Infection route of a highly endotheliotropic human cytomegalovirus (HCMV) strain in early matured endothelial progenitor cells

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We investigated the infection routes of a highly endotheliotropic recombinant HCMV virus strain (TB40E) carrying the enhanced yellow fluorescent protein (EYFP) fused with the viral protein UL83 in early matured endothelial progenitor cells derived from human hematopoetic cord blood stem cells [1]. In mature endothelial cells, highly endotheliotropic HCMV strains may enter by different pathways such as receptor-mediated endocytosis and uptake into macropinosomes. The pivotal factors for endotheliotropism of a particular HCMV strain are the initial replication events prior to viral uncoating [2].

In order to study whether endothelial progenitor derived cells (EPDC) are also permissive for HCMV, we infected EPDC with a wild type strain as well as with a recombinant strain transfected with the EYFP tagged UL83. UL83 (pp65) is a tegument protein located between the nuclear envelop and the nucleocapsid. It is an acceptor for phosphorylation and is transported to the cell nucleus immediately after infection.

The expression of this protein during virus replication was visualized by means of fluorescence microscopy. Subsequently, the fluorescence signal was converted to an electron dense diaminobenzidine (DAB) precipitate. Cells were dehydrated and embedded in Epon 612. 70nm ultrathin sections were performed. Using transmission electron microscopy, virus particles, producing UL83 could be demonstrated both in nucleus as well as in cytoplasm.

Hematopoetic stem cells were separated using paramagnetic CD133-coated particles by magnetic cell separation (MACS, Miltenyi) and cultivated in fibronectin-coated flasks in ECGM-MV medium (Promocell), supplemented with basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). Cultures were maintained in an incubator at 37°C and 5% CO_2 tension. After the formation of Weibel-Palade bodies containing cell clusters, the cultures were expanded and frozen until their use for the virus infection experiments. Photooxidation was carried out as previously described [3]. Expression of EYFP tagged UL83 was localized intracellularly by oxidative conversion of DAB into an electron dense precipitate. This reaction is initiated by formation of oxygen radicals during irradiation at 488nm excitation wavelength. The electron dense labeling was visualized using a Tecnai-20 transmission electron microscope.

Seven days after infection, about 10% of EPDC exhibited a bright UL83-EYFP specific speckled fluorescence pattern (Figure 1a). The equivalent phase contrast image shows that the UL83 positive cells appear altered and rounded up (Figure 1b). The cell nucleus is highly invaginated in order to deliver the HCMV nucleocapsids. Tegumented virus particles could be demonstrated in the cytoplasm but mainly in the nucleus (Figure 2a).

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There, noninfectious enveloped virus particles (A) lacking the central DNA-containing part [4], UL83 positive dense bodies (B) and mature virus particles (C) could be demonstrated (Figures 2a and b). UL83 is concentrated in amorphous bodies surrounded by non-enveloped virus particles (Figure 2c). Envelopment of tegumented nucleocapsids occurs in exocytic vesicles released by microparticles (M) at the cell surface (Figure 2d). The acquisition of virus envelopes occurs in exocytic vacuoles produced by dilatated Golgi compartments.

The results demonstrate that human EPDC are permissive for the endotheliotropic HCMV strain TB40E. In this system it has been shown for the first time that the DAB photooxidation technique is suitable for monitoring the infection route of UL83-EYFP in these cells.

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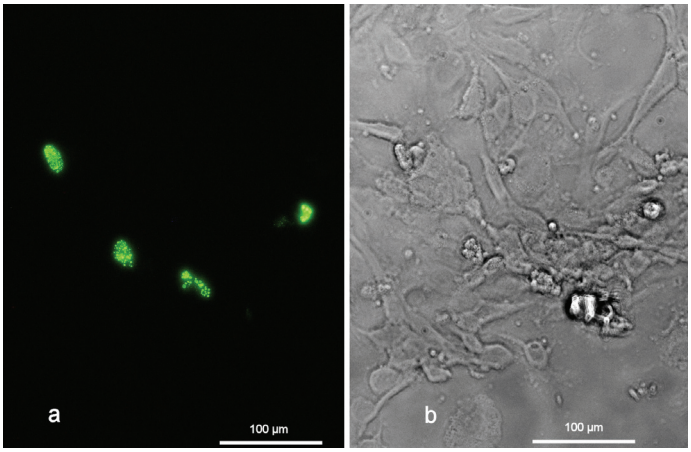

Figure 1. UL83 expression in EPDC. The fluorescence image **(a)** appears as a strong fluorescent speckled pattern. The equivalent phase contrast image is shown in **b**.

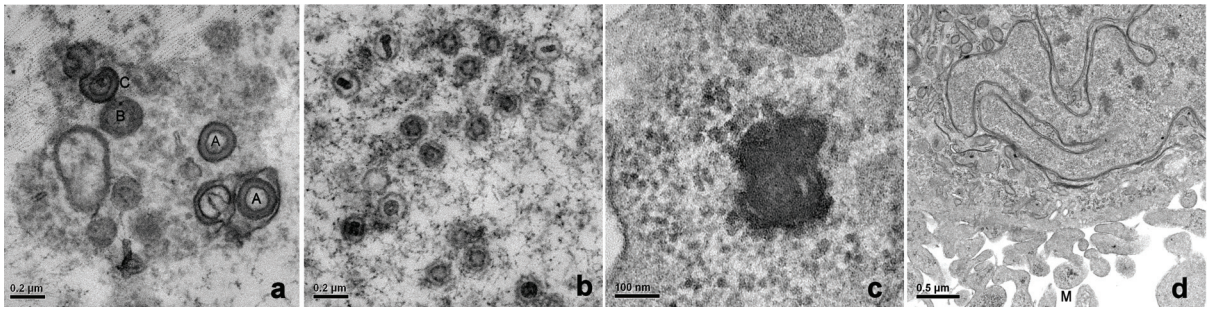

Figure 2. TEM images after photooxidation, demonstrating the entry route of an UL83-YFP transfected endotheliotropic HCMV virus strain into human EPDC. Explanation is indicated in the text.

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