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Expression of Alpha/Beta Interferons (IFN-α/β) and Their Relationship to IFN-α/β-Induced Genes in Lymphocytic Choriomeningitis†

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Expression of alpha interferon (IFN-α)-, IFN-β-, and IFN-α/β-induced genes was monitored during the development of lymphocytic choriomeningitis (LCM) to assess whether a restricted influence of these antiviral cytokines could be found in the central nervous system (CNS). High levels of IFN-α (83 ± 42 U/ml) were present in the blood of LCM virus-infected mice 3 days postinfection, whereas IFN-β was not detected (<1.0 U/ml) at any time point. Spleens contained high levels of IFN-α and IFN-β mRNAs at days 1 and 3 postinfection, whereas no IFN-α mRNA and only low levels of IFN-β mRNA were detected in brains. In situ hybridization showed IFN-α mRNA-expressing cells in the marginal zones of the spleen and in the subcapsular sinus and outer cortex of cervical lymph nodes. The expression of 2′,5′-oligoadenylate synthetase (2′,5′-OAS) mRNA followed the expression of IFN-β mRNA in the brain, whereas 2′,5′-OAS mRNA in the periphery was associated with systemic IFN-α. The localization of IFN-α-expressing cells in the spleen and lymph nodes in proximity to T- and B-cell compartments is consistent with a role for these cytokines in immune regulation. Furthermore, the absence of IFN-α and the relatively low level and delayed expression of IFN-β in the brain suggest that the CNS is an especially vulnerable organ for virus replication. With certain strains of LCM virus, the absence of early antiviral IFN-α/β activity and preferential virus growth in the brain might lead to targeted T-cell inflammation of the CNS, resulting in death of the animal.

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus which infects rodents as its natural hosts (4). LCMV infection can occur in humans, usually with subclinical symptoms but also sometimes with serious meningitis. Intracerebral (i.c.) inoculation of LCMV into adult, immunocompetent mice is followed a few days later by a delayed-type hypersensitivity reaction developing in the choroid plexus and meninges of the brain, which ultimately leads to death of the animals (9). Infiltration of leukocytes into virus-infected organs, including the central nervous system (CNS), coincides with high levels of cytokine gene expression (5).

The molecular mechanisms underlying the host response in acute LCMV infection is largely unknown. It is likely that cytokines are involved in regulation of the immunological development of inflammation, but it is also conceivable that high levels of cytokines could directly affect the CNS (5, 13). Thus, expression of cytokines may be pivotal in the development of LCM and many of the clinical signs of the disease. Previous studies of the interferon (IFN) system in acute LCM have shown that expression of IFN-α/β and IFN-γ is associated with fatal disease outcome (30, 35, 40, 42, 53). Hence, regulation of the IFN system appears to be of central importance for the development of viral meningitis. In general, IFNs have been noted to elicit three broad categories of antiviral responses: (i) establishment of the cellular antiviral state, (ii) growth inhibition, and (iii) immunomodulation (43). Focusing on type I IFN (IFN-α/β), the contribution to the host defense against viruses has been attributed mainly to the strong cellular antiviral responses induced by these mediators. However, recent studies have shown considerable immunomodulatory effects of IFN-α/β, especially concerning T- and B-cell differentiation (11, 18, 34). Such immunomodulatory activities of IFN-α/β may also be essential for successful clearance of the viral infection and might also be the primary effector mechanism by which IFN-α/β are able to control certain IFN-resistant viruses (1).

Although all major cell types in the body have been shown to be able to produce one or another type I IFN, there is considerable variation in production among various cell types and organs. With regard to LCM, modulation of type I IFNs dramatically alters the development of this disease, which appears to be related to a marked change in the distribution of LCMV replication from the CNS to visceral organs. Despite the obvious central importance of the IFN-α/β system in the natural history of this viral disease, little is known concerning the identity or spatiotemporal expression of type I IFNs in the development of LCM. It is possible that discrete secretion of IFN-α and IFN-β in various tissues is important for the course of LCMV infection. Therefore, in this study, we analyzed the expression of IFN-α/β, IFN-α/β-inducible genes, and viral RNA in the organs of LCMV-infected mice to localize where and when IFN-α and IFN-β are produced. Thus, this study constitutes a framework to better understand the impact of IFN-α and IFN-β production on the development of LCM.

MATERIALS AND METHODS

Mice and infection with LCMV. Male BALB/cByJ mice were maintained under pathogen-free conditions in the closed

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breeding colony of the Research Institute of Scripps Clinic and used at 8 to 12 weeks of age. An LCMV-Arm53b stock was obtained from a triple plaque-purified clone subsequently passaged twice in BHK cells (10). The virus was titrated in vitro on Vero-76 cells. For induction of LCM, mice were inoculated i.c. with either 30 μl of serum-free Dulbecco modified Eagle medium (control, noninfected) or serum-free Dulbecco modified Eagle medium containing 1,000 PFU of LCMV. At this dose of LCMV, all of the mice died by day 6.

Murine IFN-α and IFN-β dissociation-enhanced lanthanide fluoroimmunoassay. Specific and sensitive dissociation-enhanced lanthanide fluoroimmunoassays were designed to measure mouse IFN-α or IFN-β (10a). Briefly, purified sheep anti-mouse IFN α/β immunoglobulin G (a kind gift of Michael Tovey, Centre National de la Recherche Scientifique, Paris, France) was adsorbed overnight to 96-well microtiter plates (Nunc Immuno Plate Maxisorp; Nunc, Roskilde, Denmark) in 200 mM NaH_2PO_4 (pH 4.5)–0.005% NaN_3 and treated with postcoating solution (6% sorbitol, 0.9% NaCl, 0.1% bovine serum albumin, 0.1 mM $CaCl_2$–$2H_2O$, 4 μM EDTA, 0.005% NaN_3, 50 mM NaH_2PO_4 [pH 4.8]) for 3 h to avoid nonspecific binding of protein to the wells.

Immediately before use, the plates were washed (50 mM Tris-HCl [pH 7.8], 0.9% NaCl, 0.05% Tween 20, 0.005% NaN_3) and plasma samples were diluted in sample buffer (50 mM Tris-HCl [pH 7.8], 0.9% NaCl, 0.005% NaN_3, 0.5 mM $CaCl_2$–$2H_2O$, 20 μM EDTA, 0.5% bovine serum albumin) and incubated for 2 h. The wells were washed, europium-labelled rat anti-mouse IFN-α (clone 4E-A1; Seikagaku America, Inc., Rockville, Md.) or europium-labelled rat anti-mouse IFN-β (clone 7F-D3; Seikagaku) was added, and the wells were incubated for 1 h. Finally, the wells were washed, enhancement solution (Wallac Oy, Turku, Finland) was added, the wells were incubated at room temperature for 20 min, and fluorescence emission was counted in a 1234 Delfia Research Fluorometer (Wallac). Murine natural leukocyte-derived IFN-α and IFN-β calibrated against National Institutes of Health references (mouse IFN-α standard Ga02-901-511 and mouse IFN-β standard Gb02-902-511) were used as standards.

Preparation of poly(A)$^+$ RNA. Mice were killed at various times postinoculation, and organs were immediately removed, snap frozen in liquid nitrogen, and stored at −85°C. Poly(A)$^+$ RNA was prepared as previously described (2). Briefly, frozen tissue was placed in 10 ml of lysis buffer (0.2 M NaCl, 0.2 M Tris-HCl [pH 7.5], 1.5 mM $MgCl_2$, 2% sodium dodecyl sulfate, 200 μg of proteinase K per ml) and immediately homogenized. After 60 min of incubation at 45°C, the lysate was adjusted to 0.5 M NaCl and mixed with 40 mg of oligo(dT)-cellulose (Invitrogen, San Diego, Calif.) that had been pre-equilibrated and swollen in binding buffer (0.5 M NaCl, 0.01 M Tris-HCl [pH 7.5]). The mixture was then incubated at room temperature for 60 min with gentle rocking. Following three washes in binding buffer, poly(A)$^+$ RNA was eluted from the oligo(dT)-cellulose with 0.35 ml of elution buffer (0.01 M Tris-HCl [pH 7.5]), precipitated in ethanol, dried, and resuspended in 25 μl of elution buffer. The concentration of RNA was determined by UV spectroscopy at 260 nm.

RNase protection assay for IFN-α and IFN-β mRNAs. An RNase protection assay (RPA) for IFN-α and IFN-β mRNAs was performed essentially as previously described (23). Briefly, the *Bst*YI-*Hin*dIII fragment of a cDNA for murine IFN-α_1 (a kind gift of E. Zwarthoff, Erasmus University, Rotterdam, The Netherlands) was subcloned into the *Bam*HI-*Hin*dIII sites of pBSIIKS+/− (Stratagene). Linearization with *Hin*dIII and transcription with T7 RNA polymerase yields an antisense RNA probe of 322 bp, of which 276 bp were protected in the RPA. A 560-bp genomic *Pvu*II fragment of murine IFN-β (51) cloned into the *Sma*I site of pGEM4 (a kind gift of G. Vodjdani, Centre National de la Recherch Scientifique, Paris, France) and linearized with *Hin*dIII was used to generate a 610-bp antisense RNA following in vitro transcription with SP6 RNA polymerase. The fragment protected in the RPA was 208 bp long. For RPA of the LCMV nuclear protein (NP) RNA, a 461-bp *Bam*HI-*Bgl*II fragment of the cDNA for LCMV NP was ligated into the *Bam*HI site of pGEM3 (pG3NP). Linearization with *Eco*RI and transcription with SP6 RNA polymerase yields an antisense RNA probe for LCMV NP. Expression of the mRNA for ribosomal protein light 32 was used as a control of constitutively expressed mRNA as described previously (23). The hybridization reactions [2×10^5 cpm/5 μg of target poly(A)$^+$ RNA], RNase treatments, and isolations of protected RNA-RNA duplexes were as previously described (23). Finally, samples were dissolved in sequencing loading buffer, denatured (90°C, 2 min), and electrophoresed in 5% acrylamide–8 M urea sequencing gels. Dried gels were placed on XAR-5 film (Kodak, Rochester, N.Y.) with intensifying screens at −85°C, and the films were developed after various periods of exposure. Within each assay, a control hybridization included the probe set plus 1 pg of in vitro-transcribed sense IFN-α_1 and IFN-β RNAs as size standards and for assessment of assay performance, and the probes were run without a target as a degradation control. Densitometric scanning was performed on X-ray films exposed for various times (9 to 96 h) with an Ultroscan XL (Pharmacia LKB, Bromma, Sweden).

Northern (RNA) blot hybridization. Poly(A)$^+$ RNA (5 μg) prepared as described above was denatured, separated by electrophoresis in 1% agarose–2.2 M formaldehyde gels, transferred to nylon membranes, and hybridized overnight at 45°C with ^{32}P-labelled cDNA probes. The probes used were a 2-kb *Eco*RI fragment of mouse 2′,5′-oligoadenylate synthetase (2′,5′-OAS) cDNA (cDNA m2; provided by B. Williams, Cleveland Clinic Foundation, Cleveland, Ohio), a 0.6-kb *Kpn*I-*Sac*I fragment of the histocompatibility 2Dβ cDNA (H2Dβ) (provided by P. Petterson, Scripps Research Institute, La Jolla, Calif.), and a 0.26-kb fragment of the murine β-actin gene (49) generated by PCR and cloned into pGEM4 (provided by M. Nerenberg, Scripps Research Institute). Following hybridization, membranes were washed, placed on XAR-5 film with intensifying screens, and exposed for various times at −85°C. Even loading and transfer of mRNA were verified by ethidium bromide staining of agarose gels and nylon membranes and by hybridization to β-actin mRNA.

In situ hybridization. Anesthetized control and infected mice were perfused transcardially with ice-cold saline followed by 4% paraformaldehyde in phosphate-buffered saline (pH 7.4). Organs were removed, postfixed in the same fixative overnight at 4°C, divided along the midline, processed, and embedded in paraffin. Sagittal 10-μm sections were cut onto silanized (2% 3-aminopropyltriethoxysilane in acetone; Sigma) slides and used for in situ hybridization as previously described (45), with minor modifications. Briefly, sections were hydrated and treated sequentially with 4% formaldehyde in phosphate-buffered saline for 5 min, proteinase K (5 μg/ml in 0.1 M Tris–10 mM EDTA [pH 7.5]) for 10 min at 37°C, and 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min. Sections were washed twice for 2 min each time between the formaldehyde and proteinase treatments and once in 0.1 M triethanolamine before treatment with acetic anhydride. After acetic anhydride treatment, the sections were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), dehydrated in graded (50, 70, 95, 100, and 100%) ethanol, and dried in a vacuum for 1 h at room temperature. Hybridization

buffer (50% formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt's solution, 1 mg of *Escherichia coli* tRNA per ml, 1 mM EDTA, 10 mM dithiothreitol [DTT]) containing 10×10^6 cpm of probe per ml (approximately 5 ng of probe per ml) was added to each slide, which was then covered with a coverslip and sealed with rubber cement. Hybridization was performed at 56°C for 18 h. Free probe was removed by four washes in 4× SSC containing 0.3 M 2-mercaptoethanol for 5 min followed by one wash in 2× SSC for 5 min. Unspecific, low-stringency-hybridizing probes were removed by treatment with 0.1 μg of RNase A per ml in 0.5 M NaCl–10 mM Tris (pH 8)–4 mM EDTA for 30 min at 37°C. Remaining free radioactivity was removed by washing twice in 2× SSC–1 mM DTT for 5 min, once in 1× SSC–1 mM DTT for 10 min, once in 0.5× SSC–1 mM DTT for 10 min, once in 0.1× SSC–1 mM DTT at 56°C for 30 min, and once in 0.1× SSC–1 mM DTT at room temperature for 5 min. The sections were rapidly dehydrated in graded ethanol (50% ethanol–1 mM DTT, 70% ethanol–1 mM DTT, 95% ethanol, and 3 × 100% ethanol), dried in a vacuum for 30 min, dipped in Kodak NTB-2 emulsion containing 1% glycerol, dried, and exposed for 1 to 5 weeks at 4°C. The slides were developed and counterstained with hematoxylin and examined by dark- and bright-field microscopy.

The ^{35}S-labelled cRNA probes used for in situ hybridization were generated as described by Johnson and Johnson (26). A 0.7-kb *Eco*RI-*Hin*dIII fragment of the murine cDNA for IFN-α_1 (provided by E. Zwarthoff) was subcloned into the *Eco*RI-*Hin*dIII sites of pGEM4, linearized with *Hin*dIII, and transcribed with SP6 to yield a 0.7-kb antisense probe. Single-stranded RNA probes for murine IFN-β and LCMV NP were generated from the same templates as described for the RPA. Hybridization with sense RNA probes served as a control for low-stringency hybridization and remained negative throughout the experiments.

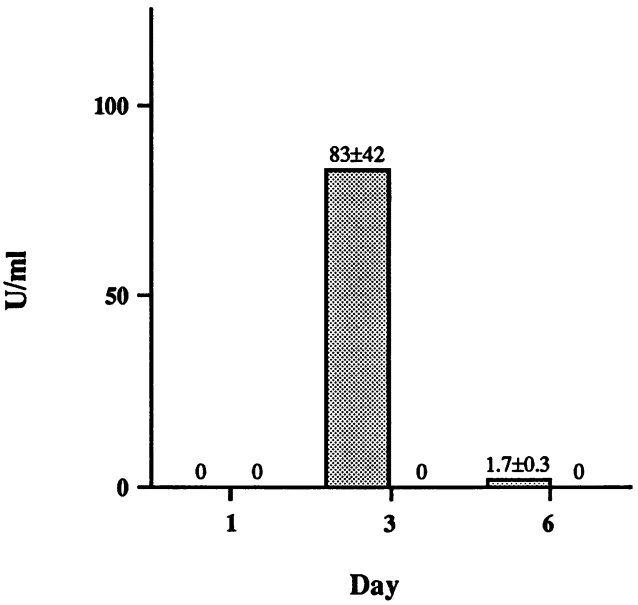

FIG. 1. Levels of IFN-α (▨) and IFN-β (■) in blood of LCMV-infected mice. Blood was collected from two LCMV-infected mice on days 1, 3, and 6 postinfection and analyzed by dissociation-enhanced lanthanide fluoroimmunoassay for murine IFN-α and IFN-β as described in Materials and Methods. Concentrations of IFN-α and IFN-β in plasma are expressed as means ± standard deviations. Samples below the lower limit for detection (<1.0 U/ml) were given the value 0.

REULTS

High levels of IFN-α, but no IFN-β, are present in blood following i.c. inoculation of LCMV. Adult, immunocompetent BALB/c mice were inoculated i.c. with 1,000 PFU of LCMV, and blood was collected by heart puncture at days 1, 3, and 6 postinfection. As seen in Fig. 1, high levels of IFN-α (83 ± 42 U/ml) were found in LCMV-infected mice by day 3. IFN-α was not detected on day 1 (<1.0 U/ml), whereas low levels of IFN-α were present in plasma on day 6 (1.7 ± 0.3 U/ml). IFN-β was not detected (<1.0 U/ml) at any time in the blood of these mice.

Expression of IFN-α mRNA is restricted to the spleen and lymph nodes. Brains, spleens, and kidneys were collected on days 1, 3, and 6 postinfection, and poly(A)$^+$ mRNA was extracted for RPA. As shown in Fig. 2A, IFN-α mRNA was readily detected in spleen tissue on days 1 and 3 but absent on day 6 postinfection. In contrast, even after extended autoradiographic exposure, no IFN-α mRNA was detected in brain tissue at any time point (Fig. 2C). When IFN-β was analyzed by RPA in the same animals, a distinct hybridization band was obtained from spleen tissue on days 1 and 3 but not on day 6 postinfection (Fig. 2B). Furthermore, IFN-β was also detected in brain tissue after extended autoradiographic exposure (Fig. 2D). The bands corresponding to IFN-β were barely visible on day 3 and further increased by day 6. In kidney tissue, no IFN-α transcript was detected at any time point (Fig. 2E) whereas a weak IFN-β mRNA signal was detected by day 3 (Fig. 2F).

In situ hybridization was performed to analyze the precise localization of the IFN-α-producing cells during LCM. Spleens, brains, and cervical lymph nodes were collected from LCMV-infected mice on days 1, 3, and 6 postinfection, and the organs were processed for in situ hybridization as described in Materials and Methods. As seen in Fig. 3A, the choroid plexus and ependymal cells in brains of LCMV-infected mice contained LCMV NP RNA. Expression of LCMV NP RNA was evident by day 3 and further increased by day 6 (Fig. 3A). No IFN-α mRNA-containing cells were found in brains of LCMV-infected animals at any time point despite excessive infiltration of mononuclear cells and inflammation of the choriomeninges (Fig. 3B). Cells expressing high levels of LCMV NP RNA were detected in the subcapsular sinus and outer cortex of cervical lymph nodes by day 3 postinfection (Fig. 3C), whereas no LCMV NP RNA was detected on day 1 or 6 postinfection. A few IFN-α mRNA-containing cells were localized to the same area in cervical lymph nodes, where LCMV NP RNA-containing cells were also demonstrated (Fig. 3D).

In spleen tissue, only a few cells expressed LCMV NP RNA by day 1 postinfection whereas many cells in the white pulp and marginal zone and a few cells in the red pulp expressed LCMV NP RNA by day 3 (Fig. 3E). At 6 days postinfection, only occasional cells which expressed LCMV NP RNA were detected. Many cells expressing IFN-α mRNA were demonstrated preferentially in the marginal zones on days 1 and 3 (Fig. 3F), whereas no IFN-α mRNA-expressing cells were found on 6 days postinfection.

Consecutive sections hybridized with LCMV NP and IFN-α_1 cRNA probes showed that cells containing IFN-α mRNA were usually situated in the same area as LCMV NP RNA-expressing cells (Fig. 3E and F). Examination of 20 visual fields of two such consecutive sections showed 419 LCMV NP RNA-containing cells, of which 50 also expressed IFN-α mRNA. Furthermore, in these 20 visual fields, 24 cells expressed IFN-α

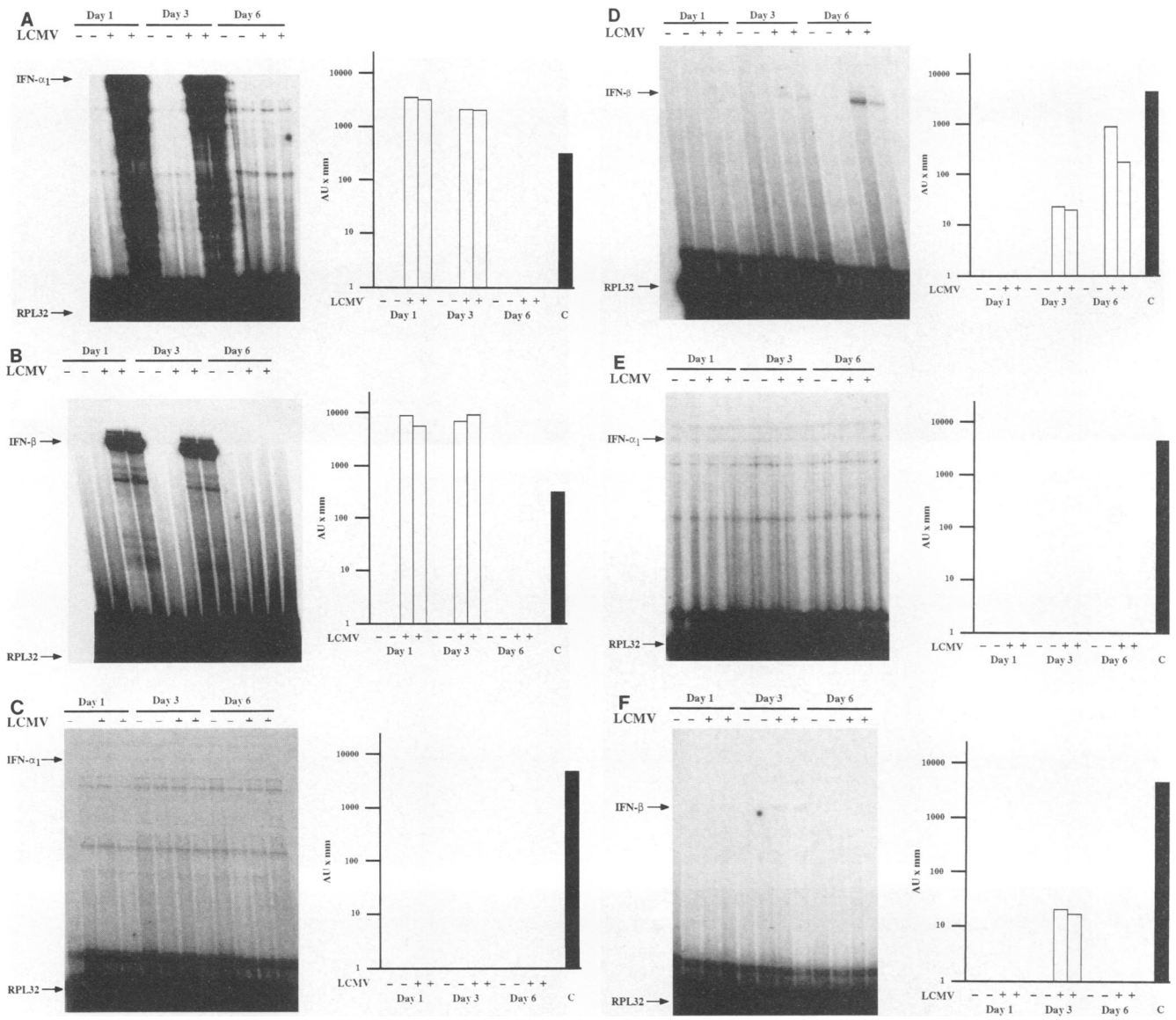

FIG. 2. Expression of IFN-α and IFN-β mRNAs in the spleens, brains, and kidneys of LCMV-infected mice. Poly(A)$^+$ RNA was extracted from spleens (A and B), brains (C and D), and kidneys (E and F) by days 1, 3, and 6 postinfection, and 5 μg of RNA from each of two mock-infected and two LCMV-infected mice was analyzed by RPA as described in Materials and Methods. The positions of protected RNA probes are indicated (A, C, and E, IFN-α and ribosomal protein light 32 [RPL32]; B, D, and F, IFN-β and ribosomal protein light 32). Sequentially loaded samples taken from two mock-infected and two LCMV-infected mice on days 1, 3, and 6 postinfection and then exposed to X-ray film for 96 h are shown. Densitometric scanning of protected RNA probes was performed as described in Materials and Methods, and the results are displayed as absorbance units × millimeters (AU × mm). The absorbance of 1 pg of in vitro-transcribed sense RNA for IFN-α_1 and IFN-β (C) is included.

mRNA in the absence of detectable LCMV NP RNA. Thus, not all of the cells expressing IFN-α mRNA expressed LCMV NP RNA.

Kinetics of appearance of mRNA for 2′,5′-OAS and H2D. An increase in the cellular level of 2′,5′-OAS has been considered a reliable and specific marker for the biological effects of IFN-α/β (44, 52). To assess the biological effects of IFN-α/β in various organs, we analyzed the induction of mRNA for 2′,5′-OAS (Fig. 4). Mice infected with LCMV displayed slightly increased levels of 2′,5′-OAS in their spleens on day 1, which further increased on day 3 and declined markedly at day 6. In the kidneys, 2′,5′-OAS mRNA levels were slightly increased on day 1 and further increased on day 3 and remained at a high level on day 6. In brain tissue, 2′,5′-OAS expression was detectable at a very low level by day 3 but increased at day 6.

Major histocompatibility complex class I is an immunologically important molecule which is positively regulated by IFN-α/β (50). Northern blot analysis showed that H2D mRNA expression was elevated on day 1, further increased on day 3, and remained high on day 6 in both spleens and kidneys (Fig. 5). In brains, H2D mRNA expression increased gradually on days 1, 3, and 6.

DISCUSSION

Production of IFN-α/β plays a pivotal role in the host defense against viral infections (19, 20, 33). However, use of

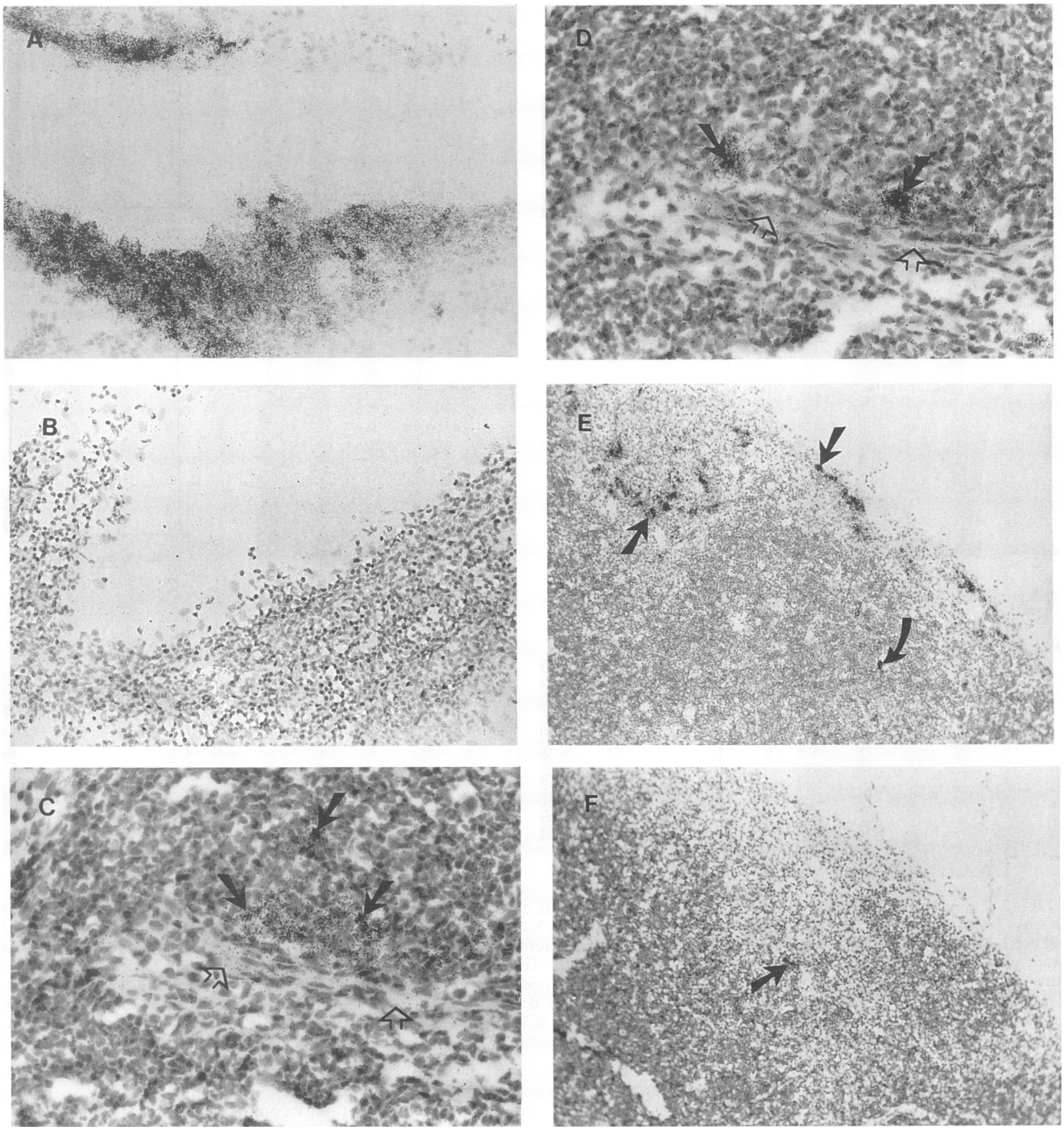

FIG. 3. Expression of LCMV NP RNA and IFN-α mRNA in the brains, spleens, and cervical lymph nodes of mice infected i.c. with LCMV. Mice were infected i.c. with LCMV, and the brains (A and B), cervical lymph nodes (C and D), and spleens (E and F) were removed from the animals as described in Materials and Methods. (A and B) Sagittal brain sections (20 μm) at a magnification of about ×74 showing the choroid plexus and ependymal cells in the fourth ventricle at 6 days postinfection hybridized with probes for LCMV NP (A) and IFN-α_1 (B). (C and D) Consecutive sections (6 μm) (magnification, about ×296) of spleen 3 days postinfection showing parts of red and white pulp hybridized with probes for LCMV NP (C) and IFN-α_1 (D). The marginal zone is indicated by the open arrows. The closed arrows indicate radioactively labelled cells. (E and F) Consecutive sections (6 μm) of cervical lymph nodes at 3 days postinfection (magnification, about ×148) showing the peripheral part of the cortex, subcapsular sinus, and trabeculae hybridized with probes for LCMV NP (E) and IFN-α_1 (F). The closed arrows indicate cells expressing mRNAs for LCMV NP and IFN-α. Representative sections from in situ hybridization performed on three LCMV-infected mice are shown. Hybridization with sense RNA probes was negative throughout the experiments.

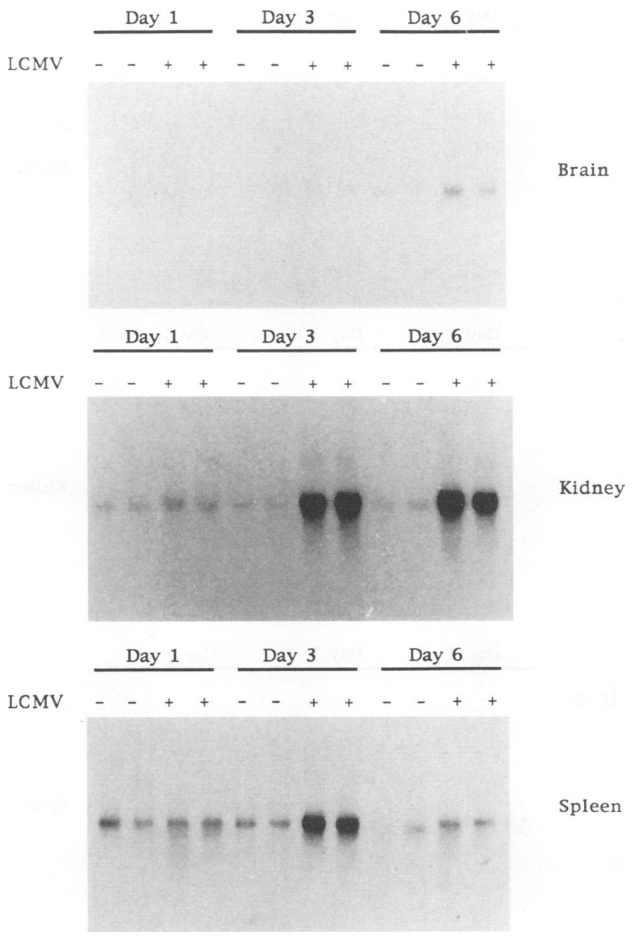

FIG. 4. Expression of 2′,5′-OAS mRNA in the brains, spleens, and kidneys of LCMV-infected mice. Poly(A)$^+$ RNA was extracted from the brains, spleens, and kidneys on days 1, 3, and day 6 postinfection, and 5 μg of poly(A)$^+$ RNA from each of two mock-infected (−) and two LCMV-infected (+) mice was analyzed by Northern blot hybridization using a ^{32}P-labelled 2′,5′-OAS DNA probe.

specific anti-IFN-α/β serum to neutralize IFN-α/β in i.c. LCMV-infected mice has shown a paradoxically lethal role of IFN-α/β in LCM (35, 40, 42). To achieve a deeper understanding of this phenomenon and the relationship of type I IFN production with LCMV infection, we analyzed the expression of IFN-α/β and the IFN-α/β-inducible genes for 2′,5′-OAS and H2D in adult, immunocompetent mice following i.c. injection of LCMV.

Earlier studies have reported that mice receiving i.c. inoculations of various strains of LCMV exhibited the highest levels of type I IFN in blood on days 2 to 4 and then a gradual decline (29, 32). However, the identity of this type I IFN activity was not identified. Our results revealed similar kinetics for the production of IFN in blood and showed that most of this type I IFN was IFN-α, with only low levels (if any) of IFN-β in the circulation.

A sensitive RPA was performed on poly(A)$^+$ RNA extracted from these organs to analyze the expression of IFN-α and IFN-β mRNAs. Surprisingly, IFN-α mRNA was not detected in brain tissue despite the presence of virally infected cells, excessive infiltration of mononuclear leukocytes, and inflammation of the meninges (Fig. 2 and 3). In contrast, IFN-α mRNA and IFN-α mRNA-containing cells were readily detectable in spleens and cervical lymph nodes. Such a restriction of expression of IFN-α to peripheral lymphoid organs has also been observed with other viral inducers (Aujeszky's disease virus [1a] and herpes simplex virus type 1 [10a]) and is probably not due to immunosuppressive properties of LCMV. Careful examination of consecutive sections of the spleens and cervical lymph nodes of LCMV-infected mice hybridized with IFN-α_1 and LCMV NP cRNA probes showed that only 50 of 416 LCMV NP RNA-containing cells produced IFN-α. Furthermore, 24 of 50 of the IFN-α mRNA-expressing cells did not appear to contain RNA for LCMV NP. However, these IFN-α mRNA-expressing cells were almost always localized close to areas of spleens and lymph nodes infected by LCMV. Although no IFN-β was detected in plasma, in situ hybridization with IFN-α_1 and IFN-β cRNA probes of consecutive spleen sections of LCMV-infected mice showed that the same cells expressed both types of IFN type I mRNA in this organ (results not shown).

Following i.c. administration of aggressive strains of LCMV, such as LCMV-Arm53b, virus replication is limited to the brain and, for the first 3 to 5 days, to the spleen (35). The absence of IFN-α-producing cells in the brain may therefore provide a selective advantage for viral replication in this organ. Such a selective advantage for viral growth could be manifested by the well-known neurotropic and visceral variants of LCMV. Two modes of action could be operative: (i) variability in the IFN-α/β-inducing capacity of LCMV and (ii) variability in sensitivity to the antiviral activity of IFN-α/β. Previous studies have shown that different strains of LCMV show variability in IFN-α/β-inducing capacity in vivo (25). However, no correlation has been found between IFN-α/β-inducing capacity and the classification of neurotropic and visceral strains of LCMV (29). Interestingly, a recent study by Moskophidis et al. shows variability in sensitivity to IFN-α/β between different strains of LCMV (32a).

Although no IFN-α mRNA was detected in brains of LCMV-infected mice, weak but distinct expression of IFN-β mRNA was found by RNase protection analysis. The kinetics of the appearance of mRNA for IFN-β in the brain was slower than that of IFN-α and IFN-β mRNAs in the spleen (Fig. 2C and D). The cellular source of IFN-β mRNA in the brain was not detected by in situ hybridization. The explanation for this could be that the IFN-β mRNA is expressed by a large number of cells in an LCMV-infected brain at low levels, below the limit of detection by in situ hybridization, or that IFN-β mRNA-expressing cells in the brain are extremely infrequent.

Basal expression of 2′,5′-OAS mRNA has been demonstrated in both the brains and spleens of normal mice (12) and is probably dependent on a low level of IFN-α/β synthesis (15). Following stimulation with IFN-α/β, the level of 2′,5′-OAS mRNA rapidly increases whereas its subsequent down regulation, at least in vitro, appears to be closely linked to the loss of IFN-α/β receptors on the cell surface (21). Therefore, elevated expression of 2′,5′-OAS mRNA may serve as a sensitive indicator of low levels of IFN-α/β. In support of this assumption, the levels of 2′,5′-OAS mRNA in spleens and brains followed the expression of IFN-α or IFN-β mRNA with a delay of 2 to 3 days (Fig. 2 and 4). The kidneys are probably affected by both IFN-α present in the circulation and locally produced IFN-β, which could account for the prolonged expression of 2′,5′-OAS mRNA in this organ. It is plausible that expression of 2′,5′-OAS mRNA in the brain is regulated by local production of IFN-β because systemic IFN-α is inefficient at increasing 2′,5′-OAS in the brain (12). On the other hand, the blood-brain barrier is known to be damaged by around day 3

postinfection with LCMV (22), which could result in leakage of IFN-α from blood to the CNS with a subsequent increase of 2′,5′-OAS mRNA. Furthermore, expression of 2′,5′-OAS mRNA could be affected by IFN-α/β (54), mRNAs for both of which are produced in the brain during acute LCM (5).

The expression of mRNA for H2D appears to be regulated also by factors other than IFN-α and IFN-β in LCMV-infected mice because the kinetics of expression was not strictly associated with IFN-α and IFN-β mRNA expression (Fig. 2 and 5). Factors other than IFN reported to regulate major histocompatibility complex class I include cytokines such as tumor necrosis factor alpha, interleukin 1, IFN-γ (36, 37), and also LCMV (14). Tumor necrosis factor alpha and interleukin 1 mRNAs are expressed in brains of i.c. LCMV-infected mice commencing at day 3 postinfection (5) and might therefore, together with the virus and IFN-β, influence the expression of H2D. Such an interaction in a cytokine network is probably very important in resistance to infections in the brain and may be a prerequisite for the development of immune reactions in the CNS. However, the role of specific cytokines can be difficult to assess because of regulatory feedback loops between the cytokines involved.

The absence of IFN-α mRNA-expressing cells among inflammatory cells in the meninges and choroid plexus in brains of LCMV-infected mice might suggest that IFN-α-producing cells are not involved in the inflammatory process in virus-infected tissues. On the contrary, the production of IFN-α in the spleen and cervical lymph nodes suggests a role for IFN-α in immune regulation, perhaps by directing lymphocyte migration (24, 27, 28) or regulation of T- and B-cell differentiation (11, 18, 34). On the other hand, the IFN-α-producing cells localized in the spleen and lymph nodes may simply have the function of rapidly producing large quantities of IFN-α to achieve early, systemic antiviral responses. Regardless of the function of secreted IFN-α, the characteristic expression of IFN-α mRNA suggests that a specialized cell type, present in the marginal zones of the spleen and lining the cortex of the lymph nodes, is the cellular source responsible for the massive production of circulating IFN-α early in viral infections. A possible candidate for the cell responsible for this IFN-α production is the antigen-presenting dendritic cell. This cell type is scattered throughout the body, with the possible exception of the CNS, and migrates via afferent lymph or the bloodstream to T-dependent areas in lymph nodes and the spleen (47), where it is involved in the initiation of several immune responses. In human (3, 6, 38, 39, 41) and pig (8) peripheral blood, an infrequent but highly efficient IFN-α-producing cell (the natural IFN-α-producing NIP cell) has been demonstrated which could correspond to the cell producing IFN-α in the spleens and cervical lymph nodes of LCMV-infected mice. The NIP cell shows many similarities to, but also differences from, antigen-presenting dendritic cells (46). However, its biological relevance is still not understood and further studies are needed to elucidate the impact of NIP cells on inflammatory and immunological reactions.

In comparison with IFN-α, IFN-β gene expression in the brain and other tissues showed a slower time of appearance in LCMV-infected mice. A difference in the kinetics of appearance for IFN-α and IFN-β mRNAs, depending on the inducing virus (17), different requirements for protein synthesis for gene expression of IFN-α and IFN-β (7, 16), and usage of different transcription factors and different DNA-binding motifs in the regulatory sequences of IFN-α and IFN-β genes (31, 48), has been reported. It is possible that these discrepancies between IFN-α and IFN-β, both in cellular and organic expression and in the requirements for transcription, reflect two partly different biological functions.

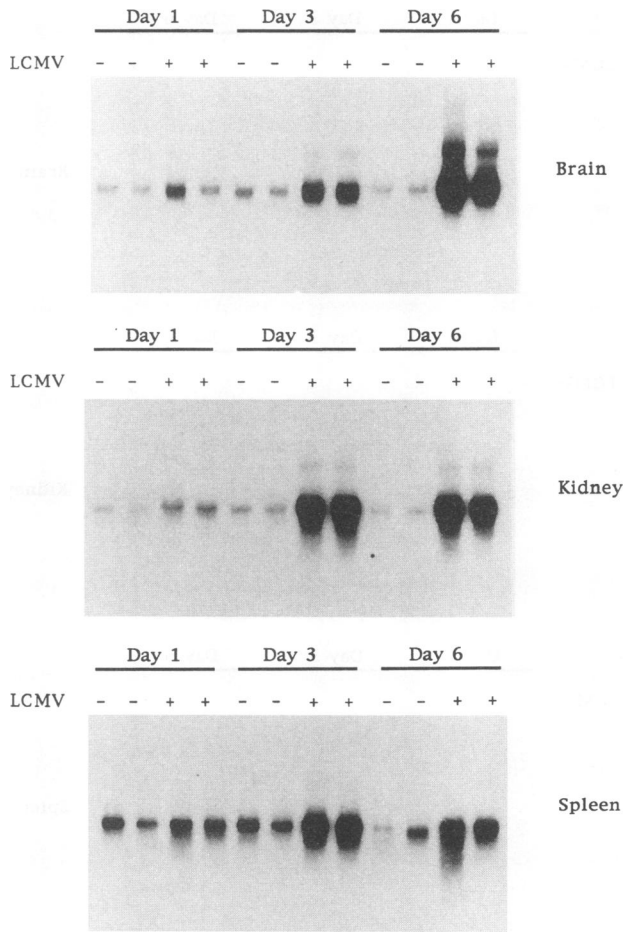

FIG. 5. Expression of H2D mRNA in the brains, spleens, and kidneys of LCMV-infected mice. Poly(A)$^+$ RNA was extracted from the brains, spleens, and kidneys on days 1, 3, and day 6 postinfection, and 5 μg of poly(A)$^+$ RNA from each of two mock-infected (−) and two LCMV-infected (+) mice was analyzed by Northern blot hybridization using a ^{32}P-labelled H2Dβ DNA probe.

In summary, we showed that high levels of circulating IFN-α, but not IFN-β, are produced soon after i.c. inoculation of mice with LCMV. The cells which likely produce the bulk of IFN-α during LCM have been shown to be localized to the marginal zone of the spleen on days 1 and 3 postinfection and, to a lesser degree, to the subcapsular sinus lining the cortex of cervical lymph nodes on day 3 postinfection. No IFN-α mRNA was detected in the brain, whereas extremely low but increasing levels of IFN-β mRNA were detected in this tissue commencing on day 3. The absence of IFN-α/β mRNA-containing cells among the infiltrating leukocytes in the CNS despite high levels of virus is an important observation because these cytokines are thought to be of central importance in limiting the spread of LCMV in infected animals (35, 40). The absence of IFN-α-producing cells in the CNS may therefore provide a selective advantage for viral replication that could, in part, account for the well-known tropism of LCMV-Arm53b for the brain, which ultimately leads to the targeted inflammation of the choriomeninges and choroid plexus in acute LCM.

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