Formation and Characterization of an Intertypic Lymphocytic Choriomeningitis Recombinant Virus

(Accepted 10 June 1980)

SUMMARY

An intertypic reassortant arenavirus has been obtained from co-infection of BHK-21 cells by two strains of lymphocytic choriomeningitis virus (LCM virus), WE and Armstrong (ARM), having the large/small (L/S) viral RNA genotype of WE/ARM. The two parental viruses have different virulence characteristics in hamsters and guinea-pigs. The reassortant virus induces LCM-WE-type plaques in Vero cell monolayers, but has the avirulent characteristics of LCM-ARM in hamsters and two strains of guinea-pigs. These results suggest that the LCM plaque phenotype is determined by L RNA gene products, while the pathogenic potential in guinea-pigs may be determined by S RNA gene products.

The genome of lymphocytic choriomeningitis virus (LCM virus), the prototype virus of the Arenaviridae family of RNA viruses (Rowe et al. 1970), consists of two ssRNA species (Pedersen, 1970, 1973). The two genome RNA species can be separated by polyacrylamide gel electrophoresis, or by sucrose density gradient centrifugation and have been designated large RNA (L, 31S, apparent mol. wt. $2.1 \times 10^6$) and small RNA (S, 23S, apparent mol. wt. $1.1 \times 10^6$). The two RNA species are considered to contain unique genetic information (Pedersen, 1973). Two additional high mol. wt. RNA species isolated from purified LCM virus preparations, having sedimentation coefficients of 28S and 18S, are believed to originate from host cell ribosomes incorporated into virus particles (Pedersen, 1971). Low mol. wt. RNA species (4S, 5S and 5.5S) associated with LCM virions, are also believed to be of host origin. The L and S RNA species extracted from purified virions of Pichinde virus, another member of the Arenaviridae family, have been shown by oligonucleotide fingerprint analyses to have unique sequences (Vezza et al. 1978). Such analyses also confirmed that the virion-associated 28S and 18S RNA species are of ribosomal origin. In this study we present data demonstrating that the L and S viral RNA species of LCM virus (strain WE) contain unique sequences as demonstrated by oligonucleotide fingerprint analyses and that these two RNAs are also distinguishable from those of a second LCM isolate, Armstrong (ARM). In addition, we have derived an intertypic recombinant LCM virus by dual infections of cells with the two LCM strains and have shown that it has: (i) the L/S RNA genotype of WE/ARM; (ii) the plaque phenotype of WE virus; and (iii) by subcutaneous inoculation in hamsters or strain 13 and Hartley guinea-pigs, the avirulent characteristics of LCM-ARM.

The L and S virion RNA species of two strains of LCM virus, LCM-ARM (Armstrong & Lillie, 1934) obtained from the American Type Culture Collection and LCM-WE (Rivers & Scott, 1936) received from Dr J. C. Winn, CDC, Atlanta, Ga., U.S.A., have been examined by oligonucleotide fingerprint analyses. The passage history of the WE strain of LCM used included one passage in mouse brain, 11 passages in guinea-pig brain, and two passages in mouse brain, before two passages in BHK-21 cells. The passage history of the LCM-ARM strain used included six passages in monkeys and 211 passages in mice, before two passages in BHK-21 cells.
Monolayers of BHK-21 cells (1.5 x 10^8 cells) were infected with LCM-ARM (or LCM-WE) virus at an m.o.i. of 0.1 and incubated at 35 °C in Eagle's medium containing 2% newborn calf serum. At 20 h p.i., the culture medium was replaced with fresh medium containing 200 μCi/ml of carrier-free ^32P-orthophosphate and incubation continued for a further 48 to 60 h at 35 °C. Cell-released virus was purified and the virion RNA extracted as described previously (Vezza et al. 1978). When the virion RNA species of each virus was analysed by electrophoresis on a slab gel of 2.4% polyacrylamide, four high mol. wt. RNA species were observed, two of which had electrophoretic mobilities identical to cellular 18S and 28S ribosomal RNA species, while the other two had mobilities similar to the L and S RNA species of Pichinde virus (data not shown; see Vezza et al. 1978). Since we recover larger amounts of high mol. wt. RNA (85 to 95%) from agarose gels than from polyacrylamide gels (Vezza et al. 1978), with no loss of resolution of the viral RNA species, the ^32P-labelled viral RNA species of LCM-ARM and LCM-WE were resolved by agarose gel electrophoresis (Wieslander, 1979) and the four high mol. wt. RNA species recovered.

The oligonucleotide fingerprints of the individual L and S RNA segments of LCM-WE were obtained as described by Clewley et al. (1977), with the results shown in the left-hand and centre panels, respectively, of Fig. 1(a). Comparison of these two fingerprints showed that the LCM-WE L and S RNA species each contained unique oligonucleotides, some of which are indicated by arrows. The fingerprints of the 28S and 18S RNA species obtained from the virus preparations corresponded, as shown previously (Vezza et al. 1978), to those of host cell ribosomal RNA (data not shown). The right-hand panel of Fig. 1(a) shows the oligonucleotide fingerprint of a mixture of LCM-WE L and S RNA species. In this finger- print most of the largest unique oligonucleotides of the viral L and S RNA segments were resolved and could be easily identified, some of which are indicated by arrows.

Similar analyses were carried out on the virion RNA species of LCM-ARM virus (Fig. 1b). A comparison of the individual L RNA fingerprints (or those of the S RNA, or L and S RNA species), of each LCM strain showed that they could be easily distinguished.

Dual infections of cells with ts mutants of viruses having a segmented, ssRNA genome (e.g. orthomyxoviruses, bunyaviruses or arenaviruses), can yield wild-type progeny viruses at a high frequency (Simpson & Hirst, 1968; Gentsch & Bishop, 1976; Vezza & Bishop, 1977). The genomes of such wild-type progeny viruses have been shown to be composed of RNA species derived from both parents. To investigate the possibility of forming recombinants between LCM-WE and LCM-ARM viruses, monolayers of BHK-21 cells were infected with both LCM-ARM (m.o.i. of 1) and LCM-WE (m.o.i. of 0.2) viruses and incubated at 35 °C for 2 days, at which time the culture fluids were harvested. These conditions were chosen since preliminary experiments with our stocks of virus indicated that these input multiplicities would provide equivalent numbers of each progeny virus by 2 days post-inoculation. The culture fluids from the mixed infection were plated on Vero cells using a semi-solid overlay (Vezza & Bishop, 1977). After 5 days of incubation at 35 °C, the cells were
stained by neutral red and several well-isolated plaques recovered. The viruses in these plaques were grown into stocks using BHK-21 cells and their virion RNA genotypes determined by oligonucleotide fingerprinting of the total RNA extracted from 32P-labelled virus preparations. By comparison with the individual L, S and composite fingerprints of LCM-WE and LCM-ARM viruses, it was determined that of four progeny viruses analysed, two had the L and S RNA genotypes of LCM-ARM (i.e. ARM/ARM, data not shown; see Fig. 1b, right-hand panel), one had the L and S RNA genotype of LCM-WE (i.e. WE/WE, data not shown; see Fig. 1a, right-hand panel), and one, as indicated in Fig. 2, had a genotype consisting of the L RNA of WE and the S RNA of ARM (i.e. WE/ARM). The largest oligonucleotides of the ribosomal 28S and 18S RNA species are smaller than those of LCM (or Pichinde) and so do not appear in the lower part of the Fig. 2 composite RNA electropherogram (Vezza et al. 1978).

The plaque phenotypes (on Vero cells) of the two parental LCM strains and the recombinant virus have been compared. After 5 days growth, LCM-ARM plaques were found to be generally smaller (0.4 cm) than those induced by LCM-WE (1 cm). The plaque sizes induced by LCM-WE/ARM virus were similar in size to those of LCM-WE virus (i.e. 1 cm), suggesting that the L RNA gene products of these strains of LCM virus are a prime determinant of the plaque phenotype. No significant differences in the time course of virus production, or final virus titres that are attained by low or high multiplicity of infection, have been noted for the WE, ARM or WE/ARM virus strains.

The abilities of the WE and ARM LCM virus stocks, as well as the WE/ARM recombinant, to infect three species of experimental animal (hamsters, guinea-pigs and mice) have been investigated. When 10⁴ p.f.u. of each virus were inoculated subcutaneously into groups of five 6 week-old female hamsters (Charles Rivers Breeding Laboratories, Wilmington, Mass., U.S.A.), none of the animals that received the ARM or WE/ARM viruses died, and indirect immunofluorescence studies indicated that all had seroconverted by 6 weeks post-inoculation. Of the five hamsters that received 10⁴ p.f.u. of LCM-WE virus, four died (mean day of death 21.8 ± 2.6).

Subcutaneous inoculation of 10⁴ p.f.u. of LCM-WE virus into groups of either five
female 350 g Hartley guinea-pigs (Buckberg Laboratory Animals, Tomkins Cove, N.Y., U.S.A.), or six female 350 g strain 13 guinea-pigs (Crest Caviary, Raymond, Calif, U.S.A.) resulted in death in all the inoculated animals by 2 weeks post-inoculation (mean day of death: Hartley guinea-pigs, 9.0 ± 0.7; strain 13 guinea-pigs, 11.5 ± 0.6). Subcutaneous inoculation of 10^4 p.f.u. of LCM-ARM virus into similar numbers of Hartley and strain 13 guinea-pigs did not result in death of the inoculated animals and by 6 weeks post-inoculation it was determined by indirect immunofluorescence analyses that all the inoculated animals had seroconverted. Subcutaneous inoculation of the WE/ARM reassortant virus into six Hartley guinea-pigs did not kill any of the animals, although one of six strain 13 guinea-pigs died after inoculation with 10^4 p.f.u. of virus. Again it was determined that all the surviving animals had seroconverted by 6 weeks post-inoculation. The log LD_{50} values for the ARM and WE/ARM viruses were determined to be greater than 5. The log LD_{50} value for the WE strain was determined to be 0.6.

After intracerebral inoculation of the viruses into groups of six male 11 to 13 week-old ICR Swiss mice (WRAIR colony, Washington D.C., U.S.A.), it was calculated that the LD_{50} values of the LCM-ARM, LCM-WE or LCM-WE/ARM virus stocks corresponded to approx. 31, 0.7 and 0.44 p.f.u., respectively. Subcutaneous inoculation of 10^4, 10^3, 10^2 or 10 p.f.u. of the three virus stocks into groups of six male 11 to 13 week-old ICR Swiss mice did not result in death of any of the inoculated animals by 6 weeks post-inoculation. Indirect immunofluorescence studies on sera obtained from the mice which received 10^4 p.f.u. of either virus indicated that the animals had seroconverted.

If these animal data are borne out in more extensive testing, then the results suggest that the S RNA segment of LCM may code for gene products that are responsible for the pathogenic potential of LCM in hamsters and guinea-pigs. Whether there are also differences in the p.f.u.:LD_{50} ratios in mice (intracerebral inoculation) between LCM-WE, LCM-ARM and the reassortant LCM-WE/ARM, requires further analyses with other strains of mice and other stocks of virus.

This study was supported by USPHS Research Grant AI 14183.

1Department of Microbiology
The Medical School
University of Alabama in Birmingham
Birmingham, Alabama 35294, U.S.A.

2U.S. Army Medical Research Institute
of Infectious Diseases, Fort Detrick
Frederick, Maryland 21701, U.S.A.

REFERENCES


* Present address: Department of Microbiology, Medical Center, University of West Virginia, Morgantown, West Virginia, 2505, U.S.A.
† Present address: Department of Bacteriology, University of Aberdeen, Forsterhill, Aberdeen, Scotland.
Short communications


(Received 18 April 1980)