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High Sensitivity of a C3H Mouse Embryo-Derived Cell Line to the Antiviral Activity of Interferon (39824)

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Introduction. Quantitative variations in the response to interferon action are found in different cell lines derived from a single species (1, 2). Therefore, the choice of the cell system as well as the choice of test virus are important considerations to ensure maximum sensitivity for the detection of small amounts of interferon. We report herein that a C3H mouse embryo-derived cell line is highly sensitive to the antiviral activity of interferon as compared with a continuous L cell line or secondary cultures of mouse embryo fibroblasts.

Materials and Methods. Cells. The cloned line of C3H mouse embryo fibroblasts, designated 10 T1/2.C18, was a gift to Dr. J. B. Little from Drs. C. Reznikoff and C. Heidelberger, McArdle Institute for Cancer Research.[1] These cells are highly susceptible to postconfluence inhibition of cell division. A detailed description of their origin and their maintenance in culture has previously been reported (3). Cells used for these experiments were between the 8th and 30th passage *in vitro*. In a later passage (32nd), spontaneous transformation occurred in one culture. These transformed cells were transferred for more than two passages. The densities at confluency were higher (3×10^6/25-cm^2 Falcon flasks) than those of nontransformed cells (1.2×10^6/flask), and cells presented a different morphology. The origin of the continuous L cell line employed has previously been described (4). The secondary cultures of Swiss mouse embryo fibroblasts (MEF) were prepared by standard techniques.

All cells were cultivated in Eagle's minimal essential medium (MEM) with 10% heat-inactivated calf serum (L and MEF cells) or fetal calf serum (10 T1/2.C18 cells). However, as it has been reported that MEM favored spontaneous transformation in C3H/10 T1/2 cell line (5), it is preferable to use Eagle's basal medium for the routine maintenance of the cells.

Interferon preparations. The mouse interferon standard from our laboratory was prepared from the brains of Ic and Swiss mice inoculated intracerebrally with West-Nile virus (6). In one experiment, an NIH reference mouse interferon (n° G002-904-511, National Institutes of Health, Bethesda, Md.) was included. The mitogen-induced interferon was prepared from mouse spleen lymphocyte suspensions stimulated with phytohemagglutinin (PHA) (7).

Interferons were assayed by the method of inhibition of the cytopathic effect of 100 $TCID_{50}$ of vesicular stomatitis virus (VSV), encephalomyocarditis (EMC) virus, or Semliki Forest virus (SFV) in monolayer cultures of cells in plastic Microtest II trays (Falcon) (8). Dilutions of interferon were incubated with cell cultures for 18 hr before the challenge virus. Interferon titers are expressed as the highest twofold dilutions giving approximately 50-70% protection of the cell sheet at a time when virus-infected control cultures showed complete destruction. One of our mouse interferon standard units equals four NIH International Reference units.

Results and discussion. The data presented in Table I show that interferon titers are consistently greater in 10 T1/2.C18 cells than in the two other homologous cells, L cells, and secondary mouse embryo fibroblasts. In a total of 10 determinations, the mean titer of our interferon reference preparation assayed on 10 T1/2.C18 was 1:20,480 (SD = 1:8010), which was significantly greater than the mean titer of 1:1480 (SD = 1:193) as assayed on L cells. The high sensitivity of 10 T1/2.C18 cells to the

[1] Requests for the C3H/10 T1/2 cells should be sent to Dr. C. Heidelberger, Los Angeles County-University of Southern California Comprehensive Cancer Center, Los Angeles, Calif. 90031.

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TABLE I. SENSITIVITY OF DIFFERENT MOUSE CELLS TO THE ANTIVIRAL ACTIVITY OF INTERFERON.

Interferon	Origin of interferon preparation	Challenge virus	Interferon titer[a] (Units/0.2 ml) L	MEF	10 T1/2.C18
Virus induced	Our laboratory[b]	VSV	1,600	4,800	16,000
		SFV	800	–	24,000
		EMC	400	–	2,000
	NIH[c]	VSV	3,000	9,600	24,000
Mitogen induced	Our laboratory[d]	VSV	10	–	320

[a] Titers are given as the reciprocal.
[b] Mouse brain interferon (see Materials and methods).
[c] Assigned titer = 12,000.
[d] PHA-stimulated lymphocyte interferon (see Materials and Methods).

TABLE II. INTERFERON TITERS IN NORMAL AND SPONTANEOUSLY TRANSFORMED 10 T1/2.C18 CELLS.

Cells	Passage used	Antiviral titer[a]/0.2 ml with VSV as challenge virus
Normal 10 T1/2.C18	8	32,000
	10	24,000
	14	32,000
	23	16,000
	24	12,800
	30	16,000
Spontaneously transformed 10 T1/2.C18	1	1,600
	2	1,600

[a] Titers are given as the reciprocal. The mouse brain interferon assayed was from our laboratory (see Materials and methods).

action of interferon is observed when three different challenge viruses are used. However, the interferon titers obtained with VSV and SFV are superior to those obtained with EMC virus.

Furthermore, the pronounced sensitivity of 10 T1/2.C18 cells is not restricted to virus-induced interferon. The antiviral titer of interferon prepared from PHA-stimulated mouse lymphocytes was 30 times higher in 10 T1/2.C18 cells than in L cells when VSV was used as challenge virus (Table I).

It is interesting to note that 10 T1/2.C18 cells remain very sensitive to interferon only while they are susceptible to postconfluence inhibition of division. As shown in Table II, interferon titers, assayed in a spontaneously transformed 10 T1/2.C18 culture, are reduced to a value usually obtained in the continuous L cell line. This difference in interferon sensitivity between normal and transformed cells has already been reported (9).

In conclusion, the C3H/10 T1/2.C18 cell line provides a sensitive tissue culture system in the assay of interferon, and utilization of these cells may prove of value in detecting small amounts of interferon.

Summary. A C3H mouse embryo-derived cell line was found to be consistently 5- to 30-fold more sensitive to the antiviral activity of mouse interferon than two other homologous cells routinely used in the assay of mouse interferon.

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