Tissue-Mediated Selection of Viral Variants: Correlation between Glycoprotein Mutation and Growth in Neuronal Cells

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Received 23 March 1994/Accepted 25 June 1994

Viral variants with different biological properties predominate in the central nervous system (CNS) and lymphoid tissues of carrier mice infected at birth with the Armstrong strain of lymphocytic choriomeningitis virus. The CNS isolates have the same phenotype as the parental strain and cause acute infections in adult mice, while the spleen-derived isolates cause chronic infections associated with suppressed T-cell responses and susceptibility to opportunistic infections. Our previous studies have identified a single amino acid change in the viral glycoprotein, a phenylalanine-to-leucine (F→L) mutation at residue 260, that correlates with the tissue-specific selection and the persistent and immunosuppressive phenotype of the spleen isolates (R. Ahmed, C. S. Hahn, T. Somasundaram, L. Villarete, M. Matloubian, and J. H. Strauss, *J. Virol.* 65:4242–4247, 1991). In this study, we screened viral isolates obtained from the spleen, liver, kidney, and brain of carrier mice for the presence of this mutation and determined the temporal selection of variants as they appear in these organs. We found that this F→L amino acid change is common to >90% of the spleen and liver isolates and is selected for rapidly by day 32 postinfection (p.i.). Although the kinetics observed in the kidney are relatively slower than in the spleen and liver, this F→L mutation predominates in the kidney-derived isolates by 250 days p.i. In contrast, the majority of the CNS isolates retain the parental sequence up to 250 days p.i. In addition, most of the brain isolates replicated efficiently in a neuronal cell line, and this enhanced growth phenotype in neurons correlated with the parental F genotype. This linkage with neurotropism, along with our earlier finding that the F→L mutation is necessary for enhanced infection of macrophages (M. Matloubian, S. R. Kolhekar, T. Somasundaram, and R. Ahmed, *J. Virol.* 67:7340–7349, 1993), provides a cellular basis for the molecular changes associated with tissue-specific selection. Taken together, these results suggest that tropism for macrophages is a critical determinant in selection of variants with the F→L mutation in tissues such as spleen and liver, and tropism for neurons is important in retention of the F genotype in the CNS.

The emergence of viral variants during the course of persistent viral infections is firmly established (2, 5, 6, 8, 32, 36–38, 41). This is not surprising since viruses, especially RNA viruses, can undergo rapid mutation. These mutations in the viral genome often produce changes in pathogenicity. Therefore, an understanding of the selective pressures involved in the emergence of viral mutants and identification of the associated genetic changes will provide insights on viral evolution and pathogenesis. To address these issues, we have been studying the evolution of lymphocytic choriomeningitis virus (LCMV) in its natural host. Our previous studies have documented the profound influence of host tissues in the selection of viral variants in carrier mice infected at birth with LCMV (Armstrong strain) (2, 4, 5, 23). These variants have different biological properties which correlate with their respective tissues of isolation. Most of the central nervous system (CNS) isolates, like the parental Armstrong strain, cause acute infections in adult mice. The virus infection is resolved in ~2 weeks as a result of an LCMV-specific cytotoxic T-cell response. In contrast, the majority of the spleen isolates cause chronic infections in adult mice associated with decreased T-cell responses and susceptibility to opportunistic infections (23, 55). The genomes of these LCMV variants are remarkably conserved (2, 45, 51, 52). Sequence analysis of several independently derived spleen variants showed >99.8% identity with the parental virus. Very few nucleotide changes leading to

amino acid changes were found in both the L and S segments. However, one common change among the spleen isolates was a phenylalanine-to-leucine (F→L) mutation at residue 260 of the glycoprotein which was shown to correlate with the tissue-specific selection and the persistent and immunosuppressive phenotypes of these isolates (2, 28, 29, 44).

In this study, we have examined the kinetics of the emergence of variants with this F→L amino acid change in the brain, spleen, liver, and kidney of carrier mice infected at birth with the Armstrong strain. We show that there is rapid selection of variants with this mutation in the spleen and liver and a relatively slower selection in the kidney. In contrast, the parental sequence is retained in the majority of the brain isolates analyzed. In addition, most of the isolates with the parental F genotype had a growth advantage in a neuronal cell line GT1-7 compared with isolates with L at glycoprotein residue 260. Thus, these results not only confirm the tissue-specific selection of LCMV variants during chronic infection but also suggest a strong molecular correlation of a single amino acid residue in the glycoprotein with the ability to grow in neuronal cells.

MATERIALS AND METHODS

Mice. BALB/c mice were bred at the University of California at Los Angeles. Neonatally infected carriers were made by injecting 10^4 PFU of LCMV intracerebrally into mice within 24 h of birth.

Virus. The Armstrong CA1371 strain of LCMV was used in these studies (18). This original laboratory stock of CA1371

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will be referred to as the parental Armstrong strain. The splenic isolate, clone 13, was isolated from an 8-week-old BALB/c LCMV carrier mouse infected at birth with the Armstrong strain (5). In this study, LCMV variants from the brain, spleen, liver, and kidney were isolated at different times postinfection (p.i.) from mice infected at birth with the parental Armstrong strain. The organs were harvested, homogenized individually, and titrated on Vero cell monolayers. Well-isolated plaques were picked from each sample and recloned one more time. Virus stocks were grown in BHK-21 cells and used in all experiments at the passage 1 or 2 level.

Virus titration. Infectious LCMV was quantitated by plaque assay on Vero cell monolayers as previously described (5). Briefly, Vero cells (7.5×10^5) were plated in 35-mm-diameter wells in six-well dishes (Costar, Cambridge, Mass.). The plates were incubated at 37°C and used the following day for the assay when the cell monolayers were confluent. The medium was removed, and the samples to be titrated were added to the cells (0.2-ml volume). After adsorption for 60 min at 37°C, the cells were overlaid with 3 ml of 0.5% Seakem agarose (FMC Corp., Marine Colloids Div., Rockland, Maine) in medium 199 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% heat-inactivated fetal calf serum, antibiotics, and L-glutamine. The plates were incubated for 4 days at 37°C and then overlaid with 2.0 ml of 0.5% agarose in medium 199 containing 0.01% neutral red (GIBCO). Plaques were scored the following day.

Cell lines. The GT1-7 cell line was derived from a hypothalamic neuronal tumor in transgenic mice (30). The gene construct utilized the promoter region of the gonadotropin-releasing hormone (GnRH) gene to express the simian virus 40 T-antigen oncogene. This clonal cell line retains the characteristics of its normal differentiated hypothalamic neuron progenitors since the cells extend neurites, express the endogenous mouse GnRH mRNA, release GnRH in response to polarization, have regulated fast Na^+ channels found in neurons, and express neuronal, but not glial, cell markers. The GT1-7 cell line was infected in suspension with LCMV at a multiplicity of infection of 0.2 (10^7 cells were pelleted and suspended in less than 0.4 ml of virus). After 1 h of adsorption, 2×10^5 cells per well were plated onto 24-well plates. These cells were grown in Dulbecco's modified essential medium with 10% non-heat-inactivated fetal calf serum, antibiotics, and L-glutamine. Supernatants were stored and virus titers were determined as described above.

Oligonucleotide hybridization. Virus stocks at the passage 2 level were used to infect BHK-21 cells for guanidinium thiocyanate-CsCl RNA extraction (46). Total cellular RNAs from these infected cells were used to prepare dot blots and Northern (RNA) blots. For dot blots, 10 μg of RNA was denatured with formaldehyde and dotted onto nitrocellulose paper with a 96-hole Bio-Dot apparatus. For Northern blots, 10 μg of each of the RNA samples was denatured with formaldehyde and formamide, separated according to size by electrophoresis using a 1.5% agarose gel, and then transferred onto nitrocellulose. Duplicate blots of both preparations were probed with two 20-base-long oligonucleotides (20-mers) which differed only at nucleotide residue 855 of the glycoprotein gene corresponding to amino acid residue 260. These 20-mers are specific for either the parental Armstrong sequence (5'-GTCTCCTAGTGAAGAACTTA-3') or spleen variant clone 13 sequence (5'-GTCTCCTAGTGAGGAAC TTA-3'). They were used to determine the viral RNA sequence at nucleotide residue 855 of all isolates cloned in this study. Both 20-mers were 5' end labeled with [γ - ^{32}P]ATP (New England Nuclear), using bacteriophage T4 polynucleotide ki-

FIG. 1. Use of oligonucleotide probes specific for the parental Armstrong or spleen variant clone 13 to screen variants at nucleotide residue 855 of the viral glycoprotein gene. Total cellular RNA was extracted from virus infected BHK-21 cells and dotted onto nitrocellulose paper. Duplicate filters of each sample were made and hybridized with γ - ^{32}P -labeled oligonucleotides (20-mers), with one base mismatch, that are specific for either the parental sequence (A-like) or the variant sequence (13-like) (see Materials and Methods). Samples A, 13, and C designate RNA from Armstrong-infected, clone 13-infected, and uninfected BHK cells, respectively. Samples 253a and 329a are individually derived spleen isolates (from different mice). 253a was isolated at 150 days p.i., and 329a was isolated at 250 days p.i. Sample 116a was derived from the brain of the same carrier mouse as clone 253a.

nase. Dot blots and Northern blots were prehybridized at 42°C for at least 2 h with buffer containing $5\times$ SSPE ($1\times$ SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4 , and 1 mM EDTA [pH 7.7]), $5\times$ Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 0.1 mg of salmon sperm DNA per ml. Duplicate blots were hybridized with either probe for 24 h at 42°C. These blots were washed with $2\times$ SSPE-0.1% SDS three times for 20 min each time at room temperature and three times for 20 min each time at 54°C, which resulted in complete loss of hybridization for the blots probed with the single mismatch. Viral clones were scored Armstrong-like (F at residue 260) or clone 13-like (L at residue 260), depending on which oligonucleotide probe gave the positive signal.

RESULTS

Screening of isolates for the F→L mutation using oligonucleotide probes with one base mismatch. Mice were injected intracerebrally at birth with 10^4 PFU of LCMV Armstrong CA1371. These mice were sacrificed on days 7, 16, 32, 150, and 250 p.i., and the brains, spleens, livers, and kidneys were harvested. These tissues were individually homogenized and titrated on Vero cell monolayers. Well-isolated plaques were picked from the plates, and these viral clones were subjected to another cycle of plaque-to-plaque purification. Virus stocks were grown, and total cellular RNA was prepared by the guanidinium isothiocyanate-CsCl procedure. Total cellular RNA was prepared as dot blots or Northern blots. Duplicate blots were probed with 20-base-long oligonucleotides which differed at one position. As shown in Fig. 1, the probe specific for the parental Armstrong sequence (with F at residue 260) hybridized to the RNA sample from cells infected with the parental virus. On the other hand, the probe specific for the variant sequence (with L at residue 260) hybridized with the RNA samples from cells infected with the splenic variant clone 13. Both probes hybridized specifically to their respective sequences, since each clone showed positive hybridization with one oligonucleotide but no hybridization with the other oligonucleotide on the duplicate blot. RNA from uninfected cells gave no signal with either probe. The LCMV isolates from the brains, spleens, livers, and kidneys of carrier mice were screened by this technique.

Kinetics of emergence of viral variants with the L mutation at amino acid residue 260 of the glycoprotein. We previously

FIG. 2. Tissue-specific selection of viral variants. Mice were injected intracerebrally at birth with 10^4 PFU of the parental LCMV Armstrong strain. Groups of mice were sacrificed on days 7, 16, 32, 150, and 250 p.i. At these times p.i., the brains, spleens, livers, and kidneys were homogenized and titrated on Vero cell monolayers. Individual viral plaques were isolated from each tissue and screened for the F→L mutation as described in the legend to Fig. 1.

showed that the mutation at nucleotide position 855 of the glycoprotein gene, which changes the amino acid at residue 260 from F to L, was found in the vast majority of spleen isolates but not CNS isolates at around 60 days p.i. (2). Here we report that variants with this mutation also predominate in other visceral organs such as the liver, lung, and kidneys of persistently infected mice. However, different kinetic patterns of emergence were observed in these tissues. There was rapid emergence of variants in the spleen, liver, and lung, relatively slower kinetics in the kidney, and retention of the parental F genotype in the brain. Data for the spleen, liver, kidney, and brain are summarized in Fig. 2 and Table 1. No variants were detected among the 42 isolates obtained at day 7 p.i. from any of the tissues analyzed. At day 16, 1 of 24 spleen isolates screened had L (Table 1). However, by 32 days p.i., virus clones with the parental genotype virtually disappeared from the spleen, liver, and lung, and variants with L became the

predominant population in these organs. This trend continued until day 250 p.i., the last time point tested. A similar pattern was also seen in the blood; at day 7, all (10 of 10) isolates had F, but by day 30, all 15 isolates tested had the variant genotype. In striking contrast, the parental genotype was retained in the brains of these carrier mice. At day 32 p.i., 20 of 20 brain-derived isolates still retained F at position 260. At day 150 p.i., 6 of 28 (21%) of the brain isolates had the variant L genotype. The percentage of the brain-derived isolates which had the variant amino acid increased to about 37% by day 250 p.i. This is not surprising since at these late time points, contamination of the brain with virus from the visceral organs via the blood is likely to occur. The variant genotype took longer to predominate in the kidney. At day 32 p.i., approximately half of the isolates still had the parental genotype. This distribution was maintained up to day 150 p.i. By day 250 p.i., the L mutation predominated (90%) among the kidney-derived isolates.

TABLE 1. Kinetics of the emergence of viral variants in different tissues of LCMV carrier mice^a

Day p.i.	No. of isolates with sequence/no. tested							
	Brain		Spleen		Liver		Kidney	
	F	L	F	L	F	L	F	L
7	9/9	0/9	13/13	0/13	10/10	0/10	10/10	0/10
16	7/7	0/7	23/24	1/24	ND	ND	ND	ND
32	20/20	0/20	3/23	20/23	0/8	8/8	5/9	4/9
150	22/28	6/28	0/14	14/14	0/10	10/10	6/12	6/12
250	5/8	3/8	1/10	9/10	0/6	6/6	1/9	8/9

^a Mice were injected intracerebrally within 24 h of birth with 10^4 PFU of LCMV Armstrong. These carrier mice were sacrificed at various times p.i., and virus was isolated from the indicated tissues. Plaque-purified isolates were screened for the F→L mutation as described in the legend to Fig. 1. F, parental Armstrong sequence; L, clone 13 sequence; ND, not done.

TABLE 2. Genotypes of virus derived from tissues of neonatal mice coinfecting with the parental and variant viruses^a

Day p.i.	Clonal ratios (%) of isolates							
	Brain		Spleen		Liver		Kidney	
	F	L	F	L	F	L	F	L
7	5/12 (42)	7/12 (58)	0/6 (0)	6/6 (100)	0/6 (0)	6/6 (100)	2/9 (22)	7/9 (78)
27	5/10 (50)	5/10 (50)	0/7 (0)	7/7 (100)	0/6 (0)	6/6 (100)	0/7 (0)	7/7 (100)

^a Mice were injected intracerebrally at birth with a 1:1 mixture, containing 10^4 PFU each, of the parental Armstrong and splenic variant clone 13. Viral clones were derived from the brain, spleen, liver, and kidney of carrier mice at days 7 and 27 p.i. The clones were scored as F (parental Armstrong sequence) or L (clone 13 sequence), using the oligonucleotide hybridization technique shown in Fig. 1.

The level of infectious virus in the spleen and brain at various times after infection was also investigated. At days 7 and 16 p.i., the brain had an approximately 10-fold-greater viral titer compared with the spleen (Fig. 3). At day 32 p.i., however, the titers switched so that the spleen virus titer became 10-fold greater than the brain titer. This increase of virus levels in the spleen paralleled the emergence of variants with the F→L mutation.

The strong selection for L in the visceral organs was further demonstrated by results obtained from screening isolates from mice coinfecting with a 1:1 mixture of the parental Armstrong and spleen variant clone 13 (Table 2). As early as 7 days p.i., 100% of the spleen and liver isolates and 78% of the kidney isolates had L at residue 260 of GP-1. By day 27 p.i., all kidney isolates had L. The brain isolates, however, had a 50/50 distribution of the parental and variant genotype up to day 27.

Collectively, these results provide direct evidence for the disappearance of one genotype and the emergence of a variant genotype in an organ-specific manner during chronic infection of the natural host.

Growth phenotype of LCMV variants in neuronal cells. Previous studies have shown that LCMV is found predominantly in neurons of persistently infected carrier mice (7, 20, 26, 34, 43). Therefore, it was of interest to determine if the CNS-derived isolates grew better than the splenic-derived isolates in neuronal cells. A hypothalamic neuronal cell line,

GT1-7, was infected with various LCMV isolates, and the amount of infectious virus released in the supernatant was quantitated by a plaque assay. A summary of the data from these experiments is shown in Table 3. The parental Armstrong strain (a brain-adapted virus) grows well in the neuronal cell line. The majority (five of eight) of the brain isolates analyzed also grew as well as or even better than the parental strain in these cells ($P < 0.05$). In contrast, the majority (7 of 10) of the spleen isolates analyzed grew poorly in neuronal cells ($P < 0.05$). Approximately 10- to 80-fold-lower levels of infectious virus were present in these cultures than in those infected with the parental Armstrong strain.

We next determined whether the mutation at residue 260 of the glycoprotein had an effect on the variant's ability to grow in this neuronal cell line. As shown in Table 3, there is a strong correlation between the presence of parental amino acid F at residue 260 of GP-1 and the ability to grow in neuronal cells. Seven of nine (78%) of the isolates with F replicated efficiently in GT1-7 cells. In contrast, eight of nine (89%) of the isolates with the L mutation had lower virus yield in this neuronal cell

FIG. 3. Kinetics of infectious virus in the brain and spleen. At the indicated time points, carrier mice were sacrificed and the amount of infectious LCMV in the brains and spleens was determined by a plaque assay on Vero cells. The data shown represent the average of two to four mice at each time point, with vertical bars indicating ranges.

TABLE 3. Growth phenotypes of brain- and spleen-derived LCMV isolates in neuronal cells: correlation with glycoprotein mutation at residue 260^a

Viral isolate	Tissue and day of isolation	Ratio of isolate titer/wild-type Armstrong titer		Growth phenotype	Amino acid at position 260 of GP-1
		Day 1 p.i.	Day 2 p.i.		
59a	Brain, 16	1.15	2.08	+	F
53a	Spleen, 16	0.50	10.80	+	F
56a	Spleen, 16	0.47	1.37	+	F
78c	Brain, 32	0.07	0.12	—	F
116a	Brain, 150	1.58	2.00	+	F
117a	Brain, 150	1.46	3.81	+	F
121a	Brain, 150	0.91	1.10	+	F
308	Brain, 250	0.59	1.02	+	F
327a	Spleen, 250	0.01	0.07	—	F
51a	Spleen, 16	<0.01	0.01	—	L
73a	Spleen, 32	0.07	0.05	—	L
83a	Spleen, 32	0.09	0.06	—	L
253a	Spleen, 150	0.10	0.04	—	L
257a	Spleen, 150	0.08	0.14	—	L
311	Brain, 250	0.08	0.04	—	L
317a	Brain, 250	0.01	0.04	—	L
320	Spleen, 250	1.25	1.70	+	L
329	Spleen, 250	0.06	0.04	—	L

^a The indicated isolates, derived from either the brains or spleens of carrier mice at different times p.i., were checked for the ability to grow in a hypothalamic neuronal cell line, GT1-7. Ratios shown are the means of two to four experiments. Viral isolates that grew to titers as high as or higher than those of the parental Armstrong strain (with ratios of ≥ 0.5) were scored as +, and those that grew to titers 10-fold lower than those of the parental strain were scored as —.

FIG. 4. Growth of LCMV isolates containing either amino acid F or L at glycoprotein position 260 in a hypothalamic neuronal cell line, GT1-7. GT1-7 cells were infected with the indicated LCMV isolates, and the amount of infectious virus released in the supernatant was quantitated by a plaque assay. Clones 56a and 51a were derived from the same mouse at 16 days p.i., and clones 308 and 311 were isolated from another mouse at 250 days p.i.

line. The correlation was not absolute but was statistically significant ($P < 0.01$). The 3 of 18 variants that did not match the projected phenotype (2 from the brain and 1 from the spleen) may have acquired other biologically significant mutations.

Comparison of the growth kinetics between pairs of LCMV isolates derived from the same carrier mouse further document the strong correlation between the amino acid at residue 260 of the glycoprotein with the variant's neuronal growth phenotype (Fig. 4). Clones 51a (L) and 56a (F) were isolated from the same mouse at 16 days p.i., and clones 308 (F) and 311 (L) were isolated from another mouse at 250 days p.i. The isolates that have F (clones 56a and 308) exhibited enhanced growth in neuronal cells, while those with L (clones 51a and 311) did not grow as well. The difference in titer is approximately 5- to 10-fold between clones 56a and 51a and >50-fold between clones 308 and 311. It is worth noting that LCMV clones 51a and 56a, which differ at residue 260, were isolated just 16 days after infection of neonatal mice and are likely to be isogenic at most other loci. These results suggest a strong molecular correlation of a single amino acid residue with ability of a virus to grow in neuronal cells.

DISCUSSION

This study confirms and extends our previous reports documenting the importance of a single amino acid change (F→L at residue 260 of GP-1) in the organ-specific selection of LCMV variants during persistent infection of mice (2, 28, 29). In this study, we have determined the temporal selection of variants with this mutation in several tissues of carrier mice infected at birth with the parental Armstrong strain of LCMV. We found that there is rapid selection of variants with the F→L mutation in the spleen and liver, a relatively slower selection in the kidney, and retention of the parental F genotype in the brain. Our earlier studies have shown that presence of amino acid L at 260 is necessary for enhanced infection of macrophages (3, 23, 28, 29). In this report, we show that LCMV isolates with F at GP-1 residue 260 replicate more efficiently in neurons. Taken together, these results suggest that tropism for macrophages is a critical determinant in selection of the L genotype

in the spleen and liver (Kupffer cells), and tropism for neurons is important in retention of the F genotype in the CNS.

The F→L mutation is close to the putative cleavage site of the glycoprotein (9, 10, 12, 13). Proper proteolytic cleavage is essential for infectivity, and alterations in the processing and/or stability of the viral glycoprotein are likely to affect the growth rate of the virus. Moreover, precise glycosylation is also needed for proper folding, transport, and cleavage as well as production of infectious virus. Although the F→L mutation is not within any of the glycosylation motifs, this single amino acid change may indirectly affect glycosylation at a nearby consensus signal. In addition, the F→L change may also directly affect the proper folding and/or tertiary structure of either the glycoprotein precursor or GP-1, since F has an aromatic side chain whereas L does not. This mutation may also affect the noncovalent interaction of GP-1 and GP-2 such that viral budding and/or stability of the membrane GP-1-GP-2 complex is affected. The need for proper posttranslational processing of the virus in determining viral infectivity lends itself to the possibility that the presence of different cellular proteases, glycosylases, and sialases in different cell types accounts for the cell-specific tropism of LCMV variants. Clearly, further experiments addressing these various possibilities are needed.

Tissue-specific selection of variants with growth advantage in certain cell types may be a critical factor by which the virus establishes persistent infection and also coexists without causing extensive damage to the host. For example, the rapid selection of variants capable of enhanced replication in macrophages will result in greater dissemination of the virus. The emergence of viral mutants that can produce greater dissemination and higher antigen load could thus be a possible mechanism by which the virus causes peripheral deletion of LCMV-specific T cells that have escaped clonal deletion within the thymus (5, 21, 22, 24, 28, 33, 40). This will not only favor viral persistence but also reduce immunopathological damage to the host.

The kinetics of emergence of kidney-derived variants with the L mutation is of interest. The kinetics of this mutation is slower than that of the spleen and liver, yet that the kidney receives one-third of the cardiac output and the blood at this time contains mostly variants with the L mutation (1a, 32). At day 32 p.i., approximately half of the kidney isolates still retain the F genotype, whereas all of the spleen and liver isolates have the L genotype. In the kidney, the virus is located mostly in the glomeruli and tubules (11, 31, 35-37, 54). Virus present in the glomeruli is in the form of infectious virus-immunoglobulin G complexes. Carrier mice contain LCMV-specific antibodies which complex with the variants in the circulation, resulting in deposition and entrapment of these infectious virus-immunoglobulin G complexes in the glomeruli. Alternatively, growth of the virus in the renal tubules may also be important in the selection of these variants. The tubules are of particular interest, since the presence of different pHs in the ascending and descending limbs may affect the growth of particular variants. In this context, it is worth noting that the F→L mutation is near the putative cleavage site of the glycoprotein precursor and that the GP-1 and GP-2 are homotetramers that are noncovalently linked and contain no disulfide bonds (12, 13). The L mutation may affect the noncovalent binding of these two proteins such that a change in pH may favor better growth of isolates with the parental genotype over the variant or vice versa, depending on where the virus is present in the tubules.

The organ-specific selection of viral variants that we have described in the LCMV model system is likely to be a general

phenomenon that occurs in other chronic viral infections. Various reports have shown that persistent infection with human immunodeficiency virus (HIV) results in the emergence of variants in the CNS which differ from those isolated in peripheral blood mononuclear cells in replication efficiency, in cell tropism, and in their pattern of neutralization by human sera (15, 17, 19, 25, 48, 53). Moreover, the replicative capacity and cell tropism of HIV have also been shown to change after passage through some established cell lines (16, 39). This altered host range is linked to differences in the glycosylation pattern of the viral envelope glycoprotein. In fact, amino acid changes in the V3 loop of HIV gp120 have been shown to be involved in both macrophage and T-cell tropism (14, 49, 50). Other reports have shown the presence of both lymphocyte- and macrophage-tropic variants that are localized in different tissues of macaques infected with simian immunodeficiency virus (47). The lymphotropic variants are selected in the spleen, lymph nodes, and plasma of infected animals. In contrast, the macrophage-tropic variants are localized in the brain and lungs. Distinct variants of Epstein-Barr virus have also been detected in epithelial but not lymphoid malignancies (1). These variants transform lymphocytes less efficiently, and the cell lines which can be established grow poorly in culture (27, 42). The prevalence of these variants in epithelial malignancies strongly implies an epithelial cell tropism and indicate that particular variants of Epstein-Barr virus may preferentially establish infection in epithelial cells. Taken together, these examples provide further evidence documenting the important influence of host tissues and cell types in the selection of viral variants during chronic infection.

In summary, the host provides the virus with a rich milieu of different tissues, with a variety of specialized functions, to which the virus can adapt and multiply. In this study, we present direct evidence that a single amino acid change can result in rapid emergence of a mutant virus in one tissue, whereas the parental genotype persists in another tissue of the same individual. This rapid emergence and tissue-specific selection of viral variants represents a mechanism by which viruses evolve in nature.

ACKNOWLEDGMENTS

We thank Rita J. Concepcion for excellent technical assistance.

This work was supported by Public Health Service grant NS 21496 from the National Institutes of Health and an award (R91LA166) from UC Universitywide Task Force on AIDS.

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