The Immediate Early 2 Protein of Human Cytomegalovirus (HCMV) Mediates the Apoptotic Control in HCMV Retinitis through Up-Regulation of the Cellular FLICE-Inhibitory Protein Expression

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The Immediate Early 2 Protein of Human Cytomegalovirus (HCMV) Mediates the Apoptotic Control in HCMV Retinitis through Up-Regulation of the Cellular FLICE-Inhibitory Protein Expression


Human CMV (HCMV) is a widespread human pathogen that causes blindness by inducing retinitis in AIDS patients. Previously, we showed that viral immediate early 2 (IE2) protein may allow HCMV to evade the immune control by killing the Fas receptor-positive T lymphocytes attracted to the infected retina with increased secretion of Fas ligand (FasL). In this study, we further demonstrate that the secreted Fasl also kills uninfected Fas-rich bystander retinal cells and that IE2 simultaneously protects the infected cells from undergoing apoptotic death, in part, by activating the expression of cellular FLIP (c-FLIP), an antiapoptotic molecule that blocks the direct downstream executor caspase 8 of the FasL/Fas pathway. c-FLIP induction requires the N-terminal 98 residues of IE2 and the c-FLIP promoter region spanning nucleotides ~978 to ~696. In vivo association of IE2 to this region, IE2-specific c-FLIP activation, and decrease of FasL-up-regulated activities of caspases 8 and 3 were all demonstrated in HCMV-infected human retinal cells. Moreover, c-FLIP up-regulation by IE2 appeared to involve PI3K and might also render cells resistant to TRAIL-mediated death. Finally, enhanced c-FLIP signals were immunohistochemically detected in IE-positive cells in the HCMV-infected lesions of the human retina. Taken together, these data demonstrate specific activation of c-FLIP by HCMV IE2 and indicate a novel role for c-FLIP in the pathogenesis of HCMV retinitis.

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well as in the human retina of AIDS patients with HCMV retinitis. Our study further demonstrated that, when released into the culture medium, the increase in FasL-induced apoptosis in the Fas-positive T cell line, Jurkat. Apoptosis was also detected in the HCMV-infected retina of AIDS patients (11). Together with the earlier observation (12), it was proposed that FasL/Fas-mediated apoptosis might play an important role in the pathogenesis of HCMV retinitis (7).

The FasL/Fas-mediated apoptotic cascade basically involves the proteolytic maturation of the executor caspase 8 from its proform (55 kDa) to an active form (20 kDa), followed by activation of caspase 3 (proform, 36 kDa; active form, 17 kDa). Caspasess contribute to programmed cell death by cleaving key protein substrates inside the cell (13). Several distinct mechanisms, including activation of the cellular FLIP (c-FLIP), interfere with this pathway. c-FLIP is expressed as multiple splice variants. Among them, both c-FLIP\textsubscript{L} (long form, 480 aa) and c-FLIP\textsubscript{S} (short form, 221 aa) block the apoptotic pathway by interacting with caspase 8 at the death-inducing signaling complex (13). It is known that signal pathways such as MEK, IL-12, stem cell factor, and PI3K/Akt increase c-FLIP expression, whereas reactive oxygen species decrease its expression (13). The c-FLIP promoter can be up-regulated by p53, c-Jun, CREB, and NF-\kappaB, and down-regulated by peroxisome proliferator-activated receptor-\gamma, c-myc, and FOXO3a (13). c-FLIP is also targeted by viral proteins. The adenoviral E1A (14) and hepatitis B virus X protein (15) modulate c-FLIP function by regulating its degradation or by directly binding to it, respectively. Hepatitis C virus core protein blocks apoptosis of hepatoma cells by sustaining the c-FLIP protein level (16). Human T cell leukemia virus-I tax protein directly increases c-FLIP gene expression (17).

In this study, using in vitro HRPE cells and in vivo HCMV-infected retina tissues from AIDS patients as working models, we discovered that, in addition to activating FasL secretion, HCMV IE2 could also induce the death of nearby uninfected retina cells and simultaneously protect the infected cells from undergoing early death, in part, by activating c-FLIP, a direct apoptotic inhibitor downstream of the FasL/Fas cascade.

Materials and Methods

Plasmids and Constructs

The following plasmids have been described previously: pSEP7IE1 and pSEP7IE2 (see Fig. 1A–C) (11); pRL-SV40 (see Figs. 2 and 3) (Dual-Luciferase Reporter Assay System; Promega). The pSEI2-F series (see Figs. 1E and 2, D–F) were generated by insertion of each of the corresponding IE2 cDNAs into pSG424IE2 (18) between the BamHI sites. The pGL3-FLIP (see Figs. 2 and 3) was a gift from Dr. H. Wajant (Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany) (19). The c-FLIP promoter deletion constructs pGL3-FLIP978, pGL3-FLIP96, pGL3-FLIP414, and pGL3-FLIP132 were generated by PCR amplification of the corresponding DNA sequences from pGL3-FLIP using the following forward primers: 5'-GGAGAAGCTTTGCAGCTCGGTAATCCT(-3'-414), and 5'-GGAGAAGCTTTGCAGCTCGGTAATCCT(-3'-132) with the BglII site underlined, and the reverse primer 5'-CCAGGAGCTCGAGCTCC-3' with the HindIII site underlined and insertion of each resulting PCR product into pGEMT vectors (Promega) through TA cloning. The corresponding PCR products were then digested with BglII and HindIII and cloned into pGL3-basic (19) between these two sites.

Antibodies

Fas (F22120, Transduction Laboratories (see Fig. 1A)); B-10, Santa Cruz Biotechnology (see Fig. 1B); CH11, Upstate Biotechnology (see Fig. 1C); ZB4, MBL (see Fig. 1D), \(\beta\)-actin (MAB1501; Chemicon International), caspase 3 (IMG-144A; IMGENEX), FasL (clone 4H9; MBL, Japan), IE2 (MAB810; Chemicon International); see Figs. 1E, 2, A and C, 3A, 4, B–D and F, and 6); rabbit, polyclonal, described in Ref. 5 (see Fig. 3B)); FLAG epitope (M2; Sigma-Aldrich), c-FLIP (sc-8347; Santa Cruz Biotechnology (see Fig. 4, B, C, and F); ab402 (Abcam; see Figs. 4D and 6), caspase 8 (sc-7890; Santa Cruz Biotechnology). The UL56 Ab was provided by Dr. T. Shenk (Department of Molecular Biology, Princeton University, NJ) (20).

**Cells, HCMV infection, transfection, luciferase assay, Western blotting, and IE2 short-interfering RNA (siRNA)**

The HRPE cell line, ARPE-19, the human colon cancer cell line, RKO, and HCMV strain, AD169, were obtained from, and maintained as instructed by, the American Type Culture Collection. Cell-free virus stock and infections were prepared as described previously (11). All infections were conducted at a multiplicity of infection (MOI) of 5, except in Fig. 5B. Transfections, luciferase assays, and Western blotting were all performed as described previously (11). For reporter assays using the Plik inhibitor, the indicated amounts of LY294002 were added to the culture medium 8 h before harvesting cells. IE2 siRNA experiments were conducted according to the procedures described by Zhang et al. (21).

**Real-time RT-PCR**

Total RNA extraction was conducted as described previously (11). One-step real-time RT-PCR was conducted with the LightCycler (Roche Applied Science) using the DNA binding dye SYBR Green I for detection of PCR products. A total of 500 ng of total RNA was reverse transcribed and amplified in a 20-\(\mu\)l reaction mixture containing 1 \(\mu\)M specific primers: c-FLIP sense, 5'-CCAAAGACTTGAGCCTGTCAGCTC-3' and antisense, 5'-CATACTGAGATCTGATGAAGATTT-3'; IE2 sense, 5'-AGAAGCTTGGTACATTCTTCCTCC-3' and antisense, 5'-TATGGTATGAAAAATAGGTCCTT-3'; and antisense, 5'-AAGGACGCGCTTCGATAAACC-3' and antisense, 5'-AGCAGACCTCATCTCCTCTT-3'. The expression level of c-FLIP was evaluated as the ratio of its mRNA to that of \(\beta\)-actin. The expected sizes of the PCR products for c-FLIP, major IE gene (MIE), caspase 8, and \(\beta\)-actin are 228 bp, 435 bp, 165 bp, and 344 bp, respectively.

**Chromatin immunoprecipitation (Chip) assay**

ChIP assays were performed as previously described (5) with minor modifications. The final DNA samples were analyzed with 25–35 cycles of PCR to amplify indicated promoter sequences. The primers and the annealing temperature used for amplifying the c-FLIP promoter were as follows: 5'-TTTTTGTTCCTTCTTCTGAG-3' and 5'-TTATCTCTAATACCTTCTTCTTCT-3' (978 bp to 829 bp, annealed at 45°C); 5'-AAGTGTATTAGGAGATAAA-3' and 5'-GTAGAGGTGTTTGCTACCA-3' (846 bp to 460 bp, annealed at 48°C); 5'-CAAAAAAATAGATTTTGGGAC-3' (509 bp to 509 bp, annealed at 43°C); 5'-AAACAAAAAACACTC-3' and 5'-GTTGTTCTCTGTGCTGGACCA-3' (508 bp to 323 bp, annealed at 48°C); 5'-CCGTTTCTCTAACAACAAACA-3' and 5'-TTTTCCTCTTCTTGTGG-3' (320 bp to 133 bp, annealed at 48°C); 5'-AGAAAGAAAAACACTC-3' and 5'-GGTACCAGGCTGCGTACC-3' (320 bp to +150 bp, annealed at 51°C).

**ELISA and flow cytometry**

The activities of caspases 8 and 3 were determined by ELISA kit (MBL) and quantified at 490 nm (MRX; Dynatech Laboratories). Each individual sample was analyzed in triplicate. Flow cytometry was performed as described previously (11).

**Immunostaining, TUNEL, and immunohistochemistry**

For the combined scheme of immunostaining and TUNEL assays (see Fig. 1E), 1\(\times\)10\(^5\) HRPE cells were seeded onto 3.5-cm dishes with coverslips and transfected with the indicated vectors. Forty-eight hours later, TUNEL was performed with an in situ Cell Death Detection kit (Roche Molecular Biochemicals), followed by immunostaining. Briefly, cells with coverslips were washed with PBS, fixed with 4% (v/v) paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 5 min, and incubated with the TUNEL reagent provided for 1 h. The cells were then blocked in PBS containing 5% goat serum for 30 min, incubated with IE2 Ab for 1 h in blocking solution, incubated with the secondary Ab and 4',6'-diamidino-2-phenylindole (DAPI) for 1 h, followed by extensive washing and fixation with the FluorSave Reagent (Calbiochem) and visualization by fluorescent microscopy. The immunostaining in Fig. 4D was performed as described above except that the TUNEL reagent was omitted and cells were fixed.
with ice-cold acetone. Immunohistochemistry was performed as described previously (11). The research followed the tenets of the Declaration of Helsinki. All samples were obtained after informed consent from the patients. Apoptotic cells in tissue sections were identified as described previ-ously (11). Chromogenic development was applied with 3-amino-9-ethyl-carbazole (brownish-red color) for TUNEL and c-FLIP signals and tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (dark purple color) for HCMV IE staining.

Results

Fas receptor-rich human retina cells containing HCMV IE2 are resistant to apoptosis

Using immunostaining coupled with confocal microscopy, we first confirmed that Fas was expressed on the surface of human retina cell line, HRPE, as revealed by the ring-shape staining pattern (Fig. 1A, arrows). This result agrees with previous observations obtained with different approaches or from human retina sections (11, 22). Fas expression was specific to HRPE cells because it was not detectable in colon cancer cells RKO (Fig. 1B). Consistently, HRPE cells treated with Fas Ab (clone Ch11; Upstate Biotechnology), an inducer of apoptosis, generated the active form of caspase 3 (17 kDa) (proform, 36 kDa) (Fig. 1C, lane 3), suggesting that ligation of Fas to FasL could signal HRPE cells to undergo programmed cell death. PMA, an inhibitor of the Fas-dependent apoptotic pathway (23), was used as a negative control. Next, we examined whether secreted FasL from HCMV-infected HRPE cells caused apoptosis of uninfected HRPE cells. Uninfected HRPE cells were cultured in medium supplemented with 50% (v/v) of supernatants from mock or HCMV-infected HRPE cell cultures, respectively. The percentage of apoptotic cells in the cultures was determined by flow cytometry using Ab against annexin V that binds to phosphatidylserine exposed at the outer leaflet of the cytoplasmatic membrane at the early stages of apoptosis. We found that cells exposed to HCMV-infected supernatants exhibited a higher percentage of apoptosis as compared with those exposed to mock supernatants (Fig. 1D, □). Importantly, functional blocking of Fas (light gray bar) or FasL (dark gray bar) with respective mAbs, but not an IgG control Ab (■), prevented HCMV-induced apoptosis of uninfected HRPE cells. Because HCMV IE2 has been reported to activate FasL expression (10, 11), we then directly evaluated whether IE2 mediated the death of uninfected HRPE cells. To this end, a combined scheme of TUNEL and immunostaining was performed. As shown in Fig. 1E, transfected IE2 gave rise to positive nuclear staining (compare e to a, green) and greatly increased the number of TUNEL-positive cells (compare f to b, red). Interestingly, most IE2-specific apoptosis signals did not colocalize with that of apoptotic cells when IE2- and TUNEL-stained images were superimposed (g). Instead, almost all apoptosis occurred in IE2-negative cells adjacent to IE2-positive cells. The mutually exclusive exhibition of apoptotic and HCMV IE2-positive cells suggested that HRPE cells expressing IE2 resisted cell death, which is consistent with previous studies in other cell models (4–6).

IE2 activates the c-FLIP promoter

Using reporter assays, we demonstrated that HCMV IE2, in addition to inhibiting p53 function (5), might also antagonize apoptosis by up-regulating c-FLIP expression. As shown in Fig. 2A, HRPE cells transfected with IE2 (lane 3), but not IE protein 1 (IE1) (lane 2), a splicing variant of IE2, significantly increased the c-FLIP promoter (~978 bp to +150 bp)-driven luciferase activity. Western blot analysis using an Ab that specifically recognizes exon 3, shared by IE1 and IE2, showed that IE1 and IE2 expressed similarly in transfected HRPE cells (Fig. 2A, bottom panel). Using DNA primers against the C-terminal region unique to c-FLIP, we found using real-time RT-PCR that cells with IE2 (lane 3), but not IE1 (lane 2) or vector alone (lane 1), contained increased levels of the endogenous mRNA of c-FLIP (Fig. 2B). Importantly, the IE2-specific c-FLIP expression was further found to depend on
P13K, as increasing amounts of the P13K inhibitor, LY294002, gradually reduced the IE2-mediated activation of c-FLIP promoter (Fig. 2C). Note that the protein level of transfected IE2 remained unchanged in the presence of LY294002 (Fig. 2C, lower panel), suggesting posttranslational regulation of IE2 function by P13K. Again, although the protein level of IE1 was higher than that of IE2, the IE1’s effect in activating c-FLIP promoter was very limited (lane 2). We then characterized the region of IE2 necessary for c-FLIP stimulation. To this end, deletion mutants of IE2 with a C-terminal FLAG tag were constructed. Western analyses using FLAG Ab showed the successful expression of all these mutant IE2 proteins (Fig. 2D). It was found that IE2 without its N-terminal 98 residues (IE2Δ98-F) totally lost its ability to activate c-FLIP promoter (Fig. 2, E and F). IE2 without its N-terminal 169 residues (IE2Δ169-F) seemed to contain some activity as well. However, the possibility was excluded when the luciferase activity was normalized to the protein level expressed in cells (Fig. 2D, lane 5). These experiments indicated the specific activation of the anti-apoptotic molecule c-FLIP by the N-terminal 98 residues of HCMV IE2.

−978 bp to −697 bp of c-FLIP promoter is important for IE2-mediated activation

Using serially deleted c-FLIP promoter mutants, we found that the region from −978 bp to −697 bp was necessary for its activation by IE2 (Fig. 3A, compare lanes 5–10 to 3 and 4). The in vivo association of IE2 to this particular region of the endogenous c-FLIP promoter was further confirmed by ChIP assays. In these assays, the chromatinized DNA associated with IE2 was sonicated...
IE2-specific up-regulation of c-FLIP in HCMV-infected HRPE cells depends on PI3K

We then determined whether HCMV infection increased the mRNA level of c-FLIP in host cells. Total RNAs were collected at different times pi for real-time RT-PCR. As shown in Fig. 4A, the expression of c-FLIP, mRNA was clearly enhanced since day 2 pi (lane 3). The expression of the c-FLIP gene correlated with that of the HCMV MIE gene that encodes proteins including IE2 (Fig. 4A, bottom panel). In contrast, the mRNA levels of both caspase 8 and β-actin remained unchanged with or without HCMV infection (Fig. 4A, bottom panel). Consistently, the synthesis of c-FLIP protein was also induced early after infection (Fig. 4B). The induction pattern was similar to that of IE2, but not IE1, as revealed by the HCMV IE Ab MAB810 (Chemicon International). Moreover, we found that the protein levels of inactive caspases 8 and 3 (proform) were not decreased during HCMV infection. In the same blots, we failed to detect any signal for processed caspases 8 and 3 (data not shown). These results suggest that c-FLIP might be responsible for inhibiting the proteolytic maturation of caspases downstream of Fas. Using siRNA to specifically knock down IE2, we found that the HCMV-induced c-FLIP, protein level (Fig. 4C, lanes 6-8) was completely abolished (lanes 2-4), indicating that IE2 was indeed responsible for the observed increase of c-FLIP protein expression during HCMV infection. The dual-immunostaining assays following HCMV infection further pointed out that the c-FLIP signal on day 3 pi was exclusively present in the cytoplasm of IE (IE1 or IE2)-positive HRPE cells (Fig. 4D). HRPE cells without IE expression failed to stimulate c-FLIP synthesis (arrows). However, by DAPI staining of the nucleus, these neighbor cells without IE2 and c-FLIP did not seem to undergo apoptosis, which in theory should happen. We suspected that other HCMV-encoded antiapoptotic proteins might be involved and protect them from FasL-dependent cell death. As expected, in these cells we detected the expression of HCMV UL36 (Fig. 4D, f, red), which has been reported to inhibit the activation of caspase 8 (24). Next, we demonstrated that HCMV-induced c-FLIP, expression (Fig. 4E, RT-PCR and Fig. 4F, Western blot, lane 2) was totally blocked in cells infected by UV-irradiated HCMV (lane 3) or cells treated with the protein synthesis inhibitor, cycloheximide (CHX) (Fig. 4F, lane 4), or PI3K inhibitor LY294002 (lane 5). Note that CHX did not reduce the mRNA synthesis of viral IE genes (Fig. 4E, lane 4, top panel). These results indicated that c-FLIP was activated only in the presence of the viral major IE proteins.
HCMV infection at high MOI decreases the FasL- and TRAIL-dependent activities of caspases 8 and 3

We then assayed the activities of both caspases 8 and 3 to investigate whether IE2-mediated c-FLIP activation caused antiapoptosis during HCMV infection. HRPE cells were mock- or HCMV-infected and treated with FasL at 48 h pi. The cell lysates were then collected on day 1, day 2, or day 3 (D1, D2, D3, respectively) post-FasL treatment for ELISA. As shown in Fig. 5A, FasL induced the caspase 8 activity in HCMV-noninfected cells. Consistent with our hypothesis, the FasL-dependent caspase 8 activity was reduced in the presence of HCMV. The phenomenon was more evident when cells were collected on day 2 or day 3 post-FasL treatment. HCMV infection also reduced FasL-mediated caspase 3 activity (data not shown). These results further imply that if the caspase activity is indeed reduced by HCMV through activation of c-FLIP within infected cells, in theory, the FasL-induced activities of these caspases should be negatively correlated with the number of cells infected by HCMV. We tested this hypothesis by assaying the activities of caspases 8 and 3 in cells without or with HCMV infection using different MOI ranging from 0.01, 0.1, 1, to 5 (Fig. 5B). We found that HCMV infection at the highest MOI (MOI = 5, same as in Fig. 5A) inhibited FasL-mediated caspase 8 activity to the most extent. In contrast, low MOI (MOI = 0.01 or 0.1) of HCMV did not result in significant reduction of caspase 8 activity. Similar results were observed when caspase 3 was assayed (data not shown). Because both PI3K (25) and c-FLIP (26) inhibit TRAIL-mediated apoptosis and TRAIL is constitutively expressed in retina (8), we also examined HCMV’s effect on TRAIL-dependent cell death. As shown in Fig. 5C, although the effect was not as strong as in cells treated with FasL, HCMV was still capable of inhibiting TRAIL-dependent caspase 8 activity. Next, we showed that when HCMV infected HRPE cells pretreated with IE2 siRNA, it failed to down-regulate the FasL-induced caspase 8 activity (compare with Fig. 5D, D1). Together with the finding that both the mRNA and protein levels of caspases 8 and 3 remained unchanged during HCMV infection (Fig. 4, A and B), these experiments indicated that HCMV IE2 specifically down-regulated the activity of caspases 8 and 3, most likely through the activation of the c-FLIP expression.
surprised to find that HCMV inhibits TRAIL-dependent death of HRPE cells as well (Fig. 5D).

In this study, IE2 was demonstrated by ChIP assays to physically associate with the c-FLIP promoter from −978 bp to −696 bp, in vivo (Fig. 3B). However, it seems unlikely that IE2 stimulated the transcriptional activity of c-FLIP by direct DNA-binding. As shown in Fig. 2E, the IE2 domain involved in c-FLIP activation was mapped to the N-terminal 98 residues, rather than the C-terminal DNA-binding motif. As mentioned, the N-terminal region of IE2 is involved in transcriptional activation and protein-protein interactions. Thus, it is more likely that IE2 was detected as binding to the c-FLIP promoter via its association with other transcription factors that directly bind to the promoter region from −978 bp to −696 bp. Sequence analysis has predicted many potential binding sites for a variety of transcription factors in this region (data not shown). Among them, the GC box-binding protein, Sp1, is likely to be one of the candidates because its expression is also up-regulated during HCMV infection in HRPE cells (data not shown). Indeed, in glioblastoma U373MG cells, IE2 synergistically stimulates the DNA-binding activity of Sp1 through direct interaction (27). Thus, whether IE2 activates c-FLIP through Sp1 remains an intriguing question.

Interestingly, the N-terminal 85 residues of IE2 that are within the crucial region necessary for c-FLIP activation are indeed shared by IE1, a splicing variant of IE2. However, IE1 has very limited ability to activate the c-FLIP promoter (Figs. 2, A–C, and 4, B and C). Similar to the current study, we demonstrated in 2004 (5) that through the N-terminal 98 residues, IE2 inhibited the histone acetyltransferase activity of the p53 coactivator p300/CBP, and only IE2, but not IE1, down-regulated p53 function. It was found that the differential effect was caused by the inability of IE1 to bind p53 and p300/CBP. In contrast, IE2 formed stable complexes with these two proteins. Thus, the different behavior of IE1 and IE2 as regards c-FLIP activation might result from the very distinct nature of these two proteins beyond the N-terminal 85 aa.

In this study, we also show that HCMV-mediated up-regulation of c-FLIP activation is dependent on the PI3K pathway (Figs. 2C, and 4, E and F). The role of PI3K in both c-FLIP activation and HCMV infection has been independently reported. PI3K activates c-FLIP-mediated effects in T cells (28). It also signals the downstream pathway of the epidermal growth factor receptor, one of the cellular receptors for HCMV (29). Our findings that the PI3K inhibitor, LY294002, decreased HCMV IE gene expression in HRPE cells (Fig. 4, E and F) are consistent with the results demonstrated in fibroblasts (30). These experiments indicate that PI3K modulates the expression of HCMV IE proteins at the transcriptional level. However, the direct inhibition of IE mRNA expression by PI3K inhibitor may not be the sole reason to explain the loss of c-FLIP expression. Using reporter assays, we found that LY294002 also decreased c-FLIP activation by SV40 promoter-driven IE2 (Fig. 2C). In this experiment, LY294002 did not decrease the protein expression of IE2 (Fig. 2C, lower panel). Because IE2 phosphorylation has great impact on its function (2), we are currently interested in studying whether blocking the PI3K pathway interferes with the phosphorylation status of IE2 and whether IE2 phosphorylation is critical in c-FLIP activation.

The phenomenon that HCMV prevents the infected host cells from undergoing apoptosis is well documented. In addition to IE2, HCMV encodes several other proteins that can antagonize cell death. These include vMIA (viral mitochondrial inhibitor of apoptosis) that perturbs apoptosis-associated mitochondrial membrane permeabilization by sequestering the proapoptotic molecule, Bax, in mitochondria (31), vICA (viral inhibitor of caspase-8-induced
apoptosis, the HCMV UL36 gene product) that inhibits Fas-mediated apoptosis by binding to the procaspase of caspase 8 and prevents its activation (24), and IE1 through an unknown mechanism (4). Interestingly, an IE2-independent c-FLIP regulation during HCMV infection was reported recently (32). The cytokine IL-10 homologue encoded by HCMV is able to increase apoptosis in dendritic cells by inhibiting mRNA synthesis of c-FLIP (32). Although none of these effects has been examined in human retina, it is unlikely that c-FLIP activation is the only mechanism that prolongs the survival of HCMV-infected retina cells. HCMV retinitis involves complicated mechanisms.

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Disclosures

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