Brief Definitive Report

ORGAN-SPECIFIC SELECTION OF VIRAL VARIANTS DURING CHRONIC INFECTION

By RAFI AHMED* and M. B. A. OLDSTONE‡

*From *the Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles, California 90024; and the ‡Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037*

A fundamental aspect of viral pathogenesis is that different isolates of a particular virus can differ in their ability to cause disease. It is now firmly established that virulence is genetically determined, that viruses can undergo rapid mutation, and that changes in the viral genome can produce dramatic changes in pathogenicity (1–3). However, we know much less about the selective pressures involved in the emergence of viral mutants. The role of the immune response in selection of antigenic variants has been described in some systems (4, 5). But other possible selective forces have remained largely unexplored. In this study, we document the profound influence of host tissues in the selection of viral variants. We show that lymphocytic choriomeningitis virus (LCMV) isolates of different phenotype predominate in the central nervous system (CNS) and lymphoid tissues of persistently infected mice. Most of the CNS isolates cause acute infections in adult mice, whereas the majority of the isolates derived from the spleen cause chronic infections in adult mice. These results show that different organs (and possibly cell types) favor the selection of a particular class of variants.

Materials and Methods

Mice. BALB/c ByJ (H-2^d) mice were purchased from The Jackson Laboratory, Bar Harbor, ME, and BALB/c WEHI (H-2^d) mice were obtained from the breeding colony at Scripps Clinic and Research Foundation.

Virus. The Armstrong CA 1371 strain of LCMV was used in these studies (6). The virus was triple plaque purified on Vero cells and stocks were grown in BHK-21 cells. This original laboratory stock of CA 1371 will be referred to as "wild-type" (wt) Armstrong strain. The CNS and lymphoid isolates of CA 1371 were also plaque purified three times. Virus stocks (grown in BHK cells) at passage 1 or passage 2 levels were used in all experiments. The biological properties of the various isolates are extremely stable in tissue culture and we have had no reversion of the phenotype during the plaque purification on Vero cells or growth in BHK cells.

Virus Titration. Infectious LCMV was quantitated by plaque assay on Vero cell monolayers as previously described (6).

Cytotoxic T Lymphocyte (CTL) Assay. LCMV-specific CTL activity in the spleen and lymph nodes was determined by a 6-h ^{51}Cr-release assay as previously described (6).

This work was supported by Public Health Service grants NS-21496 to R. Ahmed and AI-09484 to M. B. A. Oldstone from the National Institutes of Health and by an award (H860623) from the University of California Systemwide Task Force on AIDS to R. Ahmed. R. Ahmed is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society. Address correspondence to R. Ahmed, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/88/05/1719–1724/06 $2.00
Volume 167 May 1988 1719–1724

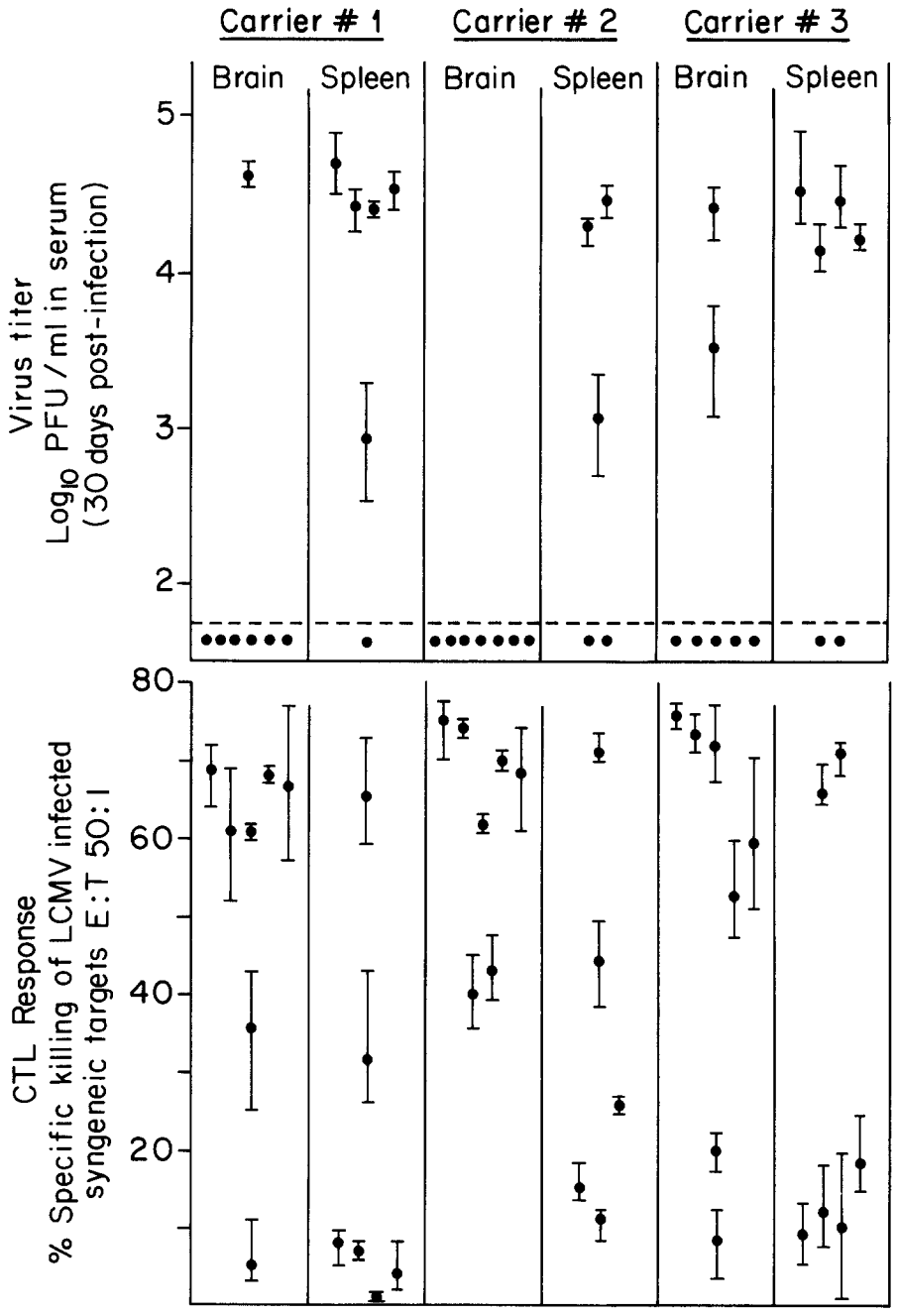

FIGURE 1. Biological characterization of LCMV isolates derived from the spleen and brain of wt Armstrong–infected carrier mice. 6–10-wk-old BALB/c mice were infected intravenously with 2×10^5 PFU of the indicated viral isolate. Virus titer in the serum was determined by a plaque assay on Vero cells. LCMV specific CTL activity in spleen and LN was determined 8 d after infection, which is the peak of the primary CTL response. Each point represents the mean value of three mice infected with a particular LCMV isolate. The bars indicate the range.

Results

LCMV Isolates of Different Phenotype Are Present in the CNS and Lymphoid Tissues of Individual Carrier Mice. 6–8-wk-old BALB/c carriers infected at birth with the wt Armstrong strain of LCMV were killed and their spleens and brains were harvested. The organs were homogenized individually and titrated on Vero cell monolayers. Well-isolated plaques were picked from each sample, recloned two more times, and then stocks were grown in BHK-21 cells. These spleen and brain isolates were authenticated as LCMV by their positive reaction with mAbs specific for the nucleoprotein and glycoproteins of wt LCMV Armstrong and by positive hybridization with Armstrong-specific cDNA probes (7, 8) (data not shown). These isolates were then tested for their ability to induce LCMV-specific CTL responses and to persist in adult mice. The data for isolates obtained from three BALB/c carrier mice are shown in Fig. 1. Striking differences were observed between the brain and spleen isolates in their ability to induce CTL responses and to cause acute or persistent infection in adult mice. Greater than 85% (18/21) of the brain isolates induced potent CTL responses and the virus was cleared within 2 wk. In contrast, the majority of the spleen isolates (12/17) induced poor CTL responses and were present in high titers in the serum at 30 d after infection. Adult mice infected with the spleen isolates harbor virus for several months. Similar results have been obtained with isolates derived from the lymph nodes (LN) of BALB/c carrier mice (data not shown). Thus, our results show that an individual carrier mouse contains at least two types of LCMV variants, one type predominating in the CNS and the other in the lymphoid tissues (spleen and LN).

Organ-specific Selection in Mice Infected with a Spleen Isolate: Isolation of Revertants from the CNS. It was of interest to determine if the "reverse" selection (spleen phenotype → CNS [wt] phenotype) also occurred during chronic LCMV infection. The spleen isolate, clone 13, was chosen for these studies. Neonatal or adult BALB/c mice were infected with clone 13 and virus isolated from the brains and spleens of these mice 65 and 172 days after infection. These isolates

TABLE I

Isolation of Revertants from the CNS of Mice Infected with Spleen Isolate Clone 13

Age at infection	Time of virus isolation (days after infection)	Mouse no.	Number of revertants/ number of brain isolates tested	Number of revertants/ number of spleen isolates tested
Neonate*	65	1	1/4	0/6
		2	0/3	0/3
		3	0/3	0/3
		4	1/3	0/6
		5	1/6	0/6
Adult‡	172	1	3/6	0/6
		2	2/7	0/5
		3	2/7	0/5

LCMV isolates derived from the brains and spleens of clone 13 infected mice were tested for their ability to persist in adult mice. Isolates that were cleared within 2 wk were scored as revertants.

* 1-day-old BALB/c mice were injected intracerebrally (i.c.) with 10^4 PFU of clone 13.

‡ 6–8-wk-old BALB/c mice were irradiated (300 rad) and then given 5×10^7 spleen cells from normal adult BALB/c mice the next day. At the time of the cell transfer, the mice were infected intravenously with 2×10^5 PFU of clone 13.

TABLE II

Biological Characterization of CNS-derived Revertants of Spleen Variant Clone 13

Virus	LCMV-specific CTL in spleen		LCMV titer $\log_{10}$ PFU/organ or ml	
	Percent specific ^{51}Cr release from BALB-7 (H-2^d) targets (E/T, 50:1)			
	Uninfected	Clone 13 infected	Spleen	Serum
wt Armstrong	8	71	<1.6	<1.6
Clone 13 Armstrong	4	6	6.2	5.1
Revertant 4a	3	57	<1.6	<1.6
Revertant 8a	9	49	2.8	2.0
Revertant 20a	2	50	2.4	2.0
Revertant 403a	3	62	<1.6	<1.6
Revertant 412a	8	43	3.0	2.1
Revertant 417a	2	64	<1.6	<1.6

Adult BALB/c mice were infected intravenously with 2×10^5 PFU of the indicated virus. CTL response and virus titer were checked 8 d after infection. The data shown are the average of two to four mice per group.

were then tested for their ability to persist in adult mice. Isolates that were cleared within 2 wk were scored as revertants. As shown in Table I, revertants were present in the CNS of the majority (6/8) of the mice examined. 10 revertants were present among the 39 brain isolates tested. In contrast, all 40 spleen isolates tested showed the clone 13 phenotype. These 10 CNS isolates were phenotypically like the original wt LCMV Armstrong and induced potent CTL responses in adult mice and the infection was rapidly cleared. The data for six independently derived (different mice) revertants of clone 13 are shown in Table II.

Discussion

This study documents the importance of host tissues in the selection of viral variants during a chronic infection. In an earlier study we showed that genetic variants of LCMV are selected in the spleens of carrier mice infected with the wt Armstrong strain (6). The present study extends these earlier observations by showing that LCMV isolates of different phenotype predominate in the lymphoid tissues and CNS of individual wt Armstrong infected carrier mice. In addition, we have shown the reverse selection (spleen phenotype → CNS [wt] phenotype) in the CNS of mice infected with a spleen isolate, thus providing unequivocal evidence for organ-specific selection during chronic LCMV infection. Adaptation of viruses by passage in a different host or in different tissues is a well-established phenomenon (1–3). However, the unique aspect of our study is the demonstration of organ-specific selection during chronic infection in the natural host. These results suggest a possible mechanism by which viral variants emerge in nature.

Neonatally or congenitally infected LCMV carrier mice contain infectious virus in most of their tissues (6, 9, 10). It will be of interest to determine if unique variants are present in different organs, and if certain cell types favor the selection of particular mutants. The presence of LCMV variants with different biological properties and plaque phenotypes in the livers and brains of car-

rier mice has been described (9, 10). In another study, Pfau et al. (11) have reported the isolation of two types of variants from the blood of a LCMV carrier mouse. However, the cell types involved in the selection of these variants is not known. We have recently shown that infection in the lymphoid tissues is confined primarily to T cells of the helper subset ($CD4^+$) and macrophages, with minimal involvement of cytotoxic T cells ($CD8^+$) and B cells (12). Our preliminary results indicate that variants are selected in both T helper cells and macrophages. Most of the spleen isolates described in this study caused a chronic infection in adult mice associated with low virus-specific T cell responses. Better replication in T helper cells and/or macrophages may be the underlying cause of this chronic infection and immunosuppression. A variety of cell types in the CNS can be infected by LCMV and the pattern of infection is different in neonatally infected carriers compared with mice infected as adults (13). It will be of interest to determine the cellular basis of the selection of variants in the CNS. Previous data indicated that virus replicated predominantly in neurons and not glial cells of persistently infected mice (14).

The various organs and cell types present in the body provide a rich milieu for the selection of viral variants. Given the high mutation rate of viruses, it is likely that tissue and cell-specific selection is an important aspect of virus evolution (15, 16). This applies in particular to viruses that cause chronic infections. Long-term persistence with continuous virus replication will permit the emergence of variants that have a growth advantage in certain cell types. In this study we have shown that LCMV variants with profound biological differences can be isolated from the lymphoid and CNS tissue of an individual carrier mouse. It will be of interest to determine if organ- and cell-specific variants are also selected in other chronic viral infections. Of particular interest is the human immunodeficiency virus (HIV), the causative agent of AIDS; HIV exhibits extensive genetic variability and is known to persist in both the lymphoid and nervous systems (17–19).

Summary

This study demonstrates organ specific selection of viral variants during chronic lymphocytic choriomeningitis virus (LCMV) infection in its natural host. Isolates with different biological properties were present in the central nervous system (CNS) and lymphoid tissues of carrier mice infected at birth with the wt Armstrong strain of LCMV. Viral isolates from the CNS were similar to the wt Armstrong strain and induced potent virus-specific cytotoxic T lymphocyte (CTL) responses in adult mice and the infection was cleared within 2 wk. In contrast, LCMV isolates derived from the lymphoid tissues caused a chronic infection in adult mice associated with suppressed CTL responses. Revertants with wt Armstrong phenotype were present in the CNS of mice infected with a spleen isolate showing unequivocally the importance of host tissues in the selection of viral variants. These results provide a possible mechanism by which viral variants emerge in nature and suggest that tissue- and cell-specific selection is an important aspect of virus evolution.

We thank Rita J. Concepcion for excellent technical assistance and Lori Kenton for help in preparing the manuscript.

References

1. Mims, C. A., and D. O. White. 1984. Viral Pathogenesis and Immunology. Blackwell Scientific Publications, Boston. 398 pp.
2. Fields, B. N. 1985. Virology, Raven Press, New York. 1614 pp.
3. Notkins, A. B., and M. B. A. Oldstone. 1987. Concepts in Viral Pathogenesis II. Springer-Verlag New York Inc., New York. 409 pp.
4. Kono, Y., K. Kobayashi, and Y. Fukunaga. 1973. Antigenic drift of equine infectious anemia virus in chronically infected horses. *Arch. Gesamte Virusforsch.* 41:1.
5. Narayan, O., D. E. Griffen, and J. Chase. 1977. Antigenic shift of visna virus in persistently infected sheep. *Science (Wash. DC).* 199:376.
6. Ahmed, R., A. Salmi, L. D. Butler, J. M. Chiller, and M. B. A. Oldstone. 1984. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice: role in suppression of cytotoxic T lymphocyte response and viral persistence. *J. Exp. Med.* 60:521.
7. Buchmeier, M. J., H. A. Lewicki, O. Tomori, and M. B. A. Oldstone. 1981. Monoclonal antibodies to lymphocytic choriomeningitis and Pichinde viruses: generation, characterization and cross-reactivity with other arenaviruses. *Virology.* 113:73.
8. Southern, P. J., M. K. Singh, Y. Riviere, D. R. Jacoby, M. J. Buchmeier, and M. B. A. Oldstone. 1987. Molecular characterization of the genomic S RNA segment from lympohcytic choriomeningitis virus. *Virology.* 157:145.
9. Hotchin, J., W. Kinch, and L. Benson. 1971. Lytic and turbid plaque-type mutants of lymphocytic choriomeningitis virus as a cause of neurological disease or persistent infection. *Infect. Immun.* 4:281.
10. Popescu, M., and F. Lehmann-Grube. 1976. Diversity of lymphocytic choriomeningitis virus: variation due to replication of the virus in the mouse. *J. Gen. Virol.* 30:113.
11. Pfau, C. J., J. K. Valenti, S. Jacobson, and D. C. Pevear. 1982. Cytotoxic T cells are induced in mice infected with lymphocytic choriomeningitis virus strains of markedly different pathogenicities. *Infect. Immun.* 36:598.
12. Ahmed, R., C.-C. King, and M. B. A. Oldstone. 1987. Virus-lymphocyte interaction: T cells of the helper subset are infected with lymphocytic choriomeningitis virus during persistent infection in vivo. *J. Virol.* 61:1571.
13. Gilden, D. H., G. A. Cole, A. A. Monjan, and N. Nathanson. 1972. Immunopathogenesis of acute central nervous system disease produced by lymphocytic choriomeningitis virus. I. Cyclophosphamide-mediated induction of the virus-carrier state in adult mice. *J. Exp. Med.* 135:860.
14. Rodriguez, M., M. J. Buchmeier, M. B. A. Oldstone, and P. W. Lampert. 1983. Ultrastructural localization of viral antigens in the CNS of mice persistently infected with lymphocytic choriomeningitis virus. *Am. J. Pathol.* 110:95.
15. Ahmed, R., W. M. Canning. R. S. Kauffman, A. H. Sharpe, J. V. Hallum, and B. N. Fields. 1981. Role of the host cell in persistent viral infection: coevolution of L cells and reovirus during persistent infection. *Cell.* 25:325.
16. Holland, J. J., K. Spindler, F. Horodyski, E. Graham, S. Nichol, and S. Vendepol. 1982. Rapid evolution of RNA genomes. *Science (Wash. DC).* 215:1577.
17. Benn, S., R. Rutledge, T. Folks, J. Gold, L. Baker, J. McCormick, P. Feorino, P. Piot, T. Quinn, and M. Martin. 1985. Genomic heterogeneity of AIDS retroviral isolates from North America and Zaire. *Science (Wash. DC).* 230:949.
18. Hahn, B., G. Shaw, M. Taylor, R. Redfield, P. Markham, S. Salahuddin, F. Wong-Staal, R. Gallo, E. Parks, and W. Parks. 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or risk for AIDS. *Science (Wash. DC).* 232:1548.
19. Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen. 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science (Wash. DC).* 236:819.