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In Vitro Replication of Varicella-Zoster Virus in Human Retinal Pigment Epithelial Cells▽

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Here we describe for the first time the productive in vitro infection of human retinal pigment epithelial cells by varicella-zoster virus (VZV), resulting in a typical cytopathic effect (CPE) that is characterized by enlarged cells with increased granularity. Depending on the CPE dissemination, high titers of up to 1.6×10^6 PFU of cell-free and cryostable VZV/ml can be recovered.

Varicella-zoster virus (VZV) is a human alphaherpesvirus (human herpesvirus 3) that causes chickenpox (varicella) during primary infection, whereas its reactivation from latency may result in shingles (zoster). VZV retinitis (VZVR) is a clinically distinct necrotizing retinitis syndrome caused by VZV that occurs often in immunocompromised patients. The pathological features of VZVR include progressive outer retinal necrosis causing retinal detachment and blindness in most patients (6). However, the mechanisms of VZVR are rarely investigated due to the lack of reliable in vitro models. Due to the difficulties to obtain cell-free infectious VZV (5), current in vitro investigations are usually based on freshly prepared, nontitrated or cell-associated VZV. However, cell-associated VZV cannot be used in neutralization assays because the virus is not accessible for the binding of antibodies present in samples to be investigated. Moreover, in antiviral susceptibility assays the use of cell-associated VZV resulted in a variable and significantly lower inhibition of plaque formation by the drugs assayed (5). Therefore, the objective of the present study was to establish a reliable reproductive in vitro infection system for generating high-titer and cryostable cell-free VZV stocks for subsequent use in different in vitro applications.

Human retinal pigment epithelial (RPE) cells were isolated from freshly enucleated bulbi for corneal transplantation according to the tenets of the Declaration of Helsinki. RPE cell isolation and culture were performed as described previously (2), with slight modifications (1). The homogeneity of cultured RPE cells was confirmed by positive immunostaining with monoclonal antibodies (MAbs) directed against cytokeratins (pan) and cellular retinaldehyde-binding protein (MAbs were donated by J. Saari, Department of Ophthalomology, University of Washington, School of Medicine, Seattle). Cells were routinely monitored for potential mycoplasma contamination and were used only until passage 5. Cells producing VZV-specific antigens were detected by biotin-streptavidin staining using MAb directed against immediate-early antigen 62 (IE62; Chemicon, Billerica, MA). Viral DNA was isolated, sequenced, and typed as described recently (10, 11). Preparation of cell-free VZV, titration of virus stocks, and antiviral susceptibility assays were performed as described elsewhere (5). Briefly, cell-free VZV was prepared as follows. The overlay medium was removed from a 75-cm^2 tissue culture flask, and the cell monolayer (1×10^7 to 2×10^7 cells) was rinsed with phosphate-buffered saline (PBS). The cell monolayer was scraped into 3 ml of PBS-sucrose-glutamate-serum (PSGC) buffer, sonicated three times for 15 s, clarified, and frozen at −70°C. PSGC buffer was prepared as described recently (5) and contains 5% (wt/vol) sucrose, 0.1% (wt/vol) sodium glutamate, and 10% heat-inactivated fetal calf serum in 10× PBS.

Vesicular fluid samples obtained from a 63-year-old male patient with zoster ophthalmicus (strain MR), from a 55-year-old male patient with zoster thoracicus (strain 06), and from a 39-year-old male patient with zoster ophthalmicus (strain PP) were inoculated into RPE cell cultures at passage 2. Within 3 days the cells developed a cytopathic effect (CPE) that was characterized by enlarged cells with increased granularity (Fig. 1A) that are absent in the uninfected parallel control cells at the same passage (Fig. 1B). VZV infection of the RPE cell culture was confirmed by immunohistochemistry using an IE62-specific MAb (Fig. 1C). VZV-specific IE62 was not detected by immunohistochemistry in the uninfected parallel control cells (data not shown). PCR amplification of the glycoprotein E (gE) gene resulted in the detection of amplification products of the expected size (Fig. 1D). Subsequent DNA sequence analysis of the novel VZV isolates MR and PP revealed genotype D strains, whereas VZV isolate 06 was found to be a genotype A strain. In addition, no nucleotide sequence variations were found in the amplified gE genes compared to the genotype D reference strain HJO (accession no. AJ871403) and the genotype A reference strain Dumas (accession no. NC001348), respectively. Therefore, it can be assumed that the three VZV isolates do not differ in their cell-to-cell spread phenotype from the reference strains as previously described

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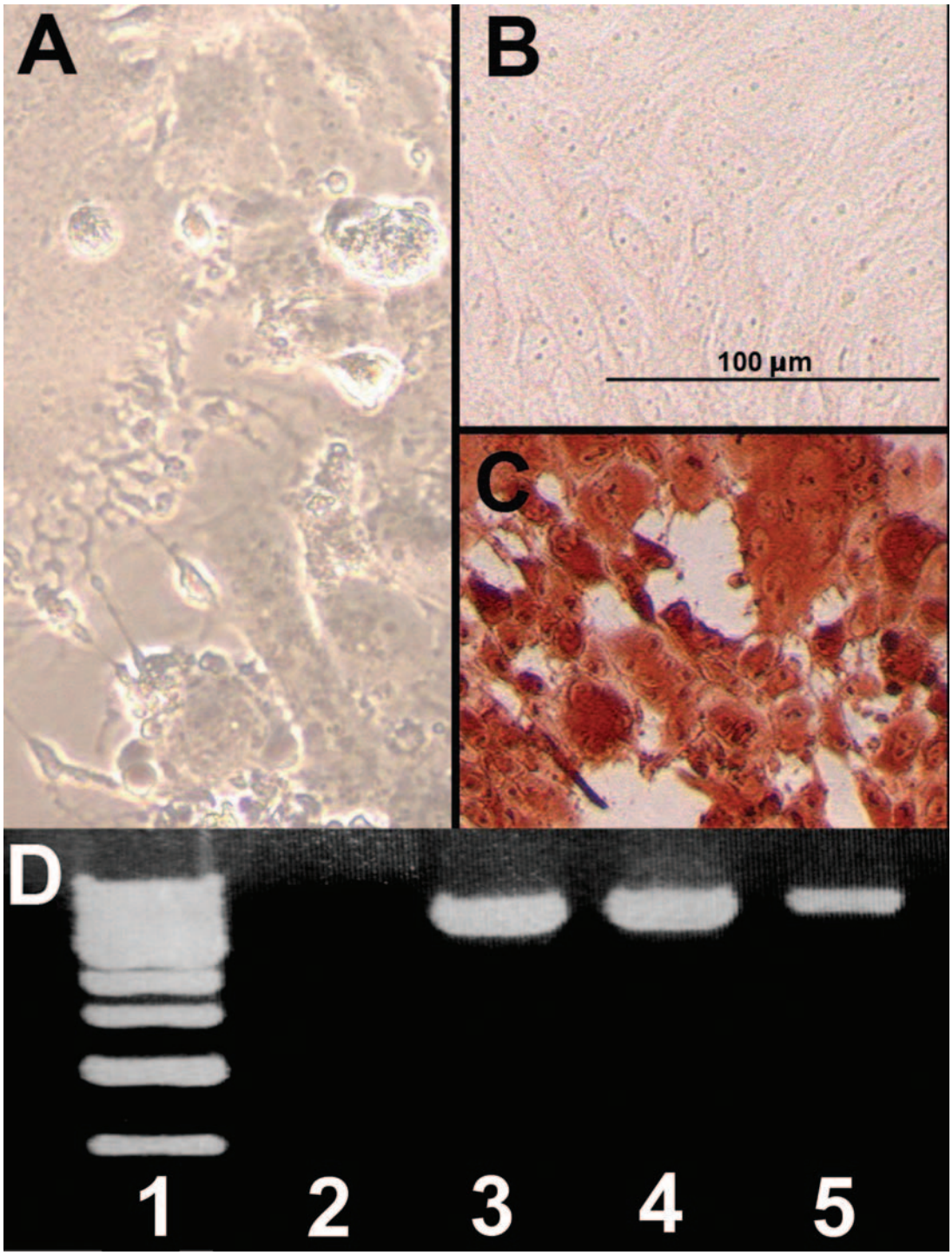

FIG. 1. Infection of RPE cells with VZV strain MR. Photomicrograph of VZV strain MR-infected RPE cells (A), uninfected parallel control cells (B), and immunohistochemistry of MR strain-infected RPE cells using biotin-streptavidin staining with a IE62-specific MAb (C). DNA fragments were obtained after PCR amplification of the gE gene and agarose gel electrophoresis as described previously (11). (D) Lane 1, molecular weight marker (GeneRuler Express DNA Ladder; Fermentas, St. Leon-Rot, Germany); lane 2, negative control (uninfected RPE cells); lane 3, VZV strain MR-infected RPE cells; lane 4, VZV strain 06-infected RPE cells; lane 5, VZV strain PP-infected RPE cells.

for the VZV gE mutant strain MSP (8). Following the kinetics of the infectious titer of VZV strain MR in the RPE cell culture (Fig. 2A), the maximum titer of cell-free VZV (1.6×10^6 PFU/ml) (Fig. 2B) was observed 3 days postinfection when the cells were inoculated with VZV strain MR at a multiplicity of infection of 0.01, when the CPE in the RPE cells reached 30 to 50%, and after sonication and 1 day of storage at −70°C in PSGC buffer. The virus titers decreased when the cytopathic

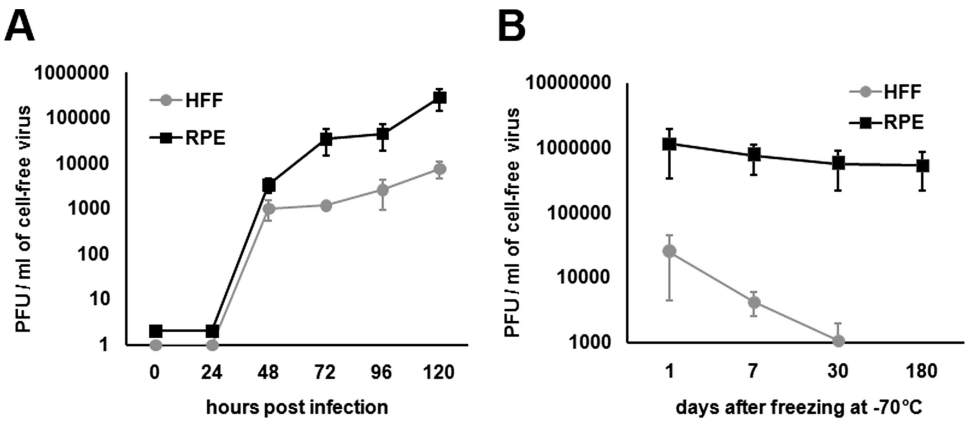

FIG. 2. Titers of cell-free VZV strain MR obtained from RPE or human foreskin fibroblast (HFF) cells over a period of 120 h postinfection without freezing (A) and 180 days after freezing (B).

effect was more advanced (data not shown). Storage of cell-free VZV at −70°C in PSGC buffer resulted in only a slight reduction of the titer (5.3×10^5 PFU/ml) after 180 days (Fig. 2B). Maximum titers of cell-free VZV obtained for the isolates PP (7.4×10^5 PFU/ml) and 06 (2.6×10^5 PFU/ml) were lower compared to the MR titer. In contrast to the infectious virus titer obtained in RPE cells, inoculation of VZV isolate MR into human foreskin fibroblast cells resulted in a maximum titer of only 1.7×10^4 PFU of cell-free VZV/ml (Fig. 2B). The susceptibility of frozen and cell-free VZV isolate MR to inhibition by acyclovir and foscarnet was found to be in the expected range (50% inhibitory concentrations, 27 μM acyclovir and 60 μM foscarnet) for a nonresistant VZV wild-type strain (7). Moreover, it was demonstrated that the prepared cell-free VZV is suitable for use in antiviral susceptibility assays (K. Bleymehl, unpublished data).

The protocol described here uses a novel cell culture system based on RPE cells and incorporates current knowledge on the generation of cell-free VZV. Thus, sonication has been described to release intracellular, nondegraded VZV from human embryonic lung fibroblasts (3) or human melanoma cells (4), and the maximum virus titer was obtained when cells are just beginning to show a CPE (9). The PSGC buffer used here was previously demonstrated to protect intracellular VZV from degradation during sonication, as well as acting as a cryopreservative (5). The novel procedure to generate cell-free wild-type VZV was found to be ~100-fold more efficient than previous approaches using fibroblasts that resulted in maximum titers of 1.9×10^4 PFU of cell-free VZV/ml (5).

In conclusion, RPE cells are susceptible to productive VZV infection and may therefore allow future in vitro investigations on the molecular mechanisms of VZV-induced retinitis. Moreover, in contrast to other cell lines, VZV infection of RPE cells is suitable to obtain high-titer and cryostable cell-free wild-type VZV stocks that can be used in subsequent plaque inhibition and antiviral susceptibility assays.

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