

IN VITRO CORRELATION BETWEEN VIRUS PRODUCTION AND CELL PROLIFERATION OF RAT LYMPHOMAS

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ABSTRACT

Kuang Dong Wu (1977). *In Vitro Correlation Between Virus Production and Cell Proliferation of Rat Lymphomas*. Bull. Inst. Zool., Academia Sinica 16(2): 123-129. Two *in vivo* sublines, LW-27 and (LW-27)B, have been developed by serial transplantation of rat lymphomas induced by mouse radiation leukemia virus (RadLV) in irradiated and non-irradiated adult W/Fu rats respectively. LW-27 is characterized by the presence of RadLV and viral specific antigen; (LW-27)B contains neither virus nor antigen. When ascitic cells were seeded in culture flasks, both LW-27 and (LW-27)B developed simultaneously as monolayer and suspension cultures. Monolayer cultures from both lines, maintained by serial subculturing, contain neither virus particles nor viral antigen. The suspension culture of LW-27 was easily established as a permanent cell line, whereas (LW-27)B survived only for a few generations. Extensive efforts (feeder layers, conditioned medium, various combinations of sera and media) to prolong the life span of (LW-27)B suspension culture failed. Both long-term and short-term suspension cultures of LW-27 contained numerous intracellular and extracellular virus particles as well as viral antigens. In contrast, neither virus nor viral antigen was found in the short-term suspension cultures of (LW-27)B. These results suggest a correlation between virus production and cell proliferation in this rat lymphoma system.

Rat lymphomas of LW-27 and (LW-27)B induced by mouse radiation leukemia virus (RadLV) have been developed in irradiated and non-irradiated adult W/Fu rats respectively. They were characterized by the presence of RadLV and virus specific antigen in the former and the absence of both in the latter⁽⁴⁾. This concomitant loss of virus and virus antigen in

(LW-27)B cells is of fundamental interest in leukemogenic studies. Apparently there is no precedent in literature for the complete and permanent loss of virus and virus antigen in virus induced leukemia such as seems to be the case in the (LW-27)B lymphomas. The following questions may be asked: 1) Do these cells still possess partial or complete Rad LV genome in an unexpressed form? If so, can it be activated by

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in vitro manipulation? 2) Why and when this loss occurred in the process of transplantation? Can this phenomenon be induced in tissue culture condition? To answer these questions, attempt was made to grow ascites of these two lines *in vitro*. After extensive efforts, it was found that success or failure of growing these cells was correlated with the presence or absence of RadLV in these cells. Some preliminary results are presented here.

MATERIALS AND METHODS

Animals:

Inbred W/Fu rats were obtained from A. R. Schmidt Co., Madison, Wisconsin.

Tumor transplantation:

1) LW-27. Pre-irradiated (4000R), six weeks old, female W/Fu rats were intraperitoneally (IP) infected with $50-200 \times 10^6$ viable ascitic cells freshly harvested or thawed from liquid nitrogen. Seven to 10 days later, animals were apparently sick and ascitic cells were collected. Two to 4 rats were transplanted each time, and a total of 31 rats were used.

2) (LW-27)B. Unirradiated, 6 months old, male W/Fu rats were injected (IP) with $7-100 \times 10^6$ cells. A total of 52 rats were used, two to 4 at a time.

Cell culture and media:

The ascitic cells were collected in the presence of panheparin, washed twice with Eagle's minimum essential medium (MEM), and then seeded at various densities in 75 cm² Falcon flasks containing one of the following media: L-15, RPMI 1640, MEM-monolayer and MEM-suspension (all from Grand Island Biological Co.). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 units/ml penicillin, 100 μ g/ml streptomycin. In some cultures of (LW-27)B cells, FBS was replaced by 25% inactivated human serum, and 1% phytohemagglutinin-P (PHA) was added to the media. Furthermore, normal rat kidney (NRK) cells (from Dr. S. S. Chang, NCI) as

well as monolayer of (LW-27)B cells isolated from the ascitic culture were irradiated with 5,000R X-ray at confluent stage, trypsinized and seeded at 1×10^6 cells per 75 cm² flasks, and used as feeder layer(2). Culture media collected from ascitic monolayer of (LW-27)B were also used as conditioned media to support the growth of (LW-27)B suspension culture⁽⁶⁾. Cell viability was checked daily in the first week of culture initiation, and then weekly until the culture was established or lost.

Chromosome analysis.

Cultured cells were treated with 0.6 μ g/ml colcemid for 2 hr at 37°C, harvested by centrifugation, resuspended in 0.075 M KCl for 10 min, fixed with methanol-acetic acid and stained with Wright-Giemsa stain. Thirty to 50 metaphase plates were counted.

Reverse transcriptase(RT) assay.

Twenty ml cultured media was clarified by centrifugation at 1,050 g for 10 min and then pelleted with Beckman Model L ultracentrifuge at 100,000 g for 2 hr. The pellets were suspended in 0.2 ml of 0.01 M Tris, pH 8.3. The remaining procedures were essentially the same as reported by Todaro *et al.*⁽⁶⁾.

Chemical activation.

Short term cultures of (LW-27)B cells as well as serially passaged monolayer cultures of both LW-27 and (LW-27)B cells were treated with 5-bromodeoxyuridine(BrdU) and 5-iododeoxyuridine(IdU) at 25, 50, and 100 μ g/ml for 1-5 days. Supernatant fluids were checked for RT and cells were examined by electron microscopy.

Electron microscopy (EM) and immunofluorescence assay (IF).

Methods for the preparation of cell cultures for EM and IF tests have been reported by Ferrer *et al.*⁽⁵⁾.

RESULTS

Cells from both LW-27 and (LW-27)B lymphomas were highly tumorigenic. Of the 31

rats transplanted with LW-27 cells and 52 rats transplanted with (LW-27)B cells, all but three developed leukemia and were sacrificed at the end of two weeks. Those three rats free from leukemia belonged to the (LW-27)B group. They were killed 8-10 days after transplantation and no leukemic symptoms were found. When ascitic cells of LW-27 and (LW-27)B were seeded in culture flask, both lines developed monolayer and suspension cultures simultaneously in the same flask. Monolayer cultures from both lines were maintained and subcultured in MEM+10% FBS. Neither virus particles, as examined by EM, nor viral expression, as checked by IF and RT, were detected in these monolayer cultures. In contrast to the similarity of these two lines in monolayer, cells grown in suspension showed different growth characteristics in the following aspects: 1) LW-27 cells could be established as permanent suspension culture in RPMI 1640+10% FBS with high frequency. As shown in Table 1, two out of three LW-27 suspension cultures resulted in permanent growth. However, none of the 20 (LW-27)B suspension cultures developed into permanent lines. Most (LW-27)B cells grew actively for the initial 3-10 days and then died out. There was only one culture which survived six weeks. When different culture conditions were tested, medium L-15 was found to be superior to RPMI 1640 in maintaining survival of (LW-27)B cells. Neither human serum nor PHA had any beneficial effect on (LW-27)B growth. Feeder layers

of NRK cells or (LW-27)B monolayers, as well as conditioned media collected from (LW-27)B monolayers, did not prolong the life span of (LW-27)B suspension cultures. In contrast, none of the extra efforts was needed in establishing permanent LW-27 suspension culture. Therefore, cells of these two sublines in subsuspension were apparently different in their *in vitro* growth characteristics. 2) Both short-term and long-term suspension cultures of LW-27 cells had numerous intracellular and extracellular virus particles as examined by EM (Figs. 1, 2) and assayed by RT (Table 1) respectively. In contrast, short-term cultures of (LW-27)B suspension cells had neither virus particles nor viral expression (Table 1). By treating these short-term cultures with 25-100 μ g/ml BrdU or IdU for 1-5 days, no viral activation was observed as checked by EM and RT. The same was true for monolayers of both LW-27 and (LW-27)B which were initiated from ascites. They were virus negative and did not respond to chemical activation.

Chromosome analyses of the suspension cultures of LW-27 and (LW-27)B indicated that they were mainly diploids, 83% and 60% of respective cells having 42 chromosomes. Structural abnormalities such as fragmentation were rare.

Our preliminary transplantation results indicated that the *in vitro* permanent suspension culture of LW-27 had the same leukemogenic potential as that of its *in vivo* parental line

TABLE 1
In Vitro Properties of Rat (W/Fu) Ascitic Cells Originated from RadLV-Induced Lymphomas

Cell Type	Cells	No. Cultures Initiated	No. Cell Lines Established	Percentage of Cells with 42 Chromosomes	Average RT CPM (No. Assays Performed)	No. Cells with Virus/Total No. Cells Observed	IF
Monolayer	LW-27	2	2	ND*	152(8)	0/53	-
	(LW-27)B	2	2	DD*	88(9)	0/31	-
Suspension	LW-27	3	2	77	105,737(20)	58/100	+
	(LW-27)B	20	0	60	637(10)	0/59	-

* ND=not done.

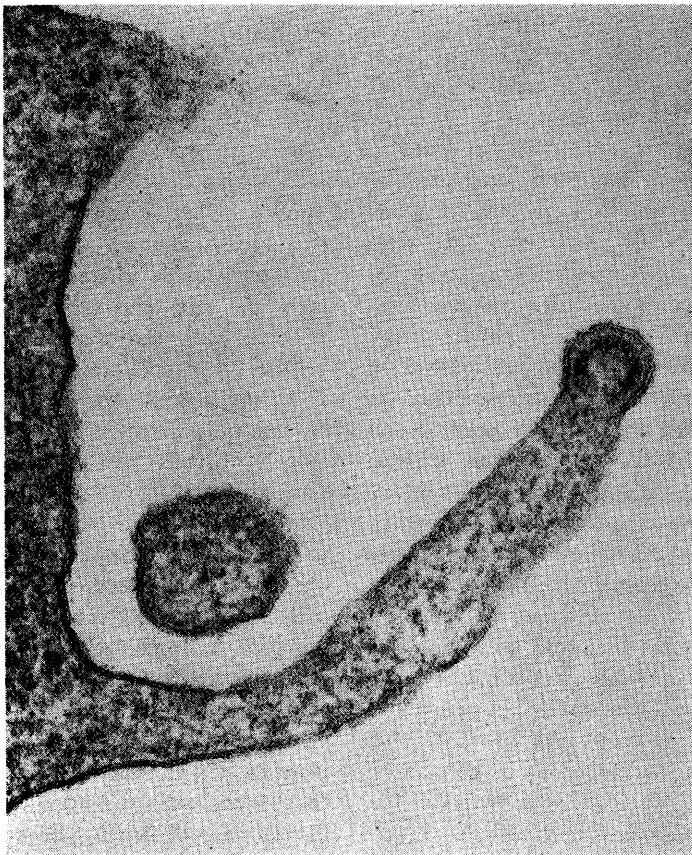


Fig. 1. Cell from LW-27 ascites cultured as suspension in RPMI 1640 plus 10% heat inactivated fetal bovine serum. Budding C viral particle is shown here. ($\times 112,500$)

Of six pre-irradiated rats injected with 200×10^6 cells/rat, all developed either thymoma or ascites within two weeks.

DISCUSSION

Attempts to grow these rat lymphomas in tissue culture are of fundamental importance in our endeavors to study the phenomenon of virus and viral antigen losses in this tumor system. Aoki and Johnson⁽⁴⁾ reported the loss of a virus specific cell surface antigen in Gross leukemia virus (GLV) induced mouse leukemia. Unlike (LW-27)B lymphoma, the antigen reappeared after the leukemic cells were maintained in the absence of specific antibody. In addition,

the suppression of the GLV antigen was not associated with the disappearance of virus particles. Losses of MuLV gs antigen and virus particles were observed in cells transformed by GLV⁽⁶⁾. Here again, the losses were temporary and therefore apparently different from that observed in the RadLV-induced rat lymphomas.

Our findings of virus and viral antigen losses in (LW-27)B tumor cells are clearly relevant to 1) the widely accepted concept that tumor specific cell surface antigen represent a unique property essential for the neoplastic behavior of cells, and 2) the hypothesis that the persistence of the viral genome, or a part of it,

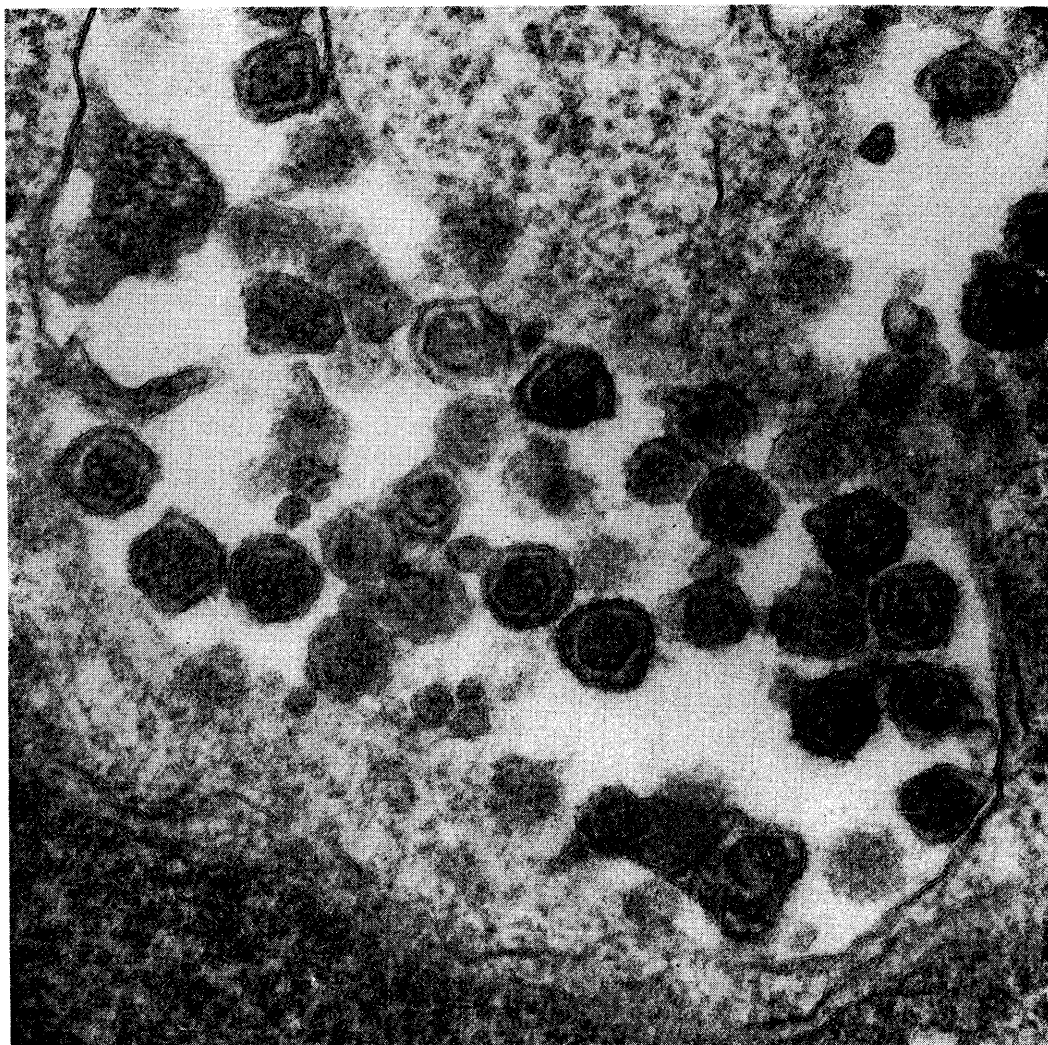


Fig. 2. Mature C viral particles found in an LW-27 ascitic cell. The culture condition was the same as in Fig. 1. ($\times 112,500$)

is a pre-requisite for the perpetuation of the leukemic nature of a cell. Although treatment of (LW-27)B cells in short-term cultures with the widely used and most effective viral activators, BrdU and IdU, failed to have any viral activation, it is of interest to note that preliminary data of molecular hybridization performed by Dr. Robert Callahan of Dr. Todaro's laboratory at NCI clearly demonstrated that

cellular DNA of (LW-27)B lymphoma had an incomplete copy complementary to MuLV DNA probe, while cellular DNA of LW-27 lymphoma had 4 or 5 complete copies complementary to MuLV DNA probe (personal communication). If these data were confirmed and extended in further studies, this suggests that the partial viral information integrated into the host genome is too small to be activated. A similar case

was observed in the endogenous viral information in normal NIH Swiss mouse cells⁽³⁾ which had its reverse transcriptase and antigen messages eliminated and lacked factor(s) to support host cell proliferation *in vitro*. This line, however, still carries the oncogenic information which dictates the host cell to be permanently neoplastic. Compare to this latter system, our study is still unique in that a cell can be leukemic in the absence of tumor specific cell surface antigen.

With the establishment of permanent *in vitro* lines of LW-27 lymphoma, an abundant source of RadLV, which until recently was found to be difficult to grow in tissue culture⁽⁷⁾, is now available. Furthermore, a number of important experiments can be carried out in the future. We are now equipped with a better tool to investigate the mechanism by which LW-27 cells can be altered to (LW-27)B. In other words, what factor(s) is(are) responsible for the loss of virus and viral antigen? If the immunologic factors play a role for the losses *in vivo*, we might be able to accomplish this *in vitro* by adding proper antiserum to the culture medium. The phenomenon of correlation between virus production and cell proliferation of these two RadLV rat lymphomas is itself interesting. Therefore with further delineation of the viral genome, we might be able to map the specific viral segment which is responsible for the growth of host cells in culture.

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大老鼠血癌細胞在人工培養劑中的細胞增殖與 毒素生產之關係

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由放射線血癌毒素 (Rad LV) 所誘致的大老鼠血癌細胞，經多代的活體移接後，分別形成 LW-27 (在經放射線處理之鼠體內) 及 (LW-27) B (在未經放射線處理之鼠體內) 兩個癌細胞系。前者在人工培養劑中，很易形成永久性細胞系，並有毒素顆粒及毒素抗原產生；後者在人工培養劑中，只能作短期增殖，無法建立永久性細胞系，細胞內無毒素顆粒，亦無毒素抗原。此等癌細胞之生長習性似與血癌毒素之有無有關。