Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9939–9943, October 1992
Microbiology

Selective disruption of growth hormone transcription machinery by viral infection

JUAN CARLOS DE LA TORRE AND MICHAEL B. A. OLDSTONE

Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Floyd E. Bloom, June 25, 1992

ABSTRACT **Viruses that establish persistent infections may show selective and unique effects on the host's transcriptional machinery. Lymphocytic choriomeningitis virus (LCMV), a noncytolytic virus, can persistently infect a rat pituitary cell line. Although the infected cells remain free of structural damage, virus markedly interferes with growth hormone (GH) but only minimally interferes with prolactin transcription. The study of GH promoter–chloramphenicol acetyltransferase-transfected cells and GH promoter deletion mutants demonstrates that the viral effect is at the level of GH promoter and is due to interference with GH transactivator factor GHF1 (Pit1). Treatment of LCMV-infected cells with the antiviral agent ribavirin cures the infection and restores normal GH mRNA levels. These results illustrate a molecular mechanism by which a virus infection can disrupt synthesis of a cell's differentiated product without perturbing vital cellular functions.**

Virus-induced disease occurs as a result of both direct cell destruction due to virus replication and damage to the infected cells by immunological assault (1–3). In addition, viruses can establish persistent infections during which the typical hallmarks of virus infection—cytolysis and inflammation—are not present, but the host's differentiated functions may be affected. This, in turn, can disrupt homeostasis and lead to disease (ref. 4; reviewed in refs. 3 and 5). We have previously described that C3H mice persistently infected with lymphocytic choriomeningitis virus (LCMV) develop a growth hormone (GH)-deficiency syndrome manifested as retarded growth and hypoglycemia (4, 6). Despite high levels of virus replication in the GH-producing cells of the anterior pituitary, there is no evidence of structural damage or inflammation (4, 6), yet production of GH mRNA and protein is significantly diminished (4, 6–9). However, the complex physiological regulation of GH biology, involving the immune hypothalamic–pituitary–adrenal axis (10), has made it difficult to answer the question of whether the reduction in GH is directly and solely caused by LCMV replication within the somatotroph cells.

To investigate the molecular mechanisms whereby GH mRNA synthesis is turned down without impairment in cellular vital functions, we established a tissue culture model with cells from a rat pituitary cell line (PC cells) that express GH and prolactin (PL) (11). This model enabled us to study the consequences of LCMV infection on the somatotroph cell program. Our results indicate that LCMV infection of somatotroph cells can cause a significant, and specific, decrease in GH transcription by interfering with the activity of the GH transactivation factor GHF1 (Pit1). This impairment on GH transcription appears to require virus replication and/or transcription since PC cells cured of the infection exhibited normal GH mRNA levels.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cells and Virus. PC cells were grown as monolayers in RPMI medium supplemented with 5% fetal bovine serum and 5% horse serum. Isolation and characterization of LCMV ARM 53B virus clonal pool, as well as procedures for LCMV infections, titrations, and immunofluorescence assays, have been described (7, 12). Infections were done at a multiplicity of three plaque-forming units per cell.

Northern Blot Analysis. Total RNA was extracted from cultured cells by the guanidinium isothiocyanate/acid phenol method (13). Poly(A)$^+$ RNA was purified through oligo(dT)-cellulose chromatography and size fractionated on 1% agarose formaldehyde gel (14). RNA was transferred to a nylon membrane and hybridized successively with specific cDNA probes for GH and actin. Labeling of probes was done with an oligolabeling kit from Pharmacia (no. 27-9250-01). Hybridization and stripping of the probes were done by standard protocols (15).

Western Blot Analysis. Whole cellular (WCE) or nuclear (NCE) extracts were prepared, separated by SDS/PAGE, and transferred to nitrocellulose membranes as described (16). Equal amounts of protein were loaded into each lane, as determined by the Bio-Rad protein assay kit. The blot was probed with rabbit antibodies against GHF1, washed, incubated with alkaline phosphatase-conjugated goat anti-rabbit antibody, and developed with the chromogenic substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

DNA Transfections and Chloramphenicol Acetyltransferase (CAT) Assays. In many independent experiments, 1–2 × 10^6 uninfected or LCMV-infected (24 hr postinfection) cells were transfected by the lipofectin method following the protocol recommended by the supplier (BRL; lipofectin reagent catalogue no. 82925A). Forty-eight hours later, cell extracts were prepared and CAT assays were performed as described (15–17).

Extract Preparation and *in Vitro* Transcription. WCE from uninfected or from LCMV-infected PC cells were prepared as described (16, 18), and the protein concentration was determined by using the Bio-Rad protein assay kit. *In vitro* transcription reactions were performed essentially as described (16–18) in a final vol of 50 μl, containing various amounts of WCE in 25 μl of 20 mM Hepes, pH 7.9 (at 30°C)/12.5 mM $MgCl_2$/0.1 M KCl/0.5 mM dithiothreitol/20% (vol/vol) glycerol/20 μl of a nucleotide mixture containing the four ribonucleotides (1 mM each) and 5% polyvinyl alcohol. Templates were added at the indicated amounts in each case in a 3-μl volume. Reaction mixtures were incubated at 30°C for 60 min and terminated by adding 2 μl of yeast tRNA (5 μg/μl) and by guanidinium isothiocyanate/acid phenol extraction of the RNA (13). The amount

Abbreviations: LCMV, lymphocytic choriomeningitis virus; GH, growth hormone; PL, prolactin; WCE, whole cell extract(s); NCE, nuclear cell extract(s); CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; RT, reverse transcriptase; NP, nucleoprotein; GP, glycoprotein; CMV, cytomegalovirus.

of correctly initiated RNA synthesized from the specific supercoiled plasmid templates was measured by primer extension with synthetic end-labeled CAT or thymidine kinase (TK) primers. Primers were labeled to the same specific activity with T4 polynucleotide kinase and [γ-^{32}P]ATP (ICN); 1×10^6 cpm of each primer was added to the corresponding RNA product from *in vitro* transcription reactions in 10 μl of 10 mM Tris·HCl, pH 7.9/1 mM EDTA/0.25 mM KCl and incubated at 55°C for 60 min. This was followed by addition of 24 μl of a mixture containing 10 mM Tris·HCl (pH 8.5), 10 mM $MgCl_2$, 5 mM dithiothreitol, actinomycin D (100 μg/ml), 0.4 mM dNTPs, and 1 μl of avian myeloblastosis virus reverse transcriptase (RT) (20 units/μl; Seikagaku America, Rockville, MD). Reaction mixtures were incubated at 37°C for 45 min and terminated by being brought to 1% SDS/200 mM NaCl/20 mM EDTA, followed by phenol/chloroform extraction and ethanol precipitation. Primer extension products [95 and 88 nucleotides for GH and cytomegalovirus (CMV) promoters, respectively] were analyzed on 8% polyacrylamide/42% urea gels (15).

RT PCR Procedures. Total RNA was purified (13) and RT PCRs were performed following the Perkin–Elmer protocol. LCMV nucleoprotein (NP) and glycoprotein (GP) RNA sequences were amplified by PCR (30 cycles), and the reaction products were resolved on 2% agarose gel electrophoresis and visualized with ethidium bromide. NP oligonucleotides were as follows: primer A, 5′-CAGTTATAGGTGCTCTTCCGC-3′ (complementary to nucleotides 1994–1974 of LCMV NP); primer B, 5′-AGATCTGGGAGCCTTGCTTTG-3′ (complementary to nucleotides 1706–1726 of LCMV NP), which amplify a 289-base-pair fragment. GP oligonucleotides were as follows: primer A, 5′-CGCCGGTCTTTGCATGTTCTAG-3′ (complementary to nucleotides 1209–1230 of LCMV GP); primer B, 5′-GCACATTCACCTGGACTTTGTC-3′ (complementary to nucleotides 1974–1994 of LCMV GP), which amplify a 357-base-pair fragment.

RESULTS AND DISCUSSION

PC cells supported LCMV replication without signs of either structural damage or impairment in cell physiology. Interestingly, the steady-state level of GH mRNA in LCMV-infected PC cells was significantly reduced (4- to 8-fold), whereas PL mRNA was only slightly reduced (0.5- to 2-fold reduction) (Fig. 1*A*). No differences were observed between uninfected and infected cells in the level of actin mRNA (Fig. 1*A*). In addition, levels of cyclophylin and glyceraldehyde-phosphate dehydrogenase mRNA were also not affected by LCMV infection (data not shown). Run-on experiments indicated a 5-fold reduction in the initiation of GH transcription (data not shown). Thus, the PC cell–LCMV model recreated the observations previously described in C3H mice persistently infected with LCMV (4, 6–9) and provided a suitable system in which to study the molecular mechanisms by which the noncytolytic LCMV interferes with GH transcription.

To explore the means by which LCMV infection was interfering with the transcriptional activity of the GH promoter, we studied the expression of the reporter gene CAT under control of the GH promoter in uninfected or in LCMV-infected PC cells. LCMV infection caused a significant decrease (average, 10.5-fold; range, 7.4- to 18.2-fold; three experiments) in CAT activity only when the reporter gene was under GH promoter control (Fig. 1*C*). This decrease was not due to a general and nonspecific impairment of cellular transcription but rather was specific for GH as illustrated by the similar levels of CAT activity obtained in uninfected and infected cells when CAT gene expression was driven by either a CMV or a simian virus 40 promoter (Fig. 1*C*). In addition, LCMV infection had a smaller but reproducible effect (2-fold decrease) on PL promoter activity (Fig. 1*C*). A common mechanism whereby this dual effect on GH and PL promoters can occur is through the same regulatory transcription factor, GHF1 (or Pit1), as it plays a key role in regulation of both GH and PL promoters (16–26).

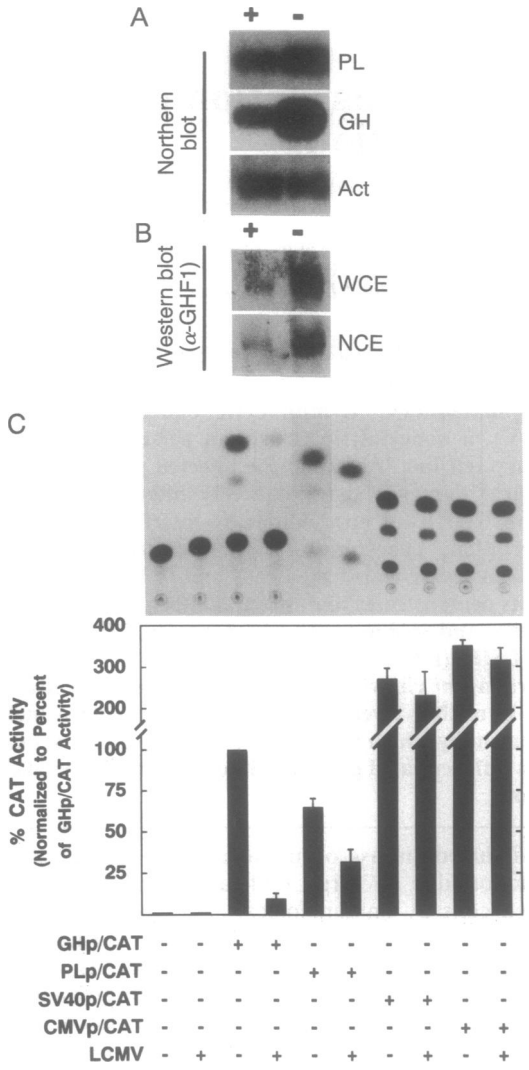

FIG. 1. Decrease in GH mRNA steady-state level in LCMV-infected PC cells is associated with a reduction in GHF1 protein level and decreased GH promoter activity. (*A*) Specific decrease in GH mRNA in LCMV-infected PC cells. RNA was extracted from uninfected (lane −) or infected (lane +) (72 hr postinfection) cells and analyzed by Northern blot hybridization using specific cDNA probes for GH and actin (Act) as described. Equal amounts (2 μg) of poly(A)$^+$ RNA from uninfected and infected cells were used. (*B*) Reduction in GHF1 protein levels in LCMV-infected PC cells. WCE or NCE were prepared from uninfected (lane −) or infected (lane +) PC cells and analyzed by Western blot with specific antibodies against GHF1 (α-GHF1) as described. (*C*) Specific decrease in GH promoter activity in LCMV-infected PC cells. Uninfected or LCMV-infected (24 hr postinfection) PC cells were transfected as described with the following plasmids: GHp/CAT (25 μg), PLp/CAT (25 μg), SV40p/CAT (25 μg), and CMVp/CAT (1 μg plus 24 μg of pBR322 plasmid DNA). Forty-eight hours later, cell extracts were prepared and CAT assays were performed as described. Protein concentration in each cell extract was measured and equal amounts of each cell extract were used for CAT assays. Levels of CAT activity were converted to percentage GHp/CAT expression in uninfected cells. Results are the averages of three independent experiments. Infections were done at a multiplicity of infection of 3, always using the same clonal pool virus. No significant differences in virus titers were observed among different infections.

Reduced GH expression in LCMV-infected PC cells could be explained by diminished availability of functional GH

promoter. In turn, GH promoter availability could be affected either by a direct interaction between a viral product and the GH promoter or by interaction between the GH promoter and cellular factors induced or modified by the viral infection. Alternatively, the decrease in GH transcription might reflect a decrease in the amount or activity of a factor(s) involved in transactivation of the GH gene. To address these possibilities, we examined the effect of GH promoter concentration on GH expression in uninfected and in LCMV-infected PC cells. We found (Fig. 2*A*) that the decrease of GHp/CAT expression caused by LCMV infection was not overcome by increasing the amount of GHp/CAT DNA used to transfect LCMV-infected PC cells. In addition, when small amounts (6 μg or less) of GHp/CAT DNA were used, uninfected and infected PC cells showed similar levels of CAT expression (Fig. 2*A*). These results suggested that a decrease in the amount of functionally active factor(s) involved in GH promoter activation likely underlies the impairment of GH transcription caused by LCMV infection.

Support for this hypothesis comes from the observation that levels of GHF1 (Pit1) protein were largely decreased in WCE as well as NCE from LCMV-infected PC cells (Fig. 1*B*). Nonetheless, as did previous investigators (14), we only found a very modest decrease (2-fold) in GHF1 mRNA steady-state level (data not shown).

GHF1 (or Pit1), a tissue-specific Pou domain transcription factor, proved to be required for activation of GH, PL, as well as GHF1 promoters (16–26). Moreover, expression of GHF1 in HeLa cells, which do not express GH, was sufficient to activate a cotransfected gene under the control of GH promoter (24, 25). To investigate the role of GHF1 in the effect exerted by LCMV on the GH transcriptional machinery, we pursued two experimental avenues. First, we studied whether LCMV infection had any effect on the ability of GHF1 to transactivate GHp/CAT expression in a non-GH-expressing cell line. Transfection of HeLa cells with GHp/CAT produced only low levels of CAT expression. In contrast, cotransfection of GHp/CAT with a DNA construct (RSVp/GHF1) that allowed GHF1 expression led to a large increase (2 orders of magnitude) in CAT activity. LCMV infection severely limited transactivation of GHp/CAT by GHF1 in HeLa cells (Fig. 2*B*), suggesting that GHF1 might

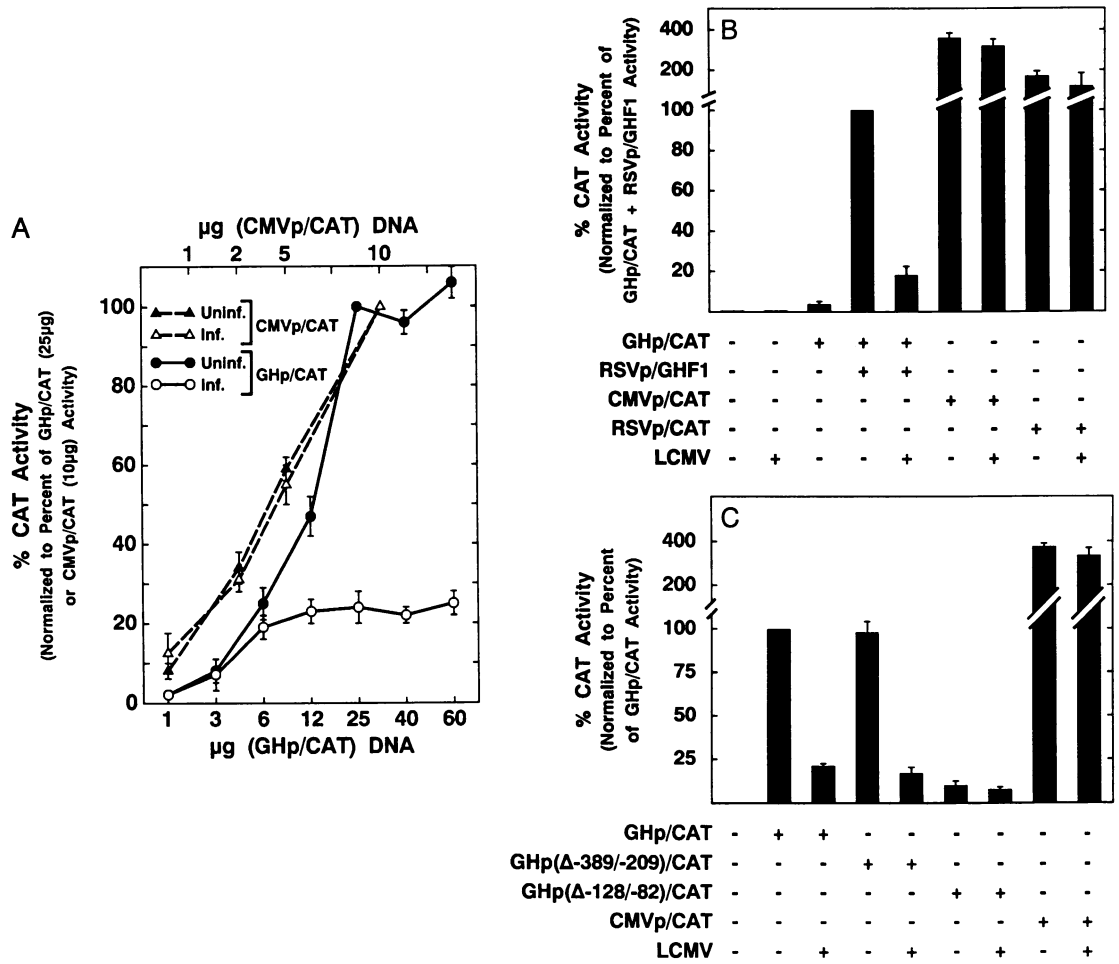

FIG. 2. Decrease in GH promoter activity caused by LCMV infection involves GHF1. (*A*) Decrease in GH promoter activity in LCMV-infected cells is not overcome by augmenting the amount of GH promoter. Uninfected or infected PC cells were transfected (24 hr postinfection) with increasing amounts of GHp/CAT or CMVp/CAT DNA. Forty-eight hours later, CAT activities were determined. Levels of CAT expression were converted to percentage of expression obtained with GHp/CAT (25 μg) or CMVp/CAT (10 μg) in uninfected PC cells. Results are averages of three independent measures for each plasmid DNA dose. (*B*) Transactivation of GH promoter by GHF1 in HeLa cells is largely impaired by LCMV infection. Uninfected or infected HeLa cells were transfected (24 hr postinfection) with the following plasmids: GHp/CAT (25 μg), GHp/CAT (25 μg) + RSVp/GHF1 (50 μg), or CMVp/CAT (1 μg). pBR322 plasmid DNA was used to adjust the final amount of DNA to 75 μg. Forty-eight hours later, CAT activities were measured and converted to percentage GHp/CAT + RSVp/GHF1 expression in uninfected HeLa cells. (*C*) Activity of a GH promoter lacking the GHF1 binding sites is not affected by LCMV infection. Uninfected or infected PC cells were transfected with the following plasmids: GHp/CAT (20 μg), GHp (Δ−389/−209)/CAT (20 μg), GHp (Δ−128/−82)/CAT, or CMVp/CAT (1 μg). Transfections, LCMV infection, and virus titrations were done as described. CAT activities were determined (15, 17) and are expressed as percentage GHp/CAT expression in uninfected cells.

play an important role in the virus–host interaction responsible for the decrease in GH synthesis. To gain further insight into the molecular basis for this virus–host interaction, the effect of LCMV infection on a GH 5′ promoter deletion mutant that lacked both binding sites for GHF1 (Δ−128/−82) was studied. This deletion limited the expression of GHp/CAT in pituitary cells (17, 26) (Fig. 2*C*), with ≈10 times less CAT activity than GH wild-type promoter. Nevertheless, although low, the CAT activity displayed by the mutant promoter (GHF1 Δ−128/−82) was ≈20 times higher than our background levels (0.1–0.4% of GHp/CAT expression in uninfected PC cells). This would allow us to measure any possible effect of the LCMV infection on the activity of a GH promoter lacking the GHF1 binding sites. Interestingly, the transcriptional activity of this promoter was not affected by LCMV infection. In contrast, the activity of another well-characterized GH 5′ promoter deletion mutant (Δ−386/−209) (17, 26) was markedly reduced by LCMV infection. The Δ−386/−209 mutant contained both GHF1 binding sites and displayed activity similar to that of the wild-type GH promoter in uninfected pituitary cells (Fig. 2) (17, 26). These results support the view that GHF1 is most likely involved in the virus–host interaction responsible for a decrease in GH transcription.

With the hypothesis that a decrease in the level of functionally active GHF1 is causing the decrease of GH transcription in LCMV-infected PC cells, some testable predictions can be made by using *in vitro* transcription systems. Extracts prepared from uninfected PC cells would provide a greater GH promoter activity than those from LCMV-infected PC cells. In contrast, extracts from uninfected or infected PC cells should support similar levels of transcription when an unrelated promoter is used as template. In addition, extracts from uninfected PC cells should complement those prepared from LCMV-infected cells and consequently restore normal levels of GH promoter activity. Extracts from LCMV-infected cells provided ≈10-fold less GH promoter activity than extracts from uninfected PC cells (Fig. 3*A*). In contrast, extracts from uninfected or infected PC cells displayed similar CMV promoter activity (Fig. 3*A*). Moreover, extracts from uninfected PC cells, but not from HeLa cells, were able to complement extracts from infected cells and restored normal levels of GH promoter activity (Fig. 3*B*). Transcription was RNA polymerase II dependent, as indicated by its sensitivity to α-amanitin (1 μg/ml) (data not shown).

Taken together, these results indicate that replication of LCMV in somatotroph cells leads to a decrease in the amount of functionally active GHF1, which in turn results in lower levels of GH promoter activity. This effect on GHF1 may significantly contribute to the GH deficiency syndrome caused by LCMV in C3H mice. That levels of PL in C3H mice persistently infected with LCMV are normal (4, 6–9) despite the fact that GHF1 has been implicated in PL expression can be explained by the lack of virus replication in PL cells (4, 6–9). In contrast, individual PC cells make both GH and PL and are uniformly infected with LCMV (>98% of the cells are positive for virus antigen after infection with LCMV; data not shown). Hence, more intriguing is the differential effect on GH and PL promoters exerted by LCMV infection in PC cells. Conceivably, in PC cells GH and PL promoters differ in their abilities to sense changes in GHF1 levels. Virus

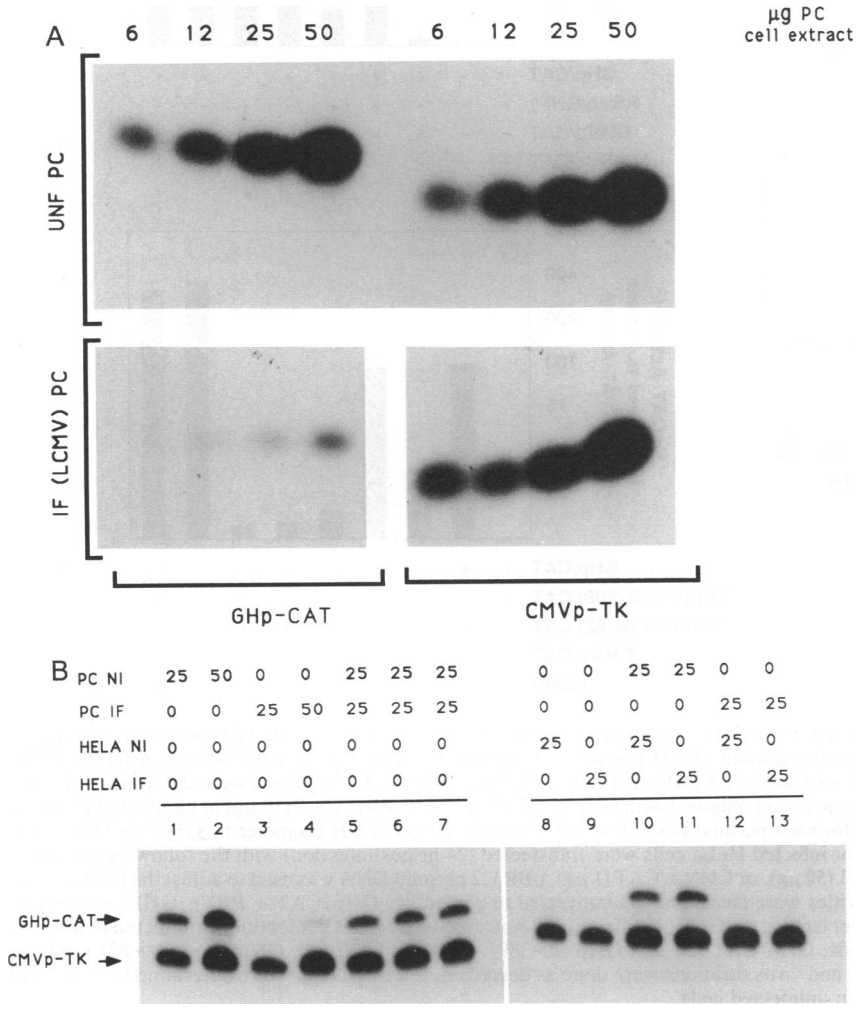

FIG. 3. Transcriptional activity of the GH promoter in cell extracts from uninfected or LCMV-infected PC cells. (*A*) Extracts from LCMV-infected (IF) PC cells support lower levels of GH promoter activity, but similar levels of CMV promoter activity compared to extracts from uninfected (UNF) PC cells. WCE were prepared as described (16, 18). *In vitro* transcriptions were performed as described. Amounts of correctly initiated RNA synthesized from the specific supercoiled plasmid templates, GHp/CAT (400 ng) or CMVp/TK (800 ng), were measured by primer extension with a synthetic end-labeled CAT or TK primer. Primer extension products (95 and 88 nucleotides for GH and CMV promoters, respectively) were analyzed on polyacrylamide/urea gels (15). (*B*) Transcriptional activity of GH promoter in mixed extracts. *In vitro* transcription reactions were performed with the indicated extract mixtures. Plasmid template amounts were 200 and 400 ng for GHp/CAT and CMVp/TK, respectively. Transcription was measured by primer extension as described in *A*. Both primers were labeled at equal specific activity and the same amounts were used to quantify the *in vitro* transcription products. Lanes: 5, 25 μg of WCE from uninfected (NI) PC cells was preincubated with the plasmid templates for 20 min (primary extract) before adding 25 μg of WCE from LCMV-infected (IF) PC cells (secondary extract); 6, 25 μg of WCE LCMV-infected PC cells was used as primary extract and 25 μg of WCE PC cells was used as secondary extract; 7, both extracts were added simultaneously to the plasmid templates; 10–13, both extracts were added simultaneously.

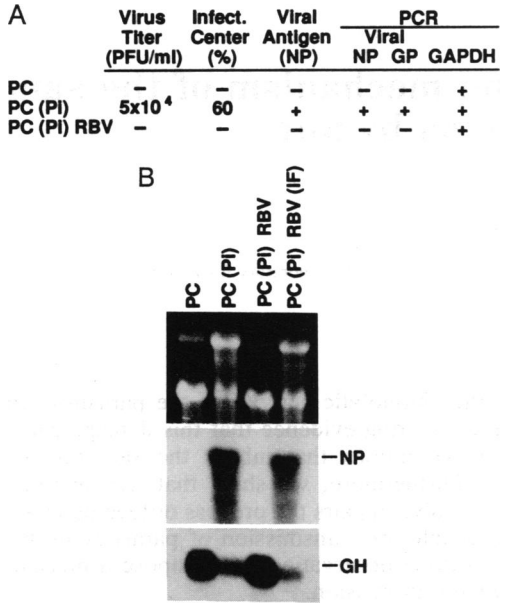

FIG. 4. (*A*) Ribavirin cures PC cells of the LCMV infection. PC cells persistently infected with LCMV [PC (Pi)] were treated with ribavirin (50 μg/ml) for 72 hr. After ribavirin treatment [PC (Pi) RBV], extracellular or intracellular LCMV infectious particles were not detected. In contrast, untreated cells had virus titers of 5×10^4 plaque-forming units (PFU)/ml and 60% of the cells in the population were carrying infectious LCMV intracellularly as determined by infectious center assay. Immunofluorescence studies showed that PC (Pi) RBV cells were negative for the NP LCMV-specific antigen. In addition, RT PCRs with specific primers for the NP and the GP LCMV mRNA failed to reveal any LCMV-specific sequences in the ribavirin-treated cells. Specific primers for glyceraldehyde-phosphate dehydrogenase (GAPDH) were used as an internal control. (*B*) Curing PC (Pi) by ribavirin restores the PC wild-type phenotype. RNA from uninfected PC cells (PC), from PC cells persistently infected with LCMV before [PC (Pi)] and after [PC (Pi) RBV] ribavirin treatment, and LCMV-infected PC (Pi) RBV cells was extracted and analyzed by Northern blot hybridization as described. (*Top*) Ethidium bromide staining of the RNA gel showing that similar amounts of total RNA were loaded. (*Middle* and *Bottom*) Hybridization with NP and GH cDNA probes, respectively. These studies showed that PC (Pi) cells after ribavirin treatment [PC (Pi) RBV] restored normal GH mRNA levels. Cells cured by ribavirin were susceptible to LCMV infection [PC (Pi) RBV (IF)] with a concomitant decrease in GH mRNA steady-state level. Virus titrations, infectious center assay, and immunofluorescence procedures were as described (7, 12). RT PCRs and PCR product analysis were performed as described. Northern blot hybridizations were done as described in Fig. 1.

infection could affect the interaction between GHF1 and other factor(s) yet to be characterized that ultimately determine differences in the promoter response. Alternatively, LCMV infection could also affect GHF1 phosphorylation, thus interfering with the interaction between GHF1 and both GH and PL promoters (27). These various possibilities remain to be examined. It is also not known whether there is a strict requirement for viral replication or whether the expression of specific viral sequences is sufficient to cause the impairment in GH transcription. Treatment with ribavirin, an antiviral agent, cured LCMV-infected PC cells of the virus infection and restored normal GH mRNA levels (Fig. 4). Production of infectious virus by LCMV-infected PC cells was abrogated by the ribavirin treatment. In addition, viral antigen and L CMV-specific nucleic acid were not detected in the ribavirin-treated cells (Fig. 4*A*). This result suggests that continuous viral replication and/or expression is needed to cause the GH deficiency.

In conclusion, the data presented here point to subtle but distinct effects viruses can have on differentiated cells. Hence, without affecting vital cellular functions, LCMV infection affects the transcription of the GH gene by its action on the GH transactivator factor GHF1. Nonlytic viruses can persistently infect cells of the endocrine, immune, or nervous system. Thus, selective defects in hormones, regulators of immune function, or neurotransmitter functions occurring in a variety of diseases of unknown etiology may be virus induced. Finally, the finding that ribavirin was able to reverse the GH deficiency suggests that this or other antiviral therapies (28) may reverse defects in such differentiated systems induced by viruses.

We thank P. Chomczynski for the PC cell line; J. L. Castrillo for the plasmids GHp/CAT, GHp (Δ−128/−82)/CAT, and GHp (Δ−386/−209)/CAT; M. G. Rosenfeld for the rabbit antibody against Pit1; and G. Schilling for excellent editorial assistance. This is Publ. No. 7115-NP from the Department of Neuropharmacology, The Scripps Research Institute. This work was supported in part by U.S. Public Health Service Grants AI-09484 and AG-04342.

1. Fenner, F., McAuslan, B., Mims, C., Sambrook, J. & White, D. (1974) *The Biology of Animal Viruses* (Academic, New York), 2nd Ed.
2. Notkins, A., ed. (1975) *Viral Immunology and Immunopathology* (Academic, New York).
3. Oldstone, M. B. A. (1989) *Sci. Am.* **260**, 42–48.
4. Oldstone, M. B. A., Sinha, Y. N., Blount, P., Tishon, A., Rodriguez, M., von Wedel, R. & Lampert, P. W. (1982) *Science* **218**, 1125–1127.
5. de la Torre, J. C., Borrow, P. & Oldstone, M. B. A. (1991) *Br. Med. Bull.* **47**, 838–851.
6. Oldstone, M. B. A., Rodriguez, M., Daughaday, W. H. & Lampert, P. (1984) *Nature (London)* **307**, 278–280.
7. Oldstone, M. B. A., Ahmed, R., Buchmeier, M. J., Blount, P. & Tishon, A. (1985) *Virology* **142**, 158–174.
8. Valsamakis, A., Riviere, Y. & Oldstone, M. B. A. (1987) *Virology* **156**, 214–220.
9. Klavinskis, L. S. & Oldstone, M. B. A. (1989) *Virology* **168**, 232–235.
10. Bateman, A., Singh, A., Kral, T. & Solomon, S. (1989) *Endocr. Rev.* **10**, 92–112.
11. Chomczynski, P., Brar, A. & Frohman, L. A. (1988) *Endocrinology* **123**, 2276–2283.
12. Oldstone, M. B. A. & Dutko, F. J. (1983) *J. Gen. Virol.* **64**, 1689–1698.
13. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
14. Castrillo, J. L., Theill, L. E. & Karin, M. (1991) *Science* **253**, 197–199.
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
16. Lefevre, C., Inayawa, M., Dana, S., Grindlay, J., Bodner, M. & Karin, M. (1987) *EMBO J.* **6**, 971–981.
17. Dana, S. & Karin, M. (1989) *Mol. Endocrinol.* **3**, 815–821.
18. Bodner, M. & Karin, M. (1987) *Cell* **50**, 267–275.
19. Bodner, M., Castrillo, J. L., Theill, L., Deerinck, T., Ellisman, M. & Karin, M. (1988) *Cell* **55**, 505–518.
20. Li, S., Crenshaw, E. B., III, Rawson, E. J., Simmons, D. M., Swanson, L. W. & Rosenfeld, M. B. (1990) *Nature (London)* **347**, 528–533.
21. Fox, S. R., Jorg, M. T. C., Casianover, J., Ye, S. F., Stanley, F. & Sammuels, H. H. (1990) *Mol. Endocrinol.* **4**, 1069–1080.
22. Dolle, P., Castrillo, J. L., Theill, L. E., Deerinck, T., Ellisman, M. & Karin, M. (1990) *Cell* **60**, 809–820.
23. Chen, R., Ingraham, H. A., Treacy, M. N., Albert, V. R., Wilson, L. & Rosenfeld, M. G. (1990) *Nature (London)* **346**, 583.
24. Ingraham, H. A., Chen, R., Mangalam, H. J., Elsholtz, H. P., Flynn, S. E., Lin, C. R., Simmons, D. M., Swanson, L. & Rosenfeld, M. G. (1988) *Cell* **55**, 519–529.
25. Mangalam, H. J., Albert, V. R., Inbraham, H. A., Kapiloff, M., Wilson, L., Nelson, C., Elsholtz, H. & Rosenfeld, M. G. (1989) *Genes Dev.* **3**, 946–958.
26. McCormick, A., Wu, D., Castrillo, J. L., Dana, S., Strobl, J., Thompson, E. B. & Karin, M. (1988) *Cell* **55**, 379–389.
27. Kapiloff, M. S., Farkash, Y., Wegner, M. & Rosenfeld. M. G (1991) *Science* **253**, 786–789.
28. Oldstone, M. B. A., Blount, P., Southern, P. J. & Lampert, P. W. (1986) *Nature (London)* **321**, 239–243.