CHAPTER I

INTRODUCTION

Cytomegalovirus (CMV), a member of the herpesvirus family, is an ubiquitous agent in nature that commonly infects man. It is the most common cause of human intrauterine and perinatal infection. CMV infections in both mother and baby, whether infected in utero or later, are mostly asymptomatic. In pregnant women, CMV can affect an individual pregnancy and infant development. Its ability to cause chronic infection of the fetus, newborn and young infant, leads in some cases to acute disease. The congenitally acquired infection, about 10%, is virulent with multiple organ involvement that is most prevalent in the central nervous system (CNS) and reticuloendothelial system. However, the significant percentage of infants born with subclinical CMV infection may develop more subtle forms of perceptual and CNS pathology later in life (Alford, 1984, Stagno et al., 1984). The infant with this disease will have a large liver, spleen and a petechial rash, eventually go on to die or to develop microcephaly and severe mental retardation. A series of excellent studies have revealed that infection with this agent is extremely common, that viral shedding is frequently detected, and that although most infected subjects are asymptomatic, other characteristic disease syndromes in infants, adults and immunocompromised subjects are now well recognized (Betts, 1984). Therefore, it is believed that pregnant women are at greater risk of CMV infections because of the influence of hormonal change

during pregnancy. It is interesting to study CMV recovery rate from the cervical excretion in various stages of gestation and to determine the prevalence of CMV-antibodies in pregnant women which provide further information on the evidence of CMV infection during pregnancy.

History of Human Cytomegalovirus

The earliest clinical description of probable CMV infection manifested as cytomegalic inclusion disease (CID) is the report of Jesionek and Kiolemenoglou in 1904 describing the first time the presence of protozoan-like cells in the lungs, kidneys, and liver of an 8-month fetus. The protozoan-like cells were 20-30 um in diameter. The nuclei were large and eccentrically placed, and each contained a central nuclear body surrounded by an outer clear zone (Ho, 1982). This was followed by several other reports describing distinctly enlarged cells with intranuclear and cytoplasmic inclusions (Sullivan and Hanshaw, 1982). In 1921, Goodpasture and Talbot suggested that these inclusions were similar to the inclusions produced by varicella virus and Lipschuetz (1921) had discovered similar inclusions that were constantly and characteristically associated with lesions produced by herpes virus. Therefore, a viral cause was postulated and on the basis of this background, von Glahn and Pappenheimer (1925) concluded that the inclusions in their case (an unusual 36-year-old white male with a liver abscess that contained large cells with prominent intranuclear inclusions, the first time case in adult) were caused by a virus identical to or closely related to the herpes group. Farber and Wolbach in 1932, noted inclusions in the salivary glands of the autopsied infants.

Wyatt and co-workers in 1950, described characteristic of CID on morphology in urine sediment. Finally, when the culture techniques became commonplace in the early 1950s, Smith (1956), Rowe et al. (1956), and Weller et al. (1957) all independently isolated CMV. During the next several years, serologic methods were developed and in the early 1960s, the studies of Weller and Hanshaw (1962), and Medearis (1964), employing viral isolation and serology, established CMV as an important pathogen in the human.

Characteristic of Human Cytomegalovirus

CMV is one of the five member of the human herpesviruses (Herpes Simplex Virus type 1 and type 2, Cytomegalovirus, Varicella-Zoster Virus and Epstein-Barr Virus) and shares many of the biologic characteristics possessed by the herpesvirus family. It has additional unique characteristics that will be delineated. Weller (1970) listed three criteria for identifying cytomegaloviruses: first, a tendency to cause infection in the salivary gland; second, a tendency to grow slowly only in cell cultures derived from their natural species; third, a tendency to form cytoplasmic inclusions in addition to intranuclear inclusions.

In 1979, the International Committee for the Nomenclature of Viruses (ICNV) swung back towards a rehabilitation of the Cytomegalovirus group which was in subfamily Betaherpesvirinae and Mathew (1979) summarized the main characteristics of the CMV group as follows:

1. Properties of the virus particle: Its DNA has a molecular weight of 130-150 X 10^6 with 50% G + C. Sequences from either or both termini may be present in an inverted form internally.

2. Replication: The CMV group have a relatively slow reproductive cycle (more than 24 hours), forming slowly progressing foci in cell culture. Enlargement of the infected cell occurs in vivo and often in vitro (cytomegalia). Inclusion bodies containing DNA may be present in the nuclei and cytoplasm late in infection. Latent virus infection is frequently present in the salivary gland and/or other tissues.

3. Biological Aspects: The host range is narrow; in cell culture, CMV usually grows best in fibroblasts, but exceptions exist.

The genus Human Cytomegalovirus Group, and type species Human (beta) Herpesvirus 5 (Human Cytomegalovirus) have the following main characteristics: DNA molecular weight = 150 X 10^6. Virus recovered only from human infections. Experimental host range narrow; grows best in human fibroblasts and less well in certain human lymphoblastoid cells.

Morphology

CMV is a double-stranded DNA virus that is approximately 200 nm in diameter, making it the largest member of the human herpesvirus family and one of the largest animal viruses. CMV consists of a 64 nm core containing the viral DNA enclosed by a 110 nm icosahedral capsid made up of 162 capsomers (Smith and de Harven, 1973, Wright et al., 1964). The complete particle is enclosed by an envelope, consisting of at least 25-30 virion-encoded protein and

glycoproteins (Sarov and Abady, 1975, Stinski, 1976, Stinski, 1977). The linear of double-stranded DNA of CMV is approximately 240 kilobases (kb) in size, making it about 50% larger than the genome of HSV (De Marchi et al., 1978, Geelen et al., 1978, Kilpatrick and Huang, 1977). The icosahedral structure surrounding the virion core contains two major structural proteins, the major and minor capsid protein (Sarov and Abady, 1975, Stinski, 1976, Stinski, 1977). Outside the virion capsid, two matrix proteins form a bridge between the capsid and virion envelope. The CMV envelope is a complex structure and consists of at least 6 glycoproteins (Stinski, 1976). It is believed that these glycoproteins express antigenic sites for neutralizing antibodies.

Properties

Because CMV is an enveloped virus, it is sensitive to fat solvents and extreme physical conditions. CMV is completely inactivated by exposure to 20% ether for 2 hours (Rowe et al., 1956), to pH of less than 5, to 56°C for 30 min (Weller et al., 1957) or to UV-light for 5 min (Albrecht and Rapp, 1973). CMV does not withstand freezing and thawing or storage at -20°C to -50°C without stabilizers (Weller et al., 1957). The addition of 5-10% serum to the diluent stabilizes the virus at 37°C but not at 4°C and infectivity is better preserved at each temperature when the virus is suspended in distilled water (Vonka and Benyesh-Melnick, 1966b), or in the medium without $NaHCO_3$ in the presence of 35% sorbitol (Vonka and Benyesh-Melnick, 1966a). Virus infected cells suspended in Eagle MEM

(Minimum Essential Medium) with 10-20% serum and 10% DMSO (Dimethyl Sulfoxide) can be stored with minimal loss of infectivity in liquid nitrogen (-190°C) (Reynolds et al., 1979).

Replication

Following absorption, possibly through specific cell surface receptors, the virus uncoats within the cytoplasm and the nucleocapsids then proceed rapidly into the nucleus (Smith and de Harven, 1974). Shortly thereafter, viral specific RNA can be found, indicating a rapid expression of the viral genome (De Marchi et al., 1980, Wather and Stinski, 1982). Viral nucleic acid replication probably begins as early as 12 hours postinfection and it is readily detectable at 24-36 hours postinfection (Stinski, 1978). Infectious particles are released approximately 72 hours after infection (McAllister et al., 1963).

Strain Variation

Antigenic variations of human cytomegaloviruses were first detected by cross-neutralization tests (Ho, 1982). Weller et al. (1960) reacted sera from two babies, named Davis and Espaillat, with isolates from themselves, with isolates from each other, and with AD 169 (an isolate from adenoids by Rowe et al., 1956). The Davis and AD 169 strains were designated types 1 and 2, representing the most diverse strains but the AD 169 strain has been widely used as the antigen in serological tests. Esp. and Kerr isolates (closely related to the Esp. strain) were designated type 3. These type designations have not been generally accepted because antigenic variations among CMV isolates are not great enough or clear enough to

warrant the designation of different types of human cytomegaloviruses. Therefore, the significance of the observed differences has been difficult to evaluate. Other methods of analysis, particularly restriction endonuclease digestion patterns and nucleic acid hybridization studies, confirm the variability of the virus genome but do not indicate that variation are great enough or uniform enough to assign different prototypes. However, there is no need to worry about type or strain variations for practical diagnostic purposes and the serologic tools for the diagnosis of CMV infection are about as good as those for any other infections.

The Detection of Cytomegalovirus Infection

The detection of CMV infection in humans has utilized both viral isolation and serologic procedures (Alford, 1984). Isolation of virus is the most specific method to establish the diagnosis of HCMV infection. The virus can be recovered from several body fluids e.g. urine, vaginal and cervical secretions, semen, stool, saliva, tears and breast milk. It can also be obtained from tissues taken at biopsy or autopsy (Reynolds et al., 1979).

Human Cytomegalovirus (HCMV) does not grow adequately in any laboratory animal (Ho, 1982). All specimens submitted for virus growth or identification must be inoculated in cell cultures. The only cells that should be used in the diagnostic laboratory to grow HCMV are human fibroblasts which may be obtained as primary cultured prepared from foreskin or embryonic skin and muscle, lung, testes or myometrium. Serially propagated diploid fibroblast cell strains, either developed in one's own laboratory or obtained commercially,

such as the WI-38 or MA-184 human embryonic fibroblasts derived from lung or foreskin, are equally satisfactory. Virus isolation is best achieved by direct inoculation of fresh materials into cultures of human fibroblasts (Ho, 1982). Storage even by rapid freezing of suspensions prepared in sorbitol may result in sufficient loss of infectious virus to prelude isolation from samples containing small amounts of virus. Infectivity of specimens is rapidly lost on drying or hard surfaces or in cloth materials such as diapers (Gold and Nankervis, 1982).

Cultures inoculated with virus-containing material may show cytopathic effect (CPE) in 3-4 days, but usually in 1-2 weeks or longer, depending on the concentration of virus in the specimen (Ho, 1982). Initially, foci of enlarged rounded refractile cells will appear in the center rather than the peripheral of the cell monolayer. Affected cells characteristically follow the linear pattern in which fibroblasts align themselves. These foci will enlarge, coalesce and may eventually destroy the monolayer, a process that may take 2-3 weeks. At times, foci of infection will enlarge but never involve the entire monolayer unless the infected cells are passed. Occasionally, CPE will not be produced in the originally inoculated cultures. When such cultures are trypsinized and planted on a fresh monolayer, CPE may occur. The pattern and progression of cytopathology is different from that of Herpes Simplex and Varicella-Zoster Viruses. That of Herpes Simplex appears sooner and progresses more rapidly. It may be visible in 24-48 hours, and cell destruction may be complete in 3-4 days. Varicella-Zoster Virus, on the other hand, produces CPE at an intermediate rate and may also grow in human epithelial cell cultures in addition to fibroblasts.

It is also rarely cultured from any source but a vesicle, which is not a lesion usually produced by CMV infections.

The various serologic assays for the diagnosis of CMV infection have been employed. Detection of antibody will identify the individual with recent or remote infection and is the gold standard to indicate frequency of latent CMV infection.

The Complement Fixation Test (CFT) is one of the more commonly used serologic tests in detecting the antibody response to CMV infection, using both glycine extract (GE) and freeze thaw (FT) preparation of CF-antigen (Betts, 1984). Initial studies using FT antigen are misleading because the reaction is insensitive. Subsequently, it has been suggested that the GE antigen provides more reproducible results (Stagno et al., 1981). The CFT with GE antigen is more sensitive for distinguishing seropositive from seronegative specimens (Kettering et al., 1977) whereas the FT antigen is slightly better for detection of fourfold or greater titer rises (Cremer et al., 1978). Choice of antigen preparation should in part depend on the purpose of the serological test. However, the CFT is the method of choice for most clinical laboratories (Starr and Friedman, 1980).

Immunofluorescent Technique using either anti-IgG or anticomplementary antibody with fluorescein label and using either whole infected cells or isolated nuclei of infected cells have proven useful (Betts, 1984). The technique of Anticomplementary Immunofluorescence (ACIF), which is read as nuclear fluorescence, is a particularly sensitive and specific test for antibodies to CMV (Rawls and Campione-Piccardo, 1981, Reeves et al., 1981).

The Indirect Immunofluorescent Antibody Test (IFA-Test) is a sensitive and broadly method for determining antibody levels to CMV. The procedure is rapid and permits measurement of antibody belonging to specific immunoglobulin classes. The IFA-Test can be used to measure CMV antibody in the IgM class of immunoglobulins, using an anti-IgM conjugate, in order to investigate the immune response in newborns when the cord sera are available (Waner et al., 1980). Furthermore, the IFA-Test has also been used for detection and identification of CMV in cell cultures, but the assay should be performed on monolayers of infected cells which are difficult to prepare (Starr and Friedman, 1980). However, the main disadvantage of this test is that the specific nuclear fluorescence which indicates a positive result is sometimes difficult to distinguish from non-specific cytoplasmic fluorescence because CMV infection of human fibroblasts induces a Fc receptor in the cytoplasm of infected cells which binds viral and non-viral IgG antibody, occurring false positive results in the IFA-Test (Keller et al., 1976). In addition, these techniques of immunofluorescence can be utilized to detect the early antigens of CMV (Stagno et al., 1975a).

Neutralization of CMV utilizing a plaque reduction assays, rarely used in routine diagnostic serology, remains a highly sensitive and specific method for the determination serologic reactivity to CMV (Reynolds et al., 1979). Indirect Hemagglutination and Immune Adherence Hemagglutination, the less common used methods, have proven useful in seroepidemiological studies of CMV (Jordan et al., 1973, Reynolds et al., 1979, Yeager, 1979).

Radioimmunoassay techniques (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA) antibody techniques have more recently been studied and have proven to be useful (Betts, 1984).

Virtually all of these methods are nearly equally sensitive at detecting the individual who is infected, identifying virtually all infected individuals, although absolute titers vary using different techniques.

Pathology

There are many gaps in knowledges about pathogenesis of human cytomegalovirus infections which are wide-spread and usually asymptomatic. The incidence and spectrum of disease in newborns establish CMV as an important human pathogen (Starr, 1979). The virus commonly produces latent infections which frequently may be activated by pregnancy, multiple blood transfusions or immunosuppression for organ transplantation (Ginsberg, 1980).

The clinical manifestations of HCMV infection vary with the age at acquisition as well as the immune competence of the individual. The great majority of persons acquiring primary infection suffer no acute illness irrespective of age, including congenital infection (Reynolds et al., 1979).

Congenital Infection

Congenital infection refers to those acquired in utero or before birth and it is transmitted transplacentally. CMV is currently recognized as the most common cause of congenital viral infections in man. It occurs in approximately 1% of all newborn

infants (Stagno et al., 1984). Fewer than 5% of those infants infected develop symptoms (typical cytomegalic inclusion disease) during the newborn period, another 5% have atypical clinical involvement and 90% have no clinical manifestation at birth (Stagno et al., 1984, Starr and Friedman, 1980). Symptomatic infants with severe intrauterine infection may die of complications within the first month of life, however, death is uncommon. Clinically apparent infections are characterized by involvement of multiple organs, in particular the reticuloendothelial and central nervous systems (Berenberg and Nankervis, 1970, Gold and Nankervis, 1982, Hanshaw, 1971, Hanshaw and Dudgeon, 1978, Hanshaw et al., 1975, Kumar et al., 1973, McCracken et al., 1969, Medearis, 1964, Pass et al., 1980, Weller and Hanshaw, 1962). A combination of petechiae, hepatosplenomegaly, and jaundice is the most frequently noted presenting signs. The organ system involvement is additionally noted by the occurrence of microcephaly with or without cerebral calcification, intrauterine growth retardation and prematurity. Inguinal hernia in males and chorioretinitis are less common (Stagno et al., 1984). Ten to 25% of the silently infected newborn may later develop sensorineural hearing deficits and 5-10% may suffer from various degrees of psychomotor disability (Hanshaw et al., 1975, Melish and Hanshaw, 1973, Reynolds et al., 1974).

Perinatal Infection

Perinatal infection refers to those acquired either during the course of delivery from exposure to infected maternal genital secretions or during the postnatal period from ingestion of breast milk that contains CMV (Stagno et al., 1984). Perinatal CMV

infection is principally a result of maternal-infant transmission, although in hospitalized infants blood transfusions are an important iatrogenic cause of CMV infection (Ballard et al., 1979, Stagno et al., 1980, Yeager et al., 1981). Stagno et al. (1980) reported that the two most efficient sources of transmission were infected breast milk, which resulted in a 63% rate of perinatal infection and the infected genital tract, particularly in late gestation, which was associated with transmission in 26% and 57% at the third trimester and, the third trimester and postpartum, respectively. Such infants begin to excrete virus at 3-12 weeks of age, but remain asymptomatic (Starr and Friedman, 1980). Perinatal infection can also be acquired through transfusion of blood and is dependent upon the number and volume of the transfusion. Yeager et al. (1981) reported a study in which 13.5% of infants of seronegative mothers who were exposed to one or more seropositive blood donors acquired CMV infection. The risk of infection increased to 24% for those patients who received more than 50 ml of packed red blood cells and who were exposed to at least one seropositive donor. The seronegative infant who is infected by blood transfusion may develop an illness characterized by the development of shock-like symptoms accompanied by a gray pallor, respiratory distress and variable involvement of the reticuloendothelial system similar to that seen with milder forms of congenital infection, although generally self-limitted death rates as high as 20% have been reported (Yeager et al., 1981).

Postnatal Infection

Postnatal infection refers to those acquired later in life. Most postnatal infections are presumably acquired close contact with individuals who are shedding virus (Starr and Friedman, 1980). The vast majority of children and adults who acquire CMV infection postnatally remain asymptomatic. Since CMV has been detected in several body fluids, including saliva, urine, breast milk, cervical secretions and semen, transmission can probably occur in a variety of ways, including by venereal disease. Infections in those patients may be due to primary infection, reactivation of latent virus (endogenous virus), or reinfection with exogenous virus.

When symptomatic primary infection does occur, whether male or female, pregnant or non-pregnant, the characteristic clinical manifestations are fever, malaise, myalgias, arthralgia, and an enlarged liver and spleen (Betts, 1984). The characteristic laboratory abnormalities are liver enzyme increases, particularly alkaline phosphatase, and atypical lymphocytosis. Viremia is detectable for a few weeks to a few months (Klemola et al., 1969, Knox et al., 1979, Rinaldo et al., 1977) and virus isolation is helpful but it is not a conclusive diagnostic aid for primary infection, since asymptomatic individual also can excrete CMV from multiple sites. Consequently, demonstration of brisk seroconversion is generally considered the best means of proving primary involvement.

Recurrent CMV infections, which in normal people are virtually always subclinical, are far more common than primary infection in most adult populations because of the high rates of

acquisition of primary infection during childhood or adolescence (Alford et al., 1980, Stagno et al., 1982a, Stagno et al., 1982b). The most popular concept for recurrent excretion of CMV in asymptomatic persons is that CMV may become latent in various organs during the primary infection and be repeatedly reactivated in later life in response to different stimuli, including pregnancy, immunologically compromised hosts, particularly recipients of organ transplantation or of multiple blood transfusion, and patients with malignancy. Because of the subclinical nature and lack of change in antibody status in normal hosts with recurrent CMV infections, this expression of infection can be detected only by mass screening for virus excretion, which is impractical.

Mononucleosis Syndrome

Acquired CMV infection may be asymptomatic, or produce a mononucleosis syndrome. Klemola and Kaariainen (1965) first described a series of patients with a mononucleosis-like syndrome characterized by fever, malaise, mild hepatitis and atypical lymphocytosis in the absence of pharyngitis, and significant adenopathy. Unlike Epstein-Barr Virus (EBV) infection (infectious mononucleosis), sore throat and lymphadenopathy are uncommon, and objective pharyngitis and exudative tonsilitis are rare. Aytpical lymphocytes are very similar in appearence to those that occur in EBV mononucleosis (Betts, 1984). CMV is responsible for approximately 70% of cases of heterophile-negative mononucleosis. Laboratory studies reveal a high percentage of atypical lymphocytes in the smears of peripheral blood, a negative Paul-Bunnell Test, an increase in cryoimmunoglobulins, and serological evidence of recent CMV

infection (Sullivan and Hanshaw, 1982). The illness generally improves within 3-6 weeks with a return of normal liver-function tests and the disappearance of atypical lymphocytes from the peripheral blood (Gold and Nankervis, 1982). Besides the characteristic mononucleosis syndrome mentioned above, there are several other manifestations of primary CMV infection. Neurologic manifestations head the list. The Guillian-Barre Syndrome has been clearly shown to be related to CMV infection (Betts, 1984).

Cytomegalovirus and Malignant Disease

The patients with malignancy are frequently severely immunosuppressed and may have protracted illness with CMV infection (Cangir and Sullivan, 1966). In most instances, this infection represents reactivation of endogenous infection. Seronegative individuals are at risk for primary infection. Clinical disease associated with CMV infection has been reported in patients with leukemia (Benyesh-Melnick et al., 1964, Henson et al., 1972), Hodgkin's disease (Hanshaw and Weller, 1961), lymphosarcoma and solid tumors (Duvall et al., 1966). The leukemia children were reported with CMV antibody in normal frequency, virus excretion in up to 25%, pneumonitis, viremia and fever (Benyesh-Melnick et al., 1964, Henson et al., 1972); including chorioretinitis, proctitis and mononucleosis (Cox and Hughes, 1975).

Epidemiology

Infection with human cytomegalovirus appears to be worldwide and common. CMV can be transmitted to individuals in a variety of different ways. It requires close contact for transmission to a

susceptible individual. It can cross the placenta and cause intrauterine infection, or pass to infants as they traverse the birth canal (Betts, 1984). CMV is acquired orally in breast milk or by person-to-person oral transmission, especially in young or elder individuals. It has also been shown to be transmitted in blood transfusion and via organ transplantation. Using antibody as a marker of CMV infection demonstrates that 40-60% of individuals have been infected by the age of 5 years, and nearly 100% by the age of 20-25 years. Various data indicate that between the age of 20 and 40 years, the majority of transmission of CMV is by sexual contact and women are at a higher risk than men. After the age of 40 years, there is probably transmission via more casual contact (Betts, 1984).

The prevalence of antibodies and of prior infection by CMV vary with the socioeconomic basis of the population group (Ho, 1982). Age also effects virus shedding. Epidemiological data from antibody prevalent studies have shown that age-related prevalence of CMV infection varies among different population groups (Alford et al., 1981, Krech et al., 1971, Lang, 1975, Pass, 1985). The age of the mother and her prior experience with CMV are certainly important factors. Younger seropositive women who breastfeed are at the greater risk for transmitting virus in early infancy, especially in lower socioeconomic groups (Stagno et al., 1984). It is interesting that in Japan (1970), Thailand (1970), Guatamala (1977), and Finland (1972, 1977); the rate of perinatal infections as shown by viruria during the first two years of life is high (39-56%). The women with primary CMV infection had cervical cultures throughout pregnancy. The frequency of isolation of CMV from the cervix of pregnant women varies with geographic locality,

socioeconomic status and sexual promiscuty. Therefore, the uterine cervix infected with CMV may be important as a source of infection during childbirth. Besides the transmissions mentioned above, the respiratory route is suspected as one method of CMV circulation. Olson et al. (1970) found CMV in the respiratory tract in 21.2% of Thai children less than 4 years of age. Recovery rate was highest from infants less than 1 year of age. Fourty percent of these with upper respiratory infection, 33% with pertussis, and 15% of well babies were respiratory carriers. This work suggests that CMV may cause or accentuate respiratory infection, or that respiratory infection by other agents may predispose to CMV infection, and crowding and living in depressed socioeconomic conditions have been shown to facilitate dissemination of CMV. However, it has been reported that CMV can be transmitted in a number of ways but none of which has been completely defined yet.

Treatment and Prevention of Cytomegalovirus Infection

At present, there is no effective treatment for CMV infections in humans. Vaccination is the main focus of attention currently (Plotkin et al., 1981) because the infection in the adult female population is silent and the possibility to protect future fetuses is necessary. Currently two attenuated (live) vaccines are available, one developed by Elek and Stern (1974) from strain AD 169 and the other developed by Plotkin and colleagues (Plotkin et al., 1975) from the Towne strain. In normal persons the vaccines are immunogenic as regards the development of antibody and lymphocyte blastogenic responses to CMV antigens at least for 2 years interval (Alford, 1984). Further studies are necessary before immunization of

normal females who may be at risk for primary infection during pregnancy can be initiated. However, the efforts to date of CMV vaccine development in humans have yielded mixed results and little reassurance that immunologic success would necessarily be paralleled by the desired protection against CMV virulence in specific hosts (Osborn, 1981).

The purpose of immunization is to produce acquired specific immunity against CMV infection. The role of antibody in the control of CMV infection is incompletely understood. Hyperimmune globulin against CMV might be effective in preventing primary infection in patients at risk such as seronegative recipients of organ transplants. It would not be expected to be effective in preventing the reactivation of latent infection in seropositive subjects, as they already have circulating antibodies. In any event, passive immunization has not been tried for the control of congenital or perinatal CMV infection. However, if it does not prevent infection, it might reduce the morbidity of infection.

Because of the unresolved problems concerning the development of a CMV vaccine, other methods of intervention are being investigated. Antiviral agents are also being used in the treatment and prevention of CMV infection in transplant recipients and other seriously affected patients. Nucleoside antiviral agents have been used in CMV infection in normal and in immunosuppressed subjects (Ho, 1982). The experience with cytosine arabinoside (ara-C) is that it will suppress virus concentration (e.g. viruria in congenital CMV disease) but rarely cure the infection (suppressed but not eliminated) (McCracken and Luby, 1972). Idoxuridine also decreased

viruria in the patients with congenital CMV disease (Barton and Tobin, 1970). Adenine arabinoside (ara-A) is ineffective in eliminating CMV in the immunosuppressed, and its transient virostatic effect appears to be without clinical benefit. Acycloguanasine (Acyclovir) is specifically activated (phosphorylated) in infected cells by virus-induced thymidine kinase (Elion et al., 1977, Schaeffer et al., 1978). It is much less effective in the laboratory against CMV, which is to be expected since this virus does not induce its own thymidine kinase.

Finally, interferon (IFN) is another method for the treatment of CMV infection. IFN, a natural antiviral protein made from human leukocytes or other virus-infected cells, is not very effective in suppressing CMV infection either in cell culture or in laboratory animals. CMV is more resistant to IFN than other viruses (Glasgow et al., 1967). IFN, at least in the doses given, was inadequate in controlling neonatal CMV infection. It would be instructive to test the effect of large doses of pure recombinant IFN, which is now available. Toxicity and variability of IFN preparations, combined with the highly variable effect on viral excretion and the lack of controls, have rendered these small studies difficult to interpret. However, the use of leukocyte IFN has yielded mixed results and further studies of IFN in the prevention and treatment of CMV infections are in progress (Rubin et al., 1979).

Cytomegalovirus During Pregnancy

Being the most ubiquitous genitourinary tract virus and causative agent of the most prevalent fetal infection, awareness and concern of CMV among pregnant women is becoming more wide-spread. The mother who has CMV infection may excrete virus in cervical excretion and urine of which the neonate may become infected during passage through the birth canal (Numazaki et al., 1970). Montgomery et al. (1972) studied the cervical and urinary excretion of CMV in pregnant women. It was recovered more frequently from the cervix (8%) than from urine (3%). The frequency with which CMV can be isolated from the pregnant woman appears to vary with age, parity, socioeconomic status, site cultured, and time during gestation but viruria has been found in 3-12% of a large number of pregnant women (Feldman, 1969, Hildebrandt et al., 1967, Kriel et al., 1970, Montgomery et al., 1972, Reynolds et al., 1973). Serological studies have shown that about 60% of women entering the childbearing years have antibody to CMV (Kriel et al., 1970, Stern and Tucker, 1973). Further studies suggest that primary CMV infection during pregnancy may carry a significant risk, the incidence is approximately 0.5% overall. Nankervis et al. (1974) reported that the women with primary CMV in the first trimester had CMV-positive urine, throat and cervical cultures throughout pregnancy, but delivered a normal uninfected infant. The infection risk of an infant born to a mother who had primary CMV infection during pregnancy was more likely to be associated with infection late in pregnancy. In another prospective study, by Stern and Tucker (1973), suggested that the delivery of an infected baby was associated with primary infection early in pregnancy and in populations in which the opportunity of exposure was

high, the small proportion of women without CMV antibody entering childbearing age appeared to have a high risk of contracting primary CMV infection during pregnancy and of delivering an infected infant. However, in both studies regardless of time of gestation maternal infection was acquired, women excreting virus at the time of delivery were most likely to have infected infants.

Asymptomatic CMV infection has been best defined in young pregnant women. It is clear that subclinical infection is common during pregnancy (Montgomery et al., 1972, Numazaki et al., 1970, Stagno et al., 1982b, Stagno et al., 1975b, Stern and Tucker, 1973) and reactivation or persistence of virus is more common than reinfection (Huang et al., 1980). With recurrent infection, virus is intermittently excreted from multiple sites. Cervical shedding has varied from 3% to 18% (average 9%) and urinary excretion has been detected in from 3% to 9% (average 3.5%) (Reynolds et al., 1973, Stagno et al., 1982a). Recovery from the cervix is more frequent than recovery from urine. The frequency of positive cultures of the cervix increases as gestation progresses (Montgomery et al., 1972, Numazaki et al., 1970, Reynolds et al., 1973) and appears to be higher in younger mothers (<25 years) with fewer than four pregnancies (Montgomery et al., 1972). Numazaki et al. (1970) first showed that CMV infection of uterine cervix varied during pregnancy. Montgomery et al. (1972) and Stagno et al. (1975b) later independently studied the cervical and urinary excretion of CMV in pregnant women and reported the results as previously described by Numazaki et al. (1970). The three studies of cervical infection in the three trimesters show that women in late pregnancy have higher rates of cervical infection. The rates increased from 1.5%

in the first trimester to 13.5% in the third trimester with an overall rate of 10%. These finding suggested an alternative hypothesis for the gestational influence, suppression of productive infection in early pregnancy that progressively wanes as gestation advances (Stagno et al., 1975b). Besides the gestational influence, age has the opposite influence on reactivation of CMV, which diminishes steadily from puberty to age 30, after which viral excretion (from these sites) is very infrequent (Knox et al., 1979). Therefore, gestation and age both have profound effects on reactivation of CMV. Reynolds et al. (1973) offered the hypothesis that the increasing rate of cervical and urinary excretion as gestation proceeded and the high rate of virus excretion in breast milk were the result of reactivation of latent CMV infection perhaps provoked by the mild immunosuppression and hormonal changes which occured during pregnancy.

In 1972, Monif et al. followed 664 pregnant women with CF-titer of less than 1:8 at bimonthly interval, and found that there was 100% correlation between primary CMV infection in the mother and congenital infection in the baby. Additionally, infection in the second trimester was more serious than infection in the third. In 1973, Stern and Tucker studied 1040 pregnant women in London and found that 56% of the white and 90% of the Asian women were seropositive. Primary infection, detected by CFT, occurred in 3% of the 254 white and 19% of the 16 Asian women. About 46% of their offsprings had viruria. In 1976, Gold and Nankervis identified 8 primary infection among more than 3000 pregnant women and found that 4 infected babies were produced with viruria but were clinically normal. These reports indicated that the rate of primary infection

of the mother would clearly vary with the immune status of the population and the intrauterine infection following primary infection in the mother was high (about 58%). In 1981, Ahlfors et al. reported a case of symptomatic congenital infection in a baby whose mother was seropositive before pregnancy.

In Thailand, only a few reports have been documented the evidence of CMV infection. Tantivanich et al. (1981) studied the prevalence of CMV antibodies in Thai population between the age of 1 day to 50 years, using Enzyme Linked Immunosorbent Assay (ELISA). The variable seropositive rates (31-64%) were found in different age groups with the highest seropositive rate (60-64%) in the age group between 6 months to 6 years, and the overall seropositive rate of 41%. In 1982, Tantivanich et al. showed 95% of seropositive rate for CMV total antibodies among Thai pregnant women, and the majority of them had positive CMV-IgM antibodies (83%). In 1983, Puthavathana et al. studied the prevalence of CMV-antibodies during various stages of gestation among pregnant women, 18-35 years of age; and showed 74.28% of CMV-antibodies in the first trimester, 81.48% in the second and 90.9% in the third, with the overall rate of 83.87%, using Complement Fixation Test (CFT). In addition, 10.2% of infants with signs of intrauterine infection (hepatomegaly with or without splenomegaly) showed antibody to CMV (Tantivanich et al., 1980).

The purpose of this study is to determine the virological and serological status of CMV in pregnant women. The recovery of CMV from the cervix of pregnant women in different periods of normal gestation was determined by virus isolation, employing human foreskin

fibroblast cell culture, African Green Monkey Kidney Cells (Vero-cells) and together with the Indirect Immunofluorescent Antibody Test (IFA-Test) for identification; and the frequency of cervical shedding of CMV related to trimesters of pregnancy was determined. Total antibodies against CMV in the sera were detected by the Complement Fixation Test (CFT) and the association of CMV recovery from the cervix with the presence of antibodies among the pregnant women were also determined. In addition, virus isolation from the cervix of married women were also performed, as group control.