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Production of B-Tropic Murine Leukemia Virus by Somatic Cell Hybrids Between Mouse Peritoneal Macrophages and Simian Virus 40-Transformed Human Cells

K. HUEBNER AND C. M. CROCE*
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

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Simian virus 40 (SV40)-transformed human cells (LN-SV) were fused with BALB/c peritoneal macrophages (BALB/c × LN-SV) and with C57BL peritoneal macrophages (C57BL × LN-SV) and hybrid clones, all of which had segregated human chromosomes and contained the entire complement of mouse chromosomes, were isolated. All 15 BALB/c × LN-SV hybrid clones were producing varying titers (10 to 10^6 plaque-forming units/ml) of B-tropic murine leukemia virus, whereas none of the nine C57BL × LN-SV hybrid clones was producing detectable ecotropic murine leukemia virus.

Naturally occurring strains of murine type C RNA virus are either ecotropic (grow preferentially on mouse cells) or xenotropic (12) (grow preferentially on cells of species other than mouse). Ecotropic viruses are either N tropic (grow preferentially on cells derived from NIH Swiss mice) or B tropic (grow preferentially on cells derived from BALB/c mice) (11).

N-tropic viruses can be induced chemically in vitro (2, 13), and are released spontaneously by some mouse embryo cells after long-term culture (1).

Isolation of B-tropic viruses in vitro is less frequent. There is a report that a BALB/3T3-derived cell line spontaneously released B-tropic virus after chronic treatment with BUdR (4) and B-tropic virus was isolated from mouse-human hybrids segregating mouse chromosomes (14). Both N- and B-tropic viruses can be isolated from mice (10) with B-tropic virus being the predominant isolate from neoplastic tissues (16).

During the investigation of the properties of somatic cell hybrids between mouse peritoneal macrophages and SV40-transformed human cells (6, 7, 8) it was observed that BALB/c × LN-SV hybrid clones were producing structural proteins (gp 69/71, p30) of RNA tumor viruses, whereas C57BL × LN-SV hybrid clones did not produce viral proteins detectable by competition radioimmunoassay (M. Strand, J. T. August, and C. M. Croce, Virology, in press).

To determine if the hybrid clones were producing ecotropic murine leukemia virus (MuLV), supernatant fluids were removed from cultures of hybrid cells and filtered (0.45-μm filter), and aliquots were tested for XC plaque-forming ability (17) on SC-1 cells, a cell line derived from a feral mouse embryo which is susceptible to both N- and B-tropic MuLV (9). The results of the XC plaque assay on SC-1 cells are presented in Table 1. Since BALB/c × LN-SV hybrids were producing ecotropic virus, the supernatants were again tested for plaque-forming ability on secondary NIH mouse embryo (ME) and BALB/c ME cells. Comparative titers of the MuLV from some hybrid clones tested in secondary NIH Swiss and BALB/c ME cultures are given in Table 2. The MuLV from each of the BALB/c × LN-SV hybrid clones has preferential growth capacity on BALB/c ME cells and is thus B tropic.

All the BALB/c × LN-SV and C57BL × LN-SV hybrid clones contain the human chromosome 7 containing the SV40 genome and in several BALB/c × LN-SV and C57BL × LN-SV hybrid clones this is the only human chromosome present (see Table 1). The mouse complement of chromosomes is, in many hybrid clones, that of normal diploid mouse cells (7). Thus, the human chromosome 7 carrying the SV40 genome (the only common feature of all the macrophage × LN-SV hybrids) is not sufficient to induce expression of B-tropic virus; nor is the SV40 genome alone sufficient since BALB/c ME cells and C57BL ME cells transformed by SV40 do not produce B-tropic MuLV (reference 1, Table 1). Since it has not been possible to obtain hybrids between normal human cells and mouse peritoneal macrophages (8), the effect of a human chromosome number 7 (without the SV40 genome) could not be assessed. Interestingly, the B-tropic virus obtained from mouse-human hybrids by Minna et

TABLE 1. *Titer on SC-1 cells of MuLV produced by mouse macrophage × LN-SV hybrid clones*[a]

Source of infecting inoculum	Titer $\log_{10}$ (PFU[b]/ml) on SC-1 cells	Human chromosomes present in hybrid clone[c]							
		No. 5	No. 6	No. 7	No. 11	No. 16	No. 17	No. 18	No. 20
Controls									
SC-1/MuLV[d]	4.6								
C57BL macrophages[e]	0								
BALB/c macrophages	0								
C57BLSV[f]	0								
BALB SV	0								
BALB/c × LN-SV hybrids[g]									
FL1 clone 10	3.0[h]	0/25		25/25	0/25				
FL1 clone 36	1.0	17/25		25/25	4/25				
FL1 clone 43	2.7	24/30		30/30	13/30				
FL1 clone 63	4.3								
FL2 clone 1	4.0	19/25		25/25	15/25				
FL2 clone 6	3.3	0/21		21/21	14/21				
FL2 clone 8	3.0	15/21		21/21	5/21				
FL2 clone 12	3.2	3/22		22/22	22/22				
FL2 clone 15	0.3	1/18		18/18	2/18				
FL2 clone 20	6.4	2/22		20/22	0/22				
C57BL × LN-SV hybrids[i]									
FL1 clone 16	0	5/20		20/20	0/20				
FL1 clone 21	0	0/22		22/22	0/22				
53-53 FL1 clone 6	0	1/20	14/20	20/20	14/20	0/20	12/20	0/20	2/20
53-53 FL1 clone 13	0	0/22	4/22	22/22	14/22	0/22	11/22	10/22	12/22
53-53 FL2 clone 5	0	2/16	7/16	16/16	11/16	4/16	4/16	0/16	1/16
53-53 FL2 clone 9	0	10/20	12/20	20/20	11/20	0/20	11/20	1/20	0/20

[a] Supernatant fluids from each of the cell lines listed on the left were taken at 2, 5, and 7 days after seeding the cells, pooled, and filtered (0.45 μm), and 0.5 ml of each sample with appropriate dilutions was inoculated into duplicate petri dishes which had been seeded for 24 h previously with 2×10^5 SC-1 cells/dish in medium containing 2 μg of polybrene per ml (19). After 1 h of adsorption, the inocula were removed and 5 ml of medium containing 5% fetal bovine serum was added; at 6 days postinfection cells were irradiated and overlaid with XC cells as described by Rowe et al. (17). Petri dishes were fixed and stained on day 4 after addition of XC cells and plaques were enumerated and the titers were calculated.

[b] PFU, Plaque-forming units.

[c] Number of metaphases containing the corresponding human chromosome over the total analyzed. Methods of isolation and karyological characterization of macrophage × LN-SV hybrids have been previously described (6, 7, 8).

[d] The SC-1 cell line was infected with Moloney MuLV at passage 73 and was in the 20th passage postinfection at the time of these experiments. The supernatant from these cells was used as a control in all XC plaque tests.

[e] C57BL and BALB/c peritoneal macrophages were prepared according to the method of Cohn and Benson (5) with some modification; supernatant medium was taken for virus assay at 2, 5, and 7 days after seeding macrophages.

[f] C57BL and BALB/c embryo cells were infected with SV40 virus and cell lines were derived which were 100% SV40 T antigen-positive (unpublished data). These cell lines had been subcultivated more than 50 times at the time of this test.

[g] In addition to the clones shown here, supernatants of five other BALB/c × LN-SV hybrid clones were tested and all contained ecotropic MuLV.

[h] These numbers are not average values; the supernatant medium of most of these clones has been assayed for virus at least three and some four or more times, and the titers obtained at different times for the same clone varied widely. For example, the supernatant medium of FL2 clone 1 was assayed in three separate experiments and the titers were 0, 2.7, 4.0. The numbers given in this table were all taken from a single experiment.

[i] Three additional C57BL × LN-SV hybrid clones which were tested were found to be producing no ecotropic MuLV. Supernatants from these hybrids were concentrated, tested for reverse transcriptase activity, and found to be negative, thus eliminating the possibility of production of xenotropic virus by these hybrids (K. Huebner and C. L. Green, unpublished data).

TABLE 2. *Comparative titers of MuLV from macrophage × LN-SV hybrid clones in secondary NIH Swiss and BALB/c ME cultures*[a]

Source of inoculum	Titer (log_{10}) PFU[b]/ml)	
	NIH-ME	BALB/c-ME
SC-1/MuLV	4.5	4.2
BALB/c × LN-SV hybrids		
FL1 clone 43	0.7	2.4
FL1 clone 63	1.0	3.3
FL2 clone 1	2.0	3.9
FL2 clone 6	0.7	3.3
FL2 clone 18	1.7	3.9
FL2 clone 20	3.9	6.2
C57BL × LN-SV hybrids		
FL1 clone 16	0	0
FL1 clone 21	0	0

[a] Plaque tests were carried out as described in the legend of Table 1, except that after adsorption of inocula, the medium was replaced with medium containing 10% fetal bovine serum.

[b] PFU, Plaque-forming units.

al. (14) was also obtained from a hybrid derived from fusion of normal mouse cells with an SV40-transformed human cell. Two out of eight hybrid clones tested were producing B-tropic virus; however, the mouse parental cell in this case was C57BL embryo brain cells. The fact that we found no B-tropic virus in C57BL × LN-SV hybrids does not necessarily conflict with the results of Minna et al. since the hybrids described by Minna et al. had segregated mouse chromosomes (14); indeed, they speculated that the loss of mouse chromosomes could be a contributing factor in the induction of the virus since hybrid clones containing a larger complement of mouse chromosomes were not producing virus.

Since C57BL mice certainly harbor B-tropic virus (14, 15), the lack of induction of B-tropic virus in our C57BL × LN-SV hybrid cells indicates a difference between genetic control of the endogenous B-tropic virus(es) in C57BL and BALB/c mice. These differences can be further explored using somatic cell hybrids between LN-SV and peritoneal macrophages derived from congenic strains of C57BL such as B6-G_{1X}+ mice (18).

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