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Viral Teratology

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INTRODUCTION

Congenital malformations account for approximately 14% of all deaths within the first year of life in the United States (19). In a recent study of 45,000 pregnancies from the Collaborative Perinatal Research Study sponsored by the National Institute of Neurological Diseases and Stroke, a rate of 414 malformations per 10,000 births (4.1%) was found (8).

Very little data are available to estimate the relative importance of viruses as causes of malformations. Certainly, several viruses have been well established as causes of severe deformities in man. The role of viruses in producing the more subtle fetal defects is just being investigated. For example, the frequency of loss of hearing as the only manifestation of the rubella syndrome was recently documented by long-term studies of patients with serologically and virologically documented infections (91).

A very significant gap in our knowledge is the role of perinatal viral infections in mental retardation. Of the 3.5 million children born each year in the U.S., approximately 3% are mentally retarded (82). It appears that as many as 10% of these cases may be attributed to infectious diseases (23). Specific studies of these preventable causes of mental retardation are needed to define this group precisely and to institute appropriate measures for treatment or prevention.

Information available on viral teratogens is still limited, but is continuously expanding as knowledge is acquired about the fetal response to infection as well as viral biology and immunology. Control and prevention of viral infections having teratogenic potential require: (i) recognition of the causal relationship between the infection and the affected fetus or child; (ii) understanding the mechanisms responsible for the infection and damage; (iii) knowledge of the biological characteristics of the viruses involved and their mechanism of transmission (88). Clinical and subclinical viral infections of the mother may lead to the invasion of the fetus. Thus, the identification of viruses which have teratogenic potential is often difficult, since many of the subclinical infections go unreported. Adequate data for both clinical

and subclinical infections can be obtained only by use of prospective studies employing both virus isolation and serological studies. The use of new animal model systems now being developed should also help in the understanding of viral effects on the fetus (90).

The frequency of clinically recognized infections during pregnancy has been determined in the Collaborative Perinatal Research Study (92). Although the figures are at best an approximation, they do provide an idea of the relative frequency of infection in the U.S. The study population included 30,000 pregnancies registered between October 1958 and February 1964. In this study population, approximately 5% of the patients reported definite or presumed viral infections. Excluding the common cold, the most frequent infections were influenza, flulike illnesses, herpesvirus infections, viral gastroenteritis, and viral tonsillitis. The specific infections of mumps, rubella, chicken pox, viral bronchitis, measles, viral conjunctivitis, viral pneumonitis, and infectious mononucleosis occurred at rates between 1 and 15 per 10,000 pregnancies. Serological tests done on serial blood specimens substantiated reported infections in about two-thirds of 45 rubella cases, one-fifth of 11 rubeola cases, and almost three-fourths of 46 mumps and varicella zoster cases. The data from clinical recognition alone frequently incorrectly estimated true frequency of infection, as indicated by the serological studies. Combined clinical reporting and serological information permitted the development of minimum estimates of frequency: thus, for 10,000 pregnancies under nonepidemic conditions, the minimum frequency of maternal infections was: mumps, 10; rubella, 8; varicella zoster, 5; and rubeola, 0.6. During epidemic years, the frequency of clinical rubella may have reached 220 per 10,000. However, since about one-third of the adult infections are subclinical, the total epidemic frequency might have been as high as 330 per 10,000.

Viral infections which are known to produce malformations in the human embryo include rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV). A number of other viruses are known to be associated with an increased incidence of illness or fetal death. Others may be implicated but, at present, data are insufficient to establish a clear causal relationship between the infection and the effects in the child. The present report summarizes some of the information now available for these agents and their effects on the developing fetus.

MECHANISMS OF THE FETAL DAMAGE

Some viruses can infect the fetus at any time during gestation, and the frequency and type of defects produced often depend upon the time of maternal infection. If morphogenesis is incomplete at time of virus infection, pregnancy may terminate in abortion, malformations, degenerative lesions of the fetus or stillbirth. If infection takes place after the fetus has completely developed, results may be abortion, stillbirth, prematurity, or pathogenic lesions. Malformations (62) such as hydrocephalus due to stenosis of the aqueduct of sylvius may be restricted to infection at a late developmental stage.

Diseases associated with congenital infection can be divided into two basic types; one resembling the disease seen in the adult and the other which is unique and characteristically different from that of the adult. In utero infections resembling the adult disease include polio, Western equine encephalitis, and smallpox. The presence or absence of receptor sites may account for this susceptibility or resistance of tissues to certain viruses (54). The unique fetal type of diseases caused by viruses which usually have limited pathogenicity in adults are notably rubella, CMV, and disseminated infections due to herpesvirus and chickenpox.

A number of factors are thought to be responsible for the increased susceptibility of the fetus to certain viruses. First, all of these viruses pass through the placenta and replicate in the fetus. The lack of antibody production until about 20 weeks of gestation and the insignificant amounts of circulating leukocytes until about the 12th week of gestation probably contribute to the dissemination of virus throughout the fetus. Rapidly dividing cells, such as those found in a developing fetus, are known to be more favorable for the cultivation of viruses. Also, these fetal tissues produce less interferon than adult tissues.

Many possible mechanisms of fetal damage have been proposed for the teratogenic capabilities of viruses. Only limited data are available to support any hypothesis. One of the mechanisms involved with fetal damage is cellular necrosis that is caused by the action of viruses in fetal tissues. Lytic viruses are associated most often with this mechanism, however, nonlytic viruses are not excluded. Herpesvirus, varicella, vaccinia, variola, and coxsackieviruses are examples of highly lytic viruses which can produce severe intrauterine or neonatal

infection, with or without malformation and death (40). Viruses such as rubella and CMV, which produce lesser degrees of cellular necrosis and cell lysis, more characteristically produce widespread malformation with variable degrees of tissue damage. These effects are thought to result from the interference of growth or maturation of tissue with persistence of infection (56). The limited replicative capacity of infected cells is probably the basis for decreased growth and specific malformations (81).

Another possible mechanism of fetal damage, although highly speculative, is that the virus may produce chromosomal injury and thereby induce congenital malformation or abortion (101). Rubella virus grown in vitro has been shown to inhibit mitosis and increase the number of chromosomal breaks (78). Herpesvirus and rubeola have also been shown to produce abnormal mitosis (72). To date, however, no such changes have been found in vivo.

Generalized vasculitis of the placenta and the fetus is another theoretical mechanism which may be associated with congenital malformations (62). In cases of congenital rubella, vasculitis of cerebral arteries and stenosis of large blood vessels have been reported (103). Vascular lesions of the cerebral circulation may lead to the production of small foci of calcification. This same mechanism may be involved in congenital cytomegalic disease, as well as in herpesvirus type II malformations (98). Intravascular coagulation may be manifested by clinical signs such as thrombocytopenia and purpura (38). This mechanism could account for the similarities of the clinical manifestations of rubella, CMV, and herpesvirus. Thrombosis and vasculitis of the small blood vessels could produce organ hypoxia, and thus reduce the rate of cellular division. This same effect could take place with placental infection and produce indirect damage without fetal involvement (62).

VIRUSES TERATOGENIC IN MAN

Rubella

Gregg, in 1941, initially reported the association between maternal rubella and congenital malformations (30). Originally, it was thought that all fetal defects were produced during infection in the first trimester. It is now clear that fetal infection following rubella in the second trimester can produce less obvious, but nevertheless severe, damage of the fetus (58, 61). The major damage to the fetus include heart lesions, cataracts, deafness, microencephaly, and mental retardation (64, 83). Approximately 50% of mothers infected in the first month of pregnancy have abnormal children, 22% in the second month, and 6 to 8% in the third through the fifth months. Defects associated with rubella in the second trimester involve problems of communication based on hearing loss, mental-motor retardation, and eye defects. Children with congenital rubella frequently remain infected for 6 months or longer after birth. Virus may be present in the naso-pharynx, lymphocytes, or spinal fluid. By 1 year of age, only about 10% are still infected. However, in one reported case, the virus was isolated from a cataract of a 3-year-old child (60). Most children shed virus while they have high circulating antibody titers, indicating a possible defect in the cellular immune mechanism.

Live vaccines produced in duck and rabbit tissue culture are now being administered to millions of children to reduce the susceptible pool and thereby reduce the exposure of pregnant women to the wild virus. Only limited data are available on the possible transmission of the vaccine strain of the virus to the fetus. Therefore, it is imperative that pregnant women do not receive the vaccines.

Cytomegalovirus

The major manifestations of congenital cytomegalic inclusion disease (CID) include microcephaly or hydrocephaly, chorioretinitis, blindness, hepatosplenomegaly, encephalitis with cerebral calcifications, and mental retardation. Occasionally, these children have pneumonia, anemia, thrombocytopenia with petechiae at birth, and rarely congenital heart disease (33).

One study has reported that congenital CID infection accounts for 1 or 2% of all infant deaths (70). A high percentage of the infants who survive often have neurological sequelae that include blindness and mental retardation (34). In one study, approximately 10% of microcephalic mentally retarded children had serological evidence of CID infection (100). Further investigations are needed to define more precisely the relative importance of CID in the production of mental retardation.

During pregnancy, approximately 3.5% of pregnant women develop significant levels of antibody to CMV (89). Other studies have demonstrated that about 3% of women excrete virus in the urine during pregnancy, and that

about 1% of the newborn infants excrete cytomegalovirus in the urine at birth (10, 39).

In a recent study, a total of 545 urine specimens were obtained from newborns within the first 24-h of life and cultured for the presence of cytomegalovirus. Three children had viruria which persisted throughout a period of 1 year of observation (10). None of the children had the classical findings of CID, although minimal abnormalities such as transient petechiae and hepatosplenomegaly were noted. After 1 year of age, two were entirely normal and one had findings of mild spasticity. It is quite evident, therefore, from these and other studies, that congenital infection with CMV is often not fatal and perhaps frequently subclinical. Large-scale longitudinal studies of infected infants are needed to define precisely the effect of perinatal CMV infection.

As with rubella infection, CMV persists in the infants for months or years in the presence of neutralizing antibody. Lymphocyte cultures from children with CID have demonstrated the presence of virus for at least several months (55).

At present no specific therapy is available. Drugs such as cytosine arabinoside have been used for treatment of children, with little effect on excretion of virus (53).

Herpes Simplex Virus

Recent studies have clearly indicated that two strains of HSV exist. Type 1 infects the oral area and type II infects the genital area (66). Type I usually causes the common cold sore or fever blister, whereas type II produces cervicitis and vaginitis and is thought to be venereally transmitted. These two strains differ considerably in their antigenic and biological properties. Approximately 95% of herpes infections of the genital area are due to type II. In a series of 28 cases of neonatal herpetic infection, 25 were found to be due to type II herpesvirus (67).

Congenital infection with HSV may produce a syndrome quite similar to that found with congenital CMV infection. Newborns may have microcephaly, cerebral calcification, chorioretinitis, and mental retardation (98). If transplacental or transamnionic infection occur near delivery, a spectrum of clinical disease in the newborn may range from a few cutaneous vesicles to a fatal generalized disease with jaundice, fever, hepatosplenomegaly, encephalitis, circulatory coagulation, and death (63, 96).

Neonatal HSV infection can occur as a fulminating or as a slow, progressive disease with delayed death or recovery with or without sequelae.

Present evidence suggests that transplacentally acquired type I does not protect infants from infection with type II virus (67). In the Collaborative Study, three cases of congenital HSV infection were found. Serological studies show that, early in pregnancy, all had antibody against type I and that seroconversion to type II occurred at the time of delivery. Thus the fetus was not protected by transplacental type I antibody.

Treatment of HSV infection in the newborn is still not possible. Drugs such as iododeoxyuridine (IUDR) has been tried without much success (107). Studies with other durgs are now in progress. The greatest risk to the fetus appears to occur when the mother develops genital herpes near the time of delivery. Caesarean section has not been thoroughly studied but may be a method of avoiding newborn infection when lesions are found in the mother.

VIRUSES IMPLICATED IN CONGENITAL INFECTIONS AND INFECTIONS IN NEWBORN INFANTS

Mumps

In a prospective study, maternal mumps infection during the first trimester was associated with increased fetal mortality. Sixty percent of the abortions occurred within 2 weeks of onset of maternal disease. However, fetal infection was not demonstrated by viral isolation (97).

Endocardial fibroelastosis in infants may be associated with congenital mumps in some cases (94). This association was based on data showing that infants with this disease have a high incidence of positive skin test to mumps antigen. Other workers, however, have not been able to confirm this association nor demonstrate hemagglutinating antibodies to mumps virus in the sera of patients with this disease (29).

In a recent animal study, pregnant Rhesus monkeys were inoculated with mumps virus during the first third of gestation. Replication of virus was detected in the fetal or placental tissues for only 1 week. Full-term infant monkeys demonstrated delayed hypersensitivity to mumps virus but no antibody (99). This split immunological response lends some support to results also found in humans (1). However, there was no report of endocardial fibroelastosis in the infant monkeys.

In the Collaborative Perinatal Research

Study, there were 10 cases of endocardial fibroelastosis, and in one there was evidence of maternal mumps.

Animal studies have been reported in which hydrocephalus was produced in suckling hamsters inoculated intracerebrally with a strain of mumps virus (42). Hydrocephalus was due to aqueductal stenosis which occurred as a late sequela several weeks after the acute infection. This was the first demonstration that mumps virus could cause a definite central nervous system anomaly. Since that report, there have been two cases of hydrocephalus noted as a late sequela occurring 27 and 20 months after acute infection in 5- and 8.5-year-old children (12, 102). Whether mumps is indeed involved in this neurological syndrome will depend upon further isolation and serological studies.

Other animal models, preferably primates, must also be developed to determine whether mumps virus is involved in utero in the production of hydrocephalic newborn infants.

An attenuated live mumps vaccine which appears to produce effective levels of mumps antibody titers has been developed.

Echoviruses

Very little information is available on the echo viruses and their capability of producing congenital infections. These viruses have been isolated from asymptomatic infants and from newborn cases of aseptic meningitis, diarrhea, febrile illnesses, and rashes (84). The echoviruses appear to have the capacity of infecting the placenta and also transmitting the infection to the fetus (9). A recent report describes three infants congenitally infected with Echo-19 (77). Pathological findings closely resemble those found with HSV which is a generalized infection with massive terminal hemorrhage involving most organs of the body.

Echo virus 14 was reported to be isolated from the liver of a newborn infant with fatal hepatic necrosis (41). High immunoglobulin M titers were present at 6 days of age, indicating that possible infection was acquired in utero.

Rubeola Virus

Only limited information is available concerning rubeola and its role in perinatal infections. Since the advent of a live rubeola vaccine, information on this virus and its relationship to congenital infection in the U.S. is practically nonexistent. However, congenital infection has been reported to be involved with a high incidence of stillbirths and abortions (24, 76). Fetal infections occurring before term may produce newborns with the clinical disease or development of disease soon after birth. Giant-cell pneumonia has also been reported in newborns with this disease (22). It has also been reported that mothers who had measles during the first trimester have increased rates of premature infants when compared to normal rates.

At the present time, there are two live measles vaccines licensed for use in the U.S. There is some evidence of reinfection of persons with low levels of antibody.

Vaccinia Virus

Congenital infection with vaccinia virus rarely occurs as a result of reimmunization of pregnant women (24). Primary maternal vaccination in the first or second trimesters may lead to fetal infection and possibly stillbirth, abortion, or prematurity and death (40). Fetal infection develops as a disseminated-type disease with focal lesions occurring on the skin, lungs, liver, and other organs of the body. Lesions may be confused with those produced by herpes or varicella virus. Complications associated with vaccination are eczema vaccinatum, vaccinia necrosium, and postvaccinial encephalitis (65). Infants or adults with exfoliative dermatitis, especially eczema, should not be vaccinated nor exposed to recently vaccinated individuals. The low incidence of smallpox in the U.S. plus the complications associated with vaccination has influenced the Public Health Service to recommend discontinuing of routine smallpox vaccinations in the U.S. as of September 1971.

Varicella-Zoster Virus

Varicella (chickenpox) and zoster (shingles) lesions are caused by the same virus. Congenital infection associated with maternal chickenpox or herpes zoster infection has been reported (2, 16). Infection early in gestation has led to prematurity, stillbirth, or abortion. Congenital infection acquired late in pregnancy is often manifested at birth or in the newborn period by the presence of the characteristic chickenpox skin lesions or severe pneumonia (3). Zoster lesions have also been observed with their characteristic segmental distribution. This disease is usually mild, although it may exist in severe disseminated forms which end in death of the infants. No vaccine is available for this disease.

Western Equine Encephalitis Virus

Transplacental passage of Western equine encephalitis virus has been demonstrated with

serological evidence by a number of investigators (21, 93). Congenital encephalitis has been found in the newborn infant at time of or shortly after delivery. No congenital abnormalities have been described.

Variola Virus (Smallpox)

The effect of variola virus on the fetus has not been considered a problem in the U.S., since it is almost completely absent from this country. It also has a very low incidence in most of the rest of the world. This virus can be transmitted to the fetus as a result of maternal infection (13, 25). The results, similar to those previously described for vaccinia virus, are death, abortion, or congenital disease with skin lesions and pneumonia in the neonatal infant. No congenital malformations have been associated with this virus.

Influenza Virus

For many years, influenza virus has been regarded as a potential teratogenic agent for the human fetus. However, there are probably just as many publications showing correlation between the disease and congenital malformations (20, 32, 36, 85, 109, 110) as there are negative reports denying this effect (18, 108). The reasons for these contradictory reports can be explained in part by the methods employed for collecting data. Many studies have depended upon the self-diagnosis of the disease and self-reporting to correlate infection with pregnancy. Often there is little or no serological backup for the histories. This method can be very inaccurate and misleading. Other studies have depended upon the evaluation of clinical symptoms and signs. With this type of approach, the inapparent or subclinical infections are overlooked. Most investigations have been made with very small numbers of patients and controls.

Some of the positive findings reported for influenza infections during pregnancy include increased incidence of fetal death, anencephaly, abortions, stillbirths, prematurity, and congenital malformations. Most of the data available on these associations are at a very low level of statistical significance.

Experimental animal studies tend to support the conclusion that maternal infection does cause fetal abnormalities (62). Abnormalities of the nervous system and fetal wastage have been demonstrated in mice infected with influenza virus early in pregnancy. Malformations in chicken embryos have been induced with influenza A virus. Abnormalities similar to those occasionally found in human fetuses were produced in chicken embryos, e.g., abnormal tube flexure and failure of neural tube closure. Viral infection was limited to extranueral tissues suggesting an indirect effect on organogenesis (43). Hydrocephalus has been reported by others as a result of influenza virus being inoculated intracerebrally into suckling hamsters and mice (45).

More extensive data with laboratory support will be required to establish the role of influenza on the course of pregnancies.

Hepatitis

Two types of hepatitis designated as A and B have been described. Type A produces infectious hepatitis of short incubation, and type B produces serum hepatitis of long incubation. The hepatitis virus responsible for these two diseases has not been isolated. A hepatitis-associated antigen shows a strong relationship to serum hepatitis (11). This antigen or its antibody may be found in serum from patients with this disease. The availability of serological tests is important in the diagnosis and in epidemiological studies of this disease (80).

Hepatitis-associated antigen can cross the placenta and appear in the cord blood (46, 72). This type B hepatitis may also be transmitted by close contact from mother to infant after birth in the neonatal period (79).

The availability of a serological test now helps to differentiate this hepatitis from other clinical hepatitis (produced by other viruses such as rubella, coxsackie, CMV, herpes, and varicella) in the newborn infant.

Congenital hepatitis may result in stillbirth or abortion of the fetus. Newborn infants can appear with jaundice, hepatosplenomegaly, hemolytic anemia with a liver function test indicating an obstructive type of jaundice. About 80% of the infants survive for at least 1 year; 50% of these exhibit a chronic persistent liver disease. There have been examples of mothers repeatedly giving birth in successive pregnancies to infants with hepatitis, thus indicating a chronic maternal infection (5).

Experimental animal model systems have been found but do not demonstrate the histopathological changes found in the human disease. Apparent viral propagation with antibody formation has been shown (57).

Coxsackieviruses

There are more than 30 serological types of coxsackieviruses which are divided into two

groups, A and B. Group B has been associated with congenital and neonatal infection. These viruses have been reported to have been isolated from stillbirths and aborted fetuses. In one study of 50 stillborn infants and neonatal deaths, coxsackie B antigen was present in the myocardium of five (17). Myocarditis is the clinical finding most frequently associated with coxsackievirus infection (14). However, disseminated infection involving multiple organ systems has been reported. In a recent prospective study of 630 mothers of anomalous infants and controls, maternal seroconversion to coxsackieviruses appeared to be increased during pregnancy with a variety of congenital defects (15). Coxsackie B_2 and B_4 were associated with urogenital anomalies, and type A9 with defects of the digestive system. Cardiovascular anomalies were associated with coxsackie B_3 and B_4 infection during pregnancy, and the likelihood of congenital heart disease was increased by maternal infection with two or more coxsackie B viruses rather than with one. Anomalous infants weighed less than their controls and were more often male. Further studies are in progress.

Maternal or postnatally acquired infection may be clinical or subclinical. Clinical manifestations can be mild or severe and include upper respiratory tract infection, pneumonitis, pleurodynia, myocarditis, pericarditis, and aseptic meningitis. Diagnosis therefore is rather difficult, and further confirmatory studies should be done with both virological and serological support.

Animal model systems have not yet been developed to help confirm the relationship of coxsackieviruses to in utero infection.

VIRUSES TERATOGENIC IN ANIMALS

Blue Tongue of Sheep

Blue tongue is an animal disease produced by a small RNA-containing virus which primarily affects sheep. The disease is transmitted naturally by an inset vector, and therefore the virus was originally grouped with the arboviruses. Blue tongue virus is now provisionally classified as a reovirus. The name of this disease is derived from the clinical picture observed in adult animals. There is hyperemia with cyanosis and edema of the tongue, mouth, eyes, and nose. Mortality usually due to pneumonia is 1 to 5%.

Malformations and other fetal damage were noted when pregnant ewes were immunized with an attenuated chicken embryo-grown vaccine. Vaccination of pregnant ewes during 5 to 7 weeks' gestation produced abnormalities in 20 to 50% of the fetuses (95). Stillbirths and neonatal deaths increased, and extensive defects such as deafness, blindness, shortened limbs, and deformities of the spine and neck were observed. Diffuse anomalies of the central nervous system were a common finding with fetal infection (31).

Additional studies demonstrated that the nature of malformations were dependent upon the gestational age when infection occurred. Direct intramuscular inoculation of the fetus in the first half of the 150-day gestation period produced consistent anomalies. Inoculation at 50 days of gestation led to hydranencephaly, at 75 days caused porencephaly, and at 100 days produced no gross malformations (74, 75). This increased susceptibility of immature cells was further demonstrated by studies with blue tongue virus vaccine adapted to newborn mice (68). However, it was shown to be a very selective infection of the germinal zones and not soley dependent on immaturity or mitotic activity.

Attempts to modify blue tongue virus-induced malformations with cyclophosphamide and antithymocyte serum did not cause mice to be more susceptible to infection (69).

An interesting observation is that the fetus can be transplacentally infected even in the presence of maternal immunity. This would indicate some intracellular transport mechanism of the virus to the fetus, possibly by circulating lymphocytes or another cellular component.

Hog Cholera Virus

Hog cholera virus is an RNA-containing virus resembling the myxovirus and is classified provisionally as a Toga virus. Initial studies demonstrated that fetal abnormalities could be produced by an attenuated rabbit-modified hog cholera virus injected into pregnant dams 10 to 16 days after breeding. These studies showed that 38% of the newborn pigs had edema, ascites, and deformities of the ears, nose, legs, and kidneys (111). Additional studies confirmed these observations, but also demonstrated anomalies of the nervous system when dams were injected at 20 to 90 days of gestation. Cerebral hypoplasia and hypomyelinogenesis with congenital tremors were produced in almost 50% of the newborns (26). Newborn pigs infected in utero also harbored a persistent virus in their viscera, making it possible to transmit infection to other members of the herd.

Recent studies indicate that malformations

are caused by a persistent, immunologically tolerant, and noncytolytic infection of the fetus. The mechanisms of malformation appears to be a selective inhibition of cell division and functions (44).

Parvoviruses

Three viruses, H-1, rat virus, and feline panleukopenia virus, belong to the group at one time called picodna but now termed parvoviruses. They are all singe-stranded DNA viruses characterized by their propensity to attack replicating cells, destructive action upon the developing cerebellum, and ability to cross transplacental barriers. H-1 was reportedly isolated from normal human fetuses and placentas (105). It has many of the characteristics of the rat virus (RV), and the two are considered closely related (106). When injected into pregnant rats, RV produces congenital infection which results in fetal death, reabsorption, fetal survival with hepatitis, and cerebellar hypoplasia (47, 50). H-1 virus produces similar effects in pregnant hamsters (104). A mongoloid-like deformity is produced in these animals which superficially resembles patients with Down's syndrome, but with the absence of chromosomal abnormalities (6).

The relationship of H-1 virus or RV to human disease is still unknown. As previously noted, isolation of this virus has been reported from human placentas and embryos. Serological studies in man, however, have failed to show any relationship between maternal antibody to H-1 or RV and pregnancies which terminate in stillbirth, abortion, mongolism, or congenital defects (71).

Feline panleukopenia virus is serologically distinct from H-1 and RV. For many years the cerebellar hypoplasia of kittens now known to be caused by feline panleukopenia virus was regarded as a common genetic disorder of cats (48). The virus selectively attacks the external germinal layer of the cerebellum during the active phase of histogenesis, resulting in a congenital cerebellar hypoplasia in cat and ferret fetuses (49).

The degree of pathogenetic damage induced by virus in the cerebellums of kittens either inoculated directly in utero or by intravenous inoculation of a pregnant cat was proportional to the duration of their infections (52). Virus can be isolated from the kidneys of infected kittens for many weeks or months after birth, even in the presence of passively acquired antibodies. The possibility that this virus or others like it being involved in production of cerebellar hypoplasia in man has not yet been thoroughly explored.

Reoviruses

Reoviruses are ubiquitous in nature, and their role in the etiology of human disease has not been well defined (27). Spontaneous disease caused by reovirus has commonly been observed in laboratory colonies, primarily in suckling mice. Congenital and neonatal infection in animals have been reported (37). Pregnant mice were inoculated with type 2 virus on 1, 3, 6, 10, and 15 days of gestation. A prolonged infection of the fetus was produced. About 25% of the newborn mice developed interstitial pneumonia and subcortical renal tubular necrosis. Virus can be isolated from all organs in high titer at 15 to 36 days of life. Another 50% show growth retardation and conjunctivitis. Developmental anomalies were not observed.

Reovirus type 1, when inoculated into suckling hamsters, ferrets, rats, and mice via cerebral or intraperitoneal routes, regularly cause obstructive hydrocephalus (51). The virus selectively attacks the ependymal and choroid plexus epithelium within 4 to 6 days after inoculation (59). Nearly 100% of the hamsters developed an ependymitis followed by gross hydrocephalus. Newborn ferrets respond similarly by tolerate infection longer before developing hydrocephalus. Rats and mice also develop hydrocephalus but have widespread systemic disease causing runting with gastrointestinal tract illness.

DIAGNOSIS

Retrospective and prospective investigations have been employed to obtain data on the frequency of various virus infections during pregnancy and to estimate the teratogenic potential of many agents. The restrospective analysis depends upon the recognition of an abnormality in the newborn and then attempts to associate this with a disease which occurred during the mother's pregnancy. The main limiting factors are the inability to document the infection and failure to recognize inapparent infections. The prospective approach provides for the observation of the mother throughout her pregnancy. This approach is usually the most preferred, even though it is more difficult and time consuming. Clinical infections can be documented, and with serology inapparent infections can be recognized.

Isolation of a virus from a newborn or fetus is the most direct method of establishing the

diagnosis of congenital infection. The development of micro tissue culture and serological techniques has reduced the cost of viral diagnosis, thus making it a feasible procedure for most diagnostic laboratories (28, 87). Virus isolation results must be evaluated with the clinical history and antibody assays in both the mother and infant. This is especially true in interpretation of the isolation of entero viruses from throat swabs or stool specimens of the mother or the newborn. Isolation of a virus from spinal fluid, urine, or lesion is usually more readily accepted as supportive evidence of infection.

Some viruses such as rubella, HSV, and CMV produce chronic infection and are excreted for many months after birth. Rubella virus has been recovered from the throats of congenital newborns from birth to 2 years of age. Virus has been isolated from cataract tissue taken from children as old as 31 months. Rubella virus has also been recovered from spinal fluid and urine. These same sites are also useful for isolation of other myxovirus such as rubeola and mumps.

CMV may be isolated from both throat cultures and urine at approximately the same rate. More recently, cervical cultures have been reported to show much higher frequencies of infection. Since CMV is very slow-growing, cultures should be kept for at least 6 weeks before considered negative. The virus is also very heat labile and thus may be lost by freezing and thawing. Specimens should be transported rapidly to the laboratory and kept cool, not frozen, before they are inoculated into human fibroblastic tissue culture.

HSV has been cultured from skin lesions, throat swabs, and spinal fluids. This virus reproduces so rapidly that cytopathic effect in tissue culture can be observed within 24 to 48 h after inoculation.

Persistence of virus shedding with these congenital infections may be a factor in transmission of disease. Therefore, precautions should be implemented regarding exposure of pregnant women, other infants, and nursing personnel caring for these infected infants.

Enteroviruses are relatively stable, and specimens can be stored at −20 C for short periods of time. They can be isolated from stool, throat swabs, and occasionally spinal fluid.

Vaccina and varicella viruses can be recovered from skin lesions. Vaccinia as well as the other poxvirus, variola, can be recovered from purulent or crusted lesions. Varicella on the other hand must be cultured before lesions become crusted. They must be obtained from the nonpurulent fluid. Isolates in human or simian cells must be passed by transferring viable infected cells.

A number of serological tests are available for determination of antibody against the viruses previously described (86). In order of widest application to the least commonly used tests are: complement fixation, hemagglutination inhibition, neutralization, passive hemagglutination, and various precipitation tests. Selection of the method depends upon the virus studied; some tests have distinctive advantages over others. The presence of antibodies provides indisputable proof of previous exposure to an antigen. However, high antibody titers in a single specimen cannot be relied upon entirely as presumptive evidence of a recent infection. The best approach is to demonstrate seroconversion with acute and convalescent sera. Seroconversion can be regarded as a change from no detectable antibody to presence of antibody or a change in antibody titer from one level to a fourfold higher level. With this approach, serological confirmation of a recent infection can be documented and also prove that it occurred during pregnancy.

Sero-immunoglobulins consist mainly of three classes; IgG, IgM, and IgA. IgG normally crosses the placenta during gestation and may be found in fetal blood (4). Thus IgG antibody present in maternal blood will also be found in approximately the same titer in the infant. Passively transferred antibody has a half-life of 3 to 4 weeks. Therefore, a serum obtained from an infant at 4 or 5 months of age should show a significant natural drop in IgG antibody titer or be completely undetectable. However, if the specific antibody level to an infectious agent remains the same or becomes elevated, it may be a clue to congenitally or neonatally acquired infection.

Since IgM antibody is not passed transplacentally from mother to fetus under normal circumstances, an elevated IgM in cord serum is indicative of immunoglobulin synthesis by the fetus in response to antigenic stimulation. Results of studies have indicated that most infants with severe congenital infections have raised IgM levels. The value of this test has been compared to a white-blood count in that it is usually increased in viral and bacterial infection but is not specific. Approximately 20% of children with increased IgM in the newborn period can be identified as having been infected with HSV, CMV, rubella virus, toxoplasmosis, or syphilis. The remaining 80% are unexplained. Long follow-up of a large number of newborns will be needed to deter-

mine the strength of correlation of in utero infection with the presence of high levels of IgM. Tests for specific IgM antibody utilizing the indirect fluorescent-antibody techniques have been developed for rubella (7), CMV (35), and HSV (67). Although the availability of these tests is still restricted to the larger laboratories, the specific IgM antibody tests should prove extremely helpful in establishing the early diagnosis of congenital infections. Appropriate treatment or other procedures could then be instituted.

Concluding Statement

The role of viruses in congenital malformation will become more clearly evident as new techniques for diagnosis become available. Further development of animal models of congenital viral infections will help contribute to our knowledge of the number of variables which influence the frequency of congenital infection and their pathogenic processes.

The possibility that viruses may remain in a latent stage after being congenitally acquired raises the question of the extent of their contribution to neurologic dysfunction in adult life. Relationship of these viruses in initiation of malignancy and immunological dysfunctions is also a possibility.

Since some viruses such as CMV and herpesvirus have the capability of latency, production of safe vaccines may be difficult. The development of chemotherapeutic agents, therefore, becomes a very important area for study. Effective control of these diseases requires increased knowledge of the parasitic relationship of viruses to cells at a molecular level.

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