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HUMAN CYTOKINE RESPONSE TO HERPES SIMPLEX VIRUS TYPE 2 INFECTION IN KERATINOCYTES: THE ANTIHERPETIC CONTRIBUTION

by

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THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Jenna N. Hickey entitled Human Cytokine Response to Herpes Simplex Virus Type 2 Infection in Keratinocytes: the Antiherpetic Contribution has been approved by the thesis committee as satisfactorily completing the thesis requirements for the degree of Masters of Science in Biology in the department of Biological Sciences.

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ABSTRACT

Human Cytokine Response to Herpes Simplex Virus Type 2 Infection in Keratinocytes: the Antiherpetic Contribution

Jenna N. Hickey

Herpes simplex virus type 2 (HSV-2) is a common genital ulcer disease with no cure. While therapeutics are available, the immune response to therapeutics and HSV-2 infection are not fully understood. In this study, cytokine expression with and without HSV-2 infection and anti-HSV-2 drugs was measured in primary keratinocytes. At the protein level, there was a marked increase in sICAM-1, IFN- γ, and Serpin E1 12 hours after HSV-2 infection; an increase in SerpinE1 and sICAM-1 was seen 6 hours post-infection with HSV-2 and acyclovir treatment; and a slight decrease in Serpin E1 was seen 6 hours post-infection with cidofovir treatment. Serpin E1 mRNA was decreased in all aforementioned groups. These results confirm that keratinocytes play a role in the initial immune response to HSV-2, that some HSV-2-induced cytokine induction occurs by 12 hours post-infection, and that some cellular or HSV-2 regulation of Serpin E1 mRNA may occur in the presence of ACV.

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CHAPTER I:

INTRODUCTION

Viruses in the herpes virus family (*Herpesviridae*) are well-characterized double-stranded DNA viruses that infect birds, mammals, reptiles, amphibians, oysters, and fish (Albà et al., 2001; Knipe et al., 2004). *Herpesviridae* consists of three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*; eight viruses within these subfamilies infect humans (Albà et al., 2001). The *Alphaherpesvirinae* subfamily includes herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). HSV-1 is most closely associated with oral cutaneous lesions, while HSV-2 usually causes genital lesions (Arduino & Porter, 2008; Davison & Wilkie, 1983). Genital herpes caused by HSV-2 is one of the most widespread viral sexually transmitted diseases (STDs) (de Jong et al., 2010) This disease affects 536 million people worldwide and 16% of the United States population between the ages of 14-49 (CDC, 2010). Additionally, within most at-risk populations around the globe, more than 50% are infected with HSV-2 (Corey, 2007).

Considering the high frequency of seropositive individuals, HSV-2's disease burden is significant, especially in immune-compromised patients and neonates who contract the disease in the birth canal. Due to the impact of HSV-2 infection, many studies have been conducted to understand viral entry and life cycle. The need for understanding genital herpes is increased by the high frequency of HSV-2 coinfection with human immunodeficiency virus (HIV). Understanding modes of infection, immune

response, and treatment mechanisms will be crucial in our attempt to prevent and treat future HSV-2 transmissions and disease.

Viral entry and life cycle

Numerous studies have sought to characterize mechanisms of HSVs' entry and infection. HSVs are enveloped viruses containing linear, double-stranded DNA, in a polyhedral capsid, and numerous associated viral glycoproteins—used to enter and infect cells through cell membrane fusion, phagocytosis, and endocytosis—in the outer envelope (Gill et al., 2008; Toma et al., 2008). To mediate cellular entry, glycoproteins gB and gC first make loose, non-specific associations with cell surface heparan sulfate proteoglycans. Next, gD induces tight association with the receptors herpesvirus entry mediator, nectin-1, and 3-O-sulfated heparan sulfate. Finally, gB, gD, and H and L complexes induce fusion with the cell membrane (Toma et al., 2008). Between the outer viral envelope and the inner capsid is an amorphous tegument (Cunningham et al., 2006). The tegument contains proteins that are released into the cytoplasm upon fusion with the cell membrane. These proteins encode regulatory elements, including virion host shutoff—the protein responsible for halting host translation by degrading host mRNAs (Smith, et al., 2002). Another tegument protein is UL36, which allows for the transport of the HSV capsid to the nucleus on microtubules. After being transported to the nucleus, the viral genome enters the nucleus and virus transcription is initiated (Shanda & Wilson, 2008).

Upon nuclear entry of the viral DNA, a prototypical cascade of gene expression ensues. Immediate early (IE), or α, genes are the first to be expressed; their expression

peaks at 3-4 hours post-infection (Honess & Roizman, 1974). One specific IE protein is ICP47, which contributes to HSV's immune evasion properties. ICP47 has been shown to inhibit the function of TAP, so that no peptides for MHC class I loading can enter the ER; this causes MHC class I molecules to remain unloaded and trapped in the ER (Tigges et al., 1996). The majority of other IE genes code for proteins that are transactivators for the second class of viral proteins, early (E), or β, genes (Boehmer & Lehman, 1997).

Early genes encode vital enzymes for viral DNA replication, and their expression peaks 5-7 hours after initial infection (Boehmer & Lehman, 1997). The final wave of viral gene expression is the late (L), or γ, genes. These encode structural viral proteins required for new virions, and these genes are increasingly expressed until at least 12 hours post-infection (Boehmer & Lehman, 1997; Honess & Roizman, 1974).

After late genes are expressed, the immature herpes virion is assembled in the nucleus (Mettenleiter, 2004). Nuclear egress occurs as the virion buds from the inner nuclear membrane and subsequently sheds this nuclear membrane as it exits the outer nuclear membrane and enters the cytoplasm (Mettenleiter, 2004). The virion then is enveloped for a final time as it buds into Golgi-derived vesicles, where it acquires tegument proteins (Mettenleiter, 2004). These vesicles fuse with the cell membrane, and the virion is released into the extracellular space to infect a new host cell (Mettenleiter, 2004).

The cells of the genital mucosa are typically the first to host HSV-2 replication, which causes cell death, leading to the characteristic lesions (Gill, Davies, & Ashkar, 2008). The virus eventually travels to the spinal cord where it establishes latency in the

dorsal root ganglia (Gill et al., 2008). Viral DNA remains in the sensory neurons for the life of the individual, and stressors can cause reactivations, where new virions are produced again (Gill et al., 2008).

HSV-2 Coinfection

HSV-2 infection can be devastating, especially in immune-compromised patients and neonates. A growing body of literature is showing the interaction between HSV-2 infection and other diseases. Human Immunodeficiency Virus (HIV) is one of the primary agents found to coinfect with HSV-2 (Corey, 2007).

HIV, the cause of AIDS, is a widespread and devastating infectious agent which currently affects 34 million people worldwide and 1.1 million people in the United States (CDC, 2013). The effects of this virus are apparent in the morbidity and mortality rates it caused in 2008 worldwide; that year, 2 million people died from AIDS, and another 2.7 million more people contracted the disease (WHO, 2013).

Dendritic cells (DCs) seem to play a prominent role in HIV infection and spread. DCs are antigen-presenting cells that recognize pathogens through DC-specific pathogen-recognition receptors. While part of the innate immune system, DCs are also important in the acquired immune system because they stimulate T-cells (Kawamura et al., 2003; van Kooyk & Geijtenbeek, 2003). HIV enters dendritic cells, which protect the virion from degradation while it is trafficked to the lymph nodes, and is released to its target T-cells in a process called *trans*-infection (Paul Cameron et al., 1992; van Kooyk & Geijtenbeek, 2003; Izquierdo-Useros et al., 2010). Studies involving simian immunodeficiency virus (SIV) in rhesus macaques confirmed that Langerhans cells (LCs) in the vaginal simple squamous epithelium are an initial target of HIV upon sexual exposure (Hu, et al., 2000;

Kawamura et al., 2003). LCs are a type of DC, but their interaction with HIV is unique from that of monocyte-derived DCs. Most DCs capture HIV using the DC-specific C-type lectin DC-SIGN, and subsequently transmit the virus to T cells via *trans*-infection (Geijtenbeek et al., 2000). However, LCs are infected with HIV through CD4 and CCR5 receptors, and may pass the virus through bona fide infection (Sugaya et al., 2004).

If an individual is infected with HSV-2, their risk of HIV infection increases by 3-three (Tan et al., 2013). Further, HSV-2 coinfects greater than 50% of all individuals infected with HIV, and HSV-2 has even been found to speed up HIV infection by increasing HIV titer in plasma (Tan et al., 2013). Additionally, greater than 50% of new cases of HIV are either attributable to or worsened by HSV-2 infection (Tan et al., 2013). Interestingly, HSV-2 treatment has been found to decrease HIV viral loads in clinical trials (Margolis, 2013). Therefore, elucidating immune responses to HSV-2 may provide methods to also reduce HIV transmission.

Consequently, researchers are exploring the connections between HIV and HSV-2 to identify the mechanism for coinfection. As expected, numerous groups have found an association of HIV coinfection with the presence of open genital sores (as are commonly caused by HSV-2); they found HIV RNA in the sores and described it as the cause of coinfection (Corey, 2007; Joan K. Kreiss et al.,1989; Schacker et al., 1998). However, the increased risk of HIV infection as a result of HSV-2 has been found even in the absence of visible lesions (Corey, 2007; de Jong et al., 2010).

In attempt to illuminate these underlying mechanisms, researchers found that HSV-2 indirectly increases LCs' susceptibility to HIV through alterations of the LCs

prior to HIV infection (de Jong et al., 2010; Ogawa et al., 2013). Some literature suggests that the human cytokine LL-37 may be of special interest in this mechanism. LL-37, or human CAP18, is a small antimicrobial peptide that is found in neutrophil granules and produced by various epithelial tissues, including the mouth and vagina (De Yang et al., 2000). Ogawa et. al. (2013) demonstrated that LL-37 increases risk of HIV infection in LCs by upregulating the production of HIV-surface receptors CD4 and CCR5, thereby increasing the chance of HIV entry into LCs (Ogawa et al., 2013). Further experiments with *ex vivo* skin explants demonstrated that the presence of viable HSV-2 increased expression of LL-37, while HIV or heat-inactivated HSV-2 did not have the same effect (Ogawa et al., 2013). Understanding the mechanisms of LL-37 and other cytokine release upon HSV-2 infection could lead to novel ways of preventing the sexual transmission of HIV in individuals already infected with HSV-2, regardless of the presence of lesions.

Immune Response to HSV-2

Similar to HIV infection, there are key immune cells that play a role in responding to HSV infection. The initial innate immune response to HSV seems to be an interplay between macrophages, NK cells, γδ T cells, and interferon (IFN) secretion (Cunningham et al., 2006). Clearance of HSV-2 infection is largely T cell-mediated. First the site is infiltrated by $CD4^+$ T cells, followed by a later CD8+ infiltrate (Mikloska et al., 1996; Milligan et al., 2004). Certain cytokines have shown to be upregulated with primary HSV-2 and HSV-1 infection, such as interleukin (IL)-2, IL-1α, and interferon (IFN)-γ (Heiligenhaus et al., 1999; Mikloska et al., 1998).

IFN-γ has been found to be of particular importance to this process. In general, IFN-γ is known to stimulate the innate and adaptive immune system to clear viral and some bacterial infections. Particularly, it activates macrophages and induces MHC class I expression (Schoenborn & Wilson, 2007). IFN-γ is also present in high levels in herpetic lesions (A. L. Cunningham et al., 1985). Also, in the absence of IFN-γ, mice were unable to clear HSV-2 infections (Milligan et al., 2004). In addition, HSV's immune evasion strategy of downregulating MHC class I was decreased when keratinocytes were pretreated with IFN-γ (Mikloska et al., 1996). Not surprisingly, pretreatment with IFN-γ in fibroblasts increased the sensitivity of HSV-2 infected cells to $CD8^+$ lysis (Tigges et al., 1996). These data confirm the ability of IFN-γ to ameliorate HSV-2-induced MHC class I downregulation and increase successful immune targeting of infected cells. Similar findings have also been seen with IFN-α and -β with HSV-1 and -2 (Hendricks et al., 1991; Kunder et al., 1993).

As keratinocytes are the major epithelial cells infected by HSV-2, resulting in visible lesions, this cell type is of importance with respect to the immune response to HSV-2 infection. While the majority of cytokine production can be attributed to peripheral blood mononuclear cells (PBMCs), keratinocytes play a central role in inducing immune and inflammatory processes early in infection (Shao et al., 2010). Their role in this process was observed by comparing cytokine quantities found in herpetic lesion fluid from HSV patients to keratinocyte cultures *in vitro*. Herpetic vesicular fluid was assayed for cytokine levels. In a separate experiment, keratinocyte cultures were infected with HSV-1, and cytokine levels were assayed and found to reach 20% of the

levels found in the patient herpetic lesion fluid. These findings support the conclusion that keratinocytes make a major contribution of specific cytokines in the immune response to HSV-1 (Mikloska et al., 1998). Specifically, keratinocytes are likely important sources of IL-12, MlP-lα, MlP-lβ, and RANTES, which they secrete within the first 24 hours of HSV-1 infection (Mikloska et al., 1998).

Keratinocyte cytokine production has also been postulated to play a role in the initial recruitment of activated T cells to the site of infection (Mikloska et al., 1998). In addition, keratinocyte-produced cytokines are thought to be important in stimulating lymphocyte precursor expansion (Mikloska et al., 1998). Thus, keratinocytes increase lymphocyte presence in the infected area, leading to increased lymphocyte-produced IFN-γ. This is crucial because IFN-γ secreted by CD4+ T cells and subsequent CD4/ CD8 T cell infiltration are vital in clearing HSV infection from infected keratinocytes (Mikloska et al., 1998).

Others have conducted similar tests by infecting kerotinocytes with HSV-2 and testing for cytokine levels. Real time PCR revealed that IFN-α, IFN-β, tumor necrosis factor α (TNF-α), colony stimulating factors (CSFs), IL-3, growth factors, defensins, and selectins are upregulated in keratinocytes after 24 hours of HSV-2 infection (Shao et al., 2010). A leukocyte proliferation assay also revealed HSV-2-infected keratinocytes' ability to increase proliferation of PBMCs, thereby demonstrating their ability to increase the response of immune cells at the site of infection (Shao et al., 2010). These findings support the claim that keratinocytes play a key role in defense against HSV-2 infection by

secreting inflammatory cytokines and recruiting immune cells to further eliminate current and future infections (Shao et al., 2010).

Antiherpetic Drugs

Other factors aside from infection can impact cytokine expression, such as therapeutics. The most common therapeutic drugs used to treat HSV infections are acyclovir (ACV), penciclovir, and ganciclovir, with ACV being one of the more commonly used therapeutics (De Clercq, 2003). ACV in a chain terminator inhibitor; it inhibits the HSV DNA polymerase, preventing replication of the viral genome (Gnann et al., 1983). Specifically, ACV is a guanosine analog that lacks a 3' hydroxyl; it is selectively toxic to HSV-2-infected cells because it is a substrate for HSV viral thymidine kinase (Gnann et al., 1983). The viral thymidine kinase, and not the cellular nucleoside kinase, adds a phosphate to ACV. This makes ACV-monophosphate a good substrate for other cellular kinases, which phosphorylate the molecule to form its triphosphate derivative (Gnann et al., 1983). Triphosphorylated ACV becomes a potent inhibitor of viral DNA synthesis because it lacks the 3' hydroxyl required to add new nucleotides to the growing DNA chain. In addition, the viral DNA polymerase has up to 100 fold more affinity for triphosphorylated ACV than cellular polymerases do, making this a targeted therapy (Gnann et al., 1983; Wood et al., 1992).

Cidofovir (CDV) is an antiherpetic drug with a function similar to that of ACV. CDV is almost exclusively used to treat the human herpes virus family cytomegalovirus (CMV) in immunocompromised AIDS patients (De Clercq, 2003). While CDV is not usually used as a primary treatment against the alphaherpesviruses, it is sometimes used

against viruses resistant to ACV and PCV (Moomaw et al., 2003). CDV is a cytosine analog that also slows down elongation of DNA (De Clercq, 2003). Unlike ACV, CDV is monophosphorylated upon administration, and therefore does not require viral thymidine kinase to be activated (De Clercq, 2003). This feature makes CDV slightly more toxic than ACV. CDV is phosphorylated to its triphosphate form by cellular kinases and then incorporated into the growing viral DNA chain by viral DNA polymerases, terminating DNA synthesis (De Clercq, 2003).

Other than producing a desired therapeutic effect, many drugs also alter gene expression. For example, it has been shown that in mice, immunomodulatory drugs show anti-tumor activity in part by stimulating DCs to secrete certain cytokines, including the murine orthologs of IFN-γ, TNF-α and MCP-1 (Reddy et al., 2008). Others have found that treatment with protease inhibitors in the anti-HIV HAART regimen increase proinflammatory cytokine production in adipocytes, which likely contributes to lipodystrophy, a major adverse side effect of these medications (Lagathu et al., 2004).

Some studies have also looked at the effect of ACV treatment on cytokine levels during HSV infection. One group examined how ACV treatment affected cytokine expression during HSV-1 latency. It was found that ACV treatment had no effect on cytokine levels at 35 and 60 days post-infection, but significantly decreased IFN-γ and TNF-α mRNA levels compared to infected controls at 120 days post-infection (Halford et al., 1997). This coincides with the finding that IFN-γ and TNF-α mRNAs are elevated in mice during HSV-1 latency for up to 120 days post-infection (Halford et al., 1997). Theses findings indicate that some amount of viral DNA replication in latently infected

cells stimulates cytokine production. As ACV inhibits viral DNA replication, it also inhibits HSV-associated latent cytokine production.

While keratinocyte cytokine release upon HSV infection has been explored, how drugs alter cytokine expression during primary HSV-2 infection has not been well developed. Also, the timing of cytokine expression for clinical application remains unclear. This is relevant because many antiherpetics halt the virus at the early stage of its life cycle, preventing the virus from entering late gene expression and subsequent egress (Honess & Roizman, 1974). Further, many studies examining cytokine response to HSV have studied HSV-1 and not HSV-2. This work aims to decipher when in the HSV-2 infectious cycle certain cytokines are produced by keratinocytes at both the protein and RNA levels. It also elucidates how anti-herpetic drugs alter the expression levels of these cytokines. The results of this study have implications for therapeutics and preventative treatments for the STD caused by HSV-2 and other agents with which it coinfects. For example, if certain cytokines associated with coinfection are upregulated with certain antiherpetics, this information could be pivotal in deciding which therapeutics to assign to which patients. Also, cytokines increased or decreased with anti-HSV-2 drugs may shed light on the mechanism by which HSV-2 induces cytokine changes in infected target cells.

Chapter II:

METHODS

Cells and Virus

Primary Normal Human Epidermal Keratinocytes (NHEK) were purchased from PromoCell (Heidelberg, Germany) and were grown in Keratinocyte Media 2 (PromoCell) supplemented with 1% antibiotic/antimycotic (Hyclone, Logan, UT). Cells were maintained in an incubator at 37 °C with 5% CO_2.

HSV-2 (MS) was purchased from ATCC (#VR-540, Manassas, VA). Virus was passed once in Vero cells (ATCC #CCL-81, gift from Prashant Desai, Johns Hopkins University School of Medicine) for amplification and subsequently stored at -80 °C.

Cytokine Protein Quantification

12 Hour Experiment

NHEKs were grown in T-75 or T-25 cell culture flasks (BD Falcon, Franklin Lakes, NJ) to 85-95% confluence. Cells were infected with HSV-2 (MS) at a multiplicity of infection (MOI) of 4.6, resulting in one synchronized round of infection (Walker et al., 2004), or with an equivalent volume of DMEM (Mediatech, Inc., Manassas, VA). At 12 hours post-infection, cells were washed with PBS then treated with 1mL lysis buffer (1% Igepal CA-630 (Sigma Aldrich #I3021, St. Lewis, MO), 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, supplemented with 1 protease inhibitor tablet (Life Technologies, Fredrick, MD, #88266) / 10mL) for every 1 x 10^7 cells. Cell lysates were rocked gently at 4 °C then scraped with a sterilized rubber policeman and centrifuged at 14,000 x g for 5 minutes at room temperature. Supernatants were analyzed

using Human Cytokine Profiler Array Panel A (R&D Systems #ARY005, Minneapolis, MN) according to manufacturer's instructions. In brief, the presence of 36 human cytokines was detected via capture antibodies spotted in duplicate on a nitrocellulose membrane. Cell lysates were incubated with biolinylated detection antibodies for 30 minutes at room temperature, and then the mixture was passed over the membrane. Finally, the membranes were treated with Streptavidin-HRP and a chemiluminescent reagent (hydrogen peroxide with luminol). Light given off in proportion to the amount of cytokine bound was quantified by exposing the membrane to X-Ray film (Thermo Scientific, Waltham, MA). Experiments were performed in triplicate.

6 Hour Experiment

NHEKs were cultured as above. Cells were pretreated for one hour with 2 μg/ mL ACV, 2.5 μg/ mL CDV, or respective vehicles. All cells were then infected with HSV-2 at an MOI of 4.6. At 6 hours post-infection, cells were washed, lysed, and analyzed using Human Cytokine Profiler Array Panel A as previously described. Experiments were performed in triplicate.

Blot Quantification

Blots were quantified using Image J (NIH). Duplicate blots were averaged, and intrinsic negative control (NC, Fig. 1) values were subtracted off each cytokine value within each blot. Corrected values in infected blots were divided by values in mock-infected blots to give fold increase (infected/ mock). In experiments with drugs, HSV-2+ drug blot values were divided by HSV-2 + vehicle blot values (infected with drug/ infected with vehicle).

RT-qPCR

NHEKs were grown in 6 well plates (BD Biosciences) to 85-95% confluence. Cells in the 12 hour experiment were infected with HSV-2 or DMEM for 12 hours, as above. Cells in the 6 hour group were pretreated with ACV, CDV or vehicle for one hour and infected with HSV-2 for 6 hours, as above. At 12 or 6 hours post-infection, cells were scraped into the medium using a sterilized rubber policeman and pelleted at 14,000 x g for 5 minutes.

Cells were lysed using a QIAshredder (Qiagen #79654, Valencia, CA) and RNA was isolated using an RNeasy mini kit (Qiagen, #74104) according to manufacturer's protocols. Isolated RNA was then treated with 1 unit DNase for 30 minutes at 37 °C, then inactivated by adding 1μL stop solution and incubating for 10 minutes at 65 °C (Promega #M6101, Madison, WI). An RNA cleanup was performed by again using an RNeasy mini kit, and RNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific).

Isolated RNA was retrotranscribed using an iScript cDNA Synthesis kit which amplified RNA using randon hexamers (Bio-Rad # 170-8891, Hercules, CA). Real time PCR was then performed (with the primers listed below) on the cDNA using an iTaq Universal SYBR Green Supermix (Bio-Rad # 172-5120). Three replicates run in duplicate were run on a Bio-Rad CFX Connect Real-Time PCR Detection System. qPCR results were analyzed using the $\Delta\Delta C_t$ method and normalized to the reference gene

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) first and then confirmed with β-actin.

Primer Sequences

Serpin E1, 5′-GTGGACTTTTCAGAGGTGGAG-3′, and 5′-GAAGTAGAGGGCATTCACCAG-3′ (Arvizo et al., 2013); GAPDH, 5′-ACCACCATGGAGAAGGCTGG-3′ and 5′-CTCAGTGTAGCCCAGGATGC-3′ (Margulies & Gibson, 2007); β-actin 5′- CTCCTTAATGTCACGCACGAT-3′ and 5′-CATGTACGTTGCTATCCAGGC3′ (Yang et al., 2015).

Statistical Analyses

Results are given as the mean ± s.d. (or range). Comparisons between groups were made using Student's unpaired two-tailed t-test. A p value of <0.05 was considered statistically significant. Fold increases of >2 and <0.5 were considered as different than 1. Calculations were performed with Excel for Mac (2011).

Chapter III:

Results

Effect of HSV-2 on cytokine levels at late stages of virus gene expression

Few experiments have examined broad cytokine profiles in response to HSV-2 infection in kerotinocytes at late stages of viral gene expression. Intracellular cytokine levels were examined in NHEKs at 12 hours post-infection because late viral genes are synthesized at increasing rates at this time point (Honess & Roizman, 1974). Cytokine arrays (similar to a multiplexed ELISA) were utilized to test a broad range of cytokines, chemokines, and acute stage proteins (Fig. 1-2). Some cytokines tested in the array are not shown here either because they were not detected or had a fold change equal to one compared to mock. For example, macrophage migration inhibitory factor (MIF) was observed to have a fold difference of approximately 1 in all treatment groups. Therefore, MIF is a good example of a cytokine that meets the null hypothesis (fold change=1). For this reason, the fold change of MIF was compared to the fold changes of other cytokines to test whether fold changes have statistical significance.

Specifically, IFN-γ had an increased (although not statistically significant) expression after 12 hours of HSV-2 infection compared to mock-infected cells also at 12 hours (>2 fold, p=0.07) (Fig. 3, Table 1). Other cytokines showed trends of increased gene expression; sICAM-1, Serpin E1, IL-2, IL-8, IL-17, IL-23, and IL-27 had elevated expression (>1.5 fold), although these expression levels did not meet the threshold of what could be consider a valid difference (Fig. 1, Table 2). It should be noted that variation within groups was high, denoted by the large standard deviations. Future

studies, perhaps ELISAs and replicates of this experiment, should be conducted with larger sample sizes to confirm the trends shown here.

Effect of anti-herpetic drug on cytokine levels after IE stage of HSV-2 infection

Protein expression was evaluated using cytokine arrays at 6 hours post HSV-2 infection, which is sufficient time for the virus to transition from the immediate early to the early stage of gene expression (Honess & Roizman, 1974). NHEKs were pretreated with ACV (or left untreated) one hour prior to a 6 hour HSV-2 infection. HSV-2 infection with ACV treatment was compared to infection with no treatment to give fold increase (HSV-2 + ACV/ HSV-2 + vehicle). Fold increases of various cytokines were again compared to MIF (which had a fold increase of 1) for statistical purposes. In 6 hour HSV-2 infection, addition of ACV led to significant increases in Serpin E1 (4 fold, $p=0.02$) (Fig. 4, Table 1). ACV treatment also slightly increased sICAM-1 GROα, IFN-γ, and IL-10 (>1.5 fold), although variation in these samples was so high that the t-test failed to reject the null hypothesis (Fig. 4, Table 1). Other tested cytokines were either undetected or not different from a fold difference of one.

A second antiherpetic drug, CDV, was tested in a similar manner. After pretreatment with CDV or vehicle for one hour, NHEKs were infected with HSV-2 and incubated for 6 hours. Intracellular cytokines were quantified using cytokine arrays. While variation within groups was high for this treatment and therefore no cytokine changes failed to reject the null hypothesis of a fold difference of 1, some trends were observed. CDV treatment during HSV-2 infection slightly increased expression of IFN-γ, sICAM-1, CD40 ligand, and GROα (Fig. 5, Table 1). Interestingly, Serpin E1 expression

showed a slight decrease with CDV treatment during HSV-2 infection (<0.5 fold) (Fig. 5, Table 1). Again, other cytokines with a fold expression of one have been omitted. It is interesting to note that certain cytokines expressed at 12 hours, such as IL-2, IL-8, IL-23, and IL-27, were not detected in the 6 hour infection under any conditions.

Serpin E1 mRNA levels at various stages of viral gene expression

As the Serpin E1 protein showed considerable expression in all experimental conditions, Serpin E1 mRNA expression levels were evaluated. Experiments for assaying Serpin E1 mRNA levels via RT-qPCR were set up using the same treatments as previously described: 12 hour HSV-2 infection, 6 hour HSV-2 infection with and without ACV, and 6 hour HSV-2 infection with and without CDV. Interestingly, Serpin E1 levels at 12 hours post HSV-2 infection showed a slight decrease when compared to mock-infected NHEKs. The fold change of infection versus non-infected ranged from 0.32-1.0 (Fig 6, Table 2). These results show the trends from two repeated RT-qPCR series with two different housekeeping genes, GAPDH and β-actin. However, it should be noted that HSV-2 infection had an impact on expression levels of both housekeeping genes, causing housekeeping gene mRNA levels to vary between infected and uninfected groups. Therefore, these data should be taken as trends seen with two unique housekeeping genes.

Interestingly, in the 6 hour HSV-2 infection with ACV and HSV-2, Serpin E1 mRNA expression was also slightly decreased compared to infected, untreated controls; fold difference for this group ranged from 0.4-1.1 (Fig. 6, Table 2). This contradicts the results observed from the intracellular Serpin E1 protein levels quantified with ACV

treatment; that is, while the mRNA levels appeared to decrease, the protein levels of Serpin E1 increased 4-fold during HSV-2 infection with ACV treatment (Fig. 4).

When NHEKs were treated with CDV and HSV-2 for 6 hours, the fold change of Serpin E1 mRNA levels did not seem profoundly different from the HSV-2 + vehicle group, meaning the fold change was not meaningfully different than one (range: 0.61-1.6 fold change). This indicates that CDV treatment did not alter Serpin E1 mRNA levels during HSV-2 infection (Fig. 6, Table 2). This lack of change in Serpin E1 levels as a result of CDV treatment matches the protein data for this protein's levels during CDV treatment (Fig. 5).

Figure 1: Cytokine and chemokine array layout. This protein array was used to quanitfy cytokine/ chemokine expression levels acorss groups in the experiment. With it, the presence of 36 human cytokines was detected via capture antibodies arranged in duplicate on a nitrocellulose membrane. Sample bound to detection antibodies was added to the membrane, which was then treated with streptavidin-HRP and a chemiluminescent reagent. Light was given off in proportion to amount of cytokine bound, and membranes were exposed to X-Ray film. PC: positive control, NC: Negative control. For each condition, this experiment was repeated three times.

PC	PC	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	PC	PC
		9	9	10	10	11	11	12	12	13	13	14	14	15	15	16	16		
		17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24		
		25	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32		
PC	PC	33	33	34	34	35	35	36	36									NC	NC

1: C5a 2: CD40 Ligand 3: G-CSF 4: GM-CSF 5: GRO-α 6: I-309 7: sICAM-1 8: IFN-γ

9: IL-1α 10: IL-1β 11: IL-1rα 12: IL-2 13: IL-4 14: IL-5 15: IL-6 16: IL-8

17: IL-10 18: IL-12 P70 19: IL-13 20: IL-16 21: IL-17 22: IL17E 23: IL-23 24: IL-27

25: IL-32α 26: IP-10 27: I-TAC 28: MCP1 29: MIF 30: MIP-1α 31: MIP-1β 32: Serpin E1

33: RANTES 34: ADF-1 35: TNF-α 36: sTREAM-1

Figure 2: Representative cytokine arrays with various treatments. Each blot, spotted in duplicate, represents a unique cytokine in the order shown in Figure 1. Blots represent intracellular cytokine quantities collected after treatments A-F: **A-B)** NHEKs were pretreated with ACV or vehicle (PBS) for 1 hour then infected with HSV-2 for 6 hours; **C-D)** NHEKs were infected with HSV-2 or mock-infected for 12 hours; **E-F)** NHEKs were pretreated with CDV or vehicle (DMSO) for 1 hour then infected with HSV-2 for 6 hours. Each set of dots in each membrane corresponds with a specific cytokine; darker blots represent increased quantity of each cytokine. Red circles highlight Serpin E1 expression, a prominent cytokine expressed among all groups.

F) 6 H HSV-2 + Vehic. **E)** 6 H HSV-2 + CDV **D)** 12 H Mock Infect. **C)**12 H HSV-2 Infect. **B)** 6 H HSV-2 + Vehic. **A)** 6 H HSV-2 + ACV

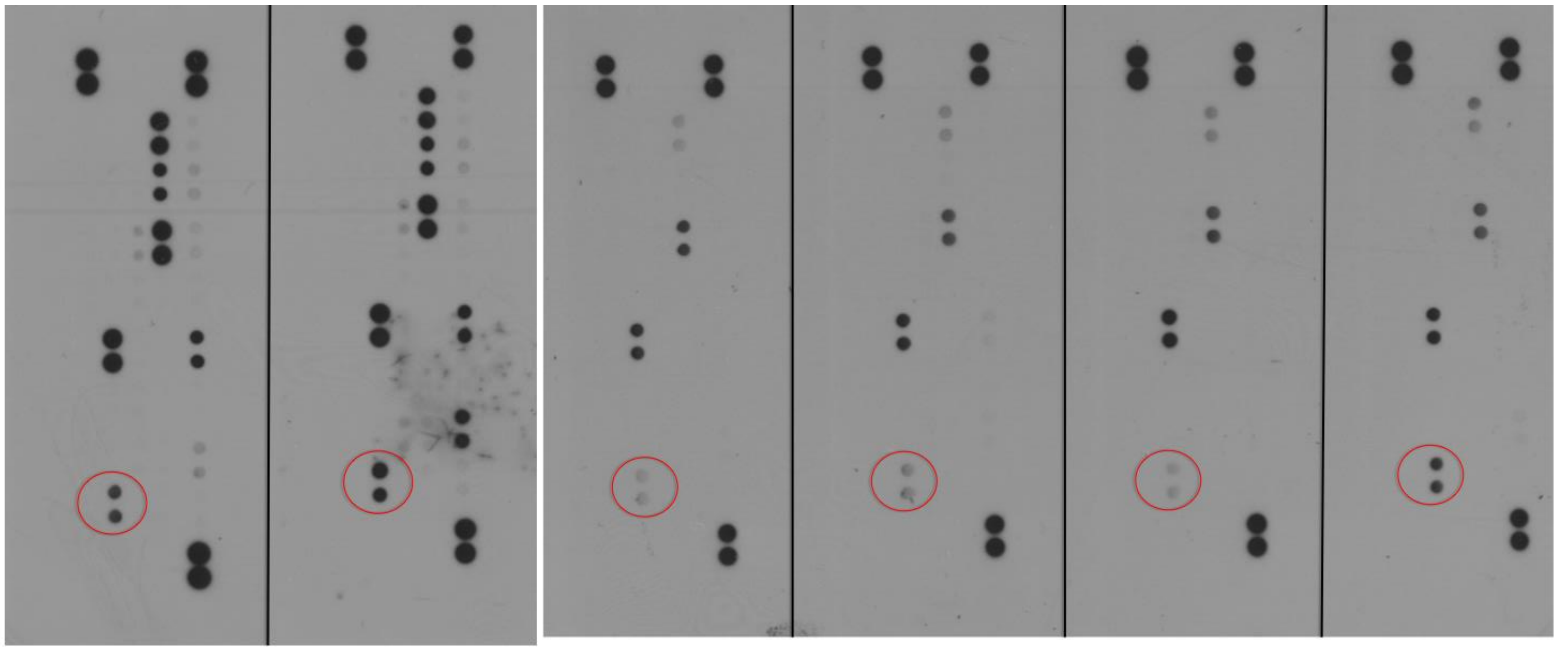


Figure 3: Fold difference in cytokine protein levels after 12 hour HSV-2 infection. Intracellular cytokines were analyzed using cytokine arrays and quantified using ImageJ (NIH). Cytokine quantities for HSV-2-infected cells were divided by the mock within each replicate (infected replicate 1/mock replicate 1) to generate fold increase. These results show the most prominent changes in protein expression within the 12-hour infection group. This figure shows the aggregate of three repetitions of the same experiment.

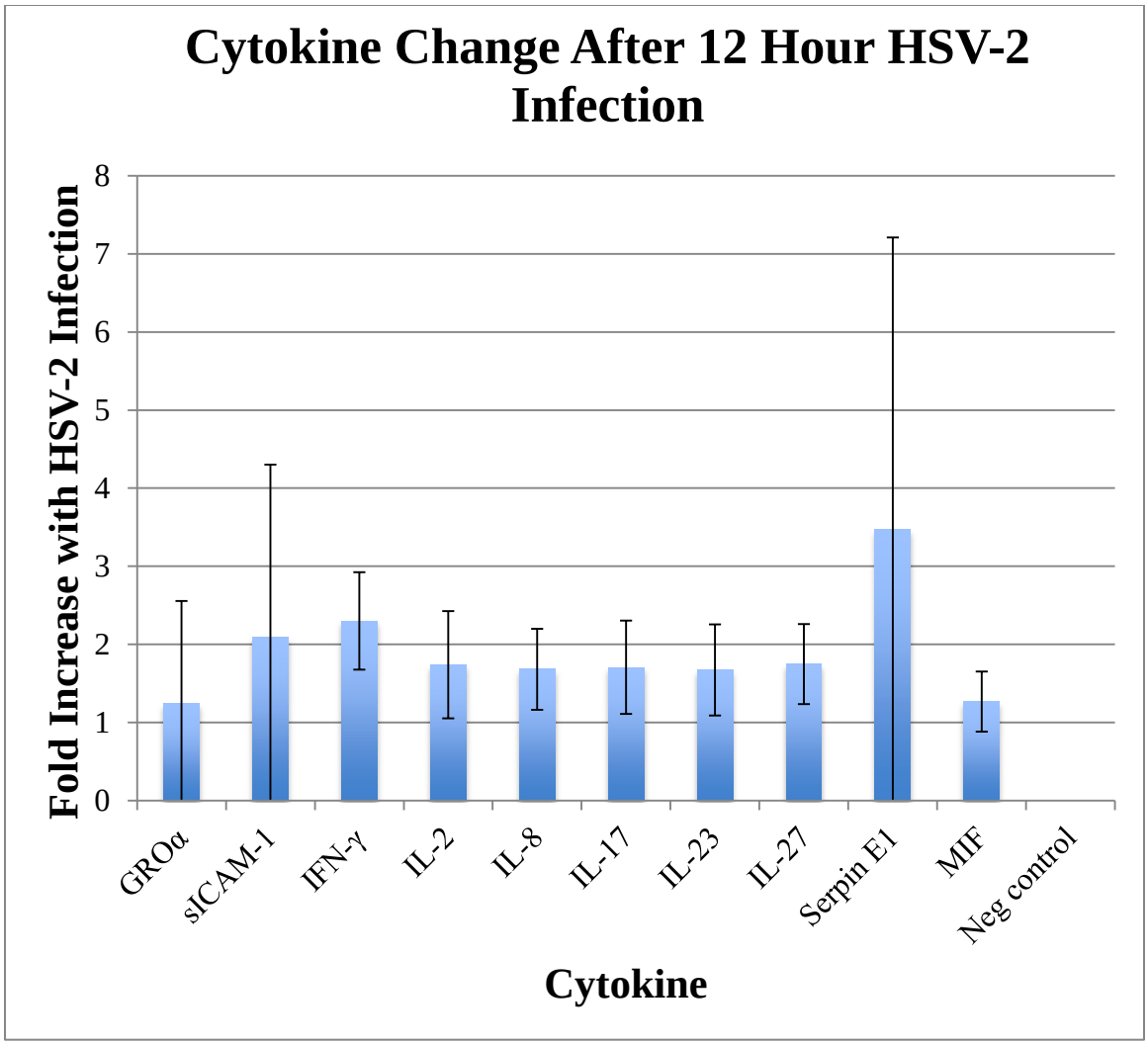

Cytokine Change After 12 Hour HSV-2 Infection
Fold Increase with HSV-2 Infection
0
1
2
3
4
5
6
7
8
GROα
sICAM-1
IFN-γ
IL-2
IL-8
IL-17
IL-23
IL-27
Serpin E1
MIF
Neg control
Cytokine

Figure 4: Fold difference in cytokine protein levels after 6 hour HSV-2 infection with or without ACV treatment. NHEKs were pretreated with ACV or vehicle for 1 hour prior to 6 hours of HSV-2 infection. Samples were taken 6 hours after infection. Intracellular cytokines were analyzed using cytokine arrays and quantified using ImageJ. Cytokine quantities for HSV-2 infected cells were divided by the mock within each replicate (HSV-2 + ACV /HSV-2 + vehicle). These results show the most prominent changes in protein expression within the 6 hour HSV-2 + ACV group. This figure shows the aggregate of three repetitions of the same experiment.

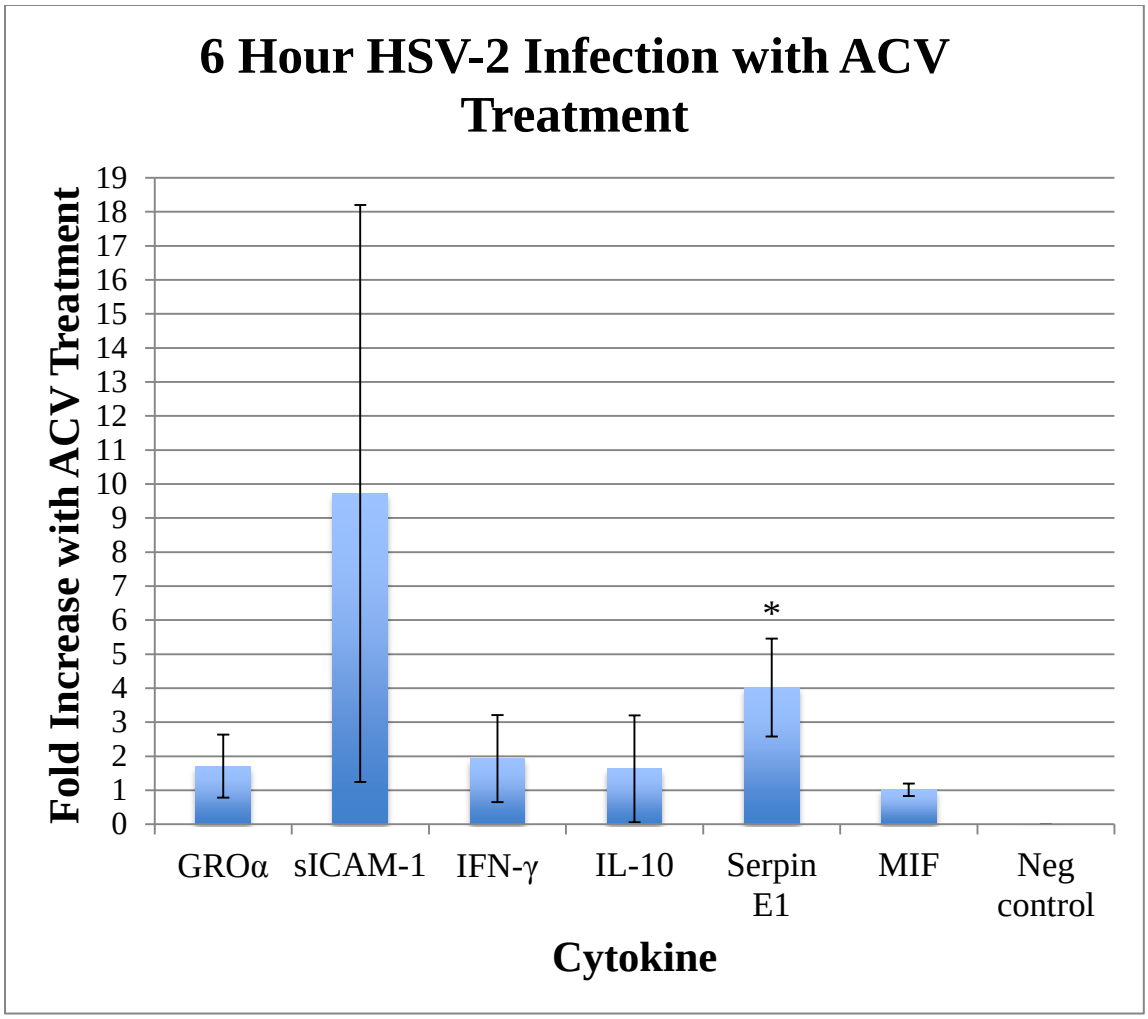

6 Hour HSV-2 Infection with ACV Treatment
Fold Increase with ACV Treatment
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
*
GROα
sICAM-1
IFN-γ
IL-10
Serpin E1
MIF
Neg control
Cytokine

Figure 5: Fold difference in cytokine protein levels after 6 hour HSV-2 infection with or without CDV treatment. NHEKs were pretreated with CDV or vehicle for 1 hour prior to 6 hour HSV-2 infection as above. Fold increase was generated by dividing infected + CDV cells/ infected + vehicle. This figure shows the aggregate of three repetitions of the same experiment.

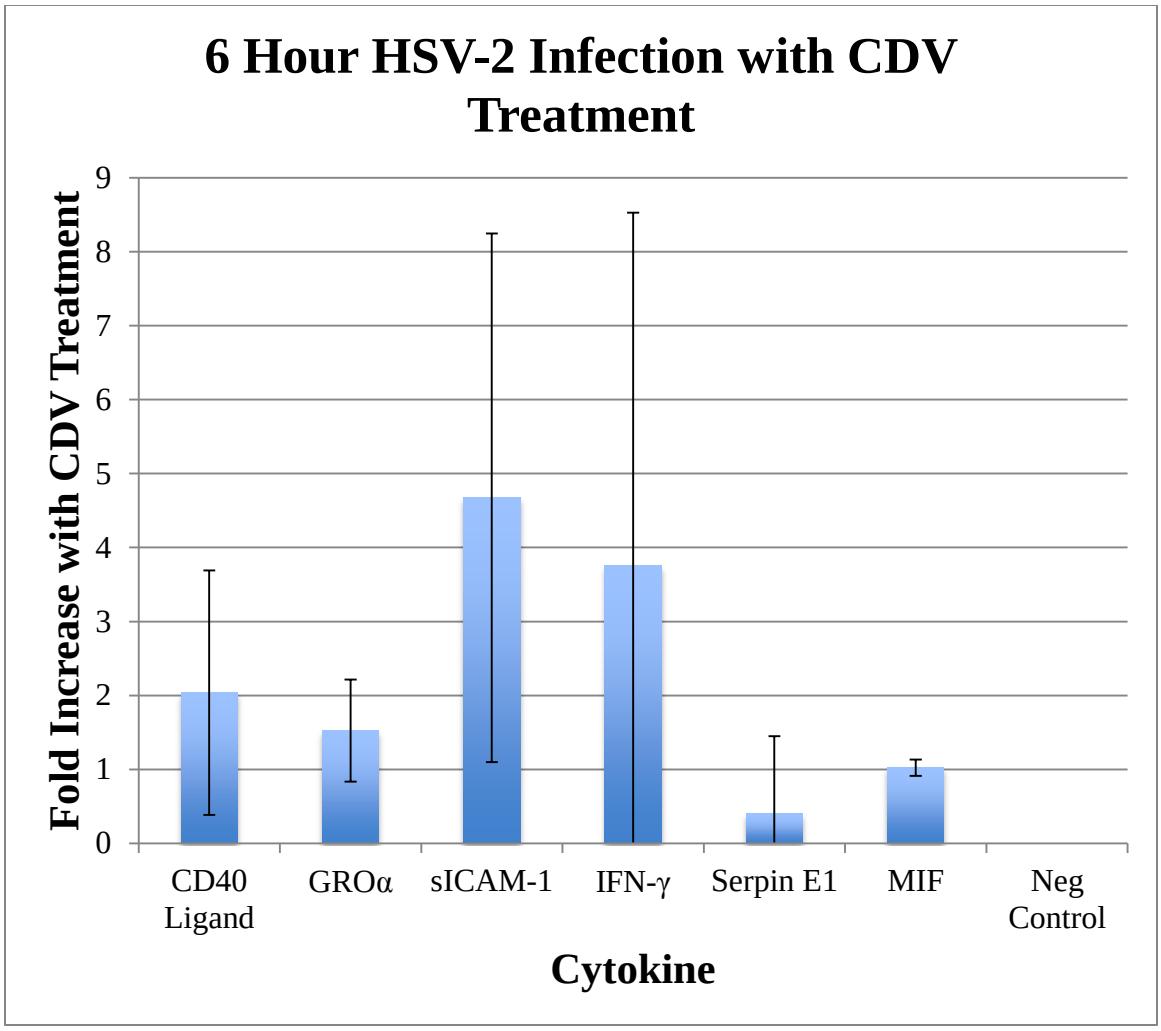

6 Hour HSV-2 Infection with CDV Treatment
Fold Increase with CDV Treatment
0
1
2
3
4
5
6
7
8
9
CD40 Ligand
GROα
sICAM-1
IFN-γ
Serpin E1
MIF
Neg Control
Cytokine

Figure 6: Fold difference in Serpin E1 mRNA as determined by RT-qPCR. NHEKs were treated with HSV-2 and ACV for 6 hours (blue), HSV-2 and CDV for 6 hours (red), or HSV-2 for 12 hours (green). Cells were collected at 6 and 12 hours, respectively, and mRNA levels of Serpin E1 were quantified using RT-qPCR**,** normalized to β-actin, and analyzed using the ΔΔCt method. Results are given as fold increase compared to mock: HSV-2 + ACV/ HSV-2 + vehicle (blue), HSV-2 + CDV/ HSV-2 + vehicle (red), HSV-2 / mock (green). This figure shows the aggregate of three repetitions of the same experiment.

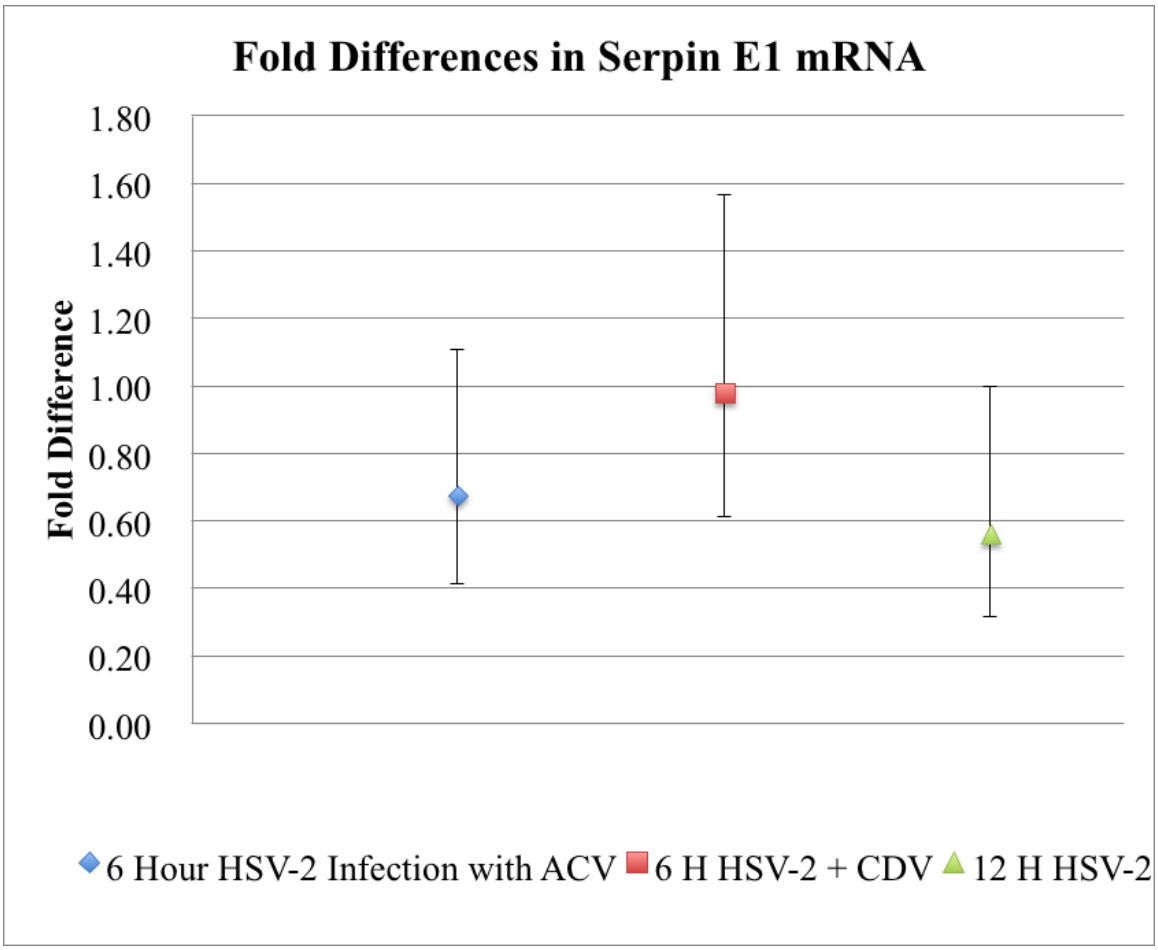

Fold Differences in Serpin E1 mRNA
Fold Difference
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
6 Hour HSV-2 Infection with ACV
6 H HSV-2 + CDV
12 H HSV-2

Table 1: Protein data from Figures 3-5. The highest cytokines among groups are compared. Dashes indicate cytokines that were undetected in that experimental group.

Name	Fold Change		
Cytokine Array	**12 Hour (Infected/Mock)**	**6 Hour ACV (ACV+ HSV-2/ HSV-2)**	**6 Hour CDV (CDV+ HSV-2/ HSV-2)**
CD40 Ligand	-	-	2.0 +/- 1.7
GROα	1.2 +/- 1.3	1.7 +/- 0.92	1.5+/- 0.69
sICAM-1	2.1 +/- 2.2	9.7 +/- 8.5	4.7 +/- 3.6
IFN-γ	2.3 +/- 0.62	1.9 +/- 1.3	3.8 +/- 4.8
Serpin E1	3.5 +/- 3.7	4.0 +/- 1.4	0.39+/- 1.1
IL-2	1.7 +/- 0.69	-	-
IL-8	1.7 +/- 0.52	-	-
IL-17	1.7 +/- 0.60	-	-
IL-23	1.7 +/- 0.58	-	-
IL-27	1.7 +/- 0.51	-	-
IL-10	-	1.6 +/- 1.6	-

Table 2: RT-qPCR data from Figure 6. Ranges for fold difference of Serpin E1 mRNA expression are given for each treatment.

Name	Fold Change		
RT-qPCR	**12 Hour (Infected/Mock)**	**6 Hour ACV (ACV+ HSV-2/ HSV-2)**	**6 Hour CDV (CDV+ HSV-2/ HSV-2)**
Serpin E1	0.32-1.0	0.41-1.1	0.61-1.6

Chapter IV:

DISCUSSION

The role of keratinocytes in HSV-2 infection

Keratinocytes are a major cell type that harbor clinical lesions associated with HSV-2 (Shao et al., 2010). Keratinocytes are the cells that make up the basal layer of the epidermis and provide a barrier to the outside world. However, HSV-2 has developed a method to compromise this barrier through endocytosis. As a result, the body's immune system responds. The initial immune response to HSV-2 is marked by infiltration of immune cells, specifically T-cells and macrophages (Cunningham et al., 2006). Keratinocytes are thought to assist in the initial recruitment and proliferation of these cells by secreting chemokines and cytokines. Keratinocytes' ability to modulate the immune system was demonstrated when HSV-2-infected keratinocytes were co-cultured with lymphocytes. Kerotinocytes increased both the proliferation and survival of the lymphocytes with which they were co-cultured (Shao et al., 2010). Thus, cytokines produced by keratinocytes play a role in the immune response to HSV-2.

Previous studies have shown that keratinocytes are capable of producing a variety of cytokines. Normal keratinocytes produce low levels of IL-α, IL-6, IL-1 β, IL-12, MlP-l α, MIP-1 β, RANTES, and TNF-α (Mikloska et al., 1998). Further, within 24 hours of HSV-2 infection, keratinocytes have been shown to secrete increased levels of IFN-α, β, TNF-α, CSFs, IL-3 (Shao et al., 2010) and lL-1β, IL-6, IL-lα, and IL-10 (Mikloska et al., 1998). Our study confirms that keratinocytes are capable of producing a variety of cytokines and chemokines (Figs. 3-5). Specifically, this study demonstrates that certain

cytokines are upregulated as early as 6-12 hours post-HSV-2 infection. Our data indicate that the virus only needs to enter the early or late phases of gene expression to initiate a cytokine response from the host cell.

In this study, we compared quantities of intracellular cytokines at certain times after infection. The timing of gene expression is directly associated with the stage in the virus life cycle triggering the cytokine response. This is relevant because different therapeutics target different stages of HSV-2 gene expression. Understanding which stage in the viral life cycle triggers an immune response will give a clearer picture of the interaction between virus, immune response, and the effects of therapeutics. The intracellular cytokine levels tested here show the immediate effects of the virus and drugs on the cell. However, additionally assaying the tissue culture fluid and comparing it with intracellular cytokine levels would have yielded a more complete picture of these interactions.

Variation in Protein Array

While some cytokines showed prominent expression in the protein array analysis, there was considerable variation among replicates in all three experimental conditions (Appendix 1-3). For example, in the 6 hour ACV experiment, sICAM-1 was markedly increased in replicates two and three, but not in replicate one (Appendix 2). Also, even though replicates two and three showed an increase in sICAM-1 expression, the magnitude of the increased expression was not identical (Appendix 2). This may have been caused by different lengths of exposure of the film to the chemiluminscent signal, which denotes the presence of the cytokine.

Another example of variation in these experiments can be observed in the third replicate of the 6 hour CDV experiment. Here, several cytokines showed signal in replicates one and two but not in replicate three (Appendix 3). Because of these anomalies, these data should be taken as preliminary for the purpose of observing trends. These trends can be applied to future experiments with more quantitative assays like ELISAs. These data will also give future experiments necessary information to use power analysis to calculate appropriate sample sizes based on the variation seen here. Also, these results help to narrow the cytokine candidates for study to ones that show expression as early as 12 hours post-infection and 6 hours post-infection in the presence of antiherpetic drugs.

HSV-2 manipulation of host gene expression

Certain cytokines were increased with HSV-2 infection. Increase in host protein synthesis during HSV-2 infection is interesting because HSV generally blocks host translation. This inhibition was prominent in this experiment because we examined primary infection and synchronized infection with every cell in the culture by using an MOI of 4.6. In general, HSV shuts down host gene expression through the HSV tegument protein virion host shutoff (vhs). This protein binds to and inactivates protein kinase R (PKR), rendering it unable to inactivate eukaryotic translation initiation factor eIF-2α (Kalvakolanu, 1999). With an inactive eIF-2α, host translation is severely inhibited, freeing cellular machinery for viral gene expression. Consequently, increased cytokine expression during the course of infection is additionally interesting because it requires overcoming the virus-induced blockade of translation.

This HSV-2-induced decrease in overall gene expression was seen in this experiment in the housekeeping genes GAPDH and β-actin. Both housekeeping genes were tested as reference genes in RT-qPCR (Appendix 5). During the 12 hour HSV-2 infection, GAPDH and β-actin mRNA expression were decreased compared to the uninfected mock (Appendix 4). This finding has been confirmed by other reports where GAPDH and β-actin mRNA levels decrease with HSV-1 infection as early as 7 hours post-infection (Hardwicke & Sandri-Goldin, 1994). This could be through vhs, or through other viral proteins such as the immediate early protein ICP27. ICP27 has been shown to interfere with host cell mRNA splicing, thus suppressing GAPDH and β-actin mRNA levels during HSV infection (Hardwicke & Sandri-Goldin, 1994). This skews the RT-qPCR data for the 12 hour group in the experiment. Because of these issues, the RT-qPCR data should only be observed as a trend, although the trend holds for both housekeeping genes (Appendix 5).

The experiment with 6 hour HSV-2 infection treated with ACV also showed higher GAPDH mRNA expression in the groups treated with ACV (Appendix 6). However, these differences were not seen when β-actin was used as the housekeeping gene (Appendix 6). This may indicate that the downregulation of β-actin as a result of HSV infection occurs later than 6 hours post-infection. The group treated with CDV showed no difference in GAPDH or β-actin expression (Appendix 7). These data seem to indicate that CDV fails to alleviate the HSV-2-induced downregulation of host protein synthesis for these genes, especially GAPDH, while ACV does.

Therefore, β-actin was an effective housekeeping gene for antiherpetic comparisons but not for 12 hour infection comparisons. Perhaps MIF would be an effective reference gene for future RT-qPCR studies since it did not change during HSV-2 infection or antiherpetic treatment (Figs. 3-5). Other commonly used reference genes we may consider in future RT-qPCR experiments are β-2-microglobulin, 18S rRNA, PPIA, HPRT1, and GUSB, to name a few (SABiosciences).

Cytokines of interest from this experiment: sICAM-1

Of the cytokines upregulated upon HSV-2 infection in our experiment, the most prominent were sICAM-1, IFN- γ, and Serpin E1. Specifically, sICAM-1 showed marked increase in all treatments, especially when infected with HSV-2 and treated with ACV for 6 hours. However, all treatments showed considerable variation (Table 1). The cytokine sICAM-1 is a circulating form of ICAM-1. ICAM-1 is expressed on endothelial cells and binds to lymphocyte function-associated antigen (LFA-1), a leukocyte integrin. sICAM-1 binding to LFA-1 can inhibit leukocyte-endothelial cell binding (Witkowska & Borawska, 2004). Thus, sICAM-1 may function to inhibit an immune response by acting as a competitor for leukocyte binding (Shapiro et al., 2003).

In other viral infections, sICAM-1 has been shown to inhibit rhinovirus-induced cytopathic effect (CPE) (Marlin et al., 1990). Rhinoviruses cause upper respiratory infections, and are usually the viruses responsible for the common cold. They utilize ICAM-1 to enter cells. Interestingly, rhinoviruses can modulate cellular mRNA to upregulate cell-surface ICAM-1, which facilitates viral entry, and downregulate the release of sICAM-1, which competes with ICAM-1 for viral binding (Whiteman et al.,

2003). However, sICAM-1 does not inhibit CPE during infection with HSV-1, which enters through ICAM-1-independent mechanisms (Marlin et al., 1990). Still, sICAM-1 is increased in patient serum during acute oral HSV-1 infection (Yamamoto et al., 1993). From the results shown in the present experiment, HSV-2 infection would likely show similar results to those seen by Yamamoto et al., (1993) (Table 1). Also, these results show that infected keratinocytes may contribute to this increase as early as 12 hours post-infection (Fig. 1). This increase in sICAM-1 during HSV-2 infection may be a result of sICAM-1 being upregulated by proinflammatory cytokines like TNF-α and IL-1 (Witkowska & Borawska, 2004).

Indeed, it remains unclear whether sICAM-1 assists in the clearance of HSV-2. It is thus interesting to find that in this study, sICAM-1 was significantly increased with the addition of the ACV (>9 fold, Table1). While the explicit function of sICAM-1 in this process has yet to be elucidated, the ability of keratinocytes to increase sICAM-1 production upon HSV-2 infection may be important. For example, since sICAM-1 functions as an inhibitor of leukocyte migration, increased presence of sICAM-1 may dampen the immune response against HSV-2 and other diseases, such as HIV. Further investigation should examine the mechanism for ACV-induced sICAM-1 upregulation, as well as the contribution of sICAM-1 to the HSV-2 immune response.

Cytokines of interest from this experiment: IFN- γ

IFN- γ was also upregulated at 12 hours post HSV-2 infection (>2 fold, Table 1). This study confirms that transition of HSV-2 into late stage of gene expression around 12 hours post-infection is sufficient for IFN- γ expression in keratinocytes. Further, these

results demonstrate that anti-herpetic drugs may increase expression of IFN- γ relative to untreated, infected cells as early as 6 hours post-infection. This suggests a possible cellular mechanism for HSV-2-induced expression of IFN- γ that is initiated somewhere before transition into the early phase of gene expression. This finding is novel because most studies examining HSV cytokine expression focus on HSV-1 at lower MOIs and at time points long after transition into the late phase of viral gene expression. This study shows an immediate response to synchronized primary HSV-2 infection.

This early contribution of IFN- γ by keratinocytes may contribute to the initial recruitment of macrophages, which are vital for later HSV-2 clearance. It also seems to make a contribution to the overall level of IFN- γ necessary for clearing HSV-2 infection (Mikloska et al., 1998). Further experiments should be done to confirm this finding. Because IFN- γ is increased with the addition of ACV, upregulation of this cytokine may contribute to the improved clinical outcomes associated with ACV treatment. This early host cell induction of IFN- γ with ACV pretreatment shows the potential benefit of prophylactic ACV treatment in HSV-2 infection.

Cytokines of interest from this experiment: Serpin E1

Another cytokine of interest in this experiment was Serpin E1, also called plasminogen activator inhibitor-1 (PAI-1). Specifically, Serpin E1 protein levels were significantly increased after 6 hours of HSV-2 infection when treated with ACV (Table 1). However, Serpin E1 protein levels were slightly decreased 6 hours post-infection when treated with the similar anti-herpetic, CDV (Table 1). While levels may have been increased after the 12 hour untreated infection as well, variation within the samples was

too high to draw a meaningful conclusion. However, all experimental conditions showed some Serpin E1 response to HSV-2 infection.

Further, when fold expression of Serpin E1 was examined on an RNA level, it was found that the 12 hour untreated infection, 6 hour infection with ACV, and 6 hour infection with CDV all exhibited slight decreases in Serpin E1 mRNA expression (Table 2). This result matches the protein assay results for 6 hour HSV-2 infection treated with CDV. However, this is in opposition to the protein results for HSV-2 treated with ACV, which showed a four-fold increase in Serpin E1 protein levels. Theses results would suggest that a unique phenomenon is occurring at the RNA or protein level in the expression of Serpin E1.

Possible explanations include rapid degradation of Serpin E1 mRNA because of the combination of cytokines induced by ACV treatment. Another explanation could be some alteration of Serpin E1 mRNA by HSV-2, such as induction of a Serpin E1-controlling miRNA. Further experiments must be conducted to determine the mechanism for the discrepancy we see between protein and mRNA levels of Serpin E1 during HSV-2 infection. While there is a chance this could have been an experimental error, the possibility remains that some regulation is occurring at the mRNA level for this gene.

The majority of gene regulation for Serpin E1 seems to occur at the transcription initiation level because the gene contains numerous binding sites for transcription factors like Smads and HIFs (Muth, et al., 2011). However, the 3' UTR of Serpin E1 mRNA contains AU-rich destabilizing elements, which could lead to high Serpin E1 mRNA turnover (Muth, et al., 2011). This may explain a portion of these curious results. While

little is known about the post-transcriptional regulation of Serpin E1, some studies have shown different mechanisms for Serpin E1 mRNA regulation. For example, it has been shown that in kidney fibroblastic cells, angiotensin II can trigger stabilization of Serpin E1 mRNA through the RNA-binding protein human-antigen R (Doller et al., 2009). Also, it has been shown that Serpin E1 is upregulated in fibroblasts under hypoxic conditions. This upregulation seems to occur because of a hypoxia-induced downregulation of a endogenous microRNAs (miRNA) targeting Serpin E1 (Muth et al., 2011). The mechanism for HSV-2-induced and ACV-induced upregulation of protein and downregulation of mRNA is yet to be determined, but it may be linked with Serpin E1 miRNA or rapid degradation of Serpin E1 mRNA, similar to those described above.

Like sICAM-1, the functions of Serpin E1 are not fully understood. However, recent findings shed light on its antiviral and coinfection possibilities. Serpin E1 is a serine protease inhibitor that exhibits constitutive expression and is stimulated by IFN. Recently, it has been shown to inhibit flu virus replication *in vitro* and *in vivo*. Specifically, Serpin E1 inhibits the proteases that cleave HA on newly synthesized influenza virions, which renders HA immature and unable to fuse with new host cells, thus limiting new influenza infections (Dittmann et al., 2015; Horvath, 2015).

Other types of serine protease inhibitors in the Serpin family have been connected with both HSV and HIV infection. For example, Serpin antithrombin III (ATIII) activated by heparin showed antiviral activity against HSV-1, HSV-2, and HIV-1 *in vitro* (Whitney et al., 2011). ATIII also decreased HSV-1-associated mortality *in vivo* from 90% (untreated) to 40% (treated with ATIII), and showed potent antiviral activity against

ACV-resistant HSV-1 strains (Quenelle et al., 2014). This inhibition seemed to occur early (within two hours) of HSV-2 infection. (Quenelle et al., 2014). These antiviral effects have been implicated to be the result of CD8+ modifying bovine ATIII to an activated form able to inhibit HIV-1 replication (Geiben-Lynn et al., 2002). A possible mechanism of ATIII action is the ability of ATIII to upregulate prostaglandin synthetase-2 (PTGS2), the overexpression of which inhibits HIV-1 replication (Whitney et al., 2011). These results show that ATIII could be a potential antiviral for HSV and HIV-1 infections. This is an especially attractive option because treatment with this cytokine shows low cytotoxicity (Quenelle et al., 2014; Whitney et al., 2011). These findings with ATIII raise the question of whether the effects of Serpin E1 will be similar. Thus far, it seems as though these tests have not been conducted with Serpin E1. If Serpin E1 also shows potent anti-HSV-2 and HIV-1 properties, this may contribute to the improved clinical outcome of HSV-2/ HIV-1 coinfected individuals with ACV treatment (Margolis, 2013).

Serpin E1 has also been linked with other complications. It has been shown that elevated levels of Serpin E1 are correlated with poor prognosis during septic shock (Pralong et al., 1989). Serpin E1 levels are also high in patients with dengue virus infection, predominantly in patients with dengue shock syndrome with poor clinical outcome (Mairuhu et al., 2005). To our knowledge, this is the first report of Serpin E1 being elevated during HSV-2 infection. Future studies should investigate whether Serpin E1 has antiviral activity against HSV-2, or whether Serpin E1 is associated with poor prognosis, especially in patients with compromised immune systems. This information

could be crucial for high-risk individuals since ACV treatment increases Serpin E1 protein levels, as was shown in this study.

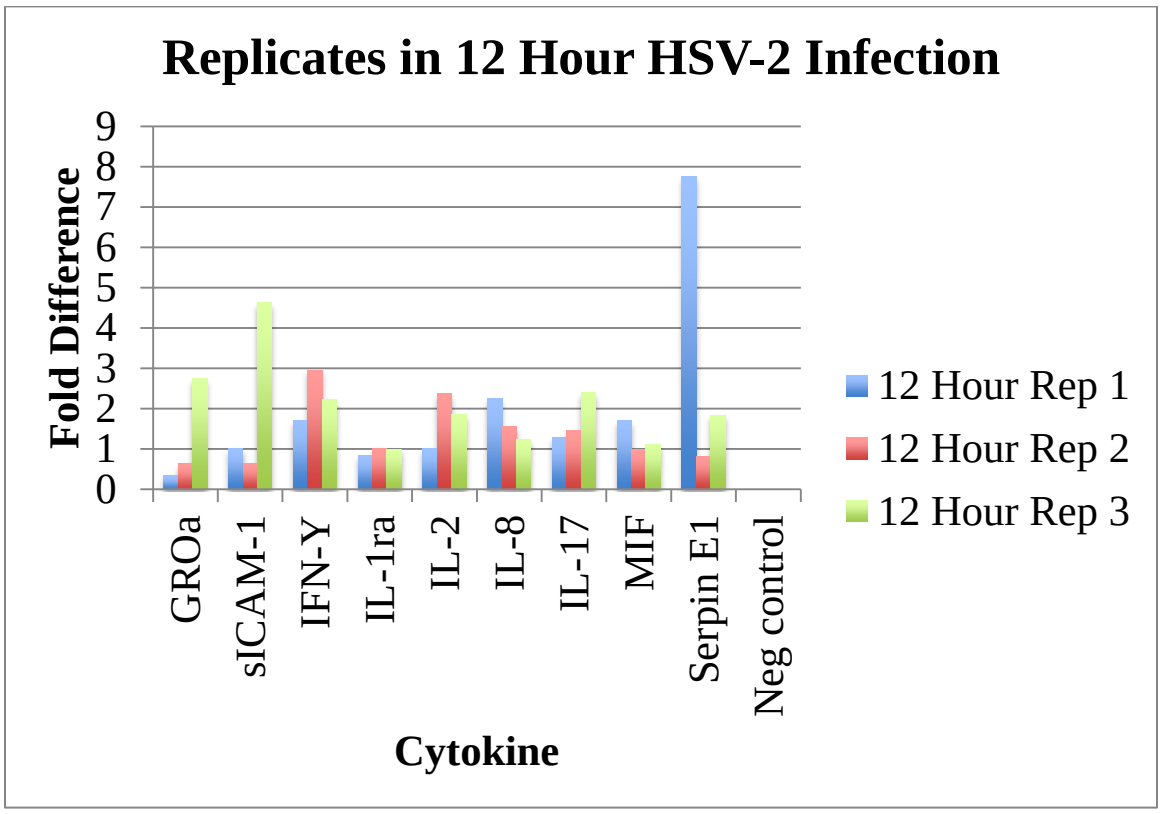
Conclusion

In conclusion, the main cytokines found to be upregulated at 6 and 12 hours post HSV-2 infection were sICAM-1, IFN- γ, and Serpin E1. While much has yet to be understood of the precise role these cytokines play in the innate immune response to HSV-2 infection, they may contribute to the initial recruitment and activation of immune cells at the site of HSV-2 infection. Specifically, for those cytokines that have been implicated to exacerbate clinical outcomes, like Serpin E1, it may be necessary to prescribe antiherpetics cautiously to avoid unwanted clinical outcomes.

Overall, this is a preliminary study preceding further investigations on the interactions between HSV-2, keratinocytes, cytokines, antiherpetics, and immune cells. There is still much to be investigated concerning these interactions. Furthering understanding of these interactions may also shed light on the mechanisms for coinfection with HSV-2 and other diseases. However, these experiments provide information toward deciphering these interactions and improving HSV-2 treatment in a clinical setting.

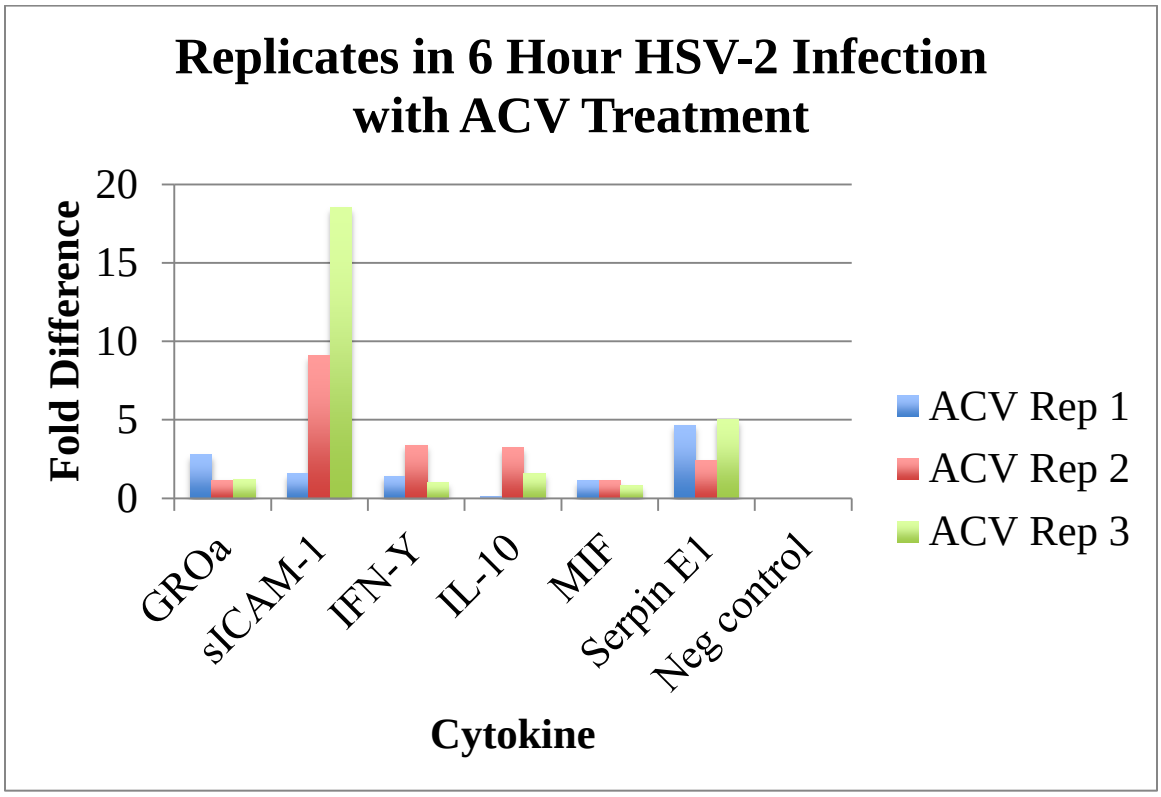
APPENDIX

Appendix 1: Individual replicates in 12 Hour HSV-2 infection—data from protein array. There was considerable variation among replicates of the same cytokine in the 12 hour HSV-2 infection. (Fold difference: infected/mock)


Replicates in 12 Hour HSV-2 Infection
Fold Difference
0
1
2
3
4
5
6
7
8
9
GROa
sICAM-1
IFN-Y
IL-1ra
IL-2
IL-8
IL-17
MIF
Serpin E1
Neg control
Cytokine
12 Hour Rep 1
12 Hour Rep 2
12 Hour Rep 3

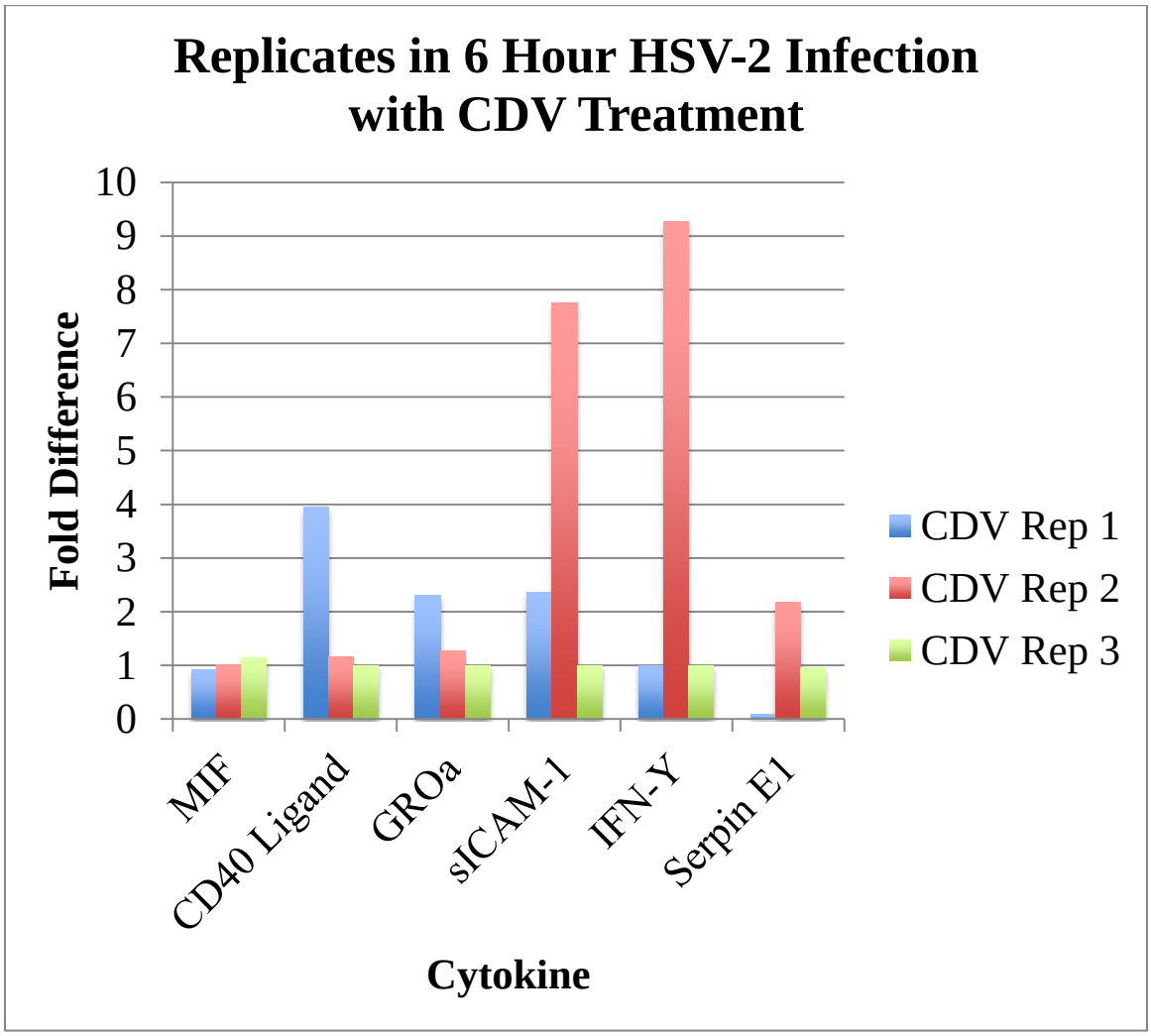
Appendix 2: Individual replicates in 6 hour HSV-2 infection with ACV treatment—data from protein array. Variation is seen among replicates of the same cytokine with the same treatment in the 6 hour HSV-2 infection with ACV treatment.

(Fold difference: HSV-2 + ACV /HSV-2 + vehicle)


Replicates in 6 Hour HSV-2 Infection with ACV Treatment
Fold Difference
20
15
10
5
0
GROa
sICAM-1
IFN-Y
IL-10
MIF
Serpin E1
Neg control
Cytokine
ACV Rep 1
ACV Rep 2
ACV Rep 3

Appendix 3: Individual replicates in 6 Hour HSV-2 infection with CDV treatment—data from protein array. Variation among replicates of the same cytokine with the same treatment in the 6 hour HSV-2 infection with CDV treatment.

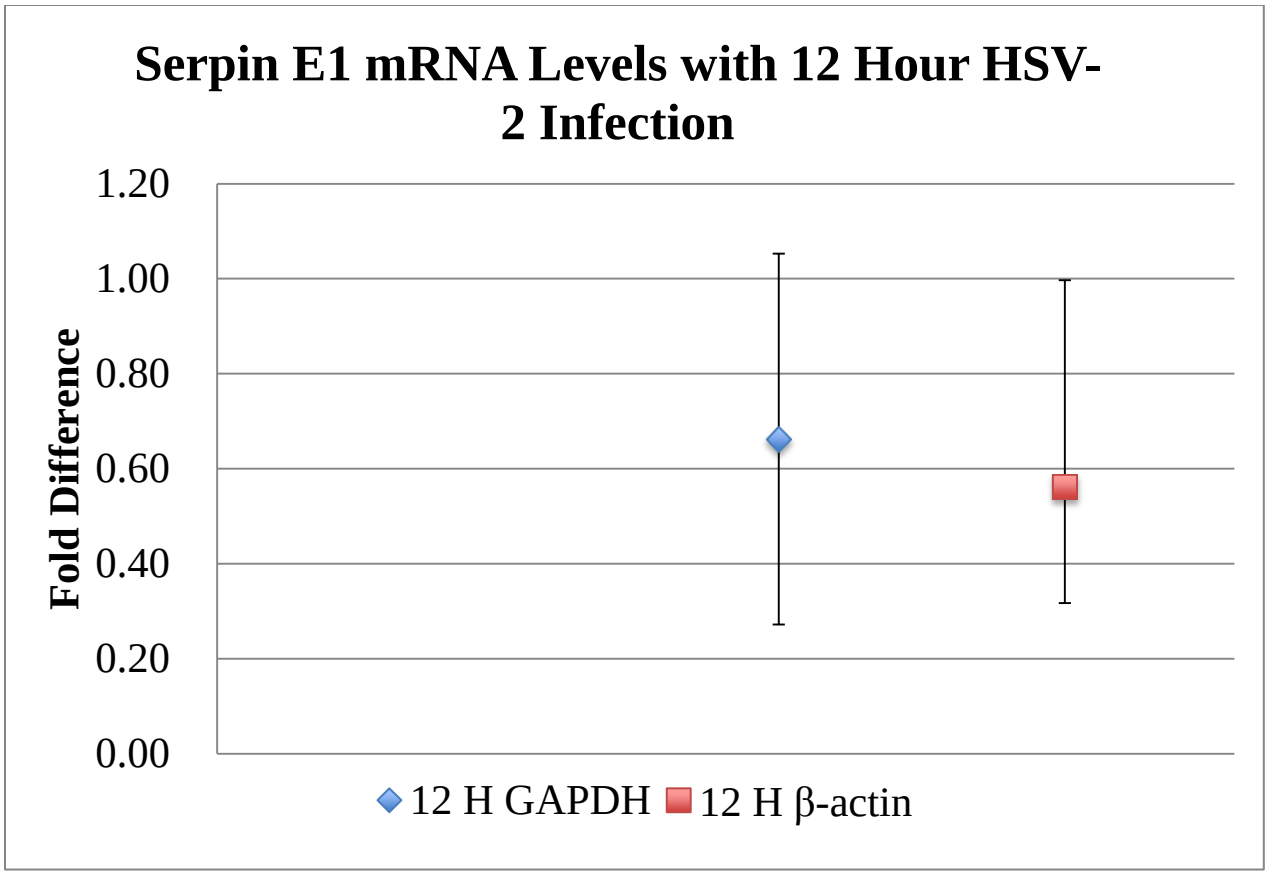
(Fold difference: HSV-2 + CDV /HSV-2 + vehicle)


Replicates in 6 Hour HSV-2 Infection with CDV Treatment
Fold Difference
10
9
8
7
6
5
4
3
2
1
0
MIF
CD40 Ligand
GROa
sICAM-1
IFN-Y
Serpin E1
Cytokine
CDV Rep 1
CDV Rep 2
CDV Rep 3

Appendix 4: Individual replicates of Cq values from qPCR of the 12 hour HSV-2 infection experiment using GAPDH and β-actin. Cq values for both housekeeping genes are lower in the mock replicates, indicating increased amount of mRNA in the groups without HSV-2 infection. This table shows three repetitions of the same treatment; each replicate was run in duplicate in RT-qPCR. Dashes indicate wells that made products different from other members in the experimental group.

Serpin E1: GAPDH	Serpin E1 Cq	GAPDH Cq	Serpin E1: β-actin	Serpin E1 Cq	β-actin Cq
Infected A1	23.80	22.03	Infected A1	25.04	21.51
Infected A2	-	22.32	Infected A2	24.68	21.67
Infected B1	24.64	23.55	Infected B1	25.70	22.97
Infected B2	-	23.92	Infected B2	25.14	23.08
Infected C1	23.85	22.51	Infected C1	24.96	21.47
Infected C2	-	22.29	Infected C2	24.92	21.84
Mock D1	18.14	17.47	Mock D1	20.06	17.28
Mock D2	-	17.55	Mock D2	19.98	17.12
Mock E1	18.64	18.28	Mock E1	19.90	18.02
Mock E2	-	18.52	Mock E2	19.82	17.81
Mock F1	17.93	18.57	Mock F1	19.84	17.89
Mock F2	19.10	-	Mock F2	19.52	18.08

Appendix 5: Results for ranges of fold difference from both housekeeping genes with the 12 hour HSV-2 infection. There is a trend toward decreased expression of Serpin E1 with 12 hour HSV-2 infection with both housekeeping genes. However, these data should only be viewed as a trend due to the downregulation of the reference gene with infection.


Serpin E1 mRNA Levels with 12 Hour HSV-2 Infection
Fold Difference
1.20
1.00
0.80
0.60
0.40
0.20
0.00
12 H GAPDH
12 H β-actin

Appendix 6: Individual replicates of Cq values from qPCR of the 6 hour HSV-2 infection treated with ACV experiment using GAPDH and β-actin. For GAPDH, the HSV-2 + vehicle group had decreased expression of GAPDH mRNA; however, β-actin mRNA levels do not change with ACV treatment, making this a favorable housekeeping gene for this experiment.

Serpin E1: GAPDH	Serpin E1 Cq	GAPDH Cq	Serpin E1: β-actin	Serpin E1 Cq	β-actin Cq
HSV-2 + ACV A1	22.80	21.04	HSV-2 + ACV A1	23.23	20.55
HSV-2 + ACV A2	23.80	21.06	HSV-2 + ACV A2	22.92	20.64
HSV-2 + ACV B1	22.81	21.11	HSV-2 + ACV B1	23.66	22.03
HSV-2 + ACV B2	23.07	21.38	HSV-2 + ACV B2	23.62	22.02
HSV-2 + ACV C1	22.79	22.03	HSV-2 + ACV C1	23.52	21.49
HSV-2 + ACV C2	23.18	21.97	HSV-2 + ACV C2	23.36	21.60
HSV-2 + Vehicle D1	24.46	25.13	HSV-2 + Vehicle D1	24.04	22.78
HSV-2 + Vehicle D2	25.01	25.39	HSV-2 + Vehicle D2	23.85	22.99
HSV-2 + Vehicle E1	23.81	24.38	HSV-2 + Vehicle E1	22.48	20.57
HSV-2 + Vehicle E2	24.11	24.71	HSV-2 + Vehicle E2	-	-
HSV-2 + Vehicle F1	27.07	29.38	HSV-2 + Vehicle F1	23.08	21.32
HSV-2 + Vehicle F2	27.58	29.55	HSV-2 + Vehicle F2	23.00	21.63

Appendix 7: Individual replicates of Cq values from qPCR of the 6 hour HSV-2 infection treated with CDV experiment using GAPDH and β-actin. Neither GAPDH nor β-actin levels change with CDV treatment, making either one an acceptable housekeeping gene for this experiment.

Serpin E1: GAPDH	Serpin E1 Cq	GAPDH Cq	Serpin E1: β-actin	Serpin E1 Cq	β-actin Cq
HSV-2 + CDV A1	22.35	20.70	HSV-2 + CDV A1	22.74	21.15
HSV-2 + CDV A2	22.06	20.41	HSV-2 + CDV A2	22.42	21.40
HSV-2 + CDV B1	22.27	21.17	HSV-2 + CDV B1	22.66	21.39
HSV-2 + CDV B2	22.24	21.01	HSV-2 + CDV B2	22.34	21.50
HSV-2 + CDV C1	23.17	21.23	HSV-2 + CDV C1	23.63	22.11
HSV-2 + CDV C2	23.08	21.21	HSV-2 + CDV C2	23.40	22.18
HSV-2 + Vehicle D1	22.09	20.03	HSV-2 + Vehicle D1	22.44	20.81
HSV-2 + Vehicle D2	21.51	20.10	HSV-2 + Vehicle D2	22.42	20.98
HSV-2 + Vehicle E1	22.52	21.44	HSV-2 + Vehicle E1	22.58	21.08
HSV-2 + Vehicle E2	22.31	20.91	HSV-2 + Vehicle E2	22.36	21.36
HSV-2 + Vehicle F1	22.73	21.55	HSV-2 + Vehicle F1	23.14	22.00
HSV-2 + Vehicle F2	22.72	21.64	HSV-2 + Vehicle F2	22.67	22.11

LITERATURE CITED

Albà, M. M., Das, R., Orengo, C. a., & Kellam, P. (2001). Genomewide function conservation and phylogeny in the Herpesviridae [2]. *Genome Research*, *11*(1), 43–54. doi:10.1101/gr.149801

Arduino, P. G., & Porter, S. R. (2008). Herpes Simplex Virus Type 1 infection: overview on relevant clinico-pathological features. *Journal of Oral Pathology & Medicine : Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *37*(2), 107–21. doi:10.1111/j.1600-0714.2007.00586.x

Arvizo, R. R., Saha, S., Wang, E., Robertson, J. D., Bhattacharya, R., Mukherjee, P., … Analysis, A. (2013). Inhibition of tumor growth and metastasis by a self-therapeutic nanoparticle. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(17), 6700–5. doi:10.1073/pnas.1214547110

Boehmer, P. E., & Lehman, I. R. (1997). Herpes simplex virus DNA replication. *Annual Review of Biochemistry*, *66*, 347–84. doi:10.1146/annurev.biochem.66.1.347

CDC. (2013). HIV in the United States : At A Glance. Retrieved from http://www.cdc.gov/hiv/statistics/basics/ataglance.html

Centers for Disease Control and Prevention (CDC) (2010). Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years--United States, 2005-2008. *MMWR. Morbidity and mortality weekly report* 59.15 (2010): 456.

Corey, L. (2007). Herpes simplex virus type 2 and HIV-1: the dialogue between the 2 organisms continues. *The Journal of Infectious Diseases*, *195*(9), 1242–4. doi:10.1086/513570

Cunningham, A. L., Diefenbach, R. J., Miranda-Saksena, M., Bosnjak, L., Kim, M., Jones, C., & Douglas, M. W. (2006). The cycle of human herpes simplex virus infection: virus transport and immune control. *The Journal of Infectious Diseases*, *194 Suppl* , S11–S18. doi:10.1086/505359

Cunningham, A. L., Turner, R. R., Miller, A. C., Para, M. F., & Merigan, T. C. (1985). Evolution of recurrent herpes simplex lesions. An immunohistologic study. *Journal of Clinical Investigation*, *75*(1), 226–233. doi:10.1172/JCI111678

Davison, a J., & Wilkie, N. M. (1983). Location and orientation of homologous sequences in the genomes of five herpesviruses. *The Journal of General Virology*, *64 (Pt 9)*, 1927–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6310033

De Clercq, E. (2003). Clinical Potential of the Acyclic Nucleoside Phosphonates Cidofovir, Adefovir, and Tenofovir in Treatment of DNA Virus and Retrovirus Infections. *Clinical Microbiology Reviews*. doi:10.1128/CMR.16.4.569-596.2003

De Jong, M. a W. P., de Witte, L., Taylor, M. E., & Geijtenbeek, T. B. H. (2010). Herpes simplex virus type 2 enhances HIV-1 susceptibility by affecting Langerhans cell function. *Journal of Immunology (Baltimore, Md. : 1950)*, *185*(3), 1633–41. doi:10.4049/jimmunol.0904137

De Yang, Chen, Q., Schmidt, a P., Anderson, G. M., Wang, J. M., Wooters, J., … Chertov, O. (2000). LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *The Journal of Experimental Medicine*, *192*(7), 1069–74. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2193321&tool=pmcentrez&rendertype=abstract

Debra Quenelle, Tracy Hartman, Robert W. Buckheit, M., & Prichard, R. L. (2014). Anti-HSV activity of serpin antithrombin III. *Int Trends Immun*, *2*(2), 87–92. doi:10.1016/j.biotechadv.2011.08.021.Secreted

Dittmann, M., Hoffmann, H., Scull, M. a, Gilmore, R. H., Bell, K. L., Ciancanelli, M., … Rice, C. M. (2015). A Serpin Shapes the Extracellular Environment to Prevent Influenza A Virus Maturation. *Cell*, *160*(4), 631–643. doi:10.1016/j.cell.2015.01.040

Doller, A., Gauer, S., Sobkowiak, E., Geiger, H., Pfeilschifter, J., & Eberhardt, W. (2009). Angiotensin II induces renal plasminogen activator inhibitor-1 and cyclooxygenase-2 expression post-transcriptionally via activation of the mRNA-stabilizing factor human-antigen R. *The American Journal of Pathology*, *174*(4), 1252–1263. doi:10.2353/ajpath.2009.080652

Geiben-Lynn, R., Brown, N., Walker, B. D., & Luster, A. D. (2002). Purification of a modified form of bovine antithrombin III as an HIV-1 CD8+ T-cell antiviral factor. *Journal of Biological Chemistry*, *277*(44), 42352–42357. doi:10.1074/jbc.M207079200

Geijtenbeek, T. B. H., Kwon, D. S., Torensma, R., Vliet, S. J. Van, Duijnhoven, G. C. F. Van, Middel, J., … Kooyk, Y. Van. (2000). DC-SIGN , a Dendritic Cell – Specific HIV-1-Binding Protein that Enhances trans -Infection of T Cells, *100*, 587–597.

Gill, N., Davies, E. J., & Ashkar, A. a. (2008). The Role of Toll-Like Receptor Ligands/Agonists in Protection Against Genital HSV-2 Infection. *American Journal*

of Reproductive Immunology (New York, N.Y. : 1989), *59*(1), 35–43. doi:10.1111/j.1600-0897.2007.00558.x

Gnann, J. W., Barton, N. H., & Whitley, R. J. (1983). Acyclovir : Mechanism of Action , Pharmacokinetics , Safety and Clinical Applications.

Halford, W. P., Gebhardt, B. M., & Carr, D. J. (1997). Acyclovir blocks cytokine gene expression in trigeminal ganglia latently infected with herpes simplex virus type 1. *Virology*, *238*(1), 53–63. doi:10.1006/viro.1997.8806

Hardwicke, M. A., & Sandri-Goldin, R. M. (1994). The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. *Journal of Virology*, *68*(8), 4797–4810.

Heiligenhaus, A., Bauer, D., Zheng, M., Mrzyk, S., & Steuhl, K. P. (1999). CD4+ T-cell type 1 and type 2 cytokines in the HSV-1 infected cornea. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *237*(5), 399–406. doi:10.1007/s004170050251

Hendricks, R. L., Weber, P. C., Taylor, J. L., Koumbis, A., Tumpey, T. M., & Glorioso, J. C. (1991). Endogenously produced interferon alpha protects mice from herpes simplex virus type 1 corneal disease. *The Journal of General Virology*, *72 (Pt 7)*, 1601–1610.

Honess, R. W., & Roizman, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *Journal of Virology*, *14*(1), 8–19. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=355471&tool=pmcentrez&rendertype=abstract

Horvath, C. M. (2015). A Serpin Takes a Bite out of the Flu. *Cell Host & Microbe*, *17*(3), 283–284. doi:10.1016/j.chom.2015.02.018

Hu, J., Gardner, M. B., & Miller, C. J. (2000). Simian Immunodeficiency Virus Rapidly Penetrates the Cervicovaginal Mucosa after Intravaginal Inoculation and Infects Intraepithelial Dendritic Cells Simian Immunodeficiency Virus Rapidly Penetrates the Cervicovaginal Mucosa after Intravaginal Inoculation. *J. Virol.*, *74*(13), 6087–6095. doi:10.1128/JVI.74.13.6087-6095.2000.Updated

Izquierdo-Useros, N., Naranjo-Gómez, M., Erkizia, I., Puertas, M. C., Borràs, F. E., Blanco, J., & Martinez-Picado, J. (2010). HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? *PLoS Pathogens*, *6*(3), e1000740. doi:10.1371/journal.ppat.1000740

Joan K. Kreiss, Robert Coombs, Francis Plummer, King K. Holmes, Beverly Nikora, William Cameron, Elizabeth Ngugi, J. O. N. A. and L. C. (1989). Isolation of human immunodeficiency virus from genital ulcers in Nairobi prostitutes. *The Journal of Infectious Diseases*, *160*(3), 380–384.

Kalvakolanu, D. V. (1999). Virus interception of cytokine-regulated pathways. *Trends in Microbiology*, *7*(4), 166–171. doi:10.1016/S0966-842X(99)01476-6

Kawamura, T., Gulden, F. O., Sugaya, M., McNamara, D. T., Borris, D. L., Lederman, M. M., … Blauvelt, A. (2003). R5 HIV productively infects Langerhans cells, and infection levels are regulated by compound CCR5 polymorphisms. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(14), 8401–6. doi:10.1073/pnas.1432450100

Knipe, Howley, Griffin, Lamb, Martin, Roizman & Straus (2001) *Fields Virology*, 4th Edition.

Kunder, S. C., Kelly, K. M., & Morahan, P. S. (1993). Biological response modifier-mediated resistance to herpesvirus infections requires induction of alpha/beta interferon. *Antiviral Research*, *21*(2), 129–139.

Lagathu, C., Bastard, J. P., Auclair, M., Maachi, M., Kornprobst, M., Copeau, J., & Caron, M. (2004). Antiretroviral drugs with adverse effects on adipocyte lipid metabolism and survival alter the expression and secretion of proinflammatory cytokines and adiponectin in vitro. *Antiviral Therapy*, *9*(6), 911–920.

Mairuhu, a T. a, Setiati, T. E., Koraka, P., Hack, C. E., Leyte, a, Faradz, S. M. H., … van Gorp, E. C. M. (2005). Increased PAI-1 plasma levels and risk of death from dengue: no association with the 4G/5G promoter polymorphism. *Thrombosis Journal*, *3*, 17. doi:10.1186/1477-9560-3-17

Margolis, L. (2013). It takes more than two for this tango. *Cell Host & Microbe*, *13*(1), 1–3. doi:10.1016/j.chom.2013.01.002

Margulies, B. J., & Gibson, W. (2007). The chemokine receptor homologue encoded by US27 of human cytomegalovirus is heavily glycosylated and is present in infected human foreskin fibroblasts and enveloped virus particles. *Virus Research*, *123*(1), 57–71. doi:10.1016/j.virusres.2006.08.003

Marlin, S. D., Staunton, D. E., Springer, T. A., Stratowa, C., Sommergruber, W., & Merluzzi, V. J. (1990). A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature*, *344*(6261), 70–72.

Mendez-Samperio, P., Miranda, E., & Trejo, A. (2008). Expression and Secretion of Cathelicidin LL-37 in Human Epithelial Cells after Infection by Mycobacterium bovis Bacillus Calmette-Guerin. *Clinical and Vaccine Immunology*. doi:10.1128/CVI.00178-08

Mettenleiter, T. C. (2004). Budding events in herpesvirus morphogenesis. *Virus Research*. doi:10.1016/j.virusres.2004.08.013

Mikloska, Z., Danis, V. A., Adams, S., Lloyd, A. R., Adrian, D. L., & Cunningham, A. L. (1998). In vivo production of cytokines and beta (C-C) chemokines in human recurrent herpes simplex lesions--do herpes simplex virus-infected keratinocytes contribute to their production? *The Journal of Infectious Diseases*, *177*(4), 827–838.

Mikloska, Z., Kesson, A. M., Penfold, M. E., & Cunningham, A. L. (1996). Herpes simplex virus protein targets for CD4 and CD8 lymphocyte cytotoxicity in cultured epidermal keratinocytes treated with interferon-gamma. *The Journal of Infectious Diseases*, *173*(1), 7–17. doi:10.1093/infdis/173.1.7

Milligan, G. N., Dudley-McClain, K. L., Young, C. G., & Chu, C.-F. (2004). T-cell-mediated mechanisms involved in resolution of genital herpes simplex virus type 2 (HSV-2) infection of mice. *Journal of Reproductive Immunology*, *61*(2), 115–127. doi:10.1016/j.jri.2003.12.002

Moomaw, M. D., Cornea, P., Rathbun, R. C., & Wendel, K. a. (2003). Review of antiviral therapy for herpes labialis, genital herpes and herpes zoster. *Expert Review of Anti-Infective Therapy*, *1*(2), 283–95. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15482124

Munk, R. B., Sugiyama, K., Ghosh, P., Sasaki, C. Y., Rezanka, L., Banerjee, K., … Longo, D. L. (2011). Antigen-independent IFN-γ production by human naïve CD4+ T cells activated by IL-12 plus IL-18. *PLoS ONE*, *6*(5). doi:10.1371/journal.pone.0018553

Muth, M., Hussein, K., Jacobi, C., Kreipe, H., & Bock, O. (2011). Hypoxia-induced down-regulation of microRNA-449a/b impairs control over targeted SERPINE1 (PAI-1) mRNA - a mechanism involved in SERPINE1 (PAI-1) overexpression. *Journal of Translational Medicine*, *9*, 24. doi:10.1186/1479-5876-9-24

Paul Cameron, Peter Freudenthal, Jeanne Barker, Stuart Gezelter, Kayo Inaba, R. S. (1992). Dendritic Cells Exposed to Human Immunodeficiency Virus Type-1 Transmit a Vigorous Cytopathic Infection to CD4+ T Cells. *Science*, *257*(No. 5068), 383–387.

Pralong, G., Calandra, T., Glauser, M. P., Schellekens, J., Verhoef, J., Bachmann, F., & Kruithof, E. K. (1989). Plasminogen activator inhibitor 1: a new prognostic marker in septic shock. *Thromb Haemost*, *61*(3), 459–462.

Reddy, N., Hernandez-Ilizaliturri, F. J., Deeb, G., Roth, M., Vaughn, M., Knight, J., … Czuczman, M. S. (2008). Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in vivo. *British Journal of Haematology*, *140*(1), 36–45. doi:10.1111/j.1365-2141.2007.06841.x

Schacker, T., Ryncarz, a J., Goddard, J., Diem, K., Shaughnessy, M., & Corey, L. (1998). Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1-infected men. *JAMA : The Journal of the American Medical Association*, *280*(1), 61–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9660365

Schoenborn, J. R., & Wilson, C. B. (2007). Regulation of interferon-gamma during innate and adaptive immune responses. *Advances in Immunology*, *96*, 41–101. doi:10.1016/S0065-2776(07)96002-2

Shanda, S. K., & Wilson, D. W. (2008). UL36p is required for efficient transport of membrane-associated herpes simplex virus type 1 along microtubules. *Journal of Virology*, *82*(15), 7388–7394. doi:10.1128/JVI.00225-08

Shao, Y., Zhang, W., Dong, X., Liu, W., Zhang, C. H., Zhang, J., … Yu, B. (2010). Keratinocytes play a role in the immunity to herpes simplex virus type 2 infection. *Acta Virologica*, *54*(4), 261–267. doi:10.4149/av_2010_04_261

Shapiro, S., Miller, A., Lahat, N., Sobel, E., & Lerner, A. (2003). Expression of matrix metalloproteinases, sICAM-1 and IL-8 in CSF from children with meningitis. *Journal of the Neurological Sciences*, *206*(1), 43–48. doi:10.1016/S0022-510X(02)00317-9

Smith, T. J., Morrison, L. A., & Leib, D. A. (2002). Pathogenesis of herpes simplex virus type 2 virion host shutoff (vhs) mutants. *Journal of Virology*, *76*(5), 2054–2061. doi:10.1128/jvi.76.5.2054-2061.2002

Sugaya, M., Loré, K., Koup, R. a, Douek, D. C., & Blauvelt, A. (2004). HIV-infected Langerhans cells preferentially transmit virus to proliferating autologous CD4+ memory T cells located within Langerhans cell-T cell clusters. *Journal of Immunology (Baltimore, Md. : 1950)*, *172*(4), 2219–24. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14764689

Tan, D. H.-S., Murphy, K., Shah, P., & Walmsley, S. L. (2013). Herpes simplex virus type 2 and HIV disease progression: a systematic review of observational studies. *BMC Infectious Diseases*, *13*(1), 502. doi:10.1186/1471-2334-13-502

Tigges, M. A., Johnson, C., & Burke, R. L. (1996). Human herpes simplex virus (HSV)-specific CD8+ CTL clones recognize HSV-2-infected fibroblasts after treatment with IFN-gamma or when virion host shutoff functions are disabled. *The Journal of Immunology*, *156 no. 10*(13), 3901–3910.

Toma, H. S., Murina, A. T., Areaux, R. G., Neumann, D. M., Bhattacharjee, P. S., Foster, T. P., … Hill, J. M. (2008). Ocular HSV-1 latency, reactivation and recurrent disease. *Seminars in Ophthalmology*, *23*(4), 249–73. doi:10.1080/08820530802111085

Van Kooyk, Y., & Geijtenbeek, T. B. H. (2003). DC-SIGN: escape mechanism for pathogens. *Nature Reviews. Immunology*, *3*(9), 697–709. doi:10.1038/nri1182

Walker, G. M., Ozers, M. S., & Beebe, D. J. (2004). Cell infection within a microfluidic device using virus gradients. *Sensors and Actuators, B: Chemical*, *98*(2-3), 347–355. doi:10.1016/j.snb.2003.10.014

Whiteman, S. C., Bianco, A., Knight, R. A., & Spiteri, M. A. (2003). Human rhinovirus selectively modulates membranous and soluble forms of its intercellular adhesion molecule-1 (ICAM-1) receptor to promote epithelial cell infectivity. *Journal of Biological Chemistry*, *278*(14), 11954–11961. doi:10.1074/jbc.M205329200

Whitney, J. B., Asmal, M., & Geiben-Lynn, R. (2011). Serpin induced antiviral activity of prostaglandin synthetase-2 against HIV-1 replication. *PLoS ONE*, *6*(4), 1–9. doi:10.1371/journal.pone.0018589

WHO. (2013). AIDS Global Statistics WHO. Retrieved from http://aids.gov/hiv-aids-basics/hiv-aids-101/global-statistics/

Witkowska, A. M., & Borawska, M. H. (2004). Soluble intercellular adhesion molecule-1 (sICAM-1): An overview. *European Cytokine Network*.

Wood, Alastair JJ, Whitley, Richard J., and J. W. G. J. (1992). Acyclovir: A Decade Later. *The New England Journal of Medicine*, *327*(11), 782–789.

Yamamoto, T., Osaki, T., Yoneda, K., & Ueta, E. (1993). Immunological investigation of adult patients with primary herpes simplex virus-1 infection. *Journal of Oral Pathology & Medicine*, *22*(6), 263–267.

Yang, Y., Zhang, Z.-X., Lian, D., Haig, A., Bhattacharjee, R. N., & Jevnikar, A. M. (2015). IL-37 inhibits IL-18-induced tubular epithelial cell expression of pro-inflammatory cytokines and renal ischemia-reperfusion injury. *Kidney International*, *87*(2), 396–408. doi:10.1038/ki.2014.29

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EDUCATION

August 2015 **Towson University**, Towson, MD
M.S. in Biological Sciences
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April 2013 **Brigham Young University,** Provo, UT
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TEACHING EXPERIENCE

08/2014-05/2015 **Towson University Biological Sciences Department**, Towson, MD
Flipped Format Co-Instructor: Led several lectures, developed learning activates, and instructed up to 75 students one-on-one in flipped format

08/2013-05/2015 **Towson University Biological Sciences Department**, Towson, MD
Teaching Assistant: Taught lab for "Introduction to Biology for Health Professions," taught several lectures, conducted review sessions, and constructed quizzes and novel material for classes of 75 students

01/2014-09/2014 **Towson University Science Education Department**, Towson, MD
Graduate Research Assistant: Researched student responses to teaching techniques for a rigorous introductory biology course with a high withdraw/ fail rate; presented research at teaching conference

04/2011-06/2011 **Brigham Young University Physiology and Developmental Biology Department**, Provo, UT
Teaching Assistant: Taught 200 students one-on-one and in review sessions; also created supplementary practice questions to enhance student learning

RESEARCH EXPERIENCE:

08/2013-08/2015 **Towson University Biological Sciences Department**, Towson, MD
Graduate Researcher (Thesis): Investigated cytokine expression in response to infection with herpes virus type 2 *in vitro*

01/2013-04/2013 **Brigham Young University Neuroscience Department**, Provo, UT
Undergraduate Researcher: Studied the effects of dopaminergic drugs on ventral tegmental area of the brain for addiction therapy applications

07/2011-11/2011 **Brigham Young University Anthropology Department**, Visakhapatnam, AP India
Undergraduate Researcher: Characterized perceptions and treatments of chronic pain in Southern Indian subpopulation; wrote a 30-page research paper entitled *Management of Chronic Pain in a More Adaptive Culture*, and wrote a grant for $1500 to fund research

WORK EXPERIENCE

01/2012-04/2013 **Brigham Young University Health Center**, Provo, UT
Physical Therapist Assistant: Instructed 40 patients per week on therapeutic exercises, and created and managed clinic's schedule and billing transactions

05/2012-08/2012 **San Jacinto Chiropractic**, Baytown, TX
Chiropractic Assistant: Treated 100 patients per week for muscle injuries and created a new scheduling procedure to improve efficiency

05/2003-08/2010 **Wylie Eye and Dental**, Glen Dale, WV
Dental Assistant: Treated 40 patients per week, assisted doctor in dental procedures (including oral surgery), took x-rays on patients in need of emergency care, performed EKGs on surgery patients, and taught patients how to improve oral hygiene

CONFERENCE PARTICIPATION

Hickey J., Margulies B. Cytokine Profile in Response to HSV-2 Infection *In Vitro*. *2015 Undergraduate and Graduate Research and Performance Expo.* Towson, MD. April 2015.
Hickey J., Margulies B. Cytokine Profile in Response to HSV-2 Infection *In Vitro*. *Host Pathogen Interactions in Biodefense and Emerging Infectious Diseases.* Manassas, VA. February 2015.
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RELEVANT COURSEWORK:

Graduate
Curriculum and Assessment
Immunology
Virology
Biotechnology
Cellular Signaling
Gene Regulation and Expression
Data Analysis and Interpretations for Biologists (Biostatistics)

Undergraduate
Anatomy with Lab
Molecular Biology with Lab
Advanced Physiology with Lab
Cell Biology
Genetics
General Microbiology
Biochemistry
General Chemistry I & II with Lab
Organic Chemistry I & II
General Statistics

TECHNICAL SKILLS:

Extensive Experience
Cloning, ELISA, Mammalian Cell Culture, Bacterial Culture, Plaque Assay, Gel Electrophoresis, PCR, RT-PCR, qPCR, Light Microscope, DNA/ RNA Miniprep, Aseptic Technique, Differential/ Selective Media Preparation, Buffer Preparation, Pipette/Micropipette Skills, Microsoft Office

Familiar Experience
Blackboard, JMP (Statistical Software), Animal-Handling