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Type C virus particles produced in human T-cell lines derived from acute lymphoblastic leukemia and a leukemic T-lymphoid malignancy.

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Abstract

Electron microscopy of four human T-cell lines revealed the production of type C virus particles in two T-cell lines: one derived from acute lymphoblastic leukemia and the other from a leukemic T-lymphoid malignancy. Virus particles isolated from these cells had reverse transcriptase activity and the major internal structural protein of 30,000 daltons (p30). The indirect immunofluorescence test of these virus-producing cells with sera of patients with adult T-cell leukemia (ATL) was negative. The data indicate that these retroviruses are different from adult T-cell leukemia virus (ATLV).

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— BRIEF NOTE —

TYPE C VIRUS PARTICLES PRODUCED IN HUMAN T-CELL LINES DERIVED FROM ACUTE LYMPHOBLASTIC LEUKEMIA AND A LEUKEMIC T-LYMPHOID MALIGNANCY

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Abstract. Electron microscopy of four human T-cell lines revealed the production of type C virus particles in two T-cell lines : one derived from acute lymphoblastic leukemia and the other from a leukemic T-lymphoid malignancy. Virus particles isolated from these cells had reverse transcriptase activity and the major internal structural protein of 30,000 daltons (p30). The indirect immunofluorescence test of these virus-producing cells with sera of patients with adult T-cell leukemia (ATL) was negative. The data indicate that these retroviruses are different from adult T-cell leukemia virus (ATLV).

Key words : type C virus particles, human T-cell lines, electron microscopy, virion proteins, immunofluorescence test.

Type C retroviruses (HTLV-I, II and ATLV) were isolated from T-cells and T-cell lines derived from patients with cutaneous T-cell lymphoma and leukemia (1-2), adult T-cell leukemia (3-5), and hairy cell leukemia (6-7). HTLV-I and ATLV have been shown to be identical or closely related (8). We recently isolated a type C retrovirus (TALV) from a human T-cell line (TALL-1) derived from acute lymphoblastic leukemia (9). TALV has the major internal structural protein of 34,000 daltons (p34) and is different from HTLV-I, II and ATLV (9-12). In the present paper, we report the production of type C retroviruses from two other human T-cell lines ; Molt-4 derived from acute lymphoblastic leukemia and HPB-MLT from a leukemic T-lymphoid malignancy. These viruses were found to be different from ATLV and TALV.

Materials and Methods. The human T-cell lines examined for the presence of virus particles were Molt-4 (13) and CCRF-CEM (14) derived from acute lymphoblastic leukemia, Jurkat from T-cell lymphoma, and HPB-MLT from a leukemic T-lymphoid malignancy (15). Molt-4 was provided by Dr. H. Hidaka of the Hayashibara Research Institute for Biological Chemistry, and other cell lines were provided by Dr. T. Tanaka of Kagawa Medical College. MT-2, an ATLV-producing T-cell line, was a generous gift of Dr. I. Miyoshi of Kochi

Medical College. These cell lines were cultured in RPMI-1640 medium supplemented with heat-inactivated 20 % fetal bovine serum at 37 ℃ in an atmosphere of 5 % CO_2 in air under conditions precluding laboratory contamination. The fetal bovine serum was free of viral particles. Cell suspensions were centrifuged at 1,000 × g for 10 min, the supernatant was removed, and the pellet was fixed with 2.5 % glutaraldehyde and 1 % osmium tetroxide, dehydrated in an ethanol series, and embedded in Epon 812. Thin-sectioned specimens were stained with 2 % uranyl acetate and lead citrate, and examined under an electron microscope. Viral particles were isolated as described (10-12). Virion proteins were analyzed by 13 % polyacrylamide-sodium dodecyl sulfate (SDS) slab gel electrophoresis according to the method of Laemmli (16). The indirect immunofluorescence test of virus-producing cells was performed as described elsewhere (3).

Results. Electron microscopy of thin sections of these human T-cells revealed the production of type C viruses in two cell lines, Molt-4 and HPB-MLT (Figs. 1 and 2). To distinguish these viruses from HTLV/ATLV and TALV, the type C virus detected in Molt-4 was tentatively designated as MOLV and that in HPB-MLT as MLTV. Many virus particles were found extracellulaly, and some in the cisternal spaces. Typical type C virus particles budding from cell membranes were also observed (Figs. 1 and 2 b-e). Most of the extracellular MOLV were typical mature type C particles measuring 118.4 nm ± 12.9 nm in diameter with a centrally located and homogenously condensed nucleoid (78.6 nm ± 11.1 nm). Intracisternal MOLV particles having budded or budding from the endoplasmic reticulum membranes were immature type A particles with a ring- or arc-form nucleoid (Fig. 1f). Most of the extracellular MLTV were mature type C particles measuring 119.4 nm ± 18.3 nm in diameter with a centrally located and unhomogenously condensed nucleoid (79.3 nm ± 12.0 nm). Intracisternal immature type A particles of MLTV having budded or budding from the endoplasmic reticulum membranes were also observed (Fig. 2f).

The indirect immunofluorescence test of MOLV-producing Molt-4 cells and MLTV-producing HPB-MLT cells using sera from patients with adult T-cell leukemia was negative, while that of ATLV-producing MT-2 cells using the same sera was strongly positive. Molt-4 cells and HPB-MLT cells were not stained with antisera to TALV, bovine leukemia virus (BLV), feline leukemia/sarcoma

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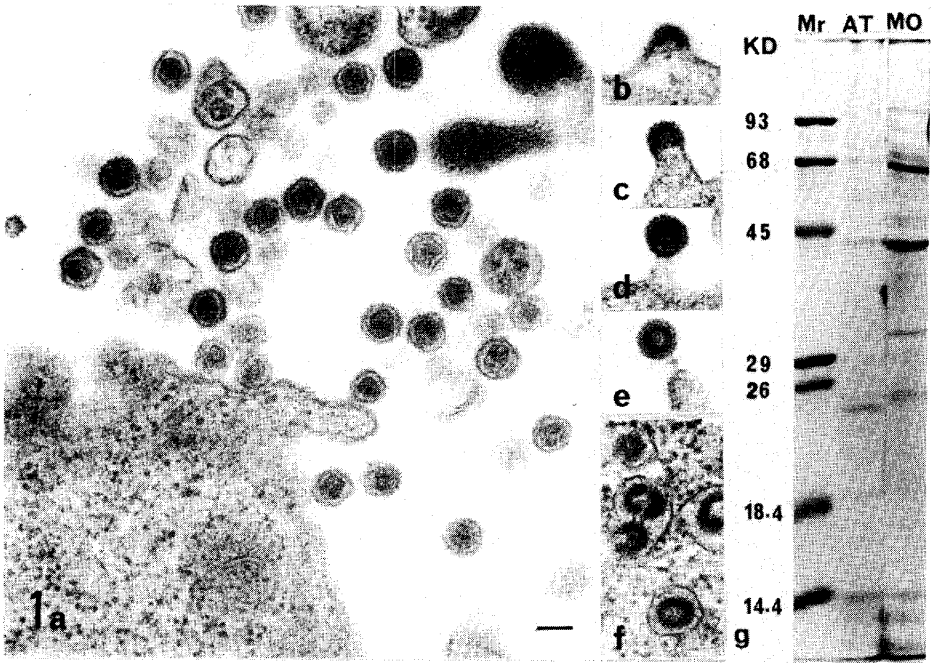
Fig. 1. Electron micrographs of MOLV-producing Molt-4 cells (a), type Cvirus particles budding from cell membranes (b, c, d and e), intracisternal budding and immature particles (f), and protein bands of partially purified MOLV electrophoresed in the SDS-polyacrylamide gel and stained with Coomassie blue (g). Mr, molecular markers in kilodaltons (KD) ; AT, ATLV proteins ; MO, MOLV proteins. a-f, × 46,600.

Fig. 2. Electron micrographs of MLTV-producing HPB-MLT cells (a), budding viruses in various stages (b, c, d and e), intracisternal budding and immature particles (f), and protein bands of partially purified MLTV electrophoresed in the SDS-polyacrylamide gel and stained with Coomassie blue (g) ML, MLTV proteins. a-f. × 46,600.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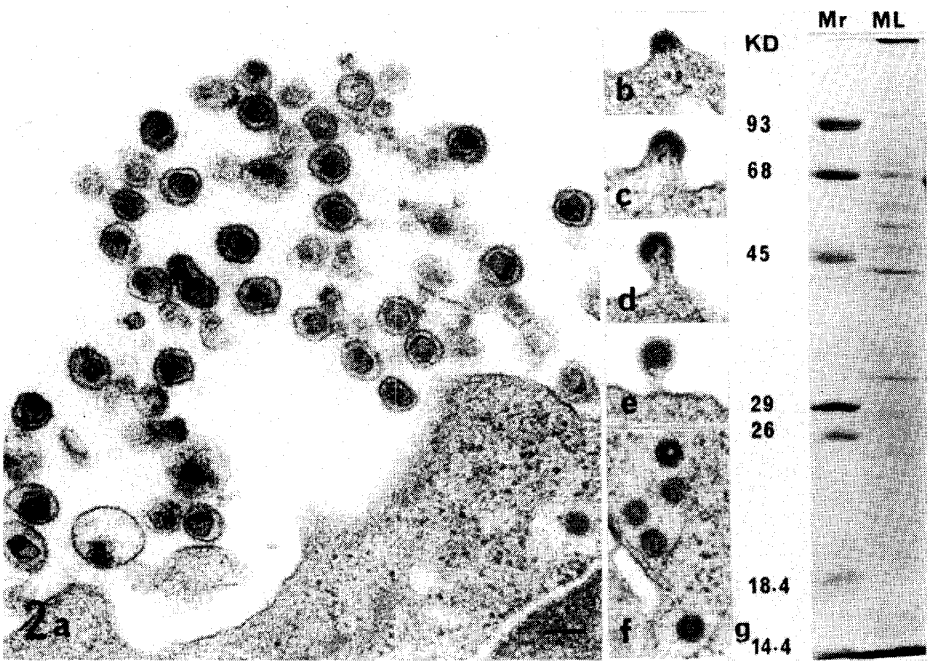
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Mr ML

viruses (FeLV/FeSV), simian sarcoma virus (SSV-1/SSAV-1), avian leukemia virus (ALV), avian myeloblastosis virus (AMV), and vesicular stomatitis virus (VSV), but were faintly stained with antisera to murine sarcoma/leukemia viruses (MSV/MLV).

Both MOLV and MLTV had the major internal structural protein of 30,000 daltons (p30) (Figs. 1 g and 2 g) and showed reverse transcriptase activity preferring Mn^{2+} in a reaction with polyriboadenylate·oligodeoxyribothymidylate [poly (A)·oligo (dT) $_{12-18}$] as a synthetic template-primer.

Discussion. The data presented in this report strongly suggest that MOLV and MLTV are different from ATLV (HTLV) and TALV. The major internal structural protein of MOLV and MLTV is p30, while that of ATLV (HTLV) and TALV is p24 and p34, respectively. Although the ultrastructures of MOLV and MLTV appear to be slightly different, electrophoretic mobilities of their virion proteins were similar. Further characterization of proteins, RNA, and immunological properties of purified particles of these retroviruses is now under way to determine the origin of these viruses. Comparative studies of the mode of integration and expression of ATLV (HTLV), TALV, MOLV and MLTV genomes in human T-cells and T-cell lines should be quite significant in obtaining further information on the viral origin of human T-cell lymphoma and leukemia.

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