Defective Interfering Particles of Encephalomyocarditis Virus

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(Accepted 14 March 1983)

SUMMARY

Encephalomyocarditis (EMC) virus and five temperature-sensitive mutants of EMC virus were serially passed numerous times in HeLa, L929 and BHK-21 cells. Equilibrium centrifugation in CsCl density gradients of virus and electrophoresis of virus RNA from many of the high number passages indicated the presence of standard EMC virus and particles containing RNA of lower molecular weight. Three virus isolates with high passage number were able to interfere with the replication of the standard virions in mixed infections. We believe our results show the generation of defective interfering particles of EMC virus.

Defective interfering (DI) particles arise upon serial undiluted passage of most viruses in permissive cell lines (Holland et al., 1980; Perrault, 1981). Within the Picornaviridae, DI particles have been detected primarily in strains of poliovirus type I (Cole et al., 1971; Lundquist et al., 1979; McClure et al., 1980; Tershak, 1982) but have also been detected after many passages with mengovirus (McClure et al., 1980). This paper describes the isolation and partial characterization of DI particles of encephalomyocarditis (EMC) virus, a cardiovirus in the family Picornaviridae. Wild-type EMC virus and several of its temperature-sensitive (ts) mutants were used in our attempts to generate the DI particles in HeLa, L929 and BHK-21 cells.

Generation of DI particles was initiated by adding 0.1 ml of wild-type or cloned ts mutant EMC virus (Radloff, 1978), in crude lysate form, to confluent monolayers of HeLa, L929 or BHK-21 cells in 35 × 10 mm Lux plastic Petri dishes to give between 10 and 100 p.f.u./cell. Mouse L929 cells from O. Baca at the University of New Mexico were propagated in suspension in siliconized Florence flasks at 37 °C in Eagle's minimum essential medium supplemented with 0.1 mM non-essential amino acids and 5% (w/v) Pluronic F68. Newborn or normal calf serum was added to the complete medium to give 9.1% (v/v). BHK-21 cells from L. C. McLaren at the University of New Mexico were propagated as monolayers in Glasgow minimum essential medium (Gibco). Newborn calf serum was added to the Glasgow medium to give 9.1% (v/v). The virus was allowed to adsorb for 30 min at room temperature before addition of the appropriate medium. After a 15 to 24 h incubation at 33 °C, the infected cells were frozen and thawed three times to release virus and the lysates were clarified by low-speed centrifugation. Subsequent virus passages used 0.1 ml of undiluted lysate from the previous passage as the inoculum. For the generation of DI particles of EMC virus in HeLa cells or L929 cells, sufficient medium was prepared so that only one batch of the appropriate medium was used during the generation of DI particles in those cells. For the generation of DI particles in BHK-21 cells, the medium was from several different preparations.

EMC virus labelled with [3H]uridine was grown essentially as described above but [5,6-3H]uridine (25 to 50 Ci/mmol; ICN Chemical and Radiosotope Division) was added to give 15 μCi/ml at 3-75 h after addition of virus to cells. Virus was partially purified by sedimentation at 45 000 rev/min for 1-5 h at 4 °C in the Spinco SW 65Ti rotor in tubes containing a 0.5 ml cushion of 30% (w/v) sucrose in phosphate-buffered saline and 0.1% bovine serum albumin. The pellet was allowed to resuspend overnight at 4 °C in 1 ml 0.1 m NaCl, 0.001 m EDTA, 0.025 m HEPES (pH 7.5), and 0.1% bovine serum albumin. Q β virus labelled with [5,6-3H]uridine was prepared.
Short communication

Fig. 1. Agarose gel electrophoresis of RNA from wild-type (wt) EMC virus and five ts mutants passed in three lines of cultured cells. (a) Virus passed in HeLa cells 41 times (ts 55a), 43 times (wt), 50 times (ts 1, ts 2 and ts 33b), or 51 times (ts 68). (b) Virus passed in L929 cells 62 times (ts 55a and wt) or 68 times (ts 1, ts 2, ts 33b and ts 68). (c) Virus passed in BHK-21 cells 61 times (wt 1), 50 times (wt 2), 62 times (ts 1 and ts 2), and 64 times (ts 33b, ts 55a and ts 68). Standard EMC virus and Qβ virus RNAs were used as markers. The RNAs were subjected to electrophoresis at room temperature for 5 h at 30 mA and about 5 V/cm. The origin (O) is shown at the top.

Wild-type EMC virus and five ts mutants were passed numerous times at 33 °C in HeLa, L929 and BHK-21 cells. The virus in selected passages was labelled with [3H]uridine and the virus RNA from partially purified virus was analysed by agarose gel electrophoresis after glyoxal denaturation. In these experiments, Qβ and EMC virus RNAs were used as markers. However, we also performed electrophoresis experiments including, in addition, poliovirus RNA and HeLa cell 18S and 28S ribosomal RNAs, and confirmed that the migration of all these markers was linear with the logarithm of their mol. wt. This linear relationship allowed us to determine the mol. wt. for each EMC virus DI particle RNA and the size of the deletion in each, relative to EMC wild-type virus RNA.

The electrophoretic profile of RNA isolated from wild-type EMC virus and five ts mutants after numerous passages of virus in HeLa cells is presented in Fig. 1 (a). Mutant ts 1 contained at least two virus RNAs of higher mobility, and therefore lower mol. wt., than standard EMC virus RNA. Mutants ts 2, ts 55a and ts 68 each contained one extra virus RNA, and wild-type virus.
contained none. We were unable to determine whether mutant \( ts \) 33b contained a smaller virus RNA, but, if so, it was very close to the band of standard RNA and was a small fraction of the total RNA in the sample. The calculated deletions for the RNA of EMC virus DI particles generated in HeLa cells were \( 6.9(\pm 0.8)\% \) and \( 10.3(\pm 0.9)\% \) (\( ts \) 1), \( 12.0(\pm 0.8)\% \) (\( ts \) 2), \( 3.7(\pm 1.0)\% \) (\( ts \) 55a), and \( 5.7(\pm 1.2)\% \) (\( ts \) 68).

Wild-type EMC virus and the same set of \( ts \) mutants passed in HeLa cells were also independently passed numerous times in L929 cells. Virus RNA from mutant \( ts \) 1 contained at least two faster migrating virus RNAs (Fig. 1 b). Sometimes the faster migrating RNA in mutant \( ts \) 33b was also resolved into at least two bands. The \( ts \) 2, \( ts \) 55a, \( ts \) 68 and wild-type preparations each contained one faster migrating virus RNA. The calculated deletions for the RNA of EMC virus DI particles passed in L929 cells were \( 7.5(\pm 0.4)\% \) and \( 9.9(\pm 1)\% \) (\( ts \) 1), \( 13.6(\pm 1.5)\% \) (\( ts \) 2), \( 14.8(\pm 1.4)\% \) (\( ts \) 33b), \( 8.9(\pm 1.5)\% \) (\( ts \) 55a), \( 13.5(\pm 1.5)\% \) (\( ts \) 68), and \( 11.4(\pm 1.4)\% \) (wild-type).

The same set of viruses was also passed in BHK-21 cells. One additional virus, wild-type EMC virus initially irradiated with a u.v. germicidal lamp to reduce the virus titre by 70\% (designated wt 2), was also passed in BHK-21 cells. The virus from these preparations was monitored for the presence of RNA smaller than wild-type RNA by agarose gel electrophoresis. In contrast to the relatively sharp bands of the faster migrating RNA seen in Fig. 1 (a, b), the faster migrating RNAs generated in BHK-21 cells formed bands which in most cases were wider and relatively indistinct (Fig. 1 c). This was reproducible from gel to gel and indicates that heterogeneous populations of DI particle RNAs were generated during passage of the viruses in BHK-21 cells. The faster migrating RNAs generated in BHK-21 cells differ in mol. wt. from the standard virion RNA by about 6\% to 15\%.

We have retained the wild-type and \( ts \) mutant designations for those samples that were passed in the three lines of cultured cells. During the sequential passages, mutant \( ts \) 1 retained its \( ts \) nature in HeLa cells and in BHK-21 cells, mutant \( ts \) 55a retained its \( ts \) characteristic in L929 cells, and mutants \( ts \) 2, \( ts \) 55a and \( ts \) 68 partly lost their \( ts \) nature with p.f.u.\( ^{35}S \) oc/p.f.u.\( ^{3}H \) ratios of \( 3 \times 10^{-2} \) (50 passages), \( 1 \times 10^{-2} \) (41 passages) and \( 8 \times 10^{-3} \) (51 passages), respectively, in HeLa cells. All other \( ts \) mutants passed in the three cell lines lost their \( ts \) characteristic after 30 passages or fewer. With some mutants, loss of the phenotype preceded the appearance of DI particles during serial passage of the virus whereas, with other mutants, DI particles appeared before any loss of the \( ts \) characteristic. In general, there is no indication that the presence of the \( ts \) mutations influenced the generation of DI particles. However, it is possible that the generation of specific DI particle species was influenced by the presence of a \( ts \) lesion in the viral genome.

We wished to determine whether DI particles generated in one cell line could be grown in the other two cell lines. Therefore, DI particles generated in each of the three lines were used to infect cells of the other two lines. All combinations of the three cell lines and DI particle samples were tried. We found that DI particles generated in each cell line were propagated for at least one growth cycle in the other cell lines.

We attempted to detect the presence of DI particles of EMC virus by separation of the DI particles from the standard virus in equilibrium density gradients of CsCl in the Spinco SW 65Ti rotor. Fig. 2 presents the distribution of \( [\text{H}] \)uridine-labelled virus in such gradients for mutant \( ts \) 68 passed in HeLa cells, mutant \( ts \) 2 passed in L929 cells and mutant \( ts \) 55a passed in BHK-21 cells. Other virus samples, represented by the corresponding RNA samples shown in Fig. 1, gave similar patterns depending upon the extent of the RNA deletion in the DI particles. Only mutant \( ts \) 2 DI particles were obviously separable from the standard virus in these experiments (Fig. 2 b). To show that the less dense peak in Fig. 2 (b) contained DI particles, we subjected partially purified mutant \( ts \) 2 virus to two successive centrifugations in CsCl equilibrium density gradients in the Spinco Type 50Ti angle head rotor (Flamm et al., 1966) rather than in the swinging bucket rotor. This gave better resolution of the two peaks. The particles in the less-dense peak contained RNA similar in size to the faster RNA shown in the \( ts \) 2 lane in Fig. 1 (b), whereas the particles with the density of standard virus contained standard-size virus RNA. We were unable to obtain a clear separation of the standard virus from the virus particles with
any of the virus samples in a single CsCl equilibrium density gradient in the Spinco SW 65Ti rotor.

Lower infectivity titres in some of the higher numbered passages of EMC ts mutants and wild-type virus in HeLa and L929 cells were observed relative to the lower numbered passages. These decreases in titre were never greater than a factor of 10 and were usually less. However, we felt the changes in titre might be due to interference in the growth of standard virus by DI particles. Because the DI particles were not easily separable from infective virus in many of our preparations, the method of McClure et al. (1980) was used to determine whether interference with virus replication occurred in mixed infections of high- and low-passage virus. In these experiments, monolayers of HeLa or L929 cells were infected with a mixture of virus from a high passage number and from the first passage, each at a multiplicity of 10. In addition, separate monolayers were infected with virus from the first passage or with virus from the high passage. Virus titres from all three cultures were determined by plaque assay. In several cases, the virus titres from DI particle stocks were not high enough to give 10 p.f.u./cell in the test cultures. In these cases, the highest possible multiplicity of infection was used. Only DI particles of mutant ts 1 virus generated in HeLa or L929 cells interfered with the replication of low passage virus. The interference observed in these experiments reduced the titre of virus in the mixed infections by 50% to 80% relative to that in single infections with only low passage or wild-type standard virus. The interference shown in these experiments was obviously weak but is comparable to that shown by McClure et al. (1980) for mengovirus. The isolates capable of interference had deletions of about 10% of the viral genome. These particles were probably both interfering and defective, assuming that all virus-coded proteins are essential and that about 90% of the RNA of picornaviruses codes for protein (Kitamura et al., 1981).

We believe our work indicates that the host cells influenced the generation of EMC virus DI particles, consistent with the results from other virus systems (Holland et al., 1980; Perrault, 1981). To summarize our evidence: (i) DI particles were generated in BHK-21 cells with all ts mutant and wild-type viruses used. The RNA present in these particles was heterogeneous relative to standard virus RNA and to the RNA of the DI particles generated in HeLa and L929 cells. (ii) DI particles were also generated in L929 cells with all ts mutant and wild-type viruses. The RNA present in these particles formed relatively sharp bands in agarose gels and only in the mutant ts 1 isolate was more than one RNA band from DI particles readily resolved. (iii)
Although DI particles were generated in HeLa cells, they were not generated with wild-type EMC virus and probably not with mutant ts 33b. The RNA of the DI particles generated in HeLa cells formed relatively sharp bands, but the deletions were not as large as in the RNA from the DI particles generated in L929 cells or BHK-21 cells.

We thank R. Tufaro and L. Brinson for their technical assistance and patience and C. Cords for helpful discussions concerning the manuscript. This research was supported by Public Health Service grant AI 15821 from the National Institute of Allergy and Infectious Disease.

REFERENCES


(Received 23 November 1982)