Genomic and Biological Variation among Commonly Used Lymphocytic Choriomeningitis Virus Strains

By FRANCIS J. DUTKO* AND MICHAEL B. A. OLDSTONE*
Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037, U.S.A.

(Accepted 27 April 1983)

SUMMARY
Six commonly used strains of lymphocytic choriomeningitis virus (LCMV) [Armstrong (Arm) CA 1371, Arm E-350, WE, UBC, Traub and Pasteur C1PV 76001] were examined for distinctive genetic and biological properties. Agarose gel electrophoresis yielded no detectable differences among the L or S RNAs of these six strains. The RNase T1 fingerprint patterns of LCMV Arm CA 1371 and E-350 RNAs were similar, but in contrast, those of the WE, UBC, Traub and Pasteur strains differed from each other and from the pattern of LCMV Arm CA 1371 and E-350. There were also differences among LCMV strains in their biological properties. LCMV Arm CA 1371, E-350 and Pasteur caused severe vasculitis and focal necrotizing hepatitis in the livers of neonatally infected BALB/WEHI mice in contrast to LCMV WE which caused minimal lesions. LCMV Arm CA 1371 and E-350 were lethal for neonatal C3H/St mice. In contrast, LCMV WE, Traub and Pasteur induced persistent infections in C3H/St mice. Adult guinea-pigs resisted infection by Arm CA 1371, E-350, Traub and Pasteur but succumbed to WE and UBC LCMV strains. Our results show a wide variation in the RNA genomes of LCMV strains commonly used in research laboratories, and these genomic differences are accompanied by variations in the biological properties of LCMV strains.

INTRODUCTION
Arising from the study of lymphocytic choriomeningitis virus (LCMV) infection of the mouse are many ideas that broaden the fields of virology and immunology. Observations on acute and persistent LCMV infections were of fundamental importance to Burnet in forming the concept of immunological tolerance (Burnet & Fenner, 1949; Burnet, 1959), a subject that has been studied in numerous laboratories (for reviews, see Hotchin & Cinits, 1958; Volkert & Larsen, 1965; Lehmann-Grube, 1971, 1982; Oldstone, 1979; Buchmeier et al., 1980).

The area of virus-induced immune response disease reported by Rowe (1954) and studied by Hotchin (1962) in acute LCMV infection was extended to persistent infections by Oldstone & Dixon (1967, 1969). Work on LCMV provided initial insight into the mechanisms of virus-induced immune complex disease (for review, see Oldstone, 1975) and the first evidence for generation of cytolytic thymus-derived lymphocytes during virus infection (Cole et al., 1972; Marker & Volkert, 1973). The phenomenon of H-2 gene restriction that governs cell-cell interaction, the assignment of a function for the K and D regions of the murine major histocompatibility complex (MHC) [and, by analogy, the A and B loci of the human MHC (Zinkernagel & Doherty, 1974, 1979)] and the virus-induced changes in homeostasis resulting from turning off differentiated (luxury) but not vital cell functions (Oldstone et al., 1977, 1982) are all concepts that stem from studies with LCMV.

LCMV has several unique biological and biochemical characteristics. These include the ability to produce non-cytolytic persistent infection in cultured cells from many species, to cause
persistent infection in mice after neonatal infection and to kill adult mice after intracerebral inoculation. Because the individual LCMV strains cannot be differentiated by conventional serological assays such as neutralization and complement fixation, in immunofluorescence tests with polyclonal antisera, by their replication strategy, polypeptide profiles in gels, or morphology by electron microscopy, all LCMVs have been classified as a homogeneous group within the Arenaviridae family (Rowe et al., 1970; Matthews, 1982). Nevertheless, considerable variability is a feature of the disease produced by different LCMV strains. For example, adult guinea-pigs are uniquely susceptible to LCMV WE strain [less than 1 plaque-forming unit (p.f.u.) per animal is lethal] but resistant to 10^6 p.f.u. of LCMV Armstrong (Arm) 1371 or E-350 (Lehmann-Grube, 1971; Buchmeier & Dutko, 1980). Kirk et al. (1980) have reported genomic differences between LCMV E-350 and LCMV WE and tentatively assigned the pathogenic effect in guinea-pigs to the small (S) RNA segment of LCMV E-350. LCMV Arm 1371 (Doyle & Dutko, 1970) is lethal but resistant to 10^6 p.f.u. of LCMV Armstrong (Arm) 1371 or E-350 guinea-pigs are uniquely susceptible to LCMV WE strain [less than 1 plaque-forming unit (p.f.u.) per animal is lethal] but resistant to 10^6 p.f.u. of LCMV Armstrong (Arm) 1371 or E-350 (Lehmann-Grube, 1971; Buchmeier & Dutko, 1980). Kirk et al. (1980) have reported genomic differences between LCMV E-350 and LCMV WE and tentatively assigned the pathogenic effect in guinea-pigs to the small (S) RNA segment of LCMV E-350. LCMV Arm 1371 (Doyle et al., 1980) and LCMV Pasteur (Riviére et al., 1980) cause lethal infection in C3H mice, whereas LCMV Traub and LCMV WE (Volkert & Larsen, 1965; Lehmann-Grube, 1971) do not kill newborn C3H mice but produce persistent life-long infection.

In this report, we systematically analyse the RNA genomes of six LCMV strains commonly used by laboratories in North and South America, Europe, Australia and Asia, by RNase T1 fingerprinting and correlate genomic patterns with biological effects caused by these strains. Our results indicate considerable variability previously not appreciated among LCMV strains and may account for many of the discrepancies in experimental observations between laboratories.

### METHODS

**Viruses and cells.** The strains of LCMV tested and their sources and passage histories appear in Table 1. The viruses were either double or triple plaque-purified in Vero cells (Mifune et al., 1971) in this laboratory before biochemical analysis. Virus stocks were grown in BHK-21 cells infected at a multiplicity of infection (m.o.i.) of 0.1. BHK-21 cells were propagated in enriched Dulbecco's medium containing 5% heat-inactivated (56 °C, 30 min) foetal bovine serum (FBS), 10% tryptose phosphate broth, 40 mM-glutamine, 4% glucose, and antibiotics, as described by Welsh et al. (1976). Vero cells were propagated in Eagle's minimal essential medium (MEM) containing 10% donor calf serum, 10 mM (final concentration) glutamine, and antibiotics.

**Materials.** Sankyo RNase T1 was purchased from CalbiochemBehring (La Jolla, Ca.), SDS, linear polyacrylamide (mol. wt. > 5 x 10^6) from Gallard-Schlesinger (Carle Place, N.Y.), proteinase K from Merck & Co. (Rahway, N.J.) and carrier-free 32P (285 Ci/mg) from ICN Chemical and Radioisotope Division (Irvine, Ca.). Autoradiography was performed using Kodak X-Omat R film and Cronex 'Lightning-plus' intensifying screens (E.I. Dupont de Nemours & Co., Wilmington, De.). Glyoxal as a 30% solution (Fischer Scientific Co., Pittsburgh, Pa.) was stored in a tube containing autoclaved, mixed ion-exchange resin (AG 501-X8 mixed bed resin, Bio-Rad). Dimethyl sulphoxide (reagent grade, J.T. Baker Chemical Company, Hayward, Ca.) was autoclaved and stored over autoclaved resin. Within 1 h of use, both glyoxal and dimethyl sulphoxide were deionized by passage through a column of mixed bed ion-exchange resin.

**Radiolabelling of virus.** BHK-21 cells were seeded into plastic flasks (1 x 10^7 cells per 175 cm^2 flask, Falcon No. 3028) containing 40 ml of enriched Dulbecco's medium and incubated at 37 °C. Two days later, the cells were infected with virus at an m.o.i. of 0.2 p.f.u./cell. After a 1.5 h adsorption period at 37 °C, 25 ml of Glasgow-modified MEM containing one-tenth the normal amount of phosphate and 5% FBS (1/10 GMEM) was added to each flask. Twenty-four h later, the medium was decanted and 20 ml of 1/10 GMEM containing 2 mCi of 32P was added to each flask. Sixteen h later, the supernatants were harvested and immediately subjected to virus purification.

**Purification of virus.** Supernatants from infected cells were first clarified by centrifugation at 10000 rev/min for 45 min at 4 °C in a Sorvall SSA rotor. Forty ml of the supernatant was then layered over 10 ml of 25% (w/v) sucrose in TNE buffer (50 mM-Tris-HCl pH 7.4, 100 mM-NaCl, 1 mM-EDTA). Virions were pelleted by centrifugation at 30000 rev/min for 3 h at 4 °C. The pellet was layered onto a linear 10 to 50% (v/v) sucrose in TNE buffer (50 mM-Tris-HCl pH 7.4, 100 mM-NaCl, 1 mM-EDTA). Virions were pelleted by centrifugation at 30000 rev/min for 3 h at 4 °C. The pellet was layered onto a linear 10 to 50% (v/v) Renografin (E.R. Squibb & Sons, Princeton, N.J.) density gradient prepared in TNE buffer. Virions were banded by equilibrium centrifugation at 30000 rev/min for 16 h at 4 °C in a Beckman SW41 rotor. The visible virion band was removed, diluted with TNE, and pelleted by centrifugation at 36000 rev/min for 45 min at 4 °C in the latter rotor. The supernatant was discarded and the pelleted virions were resuspended in TNE buffer for RNA extraction.

**Viral RNA extraction and denaturation with glyoxal.** Purified virions were disrupted by the addition of SDS and proteinase K to 2% (w/v) and 250 µg/ml respectively, and incubated at 50 °C for 5 min. Viral RNA was extracted with phenol :chloroform (1:1, v/v, saturated with TNE) and then with chloroform :isoamyl alcohol (24:1, v/v)
Variability among LCMV strains

Table 1. Source and passage histories of LCMV strains

<table>
<thead>
<tr>
<th>Virus</th>
<th>Source and reference</th>
<th>Passage history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armstrong CA 1371</td>
<td>W. P. Rowe, Bethesda, Md., U.S.A. (Rowe <em>et al.</em>, 1963)</td>
<td>Mouse brain, L cells, (BHK cells)*</td>
</tr>
<tr>
<td>Pasteur C1PV 76001</td>
<td>I. Gresser, Villejuif, France (Riviere <em>et al.</em>, 1977)</td>
<td>Mouse brain, (BHK cells)</td>
</tr>
</tbody>
</table>

* Passages done in this laboratory are indicated by parentheses.

and precipitated with cold ethanol. The RNA was denatured with glyoxal by using a modification of McMaster and Carmichael’s (1977) method. Briefly, we used 1 M-glyoxal (deionized) and 50% (v/v) deionized dimethyl sulphoxide at 60 °C for 3 min and then 50 °C for 15 min. The mixture equilibrated to room temperature before gel analysis.

Agarose gel electrophoresis

Analytical gels. Approximately 50 000 ct/min of 32P-labelled LCMV RNA (glyoxal-denatured) were electrophoresed in a 1.5% (w/v) agarose (Seakem, Marine Colloids, Rockland, Me.) horizontal slab gel for 17 h at 50 V. The buffer system consisted of 40 mM-Tris, 20 mM-sodium acetate, 5 mM-EDTA, and 0.2% SDS, pH 7.4 (TA buffer).

Preparative gels. 32P-labelled LCMV RNA (glyoxal-denatured) was electrophoresed for 17 h at 50 V in a 1.2% (w/v) Seaplaque agarose gel. The RNA was recovered by phenol extraction of melted (70 °C for 15 min) gel slices as described by Weislander (1979).

RNase T1 fingerprinting. RNA recovered after ethanol precipitation was dried in vacuo over CaCl2. The glyoxal adduct was removed by dissolving the RNA in 50 mM-Tris-HCl pH 8.2, 100 mM-NaCl, 1 mM-EDTA and 0.1% SDS and incubating at 45 °C for 48 h. RNA precipitated in cold ethanol and containing between 40 000 and 100 000 ct/min was analysed by RNase T1 oligonucleotide fingerprinting as described by De Wachter & Fiers (1972). Briefly, in the first dimension the gel was 10% acrylamide-3.25% bisacrylamide and the buffer was 0.025 M-citric acid, 6 M-urea, pH 3.5. In the second dimension, a 20% acrylamide-0.5% linear polyacrylamide gel in 0.1 M-Tris-HCl, 2.5 mM-EDTA, pH 8.3 was used.

Mice and guinea-pigs. BALB/WEHI and C3H/St mice (less than 24 h old) obtained from the breeding colony at Scripps Clinic and Research Foundation were inoculated intracerebrally with 60 p.f.u. of LCMV. For the studies on the induction of liver disease, BALB/WEHI mice infected at birth with LCMV (see above) were sacrificed at various times after infection. Liver tissue was removed, fixed in Bouin’s solution and stained with haematoxylin-eosin or periodic acid-Schiff’s reagent. Hartley guinea-pigs (350 g) were obtained from Crest Caviary (Raymond, Ca.) and were inoculated intraperitoneally with 10 or 103 p.f.u. of LCMV.

Results

Analysis of the genomic RNA of LCMV strains

We first determined whether the L (large) and S (small) RNAs from six LCMV strains (Table 1) could be distinguished by their mobility during agarose gel electrophoresis. Fig. 1 shows that no difference was detected in the electrophoretic migration of these molecules, indicating the similar molecular weights of the L and S RNAs from all of these LCMV strains. In addition, under the labelling conditions employed, virion preparations from each of the LCMV strains contained similar amounts of ribosomal RNA.

To compare these RNAs further, we prepared RNase T1 fingerprints from their isolated L and S segments. The fingerprints for the LCMV L RNAs are shown in Fig. 2. The L RNA fingerprint of LCMV Arm CA 1371 was indistinguishable from that of LCMV E-350. In contrast, the LCMV L RNAs from the four other LCMV strains (WE, UBC, Traub and Pasteur) provided distinct fingerprints that were dissimilar to Arm CA 1371 and E-350 as well as to each other.
Fig. 1. Electrophoretic patterns of LCMV RNAs. Three 175 cm² flasks of BHK-21 cells were infected with each LCMV strain at an m.o.i. of 0-2, and viral RNA was labelled by adding 32P to the infected cells. 32P-labelled RNA extracted from purified virions was denatured with glyoxal, and equivalent amounts of LCMV 32P-RNA were analysed by agarose (1.5%) gel electrophoresis as described in the text. The positions of 28S and 18S ribosomal RNAs are indicated. Lane 1, LCMV Arm CA 1371; lane 2, LCMV WE; lane 3, LCMV E-350; lane 4, LCMV Traub; lane 5, LCMV UBC; lane 6, LCMV Pasteur.

S RNAs from the six LCMV strains provided similar results (Fig. 3). The fingerprint pattern of LCMV Arm CA 1371 resembled that of E-350. On the other hand the fingerprint patterns of the other LCMV strains (WE, UBC, Traub and Pasteur) differed from each other and from that of LCMV Arm CA 1371 and E-350.

To assess more accurately the degree of similarity among these RNAs, equal amounts of 32P-labelled RNase T₁-resistant oligonucleotides from pairs of LCMV strains were mixed and then studied by two-dimensional gel electrophoresis. The percentages of shared oligonucleotides are presented in Table 2 for mixtures of LCMV RNAs. The close relatedness of LCMV Arm CA 1371 and E-350 is evident since all 47 oligonucleotides are shared by their L RNAs, and 96% (24 of 25) of the oligonucleotides are shared by their S RNAs. In comparison, LCMV Arm CA 1371 and LCMV WE, UBC, Traub and Pasteur shared only 13 to 24% of their oligonucleotides for L RNA, and 10 to 21% of their oligonucleotides for S RNA. Interestingly, LCMV WE and LCMV UBC shared only 4 of 27 and 17 of 59 oligonucleotides for the S and L RNAs respectively, indicating their lack of relatedness, even though LCMV UBC originated from LCMV WE (Hotchin & Weigand, 1961).

Experiments were performed to determine whether the unique oligonucleotide fingerprints of S and of L RNA were constant in plaque isolates from the same LCMV strain. S and L RNA fingerprints of the original LCMV CA 1371 stock used over several years (Welsh et al., 1976; Doyle et al., 1980) and two independent, triple plaque-purified isolates from this stock were identical. This indicates that the variability observed in the fingerprint patterns is probably unique for each LCMV strain.

**Analysis of the biological properties of LCMV strains**

We then determined whether the distinct differences between LCMV strains uncovered at the genome level corresponded to different biological properties. The strategy was to analyse
biological response, elicited by the genomically similar Arm CA 1371 and E-350 strains and by at least one other dissimilar strain (WE, Traub, Pasteur or UBC, Fig. 2 and 3, Table 2) in causing disease. First, we tested the ability of Arm CA 1371, E-350, WE and Pasteur to cause injury to liver cells of BALB/WEHI mice. Table 3 shows that LCMV Arm CA 1371, E-350 and Pasteur strains destroyed liver cells and elicited an inflammatory lymphoid and polymorphonuclear cell response in these mice. In contrast, LCMV WE did not cause significant liver injury. However, LCMV replicated in liver cells of mice infected with LCMV WE as determined by expression of viral antigens by immunofluorescence and generation of infectious virus. In fact the amount of infectious virus generated in the liver by LCMV WE infection was equivalent to that made following infection by LCMV Arm CA 1371, E-350 or Pasteur (F. Dutko, F. Chisari & M. B. A. Oldstone, unpublished results).
Next we assessed both the initiation of a persistent infection and the survival of C3H/St and BALB/WEHI newborn mice inoculated with LCMV. As Table 4 indicates, both LCMV Arm CA 1371 and E-350 were lethal for C3H/St mice, but neither strain killed BALB/WEHI mice, which routinely developed persistent infection. In contrast, LCMV WE and Traub readily caused persistent infection in C3H/St as well as in BALB/WEHI mice. LCMV Pasteur and UBC were intermediate, being able to generate a persistent infection in more than one-third of inoculated C3H/St mice. Persistent infection was documented by showing that sera taken from inoculated mice contained infectious LCMV that caused acute infection when transferred into adult (uninfected) mice (Lehmann-Grube, 1971; Oldstone et al., 1980).

Lastly, we analysed the ability of LCMV strains to kill adult Hartley guinea-pigs. As seen in Table 5, $10^1$ p.f.u. of LCMV WE or UBC were usually fatal. In contrast, guinea-pigs routinely survived challenge with $10^3$ p.f.u. of LCMV Arm 1371, E-350, Traub or Pasteur.
Variability among LCMV strains

Table 2. Comparison of RNase T1-resistant oligonucleotides among six LCMV strains

<table>
<thead>
<tr>
<th>Virus RNA</th>
<th>Percentage oligonucleotides shared</th>
<th>Total number of oligonucleotides used for comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 1371 + E-350 S</td>
<td>96*</td>
<td>25</td>
</tr>
<tr>
<td>CA 1371 + E-350 L</td>
<td>100</td>
<td>47</td>
</tr>
<tr>
<td>CA 1371 + Traub S</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>CA 1371 + Traub L</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>CA 1371 + WE S</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>CA 1371 + WE L</td>
<td>24</td>
<td>93</td>
</tr>
<tr>
<td>CA 1371 + UBC S</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>CA 1371 + UBC L</td>
<td>13</td>
<td>104</td>
</tr>
<tr>
<td>CA 1371 + Pasteur S</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>CA 1371 + Pasteur L</td>
<td>18</td>
<td>94</td>
</tr>
<tr>
<td>WE + UBC S</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>WE + UBC L</td>
<td>17</td>
<td>98</td>
</tr>
</tbody>
</table>

* The percentage of shared oligonucleotides was determined after fingerprinting mixtures of LCMV RNAs (see Fig. 2 and 3).

Table 3. Induction of liver disease in BALB/WEHI mice by LCMV strains*

<table>
<thead>
<tr>
<th>LCMV strain</th>
<th>Liver cell injury and death (days post-infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm CA 1371</td>
<td>4 7 21 28</td>
</tr>
<tr>
<td>E-350</td>
<td>3+† 3+ 1+ 1+</td>
</tr>
<tr>
<td>WE</td>
<td>Nil Nil Nil +</td>
</tr>
<tr>
<td>Pasteur</td>
<td>2-3+ 2-3+ + +</td>
</tr>
</tbody>
</table>

† Mice in each group were sacrificed when 4, 7, 21 and 28 days old. Haematoxylin–eosin and periodic acid–Schiff's reagent-stained sections of formalin-fixed, paraffin-embedded liver were examined microscopically and scored as follows: Nil = normal; + = ordinarily expected foci of microinfiltrates characteristic of persistent LCMV infection (see Oldstone & Dixon, 1969); 1 + = minimal areas of macronecrosis and perivascular infiltrates; 2 + = focal necrosis and perivascular infiltrates; 3 + = focal necrosis, lobular disarray, subcapsular necrosis and marked perivascular infiltrate. Results indicate findings in at least four individual mice per group.

Table 4. Survival of mice after neonatal injection of LCMV

<table>
<thead>
<tr>
<th>LCMV strain</th>
<th>Mouse strain (%) survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm CA 1371</td>
<td>C3H/St 87</td>
</tr>
<tr>
<td>E-350</td>
<td>4 95</td>
</tr>
<tr>
<td>WE</td>
<td>87 80</td>
</tr>
<tr>
<td>UBC</td>
<td>38 93</td>
</tr>
<tr>
<td>Traub</td>
<td>94 82</td>
</tr>
<tr>
<td>Pasteur</td>
<td>37 71</td>
</tr>
</tbody>
</table>

* At least 20 newborn mice of each strain were injected intracerebrally with 60 p.f.u. of LCMV. The percentage survival was calculated from the number alive at 8 weeks after injection. Survivors were persistently infected with LCMV.
Table 5. Susceptibility of adult Hartley guinea-pigs to LCMV

<table>
<thead>
<tr>
<th>LCMV strain</th>
<th>10¹ p.f.u.</th>
<th>10³ p.f.u.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm CA 1371</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>E-350</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>WE</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>UBC</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Traub</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Pasteur</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* 350 g guinea-pigs were inoculated intraperitoneally with either 10 or 1000 p.f.u. of LCMV. Death usually occurred by the 14th day after inoculation. Cumulative mortality due to LCMV infection determined at the 28th day after inoculation is given.

DISCUSSION

We have demonstrated significant differences among RNAs of the six LCMV strains (Armstrong CA 1371, E-350, WE, UBC, Traub and Pasteur) commonly used by investigators studying the pathogenesis of LCMV infection. Furthermore, these differences in the genome were associated with certain biological properties such as lethality, initiation of persistent infection and induction of liver disease. Our overall conclusions are that the LCMV strains used by several laboratories significantly differ from each other at the RNA genome level and that these differences may help to explain discrepancies in the biological properties attributed to LCMV. Furthermore, the variation among LCMV strains can be used to map the gene(s) responsible for biological activity. Since the genome of LCMV consists of two distinct RNA segments (Fig. 1 to 3), recombinant viruses between selected strains can be used to map the gene(s) controlling various disease patterns. This strategy, initially employed for reovirus (Fields & Greene, 1982), has been used by Kirk et al. (1980) to map LCMV-induced virulence in guinea-pigs and hamsters to LCMV E-350 S RNA for one type of recombinant LCMV.

Despite the classification of LCMV as a homogeneous group of viruses with LCMV representing the 'prototype virus' for the Arenaviridae family (Rowe et al., 1970; Matthews, 1982), we have found substantial differences among LCMV strains. The genomes of LCMV strains analysed here consisted of the expected two RNA segments: L RNA with an approximate mol. wt. of 2.85 x 10⁶ and S RNA of 1.35 x 10⁶. The electrophoretic mobilities of L and S RNAs in agarose were similar (Fig. 1). However, the RNA structure of the various LCMV strains differed considerably, according to RNase T₁ fingerprint analysis. This diversity is also clear at the gene product level by peptide analysis and by monoclonal antibody analysis of glycoproteins of LCMV strains (M. Buchmeier, unpublished results). At the genome level, Arm CA 1371 and E-350 showed close homology, i.e. over 95% of their oligonucleotide sequences were in common. By the same criterion, there was less than 25% homology between Traub, Pasteur, WE, UBC, or between these viruses and Arm CA 1371 or E-350 (Fig. 2 and 3, Table 2).

The significance of genome diversity among LCMV strains lies in its association with different disease states. On the basis of RNase T₁ fingerprints, LCMV Arm CA 1371 and E-350 are closely related, sharing 24 of 25 S RNA oligonucleotides and 47 of 47 L RNA oligonucleotides. Both LCMV strains had similar disease patterns in that they caused liver disease in suckling BALB/WEHI mice (Table 3), killed newborn C3H/St mice (Table 4) but failed to kill adult guinea-pigs (Table 5). The other LCMV strains (Traub, WE, UBC and Pasteur) were individually distinct in that each manifested different RNA fingerprints and biological properties. Corresponding to the structural differences, LCMV Traub, WE and Pasteur initiated persistent infection in C3H/St mice whereas Arm CA 1371 and E-350 uniformly caused a lethal disease. In other instances, LCMV strains differed significantly from each other yet showed a similar biological property (Tables 2 to 5). Hence, despite their differences, these strains must share regions that are probably responsible for the manifestation of the disease.
Variability among LCMV strains 1697

LCMV UBC and WE were lethal for guinea-pigs (Table 5). Analysis of a recombinant virus (Kirk et al., 1980) suggests that the S RNA codes for this effect and thus that the homologous S RNA sequences of these two strains were responsible for the lethal disease. The fact that portions of the RNA sequence varied between LCMV UBC and WE was unexpected because LCMV UBC was derived by passing LCMV WE in mouse brains (Hotchin & Weigand, 1961) and suggests that such variants may be generated in vivo.

There is an increasing appreciation of the generation of RNA virus variants (for review, see Holland et al., 1982) with the widening use of viral genomic analysis and monoclonal antibodies. The distinct variation reported here for LCMV suggests a correlation with unique disease patterns and offers a possible explanation for the ability of a prototype virus to cause a wide spectrum of disease manifestations. For example, Coxsackie B4 virus, which can cause pancreatitis, encephalitis or myocarditis, was recently segregated into several variants on the basis of monoclonal antibody analysis (Prabhakar et al., 1982). It will be of interest to see whether these isolated variants cause unique patterns of disease. Finally, if variants form so easily, yet prototype viruses remain, there is likely a selective pressure against the variants. It is tempting to speculate that immune responses, in the form of polyclonal antibody and cytotoxic T lymphocytes, are involved in this process. Support for this idea comes from preliminary data that, at a clonal level, cytotoxic T lymphocytes frequently recognize shared viral glycoprotein determinants among the individual LCMV strains (Arm CA 1371, WE, Traub, Pasteur and UBC) (Byrne et al., 1983).

This is Publication No. 2817 from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037. This research was supported by NIH grants AI09484 and NS12428. Francis J. Dutko is the recipient of a Special Fellowship from the Leukemia Society of America. The authors thank Michael Buchmeier, Peter Southern, Rafi Ahmed and Frank Chisari for helpful discussions and acknowledge the expert technical assistance of Antoinette Tishon, Paul Blount and Madhu Singh. We thank Ana Garcia for manuscript preparation.

REFERENCES


polyacrylamide and agarose gels by using glyoxal and acridine orange. Proceedings of the National Academy of
Sciences, U.S.A. 74, 4835–4838.


MIFUNE, K., CARTER, M. & RAWLS, W. (1971). Characterization studies of the Pichinde virus—a member of the

resulting in immunoprotection or immunologic injury – two different sides of the same coin. In Progress in


choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected


specific anti-viral antibody and Clq binding material in the circulation during persistent lymphocytic
choriomeningitis virus infection. Journal of Immunology 124, 831–838.

Virus-induced alterations in homeostasis: alterations in differentiated functions of infected cells in vivo.
Science 218, 1125–1127.

among naturally occurring human Coxsackie B4 virus isolates identified by monoclonal antibodies.

RIVERS, T. M. & SCOT, T. F. M. (1936). Meningitis in man caused by a filterable virus. II. Identification of the

choriomeningitis virus disease in suckling mice. Proceedings of the National Academy of Sciences, U.S.A. 74,
2135–2139.

Severity of lymphocytic choriomeningitis disease in different strains of suckling mice correlates with


Virology 5, 651–652.


WEISLANDER, L. (1979). A simple method to recover intact high molecular weight RNA and DNA after


polymorphic major transplantation antigens determining T cell restriction-specificity, function and
responsiveness. Advances in Immunology 27, 51–177.

(Received 19 January 1983)