Mechanisms and Consequences of Virus Persistence in the Human Nervous System

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INTRODUCTION

The central nervous system (CNS) consists of highly complex tissues. Although intracellular communication is observed throughout the body, nowhere else does this property take on such significance and reach such a level of refinement. Cells of nervous tissue possess an unusual morphology and are highly elongated or branched to maximize cell/cell contacts, and cell membranes reveal a spectacular degree of specialization. The metabolism of these neuro-functional cells is rendered subservient to cell function, and cell division does not occur. It is therefore to be expected that persistent infection by viruses could have a profound effect on cell function. The production of viral proteins and their insertion into cell membranes could disrupt vital processes, and render the cells susceptible to immunological attack. Since neuronal tissue does not divide, cells destroyed either by immune responses or directly by the virus cannot be replaced. Similarly, damage induced in supportive tissue could also affect the neurons and so the function of the CNS as a whole could be impaired. It is the purpose of this review to consider briefly the mechanisms by which viruses may persist in the CNS and to assess the effects of this process. To this end we shall consider events in the human CNS, although results obtained from animal experiments will be discussed where relevant.

In the context of this discussion, a persistent infection is any infection that is not eliminated by the host immune response. This definition therefore encompasses persistent infections that may be termed latent or slow virus infections.

MECHANISMS OF PERSISTENCE

The CNS is well protected anatomically and consequently does not normally constitute the site of primary infection. Most viruses reach the CNS by the hematogenous route, and the duration and extent of viremia are significant in the success of CNS invasion. Some viruses, such as canine distemper virus, invade the CNS through infected lymphocytes and the related human pathogen, measles virus, probably achieves invasion in the same way. Other viruses (rabies) enter the CNS through the peripheral nerves following initial replication at the site of infection.

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**DNA Viruses**

Two virus groups, herpes and papovaviruses, will be considered in this section.

Herpes viruses that most commonly establish persistent infections of the human CNS are herpes simplex (HSV) and varicella zoster. Most data have been gathered concerning HSV persistence in the sensory ganglia, but this virus may also persist in the CNS of rodents and man. Both HSV and varicella establish a persistent infection that exhibits occasional reactivation. Virus particles then migrate along peripheral nerve fibers to cause a recurrent infection of tissue served by those nerves. This results in cold sores and mouth ulcers in the case of HSV and shingles in the case of varicella. Animal experiments have shown that virus enters the nervous system through peripheral nerves and establishes a latent infection in the sensory ganglia. There is no evidence that cells in the ganglion infected with virus actually display any surface antigens recognizable by the immune system, and virus-specific intracellular inclusions cannot be found. However, virus-specific DNA and RNA can be detected by in situ hybridization, suggesting that limited virus expression may occur. Persistent virus at this site can be rescued by cultivation of explants in vitro or co-cultivation of this tissue with cells susceptible to herpes virus. Human ganglia cells subjected to this technique did not always respond by induction of infectious virus. However, ganglia that failed to respond were obtained from individuals expressing a strong anti-HSV immune response which suggested that these persons were in fact infected. This was confirmed by superinfection experiments in which temperature-sensitive (ts) mutants of HSV were used. Virus growth was then observed at the nonpermissive temperature, which was interpreted as the complementation of the ts lesion by viral genetic information residing in the cell of the ganglion, but which was itself defective in some other function. The "defectiveness" of the persistent virus may contribute to the carriage of the virus in the absence of any detectable cytopathic effect. Nondefective virus information is presumably carried in an inactive state or one in which virus replication is so slow as to be undetectable by present techniques. The manner in which this is achieved is at present unknown and it is also not certain whether the DNA is carried in an integrated or episomal form. However, unlike persistent infections, CNS cells maintained in vitro are nondividing, and therefore virus can be efficiently maintained as an episome in the absence of replication.

Papovaviruses that persist in the CNS are associated with the slow virus disease progressive multifocal leukoencephalopathy (PML). The two viruses so far associated with this condition are JC virus and SV40 PML, which are closely related both serologically and by DNA sequence. JC virus grows very poorly in tissue cultures and this has hampered research. Both viruses can be isolated from PML brain and large numbers of particles can be demonstrated in infected tissue by biochemical or electron microscopic studies. Therefore, unlike herpes virus persistence, virus antigens are expressed in the CNS. The majority of PML cases occur following some underlying immunodeficiency. This is also true of virus isolation from extraneural tissue, where successful isolation has only been possible in subjects exhibiting a reduced immune response during pregnancy or immunosuppressive regimes following organ transplantation.

**RNA Viruses**

RNA viruses establishing persistent infections in the human CNS include arena, echo, rubella and measles virus.

Rubella CNS infection is normally only important in congenital infections. However, in rare instances the virus can give rise to a slowly progressive rubella
panencephalitis (PRP) that emerges some ten years after childhood or congenital rubella. Infectious virus could be recovered from the brain both with and without co-cultivation procedures and also from infected lymphocytes. However, virus antigens could not be detected in the CNS. In vitro, rubella virus gives rise to a limited cytopathic effect and readily establishes persistently infected cultures in which defective interfering (DI) particles may be involved. The relevance of this to the CNS infection is not known.

Arena and echovirus persistence in the CNS is uncommon. Lymphocytic choriomeningitis (LCM) virus is associated with acute aseptic meningitis, meningoencephalitis or an influenza-like syndrome. The disease is normally controlled, but very occasionally it may take a chronic course. There are no data available concerning the production of this condition, nor concerning the mechanism of virus persistence. It is, however, well established that LCMV is able to produce a life-long persistent infection in congenitally or neonatally infected rodents. In this case virus persists despite a humoral anti-virus immune response. The cell-mediated immune system, however, is tolerated towards the virus and this is thought to provide the basis for virus persistence and the failure to eliminate the infection. The virus replication process may be held in check by the production of DI particles that can be detected in many organs, and whose appearance correlates with the cessation of the acute phase of the disease. However, in the rodent, this infection is generalized and there is as yet no evidence for immunological tolerance, or DI particles in the chronic human CNS infection.

Immunodeficiency is better characterized by the case of chronic echovirus CNS infections. Echovirus infections are ordinarily easily controlled by the body’s own defense mechanisms, but in patients with severe immunodeficiency and a lack of B cells a chronic echovirus infection may be produced. This infection results in a chronic meningoencephalitis and infectious virus has been recovered from the CSF. Occasionally virus can also be isolated from extraneural tissue.

Measles virus is a member of the morbilliviruses, and before the advent of vaccination procedures, was ubiquitous amongst human populations large enough to sustain it. Acute measles is a self-limiting condition controlled by a strong immune response, but involvement of neural tissue is well known. The virus exhibits a marked lymphotropism resulting in a transient hypo responsiveness during the disease, and it is thought the virus gains access to the CNS either by viremia or inside infected lymphocytes. Virus penetration may result in acute encephalitis, but occasionally a persistent infection is established that leads to a fatal slowly progressing disease, subacute sclerosing panencephalitis (SSPE). During this condition inclusion bodies are present in the CNS cells that consist of measles virus nucleocapsids. However, cell fusion that is characteristic of measles virus-induced CPE is not observed, and mature virus particles are absent. However, virus expression may sometimes be rescued from this infected tissue by co-cultivation procedures. These experiments have therefore confirmed that measles virus is the etiological agent of SSPE and also indicated that virus persistence may be based on some defect in the virus maturation process that could involve some form of host effect. Viruses rescued from SSPE tissue revealed some structural differences from wild-type measles strains. Some of these concern electrophoretic migration differences between measles and SSPE virus polypeptides but the advent of monoclonal antibodies has permitted the detection of more subtle differences in structure. Many of these concern the matrix (M) protein and may arise as a consequence of the acquisition of mutations during persistence. Indeed this process has been detected in persistent infections in vitro. Most SSPE viruses grow more poorly than wild-type measles and more readily produce persistent infections in tissue culture. This may be a consequence of the acquisition of mutations, discussed
above, and therefore there may be some selection towards a more easily carried virus than during the persistent infection itself. However, none of these mutations seems to be a common characteristic of the persistent measles virus and this suggests that this is a general process. No specific marker has yet been found that differentiates persistent measles viruses from isolates derived from acute measles. Interestingly, many of these mutations involve the M protein and this protein has been strongly implicated in the original establishment of the persistent infection of the CNS.

The SSPE patient displays a strong humoral immune response towards measles virus, and antibodies directed to virus polypeptides are present in both serum and CSF. However, whereas most virus proteins are well recognized, there are no antibodies in the CSF directed towards the matrix protein. Current evidence suggests that this situation arises through a lesion in the synthesis of this protein in the CNS, and consequently, M protein is not available to serve as an immunogen. The failure to produce large amounts of this major structural protein is thought to explain the maturation defect observed in the CNS, conferring a cell-associated phenotype of the virus. This in turn is thought to account for the slowly progressing nature of the disease. Attempts to rescue SSPE viruses from infected tissue are not always successful and occasionally a persistently infected cell line is obtained in which capacity to produce M protein is not restored. Investigation of these cell lines, known as SSPE cell lines, has shown that in one case failure to produce this protein was accomplished by a defect in translation, but in other cases, mRNA was apparently not produced or rapidly degraded. Some evidence suggests the latter mechanism also occurs in human brain (Baczko et al., in preparation). Since M protein and possibly M protein mRNA are not produced in infected brain, these molecules are presumably subject to no selection pressure. Mutations could be rapidly acquired that might explain the variation observed in matrix proteins whose expression was restored during the rescue of SSPE viruses. Other mutations could have a more profound effect, either on the ability of a rescued mRNA to function in translation reactions or on the ability of genome RNA to act as template for the production of mRNA itself. In these cases rescue of infectious virus by cocultivation techniques would be impossible and the cell-associated SSPE virus persistent infections could then result. Viewed in this way, study of SSPE cell lines may not necessarily reveal the nature of the original events driving the virus toward persistence.

Recent work with measles virus-infected tissue cultures has suggested that measles antibody may play a crucial role in the establishment of persistence. It was found that antibody could strip virus antigens from the surface of infected cells and thus protect such cells from immunological injury. Furthermore, polyvalent antiserum can alter the synthesis of intracellular virus polypeptides, a phenomenon termed antibody-induced antigenic modulation. This event is also operative in persistently infected tissue cultures, and could give rise to a virus association in which virus antigen production is apparent. This state, once established, is apparently stable in the absence of antiserum. Since it has been suggested that measles virus may enter the CNS inside invading lymphocytes, it is possible that antibody is synthesized within the CNS at the time of invasion. This antibody may then be more active in inducing a modulation process and persistence than in complement-mediated cytolysis because complement is relatively lacking in the CSF. A modulation process such as that described above is operative on all virus polypeptides, reducing the total expression of virus genetic information. Therefore, this event cannot alone account for the situation observed in SSPE where the effect predominantly concerns just one polypeptide. Other factors, perhaps host-specific, may therefore play a role in establishment and maintenance of measles virus persistence. In this connection the finding of measles virus
genetic information in the brains of apparently normal individuals by *in situ* hybridization is of interest. Measles virus may be part of the normal human CNS virological fauna, as suggested by Johnson and Carrigan. Consequently, SSPE or measles encephalitis might result from those rare instances when the modulation process was incomplete or ineffective. Further study of the involvement of the CNS during normal acute measles, and of virus expression in normal or diseased brain will be necessary to clarify this process.

It is apparent from the foregoing discussion that the methods of instigation and maintenance of a persistent infection in CNS tissue may vary, and in no case are these processes fully understood. Infections range from those truly latent infections, such as HSV, where the virus genome is only poorly, if at all, expressed to those in which virus replication is complete, and infectious virus is directly reisolable such as in PML, PRP or echovirus infections. Other infections, such as SSPE, reveal an intermediate state of expression, and although virus antigen is present, infectious virus is not produced. In all of these cases the role of the immune system has been stressed. A neuron latently infected with HSV cannot be attacked by the immune system, and the immune system itself may protect SSPE virus-infected cells from cytotoxic events by stripping off virus-specific cell surface markers. It is not known by what mechanism the herpes virus infection is converted to latency. However, suppression of cell-mediated immunity has been successful in precipitating reactivation in animals, and patients undergoing HSV recurrence often show an impairment of some aspects of cell-mediated immunity. Thus the immune system may be to some extent instrumental in controlling this process also.

Immunological dysfunction is involved in the cases of PML or echovirus infection and permits productive virus replication to occur, but in the case of PML it is not known whether the immune system deficiency has prompted the activation of a previously existing but inapparent persistent infection, or if virus invasion occurred as a consequence of a failure in immunological protection. The case of PRP is an exception; infectious virus may be isolated directly despite a strong antibody response in both serum and CSF. Similarly, the cell-mediated immune system does not show any specific deficiency. However, the site of rubella virus antigen expression has not yet been identified during this disease process. Consequently, the mechanisms underlying virus persistence in this case are as yet unknown.

**THE CONSEQUENCES OF VIRAL PERSISTENCE FOR NEURAL TISSUE**

The consequences of a persistent viral infection may be considered under two main headings. Firstly, direct effects result from the action of the persistent virus, either by promoting cell destruction or by interfering with the efficiency with which the host cell may function. Direct destruction of extraneural tissue may also be included in this heading. Secondly, indirect effects arise largely through the response of the immune system to the infection. This may involve an autoimmune reaction that could lead to tissue destruction and consequent inhibition of CNS function.

**Direct Effects**

In the case of HSV latency, virus persistence does not seem to have any direct consequences on the host cell although subtle effects have been discussed by Sequiera *et al.* Reactivation of the latent virus, however, results in the expression of virus antigens. This has been detected in explanted cervical ganglia from the mouse.
Mature virus particles are formed that migrate along cell processes to reinfect that area of the skin served by the nerve. The original host cell is presumably killed by the virus but latency may be reestablished by spread to a different neurone. Consequently, damage by these events is cumulative.

PML is associated with two papovaviruses, JC and SV40 PML. In common with other papovaviruses infection can take two courses depending upon the permissiveness of the host cell. PML lesions consist of demyelinating plaques in the white matter. Oligodendroglial cells are lost from these areas but those in the periphery are enlarged and contain large numbers of papovavirus particles. Astrocytes, which display various morphological aberrations but rarely contain virus, are observed in the foci of these lesions. This has led to the suggestion that the oligodendroglial cells and astrocytes could constitute permissive and nonpermissive cell populations, respectively. The virus is able to destroy selectively oligodendroglial cells, their cytoplasmic extensions are lost and demyelination follows with resultant damage to the CNS. The astrocytes could then undergo nonpermissive virus replication leading to transformation. Some evidence to support this has been obtained from in vitro experiments. No inflammatory lesions have been observed in PML, and since this disease is associated with immune deficiency, it seems that most tissue destruction is accomplished by a direct effect of the virus. Similarly, chronic echovirus infection is associated with immune deficiency, and although inflammatory processes are observed in extraneural tissue, it seems unlikely that CNS damage is largely caused by virus-mediated cell destruction.

However, persistent viruses are able to interfere with cell function in ways other than tissue destruction. Insertion of virus antigens into the membrane or the occurrence of virus maturation processes could well be expected to disturb the activity of structurally refined, highly specialized membrane systems. Studies in tissue culture have shown that neural cells persistently infected with LCM, measles, or even rabies virus, often display altered characteristics related to neural function. The activities of acetyltransferase and acetylcholinesterase were found to be greatly altered. Recently, it has been shown that glioma cells persistently infected with measles virus have a greatly reduced capacity to respond to catecholamine hormones and produce cyclic AMP (cAMP). Barrett and Koschel used antibody to remove virus antigens from the surface of these infected cells and found that stimulation by these hormones was restored. Furthermore, intracellular virus proteins were not apparently involved in this inhibition, since full function was restored despite the continued presence of intracellular virus antigen. In this case, the actual activity of the cAMP synthetase enzyme was not altered, but virus antigen in the membrane affected the ability of the hormone receptor on the external surface of the plasma membrane to activate the cAMP synthesizing complex on the internal surface.

In none of the above cases was cell growth rate appreciably altered, suggesting that the luxury functions of neural cells may indeed be highly susceptible to this type of interference. Such changes could have a profound effect on brain function and eventually lead to a disease with a clinically defined symptomatology.

Indirect Effects

With the exception of PML, all slow virus diseases associated with conventional viruses reveal some form of inflammatory lesion. The destruction of infected cells by immune mechanisms is therefore to be expected in every case. Although this is a normal function of the immune system, it takes on a further significance in the context of CNS infections because of the lack of regenerative capacity of this tissue. However, a further component to be considered in this context is the production of an
autoimmune response that can probably be elicited by a wide variety of viruses resulting in the destruction of uninfected CNS tissues. The most characterized model for such an immune response is experimental allergic encephalomyelitis (EAE), a disease induced by immunization with myelin basic protein or CNS-tissue extracts in combination with adjuvants. The human condition of postinfectious encephalomyelitis reveals many similarities to EAE and may arise weeks or months after virus infection (measles or mumps) or after vaccination (smallpox or rabies). The mechanism by which virus infection brings about this type of reaction has been extensively studied in the mouse and rat.

Theiler's virus (TV) infection in the mouse leads to a demyelination which is very similar to that observed in EAE. Cells surrounding lesions are free of virus antigen, indicating that direct virus-mediated tissue destruction is not important in damage production. Furthermore, immunosuppression with cyclophosphamide results in a decrease in white matter lesions, suggesting that host immune responses are involved.

Recently, the murine coronavirus JHM has been used for infection of rats to produce a condition termed subacute demyelinating encephalomyelitis (SDE) that is characterized by lesions of primary demyelination and can run a relapsing course (Wege et al., in press). An EAE-like condition could be induced in normal animals by adoptive transfer of lymphocytes from rats with SDE. Such lymphocytes were found to have been sensitized to myelin basic protein itself, during coronavirus JHM infection of the brain.

The most obvious mechanisms by which a virus could trigger an autoimmune reaction against CNS tissue would be some form of cross-reaction between distinct virus and cellular antigens. Indeed Panitch et al. have reported such a cross-reaction between measles virus and myelin basic protein (MBP). Secondly, it is possible that virus infection causes the release of CNS tissue antigens that might normally be shielded from the immune system. Alternatively, this release of tissue might mimic the action of adjuvant necessary for the induction of EAE by immunization of experimental animals against CNS tissue. Thirdly, it is possible that virus antigen physically associates with the host membrane antigen, resulting in a complex that is recognizably foreign but must also contain a "self" component.

**CONCLUSIONS**

In the foregoing discussion the immune response has been implicated in both the mechanism of virus persistence in the CNS and in the pathogenic consequences of that infection. Although the virus may be directly pathogenic, it seems likely that immunopathological mechanisms are often involved. These reactions are often similar regardless of the virus concerned, for instance PRP and SSPE show clinical and immunological similarities although the mechanisms of persistence seem to be very different. Observations such as these may explain why it has so far been impossible to identify a single viral etiology for multiple sclerosis. It is possible that a variety of agents are capable of stimulating a host-dependent autoimmune process leading to the onset and perpetuation of disease, even in the absence of viral antigens.

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DISCUSSION OF THE PAPER

W. W. TOURTELLOTTE (VA Wadsworth Medical Center, Los Angeles): We too have found it wise to hybridize and we thought that we were the first ones to find that the M genome was present in SSPE brain. I think we're all finding in our material that the M protein is not being formed but now that the genome is there, this means again as
you propose, there might be a block somehow in getting it from the gene to the mRNA. Is it possible that we're going to find nucleic acid sequences in some of these acute MS lesions?

R. T. JOHNSON (The Johns Hopkins University, Baltimore, MD): Why don't we leave that for the general discussion.

R. S. TINDALL (Dallas, TX): With reference to the CSF oligoclonal banding in the JHM-infected mice, I presume that this is an inbred strain?

V. TER MEULEN (University of Würzburg, Würzburg, FRG): Lewis rats.

TINDALL: Would you interpret from that data that those immunoglobulins were not directed to similar viral constituents because of the disparity in the response seen but indeed that some of those responses were directed at different, presumably oligo, determinants?

TER MEULEN: The point I want to raise is that in this animal model there are animals that have all viral-specific oligoclonal IgG in the CSF. Later on when they recover, this disappears as in man but then something else occurs, and this has not yet been identified. We can say quite clearly it is not viral. We have to evaluate now whether it's basic myelin protein or another antigen. Certainly, I think it is of interest that the disease process is ongoing and this is reflected by the oligoclonal bands. We can only show this by IgG activity. But it has nothing to do with the virus. I think that's the only point I wanted to make at the present time.