Human Ocular-Derived Virus-Specific CD4+ T Cells
Control Varicella Zoster Virus Replication in Human Retinal Pigment Epithelial Cells

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Purpose. Varicella zoster virus (VZV)-induced retinitis is characterized by the presence of virus-infected cells in the retinal layer and the ocular infiltration of VZV-specific T cells. Herein, the susceptibility of human retinal pigment epithelial (RPE) cells to VZV infection and the ability of virus-specific CD4+ T cells to control VZV infection in RPE cells in vitro is addressed.

Methods. Human primary RPE cell cultures (n = 2) were infected with a VZV strain expressing green fluorescent protein. The infection and viability of infected RPE cells was monitored by flow cytometry or by a fluorescent imager on RPE monolayers. RPE cells, pretreated with or without interferon-γ (IFN-γ), were infected with VZV and subsequently cultured with VZV-specific CD4+ T-cell clones (TCCs; n = 3) recognizing disparate VZV proteins presented by different HLA class II alleles. IFN-γ production and cytotoxicity of the TCCs in response to VZV-infected RPE cells was determined by flow cytometry.

Results. Human RPE cells are permissive to a productive VZV infection. VZV-infected RPE cells presented the cognate antigen to the CD4+ TCCs only if the RPE cells were pretreated with IFN-γ and expressed the appropriate HLA class II allele. VZV-specific TCCs inhibited productive VZV infection in RPE cells, which was in part attributed to TCC-mediated killing of the VZV-infected RPE cells.

Conclusions. The results presented suggest that RPE cells may play a role as retina-resident antigen-presenting cells in the intraocular, VZV-specific, T cell-mediated inflammatory response of VZV-induced uveitis. (Invest Ophthalmol Vis Sci. 2009;50:743–751) DOI:10.1167/iovs.08-2611

Herpes simplex virus (HSV) and varicella zoster virus (VZV) are human neurotropic alpha herpesviruses that are endemic worldwide. Most people become infected during childhood, and viral replication in the respiratory mucosa and skin is followed by lifelong latent infection of neurons within the sensory ganglia.1,2 Herpesvirus infections initiate the activation of the innate and adaptive immune systems to control viral replication and subsequently prevent viral reactivation to cause recrudescent disease.1–4 Antigen-presenting cells (APCs) play an important role in the induction and perpetuation of virus-specific T-cell responses. The interaction between APCs and T cells involves the processing and presentation of antigenic peptides by APCs through major histocompatibility complex class I and II and costimulatory molecules, resulting in the activation of antigen-specific T cells to exert their effector functions, such as cytokine secretion and cytolyis of the APCs.5 Depending on the site of infection and the T-cell subsets involved, T-cell-mediated inflammatory responses may result in damage to the tissue involved.

HSV-I and VZV are common etiologic agents that cause severe ocular diseases affecting all parts of the ocular globe, including the cornea and uvea.5–8 In contrast to HSV, VZV has a restricted host- and cell-type tropism impeding the elucidation of the virus and host factors involved in VZV.1,4 Hence, current knowledge on intraocular T-cell responses to human alpha herpesviruses is largely based on studies on HSV-I in humans and experimental small animal models.9–12 HSV-1-induced corneal disease herpetic stromal keratitis is a chronic inflammatory disorder orchestrated by virus-specific CD4+ Th1-like T cells that induce the infiltration and activation of granulocytes to cause irreversible damage to the corneal tissue.9,11,12 In addition to professional APCs such as macrophages and dendritic cells, corneal resident fibroblast may serve as APCs to the infiltrating keratogenic CD4+ T cells.13

In comparison, the identity and functional properties of intraocular T cells and APCs in herpetic uveitis are largely unknown.10,15,16 Ocular infiltrating virus-specific Th1-like cells and uveal-resident dendritic cells are proposed to play a pivotal role in the local inflammatory response causing disease.16,17 Herpesvirus particles and antigens have been detected in the neural retina and retinal pigment epithelium of patients with HSV- and VZV-induced posterior uveitis.18–20 The colocalization of T cells in these lesions suggests the potential role of retinal pigment epithelial (RPE) cells as APCs in the immunopathogenesis of herpetic uveitis.

The retinal pigment epithelium is a monolayer of neuroepithelium-derived pigmented cells located between the photoreceptor outer segments and the Bruch membrane.21 It is crucial for nutritional support of the photoreceptor cells, retinol metabolism, phagocytosis of the photoreceptors outer segments, and formation of the outer blood-retinal barrier (BRB).22 Additionally, RPE cells play a pivotal role in the local innate and adaptive immune responses in autoimmune and infectious uveitis.23,24 Major histocompatibility complex (MHC) class II and costimulatory molecules are expressed at low levels by RPE cells in the normal retina but at higher levels in inflamed retinas.24,25 The upregulation of these molecules along with the induction of MHC class II expression, which is largely attributed to proinflammatory cytokines such as IFN-γ and TNF-α, may transform the retinal pigment epithelium into potent retina-resident APCs.20,27
The objective of the present study was twofold: to determine the susceptibility of primary human RPE cell lines to productive VZV infection and to assay the ability of human ocular-derived VZV-specific CD4+ T cells to recognize and subsequently control VZV infection of RPE cells in vitro.

**METHODS**

**Human Cell Lines**

The generation of intraocular VZV-specific CD4+ T-cell clones (TCCs), recovered from the aqueous humor fluid sample of the affected eye of a 65-year-old woman with diagnostically proven VZV-induced anterior uveitis, has been described (patient 2 in Milikan et al.17; Table 1). TCCs were expanded and maintained every 2 to 3 weeks at 37°C in a CO2 incubator by stimulation of the T cells with 1 μg/mL phytohemagglutinin-L (Roche Applied Science, Mannheim, Germany) in T-cell medium (TCM) supplemented with 50 U/mL recombinant human interleukin-2 (rhIL-2; Eurocetus, Amsterdam, The Netherlands) and γ-irradiated (30 Gy) allogeneic peripheral blood mononuclear cells essentially as described previously.26 The TCM consisted of RPMI-1640 (Invitrogen, Breda, The Netherlands) supplemented with 10% heat-inactivated human pooled serum and antibiotics.

Two primary human RPE cell lines, designated RPE 171 and RPE 172, were isolated from the surplus eye globes from two different donors after enucleation for corneal transplantation. The cells were grown at 37°C in a CO2 incubator in medium consisting of a 1:1 ratio (vol/vol) of Dulbecco’s modified Eagle’s medium (DMEM) and Ham F12 nutrient mixture (both Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (referred to hereafter as RPE medium).25 The retinal pigment epithelium was grown in bulk in 162-cm2 flasks and cryopreserved in aliquots. RPE cultures at passages 5 to 7 were used throughout the study. In some experiments, retinal pigment epithelium was treated with a predefined optimal concentration of IFN-γ (500 U/mL; PeproTech, Rocky Hill, NJ) for 3 days in RPE medium.26,27

The HLA class II genotype of the index uveitis patient and primary RPE cell lines were defined by standard diagnostic PCR at 4-digit high resolution. The HLA profile of the index VZV uveitis patient was HLA-DRB1*0301;1501 and HLA-DQB1*0201;0602. RPE 171 expressed HLA-DRB1*0301;0701 and HLA-DQB1*0201;0201, whereas RPE 172 expressed HLA-DRB1*1201;1501 and HLA-DQB1*0301;0602 (Table 1). The present study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the institutional review board; in addition, written informed consent was obtained.

**Assessment of VZV Infection of Human RPE Cell Lines**

The recombinant VZV strain, expressing green fluorescent protein (GFP) under the human cytomegalovirus (HCMV) immediate/early promoter, used in the present study was constructed with cosmid derived from the VZV reference strain p0Ka.50 GFP expression does not affect the viral replication or integrity of infected cells significantly differently from the parental p0Ka strain.50 The VZV-GFP virus was grown in human melanoma cells (MeWo, no. HTB-65; American Type Culture Collection, Manassas, VA) cultured in RPE medium at 37°C in a CO2 incubator, as described previously.29,30 Infected MeWo monolayers, showing an approximately 60% cytotoxic effect, and noninfected cells referred to as mock-infected cells, were harvested and stored in aliquots at −15°C in DMEM supplemented with 40% fetal bovine serum and 10% dimethyl sulfoxide. Semiconfluent RPE monolayers, seeded in six-well plates, were cocultured with VZV-GFP-infected MeWo cells, or as control mock-infected MeWo cells, at different MeWo/RPE ratios for up to 3 days at 37°C in a CO2 incubator.

Two different methods were used to detect VZV-infected cells. The first method involved the application of a variable mode imager (Typhoon 9410; GE Healthcare, Diegem, Belgium). This apparatus offers the unique opportunity to determine the number of GFP-expressing cells and the GFP intensity of each plaque per well simultaneously. Because this method does not affect the viral infection and viability of the monolayer, these variables can be determined in the same well at subsequent days postinfection (PI; data not shown). GFP data were analyzed with colony-counting and image-measuring software (ImageQuant TL; Amersham Biosciences, Freiburg, Germany) and are presented as the mean relative green fluorescence intensity (MRGFI) of four or six duplicate wells per experimental condition ± SD. The MRGFI was defined by the sum of the number of plaques and the fluorescence intensity of each plaque.

The second method used to identify VZV-infected cells was flow cytometric analysis of single-cell suspensions obtained after trypsinization of the monolayers. Cells were stained with mouse monoclonal antibodies (mAbs) specific for the membrane-bound VZV glycoprotein B (gB; Advanced Biotechnologies, Columbia, MD). Alternatively, the cells were fixed and permeabilized using a commercial reagent (Cytosfix/Cytoperm; BD Biosciences). Allophycocyanin conjugated goat anti-mouse (BD Biosciences) or appropriate isotype controls were used as secondary antibody or negative controls, respectively. Cells were analyzed with a flow cytometer (FACSCalibur; BD Biosciences) and software (CellQuest Pro; BD Biosciences).

**Functional T-Cell Assays**

Confluent RPE cultures, prestimulated with or without IFN-γ (500 U/mL) in RPE medium for 3 days, were coincubated with VZV- or mock-infected MeWo cells at a MeWo/RPE cell ratio of 1:3 in six-well plates and were incubated for 3 to 4 hours at 37°C. The monolayers were washed extensively to deplete most of the MeWo cells (data not shown), and the TCCs were added at a T-cell/RPE ratio of 3:2 in TCM. Monensin (2 μM; BD Biosciences) was added throughout the incubation for 6 hours at 37°C to accumulate newly synthesized IFN-γ within the T cells. The T cells were harvested and stained with APC-conjugated anti-CD3 (UCHT1; Dako, Heverlee, The Netherlands) and fluorescence isothiocyanate conjugated anti-CD4 (MT310; Dako) mAbs, washed, fixed, and permeabilized using a commercial reagent (Cytofix/Cytoperm; BD Biosciences) and subsequently stained with intracellular IFN-γ using a phycoerythrin-conjugated specific mAb (clone B27; BD Biosciences). Experiments were performed at least twice, and appropriate isotype- and fluorochrome-matched unrelated mAbs were included as negative controls.

The ability of the TCCs to control viral replication was determined using a variable mode imager (Typhoon 9410; GE Healthcare). RPE cells were seeded and grown to confluent monolayers in flat-bottom 96-well plates, prestimulated with or without IFN-γ, and subsequently VZV or mock infected with the addition of MeWo cells at an RPE/MeWo cell ratio of 3:1 for 4 hours at 37°C. The monolayers were

| Table 1. VZV Protein Specificity and HLA Class II Compatibility of Intraocular VZV-Specific CD4+ TCCs with the RPE Lines Used |

<table>
<thead>
<tr>
<th>TCC No.</th>
<th>VZV Protein Specificity</th>
<th>HLA Class II Allele Use</th>
<th>RPE 171</th>
<th>RPE 172</th>
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</thead>
<tbody>
<tr>
<td>29</td>
<td>ORF 68</td>
<td>DRB1*1501</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>ORF 4</td>
<td>DRB1*1501</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>132</td>
<td>ORF 29</td>
<td>DRB1*1501</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* HLA class II allele used as restriction element by the respective TCCs is indicated.
† Symbols + and − represent that the HLA class II allele expressed by the indicated RPE cell lines is compatible with the HLA class II allele used by the respective TCCs.
washed extensively; subsequently, $1.5 \times 10^4$ T cells of TCCs in a total volume of 200 $\mu$L TCM were added to each well. After 1, 2, and 3 days of incubation, the MRGFI of each well was determined as outlined. Experiments were performed in four or six duplicated wells and repeated at least twice.

The cytotoxic property of the TCCs toward the RPE cells was analyzed by flow cytometry, as described recently. In these assays, membrane labeling of RPE cells by the fluorescent dye PKH-26 (2.5 mM; Sigma-Aldrich), referred to as target cells, enabled discrimination of target cells from effector cells (T cells). Cell death was monitored by the ability of a fluorescent dye (Topro-3; Molecular Probes, Breda, The Netherlands) to bind DNA on entry into cells made permeable because of target T-cell death. In brief, the effector and target cells (both at $10^4$ cells/well) were cultured in duplicate in TCM for 6 hours at 37°C at an effector/target cell ratio of 10:1. To identify dead cells, 25 nM fluorescent dye (Topro-3; Molecular Probes) was added, incubated for 20 minutes at 37°C, and immediately used for flow cytometric analyses. Forward scatter acquisition threshold and gates were set to include nonviable cells and PKH-26–positive labeled cells. Specific release was calculated using the following formula: specific lysis = ($\%$ dead target cells in tube with effector T cells) $- \%$ spontaneous death of target cells in tube without effector T cells)/[100 $- \%$ spontaneous death of target cells in tube without effector T cells].

**RESULTS**

**Primary Human RPE Cells Are Susceptible to Productive VZV Infection**

VZV is highly cell associated in cultured cells in vitro, and the generation of high titers of cell-free virus stocks is troublesome. Hence, dispersing infected cell monolayers with trypsin and passing them onto uninfected cells is commonly applied to propagate VZV. To assess the susceptibility of primary human RPE cells to VZV infection, VZV-GFP–infected MeWo cells, or mock-infected MeWo cells as control, were inoculated onto semiconfluent RPE. Intracellular GFP expression, an effortless marker to differentiate VZV-infected from uninfected cells, was monitored daily with a variable mode imager (Typhoon 9410; GE Healthcare; Fig. 1A). The MRGFI increased approximately 1 log from day 1 PI to day 3 PI (Fig. 1B). Most of the GFP$^+$ cells on day 1 PI were relatively small

**FIGURE 1.** Human primary RPE cells are susceptible to VZV infection. VZV-GFP–infected MeWo cells were seeded onto semiconfluent monolayers of two RPE cell lines, referred to as RPE 171 and RPE 172. (A) Expression of GFP was monitored by a fluorescence microscope, and (B) relative GFP expression levels were determined consecutive days (days 1–3) after infection. Arrow: Typical multinucleated syncytium formed by fusion of VZV-infected cells. Mock RPE cells co-incubated with uninfected MeWo cells. (C) VZV-GFP–infected RPE cells were analyzed by flow cytometry for the coexpression of GFP and the indicated VZV proteins. Dot plots show only viable RPE-gated cells at day 3 PI (based on a differential FSC/SSC pattern). Quadrants were set with reference to isotype controls, and the percentages of cells positive for the indicated markers are given in the upper left quadrant of each plot.
and were positioned on top of the RPE monolayer, suggesting that most of these cells were MeWo cells. However, starting on day 2 PI and increasing in numbers until day 3 PI, GFP

\[\text{GFP}^+\] giant cells, representing multinucleated syncyia formed by the fusion of VZV-infected cells, were detected throughout the monolayers (Fig. 1A). Syncytia formation is a characteristic feature of the cytopathic effect associated with VZV replication in cell culture. On day 3 PI, most GFP

\[\text{GFP}^+\] cells in the monolayer were relatively large adherent cells resembling RPE cells phenotypically. No GFP expression was detected in mock-infected RPE cultures, and comparable data were obtained with the RPE 171 and 172 cell lines (Figs. 1A, 1B; data not shown). Transfer of VZV to RPE cells was not synchronous, resulting in a variable range of GFP expression (Figs. 1A, 1C).

Analogous to HSV, VZV transcription is temporally coordinated and is characterized by the expression of transcripts encoding the immediate-early (\(\alpha\)), early (\(\beta\)), and subsequently late (\(\gamma\)) VZV proteins. To further extend the observation that human RPE cells are permissive to productive VZV infection, VZV-GFP-infected RPE cultures (day 1 PI) were subjected to flow cytometric analyses to determine the expression of individual VZV proteins. Most GFP

\[\text{GFP}^+\] cells coexpressed the viral \(\alpha\) tegument protein ORF62 and the major capsid \(\beta\) protein ORF40 intracellularly and the \(\gamma\) glycoprotein B at the cell surface (Fig. 1C). The lack of GFP expression on approximately 15% of the VZV protein–positive cells was most likely the result of the deletion of the GFP cassette in the recombinant VZV strain, especially when passaged for prolonged periods in cell culture (A. Arvin, personal communication, 2007).

**IFN-\(\gamma\) Modulates the Expression of HLA and Costimulatory Molecules on Human RPE Cells**

The expression of inflammatory cytokines, such as IFN-\(\gamma\) and TNF-\(\alpha\), in affected eyes of patients with uveitis may shape resident RPE cells to potent APCs. To address this issue, the effect of both cytokines on the RPE phenotype was determined. Naive RPE expressed no HLA class II or CD40 and only moderate levels of CD54 and HLA class I (Fig. 2A). Analogue to previous reports, IFN-\(\gamma\) stimulation induced HLA class II and CD40 expression and upregulated HLA class I and CD54 expression on RPE cells (Fig. 2A).

The high expression of HLA class I and II molecules on IFN-\(\gamma\)-stimulated RPE, compared with negligible HLA expression on MeWo cells, facilitated unequivocal differentiation between both cell types in the VZV-GFP-infected cell cultures. RPE cells were coinfected with GFP-VZV-infected MeWo cells at a MeWo/RPE ratio of 1:1, and the cells were harvested at day 3 PI. Figure 2B shows that approximately one-fourth of the RPE cells in these cell cultures, identified by the coexpression of HLA class I and II, are GFP

\[\text{GFP}^+\], emphasizing that human primary RPE cells are susceptible to VZV infection. The lower number of infected RPE cells in this experimental setting most likely resulted from the IFN-\(\gamma\)-induced antiviral state of the RPE cells. Overall, the data suggest that under inflammatory conditions, VZV-infected RPE cells express a phenotype compatible with that of functional APCs that may process and present viral antigens to ocular infiltrating T cells.

**Ocular-Derived CD4

\[\text{CD4}^+\] T-Cell Clones Recognize Human VZV-Infected RPE Cells**

Recently, we identified and characterized the phenotype and antigen specificity of VZV-reactive T cells in affected eyes of patients with VZV uveitis. The ability of RPE cells to process and present viral antigens to VZV-specific T cells was determined. Ocular-derived VZV-specific CD4

\[\text{CD4}^+\] TCCs (\(n = 3\)), directed to different VZV proteins making use of dissimilar HLA class II alleles to recognize the cognate antigen, were incubated with VZV-infected RPE 171 and RPE 172 cells pretreated with or without IFN-\(\gamma\) (500 U/mL). The TCCs used were recovered from the same eye of one patient with VZV uveitis and are directed to the viral \(\gamma\) protein glycoprotein E (gE; TCC 29), the viral transcriptional activator ORF4 (\(\alpha\) protein; TCC 45), and the viral \(\alpha\) protein major DNA-binding protein ORF29 (TCC 132) (Table 1). RPE 171 and RPE 172 expressed the appropriate HLA class II allele used as T-cell restriction element by TCC 45, TCC 29, and TCC 132, respectively (Table 1). T-cell reactivity was monitored by flow cytometric analyses of CD4, CD3, and intracellular IFN-\(\gamma\) expression. Neither the mock-infected, with and without IFN-\(\gamma\) pretreatment, nor the unstimulated VZV-GFP-infected RPE 171 and RPE 172 cell lines activated the TCCs (Fig. 3; data not shown). In contrast, marked specific T-cell responses were seen only to IFN-\(\gamma\)-pretreated VZV-GFP-infected RPE cell lines expressing the appropriate HLA class II allele (Table 1; Fig. 3). Overall, the data demonstrate that IFN-\(\gamma\)-stimulated primary human RPE cells efficiently process and present the respective VZV \(\alpha\) and \(\gamma\) proteins to VZV-specific CD4

\[\text{CD4}^+\] TCCs in an HLA class II–restricted fashion. Mock- and VZV-infected MeWo cells were not recognized by the CD4

\[\text{CD4}^+\] TCCs (data not shown).

**Ocular-Derived CD4

\[\text{CD4}^+\] T-Cell Clones Control VZV Replication in Human RPE Cells**

Next, the effect of the CD4

\[\text{CD4}^+\] T cells on VZV replication in human RPE cells was determined. TCCs were cultured on IFN-\(\gamma\)-pretreated HLA class II–matched and -mismatched VZV-GFP-infected RPE monolayers, and the expression of GFP in the wells was monitored for 3 consecutive days PI. The temporal increase of the GFP expression, indicative for VZV replication in the RPE cell cultures, was significantly inhibited in wells containing distinct TCC/RPE cell combinations (Fig. 4). Compared with infected RPE 171 without TCC, TCC 45 inhibited GFP expression to 57% at day 3 PI. Although neither TCC 29 nor TCC 132 exerted an inhibitory effect toward infected RPE 171 cells, both TCCs clearly reduced GFP expression in infected RPE 172 cell cultures. At day 3 PI, TCC 29 and TCC 132 blocked GFP expression to 77% and 57%, respectively (Fig. 4).

The differential control of temporal MRGFI expression correlated with the induction of intracellular IFN-\(\gamma\) expression of the TCCs on incubation with the respective RPE cell lines (Fig. 3). The effector mechanisms of T cells to curtail viral infections involve two main pathways. The first is secretion of cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\), which induce the activation of infected cells, resulting in the inhibition of viral replication while leaving the target cell intact. The second pathway is T-cell CD95L interaction with CD95, expressed by the APCs or exocytosis of lytic granules, or both, resulting in lysis or apoptosis of the target cell. Antigen-specific cytokine secretion is considered the main effector mechanism of human CD4

\[\text{CD4}^+\] T cells to control viral replication. Because the cytoplasmic properties of human virus-specific CD4

\[\text{CD4}^+\] T cells remain debatable, we determined whether this effector mechanism is of additional importance to control VZV replication in human RPE cells. TCC 132, used as a reference VZV-specific TCC, preferentially killed the HLA-matched but not the HLA-mismatched VZV-infected RPE cells. At an E/T ratio of 10:1, the net mean specific cell lyses of the IFN-\(\gamma\)-pretreated and VZV-infected RPE 171 and RPE 172 cells were 8.8% and 58.8%, respectively (Table 2).

**DISCUSSION**

In the present study, we determined the susceptibility of primary human RPE cells to VZV infection and the effect of human ocular-derived VZV-specific CD4

\[\text{CD4}^+\] TCCs on HLA-mis-
matched or HLA-matched VZV-infected RPE cells in vitro. By using a GFP-VZV strain, we demonstrated that human RPE cells are permissive to a productive VZV infection. IFN-γ pretreatment rendered VZV-infected RPE cells to APCs recognized by CD4+ VZV-specific TCCs, which in turn controlled VZV infection by the secretion of the antiviral cytokine IFN-γ and cytolysis of the infected RPE cells.

Alpha herpesvirus–induced uveitis can result in severe visual impairment from the direct cytopathic effect of the virus on ocular resident cells and the induction of an intraocular immune response. Most patients with herpetic uveitis have serum IgG antibodies to the triggering virus, suggesting that the disease is initiated by reactivation of the endogenous latent virus located within its ganglionic stronghold. Like other epithelial cells, RPE cells are considered a frontline defense against invading microorganisms. In vivo and in vitro studies have identified the retinal pigment epithelial cell as the target cell for RNA (West Nile virus and corona virus strain JHM) and
DNA viruses such as HSV and HCMV. Combinatory data on the increase of GFP expression in time, reflecting replication of the GFP-VZV strain applied, the expression of structural and nonstructural VZV proteins in infected RPE, the typical cytopathic effect, including syncytium formation, and the increased granularity of the infected RPE cells demonstrate the ability of VZV to infect RPE cells productively in vitro (Figs. 1, 2). The data are in agreement with a recent study on VZV infection of human RPE cells by Schmidt-Chanasit et al.33 RPE cells are located near sensory neurons and, to a limited extent, blood capillaries, making them accessible to microorganisms entering the retina by way of the axonal and hematogenous routes, respectively. Studies by Atherton et al.43 on the experimental mouse model of HSV-1 acute retinal necrosis have shown that HSV-1 preferentially enters the retina by axonal transport from ganglionic cells toward the outer layers of the retina, close to the RPE cell layer. The high similarity of both viruses and the negligible numbers of VZV-infected cells in the blood of patients with recurrent VZV infections suggest that VZV preferentially enters the retinal tissue by transaxonal spread.1,43 Like other epithelial cells carrying out secretory functions, RPE cells are polarized cells expressing an apical and a basolateral membrane.21,22 Differential protein and lipid composition at either surface affects the entry and release of viruses. Whereas viruses such as vesicular stomatitis virus and vaccinia virus preferentially infect polarized epithelial cells at the basolateral surface, the uveitogenic herpesviruses HCMV and HSV-1 predominantly infect polarized RPE at the apical membrane.44–47 In contrast to HSV, the cellular receptors mediating VZV infection and the mechanisms involved in virus entry and release of VZV from polarized cells are incompletely understood.1,48,49 The ease to generate and infect human RPE cells with VZV and the recent ability to generate relatively high titers of cell-free and cryostable VZV in human RPE cells33 advocate the in vitro VZV/RPE-culture system as an invaluable tool to address these important issues on the pathogenesis of VZV infections in future studies.

Ocular immune privilege involves a multifactorial mechanism to prevent the blinding consequences of ocular inflammation.23,24 In addition to maintaining the BRB, visual function, and the survival of photoreceptor cells, RPE cells have been identified as important participants in creating and maintaining ocular immune privilege by actively suppressing T cell–mediated ocular inflammatory responses.23,50 RPE cells express immunoregulatory proteins such as Fas ligand (CD95L) and complement factor H, and they secrete immunosuppressive cytokines such as transforming growth factor β,23–24,50,51 implicating their proinflammatory role as well. This dichotomy underscores the complex role of RPE cells in ocular T cell-mediated inflammation. Although several groups have shown that RPE cells suppress T-cell responses,58–60 others have described the opposite effect by demonstrating...
that RPE cells induced to express MHC class II efficiently process and present antigen to antigen-specific CD4+ T cells.\(^{27,61}\) However, most of these studies did not use purified or well-characterized RPE cells, T cells, or antigens. The cell cultures used might have been contaminated with conventional APCs, complicating the interpretation of the results.

The unique experimental setup of the present study enabled us to largely overcome these potential confounding factors. The data presented here demonstrate that human primary RPE cells efficiently process and present VZV antigens to human ocular-derived VZV reactive CD4+ TCCs in a HLA class II-dependent fashion. Moreover, the lack of T-cell activation using HLA-mismatched or mock-infected RPE cells strongly argues against the TCR-independent, RPE-mediated T-cell activation described previously by Liversidge et al.\(^{62,63}\) Contaminating lymph node- or spleen-derived professional APCs may, in part, be attributed to this discrepancy.\(^{62,64}\) Analogous to previous studies, IFN-γ pretreatment of the RPE cells was essential to facilitate CD4+ T-cell recognition.\(^{58–64}\) This effect involves the induction of HLA class II, the upregulation of costimulatory molecules, and the mobilization of the endocytic proteolytic machinery of RPE cells to process the cognate antigens.\(^{26,27,65}\) The initial cellular source of IFN-γ to differentiate RPE cells into CD4+ T-cell stimulatory APCs may be activated by ocular-infiltrating VZV-specific CD8+ T cells recognizing VZV-infected HLA class I+ retinal cells. Alternatively, retina-resident dendritic cells and macrophages, both of which express HLA class I and II, may present VZV antigens and subsequently activate infiltrating VZV-specific CD4+ and CD8+ T-cells to release immunostimulatory IFN-γ levels.\(^{66}\)

Because of the inability to generate high cell-free VZV stock, RPE cells were infected by coinfection with VZV-infected MeWo cells. The ability of RPE cells to phagocytose cellular debris\(^{21,22,29}\) raises the possibility that the RPE cellsprocessed and presented the cognate VZV antigens derived from the VZV-infected MeWo cells. The data implicate that the IFN-γ-pretreated RPE cells processed and presented the viral antigens actively on VZV infection, but we cannot rule out the involvement of cross-presentation of the cognate antigen by RPE cells.\(^{67}\)

Although it is widely accepted that CD4+ T cells provide helper functions for APCs and can restrict viral replication by secreting cytokines, evidence is increasing that virus-specific CD4+ T cells can directly kill infected cells.\(^{58–64}\) Among human herpesviruses, several groups, including ours, have reported on the presence of EBV-, HCMV-, HSV-, and VZV-specific CD4+ T cells in vivo and in vitro.\(^{13,28,36,69–71}\) Here, we demonstrate that CD4+ T cells controlled the productive VZV infection of RPE cells in vitro that was in part mediated by cytosis of the VZV-infected RPE cells. In conclusion, our data show that primary human RPE cells are susceptible to productive VZV infection in vitro. Rather than inducing anergy, IFN-γ-pretreated VZV-infected RPE cells are able to process and present VZV peptides and induce rapid T-cell effector functions, potentially perpetuating intraocular VZV-specific memory CD4+ T-cell responses. We postulate that RPE cells may play a role in the detrimental processes evoked on VZV infection of the retina. The direct cytopathic effect of VZV in RPE cells and the susceptibility to CD4+ T-cell-mediated cytosis may result in dysfunction of the RPE cell layer, resulting in devastating effects on retinal function and even photoreceptor cell death. Studies on the experimental monkey uveitis model of simian varicella virus, the closest relative of VZV, may provide more insight into the potentially tormented triad of VZV, RPE cells, and T cells involved in this sight-threatening disease.\(^{72}\)

### Table 2. Cytotoxic Activity of the VZV-Specific TCC 132 toward VZV-Infected RPE Cells

<table>
<thead>
<tr>
<th>Percentage Specific Lysis of Target RPE*</th>
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<tbody>
<tr>
<td><strong>RPE 171</strong></td>
</tr>
<tr>
<td>Mock</td>
</tr>
<tr>
<td><strong>RPE 172</strong></td>
</tr>
<tr>
<td>Mock</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
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<tr>
<td>-0.9 ± 0.1</td>
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<tr>
<td>IFN-γ</td>
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<td>-16.3 ± 3.1</td>
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</table>

* Shown are average percentages of specific net cell lysis ± SD of 10⁴ labeled RPE cells as target cells. The RPE were pre-treated with medium or IFN-γ and subsequently mock- or VZV-infected.

Cell lysis was the result of cocultivation in duplicate of T cells and RPE cells for 6 hours at an effector/target cell ratio of 10:1.
Acknowledgments

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