macrophages from 4-week-old mice were tested. In other experiments, it was found that the peritoneal macrophages from 8-week-old mice were also nonpermissive for VSV; but at 4 and 6 months of age, peritoneal macrophages appeared somewhat more permissive for VSV. Injection of 6-month-old mice with antibody to interferon α/β decreased the level of 2-5A synthetase and rendered these cells completely permissive for VSV. In contrast, administration of partially purified interferon α/β (10^6 U) i.p. for 2 days markedly increased the level of 2-5A synthetase and rendered the peritoneal macrophages resistant to VSV (data not shown).

Level of 2-5A synthetase and permissivity for VSV in suspensions of thymocytes and mesenteric lymph node cells from mice injected with antibody to interferon or with interferon. Thymocytes from untreated 6-week-old DBA/2 mice had very low levels of 2-5A synthetase, and the cells were fully permissive for VSV (Table 5). Injection of mice with antibody to interferon α/β did not significantly change these values, whereas administration of interferon α/β decreased the permissivity of these cells for VSV. In contrast, mesenteric lymph node cells from untreated mice were relatively nonpermissive. Injection of antibody to interferon decreased the level of 2-5A synthetase and rendered the cells more permissive, whereas injection of interferon markedly increased the level of 2-5A synthetase and rendered mesenteric lymph node cells totally resistant to VSV.

DISCUSSION

The results presented herein showed that there was an excellent inverse correlation between the level of 2-5A synthetase in freshly explanted resting peritoneal macrophages and the permissivity of these cells for VSV (Fig. 1). Thus, injection of potent antibody to interferon α/β (but not to interferon γ) from three sheep and one goat (prepared in three laboratories) resulted, in each experiment, in a clear-cut decrease in the basal level of 2-5A synthetase in peritoneal macrophages and was accompanied by a marked increase in the permissivity of these cells for VSV (Table 2). The extent of this effect of antibody on the level of 2-5A synthetase and on VSV yield correlated with the amount of antibody injected (Fig. 2). The basal level of 2-5A synthetase in macrophages varied somewhat from one experiment to another, although in each of 15 experiments, injection of antibody to interferon consistently lowered the level of 2-5A synthetase in peritoneal macrophages of 4-week-old mice compared with control macrophages within a given experiment. Similar variations in the basal level of 2-5A synthetase in human Daudi cells have been previously reported (40).

There are considerable data suggesting that the 2-5A system may play a role in the induction of the antiviral state (reviewed in references 23 and 36). It is possible, therefore, that the decrease in the basal level of 2-5A synthetase in peritoneal macrophages induced by injection of antibody to interferon α/β was causally related to the abrogation of the antiviral state in these cells. On the other hand, it is entirely possible that injection of antibody decreased the level of 2-5A synthetase in peritoneal macrophages and abrogated the antiviral state, and that these two effects were not causally related. There are, in fact, several examples of a lack of correlation between the level of 2-5A synthetase and the antiviral state (25, 36). Moreover, the work of Baglioni and Nilsen suggests that 2-5A system may not be involved at all in the inhibition of VSV multiplication in interferon-treated cells (2). However, biological effects have been ascribed to the 2-5A system other than participation in the induction of an antiviral state, such as lymphocyte proliferation (19, 20), Friend cell differentiation (18), and regeneration of rat liver after partial hepatectomy (8). In these instances, the authors suggested that the changes in 2-5A synthetase may have been related to changes in interferon levels. The point we should like to stress from this work, regardless of the biological role of 2-5A synthetase, is that the decrease in the level of 2-5A synthetase in peritoneal macrophages observed after injection of antibody to interferon is further evidence for the presence of endogenous interferon.

TABLE 5. Level of 2-5A synthetase and permissivity for VSV in suspensions of thymocytes and mesenteric lymph node cells from normal DBA/2 mice and from mice injected with antibody to interferon α/β or with interferon α/β[a]

Cells	Treatment	2-5A synthetase	VSV yield
Thymocytes	None	≤0.10	4.3 ± 0.1[b]
	Anti-interferon globulin	0.2 ± 0.1	4.2 ± 0.2[b]
	Interferon	1.2 ± 0.8	2.0 ± 0.0[b]
Mesenteric lymph node	None	4.5 ± 0.5[c]	1.9 ± 0.2
	Anti-interferon globulin	1.2 ± 0.4[c]	2.9 ± 0.1
	Interferon	24.5 ± 0.7[c]	≤0.7

[a] Six-week-old male DBA/2 mice were divided into three groups of four mice each: (i) untreated; (ii) injected i.p. with 10^5 neutralizing units of anti-interferon globulin 1-7 4 days before cells were harvested; (iii) injected i.p. with 0.2 ml of mouse interferon α/β (titer, 6×10^5) twice daily in the 2 days preceding cell harvest. There were two pools of two mice for each group. The activity of 2-5A synthetase (picomoles of ATP polymerized per hour per microgram of protein) and VSV yield ($\log_{10}$) were determined as described in the text. The results are expressed as the mean ± standard error.

[b,c] Differences among values in each of these groups were statistically significant.

Our results also show that the basal level of 2-5A synthetase in peritoneal macrophages and the permissivity of these cells for VSV changes in the early weeks of life. Thus, the level of 2-5A synthetase in peritoneal macrophages from normal 1- and 2-week-old DBA/2 mice was low, and these cells were permissive for VSV, whereas the basal levels of 2-5A synthetase in these cells increased in 3- and 4-week-old mice together with a loss of cellular permissivity for VSV (Fig. 3). It seems likely that the low levels of 2-5A synthetase activity and the permissivity for VSV in peritoneal macrophages of 1-week-old mice could be attributed to the absence of biologically active interferon in the peritoneum rather than to a resistance of these cells to interferon. Thus, injection of 1-week-old mice with antibody to interferon did not change the levels of 2-5A synthetase or the permissivity of peritoneal macrophages for VSV, whereas injection of interferon enhanced ninefold the level of 2-5A synthetase and rendered the cells completely resistant to VSV (Table 4).

It is of interest that age is also an important factor in the development of spontaneous spleen NK cell activity and resistance to parental bone marrow grafts. Both activities are low in mice under 3 weeks of age but develop rapidly after the week 3 of life (17). Interferon has been considered one of the important factors in modulating both of these activities (32). We do not know whether these different phenomena are related to developmental changes in the mouse associated with the production of interferon or whether they are dependent on environmental conditions. Among the environmental conditions, one may consider the role of bacterial flora. Antibody proved effective in axenic mice (Table 3), but this does not rule out the presence of low levels of endotoxin in the food that may have served as an inducer of low levels of interferon.

Finally, although the work we have presented concerned for the most part the state of resting peritoneal macrophages, it seems likely that a similar situation obtains for some other cells in mice. Shimizu and Sokawa (37) showed that the level of 2-5A synthetase was higher in mouse splenocytes and mesenteric lymph node cells than in thymocytes. Kimchi (18) found that there were higher levels of 2-5A synthetase in peripheral mature T lymphocytes of mice than in thymocytes, and that there were higher levels of synthetase activity in mature than in immature thymocytes. Our results show that the level of 2-5A synthetase is indeed low in thymocytes from 4-week-old DBA/2 mice and that these cells are fully permissive for VSV. Injection of antibody to interferon did not alter this state of permissivity. Thymocytes, however, were responsive to exogenous interferon, which induced an antiviral state in these cells (Table 5). In contrast, mesenteric lymph node cells, like peritoneal macrophages, exhibited higher basal levels of 2-5A synthetase and were relatively nonpermissive for VSV. Injection of antibody to interferon decreased the level of 2-5A synthetase and increased the permissivity for VSV, whereas injection of interferon induced the opposite effect. We suggest, therefore, that low levels of interferon may be produced locally and preferentially in some tissues of normal adult mice and may contribute to host defense by inducing and maintaining an antiviral state in some cells.

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LITERATURE CITED

1. **Austyn, J. M., and S. Gordon.** 1981. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. **11**:805–815.
2. **Baglioni, C., and T. W. Nilsen.** 1983. Mechanisms of antiviral action of interferon, p. 23–42. *In* I. Gresser (ed.), Interferon 5. Academic Press, Inc., London.
3. **Ball, A., and C. N. White.** 1978. Oligonucleotide inhibitor of protein synthesis made in extracts of interferon-treated chick embryo cells: comparison with the mouse low molecular weight inhibitor. Proc. Natl. Acad. Sci. U.S.A. **75**:1167–1171.
4. **Belardelli, F., F. Vignaux, E. Proietti, and I. Gresser.** 1984. Injection of mice with antibody to interferon renders peritoneal macrophages permissive for vesicular stomatitis and encephalomyocarditis virus. Proc. Natl. Acad. Sci. U.S.A. **81**:602–606.
5. **Buffet-Janvresse, C., and A. G. Hovanessian.** 1984. Enzyme markers for the presence of circulating interferon: 2-5A synthetase in blood lymphocytes and protein kinase in platelet-rich plasma. Proc. Soc. Exp. Biol. Med. **175**:169–175.
6. **Clemens, M. J., and B. R. G. Williams.** 1978. Inhibition of cell-free protein synthesis by pppA$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A: a novel oligonucleotide synthesized by interferon-treated L cell extracts. Cell **13**:565–572.
7. **De Maeyer, E., and J. De Maeyer-Guignard.** 1983. Delayed hypersensitivity to Newcastle disease virus in high and low interferon-producing mice. J. Immunol. **130**:2392–2396.
8. **Etienne-Smekens, M., P. Vandenbussche, J. Content, and J. E. Dumont.** 1983. (2′-5′) Oligoadenylate in rat liver: modulation after partial hepatectomy. Proc. Natl. Acad. Sci. U.S.A. **80**:4609–4613.
9. **Farrell, P. J., G. C. Sen, M. F. Dubois, L. Ratner, E. Slattery, and P. Lengyel.** 1978. Interferon action: two distinct pathways for inhibition of protein synthesis by double-stranded RNA. Proc. Natl. Acad. Sci. U.S.A. **75**:5893–5897.
10. **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**:1305–1315.
11. **Harboe, N., and A. Inglid.** 1973. Isolation of immunoglobulins from antisera. Scand. J. Immunol. **2**(Suppl. 1):162.
12. **Hovanessian, A. G., R. E. Brown, and I. M. Kerr.** 1977. Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells. Nature (London) **268**:537–540.
13. **Hovanessian, A. G., and I. M. Kerr.** 1978. Synthesis of an oligonucleotide inhibitor of protein synthesis in rabbit reticulocyte lysates analogous to that formed in extracts from interferon-treated cells. Eur. J. Biochem. **84**:149–159.
14. **Hovanessian, A. G., Y. Rivière, N. Robert, J. Svab, S. Chamaret, J.-C. Guillon, and L. Montagnier.** 1981. Protein kinase (pp67-IFN) in plasma and tissues of mice with high levels of circulating interferon. Ann. Virol. (Paris) **132E**:175–188.
15. **Kerr, I. M., and R. E. Brown.** 1978. PppA2′p5′A2′p5′A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc. Natl. Acad. Sci. U.S.A. **75**:256–260.
16. **Kerr, I. M., R. E. Brown, and A. G. Hovanessian.** 1977. Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA. Nature (London) **268**:540–542.
17. **Kiessling, R., P. S. Hochman, O. Haller, G. M. Shearer, H. Wigzell, and G. Cudkowicz.** 1977. Evidence for a similar or

common mechanism for natural killer cell activity and resistance to hemopoietic grafts. Eur. J. Immunol. **7**:655–663.
18. **Kimchi, A.** 1981. Increased levels of interferon-induced (2′-5′) oligo-isoadenylate synthetase in mature T-lymphocytes and in differentiated Friend-erythroleukemic cells. J. Interferon Res. **1**:559–569.
19. **Kimchi, A., H. Shure, and M. Revel.** 1979. Regulation of lymphocyte mitogenesis by (2′-5′) oligoisoadenylate. Nature (London) **282**:849–851.
20. **Kimchi, A., H. Shure, and M. Revel.** 1981. Antimitogenic function of interferon-induced (2′-5′) oligo-isoadenylate, and growth related variations in enzymes that synthesize and degrade this oligonucleotide. Eur. J. Biochem. **114**:5–10.
21. **Krishnan, I., and C. Baglioni.** 1980. 2′5′ Oligo(A) polymerase activity in serum of mice infected with EMC virus or treated with interferon. Nature (London) **285**:485–488.
22. **Krust, B., Y. Rivière, and A. G. Hovanessian.** 1982. p67K kinase in different tissues and plasma of control and interferon-treated mice. Virology **120**:240–246.
23. **Lengyel, P.** 1983. Mechanisms of interferon action: the (2′-5′) $(A)_n$ synthetase-RNase L pathway, p. 77–99. *In* I. Gresser (ed.), Interferon 3. Academic Press, Inc. London.
24. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
25. **Meurs, E., A. G. Hovanessian, and L. Montagnier.** 1981. Interferon mediated antiviral state in human MRC5 cells in the absence of detectable levels of 2-5A synthetase and protein kinase. J. Interferon Res. **1**:219–232.
26. **Mogensen, K. E., and K. Cantell.** 1979. Non-specific adsorption of interferon to immobilized serum immunoglobulin. J. Gen. Virol. **45**:171–175.
27. **Penn, L. J. Z., and B. R. G. Williams.** 1984. Interferon-induced 2-5A synthetase activity in human peripheral blood mononuclear cells after immunization with influenza virus and rubella virus vaccines. J. Virol. **49**:748–753.
28. **Preble, O. T., K. Rothko, J. H. Klippel, R. M. Friedman, and M. I. Johnston.** 1983. Interferon-induced 2′-5′ adenylate synthetase *in vivo* and interferon production *in vitro* by lymphocytes from systemic lupus erythematosus patients with and without circulating interferon. J. Exp. Med. **157**:2140–2146.
29. **Ratner, L., R. C. Wiegand, P. J. Farrell, G. C. Sen, B. Cabrer, and P. Lengyel.** 1978. Interferon, double-stranded RNA and RNA degradation. Fractionation of the endonuclease$_{Int}$ system into two macromolecular components; role of a small molecule in nuclease activation. Biochem. Biophys. Res. Commun. **81**:947–954.
30. **Revel, M., A. Schattner, D. Wallach, G. Merlin, H. Levavi, T. Hahn, and S. Levin.** 1982. Monitoring of interferon therapy, diagnosis of viral diseases, and detection of interferon deficiencies by assay of an interferon-induced enzyme in human peripheral white blood cells, p. 353–367. *In* R. Kono and J. Vilcek (ed.), The clinical potential of interferons. Tokyo University Press, Tokyo.
31. **Roberts, W. K., A. G. Hovanessian, R. E. Brown, M. J. Clemens, and I. M. Kerr.** 1976. Interferon-mediated protein kinase and low-molecular weight inhibitor of protein synthesis. Nature (London) **264**:477–480.
32. **Saksela, E.** 1981. Interferon and natural killer cells, p. 45–63. *In* I. Gresser (ed.), Interferon 3, Academic Press, London.
33. **Saron, M.-F., Y. Rivière, A. G. Hovanessian, and J.-C. Guillon.** 1982. Chronic production of interferon in carrier mice congenitally infected with lymphocytic choriomeningitis virus. Virology **117**:253–256.
34. **Schattner, A., G. Merlin, S. Levin, D. Wallach, T. Hahn, and M. Revel.** 1981. Assay of an interferon-induced enzyme in white blood cells as a diagnostic aid in viral diseases. Lancet **ii**:497–500.
35. **Schattner, A., G. Merlin, A. Shapira, M. Revel, and D. Wallach.** 1982. Comparison of (2′-5′)oligo-adenylate synthetase and interferon blood-levels in mice early after viral infection. J. Interferon Res. **2**:285–289.
36. **Sen, G. S.** 1982. Mechanism of interferon action: progress towards its understanding. Prog. Nucleic Acid Res. Mol. Biol. **27**:105–156.
37. **Shimizu, N., and Y. Sokawa.** 1979. 2′-5′ Oligoadenylate synthetase activity in lymphocytes from normal mouse. J. Biol. Chem. **254**:12034–12037.
38. **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**: 1560–1565.
39. **Tovey, M. G., J. Begon-Lours, and I. Gresser.** 1974. A method for the large scale production of potent interferon preparations. Proc. Soc. Exp. Biol. Med. **146**:809–815.
40. **Tovey, M. G., M. Dron, K. E. Mogensen, B. Lebleu, N. Mechti, and J. Begon-Lours-Guymarho.** 1983. Isolation of Daudi cells with reduced sensitivity to interferon. II. On the mechanisms of resistance. J. Gen. Virol. **64**:2649–2653.
41. **Zilberstein, A., A. Kimchi, A. Schmidt, and M. Revel.** 1978. Isolation of two interferon-induced translational inhibitors: a protein kinase and an oligo-isoadenylate synthetase. Proc. Natl. Acad. Sci. U.S.A. **75**:4734–4738.