High Molecular Weight RNA in a Murine Leukaemia Virus Helper-independent Strain of Moloney Sarcoma Virus

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SUMMARY

A murine leukaemia virus helper-independent strain of Moloney sarcoma virus (MSV), designated S+L-, was isolated from cells transformed by MSV in the absence of replicating leukaemia virus. The virus is non-infectious in tissue culture as evidenced by its failure to replicate in a variety of murine cell lines.

Sucrose gradient and gel electrophoretic analyses of the purified S+L- isolate showed that the virus contains 60 to 70 S RNA. The virus also possesses RNA-dependent DNA polymerase (RDDP) activity.

On the basis of these data it would appear that factors other than high mol. wt. RNA and RDDP may account for the lack of infectivity of this strain of MSV.

For reasons which are not as yet clearly understood, murine sarcoma viruses (MSV) have been shown to require the assistance of murine leukaemia viruses (MLV) for their replication in cell cultures (Hartley & Rowe, 1966; Bassinet al. 1968; O'Conner & Fischinger, 1968). Such dual infections by MSV and MLV of susceptible mouse cells result in easily detectable cell transformation and new growth of MSV and MLV as well as conferring upon the sarcoma virus the envelope specificity of the leukaemia virus helper (Hartley & Rowe, 1966; Bassin et al. 1968; O'Conner & Fischinger, 1968).

Certain cell types infected with and containing a rescuable MSV genome in the absence of detectable MLV are termed 'sarcoma positive, leukaemia negative (S+L-)' (Bassin, Tuttle & Fischinger, 1970). Bassin et al. (1971a) described the presence of a type C virus particle in clone C-243-3 from 3T3 (S+L-) mouse cells transformed by the Moloney strain of MSV. The virus, referred to as S+L-, incorporates uridine into nucleic acid and bands at a density of 1.16 g/ml in sucrose. This virus, however, is not infectious under a wide variety of experimental conditions, involving several different cell lines. Additional studies by these investigators (Bassin et al. 1971b) suggest that this S+L- virus has low levels of RNA-dependent DNA polymerase activity and lacks high mol. wt. ribonucleic acid (HMW-RNA). Ribonucleic acid species of 4, 18 and 28 S were, however, found to be present in the S+L- virus. They further report that competent MSV issuing from 3T3 (S+L-) transformed cells is capable of generating new transformed isolates of the S+L- phenotype, all of which produce non-infectious S+L- virus particles in the absence of MLV helper. They interpret these results to mean that the non-infectious S+L- virus particles produced by S+L- cells are products of a defective MSV genome and do not represent a form of defective leukaemia virus.

Fischinger, Schafer & Seifert (1972) reported on the antigenic analysis of non-infectious S+L- virus particles issuing from a 3T3 (S+L-) culture derived from cells transformed only by MSV (Moloney isolate). Their S+L- virus exhibited a reduced number of murine leukaemia virus group-specific antigens, and indirect evidence suggested that the envelope antigens of S+L- virus might be quite different from that of standard laboratory strains of MLV.
Thus, it is only a working hypothesis that S + L− virus is a defective sarcoma virus. For convenience, therefore, we shall refer throughout the remainder of this communication to the S + L− virus as a strain of MSV and designate it as MSV (S + L−).

We recently examined clone D-56 cells, derived from the original 3T3 (S + L−) cell line from which clone C-243-3 was derived (Bassinet al. 1970) and found that it releases a strain of MSV (S + L−) in the absence of MLV helper. Since this virus appears to be non-infectious in tissue culture, we undertook a study of its nucleic acid to determine if it also lacked a HMW-RNA component.

We first investigated the biological activity of the virus by examining its ability to replicate in cell culture. The MSV-containing 3T3 (S + L−) cell culture (clone D-56) was obtained through the courtesy of Dr R. H. Bassin (NCI, Bethesda, Maryland). This culture was derived from cells transformed by MSV (Moloney isolate) in the absence of replicating leukaemia virus and is used routinely in our laboratory for titration of MLV. The culture was grown in RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum. Preliminary observations suggested that this virus was defective since it failed to rescue the MSV genome. However, experiments to test its replication were conducted as follows:

MSV (S + L−)-containing growth medium was inoculated on to an exponentially growing NIH/Swiss mouse embryo fibroblast culture which serves as target cells for MSV. Exposure of the cells to MSV (S + L−) was carried out on 3 successive days. Throughout a period of 4 weeks and the same number of serial transfers, the cultures failed to show any visible signs of cellular alterations. Cell pellets of the cultures were then submitted for thin-sectioning and examination by electron microscopy. The MSV (S + L−)-treated cultures were negative for the presence of virus. However, the control cultures, which had been treated with RLV, showed budding virus. Our results on the lack of infectivity of this virus were confirmed by R. H. Bassin (personal communication), who found that the virus was non-infectious for a variety of murine cell lines tested, including suitable target cells of BALB/3T3 origin, even after being co-sedimented with MLV.

We then proceeded to analyse the nucleic acid composition of MSV (S + L−). The culture was grown in 32 oz prescription bottles in RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum. The virus was labelled with [5,6-3H]-uridine, harvested and purified in the manner previously described for oncornaviruses (Naso et al. 1972; Vidrine, Wang & Arlinghaus, 1972). The virus RNA was extracted with sodium dodecyl sulphate (SDS)-phenol at 20 °C (Vidrine et al. 1972). The aqueous suspension was precipitated with 95% ethanol, and the precipitate reconstituted in Na-acetate (0.1 M, pH 5.3) containing SDS (0.1%). The RNA was then stored at −95 °C until ready for use.

Sucrose gradient analyses of MSV-RNA consistently revealed the presence of 4, 18 and 28 S species of RNA. However, the presence of 60 to 70 S RNA was detected at low levels only in some preparations. This inconsistency in demonstrating HMW-RNA in all our preparations prompted us to examine the RNA extraction procedure by performing the following experiment.

The 3T3 (S + L−) cell culture was superinfected with Rauscher leukaemia virus (RLV) and carried for a 3-week period with weekly serial transfers. The culture was then labelled with 2 to 4 µCi/ml of [5,6-3H]-uridine (sp. act. 36-8 Ci/m-mol; New England Nuclear). The labelled virus synthesized by the newly infected culture was then purified and its RNA was extracted (Vidrine et al. 1972) and reconstituted in Na-acetate containing SDS (see above). Since RLV contains 60 to 70 S RNA, one would expect this species of RNA to be present in the virus synthesized by the newly infected 3T3 (S + L−) culture. Sucrose gradient analyses of the RNA from these super-infected cultures consistently showed HMW-RNA.
Fig. 1. Acrylamide-agarose electrophoresis of MSV (S+L-)−RNA and RLV-RNA. [PH]-uridine-labelled RNA, prepared as described in the text, was layered on to acrylamide-agarose gels (1.5 % acrylamide, 0.5 % agarose, 0.1 % SDS) and electrophoresed for 30 min at 150 V, 10 mA/gel. Two mm sections of the gels were fractionated and dissolved in 5 ml of Toluene-Omnifluor/NCS scintillation fluid (as 4 g Omnifluor, 100 ml NCS [Amersham-Searle] and 80 ml of 4M-ammonium hydroxide per l of Toluene). (A) Untreated MSV-RNA (●—●); MSV-RNA in 0.2 M-sodium hydroxide, heated at 85 °C for 20 min, rapidly cooled and neutralized with 3 M-sodium phosphate (○—○). (B) Untreated RLV-RNA (●—●); alkali-treated RLV-RNA (○—○).

We next examined our preparation of RNA extracted from MSV (S+L−) on agarose-acrylamide gels using the method of K. R. Harewood and J. S. Wolff, III (personal communication). This method provides excellent resolution of the virus HMW-RNA. With the enhanced sensitivity afforded by this method, we were able to demonstrate the presence of 60 to 70 S RNA in all of our preparations of MSV (S+L−)-RNA. Fig. 1A shows a profile of MSV (S+L−)-RNA analysed by gel electrophoresis. RLV-RNA extracted from virus passaged in JLS-V5 cells (Wright & Lasfarques, 1965) is shown as a control in Fig. 1B. All of the radioactive material is completely sensitive to alkaline treatment, indicating that the material is RNA and not DNA (Fig. 1A, B).

The virus described in this communication is apparently similar to the S+L− viruses described by Bassinet et al. (1971a, b) and Fischinger et al. (1972). There appear to be some differences however: our strain of MSV (S+L−) contains HMW-RNA whereas the MSV (S+L−) strain issuing from clone C-243-3 (Bassinet et al. 1971a, b) apparently does not. Moreover, our strain of MSV (S+L−) has an RNA-dependent DNA polymerase activity which is 5- to 10-fold greater than that observed for the virus issuing from clone C-243-3 (R. H. Bassin, personal communication).

To our knowledge this work represents the first demonstration of HMW-RNA in a murine leukaemia virus helper-independent strain of MSV issuing from murine cells. It should be mentioned that Gazdar et al. (1971) have demonstrated HMW-RNA in a helper-independent strain of MSV (Moloney isolate) released from MSV-transformed hamster cells. However, their strain of MSV differs from ours in that it possesses transforming ability after being co-sedimented with murine leukaemia virus, whereas our strain of MSV (S+L−) remains non-infectious when treated in a similar manner.

It would appear, therefore, that factors other than HMW-RNA and RNA-dependent DNA-polymerase may account for the lack of infectivity of this strain of MSV.
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