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Letter to the Editor

Prediction of endothelial cell tropism of human cytomegalovirus strains

Dear Editor,

Endothelial cell (EC) tropism, i.e. the ability of a virus strain to grow in EC, requires a series of virus passages in EC prior to being assessed (Hahn et al., 2004). Recently, the UL131–128 locus of HCMV genome has been reported to carry at least some genetic determinants of EC tropism, as well as leukocyte and dendritic cell tropism (Gerna et al., 2005; Hahn et al., 2004). Concomitantly, it was shown that tropism was lost when the coding sequence of one of these three genes was altered.

The major aim of this study was to develop a rapid screening assay for predicting EC tropism of HCMV strains or mutants. Short-term results achieved with rapid assays were verified by long-term propagation in EC of both endotheliotropic (EC^+) and non-endotheliotropic (EC^-) HCMV strains, eventually showing that the plaque assay is the best predictor of EC tropism.

Human embryonic lung fibroblast (HELF) and human umbilical vein EC (HUVEC) cultures were used for propagating different virus strains. The following HCMV strains were tested: (i) two HCMV clinical isolates, VR1814 and VR6340, at either low-passage or high-passage in HELF (Revello et al., 2001); (ii) the laboratory strains AD169 and Towne either grown in HELF (Revello et al., 1998, 2001) or HUVEC (Gerna et al., 2002a, 2003); (iii) the Toledo strain, which is considered a low-passage wild-type HCMV strain (Baldanti et al., 2003); (iv) two very recently isolated HCMV strains (VR7729 and VR7371) propagated in HELF five times only; (v) the FIX-BAC reconstituted virus RVFIX, derived from cloning of VR1814 as a BAC (Hahn et al., 2002), and its deletion mutants obtained by site-directed mutagenesis, as reported (Hahn et al., 2004).

In the past, EC tropism was determined during a 2–3-month period following cocultivation of infected HELF and uninfected HUVEC (Hahn et al., 2004). Following this procedure, at passage 10, EC^+ strains showed >50% HUVEC staining for viral antigens, whereas no viral antigen was detected after infection with EC^- viruses.

In view of developing a rapid assay, cell-free virus was inoculated onto HUVEC, and then tested by the indirect fluorescent assay as follows: (i) 3 h p.i. for intranuclear presence of pp65 by pp65-specific monoclonal antibodies (MAbs) (Gerna et al., 1992); (ii) 24 h p.i. for intranuclear presence of the major immediate-early (IE) protein p72 by p72-specific MAbs (Gerna et al., 1990); (iii) 4–7 days p.i. for presence of p72 (4 days) or gB plaques (7 days) by the relevant MAbs (Gerna et al., 2002b). In addition, all virus strains inoculated onto HUVEC were passaged weekly by mixing inoculated with uninoculated HUVEC. HCMV strains or mutants tested for EC tropism were tested in parallel for leukotropism (Gerna et al., 2000) and dendritic cell tropism (Gerna et al., 2005; Riegler et al., 2000).

As shown in Table 1, HUVEC were highly positive for pp65 after 3 h, for p72 after 24 h, and for plaques after 4–7 days, when inoculated with known endotheliotropic strains. HCMV strains extensively propagated in HUVEC (HUVEC-grown), like high-passage VR1814 and VR6340, or laboratory strains adapted to growth in HUVEC, like AD169 and Towne, acted as positive controls (Fig. 1a, c, and e). In addition, positive results (at a variable level of positivity) for all three assays were obtained following inoculation of HUVEC with cell-free HELF-grown virus preparations from: (i) recent HCMV isolates (low-passage VR1814, VR6340, VR7729, and VR7371); (ii) AD169 and Towne reference strains; (iii) RVFIX and its deletion mutants functionally deprived of genes other than UL131–128, such as the RVFIXΔUL45 mutant.

On the contrary, as reported in Table 1, the three assays were negative when HUVEC were inoculated with cell-free virus preparations from HELF infected with: (i) HCMV strains extensively propagated in HELF (negative controls); (ii) deletion mutants functionally deprived of one, two or all three genes of the UL131–128 locus of HCMV genome (Fig. 1b, d, and f).

Using a multiplicity of infection (moi) of 1–10, all (or most) of HUVEC infected by EC^+ viruses were pp65-, p72- and gB plaque-positive (plaques could not be counted). Using

Abbreviations: HCMV, human cytomegalovirus; EC, endothelial cells; MAbs, monoclonal antibodies; HUVEC, human umbilical vein endothelial cells; HELF, human embryonic lung fibroblasts

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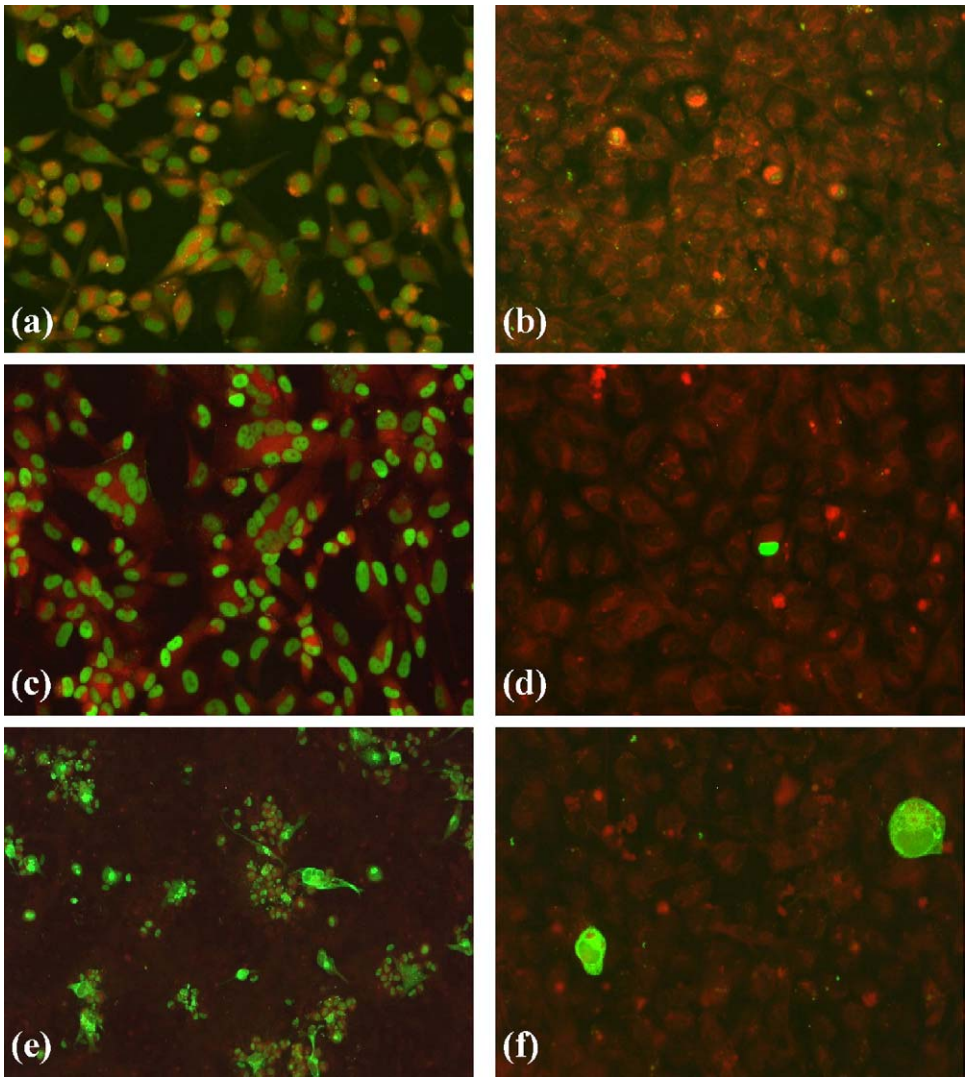

Fig. 1. HCMV pp65 immunofluorescent detection in nuclei of HUVEC 3 h p.i. with cell-free (a) EC^+ VR1814, and (b) EC^- RVFIXΔUL132–128. HCMV p72 as detected in nuclei of HUVEC 24 h p.i. with cell-free (c) EC^+ VR1814, and (d) EC^- RVFIXΔUL132–128. HCMV gB+p72 staining of HUVEC 7 days p.i. with cell-free (e) EC^+ VR1814, and (f) EC^- RVFIXΔUL132–128.

a lower moi of 0.01 and 0.1, the number of pp65- and p72-positive HUVEC ranged from 0.5–1.0% to 5–10%, while the number of plaques varied proportionally. On the contrary, HUVEC infected with EC^- HCMV strains were either both pp65- and p72-negative or showed a very small number of single pp65- and p72-positive cells, while either IE or gB plaques were never detected. However, although unable to progress to plaques, these scanty cells were able to synthesize structural antigens like gB (Fig. 1f). Thus, plaque formation was the best predictor of EC tropism. In fact, all HCMV strains displaying plaques were shown to long-term propagate in HUVEC and to be susceptible to transfer to leukocytes (Table 1) and dendritic cells.

The small number of plaques given by HELF-grown reference laboratory strains AD169 and Towne is the expression of a small number of viral variants within a mixed viral population. These variants were the basis for the successful propagation of reference strains in HUVEC.

Recently, we reported that the UL131–128 locus of the HCMV genome is indispensable for EC-tropism and that deletion of part or the entire locus as well as mutations present in natural variants interfering with the coding potential of this locus are deprived of the EC-tropic property. Although these three genes have not yet been characterized functionally, it appears reasonable to hypothesize a critical role of their gene products in the spreading process of viral infection in EC (plaque formation) or in the mechanism of transfer of virus and viral products from infected EC or HELF to leukocytes (Gerna et al., 2000; Hahn et al., 2004). Very recently, the UL131 ORF, and likely the entire UL131–128 locus, have been shown to be required also for efficient infection of epithelial cells (Wang and Shenk, 2005).