Glutamine supplementation suppresses herpes simplex virus reactivation

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**Introduction**

Approximately 60% of people in the United States are infected with herpes simplex virus type 1 (HSV-1) and 20% with HSV-2. Prophylactic therapy with acyclovir or valacyclovir reduces the rate of cold sore (herpes labialis) recurrences by 40%–60% and the rate of genital herpes reactivation by 70%–80%. Thus, other approaches to reduce reactivation of HSV are needed. Control of reactivation of HSV correlates with virus-specific T cells (1). Increased T cell function might reduce virus reactivation. Nutrients, including glucose and certain amino acids, are critical for T cell activation (2). Activated T cells require increased metabolism of glucose and glutamine for proliferation, and deprivation or inhibition of synthesis of these molecules reduces T cell proliferation (3, 4).

Glutamine serves as a nitrogen source for rapidly dividing cells including lymphocytes, in which it is important for energy production and for nucleotide synthesis. Mitogen-stimulated proliferation of peripheral blood mononuclear cells and secretion of IL-2 and IFN-γ are dose-dependent on the level of glutamine (5). Glutamine is important for activation-induced proliferation of T cells (3, 6). Glutamine transporters are increased during T cell activation, and reduction of these transporters impairs T cell effector function (6, 7). Activation of naive T cells is associated with rapid uptake of glutamine, which requires the ASCT2 amino acid transporter (8). Activated human T cells require glutamine for production of IFN-γ; depletion of glutamine inhibits T cell proliferation and reduces production of IFN-γ and IL-2 (6, 9). Reduced availability of extracellular glutamine favors a Treg phenotype over a Th1 phenotype (10). Mice that receive glutamine have lower levels of HSV-1 in vaginal fluid, higher titers of IFN-γ in vaginal fluid, and increased numbers of activated CD8 T cells in the spleen after HSV-1 infection (11). Glutamine deprivation and cellular stress have previously been shown to enhance replication of an HSV-1 mutant with deletion of virus infected cell polypeptide 0 (ICP0) (12). HSV-1 ICP0 is critical for virus reactivation. These observations suggest that low glutamine levels might be associated with increased virus reactivation, or conversely that high levels might reduce reactivation. On the basis of these findings, we postulated that glutamine supplementation might increase T cell function and improve control of a chronic virus infection.

**Results and Discussion**

UV irradiation of the eyes of latently infected mice induces reactivation of HSV-1 from mouse trigeminal ganglia in vivo (13). Therefore, we infected mice with HSV-1 by corneal scarification, and 2 weeks later we supplemented drinking water with glutamine, glycine, or no supplement. After 2 weeks of supplement (4 weeks after infection), the latently infected animals were anesthetized, the eyes were irradiated with UV light, and 2 days later the animals were euthanized and their trigeminal ganglia were homogenized and assayed for infectious virus. This assay tests for virus already reactivated from the ganglia in vivo, since the tissue is homogenized immediately after dissection. The percentage of UV-induced HSV-1 reactivation in trigeminal ganglia from mice treated with glutamine was about half that of mice treated with no supplement or with glycine.
treated with water or glycine in 3 independent experiments (Figure 1). The difference between glutamine and water was statistically significant in the first experiment ($P = 0.042$, Fisher’s exact test), but not in the second and third experiments, which had fewer animals; the difference was significant when the 3 experiments were pooled ($P = 0.0047$, Fisher’s exact test). In contrast, the difference between glutamine and water was not significant in any of the individual experiments or in the pooled experiment ($P = 0.47$, Fisher’s exact test).

The standard animal model to assess spontaneous reactivation of HSV-2 is the guinea pig model. We infected guinea pigs intravaginally with $2 \times 10^5$ PFU of HSV-2 (strain MS), monitored the HSV-2 infection of guinea pigs. We infected guinea pigs with 2 × 10^5 PFU of HSV-2 (strain MS), monitored until the end of the treatment periods, all animals were taken off therapy and then, after an additional 2 weeks, were anesthetized and their eyes exposed to UV irradiation. Two days later, animals were euthanized, trigeminal ganglia were removed and homogenized, homogenates were plated onto Vero cell monolayers, and the number of ganglia with reactivated virus was determined. Three separate experiments were performed (A–C), and results at day 5 in culture for all 3 experiments are shown (B). Experiment 1 had 25 mice in each group, and experiments 2 and 3 had 15 mice each in the glutamine and glycine groups and 7 and 10 mice in the no supplement group, respectively. Fisher’s exact test was used for statistics.

![Figure 1. Reactivation of HSV-1 in trigeminal ganglia of mice receiving glutamine, glycine, or no supplement in their drinking water. Animals were inoculated with HSV-1 by corneal scarification, received supplemental amino acid 2 weeks later, and after an additional 2 weeks, were anesthetized and their eyes exposed to UV irradiation. Two days later, animals were euthanized, trigeminal ganglia were removed and homogenized, homogenates were plated onto Vero cell monolayers, and the number of ganglia with reactivated virus was determined. Three separate experiments were performed (A–C), and results at day 5 in culture for all 3 experiments are shown (B). Experiment 1 had 25 mice in each group, and experiments 2 and 3 had 15 mice each in the glutamine and glycine groups and 7 and 10 mice in the no supplement group, respectively. Fisher’s exact test was used for statistics.](http://www.jci.org)
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Figure 2. Rate of recurrences of genital HSV-2 in guinea pigs receiving glutamine or no supplement in their drinking water. Animals were inoculated intravaginally with HSV-2, and after recovery (2 weeks later on day 15) they were divided into 2 groups (based on lesion scores after acute infection) and glutamine was added to the drinking water of 1 of the 2 groups. Animals were monitored for recurrences, and disease scores were obtained each day; the mean number of cumulative recurrences per guinea pig in each group was plotted in each of 2 separate experiments (A and B). At the end of the treatment periods, all animals were taken off therapy (washout period) and monitored for recurrences in each of the 2 separate experiments (C and D). Experiment 1 (A and C) had 9 animals per group; experiment 2 (B and D) had 15 per group. Two-tailed t test was used for statistics.

Table 1. Cellular genes upregulated in all 3 microarray assays in trigeminal ganglia of mice treated with glutamine

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>mRNA expression ratio (Gln/Gly)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Microarray assay</td>
</tr>
<tr>
<td>Ifi47</td>
<td>IFN-γ-inducible protein 47</td>
<td>1.84</td>
</tr>
<tr>
<td>Cxcl9</td>
<td>Chemokine (CXCL) ligand 9 (monokine induced by IFN-γ [Mig])</td>
<td>2.11</td>
</tr>
<tr>
<td>Pdia4</td>
<td>Protein disulfide isomerase associated 4</td>
<td>1.43</td>
</tr>
<tr>
<td>Igtg</td>
<td>IFN-γ-induced GTPase</td>
<td>1.88</td>
</tr>
<tr>
<td>Cd274</td>
<td>CD274 antigen (programmed death-ligand 1 [Pdla])</td>
<td>1.97</td>
</tr>
</tbody>
</table>

ND, not done; qRT-PCR, quantitative reverse transcriptase PCR.
Therefore, glutamine may reverse the effects of stress on CD8 T cell responses and reduce reactivation.

While glutamine reduced the rate of in vivo HSV-1 reactivation in mouse trigeminal ganglia by only 50%, this is the same level of effect that antiviral suppressive therapy has in reducing the rate of symptomatic recurrences of HSV-1 herpes labialis (24). In addition, while acyclovir partially reduces HSV-2 genital recurrences, it does not reduce the 2- to 3-fold increased risk of HIV acquisition associated with HSV-2 (25). Thus, there is a clear need for other therapies to suppress oral and genital HSV recurrences. The ability of glutamine to reduce HSV reactivation in 2 different animal models suggests a new approach to reduce reactivation of the virus in humans.

### Methods

#### Statistics

All statistics except microarray were done in JMP 7.0.2 (SAS Institute); P less than 0.05 was considered significant. Microarray data normalization and differential expression were computed using SAS and JMP/Genomics 4.0 (SAS Institute).

#### Study approval

All animal experiments were performed under protocols approved by the Animal Care and Use Committees of the National Institute of Allergy and Infectious Diseases and the Food and Drug Administration. A complete description of methods is provided in Supplemental Methods.

### Author contributions

YH, KW, KD, MS, LP, MBM, and PRK performed the animal studies. KW and YH did real-time PCR. KD quantified virus-specific...
CD8 cells in ganglia. TGM performed the microarray experiments and analyses. JJC, YH, and KW designed the study. JJC, YH, KW, TGM, and PRK wrote the paper.

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