Biochemical Composition of Lymphocytic Choriomeningitis Virus Interfering Particles

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SUMMARY

Lymphocytic choriomeningitis (LCM) virus interfering particles were enriched relative to infectious virions by ultracentrifugation in a shallow gradient made of Urografin. Electrophoretic analysis revealed that they lacked the small (‘S’) 23S RNA as well as GP-1 and GP-2 of the infectious virion and also lacked a newly characterized glycoprotein of apparent mol. wt. 85 × 10^3; instead, they contained a novel glycoprotein with mol. wt. 65 × 10^3.

Persistent infection of the mouse with lymphocytic choriomeningitis (LCM) virus appears to be a useful model for studying the mechanisms by which a foreign agent and its host may coexist for prolonged periods of time (Traub, 1939; Lehmann-Grube et al., 1981). One feature of the LCM virus carrier mouse is the lifelong equilibrium between loss of virus due to excretion and natural decay on the one hand, and replenishment by newly synthesized virus on the other. Replication of LCM virus in acutely infected cell cultures (Welsh & Pfau, 1972) and mice (Popescu & Lehmann-Grube, 1977) is accompanied by replication of a class of particles which are non-infectious but have the ability to interfere with infectious virus. Possibly it is these interfering particles (IP) which exert a feedback control on the multiplication of infectious virus in carrier mice. Unlike typical defective-interfering (DI) particles (Huang & Baltimore, 1977), LCM virus IP replicate in parallel with infectious standard virus rather than through its help (Lehmann-Grube et al., 1981). We report here that IP differ from infectious virus by lacking the 23S RNA and also the two major structural glycoproteins GP-1 and GP-2 as well as a newly characterized glycoprotein of apparent mol. wt. 85 × 10^3; instead, they appear to contain a novel GP with mol. wt. 65 × 10^3. These findings further separate LCM virus IP from characteristic DI particles which usually have the same protein composition as their standard virus (Huang & Baltimore, 1977).

The WE strain of LCM virus (Rivers & Scott, 1936) was used after it had been triple plaque-purified (Popescu & Lehmann-Grube, 1976). It was propagated and assayed in L cells, infectious virus as plaque-forming units (p.f.u.) (Lehmann-Grube & Ambrassat, 1977) and IP as interference focus-forming units (IFU) (Popescu et al., 1976). The plaque assay underestimates the number of infectious units (IU) as determined by the more sensitive mouse assay by a factor of 10. Similarly, the numbers of IFU are, on average, 100 times lower than the total numbers of IP as estimated on the basis of a yield reduction assay. Thus, to allow comparative evaluation, p.f.u. were converted to IU by multiplying the former by 10 and IFU to IP by multiplying the former by 100.

In our experience, every LCM virus preparation is a mixture of IU and IP, their ratio (IU/IP) depending on the conditions under which the virus had been produced. During its exponential growth the ratio is high but decreases when the plateau phase is reached. The ratio also depends on the multiplicity of infection (m.o.i.), being high when the latter is low and low when the m.o.i. is high. Thus, for obtaining preparations relatively rich in IU, virus was harvested from cultures 44 h after their infection with low m.o.i. (0·01 p.f.u./cell), while material relatively rich in IP was obtained after one multiplication cycle (16 h) from cultures infected with a high m.o.i. (10 p.f.u./cell).
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Fig. 1. Enrichment of LCM virus interfering particles by ultracentrifugation. Cultivated L cells were infected with LCM virus at an m.o.i. of 10 and were maintained at 37 °C under a medium containing \([3H]\)uridine. After 16 h the culture fluid was cleared by low-speed centrifugation and subsequently ultracentrifuged in a Beckman SW27 rotor at 250,000 rev/min for 3 h. The pelleted virus was resuspended, layered on top of a continuous 0 to 40% Urografin (sodium and methylglucamin diatrizoate; Schering AG, Berlin, Germany) gradient (Gschwender et al., 1975) and centrifuged to equilibrium in an SW50 rotor at 38,000 rev/min for 18 h. The radioactive material was collected, adjusted to 26.5% Urografin and centrifuged again in an SW50 rotor as before. Fractions were collected from the bottom of the tube and their biological as well as radiological activities were measured. The density was determined with a refractometer. ○, IU; ■, IP; □, density; △, radioactivity.

Virus RNA, proteins and glycoproteins were labelled by exposing infected cells for 24 h (low m.o.i.) and 16 h (high m.o.i.) to 30 μCi/ml of respectively, \([5,6-\text{H}]\)uridine, \(L-\text{[35S]}\)methionine in an otherwise methionine-free culture medium, and \(\text{D-[1-3H]}\)glucosamine hydrochloride in a medium containing fructose instead of glucose. All radioactive chemicals were purchased from Amersham/Buchler GmbH, Braunschweig, F.R.G. Virus RNA was prepared according to Spaan et al. (1981) and McMaster & Carmichael (1977). Labelled virus was disrupted by use of TES buffer containing 10 mg/ml SDS and 0.4 mg/ml proteinase K (Merck, Darmstadt, F.R.G.). RNA was extracted with phenol, precipitated with ethanol and denatured with glyoxal and dimethyl sulphoxide. Electrophoresis was in a horizontal 1% agarose gel at 150 V for 8 h at 5 °C. The gel was fixed with methanol and treated with EN3HANCE (New England Nuclear). Markers were ribosomal 18S and 28S RNAs from uninfected L cells and 42S RNA from Sindbis virus.

For the separation of structural proteins and the identification of structural glycoproteins, labelled virus was disrupted by heating for 1 min at 100 °C in the presence of 2% SDS and 5% 2-mercaptoethanol. Analytical polyacrylamide gel electrophoresis was performed essentially as described by Laemmli (1970) using slab gels prepared from a 7.5 to 20% acrylamide gradient with a 3% stacking gel and applying 18 mA for 6 h. Radioactive bands were visualized by fluorography (Bonner & Laskey, 1974). We used the [14C] methylated protein mixture from Amersham/Buchler as markers.
LCM virus IU and IP are only slightly different with respect to both size and density (Gschwender & Popescu, 1976; Pedersen, 1979) which made their separation difficult. The results were greatly improved by centrifuging the virus in 26.5% Urografin. As Fig. 1 shows, under these conditions an extremely flat gradient formed which led to a substantial enrichment of both entities. Attempts to achieve a more complete partition by re-centrifugation or by altering the conditions were not successful. Since the degree of mutual contamination varied between experiments, the biological activities of the fractions were always determined in parallel and the relative proportions of IP are presented with the data.

In Fig. 2(a) an example of the electrophoretic migration pattern of LCM virus RNA derived from IU and IP is presented. Lane 1 shows the classes of RNA as they have been described by others (Pedersen, 1979). In addition to ribosomal RNAs, there is a large component (‘L’) migrating with mol. wt. 2.6 × 10^6 and a small one (‘S’) migrating with mol. wt. 1.3 × 10^6, also known as 31S RNA and 23S RNA respectively. It should be noted that S is more abundant than L. Essentially the same pattern is seen in lane 2 (enriched for IU), although the relative preponderance of S is even more obvious. In striking contrast, in lane 3 (enriched for IP) S is all but absent, which is the more relevant when considering its dominance in preparations rich in IU.

Fig. 2 (b) illustrates the pattern of LCM virus proteins, lanes 1 and 2 containing the well known bands corresponding to ‘NP’, ‘GP-1’ and ‘GP-2’ with mol. wt. 63 × 10^3, 44 × 10^3 and 35 × 10^3 respectively (Buchmeier et al., 1978; Buchmeier & Oldstone, 1979). In addition, there is a high mol. wt. protein of approx. 200 × 10^3, not previously described for LCM virus, although it has been described for the arenavirus Pichinde virus (Harnish et al., 1981). In lane 3 (enriched for IP) NP is present and a trace of GP-2, but not GP-1, can be detected. LCM viral glycoproteins are depicted in Fig. 2(c). In lanes 1 and 2, GP-1 and GP-2 are well delineated as well as a new glycoprotein with an approx. mol. wt. of 85 × 10^3. As it is
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precipitated by anti-LCM virus antiserum, we assume that it represents a structural component of the virion. In lane 3 (enriched for IP) all glycoproteins are present in very low concentrations, and a new band has appeared with mol. wt. of about $65 \times 10^3$. All samples also contain a large glycosylated component with mol. wt. of about $230 \times 10^3$ which cannot be detected in the methionine-labelled material; whether or not it is viral has yet to be determined.

For evaluation of these observations it is mandatory to keep in mind that our procedure only enriches for IU and IP, meaning that they are still mutually contaminated. Taking this into account, we assume that the trace of 23S RNA (see Fig. 2a, lane 3) as well as the low concentrations of GP-1 and GP-2 (see Fig. 2c, lane 3) stem from residual IU. If this assumption is accepted, we propose that IP lack the S RNA of infectious virus and also GP-1 and the newly identified GP with mol. wt. $85 \times 10^3$; GP-2 is either not glycosylated or is also absent. Thus, the major structural proteins of IP appear to be NP, possibly a non-glycosylated GP-2 and a glycoprotein of mol. wt. $65 \times 10^3$.

The only previous work on LCM virus IP of which we are aware is one preliminary experiment of Pedersen (1979) who achieved separation by sucrose gradient centrifugation. IP were said to contain only one RNA which migrated slightly faster than virion L RNA. Welsh et al. (1975) and Welsh & Buchmeier (1979) have analysed defective-interfering LCM virus produced free of infectious virus by persistently infected cell lines. They found no qualitative differences either in RNA classes or in polypeptide patterns between these particles and infectious virions, which is in marked contrast to our findings for IP released (together with infectious virus) during acute infection of cells. It has previously been argued that both these interfering entities should conceptually be kept apart (Lehmann-Grube, 1981); it now appears that they are indeed different.

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