CHAPTER II

LITERATURE REVIEW

1. Herpes Simplex Viruses

1.1 Structures and Compositions of the Virus

Herpes simplex viruses (HSV) are large DNA viruses belonging to the α-sub-family of the family Herpesviridae. The herpes virion consists of four structural elements : a DNA-containing toroidal core about 75 nm in diameter ; an icosahedral capsid (95-105 nm in a diameter) that encloses the core ; a surrounding granular zone composed of globular proteins and designated as the tegument ; and an outer membrane, or envelope, which surrounds the capsid and tegument (2, 26, 27, 28).

The genome of HSV is a linear, double stranded DNA molecule, with molecular weight of $85\text{-}106 \times 10^6$ daltons which is large enough to encode 60 to 70 or more gene products (27). Eighty to ninety percents of the genome form the two largest components designated as L (long) and S(short) (13, 31). These consist each of unique sequences (Ul and Us) which are flanked by smaller reiterated sequences. Thus, the reiterated sequences constitute the molecular link between the two large components and, secondarily, allow the DNA molecule to close in a circle. Herpes

simplex DNA has a coding capacity for over 50 different proteins (2, 5, 19). Virus-coded enzymes including thymidine kinase and DNA-polymerase participate in genome replication (5, 13, 27). These enzymes have unique substrate affinities and activities that distinguish them from similar cellular enzymes. Such properties have allowed the development of nucleoside analogs that interact with and specifically inhibit the viral replicative processes in which they participate (5).

The structural features of the capsid, i.e., its 100 nm diameter and 162 capsomers, are characteristics of all herpesviruses. Within the capsid are the DNA and DNA binding proteins. The pentameric capsomers at the vertical have not been well characterized. The hexameric capsomers are 9.5 x 12.5 nm in longitudinal section ; a channel 4 nm in diameter runs partway from the surface along the long axis (26, 28).

The tegument is the structures between the capsid and envelope which composed of a number of viral proteins whose properties and function are largely unknown. The outer shell of the virus is a lipid-containing membrane, designated the envelope which derived from modified cell membrane. This envelope is acquired as the DNA-containing capsid buds through the inner nuclear membrane of host cell. The herpesvirus envelope contains numerous protrusions or spikes, which are more numerous

and shorter than those appearing on the surface of many other enveloped viruses (26, 51).

There are two serotypes of HSV, herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) which their genomes have approximately 50% sequence homology but are sufficiently different to endow each type with unique biological properties and to allow unambiguous differentiation by various techniques (2, 5, 27). HSV-1 and HSV-2 differ in the base composition and sequence homology of their DNA in electrophoretic mobility of many of the structural and non-structural polypeptides, and in many biologic properties (27).

1.2 Animal Susceptibility and Growth of the Virus

Man is the natural host of HSV, but a relatively wide range of animals are also susceptible, including mice, guinea pigs, hamsters, and rabbits. The effects of infection depend upon the route of inoculation. For example, inoculation of the cornea in the rabbit results in keratoconjunctivitis or keratitis, whereas intracerebral inoculation produces fatal encephalitis (28).

The use of cell culture as host systems for the propagation of viruses provided a relatively economical tool. Therefore, cell culture has been substituted for animals in isolation attempts or for preparation of viral antigens. Many cultured cell types, e.g. HEp-2, BHK, Vero and HeLa support multiplication of HSV, undergo extensive

cytopathic changes, and develop intranuclear inclusion bodies ; chromosomal breaks and aberrations are also observed (28, 31, 37). The response of the cells varies with the strain of virus employed ; some strains cause marked clumping of cells, producing pocklike lesions ; others produce multinucleated giant cells by fusion of membranes and recruitment of the nuclei of adjoining cells ; and some strains produce typical plaques with suitable cells.

Both primary cell cultures and established cell lines have been used extensively for the diagnosis of herpetic infections. Established cell lines of monkey kidney (Vero), baby hamster kidney (BHK 21), as well as HeLa cells and HEp-2 cells, have all been used for diagnostic purposes. The virus grows readily and produces plaques in almost any cell cultures (31). Of the established cell lines, HeLa cells have been found to be suitable in this study because it can be easy for preparing and assaying virus stocks, and for preparing antigens used in PAGE.

1.3 Viral Replication

Replication of the viruses has been examined in a number of cell systems, and the duration of successive steps in the replication cycle depends upon the type of cell, the virus strain, and the multiplicity of the infection (31). To initiate infection, the virus must attach to specific receptors on the cell surface. In the

fusion of the virus envelope and the cellular plasma membrane would bring the deenveloped capsid into the cytoplasm of the infected cell. The capsid is then transported to the nuclear pore and the viral DNA is released into the nucleus where DNA transcription and replication and assembly of the capsids takes place. Normal cellular DNA and protein synthesis virtually stop as virus replication begins (26, 27, 31).

Viral DNA is transcribed and the synthesis of viral gene products is tightly regulated, viral gene expression is coordinately regulated and sequentially ordered in a cascade fashion. The genes designated alpha genes are expressed the earliest in infection for synthesis of alpha glycoproteins. These proteins, containing one minor structural and several nonstructural polypeptides, are synthesized at highest rates from 2 to 4 h post-infection and decrease thereafter. The second class of HSV genes, designated as beta, requires prior synthesis of alpha proteins but not replication of viral DNA. The beta proteins include regulatory proteins and enzymes required for the replication of DNA such as thymidine kinase, are synthesized at highest rates from 5 to 7 h postinfection and at decreasing rates thereafter. The third classes of HSV genes is designated as gamma, expression of these genes in normal amounts depends on the replication of viral DNA. The gamma glycoproteins, containing major structural polypeptides are synthesized at increasing rates until at least 12 h postinfection (26, 31, 39).

Viral DNA replication is carried out by both viral alpha and beta proteins and host cellular enzymes but the reactions are not yet precised understood (28). After the viral genome has been replicated and following structural protiens have been synthesized, the newly synthesized viral DNA is packaged into preformed empty capsid and later bind to the inner lamella of the nuclear membrane. The virion is finally enveloped at the nuclear membrane and the virus ergess by secreted via the Golgi apparatus. Viral glycoproteins are found on the surfaces of infected cells as well as in the envelopes of the virion (3, 26, 28). In HSV-infected cells, viral DNA synthesis is detected about 3 h following infection and continues for at least another 9 to 12 h (26, 28, 32).

1.4 Virion Polypeptides

Infection of mammalian cells with HSV results in the synthesis of over 50 viral specific polypeptides, and at least 20 to 25 of them are viral structural components (26, 39, 40, 56, 58). The remainder are probably involved in the initiation and regulation of viral DNA transcription, translation,.and synthesis (26, 45). Among these polypeptides are glycosylated species which become incorporated into the cell membrane and ultimately constitute in part of the virion envelope. Herpesvirus glycoproteins represent the major target for the host immune response and are likely involved in the

process of virus attachment, penetration and maturation as well as in virus-induced cell fusion (41). Initially, the analysis of structural polypeptides by SDS-polyacrylamide gel electrophoresis has revealed the presence of approximately 12 bands containing glycosylated proteins. The major glycoproteins has been designated gA, gB, gC and gD corresponding to the virion proteins VP8, VP7,VP8.5 and VP18 respectively(2, 3, 26, 37, 61). Subsequently, it has been recognized that gA and gB are differentially glycosylated forms of the same protein (3). Later Baucke and Spear identified gE (23). Glycoproteins B, C, D and E have been shown to be distinct from each other by one or more of the following techniques : immunoprecipitation with monoclonal antibodies, tryptic peptide fingerprinting, or physical mapping (51).

The DNA of HSV-1 and HSV-2 are about 50% homologous, and viral proteins show extensive antigenic cross reactivity ; cross-reactive determinants are present on both virion and non-structural proteins. The same protein may posses both type-specific and type-common determinants, and the genes coding for many of the cross-reactive proteins map colinearly in the genomes of the virus (43, 45, 52). However, the major glycoproteins of HSV-1 have been designated gB, gC, gD and gE ; species with similar characteristics produced by HSV-2 infections have been given the same corresponding names (41, 43). An additional, Balachandran and co-workers described two

additional antigenically and structurally distinct HSV-2-specific glycoproteins (41). One of these new glycoproteins was originally designated gF but has since been shown to be encoded by an HSV-2 gene mapping colinearly with the gC gene of HSV-1 and is now referred to as gC_2 (24, 25, 41, 43). The second appearently type-specific glycoprotein is the glycoprotein gG, the previously described 92 K glycoprotein of HSV-2 which maps in the unique short region of HSV-2 DNA (43, 51).

At present, HSV-2 codes for at least five different glycoproteins designated as gB (100-130 Kd), gC_2 (75 Kd, formerly designated as gF), gD (60 Kd), gE (80 Kd), and gG (92 Kd, formerly designated as gC) (50).

1.5 Pathogenesis and Clinical Features of HSV-2 Infection

Delineation of various aspects of the pathogenesis of HSV infections in humans has been hampered by the necessity of depending on observational studies. Since a number of animals are susceptible to HSV, experimental models have been developed, and much of the informations derived from these models (1). The characteristic of HSV infection is its propensity for persisting in a quiescent or latent state, with recurrence of activity at irregular intervals. The initial infection occurs through a break in the mucous membranes (e.g., eye, mouth, throat, genitals) or skin, where local

multiplication ensues. From this focus virus spreads to regional lymph nodes, where it multiplies further (28).

The initial infection commonly occurs in children 6-18 months of age, serological surveys have demonstrated that it is most often inapparent. When the initial infection receded the virus persists, despite the presence of a high antibody titer, producing a latent infection, probably in a sensory ganglion adjacent to the major site of the primary disease. The form of the occult virus remains unknown, but the balance may be readily upset, inducing viral replication in the nerve ganglion and thus provoking the recurrent (5, 28).

HSV causes a number of diseases in man which may be manifestations either of acute exogenous infection or of recurrent endogenous infection. HSV induced disease may represent a serious problem for certain patient. The two HSV-types have generally been associated with different sites of primary infection ; HSV-1 with infection of the mouth, lips, eyes and skin above the waist, and HSV-2 with infections of the urogenital area and of skin below the waist. It has been recognized for some time, however, that in individual clinical episodes there may be exception to this general rule (3, 13).

Primary infection with HSV-1 or HSV-2 tend to be more severe than infections occuring in patients with detectable serum antibodies to the viruses. The most

common site of primary infection with HSV-1 is the oral cavity, which may result in acute gingivostomatitis (1, 27, 31). This disease is characterized by the appearance of vesicles on the mucous membranes of the mouth, fever, irritability and local lymphadenopathy. Herpes labialis or cold sore is the most common manifestation of recurrent HSV-1 infections. The lesions usually develop at the mucocutaneous junction of the lip and are often preceded by a prodome that consists of burning or a sense of irritation (1, 27). Herpetic keratoconjunctivitis, the initial infection with herpesvirus, may be in the eye and causes recurrent painful erosion, which untreated sometimes may lead to a deeper keratitis of the stroma and a following blindness (13, 31). Herpetic whitlow is a primary or recurrent herpes simplex infection of the finger and hand. Herpes encephalitis, the most common forms of severe encephalitis, is a destructive infection with a hitherto significant mortality while herpes meningitis is a self-limited disease without severe morbility. Eczema herpeticum is a wild spread cutaneous infection of the skin taking place in the partially immunodeficient atopic individual (13, 27).

Primary infection with HSV-2 is most commonly manifested as genital herpes. Humans are the sole known reservior of infection, and infection is transmitted by mucosal contact with infectd secretions (5). The primary genital infection in males as well as in females may be

associated with fever, malaise, and lymphocytic meningitis, it is painful and there will commonly be regional lymphadenopathy. Recurrent genital herpes with lesions on the penis or vulva may be painful, but is always milder and more short lasting than the primary infection. The lesion may be triggered by trauma in connection with sexual intercourse or by various stress factors (5, 13, 27, 31). Herpesvirus type 2 may be transmitted to the newborn during birth by contact with herpetic lesions in the birth canal. The spectrum of illness produced in the newborn appears to vary from subclinical or local to severe generalized disease with a fatal outcome. Severely affected infants who survive may have permanent brain damage (27).

1.6 Immunology to HSV-2 Infection

A large number of studies have been published on the immunology of primary herpetic infections in humans and experimental animals. Most commonly, virus neutralization, complement fixation, and in more recent years, radioimmunoassay and ELISA have been detection and quantitation of anti-HSV antibodies. While highly useful in detecting an immune response to the virus, these assays are of limited value in determining the fine specificity of antiviral antibodies since each assay indiscriminately detects antibodies directed against multiple viral antigens. More recently, several investigators have

employed SDS-PAGE to identify individual viral polypeptides and demonstrated the utility of immunoblot and immunoprecipitation for assessing specific aspects of host antibody response to HSV (1, 4, 46, 47, 48, 49).

Zweerink and Corey studied the sequential development of HSV-2 specific antibodies during initial genital herpes infections by radioimmunoprecipitation technique (4). The earliest detectable antibody response in HSV-2 infection was to the high-molecular weight glycoprotein complex, followed by antibodies against the major nucleocapsid polypeptide and then antibodies against a number of other viral antigens, including a polypeptide with a molecular weight of 62 Kd. Ashley and Corey reported a similar observation with genital HSV-2 patients (46). The earliest detectable antibody response in HSV-2 infection was to the major nucleocapsid protein (p 148 ; 148 Kd), and to the glycoproteins gB (110-130 Kd) and p88 (88 Kd). Seroconversion to p66 (66 Kd), gD (60 Kd), and to a complex of glycoproteins gC (80 Kd) and gE (70-75 Kd) occurred later, at a mean time of approximately 3 weeks.

Antibody response to HSV specific polypeptides have been determined with Western blot or immunoblot assays. Bernstein, Bryson, and Lovett found that serum from patient with a primary HSV-2 infection reacted with 12 HSV-2 polypeptides having molecular weights of 125, 110, 100, 92, 66, 59, 49, 45, 42 and 32 Kd

(47). Kahlon et al. reported similar findings in patient with primary genital herpes caused by HSV-2 develop antibodies first to an apparent gpG (130 Kd) (48). Subsequently antibodies to the other polypeptide complex like gpB (130), gpD (60), gpE (75-96), p35 (35) and ICP4 (40) were appeared later.

The numbers and molecular weights of HSV-2 polypeptides detected by sera from different individuals have been found to vary considerably. This may be related either to the relative sensitivity of radioimmunoprecipitation compared with that of Western blot or to differences in the antigens that are available for reaction in a given system. However, the most prominent molecule identified as an antigen in the preceding studies was a glycoprotein of about 130 Kd. Antibodies to this molecule were among the first to appear in convalescent sera and were detected in all sera.

2. Acyclovir

Antiviral chemotherapy has been used to inhibit viral replication without harm to the host. In the past, agents which were active against DNA viruses also affected the DNA of normal cells. The discovery that acyclovir [ACV, 9-2 (hydroxyethoxymethyl) guanine] was a potent inhibitor of the replication of HSV with very low toxicity to host cell (21, 29, 30). This led to an extensive investigation of its mechanism of action and selectivity.

2.1 Mechanism of Action of Acyclovir

Acyclovir is an acyclic nucleoside analogue in which an aliphatic side chain resembling a portion of the sugar moiety is attached to the 9-position of guanine. The first indication that ACV was an unusual antiherpetic agent was its very marked selectivity (9, 29, 30, 31). Although it could inhibit the replication of HSV-1 and HSV-2 in Vero cells by 50 % (ID_{50}) at concentration as low as 0.1 uM, whereas, 300 uM ACV was required to inhibit the multiplication of the Vero cells themselves. The reason for this large different effect became evident when radioactive ACV was incubated with HSV infected and non-infected cells. Whether the compound was labelled with ^{14}C in the 8-position of the guanine moiety or ^{3}H in the hydroxyethoxymethyl side chain, three new radioactive compounds were formed in readily detectable amounts only in HSV-infected cells (29, 31). These compounds were identified by their positions on high-pressure liquid chromatograms (HPLC), ultraviolet absorption spectra and enzymic conversion to ACV, as acyclovir mono-, di-, and triphosphates (ACV-MP, ACV-DP, ACV-TP) (29, 31). The amounts of ACV-MP, ACV-DP and ACV-TP formed could be quantified by HPLC and were found to depend on the strain of HSV-1 and HSV-2, the kind of host cell employed, time after infection, and the concentration of ACV employed (8, 29, 31, 33). The investigations to determine which virally induced enzymes might be responsible for the

phosphorylation of ACV revealed that the first step, the conversion of ACV to ACV-MP is catalyzed by the herpesvirus-specified-thymidine kinase (HSV-TK) (21, 29, 35) while the succeeding steps are catalized by cellular enzymes. Guanylate kinase is responsible for the conversion of the mono- to the di-phosphate while several other cellular enzymes can convert the di- to the triphosphate which is the major phosphorylated form of ACV formed in HSV-infected cells(21,31,36). The critical step in this phosphorylation sequence is the first one, since the specificity of the HSV-TK is very different from that of the normal cellular thymidine kinase of the Vero cells. The cellular TK and the HSV-TK are very similar, however, ACV not only binds 200 times better to the HSV-TK than to the Vero TK, but it is phosphorylated by the viral enzyme at a rate at least three million times faster (21, 35).

Since the timing of the addition of ACV for maximum inhibition of HSV replication was between 2 to 6 h after viral infection, it was a reasonable assumption that the compound was interfering principally with DNA synthesis (29). The effect of ACV on HSV-DNA synthesis was examined and showed that ACV-TP is a potent inhibitor of HSV-DNA-polymerases (10, 35, 38). ACV-TP can also act as a substrate for the viral DNA polymerase but this activity is selflimiting because when ACV-TP is incorporated into the growing DNA chain it acts as a chain terminator due to the absence of the 3'-OH groups on the

acyclovir molecule (11). Furthermore the enzyme is inactivated by the acyclovir terminated molecule due to light binding of the polymerase (21, 38).

The high potency and selectivity of ACV for HSV can now be understood on the basis of the selective phosphorylation of ACV by the viral specific thymidine kinase and the specific inhibition of the herpes specified DNA polymerase by the triphosphate of ACV.

2.2 Treatment

In vitro sensitivity data suggest that ACV should effect against clinical manifestations of HSV-1, HSV-2, varicella-zoster virus (VZV), and possible Epstein-Barr virus (12, 14). The clinical potential against HSV-1 and HSV-2 is further supported by results in animal models (12). These favorable properties of ACV indicate that it may be of clinical use in the treatment of herpes viral infections.

In man, ACV displays marked activity against HSV. Whether delivered topically, orally, or intravenously, ACV reduces the duration of virus shedding, local pain, itching, and lesion-healing time in primary herpes simplex virus infections though less persuasively in the recurrent attacks. Unfortunately, there appears to be no significant reduction in the establishment of latency or in the frequency of subsequent recurrences (5, 14, 15, 42).

Because acyclovir-treatment does not prevent later recurrences, investigators are currently studying the use of this drug for suppression of recurrences. Daily oral suppressive therapy has been shown to prevent most expected outbreaks in normal patients with frequent recurrences (5, 15, 42). Furthermore, since ACV has its greatest effect on HSV infections in the immunocompromised host (5). Intravenous ACV, therefore, is also used to suppress the activation of a latent herpes infection in immunosuppressed patients (44).

2.3 Effect on HSV-2 Polypeptides

Since antiviral chemotherapy is a relatively new development, little is known about the effect of acyclovir on the humoral response to HSV-specific polypeptides. In 1984, Bernstein and Lovett studied the effect of oral acyclovir on antibody response to HSV in primary genital herpetic infections by Western blot technique (17). In placebo-treated, HSV-2 infected patients responded to HSV-2 polypeptide that had molecular weights of 125, 84, 68, 59, 46 and 40 Kd. Treatment with acyclovir diminished the response to specific polypeptides especially those with molecular weight of 50-100 Kd. Ashley and Corey similary reported that systemic acyclovir treatment of first episode primary genital HSV-2 infection influences the subsequent development of precipitating antibodies to HSV-specified polypeptides (16). Both oral

and intravenous acyclovir were associated with later development of antibodies to two glycoprotein 80 and 60 Kd and one glycosylated polypeptide of 66 Kd. However, topical application of acyclovir had no effect on the immune response to HSV infection.