JOURNAL OF VIROLOGY, Dec. 1996, p. 8438–8443 Vol. 70, No. 12
0022-538X/96/$04.00+0

A Single Amino Acid Change in the Glycoprotein of Lymphocytic Choriomeningitis Virus Is Associated with the Ability To Cause Growth Hormone Deficiency Syndrome†

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Received 5 March 1996/Accepted 29 August 1996

Persistent infection of C3H/St mice with certain strains of lymphocytic choriomeningitis virus (LCMV) causes a growth hormone (GH) deficiency syndrome (GHDS) manifested as growth retardation and hypoglycemia. Infected mice show high levels of viral replication in the GH-producing cells in the anterior pituitary leading to decreased synthesis of GH mRNA and protein despite the absence of detectable virus-induced cell structural damage. Virus clones isolated from the GHDS-negative LCMV WE strain can cause the disease, while others cannot. The genetic basis of this phenotypic difference is a nucleotide substitution resulting in a single amino acid difference in the viral glycoprotein. Reassortant studies indicate that the single amino acid substitution (Ser-153 to Phe) is sufficient to allow infection of the GH-producing cells and cause GHDS. These results show that a single change in the genome can affect viral pathogenicity by altering the tropism of the virus.

Virus infection can lead to disease by disrupting host cell function in two distinct manners. First, cytolysis of the infected cell can occur either as a direct result of viral replication or as a consequence of the host's immune response against the infected cells (29). Alternatively, viruses can establish persistent infection by evading the host's immune surveillance and adopting a noncytolytic mode of replication (1, 10, 20). These persistent infections can then interfere with the differentiated function of the cell, which may lead to a perturbation in host homeostasis and thus to disease (10, 17). Evidence indicates that members of many different virus families are able to cause persistent infections that are associated with functional impairment of a variety of cell types, including cells of the immune, endocrine, and central nervous systems (10, 14, 16, 17). We have documented that neonatal infection of C3H/St mice with lymphocytic choriomeningitis virus (LCMV) leads to a persistent infection that is associated with a growth hormone (GH) deficiency syndrome (GHDS) (20). This disorder is manifested as marked growth retardation and the development of hypoglycemia, which frequently leads to the death of infected animals (19, 20). Both viral and host genetic determinants contribute to GHDS (18, 22, 27). Mice susceptible to GHDS have high levels of viral replication in the GH-producing cells in the anterior pituitary, resulting in a decrease in GH levels (19, 20). This virally induced reduction in GH synthesis has been shown to operate at the level of the GH promoter and is likely due to interference with the activity of the GH transactivation factor GHF1 (Pit-1) (9, 13, 31).

LCMV is the prototype member of the arenaviruses, which have bisegmented, ambisense RNA genomes consisting of a small (S) RNA and a large (L) RNA. The S RNA encodes the viral glycoprotein (GP) and nucleoprotein (NP), while the L RNA encodes the putative viral polymerase (L) and a small protein (Z) of unknown function (3). Reassortant studies between strains of LCMV which do (Armstrong [ARM] 5) or do not (WE) cause GHDS have shown that the ability to cause GHDS is linked to the S RNA (22). Further characterization of the viral determinants necessary for disease progression was impeded by the numerous nucleotide differences between ARM and WE S RNAs. For certain negative-stranded RNA viruses, reverse genetics systems have allowed characterization of molecular components important in influencing virus biology; however, no such system has yet been established for arenaviruses. Thus, dissection of the specific viral determinants of GHDS progression has not been possible.

Clonal virus variants which differ in the ability to cause GHDS can be derived from the same GHDS-negative LCMV WE strain (8). These clonal isolates from the same parental virus strain are expected to be very closely related on a genetic basis. Therefore, the WE variants provide a good opportunity to study the exact viral determinants necessary for the induction of GHDS. We report here that a single amino acid change (Ser to Phe) in the GP-1 subunit of the glycoprotein plays a critical role in the difference of the very closely related WE virus variants to cause GHDS. This change likely causes a difference in the virus tropism for the GH-producing cells in the pituitary.

MATERIALS AND METHODS

Mice. C3H/St mice were obtained from the vivarium of The Scripps Research Institute. Animals were inoculated intracerebrally within the first 18 h of life with 1,000 PFU of LCMV in 30 μl of diluent (20). Control mice were mock infected with the virus diluent. Body weight was measured every 3 to 4 days. Blood glucose levels were determined by Chem Strip and an AccuChek III monitor (Boehringer Mannheim, Indianapolis, Ind.). Serum viremia and levels of infectious virus in organs were measured by plaque assay on Vero cells as described previously (22). All measurements were taken between 3:00 p.m. and 5:00 p.m. to minimize differences in circadian rhythms.

Viruses and generation of reassortants. The passage histories of LCMV ARM 53b and LCMV WE clonal pools, as well as the generation of the reassortants between some of the WE clones and ARM, have been previously described (18,

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TABLE 1. PCR primer sequences

Primer	Sequence (positions)
ARM NP1	CAGTTATAGGTGCTCTTCCGC (1994–1974)
ARM NP2	AGATCTGGGAGCCTTGCTTTG (1706–1726)
ARM L1	GCTCTAGAACAGTCTCATCCAGAAGCCC (5005–5025)
ARM L2	GCTTCGAAACACAACATCTCCCATTCGG (5781–5762)
WE NP1	GCGGATCCGCCTTCAATGTCAATCCATG (2161–2180)
WE NP2	GCGAATTCGCCATACATAGCCTGTAGAAC (2377–2357)
WE S5′	GCGAATTCGATGTGAGGCAAAGCCTC (119–102)
WE S3′	GCGTCGACGACTCCTGAGTCTCTGCAAG (3108–3127)
WE IG1	GCGAATTCGGGCGGTTCATGTCCAAAGC (1472–1491)
WE IG2	GCGGATCCCCACACTGTGCACTCATGGAC (1770–1750)

22). The derivation of additional WE variants is described elsewhere (8). Reassortants between WE clone 2.5 (WE c2.5) and ARM 53b were generated by coinfection of BHK-21 cells at a multiplicity of infection (MOI) of 3 PFU per cell for each virus. An MOI ranging from 1 to 10 PFU per cell was used for WE c54 coinfection with ARM (MOI of 1) to generate new reassortants between these two viruses. At 48 h after infection, supernatants were harvested and plaqued on Vero cells. Plaques were visualized 4 days later by neutral red staining and passaged once in BHK-21 cells. The genotype of the viruses was then analyzed by Northern (RNA) hybridization of total cellular RNA isolated from infected cells by using guanidinium thiocyanate (Tri-Reagent; Molecular Research Center, Cincinnati, Ohio). RNA (10 μg) was size fractionated on 1% agarose-formaldehyde gels and transferred to nylon membranes (Magnagraph; Micron Separation, Inc.). Probes for the NP and L genes of ARM were ^{32}P labeled by random priming and hybridized to the blots as described previously (24). A probe for WE NP was also used to confirm the identity of the reassortants. In addition, reverse transcriptase PCR assays using primers specific for ARM NP (ARM NP1 and ARM NP2) and L (ARM L1 and ARM L2) were done to confirm the results obtained by Northern blot analysis. Primers for WE NP (WE NP1 and WE NP2) that cross-react with ARM NP were used to ensure the presence of viral RNA in each sample. Primer sequences are shown in Table 1. Reverse transcription was primed by random hexamers and used Moloney murine leukemia virus reverse transcriptase (Superscript II) as instructed by the manufacturer (GIBCO/BRL, Gaithersburg, Md.). PCRs were carried out with *Taq* DNA polymerase (Boehringer Mannheim) in standard buffer using the following conditions: 94°C for 1 min, 65°C for 1 min, and 72°C, 1 min for 35 cycles, followed by a 10-min extension at 72°C. PCR products were analyzed by agarose gel electrophoresis.

RNA sequencing. Direct RNA sequencing was performed basically as described previously (23). Briefly, total cellular RNA was isolated from LCMV-infected cells and hybridized to ^{32}P end-labeled oligonucleotide primers. Primer extension with avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wis.) for 1 h at 45°C in the presence of dideoxynucleotides was followed by a 15-min chase which included terminal deoxynucleotidyltransferase (Promega). Sequencing of the 3′ and 5′ termini of the S segments was done by an RNA ligase-based method (15). RNA was isolated from purified LCMV virions (23) and treated with tobacco acid pyrophosphatase (Epicentre Technologies, Madison, Wis.). The free ends were then ligated overnight with RNA ligase (New England Biolabs, Beverly, MA). After reverse transcription using random hexamers, PCR was done with primers which hybridized to the 5′ genomic and 3′ antigenomic sequences (WE S5′ and WE S3′; Table 1). To facilitate cloning, restriction enzyme sites were added to the primers. The PCR products were isolated, digested, and ligated into the polylinker of pBluescript KS (Stratagene, La Jolla, Calif.). A minimum of 10 individual colonies per virus were sequenced by using Sequenase version 2.0 (U.S. Biochemical, Cleveland, Ohio). For the intergenic region, random-primed cDNA was made from total cellular RNA at 45°C, using avian myeloblastosis virus reverse transcriptase; this was followed by PCR using primers spanning the intergenic region (WE IG1 and WE IG2; Table 1). The PCR product was then directly sequenced by Sequenase, using the primers used for PCR. Sequencing reactions were then separated on a 5% polyacrylamide–7 M urea gel (Long Ranger; FMC, Rockland, Maine) and visualized by autoradiography.

Immunofluorescence. Pituitary glands were isolated from individual mice, placed on pieces of liver from uninfected mice, and snap-frozen in liquid nitrogen. Five-micrometer sections were cut on a cryostat and fixed in acetone. Guinea pig antiserum to LCMV followed by a fluorescein-conjugated goat anti-guinea pig immunoglobulin antiserum was used to detect infected cells.

Infection of PC cells. PC cells were grown as monolayers in RPMI medium supplemented with 5% fetal bovine serum and 5% horse serum. Infection of PC cells with LCMV was done at an MOI of 5 PFU per cell as described previously (9). The number of cells productively infected was quantitated by an infectious center assay previously described (18). Levels of infectious virus released into the supernatant by infected cells were determined by plaque assay on Vero cells (18). Virus replication and transcription were assessed by Northern blot hybridization as described previously (10), using specific probes for the NP genes of ARM 53b and WEc54. Expression of viral antigen was examined by immunofluorescence, using a mouse monoclonal antibody to the LCMV nucleoprotein (NP) (7).

	GP								NP	
a.a. pos.		115			153			156		538
WE	GAC	Asp	UCC	Ser	AAA	Lys	AAG	Lys		
WE c54	. . U	Asp	. . .	Ser	. . .	Lys	. . A	Lys		
WE c2.2	. . .	Asp	. U .	Phe	. . G	Lys	. . .	Lys		
WE c2.5	. . .	Asp	. U .	Phe	. . G	Lys	. . .	Lys		

FIG. 1. Differences in S RNA sequence between clones of LCMV WE. Total RNA was isolated from BHK cells infected with the various clones of LCMV WE and directly sequenced by using oligonucleotide primers specific for the WE S segment. The nucleotide differences and resulting amino acid changes between GHDS-positive (WE c54) and GHDS-negative (WE c2.2 and c2.5) viruses are shown. Dots indicate homology with the published WE sequence (top row). a.a. pos., amino acid position.

RNase protection assay. Total RNA was isolated from pituitaries of mock-infected and virus-infected C3H/St mice by the guanidinium isothiocyanate-acid phenol method (4). Purified RNA was analyzed by RNase protection assay to determine GH and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels, using an RPA II kit as instructed by the manufacturer (Ambion, Austin, Tex.). GH and GAPDH strand-specific (antisense) ^{32}P-RNA probes were generated by T7 RNA polymerase in vitro transcription and size fractionated by electrophoresis in 5% polyacrylamide-urea sequencing gels. Full-length transcripts corresponding to the GH (425-nucleotide) and GAPDH (190-nucleotide) RNA probes were excised, extracted in elution buffer (0.5 M ammonium acetate 10 mM magnesium acetate 1 mM EDTA, 0.1% sodium dodecyl sulfate), and precipitated with ethanol. Routinely, we used 5×10^4 cpm of ^{32}P-labeled purified probe in each reaction. Protected RNAs were analyzed by electrophoresis in 5% denaturing polyacrylamide gels.

RESULTS

Sequence comparison of LCMV WE clonal variants. Clonal variants of LCMV WE parental strain which differ in their abilities to cause GHDS in newborn C3H/St mice were derived as described previously (8). Since their difference in disease potential was mapped to the S RNA, we compared the S RNA sequences of the GHDS-positive and GHDS-negative WE variant clones. Direct RNA sequencing of the entire S segments of WE c54 (GHDS negative) and WE c2.2 and WE c2.5 (GHDS positive) revealed no sequence differences between the S segments of WE c2.2 and WE c2.5. The sequence identity and similar biological properties of WE c2.2 and WE c2.5 make them virtually indistinguishable. WE c54 differed from WE c2.2 and WE c2.5 only at four nucleotide positions in the S segment (Fig. 1). All four differences were found in open reading frames, three in the glycoprotein and one in the nucleoprotein. Of these changes, only one resulted in an amino acid change. The substitution of U for C at nucleotide 535 of the glycoprotein caused a serine-to-phenylalanine (Ser to Phe) change at amino acid 153. This amino acid substitution is located in the GP-1 subunit of the glycoprotein, which has been implicated in virus attachment (5).

Generation of reassortants between WE clones and ARM. To exclude the possibility that a concurrent change in the L segment of the viral genomes, encoding for the putative L polymerase and Z polypeptides, contributed to the difference in the abilities of the WE variants to cause GHDS, we made reassortant viruses between the two WE variants and the ARM 53b clonal pool of LCMV previously shown to cause GH deficiency (22). Reassortant viruses were generated by coinfection of BHK-21 cells with both parental viruses and subsequent

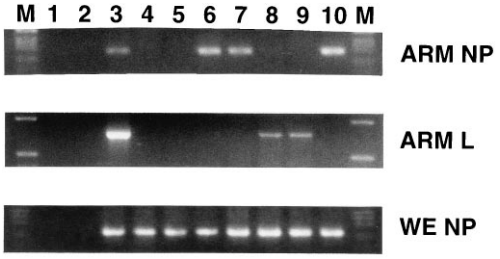
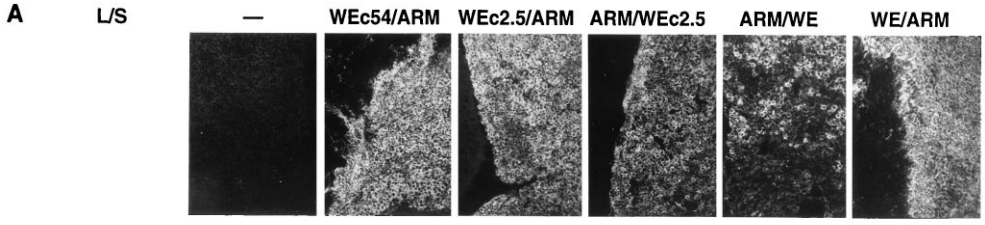


FIG. 2. Reverse transcriptase PCR analysis of reassortants. Total RNA from infected BHK-21 cells was extracted, reverse transcribed, and amplified by PCR with primers for ARM NP, ARM L, or WE NP as described in Materials and Methods. PCR products were analyzed on a 2% agarose gel. The viruses used for infection were ARM (lane 3), WE c54 (lane 4), WE c2.5 (lane 5), WE c54 (L)/ARM (S) (lane 6), WE c2.5 (L)/ARM (S) (lane 7), ARM (L)/WE c2.5 (S) (lane 8), ARM (L)/WE (S) (BC6) (lane 9), and WE (L)/ARM (S) (BC10) (lane 10). Lanes 1 and 2 contain represent PCR reactions done without cDNA and with cDNA from uninfected BHK-21 cells, respectively. Lanes M, size markers.

plaque isolation as described in Materials and Methods. Genetic characterization of the reassortants was done by Northern blot hybridization and reverse transcriptase PCR using RNA from infected BHK-21 cells and primers that specifically amplified ARM S and L RNA sequences (Fig. 2). Of the four possible combinations, three of them [WE c54 (L)/ARM (S), WE c2.5 (L)/ARM (S), and ARM (L)/WE c2.5 (S)] were readily obtained. Figure 2, lanes 3 through 5, show the specificity of the primers for the parental viruses (ARM 53b, WE c54, and WE c2.5). Lane 6 shows a WE c54 (L)/ARM (S) reassortant, while lanes 7 and 8 show reciprocal reassortants from WE c2.5 and ARM. Reassortants containing the L RNA from ARM and S RNA from WE c54 were not obtained after screening of 120 clones. To examine the effect of the WE GP containing the Ser at amino acid 153, we used reassortants between the parental WE strain and ARM, designated BC6 [ARM (L)/WE (S)] and BC10 [WE (L)/ARM (S)] (22). Sequence analysis of the GP open reading frame of the BC6 reassortant confirmed the presence of a Ser at amino acid position 153 as well as the two other silent changes found in the GP of WE c54 shown in Fig. 1 (data not shown). The genotypes of reciprocal reassortants between WE and ARM are shown in Fig. 2, lanes 9 and 10. These reassortants were used in place of ARM (L)/WE c54 (S). Reverse transcriptase PCR using primers for WE NP that cross-react between different LCMV strains shows the presence of viral RNA in all samples from infected cells (Fig. 2, bottom panel).

Biological characterization of WE:ARM reassortants. The reassortant viruses described above were inoculated intracerebrally into newborn C3H/St mice to examine their potential to cause GHDS. All reassortants containing the S segment from ARM caused GHDS, as determined by a marked growth retardation which was apparent by day 15 after birth (Fig. 3B). These animals also developed a severe hypoglycemia leading to death. Also, the ARM (L)/WE c2.5 (S) reassortant caused the growth retardation, hypoglycemia, and death characteristic of GH deficiency, similar to the phenotype of the two parental viruses, ARM and WE c2.5 (Fig. 3). Mice infected with the ARM (L)/WE (S) reassortant did not develop symptoms of GHDS; i.e., their weights, blood glucose measurements, and survival were similar to those of mock-infected animals. Immunofluorescence studies of infected animals also revealed the correlation, as previously described (18, 19), between the ability of each of the viruses to infect the GH-producing cells of the anterior pituitary and the ability to cause GHDS (Fig. 3A). All of the reassortants which caused the GH deficiency replicated to high levels in the pituitary, as evidenced by intense staining for viral proteins in >95% of cells. In contrast, the GHDS-negative ARM (L)/WE (S) reassortant infected less than 25% of the GH-producing cells in the pituitary (Fig. 3A), similar to the WE parental strain (18). Reduced numbers of viral antigen-positive cells in the anterior pituitary correlated with decreased amounts of infectious virus in whole pituitary homogenates (Table 2).

Since the serum viremia in C3H/St mice infected with the ARM (L)/WE (S) reassortant were approximately 100-fold less than with the other reassortants, it was possible that the differences between the viruses in the ability to cause GHDS were due to differences in the ability to infect the host. To exclude this possibility, we performed Northern blot analyses to determine if LCMV could replicate and transcribe in brains and



B

	—	WEc54/ARM	WEc2.5/ARM	ARM/WEc2.5	ARM/WE	WE/ARM
Weight @ d15 (g)	8.0 ± 0.30	3.1 ± 0.20	2.9 ± 0.11	4.0 ± 0.22	5.7 ± 0.17	2.9 ± 0.13
Survival (%)	100 n=19	0 n=17	0 n=27	0 n=22	100 n=21	0 n=18
Blood glucose (mg/dl)	177 ± 11	52 ± 10	87 ± 12	85 ± 6	142 ± 7	48 ± 4
Serum viremia (pfu/ml)	<500	2.7×10^5	2.0×10^5	1.2×10^5	3.3×10^3	7.0×10^5

FIG. 3. Immunofluorescence (A) and clinical measurements (B) of infected mice. Whole pituitary glands were isolated from mice persistently infected with the indicated viruses and immediately snap-frozen in liquid nitrogen. Five-micrometer sections were fixed in acetone and stained with a guinea pig antiserum to LCMV followed by fluorescein isothiocyanate-conjugated secondary antibody. Weight, survival, and blood glucose were measured as described in Materials and Methods. Shown are the means and standard errors. Serum viremia was determined by plaque assay on Vero cells; shown are the means from at least five animals per group. For the ARM (L)/WE (S) reassortant, less than 25% of cells in the pituitary were infected; shown is a particularly highly infected area.

TABLE 2. Virus titers in pituitaries, brains, and livers of C3H/St mice infected with reassortant viruses

Reassortant virus (L/S)	Virus titer (PFU/g)[a]		
	Pituitary	Brain	Liver
WE c54/ARM 5	$(8.0 \pm 2.0) \times 10^5$	$(1.5 \pm 1.0) \times 10^6$	$(7.0 \pm 3.0) \times 10^5$
WE c2.5/ARM 5	$(1.5 \pm 1.0) \times 10^6$	$(2.0 \pm 1.5) \times 10^6$	$(1.5 \pm 1.0) \times 10^6$
ARM 5/WE c2.5	$(7.5 \pm 2.0) \times 10^5$	$(8.0 \pm 2.5) \times 10^6$	$(7.5 \pm 2.5) \times 10^5$
ARM 5/WE (BC6)	$(2.1 \pm 1.5) \times 10^4$	$(2.5 \pm 1.0) \times 10^6$	$(9.0 \pm 1.5) \times 10^5$
WE/ARM 5 (BC10)	$(9.5 \pm 2.0) \times 10^5$	$(2.0 \pm 0.5) \times 10^6$	$(8.5 \pm 1.5) \times 10^5$

[a] Virus titers were determined by plaque assay on Vero cells as described previously (18). Values correspond to averages ± standard deviations for five infected mice per group.

livers from infected animals. As expected, viral S RNA (replication) and NP mRNA (transcription) were both detected in the brains and livers of animals infected neonatally with the reassortants containing the S RNA from ARM (Fig. 4, top panel). In addition, reassortants containing WE S RNAs were able to replicate and transcribe in both the brains and livers of infected mice (Fig. 4, middle panel). Levels of S RNA and NP mRNA were comparable in tissues isolated from C3H/St mice infected with the GHDS-positive ARM (L)/WE c2.5 (S) reassortant or the GHDS-negative ARM (L)/WE (S) virus (Fig. 4, lanes 4 and 10 or lanes 5 and 10, respectively). Consistent with this finding, similar titers of infectious virus were found among the brains and livers of C3H/St mice infected with each of the five different reassortant viruses (Table 2). The reasons for the low serum virus titers found in animals infected with the ARM (L)/WE (S) reassortant remain to be determined. However, ARM (L)/WE (S) reassortant exhibited a faster decay in infectivity than WE/ARM 5 and WE c54/ARM 5 reassortants when incubated at 37°C in the presence of normal serum from uninfected C3H/St mice (Fig. 5). This finding may explain the lower serum virus titers found in ARM/WE-infected C3H/St mice (Fig. 3).

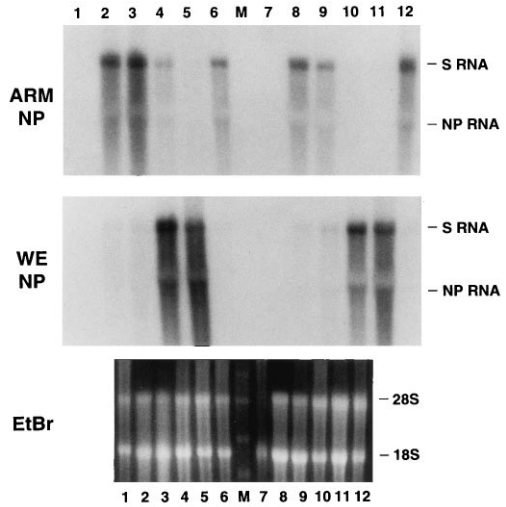


FIG. 4. Northern analysis of livers and brains from infected animals. Total RNA was isolated from the brains (lanes 1 to 6) and livers (lanes 7 to 12) of representative animals and analyzed for viral gene expression, using probes specific for NP from ARM (top) or WE (middle). Ethidium bromide staining was used to show equal loading of RNA in each lane (bottom). Samples included mock-infected controls (lanes 1 and 7) and cells infected with WE c54 (L)/ARM (S) (lanes 2 and 8), WE c2.5 (L)/ARM (S) (lanes 3 and 9), ARM (L)/WE c2.5 (S) (lanes 4 and 10), BC6 (lanes 5 and 11), and BC10 (lanes 6 and 12). Lane M, 0.4- to 9.5-kb RNA markers (GIBCO/BRL) run to determine sizes.

DISCUSSION

Clonal virus variants isolated from the parental WE strain of LCMV can differ in their abilities to cause GHDS when injected intracerebrally into neonatal C3H/St mice (8). Here, we show that the basis of this important biological difference appears to be a single nucleotide change resulting in a Ser-to-Phe substitution in the viral glycoprotein. Within the S RNAs, we found three other nucleotide substitutions, two within the GP and one within the NP. Although these changes are silent, we cannot formally exclude the possibility that they may have an effect on virus replication in GH-producing cells. However, the putative *cis*-acting regulatory sequences located at the 3′ and 5′ termini of the genomic S RNA as well as the intergenic hairpin loop (3) were conserved between the WE variants differing in their abilities to cause GHDH.

The amino acid change Ser to Phe at position 153 in the GP lies within the GP-1 subunit of the glycoprotein. Evidence indicates that this subunit is the virion attachment protein which mediates binding to host cell surface receptors at the first stage of the viral entry process (5). However, little is known about the cellular receptors utilized by arenaviruses. Previously, a 120- to 140-kDa protein was identified as the putative receptor for LCMV on rodent fibroblast cell lines, using a virus-overlay protein blot assay (VOPBA) (5). Interestingly, the single amino acid difference in the GP-1 subunits of the WE clones affects their binding to this putative receptor protein both in cultured cells and in whole tissue extracts from

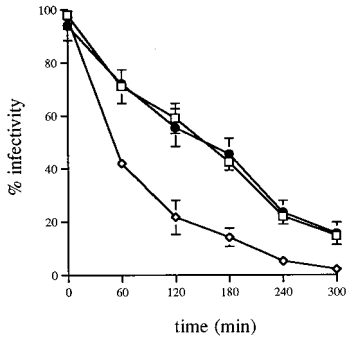


FIG. 5. Inactivation rates of ARM (L)/WE (S) (○), WE (L)/ARM (S) (●), and WE c54 (L)/ARM (S) (□) reassortant viruses at 37°C in the presence of normal serum from uninfected C3H/St mice. One thousand PFU (10 μl) of each reassortant virus was mixed with 190 μl of normal serum from uninfected C3H/St mice and incubated at 37°C for the indicated time. Serial dilutions of each sample were titrated by plaque assay on Vero cell monolayers as described in Materials and Methods. Values represent the percentage of infectivity with respect to the 1,000-PFU input. Each value represents the average with standard deviation of two independent experiments. In each experiment, each time point was titrated in duplicate.

organs: WE c54 binds to the 120- to 140-kDa protein, whereas WE c2.2 does not (not shown). These results indicate that this single amino acid substitution is associated with a biochemical change in the viral GP that could be involved in the observed difference in virus tropism for GH-producing cells in the anterior pituitary. It is interesting that LCMV ARM, which is GHDS positive, also does not bind to the putative cellular receptor in the VOPBA (4), although it has a serine at residue 153 in the GP, similar to WE c54. These data suggest that the virus-receptor interaction is not based solely on primary sequence but also involves the higher-order structure of the viral GP. There are 22 other differences between ARM and WE in the 262-amino-acid long GP-1 subunit alone, some or all of which may contribute to the difference in receptor binding by VOPBA and in GHDS phenotype. Thus, the GP of the two GHDS-positive viruses, ARM and WE c2.2, may share some biochemical properties, including an enhanced affinity for the LCMV receptor present on GH-producing cells in neonatal C3H/St mice.

The abilities of LCMV isolates to infect these cells correlates with their abilities to cause GHDS (18, 22). Viruses containing a WE c2.5-like S RNA (Phe-153) are able to infect the anterior pituitary and cause GHDS, while viruses with a WE c54-like S RNA (Ser-153) do not infect the GH-producing cells and do not cause GHDS. In addition, our reassortant studies strongly suggest that potential changes in the L RNA of the WE variants do not affect disease progression. The L RNA from WE c54 does not appear to have a dominant negative effect on replication and transcription in GH-producing cells, since the WE c54 (L)/ARM (S) reassortant was able to infect the pituitary and cause GH deficiency. All of the reassortants were able to replicate to similar levels in the brain and liver, suggesting that the viral polymerase complexes have comparable replication and transcription activities. Together, these results indicate that the difference in abilities of WE c54 and WE c2.5 to cause the GH-deficiency syndrome is dependent solely on the Ser-to-Phe change in the viral GP and not due to concomitant changes in the L RNA.

Further evidence that a change in virus tropism is sufficient to allow the induction of the GH deficiency stems from an in vitro model. We have shown that LCMV infection of PC cells, a rat somatotroph cell line, causes a decrease in transcription of GH mRNA resulting from a decrease in the amount of the GH transactivation factor GHF1 (9). As with ARM 53b, both WE c54 and WE c2.5 can infect and replicate to high levels in PC cells (Fig. 6), and both caused a decrease in the amount of GH mRNA (Fig. 7). In addition, all of the reassortants described above also replicate well in PC cells and decrease the steady-state GH mRNA in these cells (data not shown). It is not surprising that GHDS-negative, as well as GHDS-positive, viruses can infect PC cells; loss of restriction for infection is a common occurrence among cell lines adapted to tissue culture. Thus, it appears that the difference in the abilities of the WE clones to cause GHDS in the mouse lies at the level of viral entry rather than in a difference in their abilities to replicate inside the GH-producing cells. This, in turn, suggests that in addition to GP-1, which is involved in recognition and virus entry into the GH target cells, other viral determinants likely contribute to LCMV-mediated impairment of GH synthesis.

It is interesting that we were unable to obtain reassortants containing the ARM L RNA and the WE c54 S RNA, although the reciprocal reassortant was easily obtained. This result suggests that this particular combination of S and L is not viable. LCMV is not unique in that regard. Influenza virus as well as other segmented negative-stranded RNA viruses also appear to have rules for reassortment of viral RNA segments, with particular combinations being excluded (12, 21, 28, 30). However, the S RNA from the parental WE population can reassort with the L RNA from ARM. While the sequence of the glycoprotein of this reassortant is identical to that of WE c54 in the area of the nucleotide substitutions, the S RNA of the ARM (L)/WE (S) reassortant may have other compensatory changes providing it with the biological fitness required to replicate properly. Many examples showing that a single point mutation may lead to important changes in the biology of a virus have been documented for RNA viruses from diverse families, including orthomyxoviruses, rhabdoviruses, picornaviruses, and retroviruses (2, 4, 11, 23, 25, 26).

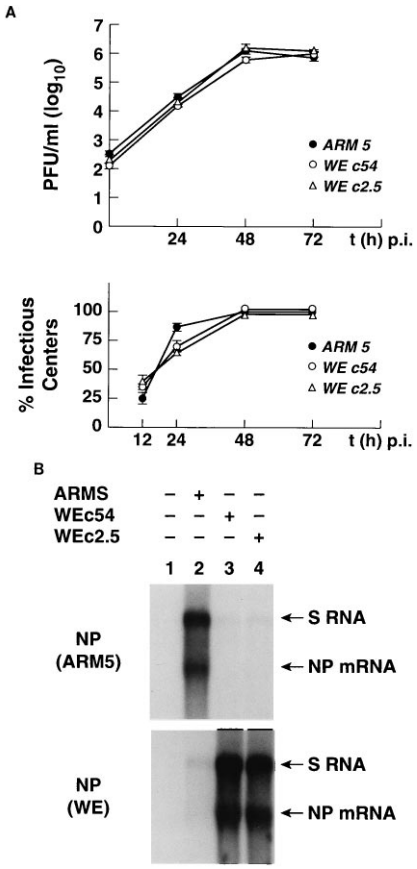


FIG. 6. Multiplication of WE c54 (GHDS-nil) and WE c2.5 (GHD+) variants in PC cells. (A) Both WE c54 and WE c2.5 variants reached similar high titers as ARM 5 (GHDS+) in the supernatant of infected PC cells. PC cells were infected with the indicated viruses at an MOI of 3 PFU per cell. After adsorption for 1 h at 37°C, the viral inoculum was removed, cell monolayers were washed three times, and fresh medium was added. At the indicated time points postinfection (p.i.), supernatant virus titers were determined by plaque assay on Vero cells (18). (B) Quantitation of infected cells. PC cells were infected with the indicated viruses at an MOI of 0.1 PFU per cell. At the indicated time points postinfection, single-cell suspensions were prepared and the number of cells productively infected was determined in an infection center assay as described previously (18). (C) Replication and transcription of WE c54 and WE c2.5 RNA genomes in PC cells. PC cells were infected with the indicated viruses as described for panel A. At the indicated time points postinfection, total cellular RNA was extracted and equal amounts (10 μg) were analyzed by Northern blot hybridization using specific probes for the viral NP of ARM and WE viruses. Replication and transcription of the viral genome were assessed on the basis of the detection of S RNA and NP mRNA, respectively.

Within even cloned viral populations, variants that have the potential, under appropriate conditions, to be selected and display new phenotypes that can be associated with disease are maintained. Understanding how such variants are kept pheno-

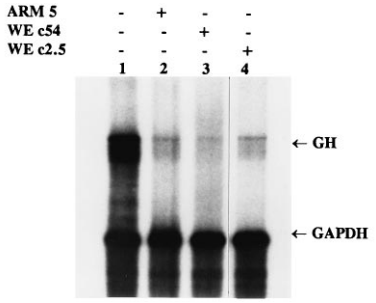


FIG. 7. Steady-state levels of GH mRNA in PC cells infected with WE c54 (GHDS-nil) and WE c2.5 (GHDS+). PC cells were infected with the indicated viruses at an MOI of 3 PFU per cell. At 72 h postinfection, total cellular RNA was isolated and analyzed by RNase protection assay using specific antisense ^{32}P-RNA probes for GH and GAPDH. Protected ^{32}P-RNA samples were analyzed by electrophoresis in denaturing polyacrylamide gels. Levels of GH protein were also reduced in infected cells compared with those in uninfected control cells, using equivalent amounts of extracts in Western blot analysis (not shown) (6).

typically silent within the population, or emerge to dominate and generate new quasispecies with new biological properties, is likely to illuminate our understanding of how the same original parental virus can be associated with a variety of diseases involving different tissues and organs.

ACKNOWLEDGMENTS

We thank Beatrice Cubitt for helpful discussion.

This research was supported by Public Health Service grants AI09484 (M.B.A.O.) and AG04342 (J.C.T.). M.N.T. was supported by postdoctoral training grant AG00080 from the National Institute of Mental Health.

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