NOTES

The S RNA Segment of Lymphocytic Choriomeningitis Virus Codes for the Nucleoprotein and Glycoproteins 1 and 2†

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The lymphocytic choriomeningitis virus (LCMV) genome consists of a large RNA segment and a small RNA segment. The three major structural proteins of this virus are an internal nucleoprotein and two surface glycoproteins. Intertypic reassortants between the Armstrong and WE strains of LCMV were made to map proteins encoded by the LCMV genome segments. Using monoclonal antibodies specific for the nucleoprotein and the glycoproteins of WE and Armstrong, we showed that the small RNA segment of LCMV codes for the three major structural polypeptides.

Lymphocytic choriomeningitis virus (LCMV) is the prototype virus of the arenavirus group (5, 11, 15). The LCMV genome consists of two segments of single-stranded RNA of negative polarity. The large segment, designated L, has a molecular weight (MW) of ca. 2×10^6 , and the small segment, designated S, has an MW of ca. 10^6 . Three major viral structural polypeptides have been identified: an internal nucleoprotein (NP) (MW, 63,000), associated with the genomic RNA, and two surface glycoproteins, GP-1 (MW, 43,000) and GP-2 (MW, 36,000) (5). An additional virus-specific glycoprotein (MW, 75,000) is found in LCMV-infected cells. This glycoprotein, termed GP-C, is the precursor of GP-1 and GP-2 (4). The genome coding assignments for the gene products of LCMV are not known. Mapping the gene products encoded by the L and S genome segments is essential for understanding the mode of viral replication and for defining the molecular basis of LCMV pathogenesis and persistence. We have approached this problem by generating reassortant viruses between the Armstrong (Arm) and WE strains of LCMV and identifying the viral proteins that segregate with the L and S segments. After coinfection of cells with these two different LCMV strains, recombinants are generated by reassortment of genome segments (10). The Arm and WE strains of LCMV were chosen as the parental viruses for generating the reassortants because the nucleoprotein and the glycoproteins of the Arm and WE strains can be distinguished readily by monoclonal antibodies and tryptic peptide mapping (2). In this report, we show that the S genome segment codes for the three major LCMV structural proteins, NP, GP-1, and GP-2.

The origins of the Arm and WE strains of LCMV have been described previously (8). The virus strains were triple plaque purified on Vero cells, and stocks were grown in BHK-21 cells. To generate reassortants between the two LCMV strains, we coinfect subconfluent monolayers of BHK-21 cells with the Arm and WE strains at multiplicities

of infection ranging between 1 and 5 PFU per cell. At 48 h postinfection, the supernatants were harvested, and infectious virus was quantitated by a plaque assay on Vero cell monolayers. After 5 days of incubation at 37°C, the cells were stained with neutral red, and several well-isolated plaques were recovered. The individual plaques were re-cloned one more time on Vero cell monolayers and then grown into stocks on BHK-21 cells. The genotype of the progeny clones derived from the coinfection was determined by Northern hybridization with cDNA probes specific for the L and S segments of the Arm strain (17) (Fig. 1). The cDNA probes specific for the L and S segments of the Arm strain showed minimal or no hybridization with the L and S segments of the WE strain. Our analysis involved S probes specific for either the GP-1 or the GP-2 coding regions. Thus, by using these cDNA probes it was possible to identify the genotype of progeny clones derived from crosses between Arm and WE. The two possible reassortants, Arm/WE (L segment of Arm/S segment of WE) and WE/Arm (L segment of WE/S segment of Arm), were identified by this method. The genotype of the WE/Arm reassortant was further confirmed by oligonucleotide T₁ mapping (data not shown). The genetic crosses from which the reassortants were obtained are summarized in Table 1. In experiment 1, 2 of 29 clones examined had the WE/Arm genotype (frequency of reassortants, 6.8%). In experiment 2, 1 clone of 21 clones was a reassortant and had the Arm/WE genotype (frequency of reassortants, 4.7%). In this second experiment, the culture fluids from the dually infected BHK-21 cells were treated with neutralizing monoclonal antibodies before the progeny clones were isolated. The monoclonal antibodies 2.11.10 and WE-40 are specific for GP-1 of the Arm and WE strains, respectively (2). The antibody selection was quite efficient, in particular with antibody 2.11.10, which has potent neutralizing activity against the Arm strain. None of the 12 progeny clones obtained after treatment with antibody 2.11.10 had the Arm/Arm genotype, 11 were WE/WE, and 1 was a reassortant and had the Arm/WE genotype.

The nucleoprotein and the glycoproteins of the WE and Arm strains can be distinguished by monoclonal antibodies (2). The reactivity of the reassortant viruses with strain-spe-

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cific monoclonal antibodies was checked to determine which LCMV genome segment(s) codes for the glycoproteins and the nucleoprotein. Monolayers of L-929 cells were infected with the two parental viruses (Arm/Arm and WE/WE) and the two reassortant viruses (Arm/WE and WE/Arm), and 48 h later the reactivity of the viral proteins was tested with a panel of monoclonal antibodies in a standard indirect immunofluorescence assay (3) (Table 2 and Fig. 2). Monoclonal antibody 1.1.3 is directed against NP and reacts with both the Arm and the WE strains (2). As expected, this antibody showed a positive reaction with both of the reassortant viruses. Similarly, monoclonal antibody 2WE-6, which is directed against GP-1 and recognizes a common determinant present in both the Arm and the WE strains, showed a positive reaction with the two reassortants. These two antibodies, 1.1.3 and 2WE-6, served as positive controls and showed that cells infected with all four viruses expressed the nucleoprotein and the glycoproteins. Monoclonal antibody WE-5-4 is specific for NP of the WE strain and does not react with the Arm strain; this antibody only stained cells infected with the Arm/WE reassortant and did not react with the WE/Arm reassortant, indicating that the S segment codes for NP. Monoclonal antibody WE-40, which is directed against GP-1 and is specific for the WE strain, showed a positive reaction with Arm/WE and did not stain cells

FIG. 1. Analysis of LCMV genotype by Northern hybridization with Arm strain-specific cDNA probes. Monolayers of BHK-21 cells (2×10^6 cells per flask) were infected with the indicated virus at multiplicity of infection of 0.2 PFU per cell, and total RNA was extracted from infected and control (noninfected) cells 40 to 44 h later. The cells were lysed with guanidinium thiocyanate, and total RNA was recovered after pelleting through cesium chloride (6). The RNA samples (50 μ g) were denatured with glyoxal, separated on the basis of size by electrophoresis in an agarose gel, and then transferred to a nitrocellulose filter. The filter was first hybridized with a 32 P-labeled cDNA probe specific for the L segment of Arm. After autoradiography, the L-specific probe was removed from the nitrocellulose filter by washing the filter under stringent conditions (80°C in $0.1 \times$ SSC [$1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.1% sodium dodecyl sulfate for 1 to 3 h). The same filter was then rehybridized with a 32 P-labeled cDNA probe specific for the GP-2 coding region (S segment) of Arm. We obtained equivalent hybridization results with probes from the Arm GP-1 coding region (data not shown). (A) L segment-specific cDNA probe. (B) S segment-specific cDNA probe. Lanes 1, RNA from cells infected with a recombinant, showing positive hybridization with the L probe but minimal hybridization with the S probe; Arm/WE genotype. Lanes 2, RNA from control (noninfected) cells. Lanes 3, RNA from Arm-infected cells, showing positive hybridization with both L- and S-specific probes; Arm/Arm genotype. Lanes 4, RNA from WE-infected cells, showing no hybridization with either probe; WE/WE genotype. Lanes 5, RNA from cells infected with a recombinant, showing minimal hybridization with the L probe but positive hybridization with the S probe; WE/Arm genotype. (The band designated x [visible in lanes 1 and 3 of panel A] is a subgenomic RNA species related to the L segment.)

TABLE 1. Summary of genetic crosses between the Arm and WE strains of LCMV

Expt	Monoclonal antibody treatment ^a	No. of progeny clones checked ^b	No. of clones with indicated genotype ^c				% of reassortants
			Arm/Arm	WE/WE	Arm/WE	WE/Arm	
1	None	29	9	18	0	2	6.8
2	WE-40	9	6	3	0	0	4.7
	2.11.10	12	0	11	1	0	

^a Culture fluids from dually infected BHK-21 cells (48 h postinfection) were treated with the indicated antibody before progeny clones were isolated. Monoclonal antibodies WE-40 and 2.11.10 are directed against GP-1 (2). WE-40 is specific for the WE strain and has neutralizing activity against WE but not Arm, whereas 2.11.10 specifically neutralizes Arm only.

^b Progeny clones derived from the coinfection were double plaque purified on Vero cell monolayers, and passage 1 stocks were made in BHK-21 cells.

^c The genotype of the LCMV progeny clones was determined by Northern transfer of viral RNA and hybridization with 32 P-labeled cDNA probes specific for the L and S segments of the Arm strain (17) (see Fig. 1). The following notation is used to designate the genotype: Arm/Arm, L segment of Arm/S segment of Arm; WE/WE, L segment of WE/S segment of WE; Arm/WE, L segment of Arm/S segment of WE; and WE/Arm, L segment of WE/S segment of Arm.

infected with WE/Arm. In contrast, monoclonal antibody 2.8.1, which is specific for GP of the Arm strain, reacted only with the WE/Arm reassortant and did not stain cells infected with the Arm/WE reassortant. The identities of viral polypeptides recognized by monoclonal antibodies 1.1.3, WE-40, and WE-5-4 were confirmed by immunoprecipitation and Western blotting analyses. Monoclonal antibody 2.8.1 bound to LCMV Arm glycoproteins expressed on the surfaces of infected cells, but, owing to its low affinity, did not immunoprecipitate either GP-1 or GP-2 (M. Buchmeier, unpublished data). These results show that the LCMV structural glycoproteins segregate with the S segment. Thus, studies with the strain-specific monoclonal antibodies and the reassortants have shown that both the nucleoprotein and the glycoproteins of LCMV are encoded by the S RNA segment. Recently, using molecular cloning, synthetic peptides, and antibodies to predetermined peptide sequences (12), we confirmed these observations (P. Southern, M. Buchmeier, and M. B. A. Oldstone, manuscript in preparation).

In the study reported here, we successfully obtained and characterized intertypic reassortants between the Arm and WE strains of LCMV. We derived the two genotypes

TABLE 2. Reactivity of the parental Arm and WE strains and the reassortant viruses with LCMV-specific monoclonal antibodies

Monoclonal antibody probe ^a	LCMV strain specificity	Polypeptide specificity	Reactivity with parental and reassortant LCMV ^b			
			Arm/Arm	WE/WE	Arm/WE	WE/Arm
1.1.3	Arm and WE	NP	+	+	+	+
2WE-6	Arm and WE	GP-1	+	+	+	+
WE-5-4	WE	NP	–	+	+	–
WE-40	WE	GP-1	–	+	+	–
2.8.1	Arm	GP-1	+	–	–	+

^a The generation and characterization of the LCMV-specific monoclonal antibodies used in this study have been described previously (2).

^b Reactivity was checked by indirect immunofluorescence staining of infected L-929 cells as previously described (3). An example of the staining is shown in Fig. 2.

FIG. 2. Indirect immunofluorescence staining of L-929 cells infected with the two parental viruses (WE/WE and Arm/Arm) and the two reassortant viruses (Arm/WE and WE/Arm). Monolayers of L-929 cells were infected with the indicated virus at a multiplicity of infection of 0.5 and checked by immunofluorescence for virus-specific proteins at 48 h postinfection. The acetone-fixed cells were stained, first with the monoclonal antibodies shown and second with rhodamine-conjugated goat antibody to mouse immunoglobulin G. Pictures were taken at equivalent exposures. The polypeptide and LCMV strain specificities of monoclonal antibodies 1.1.3, 2.8.1, WE-40, and WE-5.4 are shown in Table 2.

theoretically present, Arm/WE and WE/Arm, with a frequency of 4.7 to 6.8%, which would be expected for genetic reassortment with a segmented viral genome (14). We have shown that cDNA probes that distinguish between the RNAs of the Arm and WE strains can be used to determine the genotype of viruses obtained after dual infection. This is a convenient and rapid method for screening a large number of clones for recombinants. The generation of intertypic recombinants between the Arm and WE strains of LCMV was first reported by Kirk et al. (10) with dual wild-type crosses and by Romanowski and Bishop (16), whose dual-infection protocol involved heterologous temperature-sensitive mutants. However, only the WE/Arm reassortant was obtained in those experiments. Despite repeated attempts, these investigators were unable to generate the reassortant with the Arm/WE genotype. We were able to obtain this recombinant (Arm/WE) after antibody selection against the glycoproteins of Arm (i.e., the S segment of Arm). Thus, our results show that the Arm/WE genotype is certainly not a "forbidden" combination, although the frequency with which Arm/WE recombinants are isolated may be low. Finally, our studies show that the S genome segment codes for the three major LCMV structural proteins, NP, GP-1, and GP-2. Similar results have been reported for Pichinde virus (1, 7, 9, 13, 18), another member of the arenavirus group, suggesting that different arenaviruses may have similar gene coding strategies.

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LITERATURE CITED

1. Auperin, D. D., M. Galinski, and D. H. L. Bishop. 1984. The sequences of the N protein gene and intergenic region of the S RNA of Pichinde arenavirus. *Virology* 134:208-219.
2. Buchmeier, M. J. 1984. Antigenic and structural studies on the glycoproteins of lymphocytic choriomeningitis virus, p. 193-200. In R. W. Compans and D. H. L. Bishop (ed.), *Segmented negative strand viruses. Arenaviruses, bunyaviruses, and orthomyxoviruses*. Academic Press, Inc., New York.
3. 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* 113:73-85.
4. Buchmeier, M. J., and M. B. A. Oldstone. 1981. Molecular studies of LCM virus induced immunopathology: development and characterization of monoclonal antibodies to LCM virus, p. 71-77. In D. H. L. Bishop and R. W. Compans (ed.), *The replication of negative strand viruses*. Elsevier/North-Holland Publishing Co., New York.
5. Buchmeier, M. J., R. M. Welsh, F. J. Dutko, and M. B. A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* 30:275-331.
6. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299.
7. Compans, R. W., D. P. Boersma, P. Cash, J. P. M. Clerx, H. B. Gimenez, W. E. Kirk, C. J. Peters, A. C. Vezza, and D. H. L. Bishop. 1981. Molecular and genetic studies of Tacaribe, Pichinde and lymphocytic choriomeningitis viruses, p. 31-42. In D. H. L. Bishop and R. W. Compans (ed.), *The replication of negative strand viruses*. Elsevier/North-Holland Publishing Co., New York.
8. Dutko, F. J., and M. B. A. Oldstone. 1983. Genomic and biological variation among commonly used lymphocytic choriomeningitis virus strains. *J. Gen. Virol.* 64:1689-1698.
9. Harnish, D. G., K. Dimock, D. H. L. Bishop, and W. E. Rawls. 1983. Gene mapping in Pichinde virus: assignment of viral polypeptides to genomic L and S RNAs. *J. Virol.* 46:638-641.
10. Kirk, W. E., P. Cash, C. J. Peters, and D. H. L. Bishop. 1980. Formation and characterization of an intertypic lymphocytic choriomeningitis recombinant virus. *J. Gen. Virol.* 51:213-218.
11. Lehmann-Grube, F. 1971. Lymphocytic choriomeningitis virus. *Virology monographs*, vol. 10. Springer-Verlag, New York.
12. Lerner, R. 1982. Tapping the immunological repertoire to produce antibodies of predetermined specificity. *Nature (London)* 299:592-596.
13. Leung, W. C., D. Harnish, A. Ramsingh, K. Dimock, and W. E. Rawls. 1981. Gene mapping in Pichinde virus, p. 51-57. In D. H. L. Bishop and R. W. Compans (ed.), *The replication of negative strand viruses*. Elsevier/North-Holland Publishing Co., New York.
14. Palese, P. 1984. Reassortment continuum, p. 144-151. In A. L. Notkins and M. B. A. Oldstone (ed.), *Concepts in viral pathogenesis*. Springer-Verlag, New York.
15. Pedersen, I. R. 1979. Structural components and replication of arenavirus. *Adv. Virus Res.* 24:277-330.
16. Romanowski, V., and D. H. L. Bishop. 1983. The formation of arenaviruses that are genetically diploid. *Virology* 126:87-95.
17. Southern, P. J., and M. B. A. Oldstone. 1984. Studies on the molecular biology of lymphocytic choriomeningitis virus, p. 59-64. In R. W. Compans and D. H. L. Bishop (ed.), *Segmented negative strand viruses. Arenaviruses, bunyaviruses, and orthomyxoviruses*. Academic Press, Inc., New York.
18. Vezza, A. C., P. Cash, P. Jahrling, G. Eddy, and D. H. L. Bishop. 1980. Arenavirus recombination: the formation of recombinants between prototype Pichinde and Pichinde Munichique viruses and evidence that arenavirus S RNA codes for N polypeptide. *Virology* 106:250-260.