Expression of Interferon-Induced Genes in Different Tissues of Mice

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In vivo responses to interferon (IFN) in mice were determined by measuring the steady-state levels of induced mRNAs following injection of IFN and poly(I)-poly(C). With cDNA probes for mouse 2'-5' oligoadenylate synthetase (2-5A synthetase) and 1-8, constitutive expression of the corresponding mRNA was detectable in different organs of normal C3H/He mice. These mRNA levels were increased by as much as 15-fold over control levels in various tissues, including the brain, after IFN and poly(I)-poly(C) treatment, coincident with increases in 2-5A synthetase enzyme activity. The basal activity level of this enzyme could be reduced in normal mice by treatment with anti-mouse IFN (α+β) antibody. This treatment also reduced the levels of 2-5A synthetase and 1-8 mRNAs. Thus, physiological levels of circulating IFN maintain elevated levels of IFN-induced mRNAs in mice. Furthermore, changes in 2-5A synthetase enzyme activity reflect the changes in gene expression in vivo.

The biological activity of interferons (IFN) in cell culture is mediated largely by induction of a number of novel proteins. Whereas many of these proteins have not been characterized in detail, the enzyme 2'-5' oligoadenylate (2-5A) synthetase, has been used as an indicator of interferon activity both in vitro and in vivo (see reference 23 for a review). This IFN-induced enzyme, when activated by double-stranded RNA (dsRNA), polymerizes ATP into 2'-5'-linked oligomers which activate a latent endoribonuclease responsible for degradation of viral and cellular RNAs (5). Low basal levels of 2-5A synthetase activity are detectable in different organs of mice, and this level is enhanced severalfold by injection of IFN or inducers of IFN, such as a virus and the synthetic dsRNA poly(I)-poly(C) (10, 12, 14, 17, 24). When normal mice are injected with anti-mouse IFN (α+β) antibody, the basal levels of 2-5A synthetase activity are reduced severalfold compared with those of nontreated animals (7, 8). This result indicates that physiological levels of 2-5A synthetase activity in mice are due, at least in part, to low levels of circulating IFN (α+β). The level of IFN is generally too low to be detected in the sera of normal mice (7), possibly because circulating IFN has a very short half-life in vivo (2). The physiological role of IFN produced continuously in normal mice is unclear; however, it is possible that it is functional in different processes, such as growth and differentiation, and in mediating an antiviral activity expressed in healthy animals.

The objective of this study was to determine whether the above-mentioned changes in 2-5A synthetase enzyme activity are the result of specific changes in gene expression in vivo. It was also of interest to compare the responses of different tissues to intravenous injection of inducers of IFN and, conversely, to anti-IFN (α+β) antibodies. In vitro systems have shown that treatment of cells with IFN results in transcriptional induction of a number of mRNAs (19), including that for 2-5A synthetase (1, 21), and a family of transcripts of unknown function, called 1-8, isolated and characterized by Friedman and co-workers (6). Using transcription run-on assays, Hannigan and Williams (9) have

shown that the increase in the rate of transcription of both 2-5A synthetase and 1-8 mRNAs and their subsequent transcriptional down regulation closely follow the binding to and down-regulation of cell surface-localized IFN receptors. The murine bone marrow cell line JLSV-9 also responds to IFN with an increase in transcription of 2-5A synthetase (20) and 1-8 mRNAs (unpublished data). To address the question of in vivo responses to IFN at the level of gene expression, we cloned cDNAs of murine 2-5A synthetase and 1-8, which enabled us to examine the steady-state levels of these mRNAs after intravenous injection of IFN and poly(I)-poly(C) in normal C3H/He mice. Constitutive expression of 2-5A synthetase and 1-8 mRNAs was detectable in different organs of normal C3H/He mice. These basal levels were greatly increased after IFN and poly(I)-poly(C) treatment, as were the levels of 2-5A synthetase enzyme activity. Thus, the changes in enzyme activity reflect the changes in gene expression in vivo. Accordingly, mRNA levels of 2-5A synthetase and 1-8, in addition to 2-5A synthetase enzyme activity, may be used as markers for the presence and action of IFN.

MATERIALS AND METHODS

Mice. Pathogen-free, 6- to 8-week-old C3H/He female mice were obtained from the Institut Pasteur, Paris, France. Animals were maintained in the animal laboratory for the duration of the experiments.

IFN (α+β) treatment. Mice were injected intravenously with 10^5 U of mouse IFN (α+β) (2.5×10^8 National Institutes of Health U/mg of protein) (7). Animals were sacrificed at 2, 5, 10, 15, and 24 h after IFN injection. Control mice (0 h) were sacrificed at the time of administration of IFN.

Poly(I)-poly(C) treatment. Mice were injected intravenously with 200 µg of poly(I)-poly(C) (P-L Biochemicals, Inc., Milwaukee, Wis.) prepared as described previously by Hovanessian and Riviere (12). Control animals were injected with phosphate-buffered saline. Animals were sacrificed at 4 and 15 h after poly(I)-poly(C) injection.

Anti-mouse IFN (α+β) antibody treatment. Mice were injected intravenously with sheep anti-mouse IFN (α+β) antibody (50 µg/ml) with a neutralizing titer of 10^{-5} against 5

National Institutes of Health U of mouse IFN (α+β). Preparation of this antibody was described previously by Galabru et al. (7). Control animals were injected with unimmunized sheep serum. The anti-mouse IFN (α+β) antibody injection protocol used in these experiments is described in the table footnotes and figure legends.

Preparation of tissue extracts. Mice were sacrificed by cervical dislocation. Tissues were removed and immediately frozen in liquid nitrogen. Frozen tissues were homogenized mechanically (Polytron, type PT 10/35; Kinematica, Lucerne, Switzerland) in low-salt buffer (10 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] [pH 7.6], 10 mM KCl, 2 mM magnesium acetate, 7 mM 2-mercaptoethanol, aprotinin [Iniprol; Laboratoire Choay, Paris, France] at 100 U/ml, where 1 U = 35 ng). This suspension was left for 15 min at 4°C before addition of Nonidet P-40 to a final concentration of 0.5%. After 15 min, each suspension was sonicated for 10 s and centrifuged at 5,000 × g for 20 min. Tissue extracts were stored at −80°C until assayed for 2-5 A synthetase activity.

Assay of 2-5A synthetase. Activity of 2-5A synthetase was assayed after partial purification by poly(I)-poly(C)-Sepharose Cl-4B (prepared as described previously by Hovanessian et al. [13]) in a reaction mixture (600 μl) containing 20 mM HEPES (pH 7.6), 50 mM KCl, 25 mM magnesium acetate, 7 mM 2-mercaptoethanol, 5 mM ATP, 10 mM creatine phosphate, 0.16 mg of creatine kinase per ml, 0.1 mg of poly(I)-poly(C) per ml, and 5 μl of [³H]ATP (0.2 μCi/ml; Amersham Corp., Arlington Heights, Ill.). Reaction mixtures were incubated for 90 min at 30°C and terminated by being heated at 90°C for 5 min. ³H-labeled 2-5A was purified by DEAE-cellulose chromatography as described previously by Buffet-Janvresse et al. (3). The concentration of 2-5A in AMP equivalents was estimated from the percent incorporation of the radioactivity from input [³H]ATP into [³H]2-5A counts per minute. The 2-5A synthetase levels were calculated on this basis and are given as units corresponding to 1 nmol of 2-5A synthesized per milligram of tissue per hour.

RNA isolation and Northern (RNA) blotting. Total RNA was prepared by using the guanidinium thiocyanate method described by Chirgwin et al. (4). Total RNA samples (15 μg each) were denatured in 50% formamide–6.7% formaldehyde in MOPS buffer (400 mM MOPS [morpholine propanesulfonic acid] [pH 7], 100 mM sodium acetate, 10 mM EDTA). After being heated for 10 min at 65°C, samples were electrophoresed in a 1.5% agarose gel containing 6.4% formaldehyde in MOPS buffer. RNA was transferred by capillarity to a nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, N.H.). Hybridization to [³²P]dATP-labeled, nicktranslated cDNA probes (10⁸ cpm/μg) was performed at 42°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt solution–0.1% sodium dodecyl sulfate–150 μg of denatured salmon sperm per ml. The most stringent wash was performed in 0.1× SSC–0.1% sodium dodecyl sulfate for 30 min at 42°C. Filters were then exposed to Fuji RX film with Kyokko intensifying screens for autoradiography.

Slot blotting. Total RNA samples (10 μg each) were denatured in 7.4% formaldehyde in 6× SSC. After being heated for 15 min at 65°C, samples were slot blotted (Schleicher & Schuell Minifold II) immediately onto a nitrocellulose membrane (Schleicher & Schuell). Hybridization was performed as in the Northern blot procedure except that, where indicated, [³²P]UTP-labeled RNA probes synthesized by using the Promega riboprobe system were used in place of nick-translated probes. In this case, hybridization

was performed at 55°C in 5× SSPE (1× SSPE is 0.15 M NaCl, 10 mM sodium phosphate monobasic, 1 mM EDTA)–5× Denhardt solution–0.1% sodium dodecyl sulfate–100 μg of *Saccharomyces cerevisiae* tRNA per ml. The most stringent wash was performed at 65°C for 120 min in 0.1× SSPE–0.1% sodium dodecyl sulfate. A Joyce-Loebl Chromoscan-3 laser densitometer was used for scanning and peak integration.

Isolation of IFN-induced mouse-specific clones for 1-8 and 2-5A synthetase. Mouse 2-5A synthetase and 1-8 cDNAs were isolated from a λgt11 cDNA library constructed from IFN-treated mouse JLSV-9 cells (C. Dieffenbach, H. Jacobsen, and R. Silverman, unpublished data). Human 2-5A synthetase 6-2 cDNA (21) was nick translated and used as the probe to isolate cDNAs for murine 2-5A synthetase. A number of overlapping cDNA clones were obtained and sequenced to reveal the homology between the human and murine cDNAs. The 3' region of murine cDNA clone J-2 (20) has 67% nucleic acid sequence homology with human exons 4 and 5 present in the 3' region of human cDNA 6-2. For the studies described here, two probes derived from the cDNA clone J-2 were prepared as follows. (i) A nick-translated murine 2-5A synthetase probe was prepared from a 331-base-pair (bp) fragment of cDNA J-2 which is homologous to human exon 4. The 3' terminus corresponds to nucleotide 936, and the 5' terminus is 78 bp upstream of nucleotide 693, as described by Ichii and co-workers (15). Further analysis of several murine cDNAs indicated that this 78-bp region represents part of the intron which lies upstream of the murine exon homologous to human exon 4. (ii) An RNA probe for murine 2-5A synthetase was prepared from cDNA J-2, which had been cloned in the reverse orientation into plasmid pSP65. With SP6 polymerase, an antisense probe was prepared which included 708 bp of the 3' end of cDNA J-2, corresponding to nucleotides 693 to 1401, as described by Ichii and co-workers (15).

Mouse 1-8 cDNA was isolated by using human 1-8 cDNA as described by Friedman and co-workers (6). A partial cDNA of 360 bp was isolated which had approximately 75% nucleic acid sequence homology to human 1-8 cDNA (M. McMahon, Ph.D. thesis, University of London, England, 1985). The nick-translated mouse 1-8 probe used in this study was synthesized from a 220-bp fragment created after removal of the poly(A) tract.

RESULTS

Increased 2-5A synthetase and 1-8 mRNA levels after IFN injection. Intravenous injection of IFN has previously been shown to induce 2-5A synthetase activity over the basal levels found in many different organs of mice (12, 16). In this study, we investigated the level of 2-5A synthetase mRNA in the spleens of mice in response to treatment with exogenous IFN. In these same mice, the level of 2-5A synthetase activity in the lungs was measured to monitor the response of each mouse to the IFN. Accordingly, mice were sacrificed 2, 5, 10, 15, and 24 h after IFN administration to assay the level of 2-5A synthetase by enzyme activity and by mRNA levels in the lungs and spleens, respectively. Synthetase activity gradually increased over the study period, and at 24 h it reached a level twofold higher than that in control lung tissue at time 0 (Table 1). To determine whether this increase in enzyme activity resulted from an increase in the steady-state levels of the 2-5A synthetase message, mRNA isolated from spleen tissues at each time point was hybridized to the mouse 2-5A synthetase probe. A 1.7-kilobase message could

TABLE 1. Kinetics of enhancement of 2-5A synthetase activity
in lungs of mice treated with IFN[a]

Time (h)	Mean 2-5A synthetase activity (nmol of AMP/mg per h)
0	12.3
2	11.5
5	14.8
10	19.2
15	23.4
24	24.7

[a] Mice were injected intravenously with 10^5 Units of mouse IFN ($\alpha+\beta$) (2.5 $\times 10^8$ National Institutes of Health U/mg of protein) and sacrificed at 2, 5, 10, or 24 h post-IFN injection. Control mice were sacrificed at the time of IFN administration as represented by time zero. The 2-5A synthetase activity in the lungs was measured after partial purification on poly(I)-poly(C)-Sepharose. Each value represents the mean of three independent assays.

be visualized by Northern blot hybridization (Fig. 1). The increase in the level of expression of 2-5A synthetase mRNA was quantitated by slot blot hybridization followed by densitometry. The arbitrary densitometer numbers were modified such that the control message levels were normalized to 100. All experimental levels may be easily interpreted as
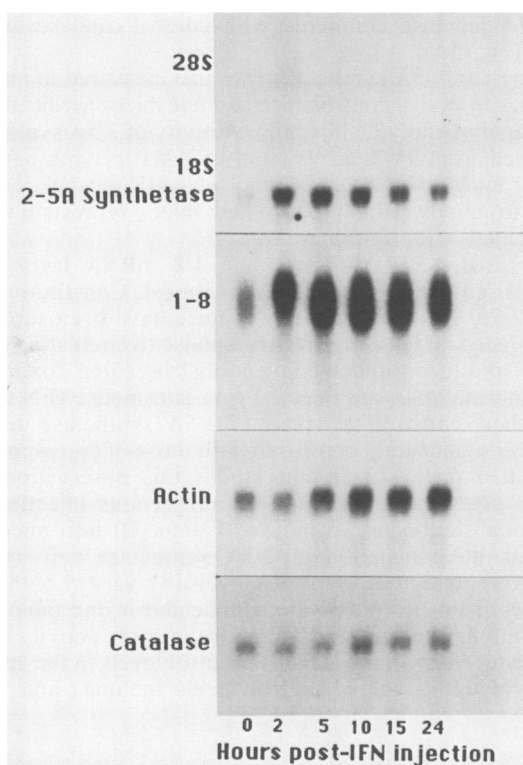

FIG. 1. Induction of 2-5A synthetase and 1-8 mRNAs in spleens of mice treated with IFN. Mice were injected with 10^5 U of mouse IFN ($\alpha+\beta$) and sacrificed at 2, 5, 10, 15, and 24 h after IFN injection. Control mice were sacrificed at the time of IFN injection as represented by time zero. Total RNA was prepared (see Materials and Methods) from three spleens at each time point and pooled. Each lane contained 15 μg of total RNA. After electrophoresis and Northern blotting (see Materials and Methods), the membrane was hybridized to a nick-translated mouse 2-5A synthetase cDNA. The same membrane was stripped and sequentially hybridized with nick-translated mouse 1-8, β-actin, and mouse catalase (R. Korneluk, unpublished data) cDNAs. rRNA markers of 28S and 18S are indicated.

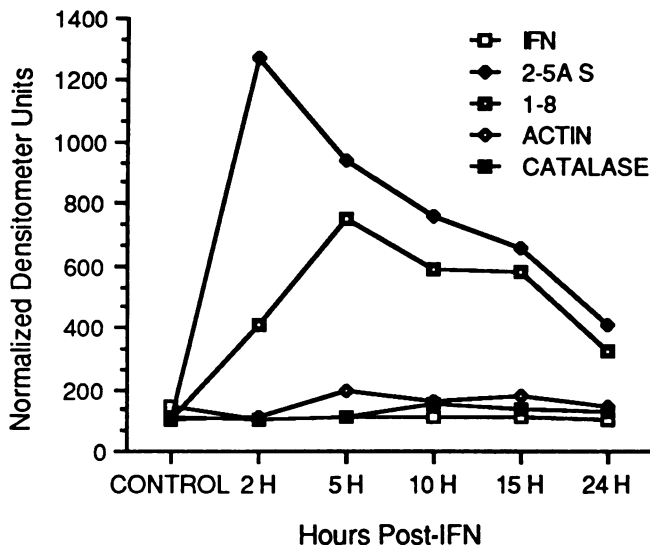FIG. 2. Quantitation of 2-5A synthetase and 1-8 mRNA levels in spleens of mice treated with mouse IFN ($\alpha+\beta$). Total RNA was prepared (see Materials and Methods) from the spleens of mice sacrificed at 0, 2, 5, 10, 15, and 24 h post-IFN injection. Total RNA was pooled from three spleen samples at each time point, and 10 μg of RNA was slot blotted (see Materials and Methods) onto a nitrocellulose membrane. Identical blots were hybridized with nick-translated mouse IFN-β (11), mouse 2-5A synthetase, mouse 1-8, β-actin, and mouse catalase cDNAs. All blots were scanned by laser densitometry, and the units were modified such that the control level of each mRNA species was normalized to 100.

x-fold increases or decreases over the control levels. The level of the 2-5A synthetase message in the spleen increased by 13-fold over the control level, peaking at 2 h postinjection, the earliest time point examined. At 24 h after IFN treatment, 2-5A synthetase mRNA levels remained at a level fourfold above the control level (Fig. 2).

Mouse 1-8 cDNA hybridized to a family of mRNAs ranging in size from 800 to 900 bp (Fig. 1). In spleen tissue, 1-8 mRNA was induced sevenfold at 5 h post-IFN. This induction slowly fell to threefold above the control level at 24 h post-IFN injection (Fig. 2).

In contrast to the 2-5A synthetase and 1-8 mRNAs, the two control mRNAs, actin and mouse catalase (the gift of Robert G. Korneluk), varied little (Fig. 1 and 2). Whereas actin mRNA levels increased by 2-fold after IFN injection, this increase was small compared with the 13-fold and 7-fold increases of 2-5A synthetase and 1-8 mRNAs, respectively. IFN mRNA levels did not fluctuate from basal levels throughout the 24-h time course (Fig. 2).

Increased 2-5A synthetase and 1-8 mRNA levels after poly(I)-poly(C) injection. Poly(I)-poly(C), a synthetic, dsRNA analog, has been shown to increase circulating levels of IFN (10, 12). Induction of the IFN message is very difficult to measure, since the message has a very short half-life (18). In this study, there was no detectable increase in hybridization of the IFN cDNA probe at 4 and 15 h after poly(I)-poly(C) injection (Fig. 3). However, the steady-state levels of IFN-induced 2-5A synthetase and 1-8 mRNAs were elevated at both 4 and 15 h postinjection in spleen, brain, and liver tissues. Initially, mRNA levels of 1-8 and a control message, actin, were measured in individual spleen and brain tissues to determine the heterogeneity of the responses of individual mice to poly(I)-poly(C) injection. The variability in 1-8 and control mRNA levels between individual mice
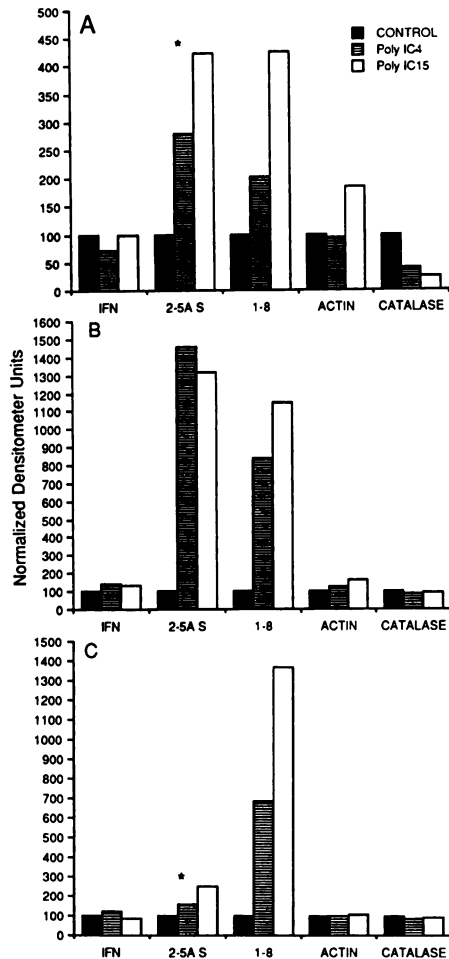
FIG. 3. Quantitation of induction of 2-5A synthetase and 1-8 mRNA levels after poly(I)-poly(C) treatment in mice. Total RNA was prepared (see Materials and Methods) from liver, spleen, and brain tissues of mice sacrificed at 4 h (Poly IC4) or 15 h (Poly IC15) post-poly(I)-poly(C) injection and from control mice injected with phosphate-buffered saline. Total RNA samples were pooled (sample sizes are indicated in parentheses) from liver (panel A: control = 8; 4 h = 4; 15 h = 3), spleen (panel B: control = 8; 4 h = 5; 15 h = 4), and brain (panel C: control = 10; 4 h = 5; 15 h = 4) tissues. Total RNA (10 μg) was slot blotted (see Materials and Methods) onto a nitrocellulose membrane. Identical blots were hybridized with nick-translated mouse IFN-β, mouse 2-5A synthetase, mouse 1-8, β-actin, and mouse catalase cDNAs. An asterisk designates a blot hybridized with a 2-5A synthetase RNA probe. The densitometer units were modified such that the control level of each mRNA species was normalized to 100 U.

of both control and IFN-treated groups was low for brain and spleen tissues (Table 2). Thus, mRNA levels were measured in pooled samples by using the sample sizes indicated in the figure legends. Responses to poly(I)-poly(C) treatment were similar when measured in individual samples (Table 2) or pooled samples (Fig. 3).

In liver tissue, levels of 2-5A synthetase and 1-8 mRNAs increased two- to threefold over control levels after 4 h and fourfold over control levels at 15 h after poly(I)-poly(C) treatment (Fig. 3A). In the spleen, 2-5A synthetase mRNA reached a level 15-fold higher in animals sacrificed at 4 h posttreatment and remained at a level 13-fold above control levels at 15-h after poly(I)-poly(C) injection. The levels of 1-8 mRNA increased to 8- and 11-fold above control levels at 4 and 15 h, respectively, postinjection (Fig. 3B).

In brain tissue, there was a dramatic difference in the magnitudes of the relative responses to poly(I)-poly(C) injection between 2-5A synthetase and 1-8 mRNAs. The level of 2-5A synthetase mRNA increased to a maximum of twofold above control levels at 15 h after poly(I)-poly(C) injection. On the other hand, 1-8 mRNA levels increased by 7-fold after 5 h and 14-fold after 15 h (Fig. 3C). In all tissues, the levels of the control mRNAs did not fluctuate significantly, except for the catalase level in the liver tissue (Fig. 3). At 15 h after poly(I)-poly(C) injection, there was a fourfold decrease compared with control catalase mRNA levels (Fig. 3A).

Activity of 2-5A synthetase was also measured in individual lungs from five control mice and five mice sacrificed at 15 h after poly(I)-poly(C) injection. Activity of 2-5A synthetase increased from 14.2 ± 5.9 nmol of AMP per mg per h in control lungs to 53.8 ± 6.4 nmol of AMP per mg per h in lungs from poly(I)-poly(C)-treated mice, representing approximately a fourfold increase.

Decreased 2-5A synthetase and 1-8 mRNA levels after anti-mouse IFN (α+β) antibody treatment. Constitutive levels of 2-5A synthetase activity in mice have been shown to be produced, at least in part, in response to circulating levels of IFN produced under conventional breeding conditions. When normal mice are injected with anti-mouse IFN (α+β) antibodies, constitutive levels of 2-5A synthetase activity decrease significantly compared with those of corresponding nontreated mice (7). In this study, this observation was further investigated by multiple intravenous injections of polyclonal antibodies to mouse IFN (α+β) into mice and subsequent examination of 2-5A synthetase activity and 2-5A synthetase and 1-8 mRNA levels in a variety of tissues. The treatment protocol was as indicated in the table footnotes and figure legends. Synthetase enzyme activity levels were reduced by fourfold below control levels in the spleens and by twofold below control levels in lungs and livers

TABLE 2. mRNA levels of 1-8 and actin in normal and poly(I)-poly(C)-treated mice[a]

Organ	Mean ± SD 1-8 mRNA level (no. of samples)			Mean ± SD actin mRNA level		
	Control mice	Treated mice at:		Control mice	Treated mice at:	
		4 h	15 h		4 h	15 h
Spleen	100 ± 22 (6)	922 ± 180 (5)	1,561 ± 61 (4)	100 ± 18	171 ± 45	235 ± 29
Brain	100 ± 17 (7)	837 ± 149 (5)	2,005 ± 176 (4)	100 ± 9	86 ± 22	103 ± 38

[a] Mice were injected intravenously with 200 μg of poly(I)-poly(C) and sacrificed at 4 or 15 h later. Control mice were injected with phosphate-buffered saline. Total RNA was prepared from frozen spleen and brain tissues as described in Materials and Methods. Individual 10 μg samples of total RNA were slotted onto a nitrocellulose membrane and the membrane was hybridized to nick-translated mouse 1-8 cDNA. The same membrane was stripped and rehybridized to nick-translated β-actin cDNA. The levels of 1-8 and actin mRNAs were determined by densitometry. The densitometer units were modified such that control levels were normalized to 100 ± the standard deviation.

TABLE 3. Effect of anti-IFN (α+β) antibody on 2-5A
synthetase activity[a]

| Organ | Mean ± SD 2-5A synthetase (nmol of AMP/mg per h) | |
	Control mice	Antibody-treated mice
Spleen	68.9 ± 20.6	15.2 ± 9.6
Lung	20.5 ± 8.7	9.2 ± 4.1
Liver	35.6 ± 10.3	14.5 ± 7.7
Thymus	3.8 ± 1.5	2.9 ± 1.4
Brain	1.7 ± 1.1	2.5 ± 1.6

[a] Anti-mouse IFN (α+β) antibody (50 μl; 50 mg/ml) with a neutralizing titer of 10^{-5} against 5 U of mouse IFN (α+β) was administered by intravenous injection on days 1, 2, 4, and 6. Control animals were injected with unimmunized sheep serum. Eight mice from each of the control and experimental groups were sacrificed on day 7. The levels of 2-5A synthetase activity were measured (see Materials and Methods) in the various organs.

(Table 3). Injection of anti-mouse IFN (α+β) antibody had little effect on levels of 2-5A synthetase activity in thymus and brain, two tissues in which control animals have low basal levels (Table 3). To determine whether injection of anti-mouse IFN (α+β) antibodies would also decrease levels of the IFN-induced 2-5A synthetase and 1-8 mRNAs, the levels of these two messages were quantitated by slot blot hybridization and densitometry in antibody-treated animals and compared with the levels in corresponding control animals. Initially, the levels of 1-8 and actin were measured in individual brain and spleen samples to determine the variation in signal intensity between samples. Standard errors remained within ±22% (Table 4); therefore, mRNA levels were analyzed in pooled total RNA samples.

Anti-mouse IFN (α+β) antibody treatment had no detectable effect on the hybridization level of the IFN cDNA probe in brains, spleens, livers, or lungs (Fig. 4). In fact, antibody treatment had little overall effect on brain tissue, since the message level of neither 2-5A synthetase nor 1-8 showed a significant decrease compared with control levels (Fig. 4A). This might reflect the inability of anti-IFN antibodies to cross the blood-brain barrier. However, these antibodies are active elsewhere, since in the spleens of treated mice, 2-5A synthetase mRNA levels dropped by five- to sixfold and the level of 1-8 mRNA decreased by twofold compared with control mice (Fig. 4B). The levels of both 2-5A synthetase and 1-8 mRNA in experimental livers decreased by approximately twofold below that of the control livers (Fig. 4C). In

TABLE 4. mRNA levels of 1-8 and actin in normal and anti-IFN (α+β) antibody-treated mice[a]

| Organ | 1-8 mRNA level (no. of samples) | | Actin mRNA level | |
	Control mice	Antibody-treated mice	Control mice	Antibody-treated mice
Spleen	100 ± 22 (6)	21 ± 4 (8)	100 ± 18	99 ± 22
Brain	100 ± 10 (7)	86 ± 15 (7)	100 ± 9	78 ± 12

[a] Anti-mouse IFN (α+β) antibody (50 μl) was administered intravenously to mice on days 0 and 3. Control mice were injected with serum from unimmunized sheep. Mice were sacrificed on day 5. Total RNA was prepared from spleen and brain tissues (see Materials and Methods). Individual samples (10 μg each) of total RNA was slotted onto a nitrocellulose membrane, and the membrane was hybridized to nick-translated mouse 1-8 cDNA. The same membrane was stripped and rehybridized to nick-translated β-actin cDNA. The levels of 1-8 and actin mRNAs were determined by densitometry. The densitometer units were modified such that control levels were normalized to 100 ± the standard deviation.
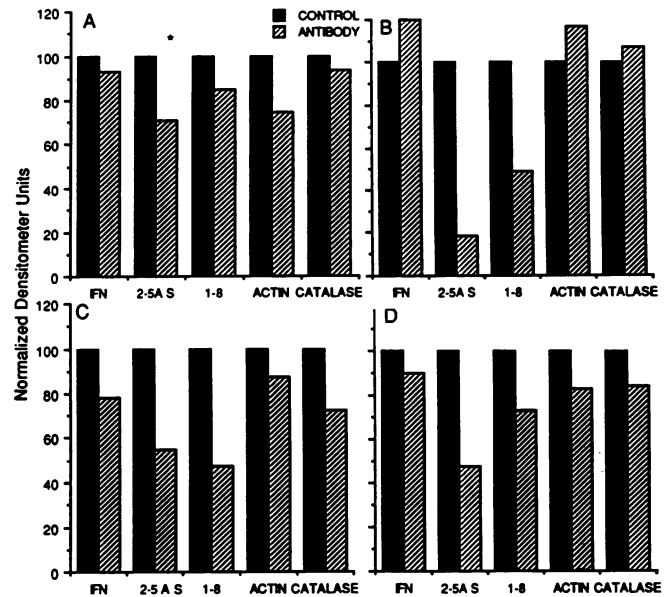

FIG. 4. Quantitation of 2-5A synthetase and 1-8 mRNA levels after anti-IFN antibody treatment in mice. Total RNA was prepared from brain, spleen, and liver tissues of mice injected intravenously with 50 μl of anti-mouse IFN (α+β) antibody on days 0 and 3 and sacrificed on day 5. Total RNA was also prepared from lung tissues of mice treated with anti-IFN antibody on days 0, 2, 4, and 6 and sacrificed on day 7. Control animals from each group were injected with unimmunized sheep serum. Total RNA samples were pooled (sample sizes are indicated in parentheses) from brain (panel A: control = 10; antibody = 10), spleen (panel B: control = 8; antibody = 10), liver (panel C: control = 8; antibody = 9), and lung (panel D: control = 6; antibody = 6) tissues, and 10 μg of RNA was slot blotted (see Materials and Methods) onto a nitrocellulose membrane. Identical blots were hybridized with nick-translated mouse IFN-β, mouse 2-5A synthetase, mouse 1-8, β-actin, and mouse catalase cDNAs. An asterisk designates a result obtained with a 2-5A synthetase RNA probe. The densitometer units were modified such that the control level of each mRNA species was normalized to 100 U.

the lung tissues of antibody-treated animals, 2-5A synthetase mRNA levels dropped by twofold; however, the levels of 1-8 mRNA were not significantly reduced (Fig. 4D). The levels of the control RNAs, actin and catalase, did not vary by more than 25% in any of the tissues examined (Fig. 4).

DISCUSSION

Transcription of some IFN genes has recently been demonstrated in different human organs of normal individuals (22). Although these results support the hypothesis that physiological levels of IFN constitute an important host defense mechanism against viral infections, the biological activity of this mRNA was not assessed nor were the levels of IFN-induced genes measured. Mice living in a normal, nonsterile environment produce low circulating levels of IFN (α+β). This low level of IFN results in increased basal activity of the IFN-induced enzyme 2-5A synthetase (7). Injection of mice with IFN or IFN inducers, such as viral and synthetic dsRNAs, has been shown to enhance the level of 2-5A synthetase activity (10, 12, 17). The increase in 2-5A synthetase activity resulting from injection of synthetic dsRNA is abrogated by preinjection of anti-mouse IFN (α+β) antibody, indicating that circulating IFN is responsi-

ble for the increased 2-5A synthetase activity (12). It was of interest to determine whether this change in enzyme activity in vivo reflected changes in gene expression rather than possible changes in substrate availability or enzyme kinetics. Mouse cDNAs corresponding to two human IFN-responsive genes, 2-5A synthetase (20) and 1-8 (unpublished data), have recently been cloned, rendering this study feasible.

The kinetics of induction of mouse 2-5A synthetase and 1-8 mRNAs in vivo was first established by injecting normal mice with mouse IFN ($\alpha+\beta$) and sacrificing mice at various intervals thereafter. The steady-state level of 2-5A synthetase mRNA in mouse spleens peaked as early as 2 h after IFN injection (Fig. 1 and 2). In contrast, steady-state 1-8 mRNA levels did not peak until 5 h post-IFN injection (Fig. 1 and 2). The difference in mRNA accumulation of 2-5A synthetase and 1-8 mRNAs was probably due to differences in posttranscriptional processing, since Hannigan and Williams (9) have shown that both messages have similar rates of synthesis after IFN treatment in vitro. Increased levels of 2-5A synthetase and 1-8 mRNAs post-IFN (Fig. 2) were detected much earlier than the increase in 2-5A synthetase enzyme activity in the lung tissues of the same mice (Table 1). Also, 2-5A synthetase mRNA levels in spleen tissues increased by 13-fold above control mRNA levels, while in lung tissues enzyme levels increased by only 2-fold. Hence, it is possible that measurement of levels of IFN-induced mRNAs is an earlier and more sensitive indicator of IFN action in vivo.

Mice were next treated with poly(I)-poly(C), a known in vivo inducer of IFN (10, 12). IFN mRNA levels did not differ from control levels at 4 or 15 h after poly(I)-poly(C) injection in spleen, brain, or liver tissues (Fig. 3). However, only mouse IFN-β_1 mRNA levels were measured in this study. It is also possible that any increase in IFN mRNA levels due to poly(I)-poly(C) treatment occurred before the 4-h time point. Although no increase in IFN mRNA levels was detected, poly(I)-poly(C) injection did result in induction of both 2-5A synthetase and 1-8 mRNA levels in spleen, liver, and brain tissues (Fig. 3). An increase in 2-5A synthetase enzyme activity was also measured in lung tissues from poly(I)-poly(C)-treated animals. The increase in 2-5A synthetase and 1-8 mRNAs in the brain (Fig. 3C) is interesting and indicates that either the systemically introduced poly(I)-poly(C) or the induced IFN was not excluded from reaching brain tissue. Levels of 2-5A synthetase mRNA in the brain increased by 2-fold at 15 h post-poly(I)-poly(C) treatment, whereas 1-8 mRNA levels were induced to almost 14-fold over the control levels (Fig. 3C). The difference between the magnitudes of response of 2-5A synthetase and 1-8 mRNAs found in the brain may be related to the fact that IFN-responsive human 1-8 cDNA was originally isolated from a glioblastoma cell line, T98G (6). Although constitutive expression has been found in all mouse tissues examined, the difference in the responses of brain tissue to IFN may reflect some tissue specificity with respect to the unknown function of the 1-8 gene.

The levels of 2-5A synthetase enzyme activity and 2-5A synthetase and 1-8 mRNAs were measured in mice treated with anti-mouse IFN ($\alpha+\beta$) antibody. The decrease in 2-5A synthetase mRNA corresponded well to the decrease in 2-5A synthetase enzyme activity as measured in the different organs. For example, levels of 2-5A synthetase mRNA (Fig. 4A) and enzyme activity (Table 3) in brain tissues were not affected by the antibody treatment. On the other hand, 2-5A synthetase mRNA levels decreased by fivefold in spleens (Fig. 4B), as did the enzyme activity, which decreased four-

to fivefold below control levels (Table 3). A twofold decrease in 2-5A synthetase mRNA levels was detected in both liver (Fig. 4C) and lung (Fig. 4D) tissues after antibody treatment, and a similar decrease was measured in 2-5A synthetase enzyme activity (Table 3). Consequently, it is evident that changes in enzyme activity in vivo are the result of specific changes in gene expression in response to levels of circulating IFN. In general, 1-8 mRNA levels were not as sensitive to anti-mouse IFN ($\alpha+\beta$) antibody treatment. However, 1-8 mRNA levels did decrease by twofold compared with control levels in both spleen and liver tissues (Fig. 4). The reduction of IFN-responsive 2-5A synthetase and 1-8 mRNA levels in the organs of normal mice treated with anti-mouse IFN ($\alpha+\beta$) antibody provides direct evidence that circulating levels of IFN are present and active under normal physiological conditions.

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