Impaired Killing of HCMV-Infected Retinal Pigment Epithelial Cells by Anti-pp65 CD8+ Cytotoxic T Cells

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PURPOSE. Host defense against infection by human cytomegalovirus (HCMV) is ensured in great part by cytotoxic CD8+ T lymphocytes (CTLs) directed against the tegument protein pp65. The hyperimmediate release of incoming pp65 into the major histocompatibility complex (MHC) class I pathway after fusion of the virus with the cell membrane provides a very early mechanism of defense. In retinal pigment epithelial (RPE) cells HCMV is known to enter through endocytosis. This study was conducted to determine whether this means of penetration into the cells would allow the virus to elude immune surveillance.

METHODS. Infection of RPE cells with HCMV AD169 was performed for 6 hours, 48 hours, and 8 days. Expression of intracellular pp65 in RPE cells and in the astrocytoma reference cell line U373MG was evaluated by flow cytometry, fluorescence microscopy, and Western blot analysis. Killing of both HCMV-infected cell lines by HLA-A2-restricted CD8+ CTLs directed against pp65 was monitored by 51Cr-release assays.

RESULTS. RPE cells were not lysed by CTLs directed against incoming pp65, contrary to U373MG. Moreover, both cell lines were not killed by anti-pp65 CTLs later after infection, because of the MHC class-I-downregulating effect of HCMV unique short (US2-11) proteins.

CONCLUSIONS. In RPE cells, both HCMV entry through endocytosis and the immunosuppressive effect of US proteins could allow the virus to evade immune surveillance at any stage of infection, which could promote viral spreading within the retina. (Invest Ophthalmol Vis Sci. 2003;44:665–671) DOI: 10.1167/iovs.02-0547

Human cytomegalovirus (HCMV), a member of the β-herpesviruses, is latent and harmless in most immunocompetent people. However, immunocompromised hosts such as transplant recipients can be severely affected by HCMV infection. It is the cause of a wide range of disabilities (mental retardation, hearing loss, developmental delay) in some children whose mothers are newly infected during pregnancy. HCMV is also among the most harmful infections faced by patients with acquired immunodeficiency syndrome (AIDS).

HCMV infection can act as a direct cause of retinitis and blindness in patients with end-stage AIDS.1–3 One major component of the very early immune control of HCMV replication is a CD8+ cytotoxic T lymphocyte (CTL) response directed against the tegument protein pp65 (pUL83).4,5 Indeed, pp65 has been shown to be internalized immediately after the fusion of both virions and dense bodies envelope with the cell membrane6 and thus is available for presentation to specific CD8+ CTLs without de novo synthesis.7 Moreover, we have shown recently that incoming pp65 contained in apoptotic infected cells can serve as an antigen source for cross-presentation by dendritic cells to CTLs.8 The observation that CTLs responsive to pp65 are present at a high frequency in blood donors suggests that this antigen is immunodominant.5 Recognition of infected cells by anti-pp65 CTLs very early after adsorption of a virus could avoid replication and spreading of the virus. The relevance of this very early presentation of pp65 to CTLs is emphasized, because HCMV develops strategies to escape from CTL responses throughout infection. Among these processes, those inducing a downregulation of major histocompatibility complex (MHC) class I molecule transport to the cell surface involve viral proteins encoded by multiple independent loci within the unique short (US) region of the HCMV genome.9,10 It has been shown that in human retinal pigment epithelial (RPE) cell lines and endothelial cells, which are two relevant targets of HCMV infection,11 the virus enters through endocytosis in vitro and is thus sequestered in vesicles.12 In light of this observation we hypothesized that RPE cells may possess characteristics that allow the virus to escape from very early control by anti-pp65 CTLs and thus favor the establishment of viral persistence and pathogenicity.

In this study, we report that HCMV-infected RPE cells were not lysed by HLA-A2-restricted cytotoxic CD8+ T-cell lines directed against incoming pp65, contrary to U373MG astrocytoma cells, although they expressed the tegument protein. We showed that a threshold amount of incoming pp65 in RPE cells was not responsible for resistance to CTL recognition. Because RPE cells that had been infected with pp65 recombinant adenovirus were sensitive to lysis by CTLs, we suggest that after infection with HCMV, incoming pp65 was not available for processing and recognition by T cells. Finally, we produce evidence that later after infection, even though amounts of pp65 increased due to de novo synthesis, both cell types were resistant to killing by anti-pp65 CTLs due to downregulation of MHC class I molecules mediated by the expression of proteins encoded in the US2-11 region of the viral genome. Together, these data suggest that control of HCMV-infected RPE is not ensured by anti-pp65 CTLs at any stage of infection, which may facilitate spread of the virus within the retina.

MATERIAL AND METHODS

Cell Cultures

Primary human RPE cell lines were established from donor eyes (Purpan Hospital, Toulouse, France), according to the tenets of the Declaration of Helsinki. RPE cells were isolated with trypsin, resuspended in
Ham-F10 medium supplemented with 20% fetal calf serum (FCS; Invitrogen, Groningen, The Netherlands) and 10 μg/mL anti-mycoplasma (Oiloctet; Roussel, Paris, France) and transferred to a 75-cm² culture flask. Cells formed monolayers and were typically hexagonal at confluence. Homogeneity was confirmed by positive immunostaining with monoclonal antibodies (mAbs) to cytokeratins (MNF 116 clone; Dako, Trappes, France). Cell surface expression of HLA-A2 on RPE cells was confirmed by flow cytometry analysis with a mouse mAb (clone B7-2, HB82; American Type Culture Collection [ATCC] Manassas, VA)). Cultures from two different primary cell lines were used at passages 4 to 11 for further experiments. HLA-A2-positive astrocytoma cells U373MG were from ATCC. Cells were maintained in RPMI medium (Invitrogen) containing 10% FCS supplemented with sodium pyruvate and antibiotics. U373MG cells transduced with IE1 cDNA were a gift from Susan Michelson (Pasteur Institute, Paris, France).

Viruses

HCMV AD169 was from ATCC, and construction of the mutant AD169, with the US2-11 genome region (HCMV AD169 US2-11) deleted, has been described.13 These viruses were propagated in human foreskin fibroblasts (HFF) in Dulbecco's modified Eagle's medium supplemented with 5% FCS. Viruses were collected when cytopathic effects were more than 90%. Supernatants were clarified by centrifugation at 15,000g for 10 minutes at 4°C and stored at −70°C until use. Virus titers were determined by plaque-forming unit titration in HFFs, according to standard procedures. A multiplicity of infection (MOI) of 3 was used for all experiments. Type 2 adenoviruses recombinant for pp65 (pUL83) sense (Ad-pp65s) or antisense (Ad-pp65as; a gift of Marina Cavazana, National Institute of Health and Medical Research, Unit 429, Paris, France) were used at 50 MOI during 24 hours before chromium-release assays.

Generation of Anti-pp65 CD8⁺ T-Cell Lines

HLA typing was performed by the Central Laboratory of Immunology (Elie Ohayon, Toulouse, France). CTLs directed against pp65 were generated from peripheral blood mononuclear cells (PBMCs) of HLA-A2 healthy HCMV-seropositive donor V as described by Arrode et al.8 Briefly, PBMC (2 × 10⁶ cells/mL) were cultured in 24-well plates in RPMI medium containing 10% human serum AB, 1% minimal essential medium with no essential amino acids, and 10 mM HEPES (all from Invitrogen). Culture was stimulated with a mixture containing 5 μg/mL of a pp65-derived peptide corresponding to a known CTL epitope (pUL83-150–165; Invitrogen), washed, and either stocked at −70°C or immediately lyed by incubation for 45 minutes on ice in lysis buffer (5 mM EDTA, 150 mM NaCl, 1 mM MgCl₂, 0.05 mM phenylmethylsulfonyl fluoride [PMSF], 50 mM Tris [pH 7.6], and 1% NP40). After a 2-hour preclaring with protein G-conjugated Sepharose beads (Amersham Pharmacia Biotech, Saclay, France), antibody was added to the lysate, and the mixture was incubated for 2 hours at 4°C. Mouse anti-pp65 mAb (gift of Giuseppe Gerna, Policlinica San Matteo, Pavia, Italy) was used. Protein G-conjugated beads were then washed and incubated overnight at 4°C. After washing, the beads were pelleted and boiled for 5 minutes in 5% β-mercaptoethanol reducing Laemmli sample buffer. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% gels. Gels were fixed, incubated in Amplify (Amersham Pharmacia Biotech), vacuum dried, and exposed to autoradiograph film (Hyperfilm-MP; Amersham Pharmacia Biotech).

Chromium Release Assay

Targets cells (U373MG or RPE cells) were seeded in six-well plates and infected with the following viruses: HCMV AD169, Ad-pp65s, Ad-pp65as, or HCMV AD169 US2-11. At various times after infection, cells were labeled with [³⁵S]methionine (+³⁵S) cysteine-free medium (Invitrogen) for 1 hour, and labeled with [³⁵S]methionine + [³⁵S]cysteine (200 μCi/mL; NEN, Cologne, Germany) for 2 hours. Monolayers were treated with trypsin (Invitrogen), washed, and either stocked at −20°C or immediately lyed by incubation for 45 minutes on ice in lysis buffer (5 mM EDTA, 150 mM NaCl, 1 mM MgCl₂, 0.05 mM phenylmethylsulfonyl fluoride [PMSF], 50 mM Tris [pH 7.6], and 1% NP40). After a 2-hour preclaring with protein G-conjugated Sepharose beads (Amersham Pharmacia Biotech, Saclay, France), antibody was added to the lysate, and the mixture was incubated for 2 hours at 4°C. Mouse anti-pp65 mAb (a gift of Giuseppe Gerna, Policlinica San Matteo, Pavia, Italy) was used. Protein G-conjugated beads were then washed and incubated overnight at 4°C. After washing, the beads were pelleted and boiled for 5 minutes in 5% β-mercaptoethanol reducing Laemmli sample buffer. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% gels. Gels were fixed, incubated in Amplify (Amersham Pharmacia Biotech), vacuum dried, and exposed to autoradiograph film (Hyperfilm-MP; Amersham Pharmacia Biotech).

Flow Cytometry

Cells were cultured in six-well plates and infected with HCMV as indicated. For pp65 labeling, cells were detached with trypsin and washed with PBS-20% human serum (PBS-HS). After fixation for 15 minutes in 1% paraformaldehyde (PFA), cells were permeabilized overnight with 100% methanol and washed again with PBS-HS. Anti-pp65 mAb (Cinapool; Argene Biosoft, Varilhes, France) was applied directly onto pelleted cells. Labeling with a mouse IgG1 (Dakopatts, A/S, Glostrup, Denmark) served as an isotypic control. After incubation for 45 minutes at 37°C and a wash with PBS-HS, cells were resuspended in goat anti-mouse F(ab’)2 conjugated to FITC (Sigma, Lyon, France), diluted 1:50, and incubated for a further 30 minutes at 4°C. Cells were then washed and analyzed with a cell sorter (EPICS Elite; Beckman Coulter, Hialeah, FL). HLA-A2 labeling was performed as follows: cells were harvested, washed with PBS and 3% FCS, and then incubated with BB7-2 mAb for 45 minutes at 4°C. Treatment with secondary antibody and cytofluorometric analysis were performed as described earlier.

Metabolic Labeling and Immunoprecipitation

Cells were infected with HCMV in six-well culture plates. At various times after infection, they were washed in PBS, incubated in methionine+cysteine-free medium (Invitrogen) for 1 hour, and labeled with [³⁵S]methionine + [³⁵S]cysteine (200 μCi/mL; NEN, Cologne, Germany) for 2 hours. Monolayers were treated with trypsin (Invitrogen), washed, and either stocked at −20°C or immediately lyed by incubation for 45 minutes on ice in lysis buffer (5 mM EDTA, 150 mM NaCl, 1 mM MgCl₂, 0.05 mM phenylmethylsulfonyl fluoride [PMSF], 50 mM Tris [pH 7.6], and 1% NP40). A 2-hour preclaring with protein G-conjugated Sepharose beads (Amersham Pharmacia Biotech, Saclay, France), antibody was added to the lysate, and the mixture was incubated for 2 hours at 4°C. Mouse anti-pp65 mAb (a gift of Giuseppe Gerna, Policlinica San Matteo, Pavia, Italy) was used. Protein G-conjugated beads were then washed and incubated overnight at 4°C. After washing, the beads were pelleted and boiled for 5 minutes in 5% β-mercaptoethanol reducing Laemmli sample buffer. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% gels. Gels were fixed, incubated in Amplify (Amersham Pharmacia Biotech), vacuum dried, and exposed to autoradiograph film (Hyperfilm-MP; Amersham Pharmacia Biotech).

Indirect Immunofluorescence Assay

One day before the assay, U373MG and RPE cells were seeded on chamber slides (Labtek; Nunc, Naperville, IL) at 6 × 10⁴ cells per well. Cells were infected with HCMV (MOI = 3) for either 6 hours or 48 hours and then processed as follows: after fixation for 10 minutes in PBS containing 2% saccharose and 5% formaldehyde, cells were permeabilized with a solution containing 10% saccharose, 0.5% NP40 and 1% FCS 1% in PBS for 5 minutes, washed and incubated (30 minutes, 37°C) with mouse mAb directed against pp65 (Cinapool 1:20; Argene Biosoft, Varilhes, France). Cells were then washed three times in PBS and incubated with a 1:200 dilution of a rhodamine-conjugated anti-mouse IgG (Coulter-Immunotech SA, Marseille, France) for 30 minutes. Slides were washed again three times, mounted, and examined, either on a fluorescence microscope (Leitz, Wetzlar, Germany) or on a confocal laser scanning microscope (model 410; Carl Zeiss, Oberkochen, Germany). Images were edited on computer (Photoshop; Adobe, San Jose, CA).

Flow Cytometry

At different times after infection, monolayers of U373MG or RPE cells were harvested, washed, and either stocked at −20°C or immediately lyed by incubation for 5 minutes on ice in radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris, 150 mM NaCl, 1% NP40, 0.5% deoxycholate, and 0.1% SDS [pH 8]). Lysates were homogenized and
RESULTS

Analysis of pp65 Expression in U373MG and RPE Cells

Before measuring the CTL activity of anti-pp65 CD8⁺ T cells, we evaluated the expression of pp65 in infected RPE and U373MG cells. Qualitative and quantitative analyses of intracellular pp65 expression were assessed by flow cytometry, fluorescence microscopy, and Western blot analysis. First, we examined the number of infected RPE cells by flow cytometric analysis of pp65 expression. This approach allowed us to detect intracellular protein only at day 8 postinfection (pi) but not at 6 hours and 48 hours pi (data not shown). Based on a previous report showing that fluorescence microscopy is more sensitive in detecting pp65 than flow cytometry,¹⁶ immunostaining of RPE cells and analysis by confocal microscopy were performed. At 6 hours pi (Fig. 1A), a granular cytoplasmic fluorescence was observed in 100% of RPE cells from which 15% exhibited a nuclear localization, as previously demonstrated by Bodaghi et al.¹² Microscopic examination and counting of fluorescent pp65-positive RPE cells revealed that the number of positive nuclei increased to 71% at 48 hours pi, suggesting that viral infection had progressed. Regarding U373MG, 100% cells were pp65 positive at 6 and 48 hours after infection (data not shown).

Kinetics of pp65 expression was analyzed by Western blot analysis and immunoprecipitation of metabolically labeled cells in both cell lines. pp65 was detected by Western blot analysis in both cell lines at 6 hours pi and increased over time, even though biosynthesis of pp65 started later in RPE cells (72 hours pi) than in U373MG cells (24 hours pi). Because no metabolic labeling was obtained after immunoprecipitation of cell lysates, at 6 hours pi we suggest that in both cell lines, pp65 originated from the virus inoculum.

Impaired Killing of RPE Cells in Response to Incoming pp65

HLA-A2 restricted CD8⁺ CTL lines directed against pp65 were raised from PBMCs of HCMV-seropositive donor V, as described in the Materials and Methods section. The cell lines used expressed CD3, were more than 80% CD8 positive, and produced IFN-γ, as described in previous publications.⁸,¹⁷ We hypothesized that CD8⁺ T cell-mediated lysis of RPE cells would be impaired compared with that of U373MG cells. Figure 2A shows specific lysis of both U373MG and RPE cells pulsed with N9V peptide in the presence of CTLs from donor V. When an irrelevant peptide I9Y known to be presented by HLA-B35 was used, no lysis of targets was observed. In Figure 2B, U373MG cells were infected with HCMV AD169 for 6 hours and 48 hours and used as targets for CTLs. U373MG cells were killed at 6 hours pi, presumably due to incoming pp65, because no protein had been synthesized at this time point. However, as will be presented in detail in the next paragraph, they became partially or totally resistant to lysis at 48 hours pi, depending on the set of experiments. Because PBMCs were expanded in the presence of the chimeric IE1-pp65 protein, we ensured that killing of infected cells was not mediated by anti-IE1 CTLs. To this end, CTLs were assayed in the presence of IE1-transfected U373MG cells as targets. In these conditions, no lysis was observed (data not shown), demonstrating that killing of HCMV-infected cells occurred through recognition by anti-pp65 CTLs. Regarding RPE cells, we took into account data from Detrik et al.¹⁸ showing that replication of HCMV progressed very slowly, compared with that in fibroblasts. Accordingly, infection of RPE cells with HCMV was performed at 6 hours and 48 hours and extended to 8 days before incubation with CTLs. Contrary to U373MG cells, HCMV-infected RPE cells were not killed by CTLs after 6 hours of infection, despite the presence of incoming pp65 (Fig. 2B). In addition, at later time points of infection (48 hours and 8 days) no more significant cytotoxicity against RPE cells was observed (Fig. 2B). Furthermore, cells were still resistant to lysis, even when infection was performed at the higher MOI of 60 (data not shown), suggesting that impaired lysis was not due to a threshold amount of pp65. It is noteworthy that the lysis observed on U373MG cells has been consistently observed on MRC5 fibroblast cells in similar conditions.⁸,¹⁷ We used U373MG cells as controls in our experiments because, contrary to fibroblasts, they constitutively expressed a sufficient amount of HLA-A2 at their surface, without treatment with IFN-γ.

To investigate whether pp65 could be processed into relevant HLA-A2-binding peptides in RPE cells, infections with...
recombinant adenoviruses containing sense or antisense pp65 gene (pp65s or pp65as) were performed and the infected cells used as targets for CTLs. Figure 3 shows that lysis occurred only when cells were infected with the sense pp65 adenovirus, demonstrating that RPE cells could present naturally processed endogenous pp65 peptides to HLA-A2–restricted CTLs.

To examine whether HCMV infection could interfere with pathways of T-cell cytotoxicity, cells were infected with HCMV, as described earlier and then pulsed with N9V peptide. Figure 4 shows that RPE and U373MG cells were lysed in these conditions by anti-pp65 CTLs, excluding that inhibition of CD8+/H11001 T-cell cytolysis pathways by HCMV infection could account for impaired killing.

Altogether, these results demonstrate that impaired killing of HCMV-infected RPE by anti-pp65 CTLs was due neither to viral interference with pathway of T-cell cytotoxicity, nor to inability of cells to process endogenous pp65 and to present peptides to CTLs.

Proteins encoded in the US2-11 genomic region are responsible for HCMV escape from anti-pp65 CTLs both in U373MG and RPE cells at late time points of infection.

As suggested earlier, even though U373MG and RPE cells had sustained amounts of pp65 in a late stage of infection, they were resistant to lysis by CTLs. It has been widely documented that HCMV inhibits MHC class I antigen presentation by a sequential multistep process involving a family of US region-encoded glycoproteins.9,10 Ectopic expression of US proteins, namely US2, US3, US6, and US11 resulted in a defective expression of MHC class I-peptide complexes at the cell surface. To adddress this question in U373MG and RPE cells, infection with either HCMV AD169 or HCMVΔ2-11 (Δ2-11) was performed and the cells used as targets for CTLs.

Figure 5A shows that in U373MG cells infected with Δ2-11, sensitivity to lysis by anti-pp65 CTLs was restored at 48 hours pi, showing that the expression of US2-11-encoded proteins was responsible for immune escape from CTL lysis. It is notable that after infection with the Δ2-11, viral lysis was more efficient at 48 hours pi than at 6 hours pi. This could be due to the presence of a higher amount of pp65, as shown in Figure 1B. Accordingly, flow cytometric analyses showed that in U373MG cells, HCMV-mediated downregulation of membranous expres-
sion of HLA-A2 was not observed in the presence of Δ2-11 (Fig. 5B). In contrast, RPE cells that had been infected with Δ2-11 for 6 and 48 hours were still resistant to CTLs, probably due to a defect in the recognition of incoming pp65 at 6 hours pi (Fig. 2B), and to a still-insufficient level of pp65 accessible to the cytosolic class I machinery at 48 hours pi, in accordance with Figure 1B. Sensitivity to CTLs was restored at day 8 pi (Fig. 5A) in accordance with the absence of MHC class I downregulation (Fig. 5B). This could be ascribed to the absence of the immunosuppressive US2-11 proteins and, in addition, to an increased amount of cytosolic pp65 caused by de novo synthesis. Flow cytometry, used as described earlier, showed that levels of pp65 expression in infected cells were identical, regardless of the presence (AD169) or absence (Δ2-11) of the US2-11 fragment in HCMV strains (data not shown). These data suggest that late after infection, anti-pp65 CTLs failed to lyse HCMV-infected U373MG and RPE cells due to the effect of the immunosuppressive proteins encoded in the US2-11 region.

**DISCUSSION**

In this study we showed impaired killing of HCMV-infected RPE cells by anti-pp65 CD8⁺ CTLs. We demonstrated that this was due neither to the absence of incoming pp65 into cells nor to their inability to process and present pp65-derived peptide to CTLs. Furthermore, we showed that later, after infection, both cell lines were resistant to killing by CTLs due to the expression of the immunosuppressive HCMV US proteins. It is well known that in fibroblasts, entry of virus through fusion leads to an efficient uptake of viral particles and to a cytosolic delivery of nucleocapsid and tegument proteins.6 Accordingly, our results showed that in the astrocytoma reference cell line U373MG, where HCMV entry by fusion exists, cytosolic delivery of incoming pp65 was responsible for activation of anti-pp65 CD8⁺ CTLs as we previously demonstrated in these cells and in MRC5 fibroblasts.8,17 We can assume that fusion entry of HCMV into cells may be a prerequisite for efficient control of infection by anti-pp65 CTLs.

According to previous studies showing that, in RPE cells, entry of HCMV occurs through an unusual endocytic pathway, followed by sequestration of viral particles,12 we made the assumption that this would be detrimental to pp65 delivery into the cytosol for processing into the MHC class I machinery. Even though we have no direct evidence that no pp65 protein at all was expressed into the cytosol of infected cells, either in native form or degraded into peptides, no CTL activation was recovered, even when very high MOIs were used, suggesting that provided cytosolic pp65 was not properly processed into HLA-A2-binding peptides.

We demonstrated that later, after infection, as expression of UL83 gene permitted an increase of intracellular pp65 level in both RPE and U373MG cells, the downregulating effect of proteins encoded in the US2-11 region on MHC class I expression was responsible for the absence of lysis of both cell lines.
by anti-p65 CTLs. It has been shown that US2-, US6-, or US11-transfected cells are unable to present endogenous antigens to CTLs and were therefore resistant to CTL lysis. However, our study is the first to show that proteins encoded by the whole US2-US11 region interfered with CTL recognition of an endogenous HCMV antigen in a context of HCMV infection. Even though we have no data demonstrating the specific effect of US2, US3, US6, and US11, as extensively reported, we demonstrated that after 6 hours of infection none of these proteins was able to efficiently inhibit presentation of incoming p65. In contrast, after 48 hours of infection the cumulative effect of the different US proteins was evidenced by using the HCMVΔ2-11 virus, especially in U373MG cells, where CTL lysis was highly restored. Regarding RPE cells, despite a partial MHC class I downregulation at 48 hours pi as shown by flow cytometric analysis (Fig. 5), cell sensitivity to CTLs was not significantly recovered by using the Δ2-11 virus, probably because of a poor level of processable p65 (Fig. 1B), because later on (8 day pi), as expression of UL83 gene increased, HCMVΔ2-11-infected cells were clearly sensitive to lysis.

Taking together our results suggest that anti-p65 CTL response within the first hours after infection could be an efficient way to control the spread of virus before expression of the immunosuppressive activity of the US proteins. In conclusion, we can assume that impaired killing by CTLs directed against incoming p65 or any other incoming tegument protein may contribute to spreading and to constitution of HCMV reservoirs within the retina.

RPE cells are in vivo targets of HCMV infection and replication, even though the in vitro replication of the virus is very delayed, compared with fibroblasts. The best known incidence of HCMV infection in immunodeficient HIV+ patients is retinitis and blindness. The pathophysiological mechanisms involved in HCMV retinopathy have not been entirely defined. The presence of HCMV in areas of retinal cotton-wool spots as well as breaches in retinal microvasculature allowing viral permeation of the blood-retina barrier have been suggested in pathogenesis. Infection of the RPE as an important part of the blood-retina barrier was also suggested to be crucial in the development of retinitis. Although the eye is an immune-privileged site, RPE cells may acquire, under inflammatory conditions, the capacity to function as antigen-presenting cells so that recognition by activated CTLs directed against viral antigens could induce cell death. We suggest that impaired killing of HCMV-infected RPE cells by CTLs directed against incoming tegument proteins could provide the virus with an additional trick for eluding host surveillance. It has also been shown recently that HCMV infection upregulates constitutive Fas ligand (FasL), which impairs neutrophil binding to infected RPE cells. The incidence of both mechanisms may contribute to viral spreading in the retina and to pathogenesis after reactivation in immunocompromised hosts. In two studies, it has been shown that growth kinetics of HCMV was altered in phagocytic macrophages and that in these cells the virus accumulated in cytoplasmic vacuoles and survived through disruption of the microtubule network to evade lysosomal fusion. The authors suggested that disruption of the microtubule network could alter the trafficking of proteins involved in antigen-presentation pathways, providing a novel mechanism for latency and evasion of immune recognition. Nevertheless, there are no data available showing that macrophages are resistant to CTLs directed against incoming p65.

Generally, T lymphocytes are dangerous to the host’s central nervous system, and these cells can be thus considered a double-edged sword for the host. Whether impaired killing by CD8+ T cells is beneficial either for the host, according to the vital functions of RPE cells, or for the persistence and spread of the virus is an open question.

Overall, it is worth hypothesizing that in cells such as epithelial cells, endothelial cells, and monocytes, both HCMV entry through endocytosis and the immunosuppressive effect of US proteins could allow the virus to elude immune surveillance at any stage of infection and to constitute reservoirs. Studies of the physiological relevance and mechanisms of viral entry in various cell types could provide new clues to fight against HCMV.

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References


