REVIEW ARTICLE

Slow Virus Infections of the Nervous System: Virological, Immunological and Pathogenetic Considerations

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INTRODUCTION

During the last two decades, after Sigurdsson's introduction of a new concept of an infectious process, slow virus infections of the central nervous system have developed into novel and attractive disease models (Sigurdsson, 1954). Through his studies of naturally occurring diseases in sheep, including Maedi, Visna and scrapie, Sigurdsson observed that these infections were characterized by an incubation period lasting for many months to years and a predictable protracted clinical course usually leading to death. Subsequent studies carried out by many research groups have basically confirmed Sigurdsson’s concept. It was found that slow virus infections are related to unconventional and conventional agents, both of which have been associated with naturally occurring diseases in animals and man (Table 1).

The unconventional agents which have not been visualized, isolated or characterized reveal unusual biological and physico-chemical properties, exceptional for any known infectious agent. The conventional viruses, isolated from diseased brain material, resemble classical viruses with typical structural, physico-chemical and biological characteristics. For the latter group of viruses, progress has been made in the understanding of the infectious process since the isolates provided an experimental basis for virological and immunological studies in these diseases. Numerous comprehensive reviews have been published dealing with many different aspects of slow virus infections (see reference Slow Virus Infections). This review, which will be restricted to the slow virus diseases of the central nervous system associated with conventional agents, summarizes the findings from recent investigations in an attempt to discuss and interpret the mechanisms which may be responsible for these disorders.

Visna

The appearance of a subacute encephalomyelitis in Icelandic sheep was first observed in 1935 (Sigurdsson et al. 1957). The disease was named Visna which refers to the clinical picture of 'shrinkage or wasting'. Apparently, this disease was brought to Iceland by Karakull sheep imported from Germany. Sigurdsson and co-workers were the first to investigate this affliction of the central nervous system (CNS) and demonstrated that the disorder was associated with a virus infection (Sigurdsson et al. 1957). Visna was readily transmissible by intracerebral inoculation of brain material obtained from diseased sheep and an RNA virus could be isolated from brain material (Sigurdsson et al. 1960). These important observations led to intensive studies of this disease.

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Table 1. *Slow virus diseases of the central nervous system in animals and man*

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**Clinical and neuropathological characteristics**

After an incubation period of several months or years the disease, insidious in its onset, usually starts with slight aberration in gait, developing into paraplegia or even total paralysis (Gudnadottir, 1974). Typical signs present in acute virus infections are missing. The clinical course is variable ranging from a slowly progressive to a rapidly deteriorating condition. In early experimental infection, in the absence of any clinical signs, a short lasting pleocytosis in the cerebrospinal fluid (CSF) is noticed. With the appearance of neurological symptoms, an elevation of CSF protein and cells often occurs in up to 100 to 300 cells/ml. A slight increase in white blood cells is sometimes found.

The neuropathological changes observed in Visna diseased sheep are of an inflammatory nature and are located in the white and grey matter of the brain and spinal cord. Severe meningitis, choroiditis and intense infiltration, and proliferation of mononuclear cells are found in the perivascular areas. The subventricular areas of the brain and white matter of the spinal cord are the main regions which are most severely affected (Sigurdsson *et al.* 1962). Moreover, a diffused destruction and demyelination occurs especially in the pons, medulla oblongata and spinal cord. Lesions are frequently located near the ventricles and aqueduct. These changes do not parallel the onset of clinical symptoms as was recently demonstrated in sheep experimentally infected with Visna virus (Petursson *et al.* 1976). Two weeks after intracerebral inoculation, a generalized encephalitis with small areas of demyelination is found in animals which do not exhibit any signs of a CNS disease.

**The virus**

Visna virus belongs to the family of retroviruses (Haase, 1975). Its size, morphology and physico-chemical properties are similar to oncornaviruses. The virus matures from infected cells by budding and contains a polyplid genome composed of 2 to 4 RNA subunits. The structural proteins are comprised of at least 15 polypeptides, one of which is phosphorylated and 2 or 3 glycosylated (Mountcastle *et al.* 1972; Haase & Baringer, 1974). The glycoproteins are located on the surface of the virion. As in oncornaviruses, most of the total Visna virion protein is in the form of small polypeptides. The major polypeptide, with a mol. wt. of approx. 30000, carries the antigenic determinants which characterize the group of lentiviruses consisting of Visna, Maedi and progressive pneumonia virus. The lentiviruses, as well as the oncornaviruses, contain an RNA-dependent DNA polymerase. However, besides comparable features, the lentiviruses also reveal distinct differences from oncornaviruses. Lentiviruses induce a chronic inflammatory disease, which leads to a pronounced cytolytic effect in cell cultures, and contain a prominent cell fusion activity. Moreover, no antigenic relationship or nucleic acid sequence homology to oncornaviruses has been demonstrated.
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(Harter, 1977). The possibility that lentiviruses possess oncogenic properties has been suggested but not unequivocally demonstrated (Takemoto & Stone, 1971).

Pathogenetic aspects

The isolation of Visna virus from infected animals, its propagation in tissue cultures and the induction of Visna in sheep with tissue culture grown virus made it possible to study the pathogenesis of this disease. Intracerebral inoculation of Visna virus in sheep leads to an infection of brain cells mainly along the needle track, the choroid plexus and ependymal cells lining the ventricular system. As a consequence of this initial inoculation and local infection of the brain, a viraemia occurs which also infects other organs, especially the lungs, lymph nodes, spleen, bone marrow, liver and kidney without the specific pathological changes attributable to Visna infection (Petursson et al. 1976). From all of these organs, virus at low titres can be consistently isolated during the long incubation period and in the course of clinical disease (Gudnadottir, 1974) mainly by explant cultivation methods. Moreover, virus can be recovered throughout the period of infection by co-cultivation of peripheral blood leukocytes with sheep choroid plexus cells. Preliminary data suggest that lymphocytes might be the site of virus replication (Petursson et al. 1976).

However, only the sheep brain seems to be the target organ for Visna virus. In response to the experimental infection, infiltration of immune and inflammatory cells occurs in the CNS leading morphologically to an acute meningo encephalitis. Despite these neuropathological changes, the animals do not develop a clinically recognizable disease at this stage. The infection of the brain does not spread as in any other known acute CNS infection because Visna virus replication is limited or restricted resulting in a persistent infection (Haase et al. 1978). This limitation of virus replication in vivo, which is not observed in vitro in infected sheep cell cultures, yielding high virus titres and a pronounced cytopathic effect, has been attributed to host factors and/or special events of virus replication. It is known from acute infection with other viral agents that the age of a host and its immune system are important factors for susceptibility and control of an infection. Experiments have been carried out by different groups to analyse the influence of age and immune status on replication and persistence of Visna virus in sheep as well as the induction of neuropathological changes.

Development of pathological lesions

Icelandic sheep between the ages of 7 and 15 months were intracerebrally infected with Visna virus and sequentially analysed for virus yield and pathological lesions in brain and other organs over a period of 12 months (Petursson et al. 1976). Low titres of free virus were often detectable in most of the tissues and fluids, but the best yield was obtained by applying tissue culture explant techniques. Virus was detectable in many organs during the whole period of observation despite a specific humoral immune response. Pathological lesions attributed to the virus infection were only detectable in the central nervous system and were relatively independent of the incubation period and age of the animals at the time of infection. However, it was particularly striking that a correlation between severity of CNS lesions and frequency of virus isolations could be established, suggesting that a certain threshold of virus replication is required for the induction of pathological lesions. Some of these animals were additionally immunosuppressed by anti-lymphocyte serum and cyclophosphamide (Nathanson et al. 1976). This regimen did not enhance virus replication in vivo but led to a prevention of histopathological lesions in some animals, suggesting that immune pathological mechanisms may play a role in the development of the 'early' lesions in this disease.
Different results were obtained in American sheep (Narayan et al. 1974, 1977a). Virus-infected foetal American lambs with and without immunosuppression by thymectomy or antilymphocyte serum failed to develop histological lesions but also revealed a depressed state of virus replication. No increase of virus was detectable in animal organs during the period of testing, indicating that the poor replicating efficiency of Visna virus in vivo is dependent on factors other than the immune status or factors associated with maturation. However, despite the lack of substantial virus growth in infected sheep, virus was present in many organs at all times during infection. The answer to the underlying mechanism of this particular virus-host relationship in Visna has come from biochemical studies of Visna virus infection in brain material and the analysis of the immune response in these animals.

**Mechanism of persistency**

In sheep choroid plexus (SCP) tissue from an infected sheep, Visna DNA was demonstrated by *in situ* hybridization in foci of many cells in the absence of detectable virus particles, seen by electron microscopy, and infectious virus recoverable from cell-free homogenates (Haase et al. 1977). Only in culture explants from SCP tissue could infectious virus be isolated. In contrast to the relatively high proportion of cells containing Visna DNA, Visna virus protein was demonstrable by immunofluorescence in only a small fraction of cells. This block in virus gene expression probably occurs at the transcriptional level, as suggested in preliminary studies of these tissues, since only few cells contained Visna RNA (Haase et al. 1978). Estimates of Visna RNA copies in these cells indicate comparable levels to those found in infected tissue cultures. Haase and co-workers (1978) and Brahic et al. (1977) showed that permissively infected cells contain approx. 1000 to 2000 Visna RNA copies per cell a few hours after infection. It can be assumed that these cells which contain Visna RNA are probably producing the minimal amount of free virus found in organ tissues. These investigations provided the first evidence that, after the initial infection in animals, a stable association between virus genetic information and the host cell is established by the formation of virus DNA copies.

**Host immunity and antigenic shift**

This mechanism does not, however, account for the finding that free Visna virus can be isolated in the presence of an antiviral immune response in an infected animal. Experimentally infected animals reveal measurable anti-Visna complement fixing and neutralizing antibodies after 1 to 2 months which are maintained during the course of infection (Petursson et al. 1976; Narayan et al. 1977a). At the level of cell-mediated immunity a Visna virus-specific reaction, measurable by the incorporation of tritiated thymidine into lymphocytes stimulated by Visna antigen, can be detected during the first weeks after infection (Griffin et al. 1978). Lymphocytes from peripheral blood, as well as from CSF, revealed such a specific response, which had its peak at 2 weeks and fell to normal 6 weeks after inoculation. The available immunological data from the initial phase of the long incubation period of Visna virus infection suggest that the immune responses at this period of infection might be similar to those observed in acute virus infections; however, they fail to control the infectious process (Griffin et al. 1978).

The possibility of isolating infectious Visna virus from peripheral blood leukocytes (PBL) by co-cultivation methods during the long incubation period and the course of the disease has provided the experimental conditions for analysing the immune responses not only to the original virus inoculum but also to the numerous isolates from PBL. In studies carried out with Icelandic sheep, Gudnadottir (1974) compared the antigenicity of re-isolated Visna
strains from different animals with the originally inoculated strain in a neutralization assay and found distinct differences which she interpreted as an antigenic drift of Visna virus in the presence of antiviral antibodies. This observation was confirmed by Narayan and co-workers (1977a) and extended by a detailed investigation on the development and characterization of mutants occurring in Visna infections (Narayan et al. 1977b, 1978). This group found that, in the course of infection in an animal, many mutants can appear months after inoculation when neutralizing antibodies to the parental virus have already developed. No mutant could be isolated in the early phase of infection. Both virus with a parental genotype and mutants persist in the presence of a humoral immune reaction and can be recovered later. Moreover, preliminary data suggest that mutants probably evolve sequentially followed by a specific antibody response (Narayan et al. 1978). In vitro studies of these mutants revealed a stable antigenicity after plaque purification and showed that the mutants differ not only antigenically from the parental strain but also from each other. The phenomenon of mutation in the presence of antiviral antibodies could also be demonstrated in tissue cultures within several passages (Narayan et al. 1977b). Mutants were derived which showed significant antigenic differences from the strain inoculated. Narayan and co-workers also infected susceptible sheep with Visna mutants. These animals developed pathological CNS lesions and an immune response to the mutant revealing a one-way cross-reaction to the parental strain from which the mutant was derived (Narayan et al. 1978).

These important observations on the occurrence of Visna mutants in the event of an infection have been interpreted as an important factor in the pathogenesis of this chronic disease. It is conceivable that each rise of progeny virus which cannot be neutralized leads to a new infection of brain cells which is accompanied by an inflammatory and an immune response resulting in CNS damage and finally in a clinically recognizable disease.

**Subacute sclerosing panencephalitis**

Subacute sclerosing panencephalitis (SSPE) is a rare, slowly progressing disease of the central nervous system which occurs primarily in children and young adults (Payne & Baublis, 1971; ter Meulen et al. 1972; Agnarsdottir, 1977). This disorder was described under different headings (Schilder, 1924; Bodechtel & Guttmann, 1929; Dawson, 1933; Pette & Döring, 1939; van Bogaert, 1945) based on neuropathological grounds but Greenfield (1950) unified the different entities under the term SSPE which is now generally accepted. In 1933 Dawson incriminated a filterable virus as the aetiological agent but the first evidence of virus involvement was reported by Bouteille et al. (1965) and Tellez-Nagel & Harter (1966) who observed tubular structures resembling paramyxovirus nucleocapsids in brain tissue from SSPE patients. The paramyxovirus was soon identified as measles virus by Connolly et al. (1967) who showed that SSPE patients had extremely high measles antibody titres in their serum and cerebrospinal fluid (CSF). Further studies (Freeman et al. 1967; Connolly et al. 1968; ter Meulen et al. 1967, 1969) using immunofluorescence methods demonstrated that measles virus antigen was present in neurons and glial cells. In spite of the detection of measles virus antigen in the brain, attempts to isolate virus by conventional methods were unsuccessful. It was not until brain cells were co-cultivated or fused with continuous cell lines that infectious measles-like virus (referred to as SSPE virus) was obtained (Horta-Barbosa et al. 1969; Payne et al. 1969; Barbanti-Brodano et al. 1970). This agent has now been repeatedly isolated from both biopsy and autopsy material and also from lymph nodes of SSPE patients (Agnarsdottir, 1977).

Although these findings incriminated measles virus as the aetiological agent in this disease, the pathogenesis still remained unexplained. If measles virus is involved, then
additional factors, either host or virus derived, must play a pathogenetic role since rarity and rural prevalence (Detels et al. 1973) of this disease cannot be correlated to the ubiquitous measles infection. Moreover, the mechanisms have to be explained whereby measles virus infection persists in the CNS. The availability of isolates from SSPE patients has initiated numerous studies to find an answer to these questions.

Clinical and neuropathological characteristics

SSPE has been observed in many ethnic groups with an incidence of approximately one case per million in children. A history of acute measles is quite often found before the age of two. The mean age of onset of SSPE is 7 to 8 years of age with an average interval of 5 to 6 years after acute measles infection. However, cases of SSPE have also been reported at below 2 and over 20 years of age.

The clinical course usually follows a stereotypic pattern beginning with insidious behavioural changes which, after a period of weeks or months, are superseded by characteristic neurological symptoms. This clinical picture is characterized by various disturbances of motor function, myoclonic jerks and epileptic seizures. At a later stage of disease, a progressive cerebral degeneration with symptoms and signs of decortication occurs. Although there have been reports of remission, the disease usually follows an ingravescent course over a period of months to years, always ending in death.

Besides this clinical picture, the patients reveal typical electroencephalogram and pathognomonic laboratory findings. Each SSPE case tested showed a marked increase in CSF IgG and extremely high measles virus antibody titres in serum and CSF specimens. Measles-specific IgG antibodies represent nearly 10 to 20% of total serum IgG and about 75% of total CSF IgG (Mehta et al. 1977). Moreover, there is always a reduced serum-CSF ratio of measles virus antibodies and the finding of oligoclonal CSF IgG, which in large part is measles virus specific, indicating a local production of measles virus antibodies within the CNS (Vandvik & Norrby, 1973).

The encephalitic process is characterized by perivascular cuffing consisting of lymphocytes and plasma cells with diffuse mononuclear infiltration of the grey and white matter. The most striking histological features of SSPE are the enormous increase in hypertrophic astrocytes and proliferation of microglia cells as well as demyelination and the presence of intranuclear Cowdry type A and B inclusion bodies (Cowdry, 1934). The inclusion bodies containing measles virus nucleocapsid are most frequently seen in oligodendroglia cells. Measles virus antigen is, however, not only detectable in cells revealing intranuclear inclusion bodies but also in the cytoplasm of many neurons and oligodendroglia cells which do not show morphological changes (ter Meulen et al. 1969). Moreover, measles antigen has been demonstrated in mononuclear cells from the CSF, in lymph nodes, spleen and liver as well as in renal glomeruli (Dayan & Stokes, 1971, 1972). However, no convincing evidence has been found for complete measles virus replication cycles in the CNS. Infectious virus could not be isolated directly from brain material by conventional methods, nor have virus particles, the process of budding, or giant cells – a characteristic cytopathic effect of measles – been seen by ultrastructural and histological investigations of brain sections.

Measles and SSPE viruses

The isolation of SSPE virus from diseased brain material has led to thorough, comparative virological investigations of measles and SSPE viruses. Biological differences between these viruses were observed regarding cell susceptibility, growth kinetics in different cell lines, including organ cultures, and neurotropism in inducing an acute or subacute encephalitis in
a variety of laboratory animals (reviewed by Agnarsdottir, 1977). However, none of these studies has convincingly ruled out that these findings are not within the range of the variability also found among different measles virus isolates. Standard serological techniques such as haemagglutination inhibition (HI), haemolysin inhibition (HLI) or complement fixing tests failed to reveal antigenic differences. However, other immunological tests indicated the existence of minor antigenic variations between measles and SSPE viruses. In neutralization tests, it appeared that SSPE viruses were less sensitive to neutralizing measles antibodies than measles virus (Payne & Baublis, 1973). By a binding inhibition test based on an indirect radioimmunoassay, it was possible to detect differences between SSPE and measles virus strains (Hall et al. 1977a). Moreover, results of this binding inhibition test suggested that SSPE viruses might be more closely related antigenically to canine distemper virus than to measles virus. While the immunological data suggest minor differences in the immunological reactivity between these viruses they are not sufficient to distinguish clearly between these virus strains. Some of the most interesting information concerning SSPE has recently come from comparisons of the biochemical properties of wild type and vaccine measles virus strains with different SSPE viruses.

Initial studies on the genetic relatedness using RNA hybridization have shown that small but distinct differences exist (Hall & ter Meulen, 1976). These findings were extended in an analysis of the mRNAs synthesized in infected cells and it was shown that the smallest mRNA was larger in SSPE virus infected cells than in measles virus infected cells (Hall et al. 1978). This RNA is thought to code for the membrane protein of the virus which would support the findings of Schluederberg et al. (1974) and more recently of Wechsler & Fields (1978) who reported that the M-proteins of SSPE strains were larger than those of measles strains. Definitive evidence that the M-proteins were different came from an immunological study of purified M-proteins of one measles and one SSPE virus strain (Hall et al. 1977b, 1978; ter Meulen et al. 1978). Antisera against M-proteins were prepared in rabbits and specific antibodies detected in a solid phase radioimmunoassay which did not react with measles or SSPE virus haemagglutinin, haemolysin or complement fixing antigens in the appropriate test system. The antigenic relatedness of the M-proteins was assayed by immunodiffusion and immunoelectrophoresis and it was shown that the two M-proteins were immunologically distinct since an immune reaction occurred only in the homologous system. These results provided a firm basis for an antigenic differentiation of measles and SSPE virus and demonstrated that SSPE virus is, in fact, closely related but not identical to measles virus.

Pathogenetic aspects

The association of the measles-like virus with a chronic CNS infection which does not in any way resemble known measles virus complications and the epidemiological observation that SSPE is preceded by an acute measles virus infection has led to many hypotheses to explain the aetiology and pathogenesis of this disease (ter Meulen et al. 1978). Measles virus infection of a sero-negative immunocompetent host results in an acute disease. During this acute illness the virus infection has a direct effect on the immune system and a typical measles virus c.p.e. consisting of giant cell formations can be found in lymph nodes and thymus (White & Boyd, 1973). Moreover, a partial or total tuberculin skin test anergy develops in tuberculous children, indicating a reduction of sensitized T lymphocytes (von Pirquet, 1908). Normally, the organism controls this infection and obtains a lifelong immunity. In contrast, however, individuals suffering from congenital or acquired T cell deficiencies often develop abnormal rash, fatal lung or CNS complications (Gatti & Good, 1970) in the course of
measles infection. These observations indicate that an intact cell-mediated immune system is vital for the host in order to overcome an infection with measles virus. Therefore, the demonstration of measles virus in SSPE brain has led to the interpretation that this CNS infection might be a late complication of measles infection in an immunocompromised host.

Host-immunity

So far, no immunological deficiencies in the humoral immunity, including the complement system, have been detected in SSPE. All standard tests employed to assess humoral immune responses are within normal limits. A single exception is a hyperimmune reaction against all demonstrable measles antigens. These antibodies neutralize infectious measles and SSPE virus and are capable of lysing measles infected target cells in the presence of complement (Kibler & ter Meulen, 1975). In addition, measles specific IgM has been demonstrated to be present in CSF and serum of some patients underlining the fact of a measles virus persistency in this disease (Kiessling et al. 1977). However, in view of the antibody responses, the normal immunoglobulin level and the normal number of B and T cells of SSPE patients, the lymph node histology in some patients with SSPE has been an unexpected finding. Lymph node biopsy material from SSPE patients, obtained after intensive immunological stimulation, showed a paucity of primary follicles and almost a complete absence of germinal centre formations (Gerson & Haslam, 1971).

In terms of cell-mediated immunity, no generalized defect has been found in patients with SSPE (reviewed by Agnarsdottir, 1977). The blastogenic and lymphokine responses are unimpaired after stimulation with mitogens or antigens unrelated to measles virus. However, anamnestic skin tests with measles antigen are often negative whereas primary sensitization with dinitrochlorobenzene, a potent skin test antigen, has always been positive. A disparity of results was obtained by using measles virus or SSPE virus antigens in assays for cell-mediated immunity (CMI). Depending on the test system employed and on the potency and purity of the virus antigens used, a minor inhibition of CMI or normal reactions in comparison to controls was observed in SSPE patients (Agnarsdottir, 1977). Moreover, a blocking factor present in serum and CSF from some SSPE patients was found to inhibit production of MIF (Sell et al, 1973) and lymphotoxin (Ahmed et al. 1974) as well as lymphocyte transformation (Allen et al. 1973) and lymphocyte cytotoxicity (Valdimarsson et al. 1974). This factor is believed to be an immune complex consisting of measles antigen, antibody and complement. However, other investigators have not been able to confirm the existence of this blocking factor in lymphocyte-mediated cytotoxicity assays (Kreth et al. 1975; Perrin et al. 1977).

An open question is the functional state of T cell dependent cytotoxicity in relation to measles-virus-infected cells in SSPE patients. Several groups have shown the presence of antibody-dependent lymphocyte cytotoxicity, but failed to detect specific T cell killing of measles virus-infected target cells (Kreth & Wiegand, 1977; Perrin et al. 1977). The failure has been attributed to the lack of matched histocompatibility antigens (HLA) between target and effector cells in the assay system. However, recently Kreth et al. (1978) provided some evidence that in spite of HLA identity within the test system no T cell killing occurred with SSPE lymphocytes, whereas in acute measles a specific cytotoxic T cell effect was observed. However, before one can accept a T cell-specific defect in SSPE, which was initially postulated by Burnet (1968) as an important pathogenetic mechanism for this disease, it has to be shown that no secondary population of effector T cells in SSPE are detectable after specific re-stimulation of memory T cells in vitro.
**Virus–cell interaction**

The virus–host relationship in SSPE obviously plays a major role in this disease. From a virological point of view, the impairment of virus replication in SSPE brain cells, as well as the biochemical and immunological differences between SSPE and measles viruses, have to be explained. Biological and biochemical characterization of tissue cultures derived from SSPE brain material and latently infected with SSPE viruses revealed a state of virus defectiveness that may represent a mechanism similar to that responsible for virus persistency in the brain of SSPE patients (Doi *et al.* 1972; Kratzsch *et al.* 1977). All attempts to initiate a reactivation of virus synthesis in those cultures were unsuccessful. Morphologically, these cultures contained nucleocapsids and revealed measles antigen. In addition, salt dependent haemagglutinin antigen was demonstrable on cell surfaces of one cell line. Biochemical analysis of intracellular RNA demonstrated that the majority of the virus RNA produced was defective or subgenomic and that the 50S RNA normally associated with infectious virus was detectable only in small amounts (Kratzsch *et al.* 1977). These findings indicate an impaired state of virus replication which could be related to the presence of defective interfering (DI) particles. It has been shown for VSV that DI particles not only interfere with the replication of infectious virus in tissue cultures, but are also capable of influencing a virulent VSV infection in animals by prolonging the incubation period and modifying, to some extent, the disease process (Huang, 1977). In measles, DI particles cannot be separated biochemically and therefore their presence cannot be directly demonstrated in infected organs but their interfering potential was indirectly shown in tissue culture (Rima *et al.* 1977a).

The observed biochemical and immunological differences of measles and SSPE virus M-proteins suggest an alternative explanation for the virus–host relationship in this disorder. If SSPE viruses have arisen from measles wild type virus it is conceivable that a mutation or modification of the gene region coding for the M-protein has occurred during latency. It is interesting to consider the implication of such a change occurring in the host. During the mutation process, it is possible that a non-functional M-protein is produced which cannot carry out its normal function in virus assembly. This may result in the initiation and maintenance of a non-productive, persistent infection in which no virus would be released. Moreover, this could explain the absence of budding virus in SSPE brain tissue and the high failure rate (about 80%) in the isolation of viruses from brain tissue, despite the fact that brain cells contain large quantities of virus antigens (Katz & Koprowski, 1973). Alternatively, if SSPE viruses represent an independent strain of the measles virus group, one would expect an epidemiological clustering of this disease which has not been observed.

To find an answer to these questions it would be essential to be able to analyse the state of SSPE virus replication in infected brain tissue as well as to have available an animal model in which the virus–host relation could be studied.

**Progressive multifocal leukoencephalopathy**

Progressive multifocal leukoencephalopathy (PML) is an uncommon subacute demyelinating disease of the human CNS. A viral aetiology of this disease was postulated by Cavanagh *et al.* (1959) and Richardson (1961) on the basis of the presence of inclusion bodies and the association of this disorder with diseases accompanied by an impaired immunological responsiveness. The first evidence of a virus involvement was provided by the electron-microscopical studies of ZuRhein & Chou (1965) and Silverman & Rubinstein (1965) who demonstrated virus structures in oligodendroglia cells which resembled papova viruses. This observation led to several attempts to isolate viruses from the brain of PML patients by
inoculation of brain material into cell cultures, small laboratory animals or primates and embryonated eggs, all without success (Schwerdt et al. 1966; Dolman et al. 1967; Gibbs et al. 1969; ZuRhein, 1969). Initial isolation of a papova virus was first done by Padgett et al. (1971) and subsequently by Weiner et al. (1972a) which made virological investigation of this disease possible.

Clinical and neuropathological characteristics

PML is characterized by multiple and varied neurological symptoms depending on the location of the CNS infection. Neurological signs such as paralysis, mental deterioration, visual loss, sensory abnormalities and ataxia take a progressive course and the disease usually leads to death in less than 1 year. The patients do not reveal any signs of inflammatory infection and have normal cerebrospinal fluid. This disease is considered to be an opportunistic papova virus infection of underlying disorders such as reticuloendothelial diseases, carcinomas, granulomatous and inflammatory diseases associated with the state of secondary immunodeficiency (Johnson et al. 1977).

In addition, PML has been seen in patients who received immunosuppressive therapy in the course of renal allografts and for the treatment of systemic lupus erythematosus. However, there have also been a few cases in which neither predisposing disease nor immunological defects were detectable (Rockwell et al. 1976; Weiner & Stohlman, 1978) and a primary PML occurred.

The neuropathological changes consist of non-inflammatory demyelinating lesions in contrast to the other diseases described in this review. The demyelinating plaques are found throughout the white matter, revealing loss of oligodendroglia cells and myelin sheaths. The oligodendroglia cells in the periphery of the plaques are often enlarged and contain intranuclear inclusion bodies which are filled with papova virus-like particles. Immunofluorescent staining of frozen sections demonstrates papova virus antigen in the nuclei of these cells in and around the lesions. Within the foci of demyelination, astrocytes occur with abnormal mitotic figures, bizarre chromatin pattern and multinucleation. These cell formations resemble neoplastic cells to a certain extent (Aström et al. 1958).

Papova viruses

Two papova virus strains, designated as JC virus (Padgett et al. 1971) and SV40-PML virus (Weiner et al. 1972a) were recovered from PML brain material. JC virus was isolated by inoculation of homogenized brain tissue in primary cultures of human foetal glial cells consisting mainly of spongioblasts. The viruses cause a c.p.e. and appear to be highly cell-associated. After this initial isolation, JC virus was isolated from many other PML cases (Padgett & Walker, 1976; Walker & Padgett, 1978). SV40-PML virus was recovered by a different technique. Cell cultures from a brain biopsy were established which showed an apparent transformation after six subcultures. Fusion of these brain cells with primary African green monkey kidney cells and BSC-1 cells yielded infectious virus which resembled SV40. So far, this strain of human papova virus has been isolated by the same laboratory applying similar techniques from only one further case.

Both PML isolates were biochemically and immunologically compared to SV40 and the third human papova virus strain, BK, which was originally recovered from the urine of a patient following a renal transplant (Gardner et al. 1971). This virus is frequently found in the urine of patients receiving immunosuppressive therapy or from children with Wiskott–Aldrich syndrome but so far has not been isolated from PML cases (Gardner, 1977). SV40-PML resembles SV40 structurally and antigenically (Weiner et al. 1972b); its DNA is a
circular duplex with approximately the same mol. wt. as SV40 DNA. Moreover, it has been shown that the DNA of both viruses has a close homology both by hybridization and comparison of the restriction enzyme cleavage patterns. Digestion of SV40-PML DNA with restriction enzymes from Haemophilus influenza resulted in 11 major fragments, 9 of which co-migrated with 9 fragments of SV40. Only in a few fragments (C and F) could a different electrophoretic mobility be observed between these two viruses, indicating that there are two small deletions in the SV40-PML genome relative to the genome of SV40 (Sack et al. 1973). However, similar variations have been seen with stable SV40 plaque morphology variants suggesting that SV40-PML could be a variant of SV40.

JC virus, which is similar to SV40 and polyoma virus with respect to size and morphology of both the virion and the DNA revealed distinct differences by comparative genome analysis. Reassociation kinetics of JC DNA in the presence of a large excess of BK or SV40 DNAs showed a polynucleotide sequence homology of 25% between the genomes of JC and BK and only 11% between the genomes of JC and SV40 viruses (Howley et al. 1976). Studies on the restriction enzyme cleavage pattern of SV40, JC and BK DNA showed no similarities in either number or size of the fragments (Osborn et al. 1974).

Immunological studies on the interrelationship between SV40-PML, JC and BK viruses have shown an antigenic relationship which can be demonstrated by HI, NT, fluorescent staining of infected cells or immune agglutination, using hyperimmune sera (Padgett & Walker, 1976). However, monospecific antisera allow identification of these virus strains (Penney & Narayan, 1973). This observation suggests that these viruses do not possess an identical group-antigen but rather one or more related V antigens which give rise to cross immunity (Padgett & Walker, 1976). A considerable cross-reactivity between the tumour (T) antigens of these viruses was found by indirect immunofluorescence (Takimoto & Mullarkey, 1973; Walker et al. 1973; Nase et al. 1975; Portolani et al. 1975; Van der Noordaa, 1976), immunoperoxidase staining and complement fixation (Dougherty, 1976). Recently Beth et al. (1977) studied the degree of relatedness between the T antigens induced by human papova viruses and were able to show that T antigens possess various subspecificities which are defined as interspecies, species and type-specific antigenic determinants. This group found that approx. 20% interspecies cross-reactivity exists among JC, BK and SV40 T antigens. On the other hand, at the level of tumour-specific transplantation immunity no cross-reaction has been observed which was demonstrated by protection experiments in hamsters. Hamsters immunized with BK virus failed to protect the animals against challenge with SV40 or JC virus-induced hamster tumour cells or vice versa (reviewed by Padgett & Walker, 1976).

Pathogenetic aspects

Transmission of papova viruses. In contrast to the rare disease, the agents associated with this CNS disorder apparently produce a widespread subclinical human infection. Seroepidemiological studies (by Padgett & Walker, 1973) have shown that during the first 14 years of life about 65% of the tested population had acquired antibodies against JC virus. At a later age, 70 to 80% revealed a humoral immune response to this agent. A similar pattern of antibody distribution was reported from different areas of the world (reviewed by Padgett & Walker, 1976). Antibodies to SV40 are less frequently found and are primarily dependent on exposure to monkeys or contact with SV40-contaminated vaccines. However, SV40 antibodies have been detected at low frequencies (2 to 4%) in humans who neither had contact with monkeys nor were immunized with contaminated polio vaccines (Shah, 1972). The observation that JC infection occurs most frequently during childhood suggests that
this virus is readily transmissible and is always present in a given population, yet it displays little virulence. No information about the origin of the infection is available. However, if the epidemiology of JC virus is similar to other papova viruses it might be speculated that JC virus is excreted with the faeces or urine of infected persons and enters the body orally. Recently, Coleman et al. (1977) demonstrated that JC virus could be recovered from the urine of three patients, two with renal transplants and one pregnant woman. Papova viruses which could be identified as JC virus were isolated. In the last case, basophilic intranuclear inclusions were observed in urothelial cells which revealed typical virus structures by electron microscopy. In this patient, a pronounced immune response to JC virus was found. After delivery, virus could not be isolated from urine specimens, the placenta nor from neonatal urine. These observations show that JC virus is active in extraneural tissues without an association with PML. On the other hand, successful isolation has been limited to persons with an immunological deficiency or with slightly reduced immune responses occurring during pregnancy.

**Virus-cell interaction.** Although JC virus infects large numbers of people, its full disease producing potential is not known. So far, the only clinically recognizable disease seems to be PML. In PML brain, large numbers of virus particles can be detected. It was estimated by electron microscopic and biochemical analysis that approx. 10^10 virus particles per gram of brain are present in areas revealing lesions (Johnson et al. 1977; Dörries et al. 1978). Ultrastructural studies indicate that JC virus particles are most frequently found in oligodendroglia cells and rarely in astrocytes. This observation, together with immunofluorescence data on papova virus antigen distribution in brain lesions, has led to the interpretation that these viruses selectively destroy the oligodendroglia cells with loss of their cytoplasmic extensions leading to demyelination (Johnson et al. 1974). Oligodendroglia cells probably represent a permissive cell population which allows virus replication and is lysed by the virus. In view of the oncogenic potential of the papova virus group an effect of these agents on astrocytes has been suggested but not yet demonstrated. After intracerebral inoculation, JC, SV40 and BK viruses produce different brain tumours in hamsters. Most of the JC derived tumours are medulloblastomas, glioblastomas, ependymomas and pineocytomas (Walker et al. 1973; Padgett & Walker, 1976; Padgett et al. 1977) whereas SV40 and BK viruses are associated with choroid plexus papillomas (Greenlee et al. 1977; Padgett et al. 1977). Therefore not only the morphological changes detectable in astrocytes within PML lesions (Aström et al. 1958) but also the simultaneous occurrence of brain tumours in a few PML cases have been associated with the papova virus infection. Two patients with PML exhibited coexisting gliomas (Richardson, 1961; Castaigne et al. 1974) which in one case appeared to correspond topographically to the demyelinating foci (Castaigne et al. 1974). In addition, the presence of BK DNA in one cerebellar spongioblastoma (Fiori & di Mayorca, 1976) and SV40 DNA in one glioblastoma multiforme (Smith et al. 1977) and of SV40 T antigen in three meningiomas (Weiss et al. 1975) have been reported. However, these reports are not confirmed and further investigations are required to demonstrate a causative relationship between papova viruses and human brain tumours.

The hypothesis that giant cell astrocytes in PML represent a non-permissive cell population which undergoes transformation as a consequence of JC or SV40 PML virus infection is only indirectly supported by experiments in cell cultures. Shein (1967) infected cultures of human foetal glial cells with SV40 and demonstrated lysis of the spongioblast and transformation of foetal brain astrocytes. Recently, this hypothesis was tested by applying hybridization in situ to localize JC virus DNA in PML brain (Dörries et al. 1978). Cryostat sections of PML brain treated with complementary RNA of JC virus revealed a heavy label on many
oligodendroglia nuclei along the periphery of lesions. However, only a minority of astrocytes in the centre of these plaques showed a specific label. These data support the observation that many oligodendroglia cells within a lesion are infected by JC virus. However, they neither prove nor disprove the hypothesis of a non-permissive infection of astrocytes since this assay is relatively insensitive compared to other hybridization techniques and therefore small quantities of virus genomic information present in astrocytes would be overlooked with this detection method.

The failure to transmit PML by inoculation of JC virus or SV40-PML virus to laboratory animals has limited the investigations on the pathogenesis of this disease. However, a natural occurrence of PML has recently been observed in Rhesus monkeys (Gribble et al. 1975; Holmberg et al. 1977). SV40 virus could be isolated from brain material of these animals and SV40 V and T antigens were demonstrated in brain lesions by immunofluorescence. It is conceivable that these animals will provide an experimental approach to the investigation of the virus–host relationship of papova viruses in brain cells which lead to PML.

Other slow virus diseases

There are two additional, naturally occurring CNS disorders associated with conventional viruses which are of interest in the search to understand the pathogenetic mechanisms in slow virus diseases: canine distemper demyelinating encephalomyelitis in dogs and progressive rubella panencephalitis in man. The available data on these two diseases are very limited in comparison to the disorders reviewed above.

**Canine distemper demyelinating encephalomyelitis**

Canine distemper is a widespread disease occurring naturally in dogs and other members of the canine family. The contagious nature of this disease was first recognized by Carré (1905) and the agent identified as a virus some 20 years later (Laidlaw & Dunkin, 1928). The virus was first placed within the group of influenza viruses by Holmes (1948), based on its characteristic clinical involvement in respiratory infections. However, Koprowski (1958) and Imagawa et al. (1960) later linked it to the measles and rinderpest viruses. The relationship of this virus to a rare CNS disorder which reveals morphologically certain similarities to SSPE, and perhaps also to multiple sclerosis (Cook et al. 1978), has recently stimulated new interest in the investigations on the mechanisms of this virus–host relationship.

**Clinical and neuropathological characteristics**

Exposure of a sero-negative dog to canine distemper virus (CDV) leads to an acute or subacute disease which is clinically characterized by pyrexia, exanthema and signs of respiratory and gastrointestinal infections. However, some dogs develop a demyelinating encephalomyelitis with severe neurological symptoms such as tremor, paralysis and convulsion. These symptoms may appear weeks or months after recovery from the acute infection (Innes & Saunders, 1962; Van Bogaert & Innes, 1962; Appel, 1969). However, this encephalitis also occurs in dogs without any preceding acute clinical illness. The CNS disorder observed in dogs was described as post-distemper encephalitis or subacute diffuse sclerosing encephalitis, demonstrating the variety of neuropathological lesions observed. In general, the neuropathological changes, either demyelinating or necrotic, primarily affect the white matter of the cerebellum, brain stem and spinal cord. The demyelinating areas are characterized by loss of myelin with relative sparing of axons and presence of gitter cells. In the neighbourhood of demyelinating plaques, perivascular cuffings consisting of lymphocytes and macrophages...
can often be observed. The necrotic plaques frequently resemble a glial scar with astrocytes forming a network in which macrophages are detectable. Moreover, intranuclear inclusion bodies of Cowdry type A containing distemper virus nucleocapsids can be observed (reviewed by Appel & Gillespie, 1972). In addition, a very rare variation of this disease may develop in aged and/or previously immunized dogs which has been called ‘old dog encephalitis’ or ‘hard-pad’ disease (Appel, 1969; Lincoln et al. 1971).

Virological and pathogenetic aspects

Recent studies have shown that CDV is antigenically related to and shares many biological and biochemical properties with measles and rinderpest virus (reviewed by Imagawa, 1968 and Kingsbury et al. 1978). As a result, these viruses have been classified as the morbillivirus subgroup of the paramyxoviruses. Virological studies on brain material from diseased animals revealed the presence of canine distemper virus antigen in neuronal glial cell elements as well as paramyxovirus nucleocapsid structures (Appel, 1969; Raine, 1972; Wisniewski et al. 1972). Attempts to isolate infectious canine distemper virus from brain material were only successful when co-cultivation methods were applied on brain tissue culture cells (Confer et al. 1975).

Studies on the pathogenesis of CDV-induced CNS disorders in experimentally infected dogs proved to be difficult since the available virus strains rarely lead to such a disease. In one such study (Appel, 1969), only two cases among 55 dogs exposed to the virus by aerosol developed a CNS affection. However, the study provided valuable information concerning the relationship between the presence of virus and the development of neutralizing antibodies. Virus antigen was first detected in macrophages with a subsequent spread to the lymphocytes. Viraemia followed and disappeared while neutralizing antibodies were produced with coincident lymphocytopenia lasting 3 to 4 weeks. The author showed that if the virus reached neuronal tissue before protective antibodies were produced, then a virus persistency may be established. This observation offers some explanation for the development of a CNS disorder associated with virus persistency.

Recently, the isolation of a neurotropic CDV strain from a naturally occurring case of demyelinating distemper eliminated many of the problems associated with the induction of this neurological disease in experimental animals (McCullough et al. 1974). Exposure of gnotobiotic dogs to this virus strain either by intracerebral inoculation or contact with diseased animals led to an acute or subacute encephalomyelitis in about 50% of the animals. The acute form was characterized by focal demyelination without perivascular cuffing but with intranuclear inclusion bodies present. All of these animals exhibited sustained lymphocytopenia throughout the course of the disease and died within 6 days. Animals having subacute encephalomyelitis with a prolonged clinical course ranging over a period of 12 weeks revealed only a transient lymphocytopenia. The neuropathological changes in this group consisted of multifocal areas of demyelination with perivascular cuffing and virus inclusion bodies. Immunological studies on these animals revealed a depression of peripheral blood-lymphocyte mitogen response probably as a result of lymphocyte infection by CDV. CDV-antibodies as well as antibodies to CNS myelin could be detected in the serum and in CSF specimens. In addition, an elevation of plasmogenase activity in the CNS was already found before clinical symptoms were detectable (Koestner & Krakowka, 1977).

The available data on this subacute demyelinating encephalomyelitis in dogs demonstrates morphological, virological and, perhaps, immunological similarities with SSPE. Since SSPE has not yet been successfully transmitted to laboratory animals by a natural route, CDV infection in dogs, especially with the neurotropic strain, could prove to be a useful experi-
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Mental model to analyse certain aspects of the human disease. Moreover, the recent suggestion of a possible relationship of CDV with multiple sclerosis makes this model even more attractive (Cook et al. 1978). It can be expected that investigations of this virus-host relationship will contribute to the understanding of virus-cell interactions in the central nervous system of paramyxoviruses.

Progressive rubella panencephalitis

Progressive rubella panencephalitis (PRP), which was first described in 1975 (Townsend et al. 1975, Weil et al. 1975a), is a chronic, inflammatory, progressive CNS disorder in man. It has been associated with rubella infection appearing either as a delayed reaction of congenital rubella or postnatal rubella infection.

Clinical and neuropathological characteristics

So far, only a few cases have been reported, four with presumptive evidence of intrauterine rubella virus exposure (Townsend et al. 1975; Weil et al. 1975a) and two as a late sequelae after early childhood rubella infection (Lebon & Lyon, 1974; Wolinsky et al. 1976). In their second decade of life, these patients developed over a period of several years a protracted clinical course consisting of seizures, myoclonus, cerebellar ataxia and corticospinal spinal tract signs. The disease starts with intellectual deterioration before neurological symptoms are recognizable. Laboratory test revealed high titres of rubella antibodies in serum and CSF ratio. Moreover, rubella-specific IgM has been observed in serum samples only at low titres (Weil et al. 1976; Wolinsky et al. 1978) indicating rubella virus persistency and an impairment of the shut-off mechanism of IgM synthesis. The CSF showed little or no pleocytosis with an increase in gamma globulin content. Moreover, in the tested cases, the occurrence of oligoclonal CSF IgG was seen (Wolinsky et al. 1976) which contains rubella specific antibodies (Vandvik et al. 1978) suggesting a local production of immunoglobulin, in the CNS. Antibodies against measles virus were normal or absent.

The neuropathological investigations showed meningeal and perivascular plasma cells and lymphocytes, gliosis and glial nodules as well as diffuse neuronal loss. Inclusion bodies or virus structures, as seen in SSPE or other virus infections of the CNS, were not detected. In contrast to SSPE, however, amorphous deposits similar to those seen in children dying with congenital rubella syndrome were present in blood vessel walls throughout white matter, in the cerebral hemispheres, basal ganglia and cerebellum (Townsend et al. 1975; Townsend et al. 1976).

Virological and pathogenetic aspects

Rubella virus was isolated in one instance from brain tissue culture with and without cocultivation of cell cultures susceptible to rubella virus propagation (Cremer et al. 1975). Moreover, rubella virus was also recovered from purified lymphocytes (Wolinsky et al. 1978) as is common in congenital rubella syndrome (Simons & Jack, 1968) as well as postnatal rubella infection (Alford, 1976). However, rubella virus antigen could not be detected in brain sections even after elution of immunoglobulins by low pH treatment (Cremer et al. 1975). The direct isolation of infectious rubella virus from brain tissue cultures suggests that infectious virus may be present in the brain in very low concentrations, although the presence of defective virus particles cannot be excluded.

Immunological studies on the humoral and cell-mediated immune responses have been carried out in few patients without revealing a consistent defect which would explain the pathogenesis of this disease (Weil et al. 1975b, 1976; Schueler et al. 1977; Wolinsky et al.

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Moreover, none of these patients has shown a recognizable immunodeficiency syndrome in relation to other diseases as was described for PML. This indicates that other mechanisms play a role in the establishment of a persistent infection of the CNS, which eventually leads to a chronic disorder.

PRP reveals many clinical, neuropathological and immunological similarities to SSPE suggesting that both diseases may have common pathogenetic factors. It seems that once a virus which has a tendency to persistency enters the CNS, a disease process may start which in its clinical/pathological features is more host than virus dependent. Although it is known from congenital rubella syndrome that rubella virus persists in human tissue for several years and has an affinity to the CNS, no unique neuropathological features are known which would explain the development of a progressive neurological disorder.

INTERPRETATIONS AND COMMENTS

In addition to the identification of the causative agents two fundamental processes are important in understanding slow virus diseases: the mechanism by which the virus persists in the host without being eliminated by the host defence systems and the events which lead to a disease process with a slowly progressing clinical course. The successful and repeated isolations of Visna, SSPE and papova viruses from brain material and their presence and localization in brain tissue by different laboratory methods very strongly suggest an aetiological relationship of these agents to the respective disease. The aetiological association of Visna virus to the CNS disorder has been clearly established. Visna can easily be induced with the agent in susceptible animals, whereas in SSPE and PML all attempts to transmit an identical disease to a laboratory animal by a natural route have failed. However, it is noteworthy that Visna can only be induced experimentally in its natural host and not in any other animal species. This is surprising considering the experiences with slow virus diseases associated with unconventional agents. These diseases, like scrapie, Kuru or Creutzfeldt-Jakob, can easily be transmitted to a variety of other laboratory animals (Gajdusek & Gibbs, 1977). It is conceivable that a restricted host range of the conventional agents responsible for slow virus diseases such as Visna or SSPE and certain host factors prevent the development of the CNS disorder in other species.

Molecular biological basis of virus persistency

Recent progress in the biochemical analysis of Visna virus infections in animal brains or tissue cultures, reported by Haase and co-workers (1978), convincingly demonstrated for the first time the molecular biological mechanism of virus persistency in one of the CNS slow virus diseases. The unique properties of Visna virus allow the agent to establish a lysogenic like relationship (Haase, 1975) and, due to the genetic restriction at the transcriptional level, an infected cell cannot be recognized by the host immune system. This peculiar virus–cell interaction accounts for the long incubation period and provides a plausible basis for virus persistency. Similar mechanisms have been suggested for SSPE (Zhdanov & Parfanovich, 1974). However, measles virus does not contain a RNA-dependent DNA polymerase as is the case with Visna virus and this enzyme would have to be provided by the cell, e.g. by endogenous virus. No laboratory evidence supports this hypothesis and the reported findings of proviral DNA in tissue culture cells persistently infected with measles virus are not yet confirmed.

In contrast to Visna, where no defective virus particles have been found, the available evidence in SSPE, derived from isolation attempts as well as biochemical characterization of
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non-producing SSPE brain tissue cultures (Kratzsch et al. 1977), suggests that an impaired replication might be responsible for virus persistency. In general, the most likely mechanisms of RNA virus persistency, other than retroviruses in tissue cultures are either the selection of resistant host cells and avirulent mutants or DI particles (Rima & Martin, 1976). However, temperature sensitive or any other isolated measles virus mutant, have not yet directly been shown to be involved in the establishment of persistent infections. On the other hand, it was recently demonstrated that undiluted passages of measles virus not only lead to the formation of DI particles containing subgenomic RNA (Hall et al. 1974) but also to a decrease in yield of infectious virus giving rise to persistent infections affecting about 80% of the cells (Rima et al. 1977b). Several hypotheses have been proposed regarding the control mechanisms by which DI particles may induce or maintain virus persistency (reviewed by Fraser & Martin, 1978). It was suggested that by different intracellular pathways in relation to DI RNA a regulatory factor is produced which limits the formation of infectious virus particles, as has been observed in a cell line persistently infected with canine distemper virus (ter Meulen & Martin, 1976). For the hypothesis that M-protein could play a pathognomic role in SSPE, preliminary evidence was reported by Hall et al. (1978) and Wechsler & Fields (1978) who found biochemical and immunological differences of SSPE and measles virus M-proteins. Further support for this interpretation could come from the analysis of M-proteins in non-producing persistently infected cell lines with measles or SSPE viruses.

The mechanism for virus persistency in PML is unknown despite the fact that the molecular biology of the papova viruses is well established and well-tested models are available to explain virus persistency (Fareed & Davoli, 1977). The difficulties in obtaining enough material from this rare disease and in handling the human strain, JC, in the laboratory has prevented a biochemical approach to the analysis of the virus cell-interaction in PML brain. The hypothesis of a permissive and non-permissive infection in different cell types of the CNS is appealing but not yet proven.

Immune response and disease process

In addition to the biochemical aspects of virus persistency in slow virus diseases, a host reaction to the infection and its role in the pathogenesis is equally important. From an immunological point of view, the three main CNS diseases discussed in this review differ widely. In Visna, the immune reactions are not impaired and probably contribute to the development of the lesions (Nathanson et al. 1976; Griffin et al. 1978). In SSPE, a humoral hyperimmune response against the aetiological agent can always be found in the presence of a functional cell-mediated immune system, whereas in PML the disease develops only in an immunologically compromised host. The puzzling finding of minimal titres of infectious Visna virus in sheep in the presence of neutralizing antibodies has been explained elegantly by the observation of the continuous occurrence of Visna virus mutants (Narayan et al. 1977; Narayan et al. 1977a, b, 1978). This phenomenon has also been seen with influenza viruses (Laver & Webster, 1968), in equine infectious anaemia (Kono et al. 1973) or in chronic protozoal infections (Vickerman, 1974). However, in influenza infections the development of mutants has never been linked to a recurrent infection in the same host, whereas in equine infectious anaemia the relapses are directly related to the occurrence of virus mutants. Similar disease mechanisms have been proposed for the Visna lesions (Narayan et al. 1978). The appearance of Visna mutants and their replication in CNS-cells may be accompanied by new neuropathological changes which eventually lead to a clinically recognizable disease when the CNS damage is incompatible with normal brain function.

In SSPE, such a phenomenon has not been observed but it could be shown that SSPE
viruses reveal a lower reactivity in neutralization assays against anti-measles and SSPE sera. This has been interpreted as being due to antigenic variation and a possible cause of SSPE (Payne & Baublis, 1973). Immunologically, SSPE patients display measles specific oligoclonal antibodies which is very unusual for a virus infection. Normally, a heterogeneous antibody response is found which is T cell-dependent for most viruses. Since no immunological data are available for the period between acute measles and the onset of SSPE, there is no way of knowing whether SSPE patients are genetically high responders to measles antigens. A genetically determined high susceptibility has been discussed but no definite histocompatibility antigen pattern has been observed in SSPE. Moreover, there are three reports on the occurrence of SSPE in one of a pair of identical twins, arguing against a genetic basis for this disease (reviewed by Agnarsdottir, 1977). The observation that measles virus infects lymphoid cells and, in acute measles, causes a marked impairment of cell-mediated immunity, suggests that a specific unresponsiveness to measles virus as found in skin tests with SSPE patients could result from a selective depression of the respective T cell clones. If suppressor cells normally controlling antibody synthesis to measles virus were affected then a hyperimmune reaction would occur as is indeed found in cases of SSPE. An explanation for the oligoclonal hyperimmune reaction could be the regulatory involvement of anti-idiotypic antibodies. Based on preliminary experimental evidence in small laboratory animals, anti-idiotypic antibodies may lead to either an inhibition of the corresponding idotype production or to a marked stimulation of its synthesis depending on the IgG subclass of anti-idotype antibodies injected (Eichmann & Rajewsky, 1975). So far, no evidence for such an immunological regulatory mechanism has been found in SSPE.

The course of SSPE, as well as the neuropathological lesions, have led to the interpretation that, as in Visna, an immunopathological component may be involved in the development of the disease. No laboratory data support this notion since humoral antibody against brain antigen could not be unequivocally detected as one would expect if an autoimmune phenomenon had been triggered by the virus infection (Agnarsdottir, 1977). Moreover, immunosuppressive treatment has not convincingly influenced the course of the disease and the possible pathogenetic role of antibodies or antigen–antibody complexes is complicated by reports that SSPE has been observed in patients with hypo gamma globulinaemia and combined immunodeficiency (Hanissian et al. 1972). On the other hand, SSPE brain tissue reveals a high content of measles antibodies which, in the presence of complement, should destroy infected CNS cells and arrest the infectious process. Obviously, this defence mechanism does not function in SSPE and it has been proposed that capping of virus antigens on membranes of infected CNS cells by anti-measles antibodies may be responsible for this failure (Joseph & Oldstone, 1975). Other reports stress the importance of measles infection in the presence of protective maternal antibodies which could lead to atypical measles infection providing a ground for the development of a chronic CNS infection (Agnarsdottir, 1977). In PML the majority of reported cases occur in persons with immunological defects, suggesting that in the absence of immunological control mechanisms the virus infection can spread. However, whether the human papovaviruses remain persistent in the CNS after childhood infection and may be activated at the onset of PML, or whether PML represents the first contact with these agents by immunosuppressed patients is unknown. On the other hand, epidemiological data indicate that JC virus and SV40-PML viruses replicate in extraneural tissue from which the virus may infect the CNS under certain conditions. So far, these viruses have not been detected in tissues other than brain.

The investigations of slow virus diseases over the last decade have yielded important
results and have demonstrated the different ways a virus–host interaction can result in a chronic CNS disorder. However, they have also pointed out where future research should be concentrated in this field. In the area of conventional virus persistency much has been learned but many aspects of these virus–cell interactions are still not understood, not only in tissue culture systems but also in a more complex host, where host factors influence virus expression. In addition, new approaches are needed in studies on the detection and rescue of persistent viruses in order to develop a methodology which can be applied to other human disorders suspected of being slow virus diseases (Johnson & ter Meulen, 1978). On the other hand, little is known about immune reactions in the CNS. From an immunological point of view, the CNS can be considered a privileged site. The immune system does not have an easy access to this compartment which could account for the failures to control an infectious process in this organ. There is little information on the flow of antibodies, lymphoid cells, macrophages, antigens and antigen-antibody complexes in and out of the CNS. Moreover, how and where the agents responsible for chronic infections enter the body, escape the general immune surveillance and reach the CNS is unknown. Obviously, many virological and immunological problems have to be studied and solved before the complexity of virus–host relationship in these diseases can be interpreted.

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