Decreased Cytomegalovirus Expression Following Proinflammatory Cytokine Treatment of Primary Human Astrocytes

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Decreased Cytomegalovirus Expression Following Proinflammatory Cytokine Treatment of Primary Human Astrocytes

Maxim C.-J. Cheeran,† Shuxian Hu,*‡ Genya Gekker,*‡ and James R. Lokensgard2*‡

Understanding the influence of immune effector mechanisms on CMV infection of the CNS may facilitate the development of immunotherapies for viral encephalitis. Using cultures of highly purified, fully permissive primary human astrocytes, proinflammatory cytokines, but not antiinflammatory cytokines or β-chemokines, were found to inhibit CMV expression, DNA synthesis, and replication. Treatment with certain proinflammatory cytokines 24 h before CMV infection markedly suppressed viral expression in astrocytes. TNF-α, IL-1β, and IFN-γ all inhibited CMV expression (70 ± 4.2%, 65 ± 3.4%, and 82 ± 3.6% inhibition of viral expression, respectively, n = 5). In contrast, no viral suppression was observed following IL-6 treatment. Suppressive activity was dependent on the addition of cytokines before CMV infection. Cytokine pretreatment did not affect CMV entry into primary astrocytes, and the observed cytokine-induced suppressive activity was not affected by the NO synthase inhibitor N(G)-monomethyl-L-arginine (N(G)MA). Instead, the suppressive effect appeared to be mediated through a mechanism involving inhibition of CMV major immediate early promoter activity. These results support the hypothesis that proinflammatory cytokines possess anti-CMV activity in brain cells and may lead to new interventions for CMV encephalitis based upon immunotherapy. The Journal of Immunology, 2000, 164: 926–933.

In the immunocompetent host, primary CMV infection is resolved by immune mechanisms before development of clinical disease, and cell-mediated immune responses appear to play a major role in successful host defense against this intracellular pathogen. However, in patients with profound defects in cell-mediated immunity, the CNS is one of the principal targets for CMV infection, where it can cause devastating encephalitis. Although CTL activity correlates with recovery from CMV infection (1), the destruction of large numbers of brain cells could inflict irreparable damage. Thus, host defense mechanisms, which protect against viral infections outside the CNS, may be destructive when operating within this “immunologically privileged” enclosed site where cell lysis, complement, and CTL responses may injure the host during the process of viral clearance.

Published studies provide evidence that lymphocytes are able to mediate clearance of viral infections from the CNS without conventional MHC expression on target cells and without massive target cell death (2). Soluble factors, derived from both endogenous brain cells and infiltrating lymphocytes may possess antiviral activity, and the blood-brain barrier may inhibit diffusion and draining of soluble antiviral factors released by specific effector T cells (3, 4). In several animal models, it appears that CD4⁺ lymphocytes are required to overcome viral CNS infections, and mounting evidence suggests that cytokines play a major role in the blockade of intracerebral viral spread (5). However, little or nothing is known about the role of soluble factors in defense of the brain against human CMV disease.

We have recently found that primary human astrocytes, the predominant cell type in the brain, support productive, cytopathic CMV replication (6). The purpose of the present study was to characterize the regulatory effects of cytokines on CMV expression in acutely infected, primary human astrocytes. We tested the hypothesis that cytokines mediate nonlytic suppression of human CMV in these brain cells.

Materials and Methods

Reagents

Recombiant human proinflammatory cytokines (IFN-γ, TNF-α, IL-1β, and IL-6), antiinflammatory cytokines (IL-4, IL-10, IL-13, and TGFB-1), and β-chemokines (RANTES, macrophage inflammatory protein (MIP)₁, 1α, as well as cytokine-specific polyclonal neutralizing Abs (goat) were obtained from R&D Systems (Minneapolis, MN). Abs to glial fibrillary acidic protein (GFAP) were obtained from DAKO (Carpinteria, CA).

Preparation of astrocyte cultures

Human fetal brain tissue was obtained from 16- to 22-week-old abortuses under a protocol approved by our Institutional Human Subjects Research Committee. Purified human fetal astrocyte cultures (>99% GFAP positive) were prepared using previously described methods (7), with minor modifications. In brief, brain tissues were cleared of meninges, minced into small fragments, and incubated with 0.25% trypsin (Sigma, St. Louis, MO) in Ca²⁺- and Mg²⁺-free Hanks’ saline (Life Technologies, Grand Island, NY) for 30 min at 37°C with gentle shaking. An equal volume of medium containing 10% FBS was added to inactivate the trypsin, and tissues were centrifuged at 1200 rpm for 5 min. After two washings with HBSS (Life Technologies), tissue fragments were replaced with fresh medium and triturated for 15 to 20 passages through a sterile Pasteur pipette. The cell suspension was then seeded into 75-cm² flasks at a density of 80 to 100 × 10⁶ cells/flask.
10^6 cells/flask in a high glucose DMEM (Sigma) containing 10% FBS, penicillin (100 U/ml), and streptomycin (100 mg/ml). These cultures were then incubated at 37°C in a water-saturated, 10% CO_2 incubator. After 72 h incubation, medium was changed with fresh DMEM containing 10% FBS. Culture medium was changed once a week thereafter. On day 21, flasks where shaken at 180 to 200 rpm for 16 h, followed by washing with the Ca^{2+} and Mg^{2+}-free HBSS containing 0.125% trypsin for 30 min at 37°C, followed by addition of 10% FBS-containing medium. After centrifugation, the cell suspension was seeded into a new flask in DMEM containing 10% FBS, and this culture medium was changed 24 h later. This subculturing procedure was repeated three times at a weekly interval. Finally, highly enriched astrocytes (>99% stain with anti-GFAP Ab) were seeded into 24-well plates for experimentation.

Quantitation of viral expression

The β-galactosidase-expressing recombinant human CMV RC256 (constructed in the laboratory of Edward Mocarski, Stanford University) was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and propagated on human foreskin fibroblasts (HFF). RC256 contains the lacZ reporter gene under the control of one copy of the major early promoter and possesses replication properties that are equivalent to wild-type CMV (8). Cytokine-treated as well as untreated astrocyte cultures were infected (multiplicity of infection (MOI) = 2.5) for 72 h. Infected astrocytes were pelleted, resuspended in 100 μL PBS, and subjected to three freeze-thaw cycles. 5-bromo-4-chloro-3-indolyl-D-β-D-galactoside (X-Gal, Boehringer Mannheim, Indianapolis, IN) was used as a substrate at a concentration of 1 mg/ml for quantitation of β-galactosidase activity in the lysates, indicative of viral expression.

Assays for viral replication

Human CMV strain AD169 (ATCC) was grown and titered by 50% tissue culture infectious dose assays (TCID_50) on HFF. For determination of viral titers from treated and untreated astrocyte cultures, the infected cells (MOI = 1) were subjected to three cycles of freeze-thaw lysis, and 4-fold serial dilutions were plated onto HFF. The cultures were assessed for cytotoxic effect after 14 days to determine TCID_50.

Assays for CMV entry into astrocytes and DNA synthesis

DNA extracted from CMV AD169-infected, cytokine-treated, as well as untreated astrocytes, at 6 h postinfection were used to determine the effect of cytokines on viral entry. DNA extracted from astrocytes at 72 h postinfection was used to determine the effect of cytokine treatment on CMV DNA synthesis. Samples from both time points were analyzed by quantitative PCR using the CMV-Quant Kit according to the manufacturer’s instructions (BioSource International, Camarillo, CA).

Measurement of NO production

Supernatants from cytokine-treated and untreated astrocytes were assayed for nitrite content, as a reflection of NO, in both the presence and absence of cytokines on viral entry. DNA extracted from astrocytes at 72 h postinfection was used to determine the effect of cytokine treatment on CMV expression (Fig. 1).

Results

Proinflammatory cytokine treatment of primary astrocytes inhibits CMV expression

Treatment of highly purified, fully permissive human astrocytes with selected proinflammatory cytokines for 24 h before infection with human CMV was found to dramatically inhibit viral expression. Addition of IL-1β (10 ng/ml) to astrocyte cultures was highly suppressive of subsequent CMV infection (Fig. 1), resulting in a 65 ± 3.4% inhibition of β-galactosidase activity, an indicator of viral expression. A similar suppressive effect was observed following the addition of TNF-α (20 ng/ml), which reduced CMV expression by 70 ± 4.2% (Fig. 1). Treatment of astrocyte cultures with IFN-γ (200 U/ml) also markedly reduced CMV expression by 82 ± 3.6% (Fig. 1). In contrast, pretreatment of astrocyte cultures with IL-6 (100 ng/ml) did not result in suppression of CMV expression (Fig. 1).

A similar pretreatment of primary human astrocytes with the antiinflammatory cytokines IL-4 (30 ng/ml), IL-10 (30 ng/ml), IL-13 (30 ng/ml), and TGF-β1 (10 ng/ml), 24 h before CMV infection, had no effect on viral expression (Fig. 1). In addition, treatment of astrocyte cultures with the β-chemokines RANTES (100 ng/ml) and MIP-1α (100 ng/ml), like antiinflammatory cytokines, had no effect on viral expression (Fig. 1).

Treatment of astrocyte cultures with anti-IL-1β, anti-TNF-α, or anti-IFN-γ Abs (10 μg/ml) 45 min before addition of the corresponding cytokine blocked the cytokine-induced suppressive effect on CMV expression (β-galactosidase levels of 159%, 144%, and 53% compared with untreated control cultures, respectively), whereas 10 μg/ml of isotype control Abs had no effect. Inclusion of each cytokine into the culture medium had no direct cytotoxic effect on astrocyte viability at the highest concentration used, as assessed by trypan blue dye exclusion (data not shown).

Inhibitory effects are cytokine concentration dependent

The doses of cytokines and chemokines used in the experiments presented in Fig. 1 are high doses that have been reported to confer
distinct effects upon astrocytes in previous experiments (7, 11–14). Dose-response studies were conducted with each of the three cytokines that suppressed expression of CMV. The suppressive effects of cytokine treatment on CMV expression were found to be dose dependent and reached maximal suppression at low concentrations (1 pg/ml, 200 pg/ml, and 0.02 U/ml for IL-1β, TNF-α, and IFN-γ, respectively, Fig. 2).

Proinflammatory cytokines inhibit viral replication in astrocytes

Astrocytes are fully permissive for CMV replication (6). To correlate levels of CMV β-promoter activity with viral replication and the production of infectious progeny, the infected cultures were collected 7 days postinfection, and plated onto HFF indicator cells for determination of viral titer by TCID₅₀ assay. IFN-γ, TNF-α, and IL-1β were all found to inhibit CMV replication with 75-, 60-, and 10-fold reductions in TCID₅₀ titers, respectively (Fig. 3). The cytokine doses used in these experiments were the same as those used in Fig. 1 (i.e., IFN-γ, 200 U/ml; TNF-α, 20 ng/ml; IL-1β, 10 ng/ml; and IL-6, 100 ng/ml).

Cytokine treatment inhibits CMV DNA synthesis but not viral entry

Cytokine-treated and untreated astrocyte cultures were infected with CMV (AD169), and viral DNA levels (number of viral genomes) were quantified by PCR. To determine the effects of proinflammatory cytokine treatment on viral entry, DNA levels were determined at 6 h postinfection. The number of CMV genomes measured in cytokine-treated astrocytes at this early time point...
(8.08 × 10^5, 8.46 × 10^5, and 2.74 × 10^6 copies for IFN-γ, TNF-α, and IL-1β treatment, respectively) was similar to that of astrocytes without cytokine treatment (8.72 × 10^5 copies, Fig. 4). However, when viral DNA levels were assessed at 72 h postinfection, the three proinflammatory cytokines markedly inhibited progeny DNA synthesis: 1.40 × 10^7 copies of the CMV genome in untreated astrocytes vs 7.42 × 10^5 genome copies for IFN-γ (94.7% inhibition), 2.61 × 10^6 copies for TNF-α (81.4% inhibition), and 6.49 × 10^6 copies for IL-1β (53.7% inhibition) treatment, whereas treatment with IL-6 had no suppressive effect (1.35 × 10^7 genome copies; Fig. 4).

Cytokine pretreatment is required for inhibition of viral expression

We next examined the effect of the timing of cytokine treatment on the induction of an antiviral state in astrocytes. For IL-1β, TNF-α, and IFN-γ, strong suppressive activity was dependent upon addition of cytokines 72 or 24 h before CMV infection, whereas simultaneous treatment had little or no suppressive effect (Fig. 5). Again, IL-6 pretreatment for either 72 or 24 h displayed no suppressive effect.

Cytokine-induced suppression of CMV expression is not mediated through NO

To evaluate the influence of NO on viral expression, the level of NO produced by astrocytes after cytokine treatment, with and without CMV infection, was examined. We found that CMV infection of astrocytes did not itself induce NO (Table I). Of the three inhibitory cytokines tested, only IL-1β had the ability to induce NO in astrocytes (52 ± 4.8 μM NO₃⁻, Table I). Addition of the NO synthase inhibitor N⁶-monomethyl-L-arginine (N⁶MA) blocked IL-1β-induced astrocyte NO production (13 ± 3.5 μM NO₃⁻ vs 48 ± 4.7 μM without N⁶MA), but did not block the suppressive effects of cytokine treatment on viral expression (0.75 ± 0.06 OD units vs 0.68 ± 0.08 OD units). Also, the addition of N⁶MA to either untreated or TNF-α- or IFN-γ-treated, CMV-infected astrocyte cultures had no effect on viral expression (Table I). Similar results were obtained in two independent experiments using astrocytes isolated from different brain specimens.
These data provide evidence that the antiviral effects of proinflammatory cytokines on astrocytes are not mediated through NO production.

**Proinflammatory cytokines inhibit CMV IE72 protein expression**

Western blot analysis of cytokine-treated, and untreated astrocyte lysates for IE72 protein expression following CMV infection was performed to determine whether cytokine treatment inhibits IE expression. Astrocytes were incubated with each proinflammatory cytokine for 72 h, infected with AD169, and analyzed for IE72 protein levels 24 h postinfection. Infected astrocytes pretreated with IL-1β, TNF-α, or IFN-γ displayed greatly reduced CMV IE72 levels when compared with untreated controls, whereas cells treated with IL-6 displayed IE protein levels similar to control cells (Fig. 6).

**Proinflammatory cytokines inhibit CMV IE1 mRNA expression**

To determine whether the effects of proinflammatory cytokines were mediated at the level of CMV IE1 transcription, RNA from cytokine-treated and untreated infected astrocyte cultures were analyzed using an IE exon 4-specific probe. Astrocytes were pretreated with either IL-1β, TNF-α, IFN-γ, or IL-6 72 h before infection with AD169, and RNA was extracted 3 h postinfection. Infected astrocytes that were pretreated with TNF-α and IFN-γ showed markedly decreased IE mRNA transcription, whereas IL-6 treatment did not influence viral expression compared with untreated controls (51.2 ± 7.7%, 49.2 ± 9.9%, and 0.9 ± 9.5%, respectively, based on densitometry, n = 3). IL-1β-treated cells, however, showed increased IE1 band density in Northern blots (163 ± 8.7%), consistent with the increase in levels of viral entry following IL-1β treatment (Fig. 6, B and C).

**Cytokine treatment inhibits CMV major IE promoter (MIEP) activity in primary human astrocytes**

To determine whether the suppressive effects of proinflammatory cytokine treatment were due to an inhibition of the activity of CMV regulatory DNA elements, a replication-defective adenovirus vector was used to transduce a CMV-MIEP-lacZ construct into our primary astrocyte cultures. This adenovirus approach was used because primary brain cells are difficult to efficiently transfect. Transduction of the construct into untreated astrocytes resulted in high β-galactosidase activity, due to CMV MIEP activity (Fig. 7). This signal was strongly reduced when the construct was transduced following pretreatment with proinflammatory cytokines, but...

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**Table 1. Role of NO in cytokine-mediated suppression of CMV expression**

<table>
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<th>Treatment</th>
<th>CMV Infection</th>
<th>NO3− (μM)</th>
<th>Viral Expressiona</th>
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<td>–</td>
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<tr>
<td>IL-1β</td>
<td>–</td>
<td>52 ± 4.8</td>
<td>1.74 ± 0.02</td>
<td>1.59 ± 0.1</td>
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<td>48 ± 4.7</td>
<td>7 ± 2.3</td>
<td>0.75 ± 0.06</td>
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<tr>
<td>IFN-γ</td>
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a OD values representing β-galactosidase activity measured using CPRG.

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**FIGURE 6.** Effect of cytokine treatment on CMV IE72 protein expression (A). Astrocytes were incubated with cytokines for 72 h, infected with CMV (AD169), and analyzed for IE72 protein levels 24 h postinfection by Western blot. Lane 1, uninfected astrocytes; lanes 2–6, CMV-infected. Lane 2, untreated; lane 3, IL-1β-treated (10 ng/ml); lane 4, TNF-α-treated (20 ng/ml); lane 5, IFN-γ-treated (200 U/ml); lane 6, IL-6-treated (100 ng/ml). Effect of cytokine treatment on CMV IE1 mRNA synthesis (B). Astrocytes were pretreated with cytokines for 72 h before infection with CMV (AD169). Total RNA was analyzed 3 h post infection by northern blot using an IE exon 4-specific probe. A Northern blot representative of three separate experiments is shown. Lane 1, uninfected astrocytes; lanes 2–6, infected astrocytes. Lane 2, untreated; lane 3, IL-1β-treated (10 ng/ml); lane 4, TNF-α-treated (20 ng/ml); lane 5, IFN-γ-treated (200 U/ml); and lane 6, IL-6-treated (100 ng/ml). GAPDH expression to control for loading. Densitometry and ImageQuant analysis (C). Ratio of IE expression over GAPDH was calculated for all the experiments performed and is expressed as an average value for each cytokine treatment. Results are expressed as an average from all blots analyzed.
not with IL-6 (Fig. 6). Pretreatment for 24 h with IFN-γ, TNF-α, or IL-1β was found to inhibit CMV MIEP activity by 76%, 70%, and 55%, respectively. Less of a suppressive effect was observed following simultaneous cytokine treatment and adenovirus infection (Fig. 7).

Discussion

Host defense responses to viral infections of the CNS must control the invading pathogen while simultaneously minimizing undesirable cytotoxic activity that may damage the brain. Immune responses within the CNS are thus different from those in other parts of the body. Noncytotoxic defense mechanisms mediated through the production of soluble, inhibitory factors most likely contribute to the control of CNS infections. Although proinflammatory cytokines, such as TNF-α and IL-1β, have been clearly implicated in neuronal damage (15–19), little is known about the regulatory effect of these cytokines on viral infections of the CNS or whether they possess beneficial, antiviral effects in brain cells.

In response to viral infection, cytokines are secreted within the CNS from both activated glial cells and T lymphocyte infiltrates (20). IFN-γ is a T lymphocyte-produced cytokine that is well known for its antiviral activity, including effects against CMV (21–23). The antiviral activity of TNF-α has also previously been reported (24–27), and IL-1 treatment decreases replication of murine CMV in vitro (28). However, the antiviral effects of particular cytokines depend on receptors present on specific infected cell types, hence the importance of using primary cells. We have previously reported on the antiviral activity of proinflammatory cytokines on HIV-1_Sf162 expression in both mixed glial/neuronal and purified microglial cell cultures (29). Davignon et al. (30) have shown that CMV IE-1 peptide-specific CD4+ T cell clones inhibit viral replication in U373 MG astrocytoma cells, and this suppression can be mimicked through synergistic interaction of IFN-γ and TNF-α. In the present study, the proinflammatory cytokines IFN-γ, TNF-α, and IL-1β, but not IL-6, were found to markedly suppress CMV expression, replication, DNA synthesis, and MIEP activity in purified cultures of primary human astrocytes. In addition, pretreatment of primary astrocytes with the antiinflammatory cytokines IL-4, IL-10, IL-13, and TGF-β1, or the β-chemokines RANTES and MIP-1α, were found not to affect viral expression.

The production and release of antiviral cytokines by glial cells may be beneficial to neighboring cells through protecting them from infection (i.e., bystander effect). Although work in our laboratory has demonstrated that IL-1β, IL-6, and TNF-α are produced by activated glial cells, we have been unable to detect IFN-γ release (using a sensitive ELISA assay; R&D Systems) from primary human microglial cell, astrocyte, or neuronal cultures in response to stimulation with LPS, IL-1β, or TNF-α (our unpublished data). Thus, infiltrating T lymphocytes would appear to be a major source of this potent antiviral cytokine in the CNS. In a healthy state, the CNS is devoid of identifiable leukocytes; however, during immune activation within the body, T lymphocytes have been shown to traffic through many organs, including the brain (31). Because CMV encephalitis usually occurs only in patients with profound defects in T cell numbers or function, the loss of the capacity of these cells to infiltrate the brain and produce IFN-γ may explain the predisposition of these patients to CMV brain infection.

Within the CNS, IL-6 has been reported to possess antiinflammatory as well as proinflammatory effects (32). In our study, IL-6 was not found to have the same profound anti-CMV effects in astrocytes as the other proinflammatory cytokines. It has recently been reported that IL-6 alone fails to initiate intracellular signaling events within astrocytes, but soluble IL-6 receptor restores the signaling function (33). Therefore, it appears likely that primary human astrocytes lack sufficient IL-6 receptors to transduce signals that induce an antiviral state, with corresponding resistance to CMV expression, following IL-6 treatment. A similar explanation could underlie the lack of antiviral activity of the β-chemokines RANTES and MIP-1β, although the presence or absence of receptors for these cytokines on astrocytes has not been defined.

Using quantitative PCR, we determined the number of viral genome copies present in infected astrocyte cultures that had been treated with inhibitory cytokines. At a 6-h postinfection time point (i.e., before viral DNA synthesis), we found similar numbers of viral genomes in cytokine-treated and untreated astrocytes, thus suggesting that the suppressive effect of cytokine treatment occurs at a postviral entry step. At 72 h postinfection, markedly less progeny viral DNA was detected in the cytokine-treated astrocytes. Taken together, these data indicate that the three antiviral proinflammatory cytokines exert their suppressive effects at a step between entry of the viral genome and DNA synthesis.

Although NO has been shown to play a role in the inhibition of replication of many viruses in response to cytokine treatment (34, 35), the cytokine-mediated inhibition of CMV in primary human astrocytes reported here was found to be NO independent. CMV has recently been reported to block NO production in IL-1β/IFN-γ-stimulated human retinal pigment epithelial cells (23). In contrast to these findings, in the present study we found that CMV infection does not block IL-1β-induced NO production in astrocytes. Furthermore, the NO synthase inhibitor Nω MA blocked IL-1β-induced astrocyte NO production but did not block the antiviral effects of cytokine treatment on viral expression. Thus, the suppressive effects of proinflammatory cytokine treatment of astrocytes are not mediated through NO production.

Using murine CMV, IFN-α treatment has been reported to directly inhibit IE gene expression in fibroblasts (36, 37). Our finding of cytokine-mediated inhibition of the CMV IE promoter-lacZ

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**FIGURE 7.** Cytokine-induced inhibition of MIEP activity. Astrocytes were treated with IL-1β (10 ng/ml), TNF-α (20 ng/ml), or IFN-γ (200 U/ml) either 24 h before (pre), at the same time (sim), or 24 h following (post) infection with a recombinant adenovirus vector (MOI = 10) containing a CMV MIEP-lacZ reporter gene construct. Seventy-two hours following adenovirus infection, β-galactosidase activity in astrocyte lysates was determined by CPRG assay (nd, not done).
construct in a recombinant adenovirus indicates that the antiviral mechanism in astrocytes, at least in part, involves inhibition of MIEP activity. The Northern blot analysis demonstrated that IFN-γ and TNF-α also inhibit activity of the CMV MIEP when positioned at its normal locus within the context of the viral genome. Based upon these Northern blots, it appears that IL-1 may mediate its suppressive effect through additional mechanisms. These data, along with those obtained by quantitative PCR and Western blot, indicate that the block in CMV expression is not at the level of viral entry, but rather at the level of viral gene expression.

The molecular basis for the cytokine-mediated viral inhibition reported here may involve inhibition of the activation of cellular transcription factors that bind to the CMV IE promoter and trans-activate transcription. In the murine CMV system, for example, IFN-α inhibits IE gene expression by down-regulating activity of the NF-κB transcription factor (38). Multiple binding sites for this transcription factor have also been identified in the human CMV IE promoter/enhancer region, and NF-κB is activated during CMV infection of HHF (39, 40). We have previously found that TNF-α and IFN-γ, as well as IL-1β, activate NF-κB in primary human astrocytes (41). Additionally, we have found that NF-κB is activated in primary astrocytes in response to CMV infection (our unpublished data). It is possible that 24 h pretreatment of astrocytes with proinflammatory cytokines desensitizes the cell to subsequent CMV-induced NF-κB activation, leading to decreased IE promoter activity and decreased viral expression. This desensitization could be mediated through increased nuclear levels of newly synthesized β-βs following treatment with inhibitory, proinflammatory cytokines (42).

In summary, the findings of this study demonstrate that the proinflammatory cytokines IL-1β, TNF-α, and IFN-γ induce an antiviral state in human astrocytes that results in suppression of CMV expression and suggest that cytokines play a role in host defense of the CNS against CMV. These findings may lead to the development of immune-based therapies for management of CMV encephalitis in immunocompromised patients.

Acknowledgments

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