

Rescue *in Vivo* in Adult Mice of Murine Sarcoma Virus (MSV) from a Nonproducer Rat Neoplasm (34019)

L. W. LAW AND R. C. TING

National Cancer Institute, Bethesda, Maryland 20014

Certain preparations of the Moloney strain of murine sarcoma virus (MSV) are known to behave as a defective virus. Focus-forming particles require "helper" viruses for their replication *in vitro* (1). The defective genome of MSV is known to exist in certain infectious virus-free hamster (2), rat (3), and mouse neoplasms (4) from which fully infectious MSV can be "rescued." Rescue is achieved if neoplastic cells carrying the defective genome are grown in contact with mouse cells in mixed tissue cultures or by inoculation into newborn mice in the presence of leukemogenic "helper" virus. Although the fully infectious pseudotype viruses recovered in this manner have the envelope antigens of the helper virus and may have restricted host range in tumorigenic capacities, the pathogenic properties of the virus pseudotypes appear to be unaffected. Thus, all of the pseudotype MSVs tested in our laboratory (5, 6) induce in mice infected during the neonatal period or in mice rendered immunologically deficient, lesions classified as atypical granulomas. Such tumors do not usually grow progressively upon transplantation to adult mice of the syngeneic strain. MSV does, however, induce several morphologic forms of simple autonomous neoplasms in inbred strains of rats (7).

In this report we describe rescue of fully infectious MSV by inoculation of cells from the MSB-1 neoplasm of a BN strain rat into adult BALB/c mice previously infected with Moloney leukemogenic virus (MLV). The BALB/c mice were rendered immunologically deficient through surgical removal of the thymus at 3 days of age.

Materials and Methods. MLV, the murine leukemogenic strain of virus used in this study, was obtained from Melpar, Inc. It was prepared from BALB/c passaged virus. This 1 g/ml concentrate was found negative prior to use for focus-forming ability on

mouse embryo cells and for its capacity to produce tumors after intramuscular inoculation in newborn BALB/c mice. MLV was inoculated intraperitoneally at 10^{-1} dilution in 0.1-ml volume into 3-week-old BALB/c recipients.

MSB-1 cells (3) passaged in tissue culture were inoculated intramuscularly after dispersion with trypsin into BALB/c mice 7 weeks of age, 4 weeks after infection with MLV. In one experiment 3×10^5 cells/mouse and in the second, 1.4×10^5 cells/mouse in 0.1-ml volume were used. (Table I). The epithelial neoplasm MSB-1 was induced by MSV in a thymectomized BN strain rat. A variety of tests were used, unsuccessfully, in attempts to demonstrate infectious MSV in these neoplastic cells. Thus, extracts of MSB-1 tumor and cultured cells consistently failed to induce tumors in newborn mice and did not induce foci on mouse embryo cells; nor did MSB-1 cells react in the CF test with broadly reactive sera of rats immunized with leukemogenic virus. MSB-1 neoplastic cells, in contrast to HT-1 cells of the hamster (2), nevertheless contain particles resembling type C virus; the serologic and biologic properties of these particles MSV(O) in MSB-1 cells have been described (8).

BALB/c An mice obtained from our inbred colony were used throughout for rescue attempts. Thymectomy was performed at 3 days of age and intact littermates served as controls. In tests for infectivity of the tumorous preparations, 3-day-old NIH Swiss mice were used.

To test for recovery of infectious MSV, tumorous growths were removed at various times from BALB/c recipients, homogenized in a Ten-Broeck homogenizer with medium 199 and centrifuged at 3000 rpm for 10 min. A 20% extract (w/v), or in several cases filtrates obtained by passage through a 0.45- μ Millipore filter, were injected intramuscular-

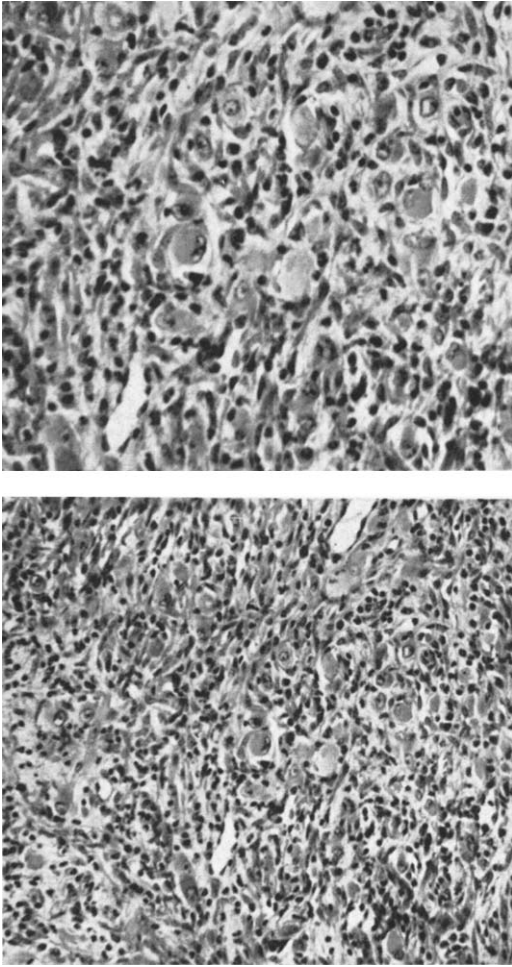


FIG. 1. Representative section through tumor (atypical granuloma) in BALB/c mouse, thymectomized at 3 days, infected with MLV at 21 days, and inoculated intramuscularly at 49 days with "non-producer" rat MSB-1 cells. Tumor appeared 81 days after MSB-1 inoculation and grew to a size of 40×20 mm at sacrifice. Above, $\times 225$; below, $\times 155$.

ly in 0.05-ml amounts into 1- to 3-day-old NIH Swiss mice.

Several pieces of each tumor mass in BALB/c and of NIH Swiss, recipients of extracts of primary and subsequent passages, were fixed in Tellysniczky's fluid and stained with hematoxylin and eosin. Spleen, liver, and lymph nodes were similarly prepared.

Results. Tumors in the muscles of thymec-

tomized BALB/c mice inoculated at 3 weeks of age with MLV and at 7 weeks with MSB-1 cells appeared as early as 23 days and as late as 85 days after inoculation of the virus-free neoplastic cells. No growths were observed in those thymectomized mice receiving MLV alone nor in those receiving the neoplastic rat cells alone; all intact mice also remained tumor-free (See Table I). Two tumors regressed, two mice with tumors were sacrificed at 40 days and 3 at 50 days after palpable intramuscular growths in order to prepare extracts; two died at 65 and 144 days respectively with progressively growing tumors and the remaining eight were sacrificed $3\frac{1}{2}$ months after tumor appearance. At this time the average size of the intramuscular growth was $40 \times 25 \times 20$ mm. Histopathologic study of several areas of each growth revealed these growths to be atypical granulomas as previously described (6). (See Fig. 1.) There exists a complex structure of a mixture of inflammatory and granulation components; lesions obtained after considerable growth in the primary BALB/c hosts were organized, irregular masses of granulation tissue. There were observed within most growths also a small number of atypical mesenchymal cells that probably represent the neoplastic component. Tumors were restricted for the most part to the muscle with only slight extensions into surrounding subcutaneous connective tissue. In several mice similar small lesions were observed in the spleen. We have not observed induced growths typical of the fibrosarcomas or rhabdomyosarcomas arising spontaneously in inbred strains of mice. Three separate tumors, taken at 40, 50, and 195 days after first palpation, failed to grow progressively in syngeneic 6 week old BALB/c recipients.

Extracts made from the tumors arising in BALB/c mice consistently contained infectious MSV and represented rescued virus as determined by intramuscular inoculations of the clarified material into NIH Swiss neonates (See Table II). The tumor dose 50 (TD 50) titer of a second-passaged extract was relatively high ($> 10^4$). The tumors induced in NIH Swiss mice upon reinoculation

TABLE I. Rescue of Infectious MSV in Adult BALB/c Mice from Rat Neoplasm MSB-1. MLV Infection at 3 Weeks; Neoplastic Cells Transferred at 7 Weeks.

Group	No. with tumors/total no. ^a		Latent period (days) ^b	Remarks
Thymectomized	(a)	(b)		
MLV + MSB-1 cells	9/17		43.7 (23-85)	Extracts of tumors (+) in test mice (see Table II)
MLV + MSB-1 cells		6/8	57.0 (33-68)	
MLV	0/6	0/6	—	
MSB-1 cells	0/7	—	—	
Intact				
MLS + MSB-1 cells	0/14	0/10	—	Extracts (—) in test mice
MLV	0/8	0/8 ^c	—	
MSB-1 cells	—	0/8	—	

^a In (a) 3×10^5 MSB-1 cells per mouse inoculated intramuscularly and in (b) 1.4×10^6 cells/mouse inoculated from tissue culture preparations.

^b Latent period = days to palpable tumor formation after inoculation of MSB-1 cells.

^c Five of 16 intact mice receiving MLV at 21 days developed generalized lymphocytic neoplasms from 2 to 5 months later.

of *in vivo* rescued virus were similar structurally to those described in BALB/c mice; all were classified as "atypical granulomas."

Discussion. The ability of leukemogenic viruses to rescue MSV directly *in vivo* was observed by Huebner *et al.* (2) using the nonproducer HT 1 hamster tumor; we have also been able to retrieve the MSV genome

by direct injections of virus-free MSB-1 neoplastic rat cells into mice also receiving a leukemogenic virus or congenitally infected with virus (strains AKR or C58) providing injections were accomplished in newborn mice. The success of *in vivo* rescue in 7-week-old BALB/c mice observed here is most likely explained on the basis of the

TABLE II. Tumor Induction in NIH 3-Day-Old Swiss Test Mice by Extracts of BALB/c Tumors Containing Recovered MSV (MLV) Pseudotype Virus.

Inoculum ^a	Dilution	Animal passage	Route of inoculation	No. of tumors	Latent period ^b (days)
Centrifuged 20% extract (40-day tumor)	10 ⁰	P ₁	Intramuscular	22/23	16.2 (14-19)
Centrifuged 20% extract (47-day tumor)	10 ⁰	P ₁	Intramuscular	9/9	33 (31-42)
Centrifuged 20% extract (50-day tumor)	10 ⁰	P ₁	Intramuscular	6/7	25.6 (10-39)
Centrifuged 20% extract (40-day muscle tissue)	10 ⁰	P ₁	Intramuscular	0/17	—
Centrifuged 20% extract (15-day P ₁ tumor)	10 ⁰	P ₂	Intramuscular	17/18	20.0 (13-28)
	10 ⁻²	P ₂	Intramuscular	14/19	35.0 (16-50)
	10 ⁻⁴	P ₂	Intramuscular	5/20	All regressed
Centrifuged 20% extract (12-day P ₂ tumor)	10 ⁻⁶	P ₂	Intramuscular	0/10	—
	10 ⁻¹	P ₃	Intramuscular	6/6	32 (20-42)

^a P₁ = First passage of extract from tumors induced in BALB/c adults with rescued pseudotype virus; P₂, P₃ = subsequent passages in NIH Swiss mice.

^b Latent period = time to first palpable growth.

immunosuppressive effects of neonatal thymectomy, allowing MSV-induced tumorous growths, known to contain transplantation antigens, to grow to a large size with resultant deaths of many of the recipients. Thymectomy and antilymphocyte serum (ALS) both provide sensitivity in otherwise resistant adult mice to induction of tumors by infectious MSV (5, 9). The mechanism involved by which adult mice resist the tumor-inducing effects of MSV is not known precisely but information obtained in this study supports our previous suggestion (5) that anti-MSV antibodies play a role. Sera obtained from tumorous BALB/c mice 4 months after intramuscular inoculation of nonproducer MSB-1 rat cells were relatively ineffective in inhibiting the focus-forming capacity of MSV in mouse embryo cells as compared with sera of nontumorous mice and those BALB/c controls that did not receive injections of MSB-1 cells. Although it is known that host range as well as certain serologic properties of pseudotype MSVs are apparently, determined by the envelope furnished by helper leukemogenic viruses, the pathogenic behavior of the rescued genome appears to be independent of this influence. The unusual tumors characteristic of MSV infection observed earlier (6) were observed with the rescued MSV described here.

Summary. Infectious MSV was rescued from "nonproducer" MSB-1 rat neoplastic cells in MLV-infected 7-week-old BALB/c strain mice. BALB/c mice were first made immunologically deficient by thymectomy at 3 days of age.

The rescued MSV (MLV) pseudotype virus-induced tumors were classified as atypical granulomas. Extracts and/or filtrates prepared from the primary lesions continued to yield infectious virus.

-
1. Hartley, J. W. and Rowe, W. P., Proc. Natl. Acad. Sci. U.S. **55**, 780 (1966).
 2. Huebner, R. J., Hartley, J. W., Rowe, W. P., Lane, W. T., and Capps, W. T., Proc. Natl. Acad. Sci. U. S. **56**, 1164 (1966).
 3. Ting, R. C., Proc. Soc. Exptl. Biol. Med. **126**, 778 (1967).
 4. Law, L. W., Ting, R. C., and Duc-Nuigee, H. (In preparation).
 5. Law, L. W., Ting, R. C., and Stanton, M. F., J. Natl. Cancer Inst. **40**, 1101 (1968).
 6. Stanton, M. F., Law, L. W., and Ting, R. C., J. Natl. Cancer Inst. **40**, 1113 (1968).
 7. Stanton, M. F. and Ting, R. C. (In preparation).
 8. Ting, R. C., Virology **2**, 865 (1968).
 9. Law, L. W., Ting, R. C., and Allison, A. C., Nature **220**, 611 (1968).

Received Feb. 17, 1969. P.S.E.B.M., 1969, Vol. 131.