A novel monolayer cell line derived from human umbilical cord blood cells shows high sensitivity to measles virus

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Measles virus (MeV) research is largely dependent on the B95a cell line, which is derived from marmoset B lymphocytes. As this cell line is persistently infected with Epstein–Barr virus (EBV), a novel cell line, COBL-a, was established from human umbilical cord blood. COBL-a cells have a significant advantage over B95a cells because they are of human origin, are free from EBV and have higher sensitivity to wild-type MeV. Thus, COBL-a cells should prove very valuable for both epidemiological and basic studies of MeV.

Over the past 40 years, measles virus (MeV) research has been conducted by using established cell lines (mostly Vero cells), but epidemiological and pathogenesis research of MeV has been limited by the difficulty in isolating MeV from patients and the failure to produce measles-specific disease in monkeys. In 1990, we established a subline of B95-8 cells (Miller et al., 1972) from marmoset B lymphocytes, named B95a (Kobune et al., 1990), which is highly sensitive to wild-type (wt) MeV. The B95a cell line not only overcame the lengthy isolation problems associated with Vero cells, but also solved mutation- and virus-selection difficulties. As a result, the B95a cell line has contributed to the construction of a phylogenetic tree of MeV prevalence across the globe (Rota & Bellini, 2003; Nakayama et al., 2004). In addition, MeV isolated from B95a cells maintains its virulence in monkey models, in contrast to the Edmonston strain of MeV that had been used as a standard for nearly 50 years, but was avirulent in monkeys. However, B95a cells are of non-human primate origin and are persistently infected with Epstein–Barr virus (EBV). To overcome these drawbacks, we attempted to establish a novel cell line from human umbilical cord blood.

Heparinized umbilical cord blood was obtained from a healthy Japanese patient in an obstetrics and gynaecology hospital, who provided informed consent. The lymphocyte-rich fraction was separated and suspended in culture medium consisting of RPMI 1640 medium supplemented with 10% fetal calf serum in the presence of concanavalin A. The cell suspension was maintained in a 5% CO₂ incubator at 37°C. One cell colony that appeared spontaneously was retrieved, transposed into a single-cell suspension and subcultured in a plastic flask. Adapted cells that grew by adhering to the vessel surface were selected and designated COBL-a (adherent cord blood) cells. COBL-a cells have been maintained in RPMI 1640 medium containing 5% fetal calf serum without concanavalin A.

The structure of the cell surface was observed by electron microscopy. COBL-a cells were fixed in glutaraldehyde and OsO₄, dehydrated by ethanol and propylene oxide and embedded in an Epon 812–propylene oxide mixture. Ultrathin sections stained with uranyl acetate and lead citrate were examined by using an electron microscope (model JEM-1220; JEOL DATUM Ltd). COBL-a cells resemble lymphoid cells and have either densely packed microvilli or a smooth surface (Fig. 1a). Next, to characterize the cell-surface antigens of COBL-a cells, cells were stained with fluorescein isothiocyanate (FITC)-labelled mouse monoclonal antibodies to CD2, CD4, CD8, CD16, CD19, CD34, CD45, CD46, CD69 (PharMingen) or CD150 (BioLegend Company) and then subjected to flow cytometry. Positive staining for the lymphoid cell-surface antigens CD4 (helper/inducer T
cells), CD38 (plasma cells, thymocytes, activated T cells, early T/B cells and monocytes), CD45 (leukocyte common antigen), CD46 (MCP, receptor for MeV vaccine strain), CD69 (activated T/B cells, activated macrophages and NK cells) (data not shown) and CD150 (SLAM, receptor for MeV vaccine and wt strains; thymocytes, T/B cells) (Fig. 1b) was observed. Therefore, COBL-a cells were considered to be a T-cell lineage.

To identify the origin of COBL-a cells, the sequence of the β-actin gene was compared to that of B95a cells. Briefly, total RNA of B95a and COBL-a cells was reverse-transcribed by using SuperScript II reverse transcriptase (Gibco-BRL) and the portion of the β-actin gene that corresponded to nt 2908–3108 in the open reading frame (ORF) was amplified by PCR using Thermo-Start Taq DNA polymerase (ABgene) and specific primer pairs (sense, 5'-CCTTCCCTGGCAGGTCC-3'; antisense, 5'-GAGCATGACTTGCATC-3') for 30 cycles according to the manufacturer’s instructions (ABgene). The PCR product sequences were confirmed by using a DNA sequencer. The nucleotide sequence of the β-actin gene in COBL-a cells was identical to the human sequence (data not shown).

Further, the absence of endogenous EBV and retroviruses in COBL-a cells was confirmed. The EBV BamHI-W fragment and the BARF1 ORF were amplified by PCR with a specific primer pair (Teng et al., 1996) using a cDNA template from COBL-a or B95a cells and the same PCR conditions as described above. EBV-specific DNA sequences were not detected by PCR (Fig. 1c). In addition, cell-associated internal and external virus-like particles were not observed by electron microscopy and reverse transcriptase was not detected in the cell extracts by using non-radioactive RT assay ELISA kits (Roche) (data not shown). Thus, COBL-a cells were considered to be free of both EBV and retroviruses.

Susceptibility of COBL-a cells to a typical wt MeV isolate, the Ichinose B (IB) strain (Kobune et al., 1990), was tested. After 12 h infection with the IB strain, the formation of small syncytia was induced in COBL-a cells (Fig. 2a, b). By 72 h, the syncytia had spread rapidly and caused cell detachment. The maximum titre of infectious virus was obtained from the COBL-a cells after approximately 48 h infection (Fig. 2c). The sensitivity of COBL-a cells to MeV

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Fig. 1. (a) Electron micrographs of COBL-a cells (magnification: left, x2500; right, x8000). Bars, 2 μm (left); 1 μm (right). (b) Flow-cytometry analysis of the cell-surface expression of CD150. COBL-a cells were incubated with a monoclonal antibody against CD150, followed by staining with FITC-labelled anti-mouse IgG. (c) Detection of EBV by PCR. PCR was carried out as described in the text. Amplified fragments corresponding to the EBV BamHI-W fragment (121 bp), EBV BARF1 ORF (697 bp) and cellular β-actin (201 bp) were subjected to agarose-gel electrophoresis.

Fig. 2. Syncytium formation by MeV. COBL-a cells (a) were infected with MeV IB at an m.o.i. of 0.001. (b) Cells were photographed under a microscope 12 h after inoculation. (c) Comparison of MeV IB replication in COBL-a cells and B95a cells. After infection at an m.o.i. of 0.001, the titres of cell extracts (labelled ‘cell’) and culture fluids (labelled ‘sup.’) were determined as log(TCID₅₀) ml⁻¹ at the indicated time points.
was further compared with that of B95a and Vero/h-SLAM cells (Tatsuo et al., 2000), both of which are monkey cell lines that are highly sensitive to wt MeV, using five blood specimens from measles patients in addition to the IB strain. The infectivity titres of the IB strain and the five blood specimens are shown in Fig. 3(a). The same titre of the IB strain was obtained from the COBL-a, B95a and Vero/h-SLAM cells. A higher titre was observed on four of five blood specimens from the COBL-a cells than from the other two cell lines. By using TaqMan RT-PCR analysis, the minimum quantity required for virus isolation from the specimens was estimated to be >400 copies per 100 μl from the COBL-a cells and >1000 copies per 100 μl from the B95a and Vero/h-SLAM cells (data not shown). wt MeV isolated in COBL-a cells from a throat-swab specimen of a patient with typical measles was subcultured once in the cells and was used for testing virulence in two cynomolgus monkeys, Macaca fascicularis, that were confirmed to have no anti-MeV antibodies. The monkeys developed measles-specific maculopapular rashes on their faces, abdomens and backs by day 8. Both monkeys showed a decrease in the number of leukocytes to <40% from day 4 to day 10 (time of autopsy) (Fig. 3b). MeV-specific giant cells were observed in the thymus.

From these data, COBL-a cells were confirmed to be of human origin, free of EBV and highly sensitive to wt MeV infection. Importantly, wt MeV that was isolated from COBL-a cells maintained its virulence in monkeys just as effectively as wt MeV that was isolated from B95a cells (Kobune et al., 1996). As a result, COBL-a cells will probably be valuable for both epidemiological and fundamental MeV studies. In particular, COBL-a cells will contribute to the application of Human Genome Project results into microarray analysis, as this requires the exclusive use of human cells that need to be free of other virus contamination.

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