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Resistance of Cultures of Normal T Cells to Infection with Murine Type C Viruses

IVAN HORAK,† LUIS ENJUANES,‡ JOHN C. LEE, AND JAMES N. IHLE*

Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland 21701

Long-term continuous cultures of normal T cells were established from C57BL/6 and BALB/c mice by using conditioned medium from concanavalin A-stimulated lymphocytes. The ability of various murine type C viruses to infect these normal T cell cultures was examined and compared with their ability to infect transformed T cells. All of the viruses examined, including a thymotropic radiation leukemia virus, were unable to infect and replicate in normal T cells but readily did so in transformed T cells.

Murine ecotropic type C viruses are commonly associated with the induction of T cell lymphomas. Although the replication of these viruses in a variety of tissue culture lines has been extensively studied, their replication in T cells, the presumed in vivo target for transformation, has not been examined. This has been due to the lack of appropriate culture conditions required to sustain normal T cell viability and proliferation in vitro. Recently, however, techniques have been developed to obtain continuous cultures of normal mouse T cells. Several studies have demonstrated the long-term growth of activated T cells by using conditioned media from concanavalin A-stimulated normal lymphocytes (6, 7, 17). The ability of such conditioned media to sustain T cell growth appears to be associated with a specific lymphokine which has been partially purified (8, 19) and is termed either T cell growth factor or interleukin 2. Long-term cultures of normal T cells have been shown to retain functional characteristics and to contain functionally distinct subpopulations (6, 7). Therefore, we have established several normal T cell cultures by using conditioned media to specifically examine their ability to replicate type C viruses.

To establish continuous T cell lines, we used conditioned media from concanavalin A-activated normal BALB/c splenic lymphocytes and splenic lymphocytes from either BALB/c or C57BL/6 mice. To enrich for T cells before culture, the spleen cells were initially fractionated on nylon wool columns (11) which preferentially remove macrophages and B cells, giving a population of lymphocytes which is approximately 80 to 90% Thy-1-positive T cells. Typical growth curves for the establishment of lines are shown in Fig. 1. In this experiment, BALB/c splenic T cells were cultured in either medium alone or in conditioned medium. When cultured in medium alone, the cultures rapidly died out, whereas in conditioned medium, proliferation was detectable after 2 weeks, and the cultures subsequently grew exponentially with a doubling time of 2 to 3 days. Such cultures grow continuously, and several have been maintained for over 1 year. The continuous growth of the cultures is dependent upon the use of conditioned medium. The typical morphology of a normal T cell culture after approximately 2 months is shown in Fig. 2. The cultures consist of a fairly homogeneous nonadherent population of lymphoblastic type cells. As determined by using monoclonal antibodies in cytotoxicity assays, the cultures generally consist of >90% Thy-1-positive cells, demonstrating their T cell lineage.

By using both BALB/c and C57BL/6 T cell cultures, we next examined the infectivity of various viruses in these cells. Initially we examined the ability of Moloney leukemia virus (MoLV) to infect cultures of normal BALB/c T cells, since this virus readily induces T cell lymphomas of both mature and immature T cell subpopulations in vivo in BALB/c mice (18). The results are shown in Table 1. In this set of experiments, a continuous T cell culture was established and infected with MoLV at 2 weeks. Polybrene was included to increase the efficiency of infection. As a control, RL-12 cells were also included. The RL-12 cell line was established from a radiation-induced lymphoma of a C57BL/Ka mouse and expresses no detectable type C viruses (13). As shown in Table 1, MoLV was infectious in RL-12 cells, and at 14 days postinfection, virus replication was detectable by immunofluorescence for p30, competition radioimmunoassays for p30, and by recov-

† Present address: Institute of Virology and Immunobiology, University of Wuerzburg, 87 Wuerzburg, West Germany.
‡ Present address: Institute de Biologia del Desarollo CSIC-Universidad Autonoma de Madrid, Spain.

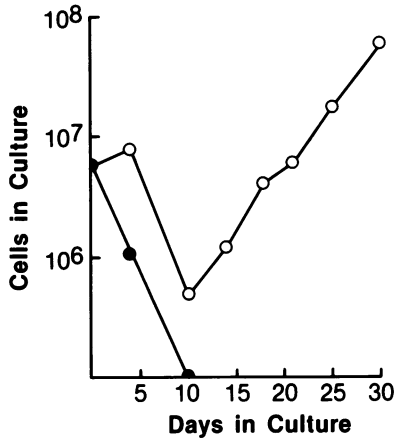

FIG. 1. *Initial growth characteristics of normal T cell cultures in the presence or absence of conditioned medium. Splenic lymphocytes were obtained from a normal 6-week-old BALB/c mouse, and the T cell subpopulation was partially purified by nylon wool column chromatography. The cells were subsequently cultured at 2 × 10^6 cells per ml in 30 ml of medium in T flasks at 37°C. Media consisted of either RPMI 1640 medium with 10% fetal calf serum (●) or 50% RPMI 1640 medium with 10% fetal calf serum and 50% conditioned medium (○). Conditioned medium was obtained by culturing 2 × 10^6 BALB/c splenic lymphocytes per ml in RPMI 1640 with 10% fetal calf serum and 5 μg of concanavalin A for 48 h at 37°C. The cells were subsequently removed by centrifugation, and the conditioned medium was filtered through a 0.45-μm Millipore filter. Conditioned medium was stored at 4°C until needed. Cell numbers were determined at the indicated times by removing samples and counting viable cells as determined by exclusion of trypan blue.*

ery of infectious virus. In contrast, MoLV infection of normal BALB/c T cell cultures resulted in no viral replication as detected by these assays at 30 or 66 days after infection. We have also examined the effects of infecting the cultures at various times during and after the establishment of the T cell lines and of pretreating the cultures with DEAE-dextran to increase infectivity. In none of these experiments did we detect infection of the cultures (data not shown). Another potential problem could have been the conditioned medium required for maintenance of the cultures. We therefore examined the titer of the MoLV stock on FG-10 cells in the presence or absence of conditioned medium and found no difference (data not shown). Thus, these results show that MoLV is not infectious in normal BALB/c T cells in culture.

We next examined the ability of an MoLV and different C57BL/6 ecotropic viruses to infect two independently established T cell cultures derived from C57BL/6 mice. The cultures were established as in Fig. 1, although the lymphocytes used were derived from mice immune to MoLV-murine sarcoma virus and initially stimulated in vitro with MoLV gp71 to activate T cell proliferation. As controls, RL-12 cells and a virus-free T cell line established from a radiation-induced lymphoma in an NIH Swiss mouse were used. As shown in Table 2, all the viruses examined readily infected both RL-12 cells and the NIH Swiss lymphoma cell line. The viruses examined included MoLV and a B-tropic ecotropic virus from C57BL mice (2). In addition, we examined the infectivity of virus obtained from an MoLV-induced lymphoma in C3H mice and viral preparations from MoLV-induced thymic and splenic lymphomas of BALB/c mice (18). Finally, we examined the infectivity of VL-3 virus which is a derivative of the radiation leukemia virus. This virus is unique in that it poorly replicates on fibroblasts although, as shown in Table 2, it replicates to high titers in RL-12 cells (3, 14). When the infectivity of these viruses was examined with two independently established T cell cultures, no infectious virus was recovered from series 11 at 20 or 35 days after infection or from series 13 at 19 or 60 days after infection. Therefore, by using standard viruses, viruses derived from lymphomas, or a "T cell"-tropic virus, we could not demonstrate infectivity of these viruses in normal T cells in vitro.

These results demonstrate a striking difference in the ability of ecotropic viruses to repli-

TABLE 1. *Infectivity of MoLV in continuous cultures of normal BALB/c T cells*

Cell line	Days p.i.[b]	Virus assay[a]			
		IMF (%)	EM	cRIA (ng/mg)	FFU/ml
Series 16 BALB/c T cells	30	<1	ND[c]	ND	<10
	66	<1	Negative	<10	<10
RL-12	14	>90	ND	1,200	1.6×10^3

[a] Continuous cultures of normal BALB/c T cells were established as described in the legend to Fig. 1. After 2 weeks, the cultures were infected with an undiluted stock of MoLV which had a titer of 8 × 10^5 focus-forming units (FFU) per ml in the FG-10 (S^+ L^-) assay (1). Potential enhancement of infectivity was attempted by using 5 μg of polybrene per ml. As a control, RL-12 cells were infected with MoLV in a comparable manner. Viral infectivity was determined at the times indicated by several criteria. Immunofluorescence (IMF) was done with a monoclonal antibody againt MoLV p30. Electron microscopy (EM) was done to attempt the detection of budding viral particles. Competition radioimmunoassays (cRIA) for p30 were performed as previously described (2) and are given as the amount of competing antigen per milligram of cell extract. Infectious virus was assayed by using the FG-10 (S^+ L^-) assay.

[b] p.i., Postinoculation.

[c] ND, Not done.

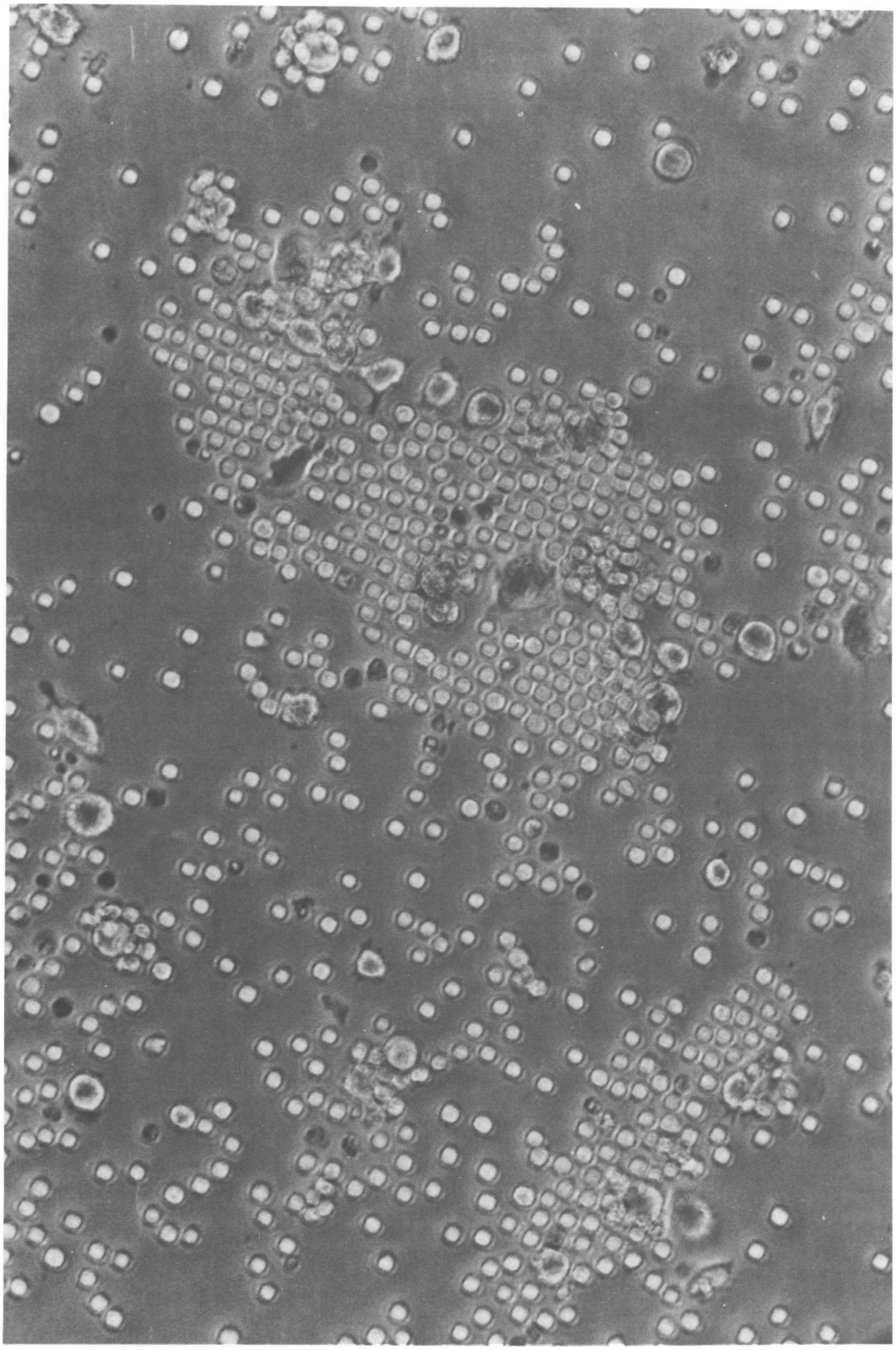

FIG. 2. *Morphology of continuous cultures of normal T cells. The culture shown is a continuous T cell culture obtained with splenic T cells from a C57BL/6 mouse which was previously immunized with MoLV-murine sarcoma virus. Cultures were established as described in the legend to Fig. 1 with the exception that the T cells were initially activated in vitro with MoLV gp71 to promote cell growth. The photomicrograph shows the cultures at approximately 2 months of age. Cultures established from nonimmune normal BALB/c mice by using a conditioned medium had comparable morphologies.*

TABLE 2. *Infectivity of various ecotropic viruses in continuous cultures of C57BL/6 T cells*

Cell line	Days pi[b]	FFU/ml[a] MoLV	B-tropic	C3H-MoLV	BALB-T-MoLV	BALB-S-MoLV	VL-3
RL-12	14	1.6×10^3	4.4×10^3	1.3×10^4	1.1×10^5	1.3×10^5	2.0×10^5
NIH Swiss lymphoma	14	7.0×10^4	4.0×10^3	1.2×10^3	6.0×10^3	1.8×10^3	ND[c]
Series 11 C57BL/6	20	0	0	0	0	0	0
	35	0	0	0	0	0	0
Series 13 C57BL/6	19	0	0	0	0	0	0
	60	0	0	0	0	0	0

[a] FFU, Focus-forming unit. Continuous T cell cultures were established from C57BL/6 mice as described in the legends to Fig. 1 and 2. After initiation, the cultures were infected with the various viruses in the presence of polybrene. RL-12 and the NIH Swiss lymphoma cell lines were comparably infected as controls. In all cases, undiluted stocks of virus were used. MoLV and B-tropic viruses were obtained from productively infected cell lines. C3H MoLV was obtained from a lymphoma cell line of C3H origin originally induced by MoLV. BALB-T-MoLV and BALB-S-MoLV were virus preparations obtained by culturing cells from thymic and splenic lymphomas induced by MoLV in BALB/c mice in vitro for 24 h. VL-3 virus was obtained from supernatants of cultures of the VL-3 cell line, a lymphoma cell line established from a C57BL/Ka mouse which had a radiation leukemia virus-induced lymphoma. Virus titers were determined at the indicated times by either the FG-10 assay or infectivity on RL-12 cells by immunofluorescence for the VL-3 virus.

[b] p.i., Postinoculation.

[c] ND, Not done.

cate in normal and transformed T cells. Under a variety of conditions, with different viruses, we were unable to demonstrate infectivity in continuous cultures of normal T cells. Previous results with T cells in short-term in vitro cultures have suggested that T cells may be less efficient for viral replication than fibroblasts (9); however, the general viability of the cells precluded a definitive conclusion. By using conditioned medium, it has been possible to examine this aspect directly. Under the conditions used, normal T cells proliferate in vitro, and viabilities are generally greater than 95%. Therefore, the lack of infectivity is not simply due to proliferation or viability. Occasionally, in the types of cultures used here, we have detected low levels of recovery of infectious viruses, especially soon after establishing the cultures. Invariably, such cultures contained adherent cells as well as T cells, and, by immunofluorescence, only the adherent non-T cells were virus positive.

The basis for the inability of ecotropic viruses to infect continuous cultures of normal T cells is not known. Factors such as the conditioned medium, time of infection after establishment, and the use of polybrene or DEAE-dextran have been examined. One factor not examined is the possible effect of immune interferon; however, interferon only appears to block type C virus maturation and not infectivity (5). Consequently, the inability to detect viral antigen-positive cells tends to negate this possibility.

The inability of ecotropic viruses to infect continuous normal T cell cultures in vitro appears to correlate with the pattern of virus infection in vivo. In a recent study, it was demonstrated that, for the most part, AKR thymocytes are not viral antigen positive throughout the majority of the preleukemic period (16). In addition, we examined the course of infection of T cells in BALB/c mice infected with MoLV (unpublished data) and observed that throughout the preleukemic period the T cell population is viral antigen negative. The majority of viral replication in the spleen is apparently in B cells and macrophage-monocyte cells. Viral antigen-positive T cells were only observed when overt leukemias developed. Thus, these data suggest that there is a correlation between our in vitro results and viral infectivity in vivo.

The lack of detectable infectivity in our experiments could be a function of the viruses examined. Considerable data have been accumulated suggesting that recombinant viruses such as the MCF or thymotropic radiation leukemia virus are essential for leukemogenesis (4, 10). This could be due to their ability to infect T cells. As shown by our results, the VL-3 virus, although being T cell specific when comparing infectivity on fibroblasts versus transformed T cells, was not infectious in normal T cells. In preliminary experiments, we also examined several isolates of MCF viruses obtained from J. Hartley (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) for infectivity in normal T cells and similarly were unable to detect replication. Therefore, it appears that normal T cells may be generally refractile to type C viruses.

The striking difference between infectivity of normal versus transformed cells was quite surprising. It is conceivable that the ability of T

cells to replicate virus is dependent upon transformation. If this is the case, determining the mechanisms involved may give some insight into the processes involved in transformation. In vivo transformation itself may be associated with chronic immune stimulation as previously suggested (12) and not require viral infection per se. A second possibility has been suggested by the work of McGrath et al. and McGrath and Weissman (15, 16), who have hypothesized that viral infection and transformation of T cells requires the presence of antigen-specific immune receptors. By using the types of cultures described here, we would not expect to detect such a subpopulation, although cloning of normal T cells may allow this. Nevertheless, the use of continuous cultures of normal T cells should provide approaches to further study the biology of type C viruses and their role in leukemogenesis.

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