Isolation and elimination of Latent and Productive Herpes Simplex Virus from the Sacral and Trigeminal Ganglions

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There is an immediate need for alternative anti-herpetic treatment options effective for both primary infections and reoccurring reactivations of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2). Existing options include antivirals that have been approved for clinical administration and a limited number of nucleoside analogues. The present article tests a new treatment based on a systemic understanding of how the herpes virus affects cell inhibition and breakdown, and targets different phases of the viral cycle, including the entry stage, reproductive cross mutation, and cell-to-cell infection. The treatment consisted of five immunotherapeutic core compounds (5CC), which were hypothesized to be capable of neutralizing human monoclonal antibodies. These 5CC are effective inhibitors of herpes viral DNA synthesis and interferon (IFN)-induced cellular antiviral response, and they were here found to neutralize antiviral reproduction by blocking cell-to-cell infection. Antiviral activity of the 5CC against HSV-1 and HSV-2 was tested on RC-37 cells in vitro using a plaque reduction assay. The 50% inhibitory concentration (IC50) of 5CC was 0.0009% for HSV-1 plaque formation and 0.0008% for HSV-2 plaque formation. Further tests comprising of a phenotypic assay, PEA, were performed to evaluate the susceptibility of HSV-1 and HSV-2 to antiherpetic drugs in Vero cells after virus entry. Indicators of the 5CC found that the combination exhibited high levels of virucidal activity against HSV-1 and HSV-2 in viral suspension. These concentrations of the 5CC are nontoxic and reduced plaque formation by 98.2% for HSV-1 and 93.0% for HSV-2. Virus HSV-1 and HSV-2 titers were reduced significantly by 5CC to the point of being negative, ranging 0.01–0.09 in 72%. These results suggest that the 5CC are strong alternative candidates for treating herpes simplex.

Alpha herpesviruses are common pathogens of mammals. They establish a productive infection in many cell types, but a life-long latent infection occurs in PNS neurons. A vast majority of the human population has latent HSV-1 infections. Currently, there is no cure to clear latent infections. HSV-1 and HSV-2 appear to share all or most features of their replication; however, HSV-1 is the prototype and best-studied representative of the alpha herpesvirus group, and its replication will serve as a model for both. HSV-1 is neurotropic and establishes latent infections in sensory and autonomic neurons. It is characterized by an extremely rapid productive replication cycle compared to many other types of herpesviruses as well as smaller nucleus-replicating DNA viruses such as adenoviruses and papovaviruses. Furthermore, both types of HSV are able to replicate in a wide selection of animals, tissues, and cultured cells. The virion contains two important host-modifying proteins: the a-trans-inducing factor (a-TIF; also known as VP16, VMW65, UL48, or the virionstimulatory protein and UL41 (virion host shutoff protein); the functions of these two proteins are discussed briefly below. Productive infection. The specifics of productive infection of a cell by HSV have been established in cultured cells. Although there may be specific and/or minor differences in the process in differentiated neuronal cells in vivo, no obvious differences are seen in infections in cultured neuronal cell lines. Replication involves a number of stages representing different levels of viral gene expression and interaction of viral gene products with host machinery—this process has been recently reviewed in some detail. Virus entry requires sequential interaction between specific viral membrane glycoproteins and cellular receptors; upon entry, the nucleocapsid is transported to the nuclear pores, where viral DNA is released into the nucleus. The viral genome is accompanied by the a-TIF protein, which functions in enhancing immediate-early viral transcription via cellular transcription factors. UL41 appears to remain in the cytoplasm, where it causes the disaggregation of polyribosomes and degradation of cellular and viral RNA. Five HSV genes (a4 [ICP4], a0 [ICP0], a27 [ICP27/UL54], a22 [ICP22/US1], and a47 [ICP47/US12]) are expressed and function in the earliest stages of the productive infection cycle.

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This stage of infection is termed the immediate-early or a phase of gene expression and is mediated by the action of a-TIF through its interaction with cellular transcription factors at specific enhancer elements associated with the individual transcript promoters. In the absence of virus-encoded protein synthesis, only a transcripts are expressed. Since promoters controlling the expression of all kinetic classes of HSV transcripts have features of cellular promoters and can be expressed by unmodified cellular transcription systems, the restriction of viral transcription in the absence of virus-induced protein synthesis is, in itself, sufficient to imply that the nature of the viral genome as a transcription template plays a critical role in subsequent viral gene expression. Proteins encoded by the a4, a0, and a27 transcripts play clear roles in the regulation of viral gene expression at the level of transcription or, at least, mRNA expression. They functionally interact to form nuclear complexes with viral genomes (183, 217, 328), and the role of these interactions in the global aspects of HSV transcription is a question of critical interest. Surprisingly, only two (a4 and a27) have extensive areas of sequence similarity among a large number of alpha herpesviruses, and only amino acid sequences in a27 appear to be extensively conserved among the more distantly related beta and gamma herpesviruses. Less is known about the function of the two other a proteins, a22 and a47. Both are dispensable for virus replication in many types of cultured cells, but a22 is required for HSV replication in others and may play a role in maintaining the ability of the virus to replicate in a broad range of cells in the host—perhaps by mediating the expression of a set of late transcripts. The a47 protein has recently been inferred to play a role in modulating host response to infection by specifically interfering with the presentation of viral antigens on the surface of infected cells by MHC-I (292, 323). Activation of the host cell transcriptional machinery by the action of a gene products results in the expression of the early or b genes. Seven of these are necessary and sufficient for viral replication under all conditions: DNA polymerase (UL30), DNA binding proteins (UL42 and UL29 or ICP8), ORI binding protein (UL9), and the helicase/primase complex (UL5, UL8, and UL52). When sufficient levels of these proteins have accumulated within the infected cell, viral DNA replication ensues. Other early proteins are involved in increasing the deoxy ribonucleotide pools of the infected cells, while still others appear to function as repair enzymes for the newly synthesized viral genomes. These accessory proteins are “nonessential “for virus replication in that cellular products can substitute for their function in one or another cell type or upon replication of previously quiescent cells; however, disruptions of such genes often have a profound effect upon viral pathogenesis and/or ability to replicate in specific cells. Thus, any deficiencies in these genes greatly impair virus replication in the natural host. The vegetative replication of viral DNA represents a critical and central event in the viral replication cycle. High levels of DNA replication irreversibly commit a cell to producing virus, which eventually results in cell destruction. DNA replication also has a significant influence on viral gene expression. Early expression is significantly reduced or shut off following the start of DNA replication, while late genes begin to be exVOL. 10, 1997 HERPES SIMPLEX VIRUS LATENCY 421 on August 23, 2020 by guest http://cmr.asm.org/ Downloaded from pressed at high levels. These late genes can be divided into two subclasses: “leaky-late” (BG) and “strict late” (g). The BG transcripts are expressed at low levels prior to DNA replication but reach maximum expression after viral DNA replication has been initiated. In contrast, g transcripts are difficult to detect until the onset of viral DNA replication.

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