Role of the Hypothalamic Pituitary Adrenal Axis and IL-6 in Stress-Induced Reactivation of Latent Herpes Simplex Virus Type 1

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Role of the Hypothalamic Pituitary Adrenal Axis and IL-6 in Stress-Induced Reactivation of Latent Herpes Simplex Virus Type 1

Sansanee Noisakran, William P. Halford, Livia Veress, and Daniel J. J. Carr

Hyperthermic stress induces reactivation of herpes simplex virus type 1 (HSV-1) in latently infected mice and also stimulates corticosterone release from the adrenals via activation of the hypothalamic pituitary adrenal axis. In the present study, we tested the hypothesis that stress-induced elevation of corticosterone potentiates HSV-1 reactivation in latently infected mice. Because of the putative role of IL-6 in facilitating HSV-1 reactivation in mice, the effect of hyperthermic stress and cyanoketone treatment on IL-6 expression in the trigeminal ganglion was also measured. Preadministration of cyanoketone, a glucocorticoid synthesis inhibitor, blocked the stress-induced elevation of corticosterone in a dose-dependent manner. Furthermore, inhibition of corticosterone synthesis was correlated with reduced levels of HSV-1 reactivation in latently infected mice. Hyperthermic stress elicited a transient rise in IL-6 mRNA levels in the trigeminal ganglion, but not other cytokine transcripts investigated. In addition, there was a significant reduction in MAC-3, CD8, and DX5 (NK cell marker) cells in the trigeminal ganglion of latently HSV-1-infected mice 24 h after stress. Cyanoketone blocked the stress-induced rise in IL-6 mRNA and protein expression in the trigeminal ganglion latently infected with HSV-1. Collectively, the results indicate that the activation of the hypothalamic pituitary adrenal axis plays an important role in stimulating IL-6 expression and HSV-1 reactivation in the trigeminal ganglion following hyperthermic stress of mice. The Journal of Immunology, 1998, 160: 5441–5447.

The activation of the hypothalamic pituitary adrenal (HPA) axis and the sympathetic nervous system (SNS) following stress are two major pathways with a significant impact on the immune system. Catecholamines as a major product of SNS activation have been shown to affect the circulation of leukocytes and Ab synthesis (1–4) through G-protein linked membrane bound receptors on the target lymphocytes (5). Glucocorticoids as the major product of HPA axis activation are potent immunosuppressive hormones (6–9) that target the transcription of 1XβR and thus block NFκB translocation to the nucleus and markedly reduce cytokine gene expression (10, 11). The result of the suppressive effects of stress on the immune system has been illustrated through experimental studies. Recent studies have shown that psychologic and physical stress can contribute to changes in susceptibility to viral pathogenesis and invasiveness (12–15), metastatic spread (16), and active immunization (17).

One viral pathogen that has achieved remarkable success in the human population, herpes simplex virus type 1 (HSV-1), is sensitive to stressors and can reactivate following the establishment of latency in response to UV irradiation (18), epinephrine iontophoresis (19), and transient hyperthermia (20) in animal models. Moreover, the administration of dexamethasone alone has been shown to induce modest reactivation of cell cultures from the trigeminal ganglion (TG) latently infected with HSV-1 and facilitate reactivation following hyperthermic stress (21). Taken together, these results suggest that the activation of the HPA and SNS pathways either independently or in concert induces reactivation of latent HSV-1 by an undefined mechanism.

While HSV-1 latency in mice is defined through the sole expression of latency-associated transcript (LAT) RNAs in the infected tissue and the lack of a detectable HSV-1-encoded protein, there is a persistent immune response during the latent period including infiltrating inflammatory cells (22), cytokine gene and protein expression (23–25), and a continuous increase in Ab titer to viral glycoproteins (26). Since spontaneous reactivation of HSV-1 rarely occurs in mice, the presence of the immune effector cells or their cytokines during latency may, in part, block viral replication. To this end, cytokines present during latency (23–25) (our unpublished observation) and implicated in antagonizing HSV-1 replication include IFN-α (27, 28), IFN-β (29), and TNF-α along with IFN-γ (30, 31). Accordingly, stress may suppress the levels of one or more of these cytokines or effector T cells eliciting these cytokines which, in turn, allows the virus to replicate yielding infectious virions. Restraint stress has previously been shown to reduce HSV-specific memory CTL activation in the spleen by mechanisms that suppress selective cytokines including IL-2, IL-4, IL-6, and IFN-γ (32, 33). These findings are consistent with the central role of CD8 lymphocytes in the control of viral replication (34, 35). However, these studies have not addressed the immune events during the reactivation of HSV-1 in the sensory ganglia. Conversely, rather than reducing the presence of effector cells...
or cytokines, stress may elicit or increase the synthesis of other factors that promote viral reactivation locally within the sensory ganglia.

The present study was undertaken to study the effects of hyperthermic stress and the activation of the HPA axis on HSV-1 reactivation and cytokine gene expression. The glucocorticoid synthesis inhibitor 2α-cyano-4,4,17α-trimethylandrosten-5-en-17β-ol-3-one (cyanoctetone (CK)), which blocks the production of active steroids (36, 37), was used to block stress-induced increases in corticosterone. We hypothesized that the HPA axis was the primary means by which latent HSV-1 is signaled (via corticosterone) to reactivate and consequently, CK would block HSV-1 reactivation in latent mice. Finally, we anticipated that hyperthermic stress would modify the expression of cytokine genes in the TG. Accordingly, we measured the expression of cytokine genes before and after hyperthermic stress in an attempt to correlate the levels of their expression with HSV-1 reactivation.

Materials and Methods

**Virus and cells**

Vero and CV-1 African monkey kidney cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 (Mediatech, Washington, D.C.) containing 5% FBS (Life Technologies, Gaithersburg, MD) and an antibiotic/antimycotic solution (Sigma Chemical, St. Louis, MO). Cells were incubated at 37°C, 5% CO₂, and 95% humidity. HSV-1 was grown up and harvested as previously described (25).

**Reverse transcription-PCR**

RT-PCR of TG was performed as described (25). Briefly, TG RNA was extracted in Ultraspec RNA isolation reagent (Biotex, Houston, TX). First-strand cDNA was synthesized using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). PCR was performed in a thermal cycler (MJ Research, Watertown, MA) with 35 cycles of 94°C (1 min), 15 s)→57–60°C (1 min, 15 s)→72°C (30 s). PCR primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), TNF-α, LAT, and RANTES were as previously described (25). IFN-β and CD8 primer sequences were obtained from Clontech Laboratories (Palo Alto, CA). Primers for IL-1α were 5'-ATGGCAAGAGTTCCGTAGTTT-3' (sense) and 5'-CCT TCAGGAAACCQGGCCTGTTG-3' (antisense) yielding a 620 bp product. Primers for IL-6 were 5'-TTCAATCATGCCTTCTCGG-3' (sense) and 5'-TTCAATGAGTCCCTAGGA-3' (antisense) yielding a 359-bp product. Following electrophoresis of the amplified product, ethidium bromide-stained PCR products were visualized with an Bio-Rad 1000 gel documentation system (Bio-Rad, Hercules, CA). Densitometric analysis of gel images was performed using Molecular analysis software 3.3 software (Bio-Rad).

**Competitive RT-PCR**

Our method involved the use of an external standard curve generated for each set of samples. The standard curve consisted of four PCR reactions containing a known amount of cloned target IL-6 or GAPDH ranging from 960 copies down to 15 copies per reaction. Three microtiters of each undiluted sample was aliquoted into separate reaction tubes. Primer competition was produced by the addition of 3 μl containing 15 copies of IL-6 or 50 copies of GAPDH to both standard and sample reactions. After completion of the PCR, the amplified products were electrophoresed and analyzed densitometrically. Copy equivalence was determined by constructing a standard curve plotting the mimetic-to-standard ratio of intensities against the number of copies in each reaction. In this way, a mimetic-to-sample ratio of intensities could be used to determine the number of copies in each sample.

**Infection and treatment of mice**

All mice (female CD1 mice, 25–34 g, Harlan Sprague Dawley, Indianapolis, IN) were infected and monitored for the success of the infection as previously described (25). Mice were hyperthermically stressed by a published protocol (20) with minor modifications. Specifically, a Brinkman RC3 circulating water bath with feedthrough copper tubing placed in an acrylic bath designed to hold up to six 50-ml tubes at any one time was used to regulate the temperature of the water bath to within 0.1°C. Mice were placed in 50-ml restraining tubes with 5-mm-diameter holes drilled throughout each tube. The mice were gently placed into the bath of water at 43°C. The mice were situated such that the water level did not exceed the neck region such that no physical effort was required by the animal to remain above the water level. However, the mice were constrained in the tube, which limited their movement suggesting some degree of restraint. Following a 10-min bath, the mice were removed, gently botted with paper towels, and placed in a warm room (34°C) for 30 min to prevent hypothermia. All animals were housed and cared for in accordance with National Institute of Health Guidelines on the Care and Use of Laboratory Animals (38). All procedures were approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee. In those experiments in which CK was used, mice received 50 to 100 mg/kg CK i.p. 24 and 2 h before the stress episode. CK (gift from Sanofi- Winthrop Research Division, Malvern, PA) was reconstituted in DMSO, served as the vehicle in control animals. This regimen has previously been shown to block LPS-induced elevations in corticosterone (39).

Dexamethasone (ICN, Aurora, OH) was reconstituted in ethanol and added to the drinking water in a 0.5% ethanol solution for a final concentration of 1 mg/ml. Mice were exposed to the glucocorticoid for 24 h before the stress event. Blood was obtained from the retroorbital plexus at the indicated time for analysis of circulating corticosterone levels.

**Detection of virus reactivated in vivo**

Ganglia from hyperthermically stressed mice were removed aseptically and homogenized with the Pro-200 tissue homogenizer (ProScientific, Monroe, CT) in 1.0 ml of RPMI 1640 containing 5% FBS. Homogenates were centrifuged (1 min, 10,000 × g) to remove cellular debris and the supernatant was layered onto CV-1 monolayers. The supernatants were incubated with the indicator cells for 45 min at 37°C in a 5% CO₂ incubator. Monolayers were then rinsed with PBS (pH 7.2), fresh RPMI 1640 containing 5% FBS and antibiotic/antimycotic solution was added, and the cultures were then plated in a 5% CO₂ incubator and monitored daily for cytopathic effect for 7 days. Plaques generally appeared 72 to 96 h following supernatant addition ranging from 2 to 5 plaques/well.

**Corticosterone determination**

Sera from killed animals were assayed for corticosterone levels by RIA (ICN Biomedicals, Costa Mesa, CA). All samples were assayed in duplicate. The corticosterone levels were extrapolated from the standard curve (R² = 0.9900).

**Cell tissue dissociation and flow cytometric analysis**

Single-cell suspensions of TG cells were obtained by placing isolated TG in 0.5 ml of calcium- and magnesium-free HBSS (pH 7.0) containing collagenase type XI (1 mg/ml, Sigma) and collagenase type IV (1 mg/ml, Sigma). Tissue was triturated every 20 min for 1 h at 37°C with a 1-ml serologic pipette. Dissociated cells were washed twice in PBS (pH 7.4) containing 0.5% BSA (PBS-BSA) by centrifugation (300 × g, 5 min). Following the second wash, the cells were resuspended in 1.0 ml of PBS-BSA. Dissociated cells (in 0.1-ml aliquots) were subsequently labeled with FITC- or phycoerythrin-conjugated Ab to dendritic cells (40) (Leuco Technologies, Ballwin, MO), macrophages (41) (MAC-3, PharMingen, San Diego, CA), NK cells (42) (DX-5, PharMingen), or CD8⁺ cells (PharMingen). Cells were incubated for 25 min on ice in the dark and subsequently washed twice with PBS-BSA by centrifugation (300 × g, 5 min). Cells were resuspended in PBS containing 1% paraformaldehyde and analyzed on a Coulter Elite FACS (Coulter, Hialeah, FL). Log forward scatter vs log side scatter plot was assembled to gate viable cells for analysis to separate cells from debris. Light scatter was collected at 488 nm, and emitted light was passed through a long pass filter followed by narrow band filter and analyzed at 525 nm (FITC) and 575 nm (phycoerythrin). Five thousand gated events were collected and analyzed per sample. Isotypic controls (PharMingen) were used to subtract nonspecific labeling of cells. Compensation of signal noise was 38%.

**Statistics**

One-way analysis of variance and the Scheffé multiple comparison test were used to determine significant (p < 0.05) differences between the indicated groups using the GBSTAT program (Dynamic Microsystems, Silver Spring, MD).
Results

Hyperthermic stress increases IL-6 but reduces CD8 transcript expression in the trigeminal ganglion of latent HSV-1-infected mice

A study was conducted to determine the effects of hyperthermic stress on cytokine gene expression and the reactivation of virus from the TG in mice latently infected with HSV-1. The results show that hyperthermic stress significantly increased the expression of IL-6 mRNA in the TG of latently infected mice in a time-dependent fashion, peaking 2 h poststress (Fig. 1, Table I). Although there was an indication that IL-6 levels were elevated in the uninfected, stressed animals, the levels did not reach significance. The stress episode was found to decrease CD8 transcript expression in the TG becoming significant 24 h poststress. No other cytokine mRNA was found to be modified in the TG, suggesting that the outcome of the stress response was specific for IL-6 and CD8. While there were significant differences comparing the levels of LAT, CD8, RANTES, and IL-1α transcripts in the TG between infected and uninfected mice, there were no differences in IL-6, TNF-α, and IFN-β.

Since qualitative changes were observed in the RT-PCR for IL-6, we assessed IL-6 mRNA using a quantitative RT-PCR method. Using competitive RT-PCR, we could confirm the RT-PCR results by determining the copy numbers of IL-6 mRNA as well as the constitutively expressed gene, GAPDH, from TG of latently infected mice following stress. Consistent with the previous observations, IL-6 mRNA copy numbers were significantly elevated in the TG of infected mice 2 h poststress but returned to prestress levels by 6 h poststress (Fig. 2). Uninfected mice had a delayed elevation in IL-6 mRNA in the TG peaking 6 h poststress but never attaining the significant level observed in the infected animals (Fig. 2). Competitive RT-PCR for GAPDH using the identical templates as the IL-6 competitive RT-PCR showed similar levels of this transcript in both infected and uninfected mice at all time points tested, suggesting that the elevation observed in IL-6 mRNA 2 h poststress was due to a specific increase in the target transcript.

Table I. Summary of cytokine and CD8 transcript expression in the TG of mice following hyperthermic stress

<table>
<thead>
<tr>
<th>Transcript</th>
<th>0 h</th>
<th>2 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAT inf</td>
<td>0.21 ± 0.09</td>
<td>0.35 ± 0.14</td>
<td>0.26 ± 0.06</td>
<td>0.20 ± 0.05</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>Lat uninfl</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-6 inf</td>
<td>0.05 ± 0.1</td>
<td>0.22 ± 0.06*</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.03</td>
<td>0.07 ± 0.1</td>
</tr>
<tr>
<td>IL-6 uninfl</td>
<td>0.06 ± 0.1</td>
<td>0.13 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.04</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>CD8 inf</td>
<td>0.78 ± 0.08</td>
<td>0.58 ± 0.10</td>
<td>0.65 ± 0.06</td>
<td>0.54 ± 0.12</td>
<td>0.31 ± 0.08*</td>
</tr>
<tr>
<td>CD8 uninfl</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.07 ± 0.03</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>TNF-α inf</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>TNF-α uninfl</td>
<td>0.05 ± 0.04</td>
<td>0.06 ± 0.04</td>
<td>0.07 ± 0.03</td>
<td>0.07 ± 0.05</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>IL-1α inf</td>
<td>0.26 ± 0.15</td>
<td>0.30 ± 0.26</td>
<td>0.21 ± 0.09</td>
<td>0.29 ± 0.17</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>IL-1α uninfl</td>
<td>0</td>
<td>0.1 ± 0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IFN-β inf</td>
<td>0.63 ± 0.16</td>
<td>0.70 ± 0.13</td>
<td>0.71 ± 0.10</td>
<td>0.66 ± 0.21</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td>IFN-β uninfl</td>
<td>0.38 ± 0.18</td>
<td>0.64 ± 0.08</td>
<td>0.60 ± 0.14</td>
<td>0.66 ± 0.26</td>
<td>0.52 ± 0.25</td>
</tr>
<tr>
<td>RANTES inf</td>
<td>0.95 ± 0.04</td>
<td>0.97 ± 0.06</td>
<td>1.02 ± 0.04</td>
<td>0.94 ± 0.08</td>
<td>0.86 ± 0.09</td>
</tr>
<tr>
<td>RANTES uninfl</td>
<td>0.67 ± 0.08</td>
<td>0.41 ± 0.16</td>
<td>0.76 ± 0.16</td>
<td>0.77 ± 0.21</td>
<td>0.52 ± 0.27</td>
</tr>
</tbody>
</table>

*RT-PCR was performed on total RNA isolated from the TGs of uninfected mice ( uninfl) and mice latently infected with HSV-1. RT-PCR product yield is expressed in terms of the integrated volume of pixels associated with each ethidium bromide-stained band. Results are expressed as a ratio of the target gene of interest and the housekeeping gene, GAPDH.

0 h defines basal levels of the expressed gene in the infected and uninfected mice. Other time points indicate the time points in which the mice were sacrificed post-hyperthermic stress.

*p < 0.05 comparing the transcript at the designated time point with all other groups (for IL-6) or with 0-, 2-, and 6-h time points (for CD8).
An association between stress and HSV-1 reactivation suggested that the activation of the HPA axis might be directly involved in this process. To investigate this possibility, the corticosterone synthesis inhibitor CK was administered to mice before hyperthermic stress. Consistent with previous observations, CK blocked stress-induced increases in circulating corticosterone levels in a dose-dependent fashion (Table III). Likewise, CK blocked HSV-1 reactivation in latent mice in a dose-dependent fashion (Table III). Since the results showed that increases in corticosterone coincided with viral reactivation, we next asked whether the exogenous administration of dexamethasone (a glucocorticoid analogue) alone and not discrepancies in the quantity or quality of total template (Fig. 3).

Hyperthermic stress reduces CD8\(^+\), MAC-3\(^+\) (macrophages), and DX5\(^+\) (NK cells) cells in the TG

Since the RT-PCR analysis showed a decrease in the expression of CD8 transcript 24 h poststress, immune cell composition before and after stress in latently infected and uninfected mice was investigated. There was a significant increase in the percentage of CD8\(^+\) and DX5\(^+\) cells in the TG of latently infected animals following stress. The dendritic cell population (as measured by a percentage of the total population) was not affected by hyperthermic stress in the latent HSV-1-infected mice. Likewise, none of the immune cell populations in the uninfected mouse TG was modified following stress (Table II).

CK blocks stress-induced increases in corticosterone and HSV-1 reactivation

An association between stress and HSV-1 reactivation suggested that the activation of the HPA axis might be directly involved in this process. To investigate this possibility, the corticosterone synthesis inhibitor CK was administered to mice before hyperthermic stress. Consistent with previous observations, CK blocked stress-induced increases in circulating corticosterone levels in a dose-dependent fashion (Table III). Likewise, CK blocked HSV-1 reactivation in latent mice in a dose-dependent fashion (Table III). Since the results showed that increases in corticosterone coincided with viral reactivation, we next asked whether the exogenous administration of dexamethasone (a glucocorticoid analogue) alone

Table II. Hyperthermic stress reduces the percentage of CD8\(^+\), MAC-3\(^+\), and DX5\(^+\) cells in the TG 24 h poststress

<table>
<thead>
<tr>
<th>Group and Cell Phenotype</th>
<th>Prestress</th>
<th>Poststress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dentritic cell</td>
<td>1.2 ± 0.60</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>2. Macrophage</td>
<td>1.4 ± 0.4</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>3. NK cell</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>4. CD8(^+) cell</td>
<td>0.6 ± 0.04</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>HSV-1-infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dentritic cell</td>
<td>0.6 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>2. Macrophage</td>
<td>3.0 ± 0.2</td>
<td>1.0 ± 0.3**</td>
</tr>
<tr>
<td>3. NK cell</td>
<td>0.9 ± 0.1</td>
<td>0 ± 0.0**</td>
</tr>
<tr>
<td>4. CD8(^+) cell</td>
<td>1.8 ± 0.3</td>
<td>0.4 ± 0.3*</td>
</tr>
</tbody>
</table>

* Mice latently infected with HSV-1 or uninfected mice (n = 3/group) were sacrificed 0 (non-stressed) or 24 h following hyperthermic stress. The TG was removed and processed. Single-cell suspensions were analyzed for dendritic cell, macrophage (MAC-3\(^+\)), NK cell (DX5\(^+\)), and CD8\(^+\) cell content by flow cytometry.

** Numbers represent the mean percent positive ± SEM. *p < 0.05, **p < 0.01, ***indicates a significant difference (p < 0.005) comparing the matched infected to uninfected group as determined by ANOVA and Scheffe multiple comparison test. **p < 0.01, *p < 0.05 comparing the percent positive cells before and after hyperthermic stress as determined by ANOVA and Scheffe multiple comparison test.

Table III. CK blocks stress-induced reactivation of HSV-1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hyperthermic Stress</th>
<th>Corticosterone (ng/ml)</th>
<th>HSV-1 Reactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>No</td>
<td>61 ± 15***</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Yes</td>
<td>798 ± 38</td>
<td>6/21 (29%)</td>
</tr>
<tr>
<td>CK (50 mg/kg)</td>
<td>Yes</td>
<td>429 ± 25**</td>
<td>3/14 (21%)</td>
</tr>
<tr>
<td>CK (75 mg/kg)</td>
<td>Yes</td>
<td>250 ± 20**</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>CK (100 mg/kg)</td>
<td>Yes</td>
<td>70 ± 56**</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>Dexamethasone (1.0 mg/ml)</td>
<td>No</td>
<td>22 ± 2**</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>Dexamethasone (1.0 mg/ml)</td>
<td>Yes</td>
<td>283 ± 168*</td>
<td>1/9 (11%)</td>
</tr>
</tbody>
</table>

* Mice latently infected with HSV-1 were given vehicle, dexamethasone, or CK at the indicated concentration as described (see Materials and Methods). Mice were or were not exposed to hyperthermic stress, and blood was obtained 1 h following the stressor to determine corticosterone levels. The mice were subsequently killed 24 h poststress, and the TGs were removed and assessed for viral reactivation (i.e., the recovery of infectious virus). CK alone was not found to induce reactivation (n = 5 for 50 mg/kg and 100 mg/kg; not tested for 75 mg/kg).

** Numbers are mean ± SEM. *p < 0.01, **p < 0.05 comparing vehicle, hyperthermic stress group with all other groups as determined by ANOVA and Tukey’s t test.
Moreover, there is clinical precedence for herpes virus reactivation following systemic glucocorticoid administration (44, 45). Previ-ously, dexamethasone alone did not induce reactivation nor did it aug ment hyperthermic stress-induced reactivation (Table III). However, dexamethasone did block stress-induced increases in corticosterone levels, suggesting that it was at a concentration that induced a negative feedback loop on the HPA axis (Table III).

CK treatment antagonizes the stress-induced increase in IL-6 mRNA and protein in the TG of mice latently infected with HSV-1

Since hyperthermic stress induced reactivation of latent HSV-1 and transiently increased the expression of IL-6 in the TG and CK blocked HSV-1 reactivation, a study was conducted to determine whether CK could block the stress-induced elevation in IL-6 mRNA expression. CK treatment (100 mg/kg) of mice was found to partially block the increase in IL-6 gene expression 2 h poststress compared with the vehicle-treated group (Fig. 4). Consistent with the mRNA expression, there was a reduction in IL-6 protein measured in the TG of the 100 mg/kg CK-treated mice compared with the vehicle-treated controls 12 h poststress (Fig. 5). The effect mediated by CK treatment on IL-6 mRNA expression was specific, since there were no changes in the expression in the housekeeping gene, GAPDH (Fig. 4). However, a lower concentration of CK (50 mg/kg) had no effect on IL-6 mRNA expression 2 h poststress. Specifically, pretreat IL-6:GAPDH image pixel ratios were 0.31 ± 0.02. Two hours after hyperthermic stress, IL-6:GAPDH image pixel ratio of the vehicle-treated group was 0.48 ± 0.05, while the IL-6:GAPDH ratio of the 50.0 mg/kg CK-treated group was 0.52 ± 0.07.

Discussion

Glucocorticoids have previously been shown to modestly induce reactivation of latent HSV-1 both in vitro (21) and in vivo (43). Moreover, there is clinical precedence for herpes virus reactivation following systemic glucocorticoid administration (44, 45). Previ-ous studies have shown the addition of dexamethasone (10^{-7} M) added to in vitro TG cell cultures enhanced the kinetics of reactivation of HSV-1 (25). However, data in the present study showed the lack of an effect in viral reactivation following dexamethasone treatment in vivo. The concentration administered to mice (1 mg/ml) significantly reduced circulating levels of corticosterone and may have interfered with other endogenous hormonal pathways that influence HSV-1 reactivation including β-adrenoceptor-sensitive pathways (46). A previous study has shown that 10 μg/ml dexamethasone in the drinking water of rats occupies 80% of the type II glucocorticoid receptor, reducing the affinity by 10-fold following an overnight exposure to the drug (47). Alternatively, the glucocorticoid may have suppressed the production of factors/ cytokines that promote HSV-1 reactivation. Cytokines that are sensitive to the effects of glucocorticoids include IL-6 (48) which is known to have glucocorticoid-responsive elements in the 5′-flanking region of the IL-6 gene (49) as well as TNF-α (50, 51). TNF-α also augments HSV-1 reactivation and replication in TG explants (52). While TNF-α has previously been shown to elicit IL-6 production by astrocytes (53, 54), the relationship between IL-6 and TNF-α has not been established in the peripheral nervous system including the TG.

The coincidental occurrence of IL-6 and HSV-1 has previously been observed. For example, UV light which reacti vates HSV-1 has been shown to induce IL-6 (55). Likewise, high levels of IL-6 in the cerebrospinal fluid of patients with HSV-1 encephalitis during the acute stage of the infection have been reported (56). Furthermore, the selective expression of the IL-6 gene in an HSV-1 permissive cell line following infection has been described (57). While the appearance of IL-6 may simply be due to its proinflammatory nature, a recent study suggests that IL-6 may, in part, facilitate HSV-1 replication or reactivation. Specifically, pretreatment of mice latently infected with HSV-1 with a mAb to IL-6 significantly blocked recoverable virus from the eye following hyperthermic stress (58). These authors suggest that IL-6 induces the activation of the transcription factors STAT3/APRF and nuclear factor-IL-6, which in turn promotes viral DNA replication. Neutralizing Ab to IL-6 has been found to partially block (50%)
HSV-1 reactivation in TG explant cultures again supporting a role for IL-6 in viral reactivation.3

In the present study, hyperthermic stress induced HSV-1 reactivation and elicitation of a transient increase in IL-6 mRNA in the TG of latent mice. In addition, CK, which blocked stress-induced HSV-1 reactivation in a dose-dependent manner, also partially blocked the expression of the IL-6 transcript and protein. Taken together, these observations seem to support Kriesel's work suggesting a role for IL-6 and HSV-1 reactivation (58). Hyperthermic stress was found to specifically elevate IL-6 mRNA but not other proinflammatory cytokines including IL-1α or TNF-α assessed during latency. However, CD8 transcript levels were significantly decreased 24 h poststress in the TG compared with the 0- to 12-h poststress time points. Consistent with these observations, hyperthermic stress of in vitro latently infected TG cell cultures results in the disappearance of CD8 transcripts 24 h poststress (59). The reduction of CD8 mRNA following stress and reduction of CD8+ lymphocytes, NK cells, and macrophages in the TG may allow viral replication and assembly to occur without interference by the would-be resident effector cells. However, the time frame of the absence of these immune cells is short lived (24–48 h) with the recruitment of infiltrating cells following viral reactivation as has been reported (60). The infiltrating CD8+ effector cells would limit the spread of the virus from the TG to the eye as suggested by the difference in frequency of detection of HSV-1 comparing the TG and eye following hyperthermic stress (20).

Changes in the immune cell constituency within the TG of latently infected mice with HSV-1 following hyperthermic stress may be due to corticosteroids. Previous studies have shown that stress-induced increases in corticosterone was accompanied by decreases in the circulating population of immune cells including B lymphocytes, NK cells, and monocytes (61). The changes in the circulating population of cells following stress were negated in adrenalectomized animals but mimicked by type 1 but not type 2 glucocorticoid receptor agonists suggesting that adrenal hormones are the primary mediators altering lymphoid and myeloid cell trafficking (61, 62). Consequently, one possible scenario is that by blocking the stress-induced increase in corticosterone with CK the subsequent antagonism of corticosterone modulation of the immune cell profile within the TG is achieved and viral reactivation/repli-
cation is limited.

The proinflammatory cytokines including TNF-α, IL-1, and IL-6 are known to activate the HPA axis (63, 64). A recent study has found that IL-6 is required for glucocorticoid production during murine CMV infection through the induction of IL-1α (65). However, in the present study hyperthermic stress was found to specifically alter IL-6 mRNA expression but not other cytokines including IL-1α in the TG of latently infected mice, suggesting that the induction of IL-6 is IL-1α independent in this stress paradigm. Yet, in another study, changes in the immune profile of restrained mice infected with influenza A virus (strain PR834) included a selective rise in IL-6 by cells from the regional lymph node (66). The restraint stress-induced rise in IL-6 could be prevented by the administration of the type II glucocorticoid antagonist RU486, suggesting the involvement of corticosteroids. This notion was further supported by data showing that low concentrations (10–10 M) of corticosterone could augment IL-6 but not IL-2, IL-10, or IFN-γ production by splenocytes (66). However, in the present study, it is difficult to determine the mechanism for IL-6 induction. Since there is no significant rise in IL-6 mRNA in uninfected TG following hyperthermic stress, the presence of latent HSV-1 would appear to be involved in the augmentation of this cytokine. Similar to the findings of Dobbs et al. (66), it is conceivable that modest concentrations of corticosterone reach the TG immediately following hyperthermic stress that through either an additive or a synergistic effect with HSV-1 reactivation augment IL-6 production. The transient rise and fall of IL-6 mRNA expression in the TG is consistent with the continued rise in circulating corticosterone that ultimately reaches a critical level and acts as a feedback inhibitor to IL-6 production (64).

The cells that express IL-6 in the TG are presently unknown but could include resident dendritic cells and macrophages (59), Schwann cells (67), or neurons (68). Since hyperthermic stress did not change the percentage of dendritic cells in the TG of latent HSV-1-infected mice, these cells are a likely candidate for IL-6 expression. Dendritic cells are potent producers of IL-6 following HSV-1 infection (69). Likewise, Schwann cells have been shown to express IL-6 and IL-6 receptors following trauma (67). It is tempting to speculate that hyperthermic stress can induce the expression of IL-6 and IL-6 receptors by resident cells within the TG that subsequently allow the peripheral nerves to respond to IL-6 as previously suggested (67) potentially thru gp130 dimerization and subsequently cytoplasmic tyrosine kinase activation (70). Future work is required to address this tempting association more closely.

References

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