CHAPTER I

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) and HSV-2 are belong to subfamily Alphaherpesvirinae of family Herpesviridae. They are responsible for several clinically important infections (1). Two biologically distinct types, HSV-1 and HSV-2, were associated with different clinical syndromes (2). The double-stranded DNA genome of HSV comprises 152 kilobase pairs (3) and there is considerably homology between the genome of HSV-1 and HSV-2, about 50% of the sequence are highly conserved with good (85%) matching of base pairs (4). In the clinical setting, there are situations in which the typing of an isolate of HSV may provide valuable diagnostic, epidemiological, and prognostic information (5). It is also known that, depending on the HSV type, antiviral agents show differences in activity against the two types (6). Therefore, in select situations there is a need for a rapid and accurate method for distinguishing between serotypes.

HSV is a widespread infectious agent in human population, that can produce a wide range of mucocutaneous manifestations. The severity of the disease depends on the entry site of the virus, the type of infection (primary or recurrent), and the patient's immune status (healthy, elderly or immunocompromised). Clinical diagnosis is based mostly on the presence of the characteristic vesicular eruptions, but these might also resemble other viral and non-viral skin infections (7-11). After initial (primary) infection, the virus enters a latent, asymptomatic state, in which it is dormant in the nervous system. Recurrent infections at the same or adjacent sites occur when the nerve is triggered. Stress or hormonal or immunological change and some physiological conditions may stimulate replication of the virus, which is mobilized back to the target tissue. The severity of HSV infections varies widely, ranging from asymptomatic infection to an often fatal herpes encephalitis. The frequency and severity of recurrences also vary to a great extent. Considerable differences between the two antigenic types (1 and 2) of HSV are recognized, especially in their susceptibility to antiviral drugs (12,13) and the site and rate of recurrence (14). This has made the typing of HSV's

valuable tool for a better understanding of the disease. The evidence for re-infection, which virus of the same serotype is much weaker, it includes instances interpreted as mouth-to-genital transmission in young children (15) and instance of deliberate inoculation of fluid from vesicles in recurring lesion at another site with the thought that such "autoimmunization" would bolster immunity to the virus which caused the recrudescence (16-19). Although evidence of re-infection with exogenous virus of the same serotype has been obtained with both humans and animals (19,20). HSV-1 is common in general populations and is often acquired non-sexually during childhood years. HSV-2 is rarely a cause of oral lesions and serological evidence of HSV-2 is regarded as a good marker of infection acquired genitally. Whereas prior studies have indicated that HSV-1 is an infrequent cause of infections "below the waist", this asssociation is not absolute and either virus type may, in fact, cause lesions anywhere on the body. A number of genital HSV-1 and nongenital HSV-2 strains were isolated in our laboratory and this gave us the opportunity to determine if adaptation of HSV to a new replication site was accompanied by changes in the structural organization of its genome. Recent studies suggest an increasing prevalence of genital HSV-1 infection (21-23). Similarly, HSV-2 pharyngitis appears to be increasingly common. The subsequent recurrence rate of genital HSV-1 infection appears to differ from that of genital HSV-2 disease (22). This relationship between the local of herpetic lesion and the HSV type is not, however, absolute since genital HSV-1 and nongenital HSV-2 strain are occasionally found.

Although the typing of HSV isolates brings no immediate benefit to the patient it is nonetheless a useful test to perform in certain circumstances. Recent surveys have demonstrated difference from the tradition genital distribution of the two types of the virus (24), suggesting that epidemiological patterns are changing. However, there is no doubt about the value of characterizing the intratypic variants of the viruses. The ability to identify different strains has obvious applications in epidemiology, such as contact tracing in genital herpes and in analyzing apparently linked hospital cases of HSV infection. Furthermore and more speculatively, such detailed analysis might yield information on particular strains being linked with more severe

disease, greater transmissibility, increase frequency of recurrent disease, or with particular syndromes such as herpes encephalitis (25).

Typing of HSV isolates may be done by using several techniques. The two serotypes of HSV may be distinguished by a number of techniques. Isolates of HSV-1 and HSV-2 generally display different sensitivity to certain inhibitors (26-28) and different abilities to form plaques on chicken embryo cells (29,30), as well as various other biological differences of varying reliability (31,32). Methods for examining serological differences also have been widely used to distinguish the two HSV types. They include immunofluorescence staining (33-35), indirect immunoperoxidase staining (36), and an enzyme-linked immunosorbent assay (37). These methods are subject to some error owing to variability among isolates or cross-reactivity of antisera or both. Recently monoclonal antibodies to HSV-1 and HSV-2, and restriction endonuclease analysis of viral DNA have been developed (38,39). It has become evident that intratypic difference exists and can demonstrable by plaque morphology (40), differential neutralization (41), immunoperoxidase staining (36), DNA density analysis (43), guanine plus cytosine content (44), and the formation of intra-nuclear tubular structures (45). These include biological assay, such as growth of the virus on chorioallantoic membrane (46), serological methods using antisera to prototypes HSV-1 and HSV-2 (36,47), polypeptide comparisons (49-50), and analysis of viral DNA by either restriction endonuclease or DNA hybridization technique (38,39).

Polymerase chain reaction (PCR) is increasing useful tool to directly detect HSV in clinical specimens whereas conventional laboratory techniques such as viral culture, viral antigen detection, or viral serology, have been shown to have poor sensitivity (51,52). An excellent example of this trend is PCR being recognized as the best way to detect HSV cerebrospinal fluid (CSF). Because the genomes of HSV-1 and HSV-2 share considerable sequence homology, PCR primer sets for HSV typically amplify targets between the two. PCR has been used in rapid and specific detection and typing of many different viruses (53-55). Using the sequence information on the DNA polymerase coding genes of HSV-1 and HSV-2 (56,57), inexpensive restriction endonuclease based procedure could reliably type HSV PCR product from two well established HSV PCR primers sets. Methods for subsequent confirmation

of the species of the virus detected have included hybridization with a specific probe, nested PCR with species-specific primers, and followed by restriction enzyme analysis (58,59).

Restriction endonuclease (RE) cleavage of purified DNA has been shown to have many advantages over conventional biological and immunological procedures for typing HSV isclates (60-62). RE analysis of the virus genome gives positive identification of HSV strains and provides a powerful tool in epidemiolgical studies. Evidence suggesting that the HSV genome may be altered in course of transmission and adaptation to a new patient was obtained by this sensitive technique (63). Restriction site polymorphism may serve as convenient markers for identification of functional variation among HSV strains and has the potential to detect very small differences between closely related virus strains.

Restriction fragment length polymorphism (RFLP) analysis is a common and powerful method for comparison of viruses or microorganisms. This technique has been shown to be useful for molecular epidemiology of HSV (38,64). Termed RFLP, is due mostly to gain or loss of a RE cleavage site. The RFLP is stable and serves as a physical marker of HSV genome. RE analysis of HSV genomic DNA makes feasible a clear differentiation between the two antigenic types (HSV-1 and HSV-2) and individual isolates within an antigenic type can be identified (61,66). Thus, to determine the epidemiology of HSV infection, a comparison of RE-digested DNAs of HSV strains has to be done. These epidemiological studies depend on the intratypic difference in RE-digestion profiles of HSV DNAs. The RE pattern can provide unequivocal information on a wide extent of intratypic genetic variation amomg HSV strains.

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