Cytomegalovirus: A Major Cause of Birth Defects

Cytomegalovirus (CMV) is the most common viral cause of mental retardation. It surpasses even rubella virus in this regard. Investigators estimate that 1 of every 1000 infants—that equals more than 3000 per year in the United States alone—is seriously retarded as a result of congenital infection with CMV. Moreover, recent evidence indicates that the virus may cause subtle, but nonetheless significant, mental defects in children who were born infected with the virus but without the classic symptoms of cytomegalic inclusion disease.

Infection with CMV may also constitute a serious problem for immunosuppressed individuals such as cancer patients and recipients of organ transplants. For these reasons, investigators would like to develop effective methods of preventing or abrogating CMV infections. The most obvious approach to prevention is vaccination, and efforts to develop a vaccine are under way in both Europe and this country. A number of investigators, however, have expressed reservations about current efforts to prepare a vaccine for use in humans.

No one has reservations about the validity of the findings that the virus is a major cause of birth defects which include mental retardation, blindness, and deafness. Investigators who have studied the epidemiology of CMV infections include James B. Hanshaw, now at the University of Massachusetts Medical School in Worcester, Thomas Weller of the Harvard School of Public Health, H. Stern of St. George's Hospital Medical School in London, George Nankervis of Case Western Reserve University School of Medicine, and John Sever of the National Institute of Neurological and Communicative Disorders and Stroke. They have shown that about 1 percent of all infants are born infected with CMV. The exact percentage varies with socioeconomic status; it is about 0.5 percent in the high socioeconomic groups, but up to 2 percent of newborn infants from the low socioeconomic groups may be infected.

Many of these infants have inapparent infections that can be detected only because they are excreting virus in the urine or because they are making antibodies against it. But about 10 percent have the serious disseminated infection called cytomegalic inclusion disease. This is characterized by enlargement of the spleen and liver, hepatitis, blood abnormalities, and mental retardation that is frequently accompanied by microcephaly (an abnormally small head). Sometimes blindness or deafness results from the disseminated disease, which is occasionally fatal. At the cellular level, CMV infections cause enlargement of the affected cells (cytomegaly, for which the virus was named) and produce inclusion bodies, probably consisting of viral particles, in the nucleus.

Recent evidence indicates that even those infants with inapparent infections may suffer some kinds of neurological damage. Hanshaw screened 8600 newborn infants at Strong Memorial Hospital in Rochester, New York, for evidence of CMV infection. The sample included infants from all socioeconomic groups, although most were from the middle class. About 0.6 percent of the total gave positive results. Hanshaw has now studied 44 of these children for 3.5 to 7 years. The 44 children were compared with an equal number of control children matched for age, sex, race, birth weight, and social class. A second random control group consisted of 44 children selected because they were born immediately after the birth of an infected child.

Hanshaw says that the average IQ score of the test group was significantly lower than those of both control groups. Moreover, there were fewer test children with high IQ scores and more with lower ones than in the control groups. Seven of the test children had IQ's below 80, whereas none of the controls did. Charles Alford of the University of Alabama Medical Center in Birmingham found similar trends toward lowered IQ scores in a prospective study of 18 children with inapparent congenital CMV infections, although the differences between control and test children were not statistically significant. On the other hand, Nankervis detected no effect of asymptomatic CMV infections on IQ.

Both Hanshaw and Alford have found a significant increase in hearing impairments in the test children. According to Hanshaw, five of them had hearing losses in both ears; for three, the impairment was severe. Only one of the control children had bilateral hearing losses. Four children in the test group studied by Alford had definite hearing losses in one or both ears, and for two of them the decrease in auditory acuity was sufficient to require the use of hearing aids. Five more had decreased acuity only at the higher sound frequencies. The investigators think that CMV may be a major cause of congenital deafness.

Cytomegalovirus is widely distributed in human populations throughout the world. In this country, up to 80 percent of the population acquires antibodies that are indicative of infection with the virus by the age of 35 or 40 years. Again, the percentages vary from group to group with higher values found in people of lower socioeconomic classes. Spread of the virus is favored by crowding and lack of sanitation facilities. For most healthy individuals who acquire the infection after birth, its effects are minimal; they may have no symptoms at all, but they often continue to shed virus in their body secretions for several months. This also facilitates its spread.

Many investigators think that congenital infections result from primary infection of a woman during pregnancy. They hypothesize that the virus spreads from the woman's blood across the placenta to the fetus. However, this may not be the only route by which the fetus can be infected. The risk of acquiring CMV infections increases during pregnancy, but, in most of these cases, the virus appears to be located only in the cervix or the urinary tract. It is rarely found in the blood. At present, no one knows whether localized cervical CMV can infect the fetus. Also unknown is whether these local infections result from reactivation of a latent virus carried by the woman or are new infections possibly acquired venereally. Since David Lang of Duke University Medical Center has found CMV in the semen of apparently healthy men, there is evidence indicating the possibility of venereal transmission. All investigators agree that much more needs to be learned about the natural history of CMV infections.

If congenital infections do result only from primary infections, then vaccination of young nonpregnant women could prevent congenital infections and the consequent neurological damage. Vaccination has now largely controlled rubella virus, another prominent viral cause of birth defects. The investigators who are trying to develop a vaccine to protect against CMV infections include Stern and S. D. Elek, also of St. George's Hospital Medical School, and Stanley Plotkin of the Children's Hospital of Philadelphia and the Wistar Institute. Both groups are working with live virus. In order to be used in a vaccine, a live virus must be attenuated so that it will not produce serious disease. A classic way to accomplish attenuation is to grow the virus in a long series of cell cultures until it loses its capacity to produce symptoms. The British investigators and

Plotkin are using CMV cultured in this way for their vaccines.

Stern and Elek inoculated volunteers with a viral preparation that also contained disrupted cellular material. When injected subcutaneously into volunteers, this material elicited the production of antibodies against CMV in 25 of the 26 individuals inoculated. The predominant side effects they experienced included transient swelling and tenderness at the site of inoculation; two individuals also had swollen lymph glands for 1 to 3 weeks. None produced virus. Plotkin, with Max Just of the Children's Hospital of Basel, obtained similar results in 30 volunteers. These investigators prepared their vaccine from virus that had been shed into the culture medium and was thus free of cell debris.

The investigators who have expressed reservations about development of a vaccine at this time do not question the conclusion that prevention of congenital CMV infections would contribute significantly to the betterment of human health. What concerns them is what is known—and unknown—about the nature of CMV and its infections. They point out that it is difficult to determine whether CMV has been attenuated because its symptoms are usually mild, or even nonexistent, in the first place. Attenuation of human CMV cannot be assessed in animals because the virus exhibits a high degree of species specificity. Plotkin says that the mildness of infections by unattenuated CMV adds to the safety of vaccine trials and that observations that inoculated volunteers do not have systemic symptoms and do not produce virus support the conclusion that the virus has been attenuated.

Other objections center around the fact that CMV is a herpesvirus. These are large complex viruses having DNA as their genetic material. Although definitive proof is lacking, there is evidence that some of the herpesviruses, including herpes simplex viruses 1 and 2 (HSV 1 and 2) and Epstein-Barr virus (EBV), may cause certain types of cancer (*Science*, 15 March 1974). Part of the evidence derives from the capacity of these viruses to transform cultured cells to the malignant state and, also, from epidemiological studies that show a correlation between the incidences of viral infections and of the cancers. According to Fred Rapp and his colleagues at the Milton S. Hershey Medical Center of the Pennsylvania State University, CMV, which has been inactivated by irradiation with ultraviolet light, transforms hamster cells in vitro. Many investigators think that transformation by the herpesviruses requires defective virus that is not capable of reproducing.

Stimulation of the synthesis of host cell DNA is another characteristic of oncogenic DNA viruses that is shared by CMV, according to Rapp and Plotkin. Plotkin says that the virus stimulates the synthesis of mitochondrial DNA first and then that of nuclear DNA later in the reproductive cycle. One way to increase DNA synthesis is by increasing the concentrations of the DNA polymerases, the enzymes that catalyze the formation of DNA. Eng-Shang Huang and Joseph Pagano of the University of North Carolina Medical School have found that CMV stimulates the synthesis of the DNA polymerases of the host cell.

There is now no epidemiological evidence for the involvement of CMV in the etiology of cancer. Nevertheless, Rapp, Pagano, and others have expressed concern about the idea of administering intact CMV particles—with their DNA—to humans. They do think that it may be possible to prepare viral antigens that do not contain DNA for use in a vaccine. On the other hand, Plotkin points out that the capacity to transform cells in vitro does not necessarily mean that this will happen in the living animal. Adenovirus is a DNA virus with wide-spread distribution in the human population. It transforms cultured cells, but there is no evidence linking it with human cancers.

CMV and Latency

Herpesviruses are notorious for their capacity to persist in a latent state for long periods in the host and then to periodically flare up from time to time and cause recurrent disease. For example, HSV 1 and HSV 2, which produce lesions around the mouth (cold sores) and genital areas, respectively, do this. Most investigators think that CMV may behave in a similar manner. The possibility of CMV latency raises two issues. The first is whether congenital infections always result from primary infections of the pregnant woman or whether reactivation of latent virus accounts for at least some of them. This issue is controversial but if reactivated virus does infect the fetus, vaccination might do more harm than good. The second involves the observations that CMV infections can pose serious problems for immunosuppressed persons including recipients of organ transplants and patients with certain kinds of cancers. The virus may produce disseminated infections in these patients. Pneumonia, which is often severe or even fatal, may result. Since CMV is frequently found in transplanted kidneys that have been rejected, investigators have even hypothesized that the virus may play a role in eliciting attack on the transplanted kidney by the immune system.

Patients who receive large quantities of transfused blood or who have been perfused with blood while on a heart-lung machine are prone to a mononucleosis-like disease that has also been linked with CMV. All these conditions may result from reactivation of virus—whether in the patient's own cells or in those of the donor—or from new infections.

Alford does have some evidence that reactivation of CMV may result in congenital infections. He has observed 118 women who had antibodies against CMV before they became pregnant. The fact that the women had the antibodies did not prevent infection of their babies; seven of the infants borne by them were infected with CMV although none had serious symptoms. This rate of congenital infection—6 percent—is much higher than that observed in studies of random populations.

To determine whether the congenital infections resulted from reinfection of the women with a different strain of virus or from reactivation of virus from the primary infection, Alford examined the antigenic composition of viruses isolated from two infected infants born of the same mother at an interval of 3 years. He detected no major differences between them and concluded that reactivated virus infected the second infant.

Alford also doubts whether vaccination can completely protect against intrauterine transmission of CMV if natural infection does not. However, strain differences may play a role here. Several investigators, including Weller and Huang and Pagano have detected antigenic dissimilarities between different strains of CMV. Thus, a vaccine may have to contain more than one strain in order to be effective. The polio vaccines serve as a good precedent for this; the need to protect against more than one strain is not necessarily an insurmountable problem.

Lang has suggested a possible mechanism for the reactivation of CMV. He points out that all of the conditions that have been associated with CMV reactivation—namely, pregnancy, organ transplantation, transfusions, and malignancies—share two common features. These are varying degrees of immunosuppression and the presence of foreign antigens. (The fetus carries antigens foreign to the mother. Cancer cells carry antigens that differ from normal cellular antigens.) The presence of foreign antigens stimulates the lymphoid cells of the immune system to divide. If CMV is harbored in lymphoid cells, as some investigators have suggested, and if the virus replicates preferentially in dividing cells, this stimulation could reactivate the virus, according to Lang. In recipients of transplants or transfusions the virus could originate either in the recipient's cells or could have originated in those of the donor. In any event, the antibody status of the patient in regard to CMV would determine wheth-

er reactivation results in clinical illness.

Lang and his colleagues have developed an animal model for studying the effect of transfusion on activation of CMV. They take blood from mice that were infected with CMV but whose blood no longer carries infectious virus, and then administer it intraperitoneally to uninfected recipients. After 1 month they invariably detect CMV in the salivary glands of mice of different genetic composition from the donors but only rarely in mice of the same genetic composition. This suggests that the response of the immune system to foreign antigens is involved in CMV activation.

Huang and Pagano found that CMV could reproduce in vitro in B (for bone marrow–derived) lymphocytes infected with EBV but not in uninfected B cells or in T (for thymus-derived) lymphocytes. This is consistent with Lang's hypothesis because normal lymphocytes do not reproduce in culture but those transformed by EBV do. The EBV does not infect T cells.

Prevention of CMV infection is desirable and so is a method of abrogating infection when it does occur. The research performed by Huang has suggested one approach to accomplishing the latter goal. He has shown that when CMV infects human cells it induces the synthesis of a new DNA polymerase. This virus-induced enzyme differs from the normal cellular DNA polymerases of uninfected cells, the synthesis of which is also stimulated by CMV, although Huang does not know whether viral or cellular genes actually code for the new enzyme. Since phosphonoacetic acid specifically inhibits the activity of the virus-induced enzyme without significantly inhibiting that of the cellular DNA polymerases, Huang and Pagano think that this drug is a promising one for treating CMV infections.

—JEAN L. MARX

Synchrotron Radiation (II): Formidable Competition Overseas

Facilities in the United States for research in which synchrotron radiation is used may be as good as those overseas, but only for the present. Researchers in Europe have been more successful than their American counterparts in securing funding for synchrotron radiation facilities, some observers argue, and by 1980 the United States will have slipped to second class status as a synchrotron power, unless certain decisions, such as establishment of a national center for the purpose, are made quickly.

The United States has three electron storage rings that operate as synchrotron radiation sources. The newest of these is the 4-Gev SPEAR machine at the Stanford Linear Accelerator Center (SLAC). The Stanford Synchrotron Radiation Project (SSRP), which is only 1½ years old, is the only source of such radiation in the x-ray region open to users from any institution. (Cornell University's 12-Gev synchrotron also produces short wavelength x-rays, but for a limited group of researchers.) At present, the Stanford project consists of a single beam port, from which radiation is divided and guided to five experimental stations. A second beam port, to be completed next April, will accommodate four more experiments.

The synchrotron project at Stanford operates as a "parasitic" facility, because SPEAR is run strictly according to the needs of the high energy physicists at SLAC. Thus, for example, when SPEAR operates at relatively low energies, as it does when investigators study psi particles, the intensity of short wavelength x-rays becomes so small that some experiments are not possible. According to Herman Winick, deputy director of SSRP, agreement has been reached with SLAC to obtain a limited amount of time (about 5 percent) each month when SPEAR will be operated for synchrotron radiation experiments only. Such a mode of operation would ensure that conditions were optimum at least part of the time and would greatly increase the productivity of SSRP, but synchrotron radiation researchers see a need for a facility that is completely dedicated to radiation production and that can produce x-rays.

At Stoughton, Wisconsin, there is already a facility dedicated solely to synchrotron radiation, but the relatively low energy (240 Mev) of the Tantalus I storage ring there limits the useful intensity to ultraviolet wavelengths longer than about 40 angstroms. The facility services an average of seven groups at once, according to Ednor Rowe, director of the University of Wisconsin Synchrotron Radiation Center. Japan has a facility of comparable size in Tokyo, IN-SOR II, which is the first storage ring planned from the beginning to be dedicated solely to synchrotron radiation research. Like Tantalus I, the Japanese storage ring will be severely limited by its energy (300 Mev), which is not even enough to cover the entire ultraviolet range. Another similarly dedicated storage ring, for 240-Mev electrons, was recently completed at the National Bureau of Standards, Gaithersburg, Maryland. Called SURF II, it has 11 beam ports.

Competition from laboratories overseas is already formidable, and will become increasingly so in the years ahead. The most impressive facilities are now in West Germany, where the DESY laboratory in Hamburg has been conducting synchrotron radiation experiments since 1964. The synchrotron radiation program there began when the high energy physics program did, and has continued to grow with it, until there are now four laboratories with radiation available at wavelengths down to hard x-rays, each with as many as ten experimental stations. Two laboratories are located at beam ports on the 7.5-Gev DESY synchrotron, completed in 1963, and two are attached to the 4.5-Gev DORIS storage ring, which began operation last year (*Science*, 8 August, p. 444).

The original laboratory at the synchrotron was expanded in 1970, and a second building was constructed. At the same time, plans were made for the two synchrotron radiation buildings adjacent to DORIS. One has been operated exclusively for physics and chemistry experiments since DORIS began operation, and the other will soon be used for life science experiments by the European Molecular Biology Organization (EMBO) (*Science*, 19 September, p. 981). The two laboratories at the synchrotron are similarly allocated.

Originally, the synchrotron radiation users at DESY had to operate as parasites to the high energy physics research, but now a small percentage of the running time is dedicated to them alone. Another advantage to the researchers at Hamburg is that the intensity of radiation is generally higher than that at Stanford. Although the energy of the Hamburg storage ring is about the same, the current in DORIS (normally 0.3 ampere) is approximately ten times higher than that in SPEAR. The DORIS ring is clearly the most powerful of the synchrotron light factories, producing a total of 1 megawatt of radiation.

The budget for synchrotron radiation in Hamburg is $1,750,000 per year: approximately $750,000 for the physical sciences by DESY and $1 million for biological research with synchrotron radiation by EMBO. In contrast, the operating budget of the Stanford project is at present $530,000.

Great Britain is not far behind West Germany, as the United Kingdom is the first country to commit money for a multi-Gev facility dedicated to synchrotron radiation users. Construction has already begun for a 2-Gev storage ring on the site of the soon to be retired 5-Gev NINA synchro-