4

Slow and Persistent Viral Infections

Introduction

There are two types of viral infections that bring about chronic, progressive, and usually fatal diseases involving the central nervous system (CNS). Each of them poses problems in meeting the postulates of causation thus far discussed. One type is caused by a unique group of viruses with no detectable immune response. These viruses are called "slow viruses" or "lentiviruses" because of the long period between exposure and the appearance of clinical disease, which presumably reflects the primary incubation or multiplication time for the agents. The second type of infection is caused by a group of several common and ubiquitous viruses that affect the brain several years after the primary infection and that are associated with an aberrant immune response. The two groups will be discussed separately because they require different criteria to establish a causal relationship between the virus and the disease. Certain members of the retrovirus family that bear a molecular resemblance to some of the slow viruses of animals are suspected of causing chronic infections of the CNS in humans. There are also several chronic and progressive diseases of the CNS in which a viral etiology is suspected, among which is multiple sclerosis.

Unconventional or True Slow Viral Infections

Slow viral infections were first named and described in Iceland. In a series of papers in 1954, Sigurdsson *et al.* (1957) discussed three chronic diseases of sheep: Maedi, a slow, progressive pneumonia; Rida, a chronic encephalitis either closely related or identical to scrapie; and Johne's disease, a paratuberculosis. Later on, visna, a progressive, demyelinating, and transmissible viral infection

A. S. Evans, *Causation and Disease*

of sheep, was added to the list (Sigurdsson *et al.*, 1957). Scrapie and its transmissibility had already been described by Cuille as early as 1936 (Cuille, 1938).

The criteria used by Sigurdsson for slow infections were: (1) a very long initial period of latency lasting from several months to several years; (2) a rather regular protracted course after clinical signs have appeared, usually ending in serious disease or death; and (3) limitation of the infection to a single host species, and anatomical lesions in only a single organ or tissue system (though this criterion was later modified to consider a wider host susceptibility). The visna–maedi complex shares characteristics with some retroviruses, such as the human immunodeficiency virus (HIV). Visna has been called a slow virus infection because of the long incubation period, but it does not belong to the unconventional group. On the other hand, scrapie, so named because itching of the skin lesions makes the sheep scrape themselves against objects, is caused by the prototype of a group of viruses called unconventional or true slow viruses. It is noninflammatory in nature and produces no detectable immune response. Also, nucleic acid is not present in the agent.

The unconventional group includes scrapie, transmissible mink encephalopathy, and three human diseases, kuru, Creutzfeldt–Jakob disease (CJD), and Gerstmann-Straüssler-Schenker syndrome (GSS). They are referred to as the spongioform encephalitides because of the spongy appearance of the pathological changes found in the brain. The features of the agents and of the host response are shown in Table 4.1. The extraordinarily high resistance of the viruses to heat, ultraviolet light, and various chemicals, such as formalin, and the long incubation period in both animals and humans are key features, in addition to those already mentioned. There is some question about whether they should be called viruses, because they do not have nucleic acid (DNA, RNA), which characterizes all conventional viruses. Prusinger (1982) and Prusinger *et al.* (1983) claim to have identified a small, infectious protein and refer to the infectious agent as prions. This work initially needed further confirmation because the components identified might have been products of the host tissue rather than the agent itself. Currently, the results are generally accepted. From the standpoint of establishing causation, these unconventional agents pose special problems because the agent cannot be isolated and grown in culture and the infection does not result in a detectable immune response. This means that none of the criteria thus far described is applicable to the group. Before proceeding with the current criteria of causation, a description of the human diseases may be of interest.

Epidemiologically, kuru is a fascinating disease. It was first described in the medical literature by Gajdusek and Zigas (1957). They observed it in the Fore tribe, a group of primitive people living in the highlands of Papua, New Guinea, where it remains geographically restricted. Clinically, kuru is a progressive and fatal neurological disease of about 24 months' average duration.

The disease was recognized in the first or second decade of the 20th century

Table 4.1
Subacute Spongioform Virus Encephalopathies (Unconventional Viruses) of Humans and Animals and Their Characteristics[a]

Viruses	
Of humans:	Kuru (limited to New Guinea)
	Creutzfeldt–Jakob disease (familial and sporadic)
	Gerstmann–Straussler disease (familial and sporadic)
Of animals	Scrapie of sheep and goats
	Transmissible mink encephalopathy
	Chronic wasting disease of mule deer and captive elk
	Bovine spongioform encephalopathy
Characteristics:	Long incubation period
	Contain no DNA or RNA, but contain "prions"
	Not visualizable under the electron microscope
	Induce no detectable antibody response
	Multiply to high titers in appropriate tissues
	Highly resistant to heat, ultraviolet light, many chemicals
	Amyloid fibrils seen in infected brain resembling aggregated scrapie-associated protein ("prion")

[a]Derived in part from Gibbs (1989).

and rates slowly increased until they reached epidemic proportions during the 1950s (Gajdusek, 1973). In 1956, intensive studies were begun and the possibility of a simple genetic hypothesis and of an infectious origin was investigated, with no conclusive results. We owe much of our knowledge of the epidemiology and etiology of kuru to the work of Carleton Gajdusek (Figure 4.1) and his associates at the National Institutes of Health. A case registry was established. The disease was found to be confined to members of about 160 villages, with a total population of 35,000, among whom over 2500 cases have been identified since 1956, primarily in the Fore linguistic group. At one point, up to 200 deaths from kuru occurred yearly. Among preadolescents the sex ratio was about equal, but in adults there was a 3:1 female predominance, which has been dropping toward 1:1 in the last decade.

The most striking aspects of the disease are the method of transmission and the long incubation period. Ritual cannibalism was introduced in about 1920, and consumption of a dead kinsman, particularly his brain, as an act of mourning appeared to be the main method of transmission. Women prepared the body barehanded and squeezed brain tissue in bamboo cylinders, in which it was steamed. The virus is highly resistant to heat, and the low boiling temperature, 90–95 °C, in the mountainous area may have failed to inactivate it. Adult men rarely participated in this ceremony and seldom ate the flesh of the kuru victims; the

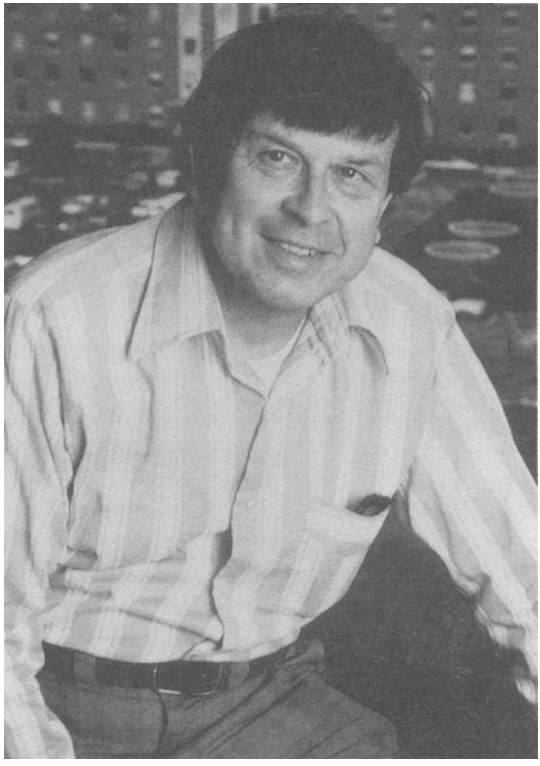

Figure 4.1. Dr Carleton Gajdusek, 1923– , Chief, Laboratory of Central Nervous System Studies, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health

women and children were primarily at risk. Both ingestion of the contaminated brain tissue and absorption through abrasions in the skin were possible mechanisms of transmission of the agent. The gradual decline in the incidence of the disease since cannibalism was discontinued from 1957 to 1962, and the observation that no child under 10 years old has developed kuru since 1967 support this idea of the means of transmission. Recent epidemiological studies of three funeral feasts and on follow-up of those who attended them, undertaken by Klitzman, as part of his Yale medical thesis and published in conjunction with Gajdusek and Alpers (Klitzman *et al.*, 1984), have indicated several important points: (1) the incubation period can range from 6 to 28 years or more (earlier evidence had suggested a period as short as 3 years), can be identical in two or more individuals infected simultaneously, and is not determined by the patient's age at the time of exposure; (2) kuru is transmitted at the time of cannibalistic mourning; (3) the attack rate among those participating in the feasts may exceed 77%. The

establishment of an infectious etiology of the disease was successful in 1966 when Gajdusek and his associates transmitted the disease to chimpanzees, based on the earlier suggestion of Hadlow (1959) that there were neuropathological similarities between scrapie and kuru. The disease developed 20 months after the intracerebral inoculation of human brain tissue from kuru patients, thus providing experimental evidence of a long incubation period. Gajdusek was awarded the Nobel Prize for his work with kuru. The disease, as well as Creutzfeldt–Jakob and scrapie, was later transmitted experimentally by the oral route to nonhuman primates, amplifying the evidence for cannibalism as the route of transmission (Gibbs *et al.*, 1980).

Creutzfeldt–Jakob disease (CJD), another chronic neurological disease caused by a slow virus, was described in the early 1920s, is worldwide in distribution, and has an incidence of about one per million (Gibbs, 1989). The agent, itself, is indistinguishable from that of kuru by current virological methods. About 90% of cases occur in adults aged 40–69 years with about an equal sex distribution. The disease was transmitted, through infected brain tissue, to chimpanzees by Gibbs (Figure 4.2) and associates (1968), and later to smaller experimental animals by Manuelides and colleagues (Manuelides *et al.*, 1976a, 1977, 1978). While the natural route of transmission in humans is unknown, transmission has been shown to occur via infected cornea (Manuelides *et al.*, 1977) and by stereotactic electroencephalographic (EEG) electrodes that had

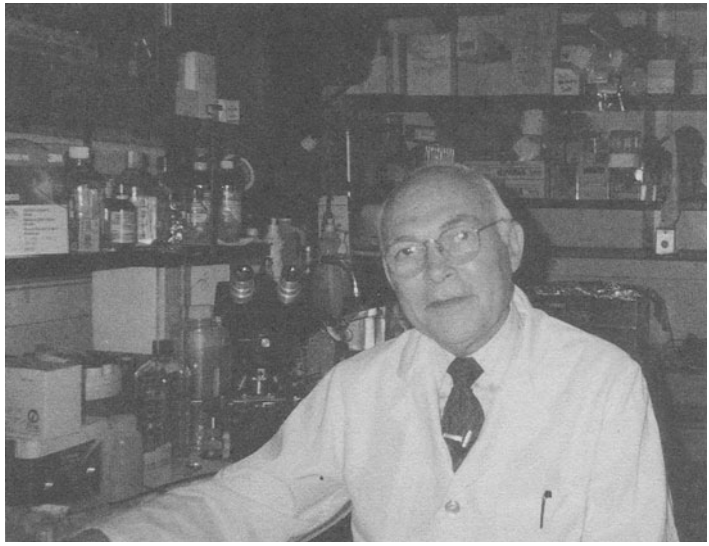

Figure 4.2. Dr. Clarence J. Gibbs, Jr., 1924– , Laboratory of Central Nervous System Studies, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health.

been previously used on a CJD patient (Bernouilli *et al.*, 1979). A neurosurgeon apparently contracted the disease after he stabbed himself with a lancet while operating on a CJD patient. More recently, two types of infection have occurred from the use of cadaver materials. One involved the use of human dura mater from a German tissue bank on persons with breaks in their dura mater needing repair (Centers for Disease Control, 1987, 1989). The other resulted from the use of growth hormone in seven children, using material derived from the pituitary gland of cadavers (Centers for Disease Control, 1985). Obviously, any cadaver tissues derived from patients with fatal chronic neurological diseases should not be used in the future, even though the estimated incidence of CJD is about one in a million. If used in a pool of materials, such as growth hormones, it might contaminate large lots and infect many persons. In addition to this, the possibility that inapparent carriers of such viruses exist has not yet been completely excluded.

The inability to isolate the agents of kuru and CJD in tissue culture or to demonstrate an immunological response to them led to the proposal of a new set of guidelines by Johnson (Figure 4.4) and Gibbs (1974) for relating slow viral infections to chronic neurological disease, as shown in Table 4.2. The consistent, serial experimental reproduction of the disease in experimental animals in more than one laboratory is the key recommendation. The demonstration of the agent

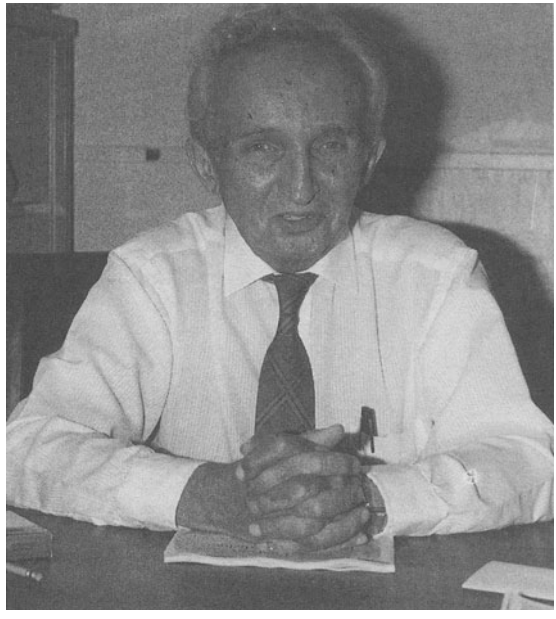

Figure 4.3. Dr Elias E Manuelides, 1918–1992, Professor of Neuropathology, emeritus, Yale University School of Medicine

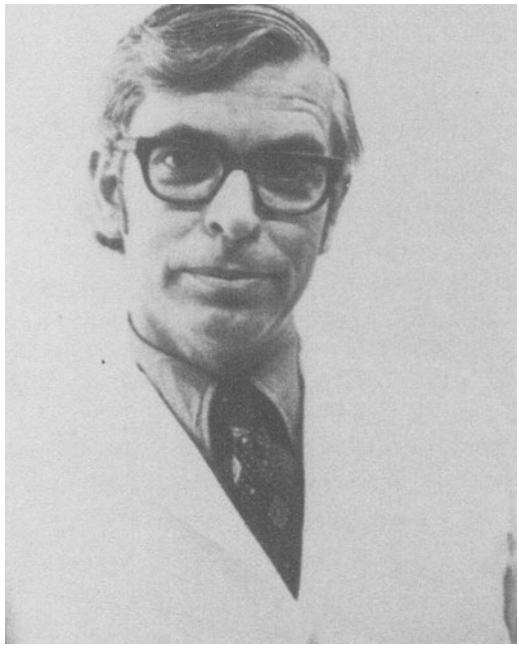

Figure 4.4. Dr. Richard T. Johnson, 1931– , Professor of Neurology, Johns Hopkins University School of Medicine.

itself in diseased tissues through the electron microscope, through immunofluorescence techniques, or even using current molecular biological methods is not yet possible for the agents of kuru or CJD. The third criterion, that tests of normal tissues or tissues from patients with other diseases should be carried out to establish that the agent is not a ubiquitous one, does not eliminate the possibility that latent, asymptomatic infections of the CNS may occur, even with the slow viruses. The isolation of the CJD agent by Manuelides in guinea pigs that had been inoculated with biopsy material from the brain of a patient who subsequently recovered indicates that recovery may occur (Manuelides *et al.*, 1978). Indeed, several viral agents, such as herpes simplex virus or varicella-zoster virus, may lie latent in the brain of normal or diseased persons. When brain tissue from a patient with some other chronic neurological disease is inoculated into an experimental animal, then these viruses might produce some other disease without being the cause of the disease under study. This problem of the carrier state was one of the limitations to the second of Koch's original postulates, that "the parasite should occur in no other disease as a fortuitous and nonpathogenic parasite." One can expect that advances in molecular virology will eventually provide modifications to Johnson's and Gibbs's guidelines and will eliminate some of these questions. Even with the limited evidence now

Table 4.2
Johnson's and Gibbs's Criteria for Relating Slow Viral Infections and Chronic Neurological Disease[a]

1. There should be consistency in the transmission of the disease to experimental animals or some consistency in the recovery of the virus in cell cultures, and this transmission or recovery should be confirmed by more than one laboratory.
2. Either serial transmission of the clinicopathological process should be accomplished using filtered material and serial dilutions to establish replication of the agent, or the recoverable agent should be demonstrated with consistency in the diseased tissue by electron microscopic, immunofluorescent, or other methods, and should be demonstrated in the appropriate cells to explain the lesions.
3. Parallel studies of normal tissues or tissues of patients with other diseases should be carried out to establish that the agent is not a ubiquitous agent or a contaminant.

[a]From Johnson and Gibbs (1974)

available, there are few who doubt that these unconventional slow viruses are the cause of kuru and CJD and are probably infectious agents called prions (Prusinger, 1992).

Chronic CNS Diseases Due to Conventional Viruses

There are several viruses or groups of viruses of the conventional type containing nucleic acids, i.e., DNA or RNA, that produce an immune response, albeit often an abnormal one, and that can result in chronic infections of the CNS. This complication is a rare event. Viruses capable of producing persistent neural infections include herpesviruses, adenoviruses, papovaviruses, rhabdoviruses, retroviruses, coronaviruses, arenaviruses, togaviruses, and picornaviruses (Johnson, 1982). The mechanism by which persistence occurs and the mechanisms involved in the destruction of myelin in the nervous system vary with different viruses. Viral infections can cause demyelination without involving the immune system, or via virus-induced immune response, or by nonspecific mechanisms, or possibly by disruption of immune regulation. Several of these viruses are capable of synthesis of antibodies in the CNS (Salmi *et al.*, 1983a,b). The causal evidence supporting a relationship between two of these viruses and the chronic nervous-system diseases with which they are associated will be discussed below.

Subacute Sclerosing Pan Encephalitis (SSPE)

This is a chronic and progressive neurological disease with onset in children, often as a reading or behavioral problem, and then leading to seizures and death. It is associated with a measleslike virus. Epidemiologically, the disease is worldwide but rare, with an incidence ranging from 0.12 to 1.4 per million; about 40 new cases are reported yearly in the United States (Gibbs, 1989). Males outnumber females about 3:1. The most striking finding is a history of measles infection early in life: in some studies, over half of the cases have such a history in the first 2 years of life. The evidence of a causal relationship includes: (1) the presence of defective measles antigen in the brains of SSPE patients, as demonstrated by the electron microscope or immunofluorescent antibody techniques, and the isolation of a measleslike virus by cocultivation from the brain; (2) the presence of high levels of measles antibody in the serum and significant titers of both IgG and IgM antibody in the cerebrospinal fluid (CSF) of most patients, indicating antibody synthesis within the CNS; and (3) the reproduction of some of the features of the disease in two experimental animal models using a hamster-adapted measles virus. This evidence establishes a firm causal link between measleslike viruses and SSPE, but one probably dependent on a defective virus and/or a defect in the immune system of the host. In addition, other viruses like rubella and Epstein–Barr virus may rarely result in the same clinical syndrome.

Progressive Multifocal Leukoencephalopathy (PML)

PML is also a rare, demyelinating disease of the CNS, with death occurring some 2–4 months after the onset of symptoms (Zu Rhein, 1982). Some 200–250 cases have been reported in the world literature since its first description by Zu Rhein (Figure 4.5) and Chow (1965), when viral particles resembling papovavirus were found in the brains of Hodgkin's disease patients dying of a progressive neurological disease. It occurs mostly in adults age 50–70, but there have been a couple of cases in children. Recently, it has occurred in adults with AIDS, appearing in from 3.8% (Krupp *et al.*, 1985) to 6.7% (Lang *et al.*, 1989) of AIDS cases. Geographically, the cases have been reported from developed countries: the United States, Canada, European countries, Australia, and Japan (Gibbs, 1989). Most cases have occurred in hypergic individuals as a late complication of a preexisting, generalized systemic disease; over half of the cases have been in patients with lymphoproliferative or myeloproliferative diseases, such as Hodgkin's disease and chronic lymphatic leukemia. The possible viral etiology of the disease was first reported by Zu Rhein and Chow (1965) when papovalike particles were found by electron microscopy in the brains of PML

Figure 4.5. Dr Gabriele Zu Rhein, 1920– , Professor of Pathology, University of Wisconsin School of Medicine

patients. The virus was isolated by Padgett and associates in 1971 in human fetal brain cells, and antibody to the agent was found to be present in over 65% of healthy children by age 14 in Wisconsin (Padgett and Walker, 1973). The viral strain was designated as JC for the patient from whom it was isolated. It is a polyomavirus in the papova family of viruses, whose other member is termed papillomavirus (which includes the wart virus and some strains associated with cervical cancer). No clinical disease has been identified in association with the primary infection with the JL strain. This strain has been found most commonly in the brains of PML patients, although another strain of the polyomavirus, SV-40 or simian virus 40, was initially implicated in a few cases, but this evidence is now questioned (Stoner, 1991).

The evidence relating the agent to the disease includes (1) the constant demonstration of the virus in the brains of PML patients by electron microscopy,

immunofluorescence, or viral isolation, and (2) the presence of antibody to the agent, often in high titer, in the serum of patients. The missing evidence is that infection with the virus and the appearance of antibody have not been shown to precede the onset of the disease. A question is thus raised whether the disease might represent a primary infection in an immunocompromised host, a reactivated and causally related viral infection occurring years after the primary infection (which seems most likely in view of the high frequency of childhood infection), or simply a reactivated infection unrelated causally to the disease. Epidemiologically, demonstration of the more frequent occurrence of the disease in persons with papovavirus antibody than in those without would be desirable. The rarity of the disease, however, precludes the possibility of showing any of these time relationships in prospective studies. Additionally, the disease has not been reproduced experimentally in animals, although inoculation of the virus intracerebrally into hamsters and monkeys has resulted in brain tumors (Walker *et al.*, 1973; London *et al.*, 1978). Thus, both PML and SSPE probably represent very rare manifestations of the reactivation of prior infection by common and ubiquitous viruses in persons with a natural or drug-induced immunodeficiency, but the factors precipitating the disease in so few persons among those infected with the virus remain unknown, except the increasing frequency of PML in patients with AIDS (Krupp *et al.*, 1985; Lang *et al.*, 1989). An increasing number of cases of progressive multifocal leukoencephalopathy (PML) in patients with HIV infection or AIDS is due in part to the longer life expectancy provided by AZT therapy. In 1987, sixteen new cases of PML were reported in HIV-infected persons, and an additional twelve reviewed from the literature (Berger *et al.*, 1987). The number has grown since then. In some cases, PML is the initial clinical manifestation of AIDS. The virus can now be identified in brain tissue using the polymerase chain reaction (PCR) (Telenti *et al.*, 1990), and newer radiological techniques, such as magnetic resonance imaging, are permitting clinical diagnosis.

New Challenges

The discovery that certain chronic diseases of the CNS are due to viruses has led to the possibility that other diseases, such as multiple sclerosis, Alzheimer's disease, and amyotrophic lateral sclerosis (Gehrig's disease) might have a similar etiology. The most extensive work in this area has been done on multiple sclerosis (MS), and the most false etiological leads have been encountered with this disease, which is the most common and best known of the demyelinating afflictions of the human CNS. It occurs worldwide but with much higher incidence in colder climates. The onset of the disease is usually after age 15 with a peak about age 30. Persons born in the higher-risk, colder areas who migrate to a

low-risk, warmer area after the age of 15 carry with them the high risk of their native area, although the disease may not develop until 20 years later; if migration occurs before the age of 15, the risk of MS is that of the low-risk area. This suggests an early environmental factor. Genetic factors may also be important because the disease is 15–20 times more common in first-degree relatives of MS patients than in unrelated persons. The evidence suggesting that MS may have an infectious disease etiology includes the different geographic distribution, familial aggregation, clustering of cases such as in the Shetland and Orkney islands (where there are 128 cases per 100,000 population), and the migrant studies already mentioned. These epidemiological features might also be associated with an environmental or genetic hypothesis with or without a viral connection. Some of the candidate infectious agents and their causal evidence will be discussed. The agents include measles virus (Norrby and Vankid, 1974; Norrby, 1978), distemper virus (Cook and Dowling, 1980), and MS-associated agent (MSSA) (Koldovsky *et al.*, 1975; Henle *et al.*, 1975). Brown and Gadjusek (1974) and Nathanson and Miller (1978) were unable to confirm the findings for MSSA, finally leading to a withdrawal of the claim by the original authors (Carp *et al.*, 1977). Then there is the agent isolated by Mitchell *et al.* (1978) which was denied by Micheletti *et al.* (1979), the virus isolated by Melnick (1982) and Melnick *et al.* (1982), and more recently the human T-cell leukemia viruses (Koprowski *et al.*, 1985).

Before proceeding further in this discussion, some of the features of the viruses and of antibody in relation to the pathogenesis of the disease deserve comment. Viruses could produce demyelinating injury to the nervous system in three ways, as pointed out by Waksman (1983) and Waksman *et al.* (1984): (1) a virus could directly infect and damage oligodendrocytes and produce myelin breakdown and perhaps some degree of inflammation; (2) an immunological response to the persistent viral antigens in the white matter of the brain can result in an inflammatory response and some degree of secondary demyelination; (3) an autoimmune response might occur from the viral infection, either as a result of cross-reactivity to some component of the oligodendrocyte or the myelin, or as a consequence of tissue damage and the release of autoantigen at the time of the initial infection. A population of helper T cells specific for the virus might provide a strong nonspecific adjuvant effect and promote the immune response against the autoantigen. It should be noted that the first of these mechanisms would require a specific etiological agent or agents with special characteristics. The second and third might occur with many viruses that could persist, immunize, and lead to immunization and/or autoimmunization. The presence of antibody to the suspected viral agent, even in high titer, and even when antibody is produced in the CNS can be a nonspecific phenomenon. This latter point deserves emphasis because the relationship to some of the candidate viruses has been based on intrathecal production of specific antibody, sometimes of the IgM type. While the CNS does not normally make antibody, it can do so in acute

infections of the CNS and in demyelinating diseases: at least 16 viruses have been shown to do this in MS and sometimes several antibodies are produced simultaneously (Salmi *et al.*, 1983a,b). Thus, the finding of antibody to a particular virus in the CNS lacks the specificity required for a strong causal relationship.

Let us now briefly review the strength of the association with some of the candidate viruses. Measles virus is perhaps the best documented. In some 25 studies of measles antibody titers in the sera of MS patients as compared to controls, modest but often significantly high titers have been found in most of the investigations. Measles antibody of the IgG type, but not the IgM type, has been found in the CSF of patients, but not in that of controls (Norrby and Vankid, 1974). Antibodies to the nucleoprotein components of the measles virus have been elevated more consistently and to higher titer than antibodies to the viral envelope. However, as noted above, other viral antibodies may also be present in the CSF of MS patients, of which rubella virus, vaccinia virus, and the herpesviruses have been the most common. The antibody findings for measles virus in MS are not nearly so impressive and specific as in SSPE, discussed previously in this chapter. While measles virus has been isolated from a case of postmeasles encephalitis, a common complication of the disease, it has not been isolated from, or demonstrated in, the brain of MS patients, in contrast to SSPE patients. A close relative of measles virus, distemper virus, has also been incriminated in an interesting epidemiological setting in the Faroe Islands. Antibody to this virus is very difficult to distinguish from that of measles virus, and there is no direct evidence that humans are naturally infected by distemper virus, although the reverse might be true in dogs (i.e., there may be evidence of measles virus infection in dogs).

Measles has been long recognized in the Faroe Islands since Panum described the first epidemic of the disease in a classic paper in 1846 (Panum, 1948). Distemper virus was apparently introduced in 1940 during the British occupation of the islands. MS had been absent from native Faroe Islanders but appeared in three epidemic waves of decreasing size beginning in 1943, three years after the start of the British occupation of the islands in 1940 during which they were accompanied by their dogs. In very careful epidemiological studies of this outbreak by Kurtzke (Kurtzke, 1980, 1987; Kurtzke and Hyllested, 1979, 1985, 1987; Kurtzke and Priester, 1979), he and his colleagues found 25 cases after 1943 in Faroe Islanders who had not been off the island and 7 cases in persons off the island less than 2 years. They feel that the first wave was due to an asymptomatic agent introduced by the British and that later waves were the result of transmission among the Faroe Islanders. They postulate that MS is due to a single infectious agent, transmissible at most from age 13 to 26, that is asymptomatic and leads in rare instances to neurological involvement (Kurtzke, 1987). In contrast to Cook and Dowling (1980), they do not believe this agent to be distemper virus because they have found no evidence of a relationship in epi-

demiological studies carried out in the United States, and others have failed to find distemper-specific antibody in MS cases. The issue remains controversial (Stephenson *et al.*, 1991).

A third candidate cause of MS, about which there was much early excitement, was MSSA, and the evidence was both virological and immunological (Koldovsky *et al.*, 1975; Henle *et al.*, 1975). A transitory depression of the leukocyte count of mice, rats, hamsters, and guinea pigs was regularly induced on inoculation of brain tissue suspension from eight cases of MS, and antibody to the filterable agent was also found frequently in high titer in the serum of these patients. It could be serially transmitted from animal to animal. No agent was isolated from control materials. Neutralizing activity present in the immunoglobulin fraction was found in sera from 20 of 22 MS patients as well as in their CSF. It was also found in sera from relatives of MS patients, as well as their nursing personnel, but very rarely in sera from patients with other diseases or from healthy American controls. Sera from over half of East African donors showed neutralizing activity, suggesting that the agent might be more common in developing countries. However, other investigators could not confirm these results (Brown and Gajdusek, 1974), and when coded specimens were submitted to the original workers, they were unable to distinguish between material from MS patients and that of controls (Nathanson and Miller, 1978). A retraction from the original authors resulted (Carp *et al.*, 1977). Newer candidate agents have been reported in a preliminary fashion by Mitchell *et al.* (1978) but then quickly denied (Mitchell *et al.*, 1979). Another virus has been apparently isolated by Melnick *et al.* (1982), but the results must be confirmed by others.

Perhaps the most interesting new possible causes are the human T-cell leukemia retroviruses (HTLV). Recent evidence has demonstrated the presence of antibodies reactive with HTLV antigens in the CSF of MS patients from Sweden, in concentrations significantly higher than in other neurological diseases or healthy controls, as well as higher titers of antibodies to the p24 and disrupted HTLV antigens than in the other groups (Koprowski *et al.*, 1985). They also found that 10 of 17 sera from MS patients in Key West, Florida, reacted at one time or another with an HTLV antigen, as compared with only 2 of 17 sera from close contacts of the patients, one of whom was a homosexual with HTLV-III antibody, and with none of 17 sera from hospital staff workers from the same area. The antibodies found varied in their occurrence in both sera and CSF, were not present in all patients' sera, and reacted differently with various HTLV antigens, suggesting that it was different from HTLV-I, -II, or -III. No virus has thus far been isolated. In other studies, however, the human immunodeficiency virus (HIV) has been identified in both acute and chronic neurological diseases with or without the usual manifestations of AIDS; thus, cells of the nervous system as well as the T4 lymphocyte may be targets for the virus. A new era of agents responsible for CNS diseases has therefore opened with the discovery of

the relation of human retroviruses to the brain. At present, much more work and confirmatory studies need to be done in different laboratories to confirm the importance of this group of persistent viruses.

In summary, the viral etiology of MS has not been established by any of the criteria so far discussed, and the strength of the association with any of the candidate agents is currently too weak to point firmly to a virus as *the* cause of MS. Even if a virus causes MS, it is not clear if it is a single agent, as suggested by Kurtzke and Hyllested (1987), or any one of several that are persistent and can invoke an autoimmune response against elements of the CNS, as Waksman (1981, 1983) and Reynolds (1984) suggest. I hold to the latter hypothesis and believe the cause may vary in different geographic locations and in different epidemiological settings. However, if there were a single cause, it would have the advantage that a vaccine might be developed against it that might prevent MS.

Summary

The information presented in this chapter provides firm evidence that kuru and CJD cause the spongioform encephalitides with which they are associated, mainly based on experimental reproduction of these fatal neurological diseases in laboratory animals. There is also strong virological and epidemiological evidence that SSPE can result from measles virus or its variant, when infection occurs very early in life, although other viruses like Epstein–Barr or rubella, on rare occasions, are also associated with this syndrome. Similarly, PML is due to a common papovavirus (the JC strain) in which reactivation occurs in the presence of immunodeficiency. To date, virus or viruses have been linked to MS in association with an aberrant immune response, but the specific agents that cause this have not been conclusively identified, and investigation of this etiology has proven to be a hazard to many fine investigators.

A new hypothesis has tried to bring together some common pathogenetic events for these chronic neurological syndromes caused by slow viruses (Gibbs, 1989). It is based on the pathological changes seen in neurofibrillary tangles (NFT) and amyloid plaques, which are similar to those seen in the aging brain. The NFT are seen in infections of the brain in SSPE and in chronic rubella infections, and the amyloid plaques have been observed with certain strains of scrapie, kuru, and CJD in the appropriate natural hosts. But similar lesions have also been found in patients suffering from aluminum intoxication, and in dialysis dementia. It is thus possible that several viruses, toxic or genetic determinants, or perhaps even trauma may lead to the lesions with NFT and amyloid plaques. The underlying mechanism is postulated to be interference with axonal transport

of neurofilaments. Modern developments in molecular virology and protein chemistry may shed light on the hypothesis that self-replicating proteins resembling "viruses" can result in the autocatalytic patterned degeneration of host precursor proteins to amyloid (Gibbs, 1989). A strong case has been presented that infectious proteins called "prions" are a major cause of these "slow viral infections" (Prusinger, 1992) and are related to amyloid materials (Prusinger *et al.*, 1983). Amyloid derivatives might thus become transformed into self-replicating agents under certain provoking determinants. Such new evidence will pose new challenges in establishing the proof of causation and the risk factors involved in these fascinating chronic neurological syndromes.

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