CHAPTER II

LITERATURE REVIEW

1. Historical Background

Certain clinical entities associated with HSV-infection have been delineated in the medical literatures for several centuries. The term herpes (the meaning is "to creep") has been used since the earliest epoch of Greek medicine to include spreading cutaneous lesions of varied etiology (22,23). In A.D. 100 Herodotus, a Roman physician was the first one who described herpetic eruptions around the mouth. In addition, 1,600 years later, herpes of the genital tract was first reported by a French physician, Astruc (16). By the nineteenth century, the generally accepted use of the term herpes was restricted to certain diseases associated with vesicular eruptions. Until the early of the twentieth century, herpes zoster was differentiated from "herpes febrilis" and "herpes genitals" on clinical and epidemiological grounds. In any case, Gruter and Lowenstein were credited for the initial successful isolation of HSV in 1912, by demonstrating transmission of the virus from human labial and keratitis lesions produced specific lesions on rabbit's cornea (16,24). Over the next 40 yrs,the experimental host-range of herpes simplex virus was widened to include other

laboratory animals such as chick embryos, and ultimately cell cultures.

It is now recognized that HSV does not differ basically from other infectious agents with respect to the primary infection of susceptible hosts. However, it is rather interesting that the viruses often remain latent after the manifestations of the initial infections subside, and recurrent lesions occur by reactivation of the latent virus. The virus remained latent in the ganglions of sensory nerves (25,26). The clinical spectrum of HSV-infections was augmented to include gingivostomatitis, pharyngitis, keratoconjunctivitis, encephalitis, aseptic meningitis, genitalia and neonatal diseases.

In the early 1961, Schneweis (16,27) and Plummer (28,29) found antigenic differences among HSV-strains. Later, in 1967 Nahmias and Dowdle (22) demonstrated that the large majority of genital and newborn infections were caused by HSV-2, and that most nongenital infections were caused by HSV-1. The most striking interest in HSV, however, is due to observations suggesting an etiologic association between HSV-2 and carcinoma of the cervix (20, 21,30,31). The application of modern biochemical and broadening clinicopathological and epidemiological observations in more recent times, has provided new approaches to laboratory diagnosis, prevention, and

therapy (32).

2. Structures and Compositions of the Virus

The genome of HSV is a linear, double-stranded DNA molecule, with molecular weight of 100×10^6 (33) which is large enough to encode 60 to 70 or more gene-products. The structure of the genome is unusual among DNA viruses in that two unique nucleotide sequences are flanked by inverted repeated sequences (17,34). The virions of HSV are made up of a DNA core, 160-200 nm in diameter, and an icosahedral capsid consisting of 162 capsomers (34,35). Within the capsid are the DNA and DNA-binding proteins (36). The outer shell of the virus is a lipid-containing membrane (37), 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 the host cell(17). The cell proteins, however, are not detectable in the virion envelope. Embedded in the lipid bilayer of the envelope are five or six viral glycoproteins which mediate attachment of virus to the host cell as well as penetration of virus into cell (38). Between the capsid and the lipid bilayer of the envelope is the tegument, composed of a number of viral proteins whose properties and functions are largely unknown.

Herpes simplex viruses contain complex DNA which have been shown to induce the synthesis of at least 50 new polypeptides in productively infected cells (39,40). The

studies of relation of HSV-1 and 2 found that some of the antigens have been shown to posses cross-reactive determinants (which react with antiserum against either HSV-1 or HSV-2) (41,42).

Several HSV induced polypeptides have been purified and immunologically chracterized with the use of monospecific antisera and a variety of immunological technics. These include the early nonstructural polypeptides VP 175 and VP 134, the envelope glycoprotein VP 123 and the nucleocapsid polypeptide VP 154. Some polypeptides, VP 154 and VP 134 had immunological reactivity common to both virus types, while others (VP 175 and VP 123) were type specific (43). Heilman et al. (44) found that a 40,000-molecular-weight polypeptide, (p40), which is a major component of nucleocapsid polypeptide of HSV, possesses both type-specific and cross-reactive antigenic determinants.

Ludwig et al. (45) studied the relatedness of DNAs of HSV-1 and HSV-2 by DNA-DNA hybridization on nitrocellulose membrane filters. Reciprocal and competition hybridization experiments performed under optimal conditions demonstrated a maximum homology of 70% between the DNAs of HSV-1 and HSV-2.

Plummer (46) reported that the identification and titration of antibodies to herpes simplex virues type 1 and 2 when both of these antibodies are presented in the

same serum have presented some problems. The delineation of these antibodies from one to another has been difficult because of the strong cross-neutralization between the two viruses.

3. Animal Susceptibility and Growth of Virus

Man is the natural host for herpes simplex virus, however, a relatively wide range of animals are also susceptible, including mice, guinea pigs, hamsters, rabbits and the chorioallantois of the embryonated egg (13,27). The pathogenesis of infections 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. While animals are now seldom used for diagnostic purposes, but rather to study the mechanism of recurrence, various aspects of immunologic reactivity , oncogenicity and effectiveness of putative therapeutic agents. The chick embryo has been a convenient host. The production of pocks on the chorioallantoic membrane affords a reproducible method for detection and assay, similar to that employed with poxviruses.

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 or embryonated eggs in isolation attempts, neutralization tests, or for preparation of viral antigens.

Many cultured cell types, e.g. HEp-2, BHK, Vero and HeLa (27,47), supported multiplication of herpes simplex virus underwent extensive cytopathic changes, and developed intranuclear inclusion bodies; chromosomal breaks and aberrations were also observed. The response of the cells varied with the strain of virus employed. Some strains caused markedly clumping of cells, producing pocklike lesions; others produced multinucleated giant cells (polykaryocytes) by fusion of membranes and recruitment of the nuclei of adjoining cells; and some strains produced typical plaques with suitable cells.

4. Viral Replication

Replication of HSV has been extensively examined in a number of cell systems, and found that the duration of successive steps in the replication cycle depends upon the cell type, the virus strain, and the multiplicity of infection. In order to initiate infection, the viral envelope must attach to cell receptors. We knew little about the initial steps of viral attachment to the cell receptor. Although the wide range of host suggests that the receptors for HSV on cell surfaces are widely distributed, these receptors have not yet been truely identified (38,48). After attachment, fusion between the viral envelope and a cell membrane must be required to liberate the nucleocapsid into the cytoplasm of the infected cell(49). This is followed by controlled

disassembly of the nucleocapsid to release the viral DNA. As soon as the viral genome reaches the nucleus of the cell, the expression of viral genes occurs in a highly regulated fashion and consequential result is on the inhibition of cell-macromolecular synthesis.

Three classes of HSV genes have been defined on the basis of the timing and requirements of their expression (50). The genes designated alpha are expressed the earliest in infection for synthesis of alpha glycoproteins. These proteins, containing one minor structural and several nonstructural polypeptides, were synthesized at higest rates from 3 to 4 h postinfection. 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, were synthesized at highest rates from 5 to 7 h postinfection. The third class 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 were synthesized at increasing rates until at least 12 h postinfection.

After the viral genome has been replicated and following strucutral proteins have been synthesized, nucleocapsid are assembled in the nucleus of the infected

cell. Envelopment occurs as the nucleocapsids bud through the inner nuclear membrane into the perinuclear space. Virions are then transported through the endoplasmic reticulum and the Golgi apparatus to the cell surface. Viral glycoproteins are found on the surfaces of infected cells as well as in the envelopes of the virion (51).

5. Pathogenesis of HSV-Infection

Delineation of various aspects of the pathogenesis of HSV infections in human has been hampered 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 (13,27). Exposure to virus at mucosal surfaces or abraded skin permits the entry of virus and the initiation of replication in cells of the epidermis and dermis. In humans, the time-duration from the inoculation of virus in peripheral tissue to the spread of virus to the ganglia is presently unknown. In mice and guinea pigs, however, HSV can be recovered from ganglia within two days after vaginal or skin-inoculation (52). During this early phase of infection, viral replication may occur in ganglia and adjacent neural tissue. Virus then spreads to other mucosal skin surfaces by mean of peripheral sensory nerves.

Latency is an interesting feature of HSV, and this subject has been recently reviewed (53). Within 2 weeks

after the productive infection at the sensory ganglia, a latent stage develops in which infectious virus cannot be detected. However, under appropiate conditions the virus may be reactivated, and infectious virus is again produced. The signal for reactivation is still not understood, but various stimuli, such as fever, stress, sunlight and local trauma, may be recognized by individuals.

The reactivated virus is generally thought to spread peripherally along the nerve axon to the epithelium, where virus replication occurs and virus is shed. Replication of virus in the epithelial cells may give rise to local lesions that may or may not be clinically apparent.

6. Clinical Spectrum of HSV-Infection

Herpes simplex virus has been isolated from nearly all visceral and mucocutaneous sites. The clinical manifestations and course of HSV infections largely depend on the route of infection, the age and immune status of the host, and the antigenic type of virus (16,24). Primary infections in most individuals is clinically inapparent and often resulted in the latent state in sensory ganglia of the host (25,26). Latent infections persist in persons with antibodies, and recurrent lesions are common.

HSV causes a number of diseases in man which may be manifestations either of acute exogenous infection or of recurrent endogenous infection. HSV induced diseases may represent a serious problem for certain patients (18,19). Most primary HSV-1 infections of the mouth in early chilhood (39%) were gingivostomatitis and pharyngitis in young adults (42%) (54,55). Herpes labialis, the so-called fever blister, a recurrent circumoral infection, is extremely common and may be clinical relevance in some patients. Herpetic kerato-conjunctivitis is a leading cause of corneal blindness (19).Acute necrotizing retinitis due to HSV is an uncommon but rather severe manifestation of HSV infection (56,57). The spectrum of ocular involvement by HSV has expanded to include not only conjunctivitis and keratitis but also cataracts, iridocyclitis and panuveitis (32) Herpes genitalis, the most commonly occurs in adolescents and young adults, is usually due to venereally transmitted HSV-2 (58). A sharp increase in the number of recognized cases has been noted recently in the United states (18,19), making genital HSV infections today are among the most common sexually transmitted diseases (59). In Thailand, it has been reported that more than 60% of population in Bangkok showed prevalance of HSV-2 infection (60). Another severe disorder caused by HSV is the generalized herpetic disease in newborns (10,11). As much as 70% of neonatal HSV cases have been related to

HSV-2 infections, almost all of which resulted from contact with infected genital secretions at the time of delivery. In the more severe form of HSV infections, there has been estimated that more than 50% of the untreated cases may terminate fatally or result in permanent neurologic sequelae. A relationship between herpesvirus and cancer was proved,especially between HSV-2 and cervical cancer (20,21,30,31). Interestingly, herpes encephalitis is the most commonly reported viral infection of the central nervous system (61,62). At present, it is not known if encephalitis is caused by endogenous or exogenous infection. More recently, it was found that HSV-2 was major cause of aseptic meningitis in adults (15,19,63).

7. The Epidemiology of HSV-Infection

HSV infections are found worldwide. The epidemiological aspects of HSV-1 and HSV-2 infections have been recently reviewed (16). There are unique features in the distribution among populations of the various clinical illness associated with infections by these viruses. Standard complement-fixation antibody assay does not distinguish between HSV-1 and HSV-2 infections. However, several serological assays, such as neutralization, indirect immunofluorescence, passive hemagglutination, radioimmunoassay, and enzyme-linked immunosorbent assay, can measure the relative selectivity of serum antibodies

between the two subtypes. Much of the humoral immune response to HSV is confined to type-common antigenic determinants; thus, it is difficult to detect HSV-2 antibodies in patients with prior HSV-1 infection and HSV-1 infection in those with prior HSV-2 infections (41,42).

The major epidemiological determinants of HSV infections are age, socioeconomic level, and geographic area. Seroepidemiological studies demonstrated that in almost all the populations studied, more than 90 percents had antibodies to HSV by the fourth decade of life (32). In underdeveloped areas, similar patterns still prevail. In many Western industrailized middle-class populations, however, the age-specific prevalence rates of HSV-1 infection appear to be decreasing (64). The most complete study detailed the occurrence of primary gingivostomatitis indicated that early childhood represents on initial high-risk age for acquiring HSV-1 infections (65).

Genital infections are acquired through sexual contact, and the risk factors associated with acquiring these infections are similar to those of acquiring other venereal diseases. The highest prevalence rates of genital infections have been found between 20 and 29 years of age (66).

Some previously infected persons who have antibody to HSV-1 or HSV-2 are asymptomatic. HSV infections occur

throughout the year. The incubation period ranges from 1 to 26 days with an average of 6 to 8 days. Contact with active ulcerative lesions or asymptomatically excreting patients can result in transmission.

8. Laboratory Diagnosis of Herpes Simplex Virus-Infection

While the incidence of many bacterial sexually transmitted diseases appears to be decreasing, the complications and frequency of viral sexually transmitted diseases in developed countries has steadily increased over 10 fold in the last decade. Significant advances in our understanding of the epidemiology, natural history, and therapy of symptomatic genital herpes has occured in the last 5 years. In order to properly utilized this information, however, more widespread availability of laboratory diagnostic testing for herpes simplex virus is needed (67). A number of biological features of the characteristic for herpes simplex virus can be utilized for the detection: the infectivity (isolation in tissue culture), the morphology (visualization by electronmicroscopy), the antigenicity of viral specified proteins produced by infected cell (IFA, ELISA and RIA and Western blot) and unique sequences in viral DNA (hybridization) (2).

Diagnosis of viral infection is often dependent upon the identification of specific antigens or antibodies in biological fluid . Viral antigens and their

corresponding antibodies can be assayed by a variety of immunological technics. Classical technics, such as gel diffusion, complement- fixation and virus-neutralization, have been extensively used, yet are limited by inherent practical-and theoretical consideration .

During the last two decades there has been a phenomenal increase in the number and variety of immunodiagnostic tests performed. One of the reasons for this has been due to the development and perfection of methods which use labeled antigens or antibodies, resulting in tests with very high levels of sensitivity and specificity. The rapid diagnosis for HSV infections are included in:

8.1 Immunofluorescent antibody technic (IFA)

The IFA technic is based on the same principles as other immunological reactions involving antibodies and antigens (68). Fluorescein labels have been attached to antibodies, and these conjugates are very useful for the rapid identification of organisms responsible for infectious diseases as well as for the measurement of antibody levels, especially in infectious and auto-immune diseases (6,7).

These IFA procedures are rapid and sensitive, and have been recommended as an alternative to radioimmunoassay, and enzyme immunoassay. The detection

of antibody, the antigen can be prepared by simplified processes, e.g. Williams et al. (69) prepared VZV-antigen in human embryonic lung fibroblasts and infected cells were washed in PBS. The detection of antibody to this membrane antigen has been used successfully to confirm the clinical diagnosis of varicella-zoster infection. Accordingly, El Falaky et al.(70) studied IgG, IgA and IgM antibodies to HSV by IFA method. In this study, the antigen was prepared by infecting HSV in Vero cells. The most pertinent finding of the present study was the marked rise in IgA antibodies following herpetic infection.

Identification and typing of HSV by immunofluorescence with conventionally used animal antisera is generally unreliable because of the extensive antigenic cross-reactivity of HSV-1 and HSV-2. Type-specific monoclonal antibodies have the potential for an unambiguous differentiation of HSV-1 and HSV-2. Balachandran and his colleagues (71) used monoclonal antibodies which reacted with type-specific antigen of HSV to detect viral antigens in cells from herpetic lesions. The results indicated that monoclonal antibodies can be used to accurately identify and type the isolates of herpes simplex virus. Goldstein et al. (72) using IFA, demonstrated that monoclonal antibodies possessed sufficient specificity and allow rapid serotyping of HSV obtained either from infected cells in culture or directly from patients.

8.2 Radioimmunoassay (RIA)

Radioimmunoassay (RIA), utilizing radiolabeled antigens and antibodies, was a major technological advancement provides much greater sensitivity than classical serological technics. RIA has been considered as the method of choice in current research, and has been used to detect fungal, bacterial and viral antigens as well as their corresponding antibodies.

In addition, the solid-phase radioimmunoassay (RIA) has been a sensitive method for the detection of IgG antibody to herpesviruses, including VZV, HSV and CMV (73,74,75) and has also been used to measure IgM antibody to CMV (75). Arvin and Koropchak (76) studied a simplified solid-phase RIA technic with the use of crude antigen and commercially available reagent was employed in measuring both IgG and IgM antibodies to VZV in unfractionated serum samples. The RIA method was compared with other assays for VZV antibody, including complement fixation, indirect immunofluorescence assay and immune adherance hemagglutination assay (IAHA). Therefore, RIA has been applied to study both IgG and IgM in the patients of primary or acute herpes zoster infection.

However, both IFA and RIA have their limitations. IFA is tedious, time consuming and not easily automated so it can be used for only small batches of tests. In contrast, RIA is particularly suitable for large-scale

operations but the short shelf-life of the reagents, the rather sophisticated expensive equipment, and the strict regulation on the use of radio-isotopes have tended to exclude RIA from many small laboratories. These problems have led workers to search for alternative labels for antibodies or antigens which will be the subject of the next topic.

8.3 Enzyme-Linked Immunosorbent Assay (ELISA)

In 1971, Engvall and Perlmann (9) described an enzyme-linked immunosorbent assay (ELISA) for the quantitation of rabbit-IgG that appeared to be as sensitive as RIA. Since this original report, the enzyme linked assays have been applied with great success in the detection of viral and bacterial antigens, antibodies, drugs, hormones and other biological substances.

Enzyme-immunoassay (EIA) is also objective and can be automated. In addition, it is free from restrictive legislation and the reagents used have a very long shelf-life. These adventages, together with the fact that only simple and inexpensive equipments need to be used, have led to the recent development of numerous enzyme-immunoassays, many of which are especially suitable for use in small laboratories and in developing countries as well.

In this assay, the antigen or antibody is usually attached to a solid-phase support allowing easy separation of bound and free reagents. It has been found that both antigens and antibodies can be covalently attached to particulate material, such as cellulose and polyacrylamide, and that satisfactory passive adsorption can also be obtained to tubes, beads, disks, or microplates; made of nylon, polystyrene, polyvinyl chloride or polypropylene. Probably the most advantage of an enzyme immunoassay is the conjugate of antibody (or antigen) linked to an enzyme. To date, the enzymes found most satisfactory are horseradish peroxidase, glucose oxidase, B-galactosidase, and alkaline phosphatase (8).

Antibodies can be detected by an indirect ELISA procedure. In this test, antigen is adsorbed to a solid phase followed by incubation with test serum. Any antibody specific for antigen binds to the antigen on the solid phase and is further detected by addition of enzyme-labeled antiglobulin and suitable substrate. The amount of specific antibody presented in the test serum is proportional to the amount of enzyme activity bound to the solid phase at the end of the assay. As with most antibody assays, results are expressed either qualitatively (i.e., positive or negative) or quantitatively(i.e., end point titer) (8,77). The indirect method is widely used for antibody measurement since only a conjugates are needed to assay antibody in a variety of

diseases states.

The ELISA technic has been found to be reliable for the detection of antibody to herpesviruses (3,78,79,80). The assay was found to be more sensitive in detecting levels of antibody than the complement-fixation assay (3,81). Furthermore, van Loon and co-workers (82) developed the ELISA for detection of IgM and IgA antibody to HSV in patient with encephalitis. The work has pointed to the usefulness of the ELISA as a noninvasive technique for diagnosing HSV encephalitis. Recently Kimmel et al. (83) described an ELISA technic for the detection of HSV-specific IgM in serum and demonstrated its specificity by which no cross-reactivity with heterologous herpesviruses was found. Consequently, the ELISA appeared to be a rapid, reproducible, sensitive and specific method for routine demonstration of HSV antibody in a clinical setting (4,84,85).

9. Preparations of Antigen for the Detection of Antibody by Solid-Phase Immunoassay

9.1 Purified and partially purified antigen

The purification is very complicate and need rather sophisticated instruments. Gilman and Docherty (3) prepared the partially purified HSV antigen from virus-infected HEL cells. The infected cells were separated by ultracentrifugation. The pellet was sonicated, layered on

to 30% sucrose and centrifuged at 100,000 g for 180 min. The final pellet was resuspended in cold saline and was extensively sonicated before use. Later, van Loon et al. (82) used Vero cells for preparing HSV antigen. The infected cells were separated and disrupted with a Dounce homogenizer. Nuclei and cytoplasm were separated in a 17% (w/w) Ficoll gradient. The cytoplasm, fraction was then layered on a 30% (w/w) sucrose gradient and centrifuged at 90,000 g for 90 minutes. The pellet was resuspended, layered on a continuous 30-60% (w/w) sucrose gradient and centrifuged overnight at 90,000 g. The band containing the viral material was collected and the sucrose gradient was removed by subsequent dialysis.

Recently, Hampar et al. (85) prepared the HSV antigen in Vero cells. The infected cells were disrupted by sonication and centrifugation. Finally, antigen was purified by immunoaffinity chromatography.

9.2 Crude antigen

The method is simple and can be done in small laboratories. Leinikki and Passila (86) prepared CMV antigen in human fibroblast cells. Infected cells were harvested in basal medium without serum and was sonicated before use.

Arvin and Koropchak (76) used human foreskin fibroblast cells to prepare VZV antigen. Infected cells

were washed and resuspended in PBS. The suspended cells were sonicated, and viral antigen was finally separated by centrifugation. Similarly, Coleman et al.(87) used the same method for preparing HSV antigen in HEp-2 cells. This antigen was used for the detection of HSV in cerebrospinal fluid by inhibition enzyme linked immunosorbent assay. Moreover, some investigators prepared crude antigen by using trypsin-EDTA (73), 0.1 M glycine-NaOH (81), and 10% Tween-40 and 10% desoxycholate (84).