Acta Medica Okayama

Volume 32, *Issue* 2 1978 *Article* 8

JUNE 1978

Absence of C-type virus production in human leukemic B cell, T cell and null cell lines.

Hajime Ogura* Shunkichi Hiraki† Sachiko Omura‡

Isao Miyoshi** Takuzo Oda††

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,

Absence of C-type virus production in human leukemic B cell, T cell and null cell lines.*

Hajime Ogura, Shunkichi Hiraki, Sachiko Omura, Isao Miyoshi, and Takuzo Oda

Abstract

Electron microscope observation of cultured human leukemic B cell, T cell and null cell lines and reverse transcriptase assay of the culture supernatants were all negative for the presence of C-type virus. Bat cell line, which propagates primate C-type viruses well, was cocultivated with the human leukemic cell lines, in the hope of amplification of virus if present. Three weeks after mixed culture, the culture supernatants were again examined for reverse transcriptase activity and the cells were tested for syncytia formation by cocultivation with rat XC, human KC and RSb cell lines. All these tests, except for the positive control using a simian sarcoma virus, were negative, suggesting that no C-type was produced from these human leukemic cell lines.

KEYWORDS: C-type virus, established human leukemic cell lines

---

*PMID: 79301 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 32, (2), 169—172 (1978)

BRIEF NOTE

ABSENCE OF C-TYPE VIRUS PRODUCTION IN HUMAN LEUKEMIC B CELL, T CELL ANE NULL CELL LINES

Hajime OGURA,[1,3] Shunkichi HIRAKI,[2] Sachiko OMURA,[1] Isao MIYOSHI,[2] and Takuzo ODA[1]

1. Department of Biochemistry, Cancer Institute:
2. Department of Medicine, Okayama Uuiversity Medical School, Okayama 700, Japan

Received March 24, 1978

Abstract. Electron microscope observation of cultured human leukemic B cell, T cell and null cell lines and reverse transcriptase assay of the culture supernatants were all negative for the presence of C-type virus. Bat cell line, which propagates primate C-type viruses well, was cocultivated with the human leukemic cell lines, in the hope of amplification of virus if present. Three weeks after mixed culture, the culture supernatants were again examined for reverse transcriptase activity and the cells were tested for syncytia formation by cocultivation with rat XC, human KC and RSb cell lines. All these tests, except for the positive control using a simian sarcoma virus, were negative, suggesting that no C-type virus was produced from these human leukemic cell lines.

Key words: C-type virus, established human leukemic cell lines.

The role of viruses with regard to leukemia has been well established in experimental animals. Recently, several reports have appeared suggesting the presence of C-type RNA virus in human leukemia also (1–5) and in human multiple myeloma (6, 7). Although the oncogenicity of these viruses in human and their original hosts are still unknown, these reports stimulated us to search for C-type RNA virus in human leukemia. Previously, we reported the establishment of human leukemic B cell (BALL-1), T cell (TALL-1) and null cell (NALL-1) lines (8–11). This paper is concerned with the search for production of C-type RNA virus in these human leukemic cell lines using electron microscopic, enzymatic and biological techniques.

The human leukemic BALL-1, TALL-1 and NALL-1 cell lines were studied by electron microscopy with the ultra-thin section method. No virus-like

3. Present Address: Department of Virology, Cancer Institute, Okayama University Medical School

particle was detected in any of these cell lines as reported previously (9–11). The culture supernatants of the cell lines were assayed for reverse transcriptase activity. The reaction mixture consisted of 40 mM Tris-HCl, 60 mM KCl, 1 mM DTT, 0.1% Triton X-100, 2 μCi ^{3}H-dTTP, 0.02 OD_{260} poly (rA)·oligo (dT_{12}), 100 μl of 100 fold-concentrated culture supernatants and as a divalent cation 0.5 mM $MnCl_2$ or 5 mM $MgCl_2$ was added. The results were negative (Table 1). The negative results in the electron microscope studies and enzyme assay, however, did not completely exclude the presence of C-type virus because of the technical limitation of the method in detecting a very low level of virus production.

TABLE 1. REVERSE TRANSCRIPTASE ASSAY OF THE CULTURE SUPERNATANTS

Cultures	^{3}H cpm[a]
BALL-1	52
TALL-1	57
NALL-1	45
BAT/88	41
BAT/88 + BALL-1	49
BAT/88 + TALL-1	33
BAT/88 + NALL-1	39
BAT/88 + SSV_1-$SSAV_1$[b]	6414

a Each of the culture supernatants was centrifuged at 10,000×g for 30 min to remove cell debris. Ten ml of the supernatants were pelleted by centrifugation through a 2.0 ml cushion of 20% (v/v) glycerol and resuspended in 0.1 ml of 0.01 M Tris-HCl buffer in order to determine the total activity of reverse transcriptase by measuring the incorporation of the radioactive precursor ^{3}H-TMP into the acid precipitable fraction. The data show manganese-requiring exogenous reaction. When magnesium instead of manganese was used, all tritium cpm were low (data not shown).

b BAT/88 cells were infected with SSV_1-$SSAV_1$ three weeks before the tests.

Bat lung cell line (BAT/88), which propagates primate C-type virus well (12), was obtained via Dr. M. Hill (Villejuif, France) from the laboratory of Dr. R. C. Gallo (Bethesda, U. S. A.). With the hope of amplifying a possible human leukemia virus by cocultivation of leukemic cells with permissive fibroblastic cells as in the case with ovine and bovine leukemia viruses (13), BALL-1, TALL-1 and NALL-1 cell lines were each cocultivated with BAT/88 cells. After three weeks of cocultivation, reverse transcriptase assay of the culture supernatants and syncytia formation assay by mixed culture were performed as described previously (13–16). The positive control for both assays was simian sarcoma virus-simian sarcoma virus associated virus (SSV_1-$SSAV_1$) complex obtained from Dr. R. R. Friis (Giessen, W. Germany). To avoid contamination of human leukemic cell lines with SSV_1-$SSAV_1$, studies on SSV_1-$SSAV_1$ production from BAT/88

cells were performed in the Department of Biochemistry, Cancer Institute, while cocultivation of the human leukemic cell lines with BAT/88 cells was done in the Department of Medicine.

As shown in Table 1, the culture supernatants of cocultivated human leukemic cell lines with BAT/88 cells were negative for reverse transcriptase activity, while that of the SSV_1-$SSAV_1$-infected BAT/88 cells showed a clearly positive result.

SSV_1-$SSAV_1$ induced syncytia formation in rat XC, human KC and RSb cells (Ocho, M., Ogura, H., Tanaka, T. and Oda, T., manuscript in preparation). Furthermore, it has been reported that the viruses isolated from human leukemic materials induced XC cell syncytia formation (4, 5). Accordingly, syncytia formation assay by mixed culture of XC, KC and RSb cells with BAT/88 cells which had been cocultivated with human leukemic cell lines or infected with SSV_1-$SSAV_1$ was performed. As Table 2 shows, only the BAT/88 cells infected

TABLE 2. SYNCYTIA FORMATION ASSAY BY COCULTIVATION[a]

	XC	KC	RSb
BAT/88	−	−	−
BAT/88 + BALL-1	−	−	−
BAT/88 + TALL-1	−	−	−
BAT/88 + NALL-1	−	−	−
BAT/88 + SSV_1-$SSAV_1$	+	+	+

a BAT/88 cells, BAT/88 cells cocultivated with each of the human leukemic cell lines or infected with SSV_1-$SSAV_1$ were each cocultivated overnight with XC, KC and RSb cells. After Giemsa staining they were examined for syncytia using a light microscope.
+ positive, or − negative for syncytia formation.

with SSV_1-$SSAV_1$ was positive for syncytia formation of XC, KC and RSb cells. From all these results, we conclude that the human leukemic cell lines produce no C-type RNA virus, although the possibility remains that C-type virus may be present in a masked form in these cell lines.

Acknowledgment. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Baxt, W., Hehlman, R. and Spiegelman, S.: Human leukemic cells contain reverse transcriptase associated with a high molecular weight virus-related RNA. *Nat. New Biol.* **240**, 72-75, 1972.
2. Sarngadharan, M. G., Sarin, P. S., Reitz, M. S. and Gallo, R. C.: Reverse transcriptase activity of human acute leukemic cells: Purification of the enzyme, responce to AMV 70 S RNA and characterization of the DNA product. *Nat. New Biol.* **240**, 67-72, 1972.

3. Mak, T. W., Manaster, J., Howatson, A. F., McCulloch, E. A. and Till, J. E.: Particles with characteristics of leukoviruses in cultures of marrow cells from leukemic patients in remission and relapse. *Proc Natl. Acad. Sci. U. S. A.* **71**, 4336-4340, 1974.
4. Teich, N. M., Weiss, R. A., Salahuddin, S. Z., Gallagher, R. E., Gillespie, D. H. and Gallo, R. C.: Infective transmission and characterization of a C-type virus released by cultured human myeloid leukemia cells. *Nature* **256**, 551-555, 1975.
5. Nooter, K., Aarssen, A. M., Bentvelzen, P., DeGroot, F. G. and Van Pelt, F. G.: Isolation of infectious C-type oncorna-virus from human leukemic bone marrow cells. *Nature* **256**, 595-597, 1975.
6. Sawada, H., Tashima, M., Nakamura, T., Uchiyama, T., Sagawa, K., Takatsuki, K., Uchino, H. and Ito, Y.: RNA-reverse transcriptase complex from cultured human myeloma-leukemia cells. *Int. J. Cancer* **20**, 15-20, 1977.
7. Grofova, M., Popovic, M., Ogura, H., Motoska, J., Lizonova, A., Nilsson, K. and Kuzela, S.: The retrovirus particles in human myeloma cells RPMI6226: Morphological, biochemical, immunological and infective transmission studies. *Neoplasma* (in press).
8. Miyoshi, I., Hiraki, S., Tsubota, T., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H., Kimura, I. and Masuji, H.: Human B cell, T cell and null cell leukaemic cell lines derived from acute lymphoblastic leukaemias. *Nature* **267**, 843-844, 1977.
9. Hiraki, S., Miyoshi, I., Masuji, H., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H., Chen, P. and Kimura, I.: Establishment of an Epstein-Barr virus-determined nuclear antigen-negative human B-cell line from acute lymphoblastic leukemia *J. Natl. Cancer Inst.* **59**, 93-94, 1977.
10. Hiraki, S., Miyoshi, I., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H. and Masuji, H.: Human leukemic "null" cell line (NALL-1) *Cancer* **40**, 2131-2135, 1977.
11. Hiraki, S., Miyoshi, I., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H., Machida, K., Masuji, H., and Kimura, I.: Establshment of a T-cell line from human lymphosarcoma. *Gann* **69**, 115-118, 1978.
12. Schnitzer, T. J., Weiss, R. A. and Zavada, J.: Pseudotypes of vesicular stomatitits virus with the envelope properties of mammalian and primate retroviruses. *J. Virol.* **23**, 449-454, 1977.
13. Ogura, H., Paulsen, J. and Bauer, H.: Cross neutralization of ovine and bovine C-type leukemia virus-induced syncytia formation. *Cancer Res.* **37**, 1486-1489, 1977.
14. Ogura, H.: XC cell fusion by murine leukemia viruses: fusion from without. *Med. Microbiol. Immunol.* **162**, 175-181, 1976.
15. Ogura, H. and Oda, T.: Studies on the human KC cell syncytia formation induced by Mason-Pfizer monkey virus. *Acta Med. Okayama* **31**, 243-247, 1977.
16. Ogura, H., Tanaka, T., Ocho, M., Kuwata, T. and Oda, T.: Detection of Mason-Pfizer monkey virus infection by syncytia formation of human cells doubly transformed by Rous sarcoma virus and simian virus 40. *Arch. Virol.* in press.