Effect of Immunosuppression on Gene Expression in the HSV-1 Latently Infected Mouse Trigeminal Ganglion

Shiro Higaki,1 Bryan M. Gebhardt,1,2,3 Walter J. Lukiv,1,5 Hilary W. Thompson,1,3 and James M. Hill1,2,5,4

PURPOSE. To determine alterations in expression of genes in herpes simples virus (HSV-1) latently infected mouse trigeminal ganglia (TGs), after treatment with cyclophosphamide and dexamethasone.

METHODS. Scarified corneas of female BALB/c mice were inoculated with HSV-1 strain McKrae. Four weeks after inoculation, cyclophosphamide and dexamethasone were intravenously injected to induce HSV-1 reactivation. Uninfected mice were also treated with the immunosuppressants. Four groups of animals were studied: uninfected, not treated; uninfected, drug treated; latently infected, not treated; and latently infected, drug treated. PolyA+ mRNA from the TGs of each group was reverse transcribed, labeled with 32P, incubated on a 1185-gene array, and analyzed by phosphorimaging. As a comparison and to confirm microarray results, semiquantitative RT-PCR was also performed for selected genes.

RESULTS. The immunosuppressive drugs significantly increased expression of two genes (calpain 1 light chain and guanine nucleotide-binding protein α-stimulating polypeptide [GNAT]) in the ganglia of infected mice compared with those in untreated uninfected mice. Ten genes were shown to be significantly increased in the latent TGs of mice treated with immunosuppressants compared with latently infected untreated mice. These genes were prostat glandin E2 receptor EP4 subtype (PTGER4), insulin promoter factor 1 (IPF1), glutathione S-transferase μ2, cyclin D2, peripherin, plasma glutathione peroxidase, methyl Cpg-binding protein 2, retinal S-antigen, ErbB2 proto-oncogene, and GNAT. Eight genes were shown to be significantly decreased in the HSV-1 latent TGs treated with the drugs, compared with untreated latently infected mice. These genes were peripheral myelin protein 22, decorin, transcription factor AP-1, dystroglycan 1, myelin protein zero, mitogen-activated protein kinase 3, prothymosin beta 4, and brain lipid-binding protein. The results obtained by semiquantitative RT-PCR were similar to those obtained by microarray analysis.

CONCLUSIONS. Those genes with expression altered by immunosuppressive drug treatment may play an important role in ocular HSV-1 recurrence. Changes in expression of genes in the prostaglandin pathway, a transcription factor, and an enzyme in the cell cycle are considered especially important in HSV-1 reactivation by immunosuppression and are reviewed. (Invest Ophthal Vis Sci. 2002;43:1862–1869)

Herpes simplex virus (HSV-1) establishes a latent state in sensory neurons and may reactivate throughout the life of the host.1–3 Herpes reactivation may involve altered expression of cellular genes, such as increased expression of certain transcriptional activators and/or reduced expression of repressor factors. The determination of the qualitative and quantitative changes in host and viral transcripts during the transition from latency to reactivation may facilitate the development of therapeutics for the prevention of viral reactivation. Some 30 cellular proteins have been suggested to be involved in HSV-1 latency and reactivation.4–8 These include transcription factors, such as cAMP response element binding protein,9–11 lumen.12,13 OCT-1 and OCT-2,14–17 and c-jun18; signal transduction factors, such as protein kinase C19 and fibroblast growth factor20; tumor necrosis factor-α21, interferons22,23; nerve growth factor24; and cycloxygenase (COX)-2.25 Reverse transcription-polymerase chain reaction (RT-PCR) and Northern blot and Western blot analyses have been used to assay for cellular responses during viral latency and reactivation, although to date only a few cell-derived proteins have been investigated in each study.9–25

Microarray analysis is a powerful tool that permits the study of many genes at one time. Stingley et al.26 prepared the first microarrays containing the 80 HSV-1 transcripts expressed during acute and latent infection. Using an HSV mutant, they found that the viral α27 gene plays a major role in the reduction of cellular gene expression. Kodarev et al.27 hybridized cDNAs derived from three separate human cell lines infected with wild-type HSV and a mutant virus to a DNA array containing probes for 588 human genes. They reported an accumulation of mRNAs that encode for transcription factors and stress response proteins in cells in which the transcription of most cellular RNAs has been reduced. Tsavachidou et al.28 examined the gene expression changes that occur in cellular mRNA levels in uninfected mouse TGs after explantation, using gene array analysis. Many genes, including B-cell lymphoma 3-encoded protein, were altered in expression in the model. These microarray investigations were either in vitro or ex vivo.29–38 We have used microarray analysis to identify nine genes in the HSV-1 latently infected mouse TGs that exhibited increased expression 1 hour after hyperthermia.29 In that in vivo study, we used a mouse stress array (Clontech, Palo Alto, CA) containing sequences for 149 genes. To our knowledge, this is the only in vivo study involving microarray analysis to assess the cellular gene response to HSV-1 latency during reactivation.

In the present study, we assessed gene expression in uninfected mouse TGs and those latently infected with HSV-1 after treatment with cyclophosphamide and dexamethasone. Our goal was to determine the cellular factors that are altered during immunosuppression-induced HSV-1 reactivation. Immunosuppression may induce transcription of a unique set of...
cellular mRNAs expressed in latently infected TGs that differ from the mRNAs expressed in latently infected TGs not exposed to the drugs.

We report that the transcription of a large number of genes was altered by treatment with immunosuppressive drugs. Using an array membrane containing the probes for 1185 known genes, including nine housekeeping genes, altered expression of at least 18 genes was noted in the ganglia of latently infected drug-treated mice. Possibly some of these genes with expression altered by treatment of latent disease with immunosuppressive drugs are key in the genetic mechanism of HSV-1 reactivation.

**Materials and Methods**

**Mouse Eye Model of Immunosuppression**

Five- to 7-week-old female BALB/c mice (National Cancer Institute, Bethesda, MD) were housed in the Louisiana State University Health Sciences Center animal care facility and maintained on laboratory chow and water ad libitum. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice were anesthetized with ketamine hydrochloride (1 mg/kg; Phoenix Scientific Inc., St. Joseph, MO), and xylazine (0.5 mg/kg; Taylor Pharmaceuticals, Decatur, IL). The corneas were scarified and inoculated with 3 μL of a viral suspension containing 2.5 × 10^6 plaque-forming units of HSV-1 strain McKrae. To verify corneal infection, slit lamp examinations and ocular swabs were performed on days 3 and 4 after inoculation.

Four weeks after inoculation, when the corneas healed, the mice were given an intravenous (IV) injection of 5 mg cyclophosphamide (Sigma, St. Louis, MO) in 0.2 mL of water, and 24 hours later an IV injection of 0.2 mg dexamethasone (Sigma) in 0.2 mL of water.29–31 Uninfected mice were also given drugs on the same schedule. One hour after the dexamethasone injection, the mice were killed, and the TGs were removed. Four groups of animals were studied: uninfected, not treated; uninfected, drug treated; latently infected, not treated; and latently infected, drug treated. Twenty-two mice (44 TGs) were examined in each group.

**Isolation of PolyA+ mRNA and cDNA Array Hybridization**

Total RNA was isolated from the TGs in extraction reagent (TRIZol; Gibco BRL, Rockville, MD) and DNase, as part of a commercial pure total RNA labeling protocol (Atlas; Clontech). The RNA was spectrophotometrically scanned, and all samples had an absorbance ratio (A260/A280) of 2.0 or more. No significant differences in the spectral purity, rate of degradation, and yield were noted among the groups. The polyA+ mRNA fraction was obtained using biotinylated oligo (dT) and streptavidin-coated paramagnetic beads. PolyA+ mRNA was reverse transcribed with Moloney murine leukemia virus (MMLV) reverse transcriptase in the presence of [32P]-dATP (10 μCi/μL; 3000 Ci/mmol; 35 μCi per reaction; Amersham, Piscataway, NJ). cDNAs were purified in a nucleopin column and hybridized onto cDNA array panels (Atlas Mouse 1.2 Array; Clontech) with hybridization solution (ExpressHyb; Clontech). cDNA array nylon membranes were washed and exposed29,32,33 according to the manufacturer's protocol (PT 3140-1; Clontech). The mouse array has 1185 genes; the complete list can be found at http://www.clontech.com/atlas/genelists/index.shtml. There was approximately 1 × 10^7 cpm associated with each cDNA that was hybridized to an array membrane. For each cDNA preparation, two determinations were conducted.

**Table 1.** Changes in Gene Transcription in Mouse TGs after Immunosuppression with Dexamethasone and Cyclophosphamide

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Intensity: Uninfected Trigeminal Ganglia</th>
<th>Intensity: HSV-Latent Trigeminal Ganglia</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpain 1 light chain</td>
<td>M16465</td>
<td>14157.5 ± 8437.9</td>
<td>29044.5 ± 11096.6</td>
<td>1.52 ± 0.12</td>
<td>0.0023</td>
</tr>
<tr>
<td>GNAS</td>
<td>Y00705</td>
<td>8517.0 ± 5762.9</td>
<td>16081.5 ± 6491.9</td>
<td>2.19 ± 0.74</td>
<td>0.0006</td>
</tr>
<tr>
<td>Glutathione S-transferase mu2</td>
<td>X74342</td>
<td>985.8 ± 12.0</td>
<td>2476.5 ± 122.3</td>
<td>25.26 ± 1.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cyclin D2</td>
<td>M83749</td>
<td>1915.5 ± 94.0</td>
<td>2676.0 ± 186.7</td>
<td>1.40 ± 0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peripherin</td>
<td>X15475</td>
<td>868.0 ± 128.7</td>
<td>1135.4 ± 157.7</td>
<td>1.71 ± 0.55</td>
<td>0.0167</td>
</tr>
<tr>
<td>Plasma glutathione peroxidase</td>
<td>U13705</td>
<td>713.5 ± 6.4</td>
<td>1137.5 ± 40.0</td>
<td>1.60 ± 0.11</td>
<td>0.0233</td>
</tr>
<tr>
<td>Methyl CpG-binding protein 2</td>
<td>AFO72251</td>
<td>1281.0 ± 206.5</td>
<td>1904.5 ± 53.0</td>
<td>1.51 ± 0.29</td>
<td>0.0011</td>
</tr>
<tr>
<td>Erg2 proto-oncogene</td>
<td>L47239</td>
<td>1582.0 ± 176.8</td>
<td>2181.0 ± 123.4</td>
<td>1.36 ± 0.06</td>
<td>0.0016</td>
</tr>
<tr>
<td>GNAS</td>
<td>Y00705</td>
<td>4195.0 ± 130.1</td>
<td>4873.0 ± 479.4</td>
<td>1.15 ± 0.09</td>
<td>0.0004</td>
</tr>
<tr>
<td>Peripheral myelin protein 22</td>
<td>M32240</td>
<td>1669.5 ± 78.5</td>
<td>747.0 ± 328.1</td>
<td>0.44 ± 0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Decorin</td>
<td>X55392</td>
<td>2975.0 ± 219.2</td>
<td>1435.0 ± 91.9</td>
<td>0.56 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Transcription factor AP-1</td>
<td>J04115</td>
<td>2990.0 ± 220.6</td>
<td>1588.0 ± 476.6</td>
<td>0.62 ± 0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dystroglycan 1</td>
<td>U45312</td>
<td>1167.5 ± 0.7</td>
<td>610.5 ± 280.7</td>
<td>0.52 ± 0.24</td>
<td>0.0043</td>
</tr>
<tr>
<td>Myelin protein zero</td>
<td>M62860</td>
<td>1938.5 ± 27.6</td>
<td>1340.5 ± 98.3</td>
<td>0.69 ± 0.04</td>
<td>0.0017</td>
</tr>
<tr>
<td>Mitogen-activated protein kinase 1</td>
<td>M6177</td>
<td>15180.0 ± 82.0</td>
<td>907.5 ± 456.1</td>
<td>0.70 ± 0.39</td>
<td>0.02793</td>
</tr>
<tr>
<td>Prothymosin beta 4</td>
<td>X16055</td>
<td>3834.5 ± 129.4</td>
<td>3513.0 ± 383.3</td>
<td>0.86 ± 0.14</td>
<td>0.0057</td>
</tr>
<tr>
<td>Brain lipid-binding protein</td>
<td>S69799</td>
<td>3948.0 ± 418.6</td>
<td>3518.0 ± 2.8</td>
<td>0.88 ± 0.88</td>
<td>0.0215</td>
</tr>
</tbody>
</table>

* Reduced transcription not seen in this group.
† Erb2 proto-oncogene and GNAS were increased less than 1.4-fold in this group, but were increased more than 1.4-fold in the uninfected TGs, and therefore are included to achieve a balanced composition in the ANOVA.
‡ Prothymosin beta 4 and brain lipid-binding protein were increased less than 1.4-fold in this group, but were increased more than 1.4-fold in the uninfected TGs, and therefore are included to achieve a balanced composition in the ANOVA.

Data are mean ± SD.

---

**Table 2.** Changes in Gene Expression in the HSV-1 Latent Ganglion

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Intensity: Uninfected Trigeminal Ganglia</th>
<th>Intensity: HSV-Latent Trigeminal Ganglia</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain lipid-binding protein</td>
<td>S69799</td>
<td>3948.0 ± 418.6</td>
<td>3518.0 ± 2.8</td>
<td>0.88 ± 0.88</td>
<td>0.0215</td>
</tr>
</tbody>
</table>

---

**Table 3.** Changes in Gene Expression in the HSV-1 Latent Ganglion

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Intensity: Uninfected Trigeminal Ganglia</th>
<th>Intensity: HSV-Latent Trigeminal Ganglia</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain lipid-binding protein</td>
<td>S69799</td>
<td>3948.0 ± 418.6</td>
<td>3518.0 ± 2.8</td>
<td>0.88 ± 0.88</td>
<td>0.0215</td>
</tr>
</tbody>
</table>
Each phosphorimage is a representative example of microarray analysis of polyA+ mRNA from the TGs of mice. (A) Uninfected mice, not treated; (B) uninfected mice, drug treated. cDNA array panels are divided into seven theme-targeted sectors, including (a) basic transcription factors, (b) cell-cycle regulation, (c) oncogenes, (d) chemokine-cytokine signaling, (e) signal transduction, (f) cytoskeleton proteins and DNA damage repair proteins, and (g) housekeeping genes. Arrows: cDNA spots that show significant increases in expression.

**Figure 1.** Each phosphorimage is a representative example of microarray analysis of polyA+ mRNA from the TGs of mice. (A) Uninfected mice, not treated; (B) uninfected mice, drug treated. cDNA array panels are divided into seven theme-targeted sectors, including (a) basic transcription factors, (b) cell-cycle regulation, (c) oncogenes, (d) chemokine-cytokine signaling, (e) signal transduction, (f) cytoskeleton proteins and DNA damage repair proteins, and (g) housekeeping genes. Arrows: cDNA spots that show significant increases in expression.

**Table 2.** Mouse-Specific Primer Sequences and Sizes of RT-PCR Products

<table>
<thead>
<tr>
<th>Gene</th>
<th>Orientation</th>
<th>5'-3' Sequence</th>
<th>Sizes of Amplified Fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GAPDH</strong></td>
<td>Sense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td>983</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-CATGGAGCCATGAGCGGACCAC-3'</td>
<td></td>
</tr>
<tr>
<td><strong>PTGER4</strong></td>
<td>Sense</td>
<td>5'-TGGCGCTGTTGAGCGTGTGCTG-3'</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td>434</td>
</tr>
<tr>
<td><strong>Cyclin D2</strong></td>
<td>Sense</td>
<td>5'-AGAGCGATCCGGCTGACTGC-3'</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td></td>
</tr>
<tr>
<td><strong>Inhibitor of DNA-binding protein 4</strong></td>
<td>Sense</td>
<td>5'-GAGGTGTGTGCTGCTGAGTCG-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td></td>
</tr>
<tr>
<td><strong>Transcription factor AP-1</strong></td>
<td>Sense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td>288</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td></td>
</tr>
<tr>
<td><strong>Myelin protein zero</strong></td>
<td>Sense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-AGGAGGTCGGTGATGAGCGGATTGCG-3'</td>
<td></td>
</tr>
</tbody>
</table>
Data Analysis

Signal intensity data were obtained from two independent cDNA array panels with four groups by phosphorimaging onto molecular imaging screens, by using a molecular imager (model GS250; Bio-Rad, Hercules, CA). The labeled cDNA was hybridized to the array membrane for 18 hours, followed by 48 hours of exposure to the storage screens. The data were analyzed using software for Atlas array analysis (AtlasImage 2.0; Clontech). The signal intensities on a control membrane (no drug treatment) and an experimental membrane (drug-treated) were normalized by a global method, according to the software user’s manual. The intensity obtained was compared between two groups (no drug treatment versus drug treated). Signal analytical parameters (signal ratio, signal difference, and common cDNA filter background correction) were adjusted so that gene targets exhibiting the largest nonimmunosuppression-to-immunosuppression changes exceeding a factor of 1.4 or more were reported. In one published study, a postnormalization cutoff of a twofold increase or decrease in measured level was used to define differential expression, although there is no established theoretical basis for selecting this level as significant. For statistical analysis, any values that were increased or decreased 1.4-fold or more in either comparative group were used. Next, analysis of variance (ANOVA) was performed to assess the statistical significance of the genes listed in Table 1. Statistical evaluations of the magnitude and direction of change in gene expression levels were conducted by two-level factorial ANOVA and followed by two-tailed protected t-tests. The factors (main effects) in this ANOVA model were immunosuppressive drug treatment and viral latency (+ or -). The Tukey method was used to correct the significance criterion levels for the number of multiple comparisons made.

Semiquantitative RT-PCR
cDNA was synthesized using reverse transcriptase (SuperScript II; Gibco BRL, Carlsbad, CA), according to the manufacturer’s recommendations. Reverse transcription was performed using 5 µg RNA and 0.5 µg oligo(dT) primer. Table 2 lists sequences of mouse-specific oligonucleotide primer pairs. The cDNA sample was amplified in a buffer containing 1.5 mM MgCl2 (Perkin-Elmer, Oceanport, NJ), 0.2 mM of each dNTP (New England BioLabs, Beverly, MA), 0.5% DNA polymerase (AmpliTaq; Perkin-Elmer), and 0.2 µM of each primer pair. The conditions for PCR were as follows: an initial 10-minute denaturing step at 94°C and exponential cycles of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. The PCR products were examined by 2% agarose gel electrophoresis with ethidium bromide staining. To determine the relative levels of gene expression, semiquantitative
analysis was performed by the method reported in Nakayama et al. and Yokoi et al. The optical density of each band was measured, and the background intensity was subtracted from the band density by using image-analysis software (Eagle Sight 3; Stratagene, La Jolla, CA). For estimation of the initial amount of the template, the equation \( y = a^x + b \) was fitted to the data in the linear portion of the graphs. The ratio of the initial amount of each gene to GAPDH was compared for each sample. These experiments were repeated at least twice for each sample and primer pair.

**RESULTS**

**Microarray**

The results of the microarray phosphorimaging of uninfected and latently infected TGs are shown in Figures 1 and 2: uninfected, nonimmunosuppression (Fig. 1A); uninfected, immunosuppression (Fig. 1B); HSV-1 latently infected, nonimmunosuppression (Fig. 2A); and HSV-1 latently infected, immunosuppression (Fig. 2B). Signals were not seen in the negative control spots, indicating the one area of specificity of the hybridization. To determine the reproducibility of the results, a series of experiments was performed twice (eight hybridizations to eight microarray membranes). The results were remarkably similar, and the signal intensity readings for each gene were averaged from the two experiments. The lists of statistically significant (ANOVA, protected t-test, \( P < 0.05 \)) genes with expression that increased or decreased greater than 1.4-fold are presented in Table 1. Two genes in the TGs of uninfected, drug-treated mice exhibited increased expression. The TGs of latently infected, drug-treated mice showed increased expression of 10 genes and reduced expression of 8 genes.

**Semiquantitative RT-PCR**

Five genes whose transcriptional level was altered by the drug treatment were analyzed by semiquantitative RT-PCR. The genes studied were inhibitor of DNA-binding protein 4 (ID4), PTGER4, cyclin D2, transcription factor activator protein (AP)-1, and myelin protein zero. Semiquantitative RT-PCR yielded results consistent with microarray results for each of the five genes studied (Figs. 3, 4; Table 3).

**DISCUSSION**

Our ultimate goal is to prevent reactivation of HSV-1 and recurrent disease by inhibiting or stimulating host genes required for ocular recurrence of HSV-1. In this study, the transcriptional activity of 10 genes was significantly increased, and the transcriptional level of 8 genes was decreased in latently infected mice treated with cyclophosphamide and dexamethasone, compared with untreated latently infected mice.

The involvement of cellular proteins in HSV latency and reactivation has been reviewed. Some of the host cell proteins thought to be important in latency and reactivation have been noted in the introduction. By microarray analysis, we showed previously that nine genes including transcription factor II, DNAJ-like heat shock protein, stress-activated c-jun kinase 3 (JNK3), and COX-2 underwent transcriptional activation 1 hour after hyperthermia.
The results of the present study and other published observations\textsuperscript{5–8, 18, 19, 25, 28, 39, 40} indicate that PTGER4, cyclin D2, transcription factor AP-1, mitogen-activated protein kinase (MAPK), and IPF1 could have important roles in HSV-1 infection and reactivation. The expression of these genes is thought to be altered in response to immunosuppressive induction of uninfected or HSV latently infected neurons or viral replication after reactivation. To determine this, we must investigate when viral and cellular genes begin to change after induction of immunosuppression. Furthermore, it is difficult to determine in the current experiments whether induction of immunosuppression was caused primarily by cyclophosphamide or primarily by dexamethasone. It is possible that neither cyclophosphamide nor dexamethasone alone could reactivate latent H SV.\textsuperscript{29} Stroop and Schaefer\textsuperscript{29} reported that injection of dexamethasone or cyclophosphamide alone did not reactivate latent HSV-1 in rabbits; only 3% and 1% of eyes were positive for HSV-1 in rabbits receiving cyclophosphamide or dexamethasone, in contrast to the reactivation frequency of 85% observed when the drugs were administered together. They suggested that these two drugs act in a synergistic manner. If changes in gene expression are examined only after injection of cyclophosphamide or dexamethasone, fewer genes may be altered than were changed in the present study. Naray-Fejes-Toth et al.\textsuperscript{41} reported that an initial effect of dexamethasone was to increase the expression of the glucocorticoid-induced

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Representative results of semiquantitative RT-PCR. IMS: immunosuppression.}
\end{figure}

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Gene & Cycle & Uninfected Latent  \\
\hline
PTGER4 & 22 23 24 25 26 27 28 29 & 3 4 5 6 7 8 9 22 23 24 25 26 27 28 29 30  \\
Cyclin D2 & 22 23 24 25 26 27 28 29 30 & 20 21 22 23 24 25 26  \\
Transcription factor AP-1 & 16 17 18 19 20 21 22 23 24 25 & 13 15 16 17 18 19 20 21 22 23 24 25  \\
Myelin protein zero & 0.14 0.16 0.18 0.20 0.22 0.24 0.26 0.28 0.30 & 0.22 0.24 0.26 0.28 0.30 0.32 0.34 0.36 0.38  \\
\hline
\end{tabular}
\caption{Density Ratios of Untreated and Drug-Treated HSV Latently Infected TGs by Semiquantitative RT-PCR and Microarray}
\end{table}

Data are mean ± SD.
kinase (SGK) gene. Time course analyses revealed that SGK mRNA levels are elevated as early as 30 minutes after exposure to the glucocorticoid, and the levels remain elevated for several hours. Using this observation, we selected the drug treatment protocol used in this study.

We noted a significant increase in expression of the PTGER4 gene, which codes for a prostaglandin receptor. In this study, expression of the PTGER4 gene was increased 25-fold in latently infected TGs after drug treatment and only 1.2-fold in uninfected TGs. Although microarray analysis showed that expression of PTGER4 was increased 25-fold in drug-treated, latently infected TGs, semiquantitative RT-PCR indicated only a 3.05-fold upregulation of gene expression. According to previous reports, the changes in gene expression obtained from an array system are usually similar to, but are not always identical with, those obtained from semiquantitative RT-PCR. Glucocorticoids attenuate expression of COX-2, and deregulated expression of COX-2 leads to an overproduction of prostaglandins. Also, perhaps the upregulation of this gene is a host response to reactivation of HSV-1, similar to that seen in acute cytomegalovirus infection.

The expression of the cyclin D2 gene was increased to similar levels after drug treatment of uninfected and latently infected mice. Cyclin D consists of three subtypes of cyclins (D1, D2, and D3). Cyclin D2 is implicated in cell cycle regulation, differentiation, and oncogenic transformation. Cellular cyclin-dependent kinases (CDKs) are required for HSV replication. Inhibitors of CDK-1, -2, and -5 inhibit viral replication. Because members of the cyclin D family bind to and activate CDK-4 and CDK-6, inhibitors of these genes may prevent HSV-1 reactivation.

In this study, for the transcription factor AP-1 and MAPK-3 exhibited reduced transcription after immunosuppression. AP-1 can regulate many aspects of cell physiology in response to environmental changes. Fos and Jun constitute the prototypic components of the AP-1 complex. MAPKs have roles in directing both the establishment and reactivation of latent HSV infection.

Of the genes with transcriptional activity reduced by drug treatment, peripheral myelin protein 22 (PMP22) underwent the largest decrease (0.44-fold in latently infected TGs, 0.42-fold in uninfected TGs). PMP22 plays a crucial role in normal nervous system function in cell growth, differentiation, and apoptosis. Further investigation is necessary to understand the significance of regulation of PMP22 by immunosuppression in the TG.

IPF1 is selectively expressed in the β cells and transactivates the insulin promoter. It is required in early development of the pancreas, and it may act either at the level of determination or the early differentiation of the pancreas. No relationship between IPF1 and herpes viruses has been reported to date, but further studies are needed.

Recently, Tsavachidou et al. reported the gene expression changes in uninfected mouse TGs after explantation using a gene array (Gene Discovery Array Mouse I; Gene Systems, Menlo Park, CA). In their studies, increased expression of genes including prostaglandin endoperoxidase, cyclin-dependent kinase, cyclin F, transcription factor SII, and glutathione S-transferase was noted. In contrast, myelin gene expression decreased. Although the investigators studied uninfected TGs and ex vivo events, some of the same genes, such as PTGER4, glutathione S-transferase μ2, cyclin D2, PMP22, and transcription factor AP-1, were altered in their study as in our present study of HSV-1-latent TGs induced in vivo.

HSV-1 can be induced to reactivate by UV irradiation, trauma, heat stress, cold stress, and adrenergic iontophoresis. To better understand the interactions between the host and the virus, we plan in future experiments to investigate changes in gene expression after the use of these other inducers.

In summary, 18 genes were significantly altered after injection of cyclophosphamide and dexamethasone in mice latently infected with HSV-1. One or more of these genes could hold the key to the prevention of recurrence of ocular herpes. If substances that regulate these genes can be shown to block viral reactivation, such agents could form a new class of antiviral medication.

Acknowledgments

The authors thank Maxine S. Evans and Will E. Close (LSU Eye Center) for technical support, Michael K. Smolek (LSU Eye Center) for computational assistance, and David C. Bloom (Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, Florida) and Craig S. Miller (Department of Oral Health Practice, University of Kentucky College of Dentistry, Louisville, Kentucky) for reading the manuscript.

References


