Zika Virus Efficiently Replicates in Human Retinal Epithelium and Disturbs Its Permeability

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Recently, the flavivirus Zika virus (ZIKV) has rapidly spread in the Americas and the Caribbean islands. While a large proportion of infected persons are subjected to mild or asymptomatic disease, neurological disorders such as Guillain-Barré syndrome and microcephaly have been linked to ZIKV infections (1). Notably, ZIKV-associated cerebral malformations can be associated with ocular disorders (2–7), and ZIKV is found in the eye anterior chamber fluid and in conjunctival swab samples of patients (8, 9). Moreover, ZIKV-infected mice develop ocular pathologies, and viral RNA is detected in the retina, optic nerve, tears, and lacrimal glands (10).

We and others have previously shown that the human induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) is morphologically and functionally characteristic of the RPE *in vivo* (11). The iPSC-derived RPE monolayer is a characteristic polarized pigmented cobblestone (Fig. 1A) that expresses specific RPE markers and tight junction proteins (e.g., ZO-1) (Fig. 1B) and is fully functional in terms of phagocytosis and apicobasal fluid transport (11). It thus represents a powerful model for studying the pathophysiology of the RPE, a tissue that is normally inaccessible for experimental manipulation. To study the ability of ZIKV to replicate in the human retina, we infected confluent iPSC-derived RPE with a clinical ZIKV of Asian lineage isolated in French Polynesia (H/PF/2013) at a multiplicity of infection (MOI) of 0.1 (12). Four days postinfection, we detected infected cells by immunofluorescence and found diffuse viral labeling inside the cells (Fig. 1C). To determine whether the infection was productive, supernatants were collected and viral titers were determined on Vero cells by the 50% tissue culture infective dose (TCID₅₀) method (13, 14). Using this approach, we found that ZIKV efficiently replicated in RPE 4 days postinfection ($1.9 \times 10^7 \pm 6.5 \times 10^6$ TCID₅₀/ml [mean $\pm$ SEM; $n = 3$]). Interestingly, 11 days postinfection, we still detected intracellular virions, although their location changed and came into close proximity to the cellular membrane (Fig. 1D).

Notably, at day 11 postinfection, the labeling of the tight junction marker ZO-1 was less marked than in the control sample (Fig. 1D), suggesting that the integrity of the cell layer could be impaired. To test whether epithelium integrity was affected upon infection, we measured the transepithelial resistance (TER) of the RPE monolayers cultured on Transwell filters in compartmentalized chambers (Fig. 2A) (15). Results showed a perturbation of the TER 7 days postinfection, consistent with an alteration of epithelium permeability (Fig. 2B). Moreover, virions were detected

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FIG 1 ZIKV efficiently infects polarized retinal pigment epithelium. (A) iPSC-derived RPE was grown to confluence at passage 3 and showed the classical pigmented cobblestone structure by light microscopy. (B, C, and D) Indirect immunofluorescence studies on paraformaldehyde-fixed RPE using an anti-ZO-1 antibody (zona occludens 1), a pan-flavivirus antibody (clone 4G2), and 4',6-diamidino-2-phenylindole (DAPI) to visualize nuclei. (B) ZO-1 labeling showed well-defined cell-cell adhesion, characteristic of polarized epithelia. NI, noninfected. (C) RPE was infected with ZIKV at an MOI of 0.1 according to established protocols (14) and fixed 4 days postinfection (D4). ZIKV-infected cells showed diffuse viral labeling consistent with viral replication. (D) RPE infected for 11 days (D11) was fixed and studied by indirect immunofluorescence. Data showed ZIKV labeling in close proximity to cellular membranes and a less defined ZO-1 location. Scale bars, 20 μ m (A) and 10 μ m (B, C, and D).

in the lower compartment at day 4 postinfection, when epithelium integrity was not yet affected, suggesting a basolateral release (Fig. 2C). Finally, electron microscopy analyses showed an impairment of cell junctions after ZIKV infection (Fig. 2C and E).

Our data underline the potential permissibility of human RPE to ZIKV infection and the associated deleterious effect on permeability. Our work also draws attention to the necessity of better investigating the ocular disorders associated with ZIKV-infected patients and to evaluate the risk of transmission by eye or tear contact, as has recently been suggested (16).

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FIG 2 ZIKV perturbs retinal pigment epithelium integrity. (A) Passage 3 iPSC-derived RPE was grown on Matrigel-coated polycarbonate Transwell filters (0.4- μ m porosity, 6.5-mm diameter). RPE integrity ensured that media from the upper compartment, in contact with the RPE apical side, and the lower compartment, in contact with the filter, cannot undergo direct exchange. (B) Measurements of RPE transepithelial resistance (TER) were performed using an epithelial volt-ohm meter before and after ZIKV infection at an MOI of 0.1. TER decreased markedly at day 7 postinfection. Results are expressed as the mean $\pm$ SEM ($n = 3$ independent experiments). Unpaired data were analyzed using the Mann-Whitney test (*, $P < 0.05$). (C) Viral titers of supernatant collected at day 4 postinfection, when epithelial integrity had not yet been affected, were determined on Vero cells by the Spearman-Kärber TCID₅₀ method (13) and showed virus in both the upper and lower compartments. Results are expressed as the mean $\pm$ SEM ($n = 3$ independent experiments). (D and E) Electron microscopy analyses were performed in glutaraldehyde-fixed RPE cultured on Transwell filters. (D, panel a) Typical polarized RPE architecture can be seen in noninfected samples. (b [zoom of panel a] and c) Tight junctions (black arrows) can be detected. Scale bars: 1 μ m (a), 500 nm (b), 200 nm (c). (E) In ZIKV-infected RPE (D11), cell-cell contact was less defined (a), with observable gaps between cells (asterisks) (b [zoom of panel a] and c). Scale bars, 1 μ m (a) and 200 nm (b and c).

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