Infection of vascular endothelial cells (ECs) is assumed to contribute to dissemination of human cytomegalovirus (HCMV). Investigation of virus–host interactions in ECs such as human umbilical vein endothelial cells (HUVECs) is limited due to the low maximal passage numbers of these primary cells. We tested a conditionally immortalized EC line (HEC-LTT) and a permanent cell line (EA.hy926) for their susceptibility to HCMV infection. Both cell lines resembled HUVECs in that they allowed for entry and immediate early protein expression of highly endotheliotropic HCMV strains but not of poorly endotheliotropic strains, rendering them suitable for analysis of the viral entry mechanism in ECs. The late phase of viral replication and release, however, was supported by growth-controlled HEC-LTT cells but not by EA.hy926 cells. HEC-LTT cells support both the early and late phase of viral replication and release infectious progeny virus at titers comparable to primary HUVECs; thus, the HEC-LTT cell line is a cell culture model representing the full viral replicative cycle of HCMV in ECs. The implementation of permanent HEC-LTT and EA.hy926 cell lines in HCMV research will facilitate long-term approaches that are not feasible in primary HUVECs.

Human cytomegalovirus (HCMV) infection of vascular endothelial cells (ECs) is thought to promote hematogenous viral dissemination (1–7) and to be a possible factor in the development of vascular lesions (5,8–10). The initial events in HCMV infection of ECs and viral EC tropism are commonly studied in primary human umbilical vein endothelial cells (HUVECs) (5). Primary HUVECs possess only a limited proliferation potential, and their life span in vitro is short. As a result, they cannot be expanded to high cell numbers and do not allow for long-term studies of cells from a single donor. Consequently, donor-to-donor variation might become an issue when working with primary HUVECs, and generation of large amounts of EC-derived virus is difficult. Since most immortalized cell lines, such as oligodendroglia cells or monocytic cell lines, allow HCMV replication only to a minor extent and exclusively after differentiation (11–14), HCMV research currently greatly depends on primary cells. As an exception, telomerase-immortalized microvascular endothelial (TIME) cells appear to support the full HCMV replicative cycle since they closely resemble
HUVECs in terms of virus release and spread pattern (15,16). Although previous work suggests that ECs from different tissues behave similarly in HCMV infection (4), a cell line derived from HUVECs would be desirable in the future to complement primary HUVECs in long-term or large-scale experiments. EA.hy926 cells could fulfill this requirement as this cell line represents a hybrid of HUVECs and the lung carcinoma cell line A-549 (17). However, analysis of virus replication in permanent cell lines is impaired by the fact that uninfected cells proliferate at higher rates and outgrow infected cells. Recently, a conditionally immortalized cell line termed HEC-LTT has been generated by introduction of doxycycline (Dox) controlled expression cassettes for human telomerase catalytic subunit (hTERT) and simian virus 40 large T-antigen (SV40-Tag) into HUVECs (18). In the presence of the inducer, hTERT and SV40-Tag expression are activated, resulting in high cell proliferation and unlimited expansion. In the absence of the inducer, cells stop proliferating and can be maintained in a growth-arrested state that reflects the status of endothelial cells in vivo. Thus, expansion of cells can be tightly controlled by addition or withdrawal of Dox. Of note, HEC-LTT cells retain the phenotypic characteristics of HUVECs, such as expression of CD31 and CD34, the capacity to take up macromolecules, and the ability to form tube-like structures (18).

In this work, we analyzed HCMV infection in HEC-LTT and EA.hy926 cells in order to find out whether these permanent cell lines could serve as cell culture models for infection of ECs. To this end, we tested the susceptibility of the cell lines to highly and poorly endo- and heterotrophic HCMV variants and investigated the cytopathogenicity and expression of viral proteins representative of the three kinetic classes of viral gene products. Finally, the released infectious virus was quantified. Our results demonstrate that EA.hy926 cells are susceptible to HCMV during the initial steps of infection, yet viral replication is subsequently halted in the early phase. HEC-LTT cells, in contrast, allow productive viral replication comparable to primary HUVECs. Therefore, while EA.hy926 cells can support the initial events of infection, HEC-LTT cells will be useful for investigating virus-host interactions throughout the full replicative cycle.

**Materials and methods**

### Cells and viruses

**Generation and characterization of HEC-LTT cells**

HEC-LTT cells are derived from HUVECs (18); in that publication, the cells were designated as “HUCV uni-Tag” and “bi-hTert.” HEC-LTT cells were cultured in endothelial growth medium (EGM BulletKit; Lonza Sales Ltd., Basel, Switzerland) supplemented with 2 µg/mL Dox (Sigma-Aldrich, Saint Louis, MO) in culture vessels coated with 0.1% gelatin (Sigma-Aldrich). If not stated otherwise, HEC-LTT cells were infected 1 day after withdrawal of Dox. HEC-LTT cells were used from passage 40 to passage >120 corresponding to >200 population doublings with no apparent changes in permissivity to infection by HCMV strains of different cell tropism. EA.hy926 cells (ATCC: CRL-2922; obtained in October 2013 from LGC Standards, Wesel, Germany) (17) were cultured in DMEM (Life Technologies GmbH, Darmstadt, Germany) with 10% FBS (PAA, Pasching, Austria) and 100 µg/mL gentamicin (Life Technologies). Human foreskin fibroblasts (HFFs) were cultured in MEM supplemented with GlutaMAX (Life Technologies), 5% FBS, 0.5 ng/mL basic fibroblast growth factor (bFGF) (Life Technologies), and 100 µg/mL gentamicin. During experiments, bFGF was omitted from the HFF medium. Human vascular endothelial cells (HUVECs) were maintained in RPMI1640 medium (Life Technologies) supplemented with 10% HCMV-seronegative human serum, 50 µg/mL endothelial cell growth supplement (BD Biosciences, Bedford, MA), 5 international units/mL heparin (Sigma-Aldrich), and 100 µg/mL gentamicin. HUVECs were kept in gelatin-coated culture vessels.

HCMV TB40/F and TB40/E strains had originally been obtained in our laboratory by propagating an isolate from a bone marrow transplant patient for 22 passages in HFFs and HUVECs, respectively (19). Virus stocks were obtained by collecting supernatant from infected HFF cultures at 5–7 days post infection (p.i.). The supernatants were centrifuged at 3345 × g for 10 min to remove cell debris and then stored at -80°C.

### Indirect immunofluorescence for detection of viral antigen

For detection of infected cells and quantification of infection rates, cells were fixed with 80% acetone (Sigma-Aldrich) and incubated with primary mouse antibody E13 (Argene, Verniolle, France) to detect immediate early antigens 1 and 2 (pUL122/123). Mouse hybridoma supernatants were used to detect pUL44 (clone 5.4; Biotest AG, Dreieich, Germany) and major capsid protein pUL86 (clone 28–4, kindly provided by W. Brit, University of Alabama at Birmingham). In all cases, a Cy3-conjugated goat polyclonal anti-mouse F(ab')2 antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was used as the secondary antibody. Cell nuclei were counterstained with DAPI (Sigma-Aldrich). Visualization was done by fluorescence microscopy with an Axio Observer D1 microscope (Zeiss, Jena, Germany).

The rate of antigen-positive cells reflecting the infection rate or expression rate was determined by counting the number of antigen-positive nuclei and the total number of DAPI-stained nuclei in 3 images per well of a 96-well plate. Two replicate wells per condition were analyzed (n = 6). To calculate the infection/ expression rates, the number of antigen-positive nuclei in an image was divided by the total number of nuclei in the same image, and the mean and standard error of the mean (SEM) of the six images were determined.

### Tropism analysis

Cells were seeded in duplicate at a density of 1 × 10⁴ cells/well in 96-well plates 1 day prior to infection. HUVECs and HEC-LTT cells were seeded in gelatin-coated wells. Following a 30 min pre-incubation of all cells in HFF medium without bFGF, cells were infected for 2 h with serial dilutions of TB40/E or TB40/F in HFF medium. After infection, cells were maintained in their respective media. Expression of pUL122/123 was detected by indirect immunofluorescence, as described above, and quantified by automated counting of stained nuclei (20). The multiplicity of infection (MOI) achieved with the respective virus dilutions was calculated on the basis of infection rates determined in HFFs.

### Documentation of cytopathic effects

For generation of infectious virus stock, HFFs were infected with TB40/E, and the supernatant was harvested 5 days p.i. by centrifugation at 3345 × g for 10 min to remove cell debris. Cells were seeded at a
density of $1 \times 10^5$ cells/well in 6-well plates 1 day prior to infection. Infection was performed with fresh infectious supernatant. The medium was replaced daily, and cell morphology was documented by phase contrast microscopy using a Zeiss Axio Observer D1 microscope.

Quantification of infectious virus release
Prior to the assay, HEC-LTT cells were cultured in EGM medium in the presence of Dox. Deprivation of Dox for at least 1 day reliably stops cell growth, as indicated by stable cell numbers (18). For the assay, cells were each seeded in 3 wells per cell type per plate in 2 replica 6-well plates at a density of 300,000 cells per well.

Cell-type specific medium was used, and Dox (HEC-LTT cells) and bFGF (HFFs) were omitted. One day after seeding, all cells were incubated in HFF medium for 30 min at 37°C prior to infection. Infection was performed with 3 serial dilutions of TB40/E in HFF medium on each plate, yielding 2 identical replica plates per cell type. After 2 h of infection with occasional swirling, the virus suspension was replaced with cell type-specific medium. Twenty-four hours post infection, one of the two replica plates of each cell type was fixed with 80% acetone and immunostained for pUL122/123, as described above, to determine the infection rates. Cultures achieving comparable infection

Figure 1. HEC-LTT and EA.hy926 cells are susceptible to TB40/E but not to TB40/F. Cells were infected with the highly endotheliotropic human cytomegalovirus (HCMV) strain TB40/E or the poorly endotheliotropic strain TB40/F, and expression of immediate early antigens 1 and 2 (pUL122/123) was detected by indirect immunofluorescence 24 h post infection (p.i.). (A) Antibodies against pUL122/123 were visualized by a Cy3-conjugated secondary antibody (red), and nuclei were stained by DAPI (blue). (B) Infection rates of TB40/E (●) and TB40/F (○) were determined by counting pUL122/123-positive nuclei. The MOIs were calculated based on the respective infection rates in human foreskin fibroblasts (HFFs). Error bars: SEM.
rates of 60%–80% (corresponding to an approximate MOI of 1 infectious units (IU)/cell) were selected for analysis of virus release. The remaining plates were washed 5 times with fresh HFF medium, and the medium was replaced daily. Starting from 3 days p.i. (when release of infectious progeny was expected to become detectable) up to 9 days p.i., the supernatants were harvested, centrifuged at 3345 × g for 10 min to remove cell debris, and stored at -80°C. Titers of infectivity were determined in HFFs by a virus dilution series (21).

Quantification of viral genome replication and immunoblotting

Cells (5 × 10^5 per sample) were mock-infected or infected with strain TB40/E. The virus dose was adjusted to achieve comparable MOIs of ~0.8 IU/cell for all cell types. Two hours post infection, cell supernatant containing the virus inoculum was removed, and cells were washed twice with medium. Cells were harvested by trypsinization at 0 (mock), 8, and 16 h p.i., and at 1, 2, 3, 5, and 7 days p.i., split into 2 aliquots (one third for DNA extraction and two thirds for immunoblotting), pelleted by centrifugation, and stored at -80°C until all samples were collected.

DNA extraction was performed using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Quantification of viral DNA was done by real-time PCR assays as described in (22).

For immunoblotting, cells were lysed in 1× Laemmli buffer (4% SDS, 20% glycerol, 2 mmol/L EDTA, 0.02% bromophenol blue, 125 mmol/L Tris, pH 6.8) (Sigma-Aldrich), heated to 95°C for 5 min, and supplemented with 2% β-mercaptoethanol (Sigma-Aldrich). Samples were separated on SDS-PAGE gels and blotted on PVDF membranes. Membranes were blocked with milk powder and incubated with primary antibodies against viral antigens or against actin diluted in PBS/0.3% Tween (viral immediate early antigens: mouse monoclonal antibody E13 by Argene; viral early antigen pUL44: mouse hybridoma supernatant, clone 5.4, Biotest AG; viral late antigen pp150: mouse monoclonal antibody XP1, Dade Behring, Schwalbach, Germany; actin: rabbit monoclonal antibody, A5060, Sigma-Aldrich). HRP-conjugated anti-

mouse Ig and anti-rabbit Ig (sc-2054 and sc-2055; Santa Cruz Biotechnology Inc., Dallas, TX) were used as secondary antibodies.

Results and discussion

Primary ECs are readily infected by highly endotheliotropic HCMV variants such as VHL/E or TB40/E, while infection by poorly endotheliotropic variants such as VHL/F or TB40/F is, in contrast, rare (19,23,24). To test whether the endothelial cell lines HEC-LTT and EA.hy926 allow viral entry and initiate viral gene expression of TB40/E and TB40/F, cells were infected with serial HCMV dilutions corresponding to multiplicities of infection (MOIs) from 1 to 10 IU/cell (Figure 1). HEC-LTT cells were deprived of Dox the day before infection to achieve a growth-arrested state and to avoid overgrowing of non-infected cells. HUVECs and HFFs were used as control cells. Expression of viral immediate early antigens 1 and 2 (pUL122/123) was analyzed 24 h p.i. by indirect immunofluorescence staining (Figure 1A). Characteristic nuclear staining indicated efficiency of initial steps of infection, including initiation of viral gene expression. Remarkably, both permanent cell lines, HEC-LTT and EA.hy926, were in principle susceptible to TB40/E, and both cell lines reached maximum infection rates comparable to primary HFFs and HUVECs (Figure 1A), although EA.hy926 cells required a higher virus dose relative to the other cell types (Figure 1B). Both, HEC-LTT and EA.hy926 cells further resembled primary HUVECs in that TB40/F failed to efficiently initiate viral gene expression. The infection rate in all ECs remained very low (<10%) even at high virus dosages (Figure 1B). Similar results concerning the susceptibility to different virus strains were obtained with the highly endotheliotropic HCMV strain VHL/E and the poorly endotheioiotropic strains VHL/F, AD169, and Towne (data not shown). Taken together, HEC-LTT and EA.hy926 cells, like HUVECs, allowed initiation of viral gene expression of the two highly endotheliotropic HCMV variants, whereas all poorly endotheioiotropic variants were ineffective in these cell lines, suggesting that HEC-LTT and EA.hy926 cells resemble HUVECs with regard to the tropism of HCMV strains.

HCMV infection of susceptible primary cell cultures leads to cytopathic effects (CPEs), distinct morphological altera-
tions indicative of an ongoing viral replication. This phenomenon was used to investigate whether viral replication in HEC-LTT and EA.hy926 cells proceeds beyond the entry phase and immediate early gene expression. To this end, HFFs, HUVECs, HEC-LTT cells, and EA.hy926 cells were infected with TB40/E (Figure 2). The respective virus dose was adjusted to account for the cell type–dependent differences in infectivity observed in the previous experiment (Figure 1) in order to reach similar initial infection rates with all cell types. Cell morphology was documented by phase contrast microscopy for up to 5 days post infection (p.i.). Arrowheads indicate nuclear inclusions.

Figure 2. Human cytomegalovirus (HCMV)-infected HEC-LTT cells but not EA.hy926 cells display characteristic cytopathic effects. Cells were infected with HCMV TB40/E, and morphological changes were documented daily by phase contrast microscopy until 5 days post infection (p.i.). Arrowheads indicate nuclear inclusions.

To analyze this further, expression of viral marker proteins representing the different kinetic classes (immediate early, early, and late) was studied by indirect immunofluorescence staining at different times post infection with TB40/E (Figure 3). Again, pUL122/123 were detected in the nuclei of all 4 cell types at 1 day p.i. The viral DNA polymerase processivity factor pUL44—a marker of the early infection phase—was detected in HFFs, HUVECs, and HEC-LTT cells as early as 1 day p.i. (data not shown), and the staining pattern reached its typical appearance 2 days p.i. (Figure 3). Expression of pUL44 was rare in EA.hy926 cells. The major capsid protein pUL86—representing the late replication phase—was detected in HFFs, HUVECs, and HEC-LTT cells at 4 days p.i. These cell types showed the typical pUL86 staining pattern resembling the morphology of viral replication compartments shown in Figure 2. In contrast, EA.hy926 cells expressed pUL86 infrequently. The number of pUL86-positive nuclei did not increase further until 7 days p.i., which argues against a mere delay in the HCMV replication cycle. The stained nuclear regions in EA.hy926 cells were tiny compared with the other cell types and not characteristic of HCMV replication compartments at that time post infection (Figure 3).

In order to obtain more quantitative data on viral protein expression in the course of infection, immunoblotting analysis was carried out. Major immediate early antigens were detectable in all four cell types (Figure 3B). HUVECs and HEC-LTT cells closely resembled each other regarding the appearance of the IE1–72 (72 KDa; detectable from 8 h p.i.) and the larger IE2–86 protein (86 KDa; detectable only at 1 day p.i.). EA.hy926 cells also showed IE1–72 and IE2–86 expression from 8 h p.i.; however, the signals continued to rise to high levels with a peak at 2 days p.i. and disappeared thereafter.

pUL44 was detectable in HEC-LTT cells at similar levels to HUVECs and HFFs. In HEC-LTT cells, emergence of pUL44 appeared slightly delayed compared with HUVECs and HFFs (from 1 to 2 days p.i. instead of 16 h or 1 day p.i., respectively). In contrast, EA.hy926 cells yielded only
a faint pUL44 signal under comparable conditions. Only after extended exposure was pUL44 clearly detectable, starting from 2 days p.i. through 5 days p.i.

pp150 (pUL32), a representative of the late kinetic class of viral proteins, was detectable in all cell types except EA.hy926 cells, again with a slight delay in HEC-LTT cells compared with HUVECs. The severely reduced level of pUL44, the disappearance of both immediate early antigens and pUL44 at 7 days p.i., and the failure to detect pp150 in EA.hy926 cells at all further support the existence of a premature block in the replicative cycle of HCMV in these cells. In contrast, HEC-LTT cells expressed all viral antigens analyzed in this study, and their expression patterns and protein levels in HEC-LTT cells closely resembled those in HUVECs.

In order to test whether HEC-LTT cells and, in particular, EA.hy926 cells replicate the viral genome, quantitative real-time PCR was performed. HEC-LTT cells replicated the viral genome to amounts comparable to HFFs and HUVECs, albeit with a slight delay (Figure 3C). EA.hy926 cells failed to efficiently produce viral DNA, however, with a minor increase occurring from 8 h p.i. to 2 days p.i. These results confirm that HEC-LTT cells fully support viral replication and that infection of EA.hy926 cells is blocked at the early stage.

Together, the HCMV replicative cycle seems to proceed normally in HEC-LTT cells as in primary HFFs and HUVECs, whereas it appears to be arrested prematurely in EA.hy926 cells.

Formation of nuclear inclusions and the characteristic expression pattern

Figure 3. Human cytomegalovirus (HCMV)-infected HEC-LTT cells but not EA.hy926 cells express representative early and late antigens of HCMV and replicate the viral genome efficiently. (A) Cells were infected with TB40/E, and expression of representative immediate early (pUL122/123), early (pUL44), and late (pUL86) antigens was analyzed. The rate of antigen-positive cells (expression rate) was determined at the indicated time points after infection. A Cy3-conjugated antibody (red) visualized viral antigens, and nuclei were stained with DAPI (blue). (B) Immunoblotting analysis of pUL122/123, pUL44, and pp150 (late viral antigen) protein levels at the indicated time points after infection. Actin served as a loading control. (C) Analysis of viral genome amplification by quantitative real-time PCR. The values shown were generated by subtracting the quantification cycle (Cq) value of each sample from that of the uninfected background. Error bars: SEM; d.p.i: days post infection.
of pUL44 and pUL86 in HEC-LTT cells suggest that viral progeny might be formed and released from these cells but not from EA.hy926 cells. In order to test this, viral growth was evaluated for all four cell types. HUVECs, HFFs, HEC-LTT cells, and EA.hy926 cells were infected with TB40/E at comparable MOIs and then washed. The cell culture supernatant was assessed 5 days p.i. for release of infectious virus. While HFFs produced the highest levels of virus (6.1 log IU/mL), HEC-LTT cells released slightly reduced but still appreciable amounts of infectious virus, similar to HUVECs (4.2 log IU/mL and 4.7 log IU/mL, respectively) (Figure 4A). Of note, in the supernatant of EA.hy926 cells, no infectious progeny virus could be detected, which is consistent with the lack of nuclear HCMV replication compartments. The data indicate that HEC-LTT cells but not EA.hy926 cells support the complete HCMV replicative cycle and produce virus with comparable efficiencies to primary HUVECs.

In order to analyze when infection in HEC-LTT cells reached maximal productivity, the cells were infected 1 day after Dox withdrawal, and supernatants were harvested daily, starting at 3 days p.i. when virus progeny was expected to become detectable. In repeated experiments, HEC-LTT cultures consistently released infectious progeny virus, albeit less than HFF control cultures (Figure 4B). Virus release became apparent on day 4 p.i. with a short delay compared with HFFs and HUVECs, and, like HFFs and HUVECs, reached a plateau at 5 days p.i. This result confirms that the experimental conditions with infection at 1 day after Dox removal are compatible with virus production in HEC-LTT cells.

In order to assess the impact of Dox treatment on HCMV replication in HEC-LTT cells, release of infectious progeny virus from infected cells in the presence or absence of Dox was quantified. No effect of Dox on the virus titer could be observed, indicating that HEC-LTT cells are as productively infected in the presence of Dox as in its absence (Supplementary Figure S1). This work demonstrates that both EC lines under investigation, HEC-LTT and EA.hy926, are efficiently infected up to the stage of immediate early gene expression by highly endotheliotropic HCMV strains but not by poorly endotheliotropic strains. Since this pattern was reproduced by several virus strains established from independent clinical isolates, the cell lines possess the potential to reveal general determinants of viral cell tropism in the initial phase of infection. Whether HCMV enters ECs by an endocytic pathway or by fusion at the plasma membrane has not been fully elucidated (25–27). Further studies will be needed to show whether HEC-LTT and/or EA.hy926 cells resemble primary HUVECs with regard to the favored entry pathway.

The expression of early and late viral antigens in HEC-LTT and EA.hy926 cells appeared consistent with the occurrence or lack, respectively, of the late cytopathic effects and release of infectious viral progeny. EA.hy926 cells failed to execute the full replicative cycle and produce viral progeny in accordance with previous findings that productive infection of permanent cell lines by HCMV is rare and usually drastically less efficient than in more differentiated cells (1,13,14,28,29). Remarkably, antigen expression, cytopathogenicity, and the productivity of HCMV infection in HEC-LTT cells was indistinguishable compared with primary HUVECs.

Primary cells are frequently used to investigate virus–cell interactions and also to amplify virus. However, the use of primary cells is impaired due to limited access and donor-to-donor variations. For the TIME cells, a permanent EC line has previously been used to study HCMV spread and release (15,16). This cell line was generated in a microvascular background. In vivo, microvascular and macrovascular ECs may both contribute to HCMV dissemination and pathogenesis in different ways (30). For that reason, HEC-LTT cells are of interest as a macrovascular in vitro model for HCMV infection. In addition, HEC-LTT cells allow the generation of high numbers of genetically identical ECs with primary cell characteristics, thereby overcoming the disadvantages of frequent HUVEC isolation from varied donors and facilitating large-scale experiments such as high-throughput screening approaches. Since HEC-LTT cells are tightly growth-controlled in the absence of Dox, cell numbers and cell density remain constant during the experiment, which facilitates experimental design and execution. Proliferating cell types used in HCMV research such as TIME and ARPE-19 (31), a human retinal pigment epithelial cell line, cannot be growth-controlled, and uninfected cells will continue to proliferate throughout the experiment. In addition to the practical advantages offered by HEC-LTT cells, growth-arrested cells might better resemble the state of endothelial cells in the physiological context. Finally, HEC-LTT cells might be especially useful for the propagation and preservation of clinical isolates of HCMV, which grow in a cell-associated manner, rapidly undergo genetic changes, and lose EC tropism if propagated in fibroblasts (19,24).

In conclusion, both cell lines, EA.hy926 and HEC-LTT, appear suitable for investigating the initial events of HCMV infection in ECs up to the initiation of viral gene expression. HEC-LTT cells, in addition,
offer an innovative, unlimitedly growing cell culture model for analyzing the advanced stages of HCMV replication in ECs as well as for the production of EC-derived HCMV progeny.

Author contributions
D.L., D.H., D.S., and C.S. executed experiments. D.L. and C.S. designed the experiments and supervised the experimental work. D.L., D.H., and C.S. performed data analysis and drafted the manuscript. D.L. wrote the manuscript. R.L. contributed intellectually and provided human serum from anti-HCMV negative individuals. T.M. and D.W. provided HEC-LTT cells and acted as consultants on HEC-LTT cell culture. D.W. and A.S. contributed to designing the study. All authors edited and approved the manuscript.

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Competing interests
Tobias May and Dagmar Wirth have filed a patent for the technology for establishing conditionally immortalized cell lines.

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