I, Anjelika Gasilina, hereby submit this original work as part of the requirements for the degree of Master of Science in Immunology.

It is entitled:
Herpes Simplex Virus-1: Crosstalk Between the Host Immunity and the Virus during Infection, Latency and Reanimation

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Herpes Simplex Virus-1: 
Crosstalk Between the Host Immunity and the Virus During Infection, Latency and Reanimation

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Abstract

Herpes Simplex Viruses 1 and 2 (HSV-1 and -2) are ubiquitous, contagious human pathogens, with estimated 776,000 new infections in the USA yearly. Majority of primary infections occur in early childhood during delivery or through transmission by parent/guardian and it is estimated that 90% of the world population is seropositive for HSV-1 by the age of 65. Primary infection presents as cold sores and is self-contained in immune-competent host. HSV-1 has ability to evade immune clearance and reside undetected in the host by establishing latency in the trigeminal ganglia. Recurrent, sporadic episodes of viral reactivation are mostly mild, but in immune-compromised individuals may cause encephalitis and keratitis. Untreated viral encephalitis has a 70% mortality rate. Early intervention with antiretroviral therapy improves the outcome, but leaves over 60% of patients with lifelong neurological morbidities. Keratitis, induced by HSV-1, is the leading cause of virally induced blindness. This review summarizes viral structure, mode of infection, and clinical presentation, as well as the role of immune system and gene remodeling in the pathogenesis of HSV-1.
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LIST OF ABBREVIATIONS

HSV-1/-2  Herpes Simplex Virus 1/2
VZV      Varicella Zoster Virus
JNK      c-Jun N-terminal Kinase
EC50     Half maximal effective concentration
TNF      Tumor Necrosis Factor
IE       Immediate early genes
L        Late genes
E        Early genes
LAT      Latency Associated Transcript
HDAC     Histone Deacetylase
HVEM     Herpesvirus Entry Mediator
PAMPs    Pathogen-associated Molecular Patterns
TLR      Toll-like Receptor
iPSC     Induced Pluripotent Stem Cells
IRF      Interferon Regulatory Factor
USP      Ubiquitin-specific Protease
ICP      Infected Cell Protein
DC       Dendritic Cell
NK cell  Natural Killer Cell
MΦ       Macrophage
IFN      Interferon
<table>
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<tr>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>HCF1</td>
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<td>Oct-1</td>
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1. Introduction

Overview and Clinical Manifestations

Herpes simplex virus 1 (HSV-1) is an enveloped, double-stranded DNA virus of alphaherpesvirus subgroup in the *Herpesviridae* family of viruses [1]. As the two other members of this subgroup HSV-2 (herpes simplex virus 2) and VZV (varicella zoster virus), HSV-1 has two stages of infection – an actively replicating cell-lytic stage and a minimally replicating non-lytic latent stage. The Latent stage of the virus is established for the lifetime of the host. Virion structure of HSV-1 consists of capsid, protecting the genome, tegument space, and envelope, consisting of lipid bilayers, polyamines and glycoproteins (Fig.1).

![Virion Structure of HSV-1](image)

**Figure 1. Virion Structure of HSV-1.** Like all herpesviruses, HSV-1 is *enveloped* and consists of an icosahedral *capsid*, protecting the viral *dsDNA* genome, surrounded by *tegument* space. Tegument proteins are required for initial viral replication in the host nucleus. *Glycoproteins*, required for recognition and initiation of infection, are expressed on the surface of the *envelope*. 
HSV-1 is strictly a human pathogen, with estimated 776,000 new infections yearly in the U.S. alone [2]. The majority of primary HSV-1 infections occur early in childhood by transmission of shed viral particles by parent/guardian [3]. Primary infection most commonly presents clinically as cold sores around the mouth area (in case of oral-oral or oral-genital transmission) or sores in genital area (after oral-genital transmission); both cases are confined to a single area. Viral infection of neonates occurs most commonly during vaginal delivery and may be disseminated across multiple areas of the body, including skin and eyes. Clinical presentation at reactivation from latency is usually confined to area of primary infection and is self-limiting [4], but sometimes presents as herpetic keratitis and sporadic encephalitis. Keratitis is the leading cause of virally induced blindness worldwide [5], and epidemiological studies estimate that 1.5 million people worldwide live with HSV-induced keratitis [6]. Sporadic encephalitis is a dangerous condition, with a 70% mortality rate if left untreated. It presents most often in neonates born to mothers with actively replicating virus, but may also occur in children and adolescents [7], [8]. Although current laboratory testing implements sensitive PCR and ELISA technology, thereby aiding medical professionals at providing diagnosis at much earlier timeframe, even early administration of antiretroviral therapy with Acyclovir (viral thymidine kinase inhibitor) leaves 60% of patients with significant neurological morbidities [9]. Moreover, in an immunocompromised host, viral replication is sustained over a prolonged period of time and recurrent infections are more frequent, leading to occurrence of acyclovir-resistant clones. Specifically, in HIV-positive individuals, prevalence of acyclovir resistance ranges from 3.5% to 7%, while in patients undergoing solid organ transplant or bone
marrow transplant, the rate of acyclovir resistance can be as high as 10% [10]. Although these outbreaks are rarely lethal, they greatly reduce patients’ quality of life. No advances in the development of antiherpetic therapeutic agents have been made since the discovery of acyclovir in 1977. Its successor with a greater oral bioavailability, valacyclovir, was introduced in the early 1980’s. Emergence of resistant strains and lack of alternative treatment options calls for discovery and development of new antitherpetics, preferably with a mechanism of action independent of the thymidine kinase inhibition, and several candidates are being evaluated for efficacy in clinical trials [11]. These potential treatment options, however, are targeted towards an active infection. Very little success has been made to identify therapeutics that would prevent viral infection or reactivation. There is a great void in understanding of the mechanism of viral latency and subsequent reactivation. Thus, in order to successfully develop novel antiviral therapies, it is instrumental to define and describe key virus – host protein interactions and molecular mechanisms behind them.

Most recent attempts in the development of novel antiviral agents that aim at preventing infection by HSV-1 produced harmine, a plant-derived molecule with potent psychedelic effects. In their paper, Chen and colleagues described potent, low-micromolar-range anti-viral effects of harmine on HSV-2 infected cells in culture. Relying on previous reports that harmine may exert its effects by perturbing the oxidative balance in the cells, the authors attempt to decipher herpes-virus specific mechanism of action of this molecule. The authors attempt to link HSV-mediated upregulation of JNK and p38 phosphorylation and their subsequent dephosphorylation induced by harmine. Interestingly, levels of harmine that were administered to show loss
of p38 and JNK phosphorylation were 20-40uM, almost 10 times the EC50 of 1.47 uM needed to abrogate viral replication. Further inquiry is certainly needed in order to establish if harmine indeed has herpes virus-specific molecular targets [12].

**Primary Infection and Escape to Latency**

During acute infection, glycoproteins gC and gB, expressed on the surface of viral envelope, bind to glycosaminoglycans on the surface of host epithelial cells (summarized in Fig 2A, pictorial representation in Fig. 2B). Next, glycoprotein gD interacts with a host receptor, which results in a conformational change of gD [13]. To date, three unrelated receptors have been shown to interact with gD – nectin-1 and nectin-2, members of the immunoglobulin superfamily, herpesvirus entry mediator (HVEM), member of tumor necrosis factor (TNF) receptor family, and 3-O-sulfated heparin sulfate glycosaminoglycan receptor [14], [15]. Trimeric complex of gD with gL and gH creates a fusion port and facilitates virion’s entry into the cell. Once inside the host cell, tegument proteins and nucleocapsid use microtubule-mediated transport to travel to and enter the nucleus through the nuclear pores. Tegument protein VP16 collaborates and forms protein-DNA complex with transcription factor Oct-1 (Octamer-binding protein 1) and transcriptional co-regulator HCF-1 (host cell factor 1) to initiate transcription of immediate early (IE) viral genes [16]. Immediate early genes activate transcription of early (E) genes, which are involved in viral genome replication and feedback suppression of immediate early genes. After viral genome is transcribed, expression of late (L) genes, which are involved in assembly of daughter virions, is initiated. Packaging of genome into the capsid and capsid assembly happens in the
nucleus, while envelope assembly and maturation of virion takes place in the cytoplasm. Release of newly formed viral particles is lytic to the host cell. Active viral replication eventually ceases and some viral particles move into the cell bodies of sensory ganglia and establish latent infection (Figure 2). A recent study shed light on how alphaherpesviruses enter neurons to establish latency. Although a key protein, glycoprotein K, was hypothesized to be responsible for this phenomenon - as this protein is not conserved in the beta- or gammaherpesviruses, which also incidentally cannot establish latency - the exact mechanism behind glycoprotein K interaction with the host neuron was not known. Deletion of amino acids 31-68, which are predicted to form a β-sheet in an in silico-constructed three-dimensional model, prevents viral entry into neuronal axons. It is not known whether this deletion prevents interaction with host cell receptors, but evidence that a single glycoprotein is responsible for successful entry of virus into the neuron could be used as a launching point to create vaccines against alphaherpes viruses [17].
The ability of HSV-1 to establish a latent infection is thought to be an evolutionary phenomenon, which allows for maintenance of viral genome in humans, this virus’ only natural reservoir. During the latency stage, viral DNA remains circular and episomal, lytic gene expression does not take place, and only latency-associated transcripts (LATs) [18], as well as a set of RNAs and miRNAs, are expressed in order to maintain viral genome [19], [20]. For reasons poorly understood, but associated with UV radiation [21], stress [22], and weakened immune system of the host, periodically HSV-1 can exit the latent stage and enter active lytic stage [23]–[25]. To date, there is no common denominator described delineating the cause of reanimation from latency.

**Figure 2. Cascade of Events during Primary Infection.** Virus particle binds to the surface of the epithelial cells via viral glycoprotein – host cell receptor recognition. Trimeric glycoprotein complex mediates viral entry into the host cell. Once inside the cell, envelope disassembles, releasing genome-containing capsid and tegument. Capsid and tegument use microtubule-mediated transport to move into the nucleus and initiate viral replication and assembly of daughter capsid. New viral particles exit the host cell through cell lysis and disseminate to infect neighboring cells. Some virions retrograde to infect sensory neurons and establish latency.
Due to the requirement to persist as a lifelong infection in the host, HSV-1 evolved to employ intricate strategies to modulate both viral and host gene expression and thus evade host immune response. HSV-1 genome expresses a number of factors that derail immune response during primary infection. During latency, virus is able to interact with chromatin and modulate gene expression processes that guarantee evasion from immune surveillance. In fact, much of newly published primary literature in the field of HSV-1 research has focused on deciphering the identity and role of key viral and human host epigenetic regulators that are involved in the interplay between latency and reanimation.

**Tools and Animal Models**

Cell culture methods, although devoid of mimicking physiological conditions and contribution of whole organism, provide a controlled and relatively inexpensive environment, in which gene expression during initial infection can be studied. The latent stage cannot be reproduced, except when cells are pre-treated with inhibitors that block active viral replication [26], [27].

Although HSV-1 is a human pathogen, it nonetheless can infect laboratory animals, such as mice and rabbits, which are most widely used *in vivo* models. Murine infection with HSV-1 results in migration of the virus and establishment of latency as in the human host and maintenance of the colony is inexpensive. Ganglia, isolated from infected mice, can be used to study reactivation *in vitro*, but this method assumes no contribution from neighboring tissue and cells. This model has a major drawback as no spontaneous viral shedding occurs after reanimation and reanimation has to be
induced. Induction of reanimation is produced by placing mice into a warm bath, but reproducibility of this method is variable [28], [29]. There is a certain degree of discrepancy depending on the mouse strain used and mode of infection, but this murine model is nonetheless useful to study persistent latent state. The rabbit infection model most closely mimics human infection and is most commonly used to study viral reanimation and ocular disease, but can be cost prohibitive [30]. Moreover, in the area of epigenetics, evolutionary divergence among players involved in epigenetic regulation between different types of laboratory animals may lead to different outcomes, which may not be useful in the context of human disease.
2. Host Immune Response

Recognition of viral glycoproteins and viral DNA by the Innate Immune System

Immediate response by the innate immune system to HSV-1 infection is similar to response against other viral pathogens (Fig 3). Toll-like Receptors (TLRs) expressed on the surface of epithelial cells can recognize pathogen-associated molecular patterns (PAMPs), such as the viral proteins and viral DNA. Glycoproteins binding to the cell surface are recognized by TLR1 and TLR2, although not all strains of HSV-1 are detected by TLR2 [31]. Once the viral particle fuses with the host cell and capsid is released, viral genome DNA is recognized by TLR9, most likely due to abundance of CpG islands in herpesvirus DNA [32]. Activation of TLR9 induces expression of IRF7 (Interferon Regulatory Factor 7), which turns on production of type I interferon, but TLR9-mediated response is only confined to infection of dendritic cells [33]. TLR3, another DNA sensing receptor, induces activation of IRF3 and production of type I interferons. The role of TLR3 in containing the viral replication appears to be non-redundant with other DNA-sensing TLRs, as children with impaired function or deletion of TLR3 have been shown to be more susceptible to herpes-induced encephalitis [34]–[37]. Moreover, mouse models and in vitro studies on patient-derived induced pluripotent stem cells (iPSCs) have shown that TLR3 is required for protection against HSV-1 specifically in the central nervous system [38]–[41].

Infection of epithelial cells and active viral replication causes production and release of type I interferons, which in turn activate macrophages and Natural Killer (NK) cells. NK cells are important in clearing infected cells by inducing apoptosis, although absence of NK cells did not affect viral titer or viral spread. IFN type I also activates
dendritic cells (DC), and in case of HSV-1 infected DCs, these secrete IL-12 to activate bystander DCs [42]. Moreover, dendritic cells play an indispensable role in boosting activation of NK cells and their IFN-γ production [43]. Other cytokines, such as TNF-α, also induce activation of NK cells and induce secretion of IFN-γ, but it had no effect on NK cell degranulation [44]. Dendritic cells and macrophages act as antigen presenting cells, processing the viral proteins and presenting the peptides to activate players of the adaptive immune system. Macrophages have been shown to limit viral replication at the initial site of infection and reduce eyelid inflammation in a mouse model of HSV-1 ocular infection [45]. The majority of innate immune response unleashed against HSV-1 is orchestrated by interferon release, therefore, the HSV has evolved to express Infected Cell Proteins (ICPs), ICP0, ICP27, and ICP34.5, that specifically target production of type I interferon [3], [46]. ICP0 is a RING domain E3 ligase and its ligase activity is directly and indirectly responsible for proteasome-mediated degradation of cellular host proteins immediately following the infection. ICP0 protects itself from degradation by interacting with ubiquitin-specific protease USP7 and this interaction leads to stabilization of ICP0 in infected cells [47], [48]. Ablation of USP7 expression by shRNA leads to poor propagation of virus in vitro. However, the exact mechanism of interaction between these two proteins has not been mapped until very recently. A biochemical inquiry into the interaction using isothermal titration calorimetry and N\textsuperscript{15}NMR using an ICP0 peptide and single or tandem UBL domains of USP7 showed that USP7 binds to CIP0 via its first two C-terminal UBL domains. The binding most likely maps to the charged pockets on domains, given highly positively and negatively charged nature of the peptide [49].
Adaptive Immunity

Major players of adaptive immune system response are T cells. Cytotoxic CD8+ T cells subset is the primary T cell population responsible for clearing the virus. Depending on mouse strain used, NK T cells are either dispensable [50] or crucial in controlling virus dissemination [51]. Studies have shown that cytotoxic T cells are capable of clearing the virus from infected epithelial cells, however they fail to clear the virus in neurons, most likely due to immune-privileged status of these cells and, therefore, low MHC I expression on their surface. Another important aspect is that the virus uses neurons as gateway to immortality within its host, and there may be key factors in play that down-regulate gene expression or shut off “Eat me” signals from being sent out by infected sensory ganglia. Tissue resident memory T cells have been detected in skin and latently infected ganglia in animal models and these may be important in suppression of reactivated virus [52], [53], but due to the model limitation, this effect may not occur in humans.

Interestingly, primary role of B cells in HSV-1 infection is not to produce neutralizing antibodies, as restoration of antibody failed to restore viral resistance in B-cell-deficient mice, but to act as antigen-presenting cells to secrete cytokines and aid in T-cell stimulation [54].
Figure 3. Immune System Response against HSV-1 Infection. Glycoproteins expressed on the surface of the virion are recognized by TLR1 and TLR2 (Toll-like Receptors), while intracellular viral DNA is sensed by TLR3 and TLR9. This leads to induction of IRF3 & 7 (Interferon-Regulatory Factors), and subsequently, production of type I interferon (IFN I). IFN I signaling activates cells of the innate and adaptive immune system – CD8+ T cells, Natural Killer (NK) cells, dendritic cells (DCs) and macrophages (MΦ). Activated cells of the immune system release cytokines (IL-6, IL-12, IFNγ, TNF-α) and granzymes to clear the infection.
**Adult vs. Neonatal Infection**

Differences in immune responses between adult and neonatal infections have not been studied extensively, but since both clinical presentation and severity of HSV-1 infection is different between these groups, it is plausible to hypothesize that response by the immune system is also different [55]. Indeed, neonatal neutrophils are more susceptible to apoptotic signals by the virus than adult neutrophils. Although the exact mechanism of apoptotic effect is yet to be determined, this phenomenon occurs 6 to 20 hours post infection and can be blocked by antiretroviral Acyclovir treatment (see Overview), suggesting requirement for actively replicating virus and, possibly, presence of early and late genes [56]. Adaptive T cell response is also diminished in neonatal infections, most likely due to weak cytokine production and stimulation of T cells [57].
3. Latency

Maintaining Lifelong infection and Natural Host: Sensory Neurons as a Reservoir

As mentioned previously, some newly formed viral particles retrograde into sensory neurons to establish latent infection. The pool of latent virus presence is restricted to sensory ganglia, which have projections in the mandibular/maxillary zone. This explains why, when reanimated from latency, the virus establishes sores and lesions at the original site of infection. It is however, unknown why latent infection is restricted to sensory neurons, but it has been hypothesized that factors necessary for a strong immune response are absent in these cells, as these cells may be considered immune-privileged and that it is advantageous for virus to maintain its genome in an environment that does not allow or does not have necessary machinery to produce an acute viral replication. It has also been hypothesized that key factors used for genome transcription, such as HCF-1 and VP16 (see Primary Infection), are not present or localized in the nucleus, and thus active replication of virus is not possible [16] and recent evidence suggests that IFI16 (IFN-γ inducible factor 16) interferes with HSV-1 ability to associate with Oct-1 [58]. It remains to be established if there is any communication between infected neurons, and if there are factors that determine which neurons are to reactivate viral replication and which are to maintain the virus in dormant state.

Gene Remodeling: Histone Modifications and Epigenetic Interplay

HSV-1 does not contain histones in its virions and must use host histone modification machinery. It has been shown to associate with histones in vivo upon establishing quiescent latent stage. Various studies noted viral reanimation upon
histone deacetylase (HDAC) inhibition [59], [60]. There is now ample evidence that chromatin modulation plays an important role in repression and reactivation of viral gene expression [30], [61]–[66]. Histones can be post-translationally modified to yield transcriptionally permissive or transcriptionally restrictive states. Due to recent improvements in technology, quantitative ChIP served as an invaluable tool to decipher post-translational modifications of histones on the HSV genome. H3K9me3 and H3K27md3, associated with gene repression have been shown to be present on a viral LAT promoter as well as promoters of immediate early and early genes [67]. On the other hand, methylation marks that are associated with gene permission are not abundant [68]. Much of ongoing research on HSV-1 latency and reanimation has concentrated on the epigenetic plasticity and virus-host crosstalk (reviewed in [28], [30], [66], [68], [69]). Further studies on HSV-1 induced regulation of chromatin and host cell feedback are much needed and will provide an explanation of events leading to reanimation of latent virus and subsequent clinical manifestations. What are the key elements that lead to reanimation? Are there genetic variations that are responsible for such disparity? Genome-Wide Association Studies (GWAS) would be helpful in discovering genetic variations that may predispose individuals to more severe progression and outcome of HSV-1 infection.
4. Conclusions

Some individuals never experience a full episode of HSV reanimation, while others suffer recurrent outbreaks. Considering that 90% of human population is seropositive for HSV-1 by the age of 65 [70], most will have mild, contained clinical presentation and for some an outbreak will be lethal and debilitating. Studies, involving discovery of variable gene expression and regulation during primary infection, escape to latency and reanimation, as well as epigenetic control of viral replication, should shed light on key gene expression and signaling pathways and provide possible targets that could be used in therapeutic intervention of HSV-1 infections.
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