A Potential Strategy to Maintain HSV-1 in a Latent State: Use of Immunoregulatory Peptide Mimetics

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A POTENTIAL STRATEGY TO MAINTAIN HSV-1 IN A LATENT STATE:

USE OF IMMUNOREGULATORY

PEPTIDE MIMETICS

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

NASRIN MAJIDI

2009

Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Nasrin Majidi ENTITLED. A potential strategy to maintain HSV-1 in a latent state: use of immunoregulatory peptide mimetics, BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Majidi, Nasrin. M.S., Department of Biological Sciences, Microbiology and Immunology Program, Wright State University, 2009. A potential strategy to maintain HSV-1 in a latent state: use of immunoregulatory peptide mimetics.

This study reviews the role of interferon-gamma (IFN-\(\gamma\)) in HSV-1 latency. CD8+ T cells inhibit the reactivation of HSV-1 in trigeminal ganglia (TG) by production of IFN-\(\gamma\). Although CD8+ T cells include all the cytotoxic apparatus for cytotoxicity, latently infected neuronal cells are not killed by CD8+ T cells. The CD94-NKG2a molecule on CD8+ T cells, binds to Qa-1^b (a MHC class I like molecule) present on neuronal cell to inhibit CD8+ T cells cytotoxicity.

Other studies have also determined that the IFN-\(\gamma\) peptide mimetic 95-132 inhibits HSV-1 replication in the same way as IFN-\(\gamma\) in infected cells. IFN-\(\gamma\) mimetic peptide binds to intracellular domain of interferon gamma receptor-1 (IFNGR-1) and initiates IFN-\(\gamma\) signal transduction to the nucleus for gene activation. IFN-\(\gamma\) mimetic also is more active than IFN-\(\gamma\) in gamma activated sequences (GAS) promoter.

Epithelial cells such as keratinocytes are the major target cell for HSV-1 replication. In a murine keratinocyte cell line (HEL-30), suppressor of cytokine signaling-1 (SOCS-1) prevents the antiviral activity of IFN-\(\gamma\) by inhibition of the JAK/STAT signaling pathway.
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ACRONYMS

\(\alpha\)-TIF = \(\alpha\)-trans-inducing factor
CD94 = inhibitory molecule
DTH = delayed type hypersensitivity
E gene = early gene
EAE = experimental allergic encephalomyelitis
ELISA = enzyme linked immune sorbent assay
Foxp = forkhead/winged helix transcription factor
GAS = IFN-\(\gamma\)-activated sequence
HSV-1 = herpes simplex virus type-1
ICP0 = infected cell protein null
ICP4 = infected cell protein 4
IRF = IFN regulatory factor
IFN\(\gamma\)R = IFN-\(\gamma\) receptor
IE gene = immediate early gene
IP-10 = IFN-\(\gamma\)-inducible protein 10
IMPs = importins
JAK = janus kinase
L gene = late gene
LAT = latency associate transcripts
Mig = monokine induced by IFN-\(\gamma\)
MBP = myelin basic protein
NO = nitric oxide
NLS = nuclear localization sequence
NKG2a = NK receptor subfamily G2a
NK cell = natural killer cell
p-STAT1α = phosphorylated-STAT1α
PI = post inoculation
Qa-1β = a MHC class I like molecule
RT = reverse transcriptase
SOCS = suppressor of cytokine signaling
STAT = signal transduction and transcription
TG = trigeminal ganglion
Tkip = tyrosine kinase inhibitory peptide
Treg cells = T regulatory cells
TNF = tumor necrosis factor
TLR = toll like receptor
TGF-β1 = transforming growth factor β1
vhs protein = virus host shut off protein
Objective

To review the literature concerning HSV-1 latency and potential strategies to prevent reactivation of HSV-1 from latency in trigeminal neurons (TGNs).

I. Herpes simplex virus infection and replication

Herpes Simplex Virus

Herpes simplex virus is an alpha herpes virus of the herpesviridae family. HSV-1 causes fever blisters or cold sore infection in humans. Most people get infected when exposed to the virus, and recover completely from primary infection. Most primary infections with HSV-1 are without visible symptoms. Neuronal cells are susceptible targets to be attacked by HSV-1 and virus will remain in them throughout the human’s life span. Reactivation of HSV-1 may cause herpes encephalitis. Some stimuli such as stress may induce viral gene reactivation. Production and assembly of viral particles overcome the body’s immune system and leads to secondary infection (6, 9, and 16).

HSV-1 structure

Herpes simplex viruses are enveloped viruses. The envelope consists of different glycoproteins (gB, gC, gD, gE, gG, gH, gI, gK, gL, gM) which are responsible for attachment to the cellular receptors. The inner layer of the virus is the capsid made of capsomeres about 100-200 nm in diameter with an icosahedral nucleocapsid. The space between the envelope and the capsid is called the tegument. The tegument contains proteins and enzymes which are involved in the virus replication (9).
Linear double stranded DNA contains three classes of genes: Immediate early (IE) genes, early (E) genes, and late (L) genes. Immediate early genes regulate expression of other herpes virus genes (9).

Infected cell protein null (ICP0) and ICP4 are two products of IE genes. ICP0 (a promiscuous transactivator of gene expression that it is required for both efficient initiation of lytic infection and reactivation from latency) and ICP4 have an important role in the early (E) and the late (L) genes expression. Early genes arrange the stage for viral DNA replication and late gene activation. Expression of late genes encodes viral structural proteins. Low-level expression of immediate early (IE) and early (E) viral genes during latency inhibits the virus replication which induces a chronic immune response. ICP0 and ICP4 could not be expressed in latently infected TGs. Latency associate transcript (LAT) with sequence antisense to ICP0 blocks ICP0-mediated virus replication and induce latency in neuronal cell. In contrast, expression of ICP0 or ICP4 in early stage of infection induces virus replication (lytic infection) in infected cell (2, 9).

**HSV-1 entry to the host cell**

As shown in Figure 1, HSV-1 infects host cells by interfering with glycoprotein gB or gC, to the cell receptor heparan sulphate. In the next step, binding of one herpes virus entry protein (HveA, HveC and HveB) to the glycoprotein gD causes conformational change. This conformational change allows gD to bind to gB or gH-gL dimmer. Following this interaction, virus fusion occurs through host cell plasma membrane (9).
HSV-1 replication

HSV-1 proteins (α,β,γ) are produced during virus replication. Virus recruits cellular RNA polymerase II for its gene transcription. Immediate early (IE) genes express α proteins, which then induce early gene expression. Tegument proteins such as α-trans-inducing factor (α-TIF) initiates immediate early gene transcription. Expression of these three gene classes results in assembly of virus particles which bud from the host cell. In contrast, induction of latency associate transcripts (LATs) leads the virus toward latency. LAT suppress IE genes during HSV-1 primary infection and facilitate latency in neuronal cells. Another tegument protein which has important role in virus replication is virus host shut off protein (vhs). This protein shuts off the host cell production machinery and
recruits it for its own replication. Late protein expression forms capsid and receptors on the surface of the virus. Assembly of virus particles including genome, core and capsid occurs in the nucleus of the cell. The virus envelope is created by budding into the inner nuclear membrane of the cell (2, 6, and 9).
II. Anti viral host defense

Cellular response in primary infection with HSV-1

HSV-1 primary infection induces an acute response during the first days of infection. Natural killer (NK) cells and macrophages are two major cell groups that produce IFNs in early defense against HSV-1 infection.

During first few hours macrophages produce cytokines such as IFN type I (IFN-α/β), TNF, and IL-12. These cytokine products stimulate more macrophages to produce reactive oxygen species (ROS) which have a direct antiviral effect. Depletion of these cytokines enhances mortality of mice treated with HSV-1. Each cytokine gene expression is induced through a particular signaling pathway. For example, signaling of IFNα/β gene activation is induced by NF-κB family transcription factors. NF-κB is an intracellular transcription factor which interacts with intracellular domain of toll like receptors (TLR2, TLR3, and TLR9), IL-1R, and TNFR and transfers the signal to the nucleus for IFNα/β gene activation (9). HSV-1 transcripts (i.e. LAT or ICP27) are related to cytokine expression in acute infection. Immediately after HSV-1 infection, IE and E genes produce their protein. Each of these proteins could induce cytokine production (2, 7, and 9).

As discussed previously, type I IFN and TNF are secreted immediately after HSV-1 infection in host cells. A few hours after these are produced, macrophages react with the HSV-1 by producing IL-12. As shown in figure 2, production of IL-12, IL-18, TNF and IFNα/β by macrophages will induce IFN-γ production in NK cells. IFN-γ later induces macrophages to produce more IFN-α/β, TNF and IL-12. IFNα/β and TNF
negatively regulate IL-12 production in macrophages. Nitric oxide (NO) produced by macrophages exerts direct antiviral activity. In Figure 2, note that IFN-α and IFN-β may have direct effect on HSV-1 infection. ELISA analysis of TGs infected with HSV-1 also demonstrated that IL-2, IL-6, IL-10, and IFN-γ proteins were present in acutely infected TGs (7, 9).


Figure 2. Production of cytokines during HSV infection in macrophages and NK cells (Adapted from 9).

**IFN signaling pathway**

Interferon plays an important role in the antiviral activity of target cells in HSV-1 infection. Interferons are produced by different cell types including NK and macrophages cells in first line of host defense. IFN-γ produced by CD8+ T cells is effective against HSV-1 infection in adaptive immunity. IFN-γ binds to its specific receptors on the target cells and stimulates gene transcription. All type I IFNs (IFN-α/β) bind only to
IFNAR1/IFNAR2 whereas the Type II (IFN-γ) receptors are composed of two peptide chains IFNGR1 and IFNGR2 (23, 24). IFN-γ signaling begins with interaction of IFN-γ with an IFN receptor complex consisting of two IFNGR1 molecules and two IFNGR2 molecules (23).

The C-terminus of IFN-γ consists of a nuclear localization sequence (NLS), which plays an important role in binding of IFN-γ to IFNGRs. Endocytosis of IFNγ into the cell occurs with interaction of IFN-γ NLS domain to IFNGR1. NLS domain also can bind to IFNGR1 intracellular domain and induce JAK2 phosphorylation in tyrosine residue (19, 24). Autophosphorylation of JAK2 will induce activation and phosphorylation of STAT1α. Following this induction, STAT1α transfers signal through mediators such as importin-α5 (IMPα5) to the nucleus. Each cytokine has a particular signaling pathway. IFNs (type I and II) select their specific pair of janus kinase (JAK) and signal transduction and transcription (STAT) group for translocation of the signal to the nucleus. For example IFN-α/β receptor complex recruit JAK1/Tyk2 for functioning, while IFN-γ employs JAK2/STAT1α for the signaling pathway (5, 9, 19, and 24).
Figure 3. IFN type I ($\alpha$, $\beta$, $\kappa$, $\epsilon$, and $\omega$) and IFN type II (IFN-$\gamma$) signaling pathway.
(Adapted from Google images)
Based on the role of IFN-γ in the inhibition of HSV-1 that is determined by various studies, investigators sought to develop an IFN mimetic peptide which may inhibit HSV-1 infection in the same manner as IFN-γ (20). Most experiments have demonstrated that IFN-γ mimetic is even more active than IFN-γ (22). IFN-γ mimetic peptide consists of N-terminal and C-terminal units, which the C-terminal consists of nuclear localization sequence (NLS) region. C-terminal of IFN-γ is necessary for IFN-γ mimetic function (19). NLS region does not have antiviral activity itself. Lipophilic group are also added to IFN-γ C-terminal for cell penetration. C-terminal of IFN-γ binds to intracellular domain of receptor while N-terminal binds to extracellular domain (19).

Viruses have some strategies to escape from immune response. For example, vaccinia virus produces B8R protein (a virulence factor of poxviruses) to evade from the host immune response (18). B8R virulence factor which is a homology of extracellular domain of IFNGR1 binds to IFN-γ and prevents its interaction with IFNGR1 on the cell surface. IFN-γ peptide mimetic is a small peptide with attached lipo-palmitate in C-terminal for the cell penetration. This peptide binds to intracellular domain of IFNGR1 and initiates the IFN-γ signaling pathway through phosphorylation of JAK2/STAT1α (20, 21).

**SOCS-1 expression suppresses the IFN-γ activity**

HSV-1 uses suppressor of cytokine signaling (SOCS) proteins to fight on immune response. These protein products are inducible when IFN-γ binds to its receptor on the cell surface and negatively regulates IFN-γ antiviral activity in HSV-1 infection. The SOCS family include 7 members; SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6,
and SOCS7. SOCS has a kinase inhibitory region (KIR) and src homology 2 (SH2) domains which are very important in binding of SOCS-1 to JAK2. The binding of KIR region to JAK2 tyrosine residue inhibits the autophosphorylation of STAT1α. Inhibition of STAT1α autophosphorylation results in IFN-γ signaling pathway shut off. Using a SOCS mimetic or tyrosine kinase inhibitory peptide (Tkip) in HSV-1 infection showed this peptide mimetic is able to bind to tyrosine residue of JAK2 and prevents STAT1α phosphorylation in IFN-γ signaling pathway. This observation led the investigators to develop a SOCS antagonist that blocks SOCS activity produced by virus. SOCS-1 peptide antagonist binds to KIR region of SOCS-1 and allows JAK2 phosphorylation and subsequently STAT1α phosphorylation and IFN-γ signaling pathway continues (23, 24, 25, 26, and 27).
III. HSV-1 latency

Cellular interactions in HSV-1 latent infection in Trigeminal Ganglia (TG)

After primary infection, HSV-1 persists in neural ganglia throughout human life span. During latent state, HSV-1 produces latency associate transcript (LAT) mRNA. Immediate early (IE) and early (E) genes expressions are very low. This low level of α and β proteins are essential to induce CD8+ T cells activity. CD8+ antigen specific T cells with IFN-γ production capacity are the major immune cells in trigeminal ganglia (TG). These cells inhibit the viral replication in neuron cells without any cell destruction (1, 6).

Chemokines such as IP-10 and RANTES are present in Trigeminal Ganglia (TG), and recruit other immune cells to defeat active HSV-1 infection (12). All these factors together overwhelm the virus replication and keep it in a latent state. The reason that neurons are susceptible to HSV-1 infection could be the low level expression of MHC molecules (1). Neuronal cells with transient expression of MHC class I molecules are able to present the virus antigen to CD8+ T cells (1).

Cellular immunity including CD8+ T cells, CD4+ T cells and γ/δ T cells are essential to fight HSV-1 infection in TG. CD8+ T cells do not eradicate HSV-1 with their cytotoxic effect in neuronal cells. CD8+ T cells also defeat HSV-1 by its non lytic activation in primary infection. In latent state, neuron cells infected with HSV-1 will not be killed by CD8+ T cells, because CD8+ T cells use their nonlytic mechanism only to overcome HSV-1 replication in latent state. Although CD8+ T cells contain lytic granules, they are not able to kill neuronal cells (1, 6).
This observation guided Suvas (1) and colleagues to ask whether other mechanisms such as cellular interaction could be involved in the CD8+ T cells function (1). For this purpose they infected female 6 to 8 weeks old C57Bl/6 (B6) mice with HSV-1. TG were removed 28 days after ocular infection and flow cytometry used for cell surface and intracellular staining of the neuron nucleus. Granzym B (induces apoptosis in viral infected cells) and perforin (cytolytic protein) were expressed in CD8+ T cells isolated from TG. They also examined the expression of NK inhibitory receptors on T-cells. The result showed 65% of Ag-specific CD8+ T cells express NKG2a (1).

In next experiment the expression of CD94 molecule (inhibitory receptor) on CD8+ T cells also examined. NKG2a functions when co-expressed with CD94 on CD8+ T cells. Results showed 80% of the CD8+ T cells positive for NKG2a expressed CD94 molecule. They hypothesized interaction of CD94/NKG2a and Qa-1b (a MHC class I like molecule) on neuronal cells may blocks cytolytic activity of CD8+ T cells. Therefore they used MC-38 (a colon carcinoma cell) as target cell to show Qa-1b expression. As shown in Figure 4a ~ 50% of MC-38 cells were able to express Qa-1b molecule (1).

In the next experiment, an immunogenic peptide derived from HSV-1 (SSIEFAR) was added in two different concentrations (2.0 and 0.2 µM) to target cells (MC-38 cells) that were co-cultured with CD8+ T cells. This system was used to determine the level of cytotoxicity of CD8+ T cells for the HSV-1 peptide. Cells treated with both concentrations showed significant cell lysis in the presence of the CD8+ T cells. As shown in Figure 4b, blocking of the CD94-NKG2a-Qa1b interaction increased CPE of target cells (using anti-Qa-1b Ab). These result demonstrated that, in spite of expression of lytic granules in HSV-specific CD8+ T cells for the treated carcinoma cell line, these
CD8+ T cells could not destroy the cultured neuronal cells due to CD94-NKG2a-Qa1b interaction (1).

Figure 4. The effect of Qa-1b-CD94/NKG2a interaction blockage on the CD8+ T cells cytotoxicity. a, Using target cells MC-38 (a colon carcinoma cell line) for the expression of Qa-1b molecule. b. The effect of anti-Qa-1b on cell lysis (Adapted from 1).

Co-expression of CD94/NKG2a on HSV-1 CD8+ specific T cells could be induced by transforming growth factor β1 (TGF-β1), which is present in TG after HSV-1 infection. The presence of CD4+CD25+ reg (regulatory) T cells in TG could be a possible source of TGF-β1. To determine whether CD4+ CD25+ T reg cells play a role in TGF-β1 expression in latent infection of TG, B6 mice were treated (28 days p.i.) with a monoclonal antibody (PC61) to deplete endogenous CD25+ T cells. The level of TGF-β1 protein was decreased in CD4+CD25+ T cells depleted TG culture. This treatment almost
completely depleted TGs of CD4+CD25+ T cells. Regulatory CD4+Foxp3+ T cells (~4%), another source of TGF-β, were expressed in the latent TG of such treated animals. Therefore CD4+CD25+ T reg cells may play an important role in induction of CD8+ T cells cytotoxicity (1).

**Cytokines in latently infected TG models**

Ocular infection with HSV-1 results a latent infection in mice. Lymphocytes persist in TG producing of cytokines that maintain the virus in non replicating state. If immune system was not able to clear HSV-1 from the body, virus moves to central nervous system and may cause fetal encephalitis (an acute inflammation of brain) (7).

Halford and colleagues (7) hypothesized that the cytokines expression in TG may have some effect on fetal encephalitis. They infected ICR mice by corneal inoculation with HSV-1 and six to nine days later 4 of 5 mice died with fetal encephalitis. Cytokine transcript expression (especially IL-10 and IFN-γ mRNAs) was strongly up-regulated in acutely infected TG 5 days post inoculation (PI). RT-PCR detection of LAT RNA was used to confirm that TGs were infected with HSV-1. ICP27 is a α gene production which is necessary for HSV-1 infection. Therefore the presence or absence of ICP27 mRNA would confirm the virus is in latent or active state, respectively in this experiment ICP27 mRNA was not detected. Thus the replication of virus was inhibited by cytokine expression in some way in TG (7).

Within TG cytokines such as TNF-α, IL-6 IFN-γ inhibit HSV-1 reactivation. TNF-α is also produced in the innate response against HSV-1 by macrophages and NK cell (8).
To study whether the depletion of this cytokine has any effect on disease severity, Minagawa and colleagues (8) examined the effect of neutralizing specific proinflammatory cytokines on HSV-1 reactivation from TG explants. They examined the effect of TNF depletion in HSV-1 reactivation both in vitro, and in vivo in TNF-knockout mice (8). BALB/C mice 5-6 weeks of age were inoculated with HSV-1 in both eyes, and after 8 weeks TG removed from mice and added to fresh medium. Samples were taken from TG cultures and added to Vero cells to monitor the cytopathic effect (CPE) in vitro. As shown in Figure 5, depletion of TNF in the cell culture resulted in virus reactivation, which was significant when compared with control. In contrast, adding anti-IL6 had no effect on virus reactivation (8).

They did the same study in vivo on B6 mice, which are more resistant to HSV-1 infection than BALB/C mice. B6 TNF knock-out mice were infected with HSV-1 and survival percentage monitored. In TNF-knockout mice the survival percentage dropped to 0-40%. Therefore using TNF-KO mice showed that TNF has an important role in the body’s immune system against HSV-1 reactivation in TG (8).
Figure 5. The effect of TNF and IL-6 depletion on reactivation of HSV-1. TG explants were depleted from IL-6 or TNF using specific antibodies and the HSV-1 reactivation detected (Adapted from 8).

**Effect of neutrophils on HSV-1 infection**

Similar to T lymphocytes and macrophages, which are thought to be the major cellular defense against HSV-1 infection, neutrophils also participate indirectly against HSV-1 infection. Neutrophils produce chemokines such as interferon-inducible protein 10 (IP-10) and monokine induced by IFN-γ (Mig) that attract the other immune cells (CD4+ and CXCR3+ expressing cells) to the site of infection. Molesworth-Kenyon and colleagues (12) hypothesized that neutrophil depletion would have an inhibition effect on cellular recruitment to the delayed type hypersensitivity (DTH) site. BALB/c mice were treated with HSV-1 via right cornea and DTH monitored by ear swelling assay. Neutrophils depleted 9 days later by i.p. administration of monoclonal antibody (RB6
8C5 Ab) in mice. On day 10, the mice were challenged in the ear pinna with HSV-1.

Figure 6 show that mAb RB6 8C5 mice treatment reduced the number of neutrophils at the DTH site by 85%. CD4+ and CD8+ T cell recruitment was reduced significantly by 90% and 70%, respectively. Depletion of neutrophils also resulted in a 73% reduction in cells expressing the chemokine receptor CXCR3. Therefore neutrophils synergize with the other immune cells such as CD4+, CD8+ T cells in preventing HSV-1 infection. (12).

![Figure 6](image-url)

Figure 6. Depletion of neutrophils, reduced the number of immune cell infiltration to the DTH site. The number of infiltrating neutrophils, CD4+ T cells, CD8+ T cells, and CXCR3+ cells determined by immunohistochemical staining using appropriate antibodies and compared with control IgA (Adapted from 12).
IV. Using peptide mimetic for antiviral activity

Antiviral activity of IFN-γ mimetic peptide

NLS domain of IFN-γ interacts with cytoplasmic domain of IFNGR1 and phosphorylates JAK2 in tyrosine residue, this autophosphorylation induces STAT1α activation. In the next step, the complex (IFN-γ/IFNGR1/JAK2/ STAT1a) associates with IMPα5 which later translocates to nucleus to activate gene transcription (22).

Fulcher and colleagues (22) hypothesized that the IFN-γ mimetic peptide has the same affinity for IMPs as does IFN-γ in signal transduction to the nucleus. They synthesized a mIFN-γ peptide mimetic with binding affinity to IMPs (Importins). Using ELISA-based binding assay they showed that both IFN-γ and mIFN-γ had affinity to IMPs (antibody specific to IMPα-GST). IFN-γ peptide mimetic even had more affinity to IMPs compared to control T-ag 111-135-β-gal (22).

In the next experiment, they used plamids to transfect of mIFN-γ mimetic and IFN-γ which were bound to firefly luciferase to activate GAS promoter. As shown in Figure 7, mIFN-γ peptide mimetic was more active than IFN-γ in activating the IFN-γ activated site (GAS) promoter (by measuring of luciferase activity). These results demonstrated that high affinity of IMPα/β for mIFN-γ mimetic leads to activation of GAS promoter and gene transcription (22).
The effect of vaccinia virus virulence factor B8R on IFN-γ

Viruses exert various strategies to escape from host immune responses. IFN-γ has an important role in host antiviral activity in various viral infections such as HSV-1 and vaccinia virus infections. B8R protein (a virulence factor and homology of extracellular domain of IFNGR1) produced by vaccinia virus binds to IFN-γ and prevents its attachment to extracellular domain of IFNGR1 on target cell. IFN-γ mimetic peptide is a small peptide with lipo-palmitate attached to C-terminal for cell penetration. This peptide is able to penetrate to the cell and bind to the intracellular domain of IFNGR1 and initiates the IFN-γ signaling pathway in vaccinia virus and other viruses such as HSV-1(18).
Ahmed and colleagues (18) hypothesized that a synthesized IFN-γ peptide mimetic which binds to intracellular domain of IFNGR1 and induce phosphorylation of JAK/STAT mediators may block the B8R activity (18).

They synthesized a small IFN-γ mimetic peptide (mIFN-γ)\textsubscript{95-132} for mice. C57BL/6 mice treated with different doses of lipo-mimetic IFN-γ\textsubscript{95-132}, non-lipomimetic (lipophilic palmitate is absent for cell penetration) and lipo control (NLS is absent for binding to intracellular domain of IFNGR1) i.p., and then the mice were challenged with vaccine virus with B8R production potential. As shown in Figure 8 mice were completely protected with 200 µg, but protection with 50, 15, and 5 µg decreased to 80, 80, and 40%, respectively. Mice were not protected with non-lipo mimetic and lipo-control treatment. This approach led the investigators to design an IFN-γ mimetic peptide therapy for humans infected with vaccinia virus and other viruses (18).

![Figure 8. mIFN-γ\textsubscript{95-132} role in the protection of mice against encephalomycarditis caused by vaccinia virus.](image)

Different doses of lipo-mimetic IFN-γ\textsubscript{95-125} were given to C57BL/6 mice (5, 15, 50, 200 µg) and then challenged with vaccinia virus and mice survival percentage followed (Adapted from 18).
Tkip negatively regulates cytokine signaling pathway

IFN-γ binds to its receptor (IFNGR1) on the cell and autophosphorylates JAK2. Suppressor of cytokine signaling 1 (SOCS-1) binds to JAK tyrosine residue and inhibits STAT1 autophosphorylation which leads to blockage of IFN-γ signaling pathway (21).

The abnormal phosphorylation of JAK tyrosine kinase region also causes inflammatory diseases such as experimental allergic encephalomyelitis (EAE) (an animal model for multiple sclerosis). To confirm this hypothesis Mujtaba and colleagues (21) developed a tyrosine kinase inhibitory peptide (Tkip) which has 12 residues (WLVFFVIFYIFYFFR) which bind to the tyrosine kinase residue of JAK2 and makes it inaccessible for binding of STAT1α. For this experiment, Tkip and IFN-γ<sub>95-125</sub> peptides were synthesized and acute EAE induced in NZW mice. Then lipo-Tkip, lipo-IFN-γ<sub>95-133</sub> was given to mice i.p. and the severity of disease was monitored. As shown in figure 9, Tkip treated mice did not develop acute EAE. In contrast mice treated only with PBS are not protected. Therefore using Tkip could be useful to control inflammatory diseases such as EAE (21).
Figure 9. Treatment of mice with the suppressor of cytokine signaling-1 mimetic peptide, Tkip, prevents development of the acute form of EAE. NZW mice were treated with Tkip i.p. Control mice received PBS. All of the BPS mice developed EAE (Adapted from 21).

SOCS-1 KIR and Tkip inhibit IFN-γ induced macrophages activity

Tkip binds to JAK2 autophosphorylation site and prevents STAT1α binding and subsequent phosphorylation (23). The prevention of STAT1α phosphorylation results in inhibition of activation of further mediators, and IFN-γ signal transduction path way will be halted. Similar to Tkip, SOCS-1 KIR recognizes the JAK2 tyrosine region for its function and follows the pathway discussed above. To confirm this hypothesis, Waiboci and associates (23) sought to show both SOCS-1-KIR and Tkip bind to JAK2 autophosphorylation site and prevent binding and autophosphorylation of STAT1α. For this purpose, they immobilized SOCS-1-KIR, Tkip, and control peptide on 96-well micro titer plates and incubated with JAK2 (1001-1013). Binding assays showed both SOCS1-KIR and Tkip bound to JAK2 autophosphorylation site (23). In the next experiment, they
showed SOCS1-KIR and Tkip inhibited cytokine signaling through blocking of STAT1α phosphorylation in murine U937 cells (immunoblot analysis of STAT1α). Macrophages have a key role in antiviral resistance, and are inducible by IFN-γ. The macrophage cell line Raw 264.7 was activated by IFN-γ and SOCS1-KIR or Tkip were then used to determine if they are able to block nitrite oxide (NO) production by macrophages. Figure 10 shows both Tkip and SOCS1-KIR inhibited the NO production. The SOCS1 antagonist may provide a therapeutic strategy for viral infections (23).

Figure 10. The role of TKip or SOCS-1 in induction of NO by various concentrations of IFN-γ in macrophages. Raw 264.7 macrophages were incubated with different concentration of IFN-γ alone or in the presence of either Lipo Tkip, Lipo SOCS1-KIR or control. Nitrite concentration determined using Griess reagent (Adapted from 23).
IFN signal transduction is inhibited by over expression of SOCS

Yokota and colleagues (24) demonstrated that SOCS3, member of SOCS family, inhibits IFN-α signal transduction through blocking of JAK/STAT path way in the same manner as SOCS-1. They used defective HSV-1 (mutated in tegument proteins UL41 and UL13) and wild type (VR3). Human amnion cell line FL was infected with HSV-1 wild type (VR3) or defective d41 or d13, and then treated with INF-α to examine the effect of SOCS3 on STAT1 phosphorylation. SOCS3 produced by wild type VR3 inhibited the phosphorylation of JAK2/STAT1. In contrast, mutated strains d41 or d13 could not prevent STAT1 phosphorylation completely (24).

They also sought to show whether over expression of SOCS3 in FL cell culture has the same result. FL cell culture was transfected with the pSOCS3 expression plasmid. Shown in Figure 11A is SOCS3 expression in the cultures transfected with pSOCS3 expression plasmid. After 24 h, transfected cells were treated with IFN-α. Western blot showed STAT1 phosphorylation was inhibited in SOCS3 over expressed cells. These results determined HSV-1 uses SOCS3 in the same strategies as SOCS1 for its antiviral function (24).

Figure 11. IFN signal transduction is inhibited by over expression of SOCS3. Over expression of SOCS3 in FL cells with pSOCS3 expression plasmid and then treated them with IFN-α. IFN-α-induced STAT1 phosphorylation was inhibited in cells transfected with pSOCS3 (Adapted from 24).
The role of IFN-$\gamma$ anti viral activity in fibroblast (L-929) or Keratinocytes (HEL-30)

Epithelial cells such as keratinocytes are the major target cells for HSV-1 replication. To determine why HSV-1 targets keratinocyte cell line for its replication, Frey and associates (27) considered keratinocyte and fibroblast cell lines for their experiments. As discussed before, IFN-$\gamma$ is an effective cytokine against HSV-1 infection. Therefore, induction of this cytokine in the cell infected with HSV-1 would have some benefits to protect cell from virus destruction. They treated keratinocytes (HEL-30) and fibroblast (L-929) cells with murine IFN-$\gamma$ or IFN-$\gamma$ peptide mimetic, and then these cell cultures were infected with HSV-1. Two to three days post infection cell survival observed. As shown in figure 12 IFN-$\gamma$ protected fibroblasts cells from HSV-1 infection in 50, 25, 12.5 and 6.25 concentration, but this protection was abrogated with the same concentration in HSV-1 infected keratinocytes (27).

![Figure 12](image)

Figure12. The effect of IFN-$\gamma$ on L929 fibroblasts (a) or HEL-30 keratinocytes (b) infected with HSV-1. Recombinant murine IFN-$\gamma$ added to the cultures at the concentration of 12.5 to 50, and cells lysis was monitored (Adapted from 27).
They hypothesized that the IFN-γ signaling may be obstructed somewhere in the cell signaling pathway. They developed a small peptide mimetic of mouse IFN-γ (95-132). This synthetic mimetic peptide could penetrate the plasma membrane via palmitate attached to C-terminal. The mimetic peptide was able to activate JAK/STAT transduction pathway in the same manner as IFN-γ (discussed in previous studies). Using mimetic IFN-γ peptide in keratinocytes (HEL-30) or fibroblasts (L-929), HSV-1 replication was inhibited only in fibroblasts, but not in keratinocytes (27).

![Figure 13](image)

Figure 13. The effect of IFN-γ mimetic peptide (95-132) on Fibroblasts cells L929 (a) and Keratinocytes HEL-30 (b). L929 and HEL-30 treated with IFN-γ mimetic at the indicated concentration. The effect of IFN-γ mimetic on L929 and HEL-30 cells determined based on cell survival (Adapted from 27).

Since several studies have determined SOCS negatively regulates the IFN-γ activity. Frey and colleagues hypothesized that SOCS-1 may inhibits IFN-γ antiviral activity in keratinocytes. HEL-30 keratinocytes and L929 fibroblasts were infected with HSV-1 and SOCS-1 expression detected by Western blot analysis. As shown in figure 14,
keratinocytes (HEL-30 cells) expressed of SOCS-1 mRNA, while infected fibroblast cells
did not show SOCS-1 mRNA expression. HSV-1 induces SOCS-1 expression in
keratinocytes (HEL-30 cells), but not in fibroblasts (L929 cells) (27).

Figure 14. RT–PCR analysis of SOCS-1 mRNA expression in HSV-1 infected fibroblasts and
keratinocytes. HEL-30 and L929 cells were infected with HSV-1. Total RNA was extracted and used as a
template for RT-PCR using primers specific for SOCS-1 or GAPDH as a control (Adapted from 27).

STAT-1α phosphorylated by IFN-γ induction in fibroblasts (L-929) but inhibited in
keratinocytes (HEL-30)

They suggested SOCS1 induced by HSV-1 in keratinocytes may bind to JAK2 by its KIR
(kinase inhibitory region) and prevents JAK2 phosphorylation and finally blocks the IFN-
γ signal transduction. To confirm this suggestion, IFN-γ -treated keratinocytes or
fibroblasts were infected with HSV-1, and STAT1α phosphorylation detected. As shown
in Figure15 L-929 cells expressed pSTAT1α phosphorylation, but HEL-30 cells did not.
This demonstrates that HSV-1 employs SOCS-1 to inhibit HSV-1 antiviral activity in
keratinocytes but not in fibroblasts (27).
A peptide antagonist of SOCS-1 reduces HSV-1 induced CPE in HEL-30

The role of SOCS-1 was established in inhibition of IFN-γ antiviral activity. SOCS-1 binds to JAK2 and prevents its phosphorylation. Subsequently, they synthesized an antagonist of SOCS-1 which binds to the kinase inhibitory region (KIR) of SOCS-1 and prevents the binding of SOCS-1 to JAK2. Therefore JAK2 phosphorylates STAT1α and IFN-γ signaling pathway will be continued. HEL-30 cells were cultured and then treated with IFN-γ. Different concentration of pJAK2 added to HEL-30 cultures and then cells were infected with HSV-1. As shown in Figure 16 HEL-30 cells treated with 35µM pJAK2 were completely survived from HSV-1 infection. HEL-30 cell survival dropped to 75% and 25% with 17 and 8 µM concentration of pJAK2, respectively (27).
Figure 16. Using a peptide antagonist of SOCS-1 in HSV-1 infected HEL-30 cells. HEL-30 cells were treated with IFN-γ alone, or different concentrations of pJAK2 with or without 100U/ml of IFN-γ. Then cells were infected with HSV-1 and HSV-1 induced CPE monitored (Adapted from 27).

Over expression of SOCS-1 inhibits the anti-viral activity of IFN-γ in HSV-1 infected L929 cells

Expression of SOCS-1α significantly decreased the antiviral activity of IFN-γ in HEL-30 keratinocytes cells infected with HSV-1. This suggests overexpression of SOCS-1α may have the same effect in the L929 cell lines. Therefore, a tyrosine kinase inhibitor peptide (Tkip) (a SOCS-1 mimetic peptide) was developed. This small peptide (WLVFFVIFYFFR) and Tkip2 (alanine was substituted with phenylalanine) was used to transfect fibroblasts which were treated with IFN-γ and infected with HSV-1. L929 fibroblasts were cultured and then were transfected with control plasmid or pFLAG-SOCS-1 plasmid. Cells then treated with IFN-γ and after 24 hours cells were infected with HSV-1. Tkip prevented antiviral activity in fibroblast in the same manner as SOCS-1 did in keratinocytes. In contrast Tkip2 did not show any effect on IFN-γ antiviral
activity (27). Therefore using SOCS-1 peptide antagonist could have some benefits in persons infected with HSV-1 infection.

![Diagram showing cell survival with different conditions](image)

**Figure 17.** Overexpression of SOCS-1 in L929 cells inhibited IFN-γ anti viral activity. L929 fibroblasts transfected with the indicated amounts of control plasmid or pFLAG-SOCS plasmid. Then cells treated with 100 U/ml IFN/γ for 24 hours. Cells infected with HSV-1. HSV-1 induced CPE was measured (Adapted from 27).
Discussion

Herpes simplex virus 1 infection induces innate and adaptive immune responses (9). NK cells and macrophages play an important role in clearing viruses from the body in the first hours of the virus attack. In the innate response, proinflammatory cytokines such as TNF-α, IFN type I (α&β) are involved (9). Nitric oxide (NO) produced by macrophages also may act directly against HSV-1 in early HSV-1 infection. If innate immunity cannot clear the virus from the body, adaptive immunity needs to be activated (9).

Cell mediated immunity, especially involving CD8+ T cells and CD4+ T cells, is important in adaptive immunity (1, 7). Like other viruses HSV-1 replication takes place in the cells. CD8+ T cells are able to destroy the cell infected with HSV-1 by cytotoxic effect (6). After primary infection, HSV-1 moves to the neuronal cells and is retained in a latent state. One reason why neuronal cells could be selected by the virus for replication is the low level expression of MHC molecules (1). Transient expression of MHC class I molecules on neuronal cells would induce CD8+ T cells to take action against HSV-1 (1).

Expression of HSV-1 immediate early genes such as ICP0, ICP4, and ICP27 induces early and late gene products, which finally leads to virus replication (7). Expression or absence of ICP0, ICP4, ICP27 and LAT proteins has a pivotal role in virus latency. Low level expression of α and β genes are essential for induction of immune response against HSV-1 (7).
CD8+ T cells produce IFN-\(\gamma\) to keep HSV-1 in a latent state without any cell destruction (1). Suvas et al. (1) showed co-expression of CD94-NKG2a on CD8+ T cells and interaction with Qa-1\(^b\) molecule on neuronal cells that prevent the neuronal cell destruction by CD8+ T cells. They showed the majority of Ag-specific CD8+ T cells express NKG2a (65%) and CD94 molecules. MC-38 cells (a colon carcinoma cell line) as target cells were examined for Qa-1\(^b\) molecule expression and 50% of these cells were able to express Qa-1\(^b\) molecule (1).

An immunogenic peptide derived from HSV-1 (SSIEFAR) was added in two different concentrations (2.0 and 0.2 \(\mu\)M) to target cells that were co-cultured with CD8+ T cells. This system was used to determine the level of cytotoxicity of CD8+T cells for the HSV-1 peptide. Both concentrations showed significant cell lysis in the presence of the CD8 +T cells. Blocking of the CD94-NKG2a-Qa1\(^b\) interaction led to increased CPE of target cells. These results demonstrated that, in spite of expression of lytic granules in HSV-specific CD8+T cells for the treated carcinoma cell line, these CD8+ T cells could not destroy the cultured neuronal cells due to CD94-NKG2a-Qa1\(^b\) interaction. This interaction inhibits CD8+ T cell cytotoxicity and keeps HSV-1 in latency (1).

CD94 molecule expression on CD8+ T cells could be induced by TGF-\(\beta\)1 present in TG (1). Depletion of CD4+ CD25+ cells resulted in severe disease in mice infected with HSV-1 and also reduction of the percentage of TGF-\(\beta\)1 produced (1). Thus CD4+CD25+ cells have a pivotal role in CD8+ T cell cytotoxicity. Although mice were depleted >90% from CD4+CD25+ T cells, the small remaining (4%) Foxp3 CD4+ cells in TG may induce TGF-\(\beta\)1 expression. Therefore Foxp3 CD4+ cells may serve as another source of TGF-\(\beta\)1 (1).
Lymphocytes with cytokine production persist in TG to maintain HSV-1 in latency. Halford et al. (7) showed that cytokine transcript expression (especially IL-10 and IFN-γ mRNAs) was strongly up-regulated in TG 5 days post inoculation in ICR mice (7). Cytokines IL-2, IL-10, IFN-γ, RANTES, and TNF-α mRNAs were detected in TG 14, 24, 35, 60 and 125 days p.i. by using RT-PCR analysis (7).

The HSV-1 infection also was confirmed by detection of ICP27 gene expression. ICP27 mRNA was not detected, but LAT RNA was expressed in TG, showing that virus was in a non-replicating state. Thus lymphocytes with production of cytokines have important role to maintain HSV-1 in latent state and prevent the reactivation of the virus in TG (7).

Expression of cytokines expression such as TNF-α, IL-6, and IFN-γ inhibits HSV-1 reactivation in TG. TNF-α is also produced in the innate response against HSV-1 by macrophages and NK cell (8). Minagawa and colleagues (8) examined the efficacy of TNF-α depletion in HSV-1 reactivation both in TG culture, and in TNF-knockout mice. They showed depletion of TNF-α in cell culture infected with HSV-1 increased virus reactivation. In the in vivo experiment, the survival of TNF-KO mice infected with HSV-1 dropped to 0% to 40%. Therefore this study confirmed that TNF-α also plays a pivotal role to inhibit HSV-1 reactivation in TG (8).

Neutrophils also participate indirectly in the immune defense against HSV-1 infection. Molesworth-Kenyan and associates (12) demonstrated that the survival percentage of BALB/c mice depleted of neutrophils significantly dropped. The number of CD8+ T cells, CD4+ T cells, neutrophils, and CXCR3+ expressing cells in DTH
decreased in neutrophil depleted mice. They also showed neutrophil depletion decreased the production of IP-10 and Mig in delayed type hypersensitivity (DTH) (12).

Viruses employ tactics to escape from the host immune defense, for example expression of virulence factor B8R by vaccinia virus impedes IFN-γ antiviral activity. This protein binds to IFN-γ and inhibits its binding to the cell surface receptor. Ahmed and colleagues (18) showed that a synthesized IFN-γ peptide mimetic bound to the intracellular domain of IFNGR1 and induced phosphorylation of JAK/STAT mediators and blocked the B8R activity. They treated C57BL/6 mice with different doses of mIFN-γ 95-132, and then challenged with vaccinia virus with B8R production potential. Results showed most mice were protected from death (18).

This approach led the investigators to design an IFN-γ mimetic peptide therapy for the persons infected with vaccinia virus and other viruses. This finding may be useful in clinical therapy to protect persons against diseases caused by vaccinia and other viruses such as HSV-1(18).

IFN-γ produced by CD8+ T cells induces gene activation through internalization to the cell by a specific signaling pathway. This pathway is initiated by binding of IFN-γ to its specific receptor IFNGR1/IFNGR2. IFN-γ interaction with IFNGR1 leads to JAK/STAT phosphorylation and finally activation of other signal mediators. This signal will be transferred to the nucleus to initiate the GAS activation. IFN-γ NLS domain has an important role in IFN-γ signaling pathway. IFN-γ and IFN-γ mimetic interact with IMPα5 in cytosol through complex of IFN-γ-IFNGR1/phosphorylated STAT1α (22).
Fulcher et al. (22) showed that the IFN-γ mimetic peptide has the same affinity for IMPs as does IFN-γ in signal transduction to the nucleus. Using ELISA- based binding assay they showed that both IFN-γ and mIFN-γ had affinity to IMPs (antibody specific to IMPα-GST). They also explained mIFN-γ peptide mimetic was more active than IFN-γ in activating the GAS promoter (by measuring of lucifrase activity). These results demonstrated that high affinity of IMPα/β for mIFN-γ mimetic leads to more activation of GAS promoter and gene activation. Thus IFN-γ mimetic peptide could be used in future studies in place of IFN-γ (22).

Suppressor of cytokine signaling (SOCS) is a strategy employed by HSV-1 to escape from immune system. SOCS binds to JAK2 autophosphorylation site via SOCS-KIR (kinase inhibitor region). This binding prevents auto phosphorylation of STAT1α and inhibits signal transduction. Abnormal phosphorylation of tyrosine region of kinases causes some diseases, for example experimental allergic encephalomyelitis (EAE). Tkip or SOCS mimetic peptide could have some benefits in protection of mice from EAE (21).

Mujtaba and colleagues (21) developed a tyrosine kinase inhibitory peptide (Tkip) which has 12 residues (WLVFFVIFYIFYFFR) which binds to the tyrosine kinase residue of JAK2 and makes it inaccessible for binding of STAT1α. They showed Tkip-treated mice survived acute EAE. Therefore using Tkip could be useful to control inflammatory diseases such as EAE (21).

SOCS-1 is used by HSV-1 to escape from body’s immune system. Similar to Tkip, SOCS-1 KIR recognizes the JAK2 tyrosine region for its function. Waiboci and associates (23) demonstrated both SOCS-1-KIR and Tkip bind to JAK2 autophosphorylation site and prevent binding and autophosphorylation of STAT1a (21).
Binding assay showed both SOCS1-KIR and Tkip bound to JAK2 autophosphorylation site. Macrophages have a key role in anti viral resistance, and are inducible by IFN-γ. They used macrophage cell line Raw 264.7 which was activated by IFN-γ and SOCS1-KIR or Tkip were then used to determine if they are able to block NO production by macrophages. Both Tkip and SOCS1-KIR inhibited the NO production. Thus SOCS1 antagonist may provide a therapeutic strategy to inhibit virus replication by prevention of SOCS1 function (23).

Using SOCS3 Yokota and colleagues (24) demonstrated that IFN-α signal transduction pathway was blocked in JAK phosphorylation site in the same manner as SOCS-1. They also showed overexpression of SOCS3 in FL cell culture had the same result. Western blot showed STAT1 phosphorylation was inhibited in SOCS3 over expressed cells. These results determined HSV-1 uses SOCS3 in the same strategies as SOCS1 for its anti viral function (24).

Keratinocyte cells are the most important site for HSV-1 replication. The SOCS family expression (SOCS1, SOCS2, SOCS5, and CIS) was highly increased in IFN-γ infected keratinocytes. Frey et al. (27) showed IFN-γ inhibited the HSV-1 infection in fibroblasts but did not prevent the viral replication in keratinocytes. This is because of SOCS-1 expression in keratinocytes cells but not in fibroblasts. Using SOCS-1 peptide antagonist in keratinocytes increased the percentage of cell survival. In the other hand overexpression of SOCS-1 in fibroblasts treated with IFN-γ and later infected with HSV-1 decreased the fibroblast survival (27).

Although SOCS proteins have important role in inhibition of body’s anti viral defense, it is not the only strategy employed by HSV-1. Rongtuanlin and colleagues (29)
have demonstrated that other strategies can be used by HSV-1 to defeat body antiviral defense, which is not IFN-γ dependent. They have shown that the HSV-1 immediate early proteins ICP0 and virus shut off protein (vhs) together act against immune response (29). Therefore, further studies are needed to verify which of these strategies are more effective to block host immune response by HSV-1. This could be considered one limitation on this approach and would be an appropriate subject for future studies.

Based on this study regarding the effect of IFN-γ and SOCS-1 role on HSV-1 reactivation and infection, I suggest using an appropriate mouse model such as the corneal infection model of Frye and colleagues (30) in BALB/c or C3H mouse strains, both of which are sensitive to HSV-1 infection. Keratinocyte and fibroblast cell cultures of these strains were used by Frey and colleagues to show the efficacy of the IFN-γ mimetic and SOCS-1 antagonist in inhibiting the cytopathic effect of HSV-1, strain 17syn+ (27) in mouse keratinocytes. In my proposed experiment, both IFN-γ peptide mimetic and SOCS-1 antagonist would be used as a drug therapy to reduce the number of corneal lesions by 3-4 days after HSV-1 reactivation (31). In this experiment, mice will be exposed to thermal stress (held for 10 minutes in a water-bath at 43 °C) 28 days after initial corneal infection (31) and subsequently, their corneas will be examine daily for 5 days for the number of lesions. At the same time thermal stress is induced, the mice will be administered (by oral or intraperitoneal route) SOCS-1 antagonist and INN-γ peptide mimetic. This treatment will be repeated on day 2 and 4 after induction of thermal stress. The mice will be examined daily for 5 days for the number of ocular lesions. It is expected that reduced numbers of ocular leasions will be seen in comparison
with control mice that had received thermal stress but no treatments. If so, the experiment will be repeated and the trigeminal neurons will be removed from the mice in each of these groups and examined immunohistochemically for the presence of phosphorylated JAK2. This observation would confirm the cell culture observations of the molecular events in which the IFN-γ peptide mimetic induces antiviral activity through binding to intracellular domain of IFNGR1, and subsequently initiates autophosphorylation of JAK2/STAT1α pathway (18, 19, And 20). In addition, SOCS-1 antagonist binds to KIR region of SOCS-1 produced by virus and inhibits its activity.
References


5. **Kstrom-Himes, J. A., R. A. Leblanc, L. Pesnicak, M. Godleski, and S. E. Straus.** 2000. Gamma interferon impedes the establishment of Herpes Simplex Virus Type 1 latent infection but has no impact on its maintenance or reactivation in mice. J. Virol. **74:**6680-6683.


