Molecular studies of viral pathogenesis in the central nervous system

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ABSTRACT—What is the molecular biological basis of viral pathogenesis in the central nervous system (CNS), ie by what molecular mechanisms do different viruses produce particular patterns of neurological disease in man and animal models, and can one use molecular techniques to ascertain the viral aetiology of certain neurological conditions? This complex subject can be approached in three different but interrelated ways. First, one may relate molecular techniques to specific biological properties such as viral spread to the CNS, to neurotropism, ie the affinity of the virus for particular neural regions and cells, and to neurovirulence, which refers to the actual ability to cause neurological disease. Second, the reverse approach can be adopted by considering these different aspects of virus–host relationships and then how the techniques have contributed to their understanding. Third, one can select specific neurotropic viruses, such as polio or herpes viruses, and then relate these to both particular techniques and pathogenetic mechanisms [1]. The second component of this paper will deal with the immunopathological mechanisms seen in three specific CNS viral infections, all of which have been the focus of study in the author's laboratory over the past six years.

Molecular techniques

A wide variety of techniques is now available to study CNS viral pathogenesis; some of the more important are shown in Table 1. The kind of information that has been obtained from such sources is indicated in Table 2 where the properties of a number of neurotropic viruses are shown. Of particular note is the frequent identification of specific target cell surface receptors which interact with specific 'cell attachment' proteins located on the virus [1,2].

Viral gene and protein detection

For many years it has been possible to localise viral proteins in pathological tissues using conventional immunocytochemical techniques such as immunoperoxidase and immunofluorescence [3,4]. But our understanding of viral involvement has been greatly enhanced by methods such as Southern blot and *in situ* hybridisation (ISH) whereby viral genetic information (DNA for Southern blot, RNA for Northern blot, and both for ISH) can be detected in infected tissues [1]. All these techniques utilise complementary binding of radiolabelled or biotin-labelled viral DNA or RNA probes to nucleic acids which have been extracted from tissues and immobilised on a hybridisation membrane following which autoradiography demonstrates the presence of viral genes on an X-ray plate or, in the case of ISH, on individual tissue sections mounted on glass slides. ISH is a very sensitive technique and can localise viral genes within small areas in a large tissue region. Recently, two additional procedures have proved to be of great value in such studies. First, ISH can be combined with immunocytochemistry using specific antibodies to co-localise viral genes and proteins in individual cells so that it becomes possible, for instance, to make a distinction between a latent and a productive viral infection or to identify viral DNA in marker-identified cell types [5,6]. Second, the amplification of viral DNA by the polymerase chain reaction (PCR) allows the detection of single copies of specific viral or other nucleic acid sequences even when infected cells comprise only a minute fraction of the total cell number in the specimen under test [7]

These techniques have been applied to tissues obtained from patients with a variety of neurological diseases in which viral infection has been thought to play a role. In some cases, such as herpes simplex virus encephalitis, the presence of viral genome is to be expected [5], whereas in many other studies the exercise takes the form of a 'fishing expedition' in which even positive results may be difficult to interpret. Even if a virus can be convincingly demonstrated in a particular disease, it is quite another matter to assume that there is a cause and effect relationship between the two. Such demonstrations also need to be repeatable in different patients, and the need for valid normal and pathological control tissues cannot be overstated.

In spite of these caveats, ISH and PCR have recently provided potentially exciting insights into the causation of a number of neurological diseases. The long history of multiple sclerosis (MS) research has been bedevilled by unconfirmed reports of apparent viral isolation. However, a recent careful study using ISH

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Table 1. Molecular techniques used in viral neuropathogenesis studies

Technique	Examples of neurological applications
Viral gene and protein detection (ISH, Southern blot, PCR, monoclonal antibodies)	Herpes virus latency and encephalitis HIV encephalitis SSPE, PML, MND
Viral reassortants and recombinants	Reovirus spread and neurotropism Herpes virus spread Bunyavirus neuroinvasiveness
Restriction enzyme analysis of viral isolates and gene sequencing	Poliovirus neurovirulence Herpes virus epidemiology and encephalitis
Viral mutants (temperature-sensitive, deletion and monoclonal antibody generated)	Herpes simplex virus latency Neurovirulence of reovirus, poliovirus, TMEV, rabies virus
Infectious viral DNA manipulation	Poliovirus neurovirulence TMEV neuropathogenicity
Transgenic mice techniques	SV40 in the CNS JC virus and PML model

Table 2. Characteristics of some neurotropic viruses [1]

Virus	Nucleic acid	Major host target cell(s)	Special features
Herpes simplex virus	dsDNA	Neurone	Latency in sensory ganglia
Varicella-zoster virus	dsDNA	Neurone? non-neuronal cells	Latency in sensory ganglia
Rabies	ssRNA	Neuromuscular junction Limbic system	? Acetylcholine receptor is cell receptor
Reovirus type 1	dsRNA	Ependymal cells Anterior pituitary gland	S1 gene determines cell and tissue tropism
Reovirus type 3	dsRNA	Neurone	S1 gene determines cell and tissue tropism Receptor is beta-adrenergic receptor
Poliovirus	ssRNA	Neurone Anterior horn spinal cord	Receptor is immunoglobulin superfamily membrane protein
Theiler's murine encephalomyelitis virus	ssRNA	Neurone oligodendrocyte	Produces acute poliomyelitis and chronic spinal cord demyelination
Human immunodeficiency virus	ssRNA	T4 lymphocyte CNS macrophages	Cell receptor is CD4 molecule to which viral gp 120 binds
Visna-maedi	ssRNA	Monocyte-macrophage lineage ? oligodendrocyte	Produces persistent infection with restricted viral replication in systemic and CNS tissues

found measles N genomic sequences in the brains of two out of eight MS cases and in one of 56 control brains [8]. This is of particular interest in view of the consistently noted and significant rise in measles antibody titres in patients with this disease [9]. It is critical that such studies be repeated in larger groups of patients and also by the PCR technique. The exquisite sensitivity of the latter technique is, however, something of a two-edged weapon in that, despite its vastly improved ability to detect very low levels of viral DNA, it is very susceptible to contamination during the procedure with nucleic acids from other sources. A similar study has recently demonstrated picornovirus RNA (polio or Theiler's murine encephalomyelitis virus (TMEV) sequences in the spinal cord from a case of motor neurone disease (MND) as well as from a normal control case [10]. This is of considerable interest in view of the possible association between this disease and previous poliovirus infection. A very recent study using ISH has reported the presence of JC viral DNA

and viral capsid antigens in 4 out of 10 brain tissues from elderly patients aged between 68 and 96 years [1]. The JC virus is known to be the cause of progressive multifocal leucoencephalopathy (PML), a progressive demyelinating disease usually seen in immunocompromised humans [12]; its detection in normal individuals was therefore unexpected despite the presence of antibody to JC virus in about 70% of normal adults. Molecular studies commonly reveal viral nucleic acid sequences in normal as well as diseased tissues, but these findings are difficult to evaluate. Some of the problems may be partially resolved by the use of very specific gene probes whereby only selected genes can be localised, thereby reducing the risk of artefacts. For example, herpes simplex virus (HSV) is well known to remain latent in the sensory ganglia of humans and animals [13,14]. Its latent state is associated with the presence of latency-associated RNA transcripts (LATs) [15]; their main role is in the viral reactivation process [13]. Detection of such transcripts in human tissues will give better insights into pathogenesis and latency than the detection of very large portions of the HSV genome. In addition, interpretable information can be gained by the combined ISH and immunocytochemical technique. Thus detection of viral nucleic acids in diseased cells is more likely to be a genuine observation if the corresponding protein products of the virus can also identified.

Reassortant viruses to study viral spread and neurotropism

Some viruses contain genomes which are arranged in segments. For example, the three serotypes of mammalian reoviruses contain 10 double-stranded (ds) RNA segments [16], and the La Crosse bunyavirus, responsible for causing Californian encephalitis in humans, contains three types of RNA segments [17]. Fields and his colleagues have exploited the segmental nature of reovirus in a large series of pioneering experiments using reassortant viruses derived from co-infection of susceptible cultured cells with two types of reovirus serotype [16]. The resulting reassortant viruses contain gene segments derived from each original virus, eg types 1 and 3, and these segments can be identified by restriction enzyme analysis. Since type 1 and 3 reoviruses have different neural cell tropisms, eg type 1 has an affinity for ependymal cells but not neurones whereas type 3 virus infects neurones but not ependymal cells and produces a lethal encephalitis in mice, it has been possible to correlate this specific neuotropism with particular regions of the genome [16]. The critical region in determining this tropism is the S1 gene which codes for the outer capsid protein sigma-1 [16] (Fig. 1). This latter protein is the viral haemagglutinin which binds to reovirus receptors on neurons. Fields and colleagues have also shown that the S1 gene determines whether the reovirus serotypes spread via haematogenous or neural pathways to the CNS [2,16,18]. The same principles have also been applied to La Crosse virus in which the gene coding for the envelope glycoprotein is correlated with the property of neuroinvasiveness [17] (Fig. 2).

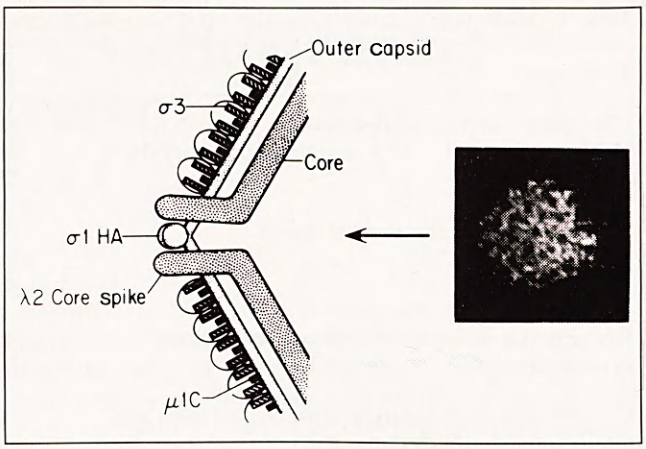


Fig. 1. *Schematic diagram of the outer capsid of mammalian serotype 1, 2, 3.* On the right is shown a negatively stained electron micrograph of an intact virion. On the left is a tentative schematic drawing showing how the three outer capsid proteins (σ1, σ3, μ1C) and the core protein (λ2) are organised on the outside of the virion (From *Nature* 1982;**300**:19–23).

Analysis of viral isolates from patients

Restriction enzyme analysis and gene mapping of viruses obtained from patients, and the use of PCR to detect low levels of virus in such isolates, has proved to be of considerable value in both pathogenetic and diagnostic studies. A classic example of the former was the demonstration by Evans *et al.* [19] that the live attenuated poliovirus vaccine (Sabin type 3) acquires increasingly neurovirulent properties on passage through the infected gastrointestinal tract, and that this property is associated with a single nucleotide change in the viral genome. The latter occurred in the 5' non-coding region of the genome and consisted of a single nucleotide change from uridine to cytidine at position 472. It is significant that this new genetic sequence is also found in naturally neurovirulent poliovirus strains. It is clearly remarkable that such a specific and minute genomic mutation in the virus should have resulted in such a profound alteration in its biological properties and calls for further studies of the precise mechanisms of this alteration.

Molecular analysis has also proved useful in the study of herpes viruses in man. Restriction enzyme analysis has led to advances in HSV epidemiology, and has also helped to clarify some aspects of HSV latency in human sensory ganglia [20] as well as the pathogenesis of HSV encephalitis [21]. In the latter study it was shown that HSV encephalitis could be the result of a primary HSV infection, a secondary infection, or a reactivation of virus that had remained latent.

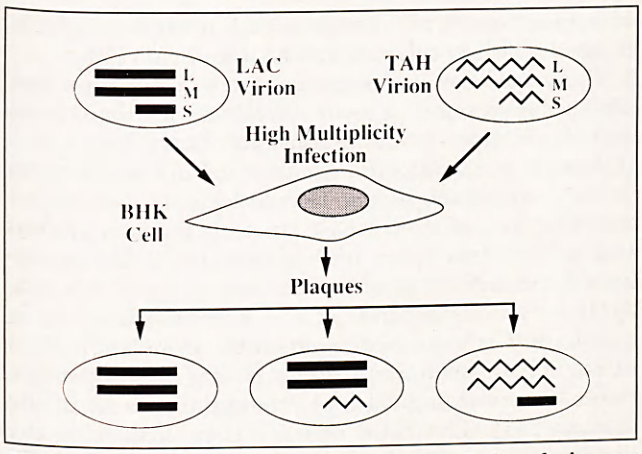


Fig. 2. *Preparation of reassortant viruses.* Related viruses with segmental genomes can be recombined to generate hybrid virions. These two strains of the California serogroup are used to co-infect a tissue culture cell line. Individual clones containing gene segments from the two strains can be typed by SDS-polyacrylamide gel electrophoresis and/or RNA–RNA hybridisation. All viruses with segmental genomes can be recombined in this fashion (From Ref. 17).

PCR has been used with increasing effectiveness in neurological and other conditions. For example, human immunodeficiency virus (HIV) has been detected in patients' blood prior to the appearance of antiviral antibody [22] and is likely to be used in monitoring the appearance of virus in the CNS in relation to neurological symptoms. The diagnosis of HSV encephalitis has also been considerably facilitated using the PCR to detect virus in cerebrospinal fluid [23]. This is likely to be of great practical value in view of the rapidity with which the virus can be detected which is of considerable importance in this condition.

Ultrastructural studies

Considerable insight into the basis of viral neurotropism has been gained by high resolution X-ray crystallography in which the three-dimensional structure of viruses such as poliovirus and rhinovirus has been studied [24,25]. Through the mapping of specific viral antigenic sites the structure–function properties of these viruses have been elucidated in detail. For example, the surface of the poliovirus has been shown to contain probable viral attachment proteins for specific target cell receptors, the former consisting of 'peaks' and 'valleys' [24].

Viral mutants as tools for pathogenetic studies

Three main types of mutant are recognised. Temperature-sensitive (ts) mutants can replicate at the 'permissive' temperature (31°C) but not at the non-permissive' temperature (38.5°C) because of a small nucleotide mutation in the DNA sequence [26]. Deletion mutants have a larger nucleotide change with a corresponding absence of particular regions of the genome [27]. Third, monoclonal antibody-derived viral mutants have been generated by treating viruses with neutralising monoclonal antibodies of varying specificities leading to *in vitro* selection of variants which are no longer neutralised owing to the emergency of codon changes leading to specific protein alterations [17]. Such mutants have applications to neurovirulence studies in a very wide range of viruses, including reovirus, bunyavirus, rabies virus, poliovirus, mouse hepatitis virus and Theiler's murine encephalomyelitis virus, as well as HSV latency [1]. The general principle is a relatively simple one and consists of attempting to correlate the absence of a particular region of the viral nucleic acid sequence with a demonstrable *in vitro* or *in vivo* property such as neurovirulence or, in the case of HSV, the ability to produce latency in neuronal cells. Once the apparent biological function has been identified, proof of the correlation can be provided by 'rescue techniques' in which the missing region of the genome is reinserted, leading to restoration of the original biological function. The latter technique has been successfully applied by Brown and her colleagues using a deletion mutant of HSV-2 with a 1,488 base pair deletion [28]. Although this mutant fails to produce neurological disease in mice, neurovirulence is restored by correction of the deletion, thereby allowing the assignment of the function of neurovirulence to this region of the genome. Similar techniques have been used to study HSV latency in mice [1,13]. Particularly elegant studies by Fields and colleagues have shown that monoclonal antibody-derived reovirus type-3 mutants with alterations of the viral haemagglutinin (sigma-1 polypeptide) have impaired neurovirulence in mice compared with wild-type viruses [2,16]. Molecular analysis of the viral mutants was then carried out and the specific nucleotide changes that were detected could then be correlated with the property of neurovirulence.

Infectious viral DNA

The use of viral infectious DNA clones has been discussed in detail by O'Hara and Roos [29]. This approach has proved to be of considerable importance in this field. The principle has been to general viral infectious cDNA clones with specific genomic alterations from positive strand RNA viruses such as poliovirus. These can then be tested for neurovirulence, thereby allowing the identification of DNA sequences relevant to disease production; in addition, the technique has been combined with other methods such as the generation of intertypic recombinant viruses.

Transgenic mice techniques

The use of transgenic animals in neurobiological studies has recently been reviewed by Messing [30]. The relevant approach has been to insert viral DNA into fertilised mouse eggs by microinjection techniques leading to integration of the DNA into the progeny cells. In a now classic study, for example, this technique has been used to insert JC virus promoter/enhancer nucleic acid sequences containing large and small T antigens into mouse embryos [31]. JC virus is the cause of PML, a human demyelinating disease. The progency mice developed a neurological disorder with many pathological changes similar to human PML, and expression of T antigens was detected in oligodendrocytes which are the cells that make CNS myelin. It is possible that in some way the T antigens interfere with the normal functioning of the oligodendrocytes, thereby leading to demyelination in the experimental animals and also possibly in the human disease. It is likely that the transgenic techniques will produce major insights into other neurological disorders including those thought to be produced by viral infection.

Viral immunopathogenesis studies

This brief survey of molecular techniques has emphasised a number of principles, but perhaps one of the most important is that relatively minor alterations of the genomic sequence of a virus may lead to profound alterations in its neurovirulence. In the second part of this overview I shall outline some recent work which has highlighted the important role of immune mechanisms in the molecular pathogenesis of viral infections. A common feature of these three conditions described is the marked alteration of immune antigen regulation in the context of restricted viral gene expression in tissues.

Visna-maedi of sheep

For many years this was regarded as an interesting area of veterinary study, if not something of an oddity, but its importance as an animal model relevant to human disease was underpinned by the recent realisation that it showed remarkable *in vitro* and *in vivo* similarities to HIV infection in man [32]. These viruses have now been classified as lentiviruses, a recently recognised taxonomic group which also includes caprine arthritis encephalitis virus (CAEV) of goats, equine infectious anaemia virus (EIAV) of horses, simian immunodeficiency virus (SIV) of nonhuman primates, feline immunodeficiency virus (FIV) of cats, and human immunodeficiency virus (HIV) of humans [33]. These viruses are all RNA-containing non-oncogenic retroviruses which after prolonged, sometimes asymptomatic, incubation periods cause persistent infections in their natural hosts. Paradoxically, these agents show restricted replication *in vivo* but produce marked cytopathic effects including cell lysis and fusion *in vitro* [33].

The visna-maedia disease complex is the prototype 'slow virus' disease, a term introduced by Sigurdsson in Iceland to describe this condition in the 1950s [34]. 'Visna' is Icelandic for wasting, whereas the term 'maedi' means dyspnoea. This reflects the two important features of the disease, namely an encephalitis and an indolent interstitial pneumonitis. The neurological manifestation of the disease, ie visna, is a relatively rare complication of the pneumonic form, ie maedi, and is seen more frequently in Icelandic than in European sheep, indicating a strong breed specificity for the neurological and pneumonic forms of the disease [35]. The third characteristic feature of the disease is an arthritis. Clinically the neurological disease is characterised by hind limb paralysis and ataxia, and the disease may show relapses and remissions. The insidious pneumonitis leads to respiratory failure and usually death. Pathologically the lesions in the CNS and systemic tissues are typified by marked inflammation, with infiltration and proliferation of lymphocytes and macrophages producing considerable lymph node enlargement. The CNS lesions occur in both white and grey matter and are characteristically found in periventricular areas with marked perivascular cuffing with lymphocytes and macrophages, leading to demyelination in some cases [33,36].

Our studies of visna were directed towards an understanding of the pathogenesis of the disease, in particular the markedly restricted viral replication seen in both systemic and neural tissues and the mode of tissue destruction. It is known that the host target cells of the visna virus are those of the monocyte-macrophage lineage. This has been gleaned from a variety of sources, the most definitive of which was the demonstration by combined immunocytochemistry and *in situ* hybridisation, both *in vitro* and *in vivo*, of viral nucleic acid in marker-identified monocytes and macrophages [6,37]. The tissue culture studies using sheep alveolar macrophages demonstrated amplification of both transcription and translation of the viral genome in the infected monocytes as they matured into macrophages. Also, such studies have allowed the identification of a unique lentivirus-induced interferon which is produced as a result of the interaction between virally infected macrophages and T lymphocytes [38]. This interferon increases MHC class II (Ia) antigen expression in macrophages and can also curtail viral replication [4].

It has been possible to correlate the *in vitro* observations with the actual disease process using experimental sheep which have been infected via the transbronchial route. Following infection is was found using *in situ* hybridisation and immunocytochemical techniques that the organs which were primarily infected, such as the spleen, lung and mediastinal lymph nodes, contained infected macrophages, although the level of this infection was limited [4]. A

small proportion of promonocytes in the bone marrow was also infected [6]. Most interestingly, the persistent and increased level of Ia antigen expression was located in the infected target organs showing lymphoproliferation and viral RNA expression, whereas this was not the case in uninfected tissues. Class II (Ia) antigens are required on the surface of macrophages in order to present viral antigen to T-helper lymphocytes. Further, double-labelling experiments demonstrated the presence of Ia antigens in a small proportion of infected macrophages in the target tissues [4].

We have interpreted these findings as representing important clues as to the pathogenesis of the persistent inflammation seen in the disease. Figure 3 shows a possible sequence of events which may occur *in vivo*. The model envisages local production of interferon within the lesions since they contain both infected macrophages and lymphocytes — prerequisites for interferon production. However, interferon release is seen as a two-edged weapon. On the one hand it may restrict viral replication thus leading to the slow progression of the disease; on the other hand it has the disadvantage of increasing macrophage Ia expression, thereby attracting T-helper lymphocytes which in turn lead to cytokine production and lymphoproliferation, and thereby cause lymphadenopathy [4]. The presence of infected promonocytes in the bone marrow might well explain the *persistent* nature of the disease [6].

These techniques have also allowed us to analyse the neurological component of the visna-maedia complex [35]. The marked breed susceptibility to the encephalitis has already been alluded to. Moroever, there is a qualitative difference in that the encephalitis seen in Icelandic sheep is characteristically chronic and progressive, contrasting with the disease in European sheep which tends to be episodic leading to focal scar formation and repair [36]. Intracranial inoculation of British sheep with visna virus resulted in the development of encephalitic lesions. However, in contrast to the systemic tissues which contain viral RNA, it was not possible to detect by *in situ* hybridisation more than an occasional virus-containing cell in the brains of these animals. This was not due to the strain of virus

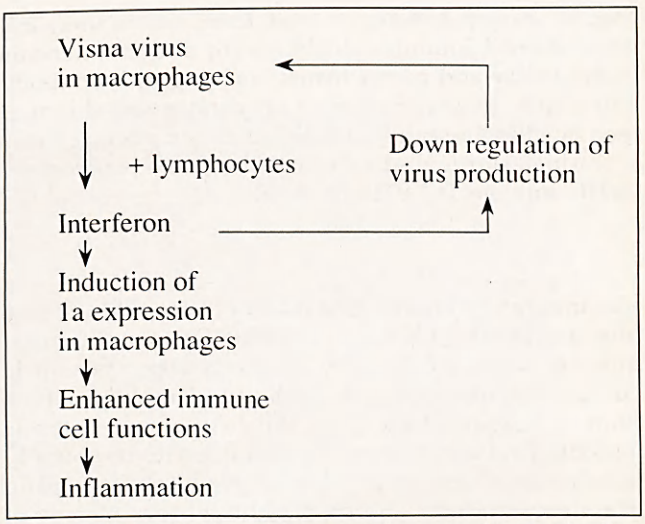


Fig. 3. *Chart showing the suggested course of events leading to restriction of visna virus replication and the development of inflammation* (From Ref. 35).

inoculated because similar results were obtained using a neurotropic Icelandic visna virus strain. However, when these techniques were applied to brain sections from an Icelandic sheep which had died with visna encephalitis, the results were very different in that abundant viral nucleic acid and increased Ia expression were detected within the inflammatory lesions [35]. Double-label experiments showed that the virus had extended its cell specificity in that both macrophages and a few oligodendrocytes contained viral RNA [39], which are summarised in Fig. 4.

These findings indicate that different genes have a role in controlling the systemic and neurological manifestations of the visna-maedia complex, and the experimental data are consistent with the characteristic features seen in the natural disease. The exact pathogenesis of the brain disease is still not known for certain, but we can suggest a possible scenario for the neurological events. Infected monocytes in the blood must cross the blood-brain barrier, possibly via the CSF — the 'Trojan horse' mechanism [40]. In the brain

Virus Life Cycle

		Pro. V.DNA	V.RNA	Protein	Virus	Lesion
Icelandic Sheep	Lung	+	+	+	+	+
	Brain	+	+	+	±	+
	Liver	0	0	0	0	0
Monocyte (V.RNA +)						
British Sheep	Lung	+	+	+	±	+
	Brain	+	0	0	0	0
	Liver	0	0	0	0	0

Fig. 4. *Chart showing that the degree of restricted visna virus replication in the animal depends on the tissue as well as the breed of animal* (From Ref. 35).

Fig. 5. *Antigen labelling on brain tissue sections using avidin biotin peroxidase technique.* Cerebral hemisphere sections were stained immunocytochemically and counterstained with haematoxylin. (a) HIV encephalitis case showing perivascular and parenchymal labelling with antibody against class II MHC antigens (×428). This contrasts with (b) which shows the absence of staining with this antibody on a normal control brain (×428). In (c) class II antigen labelling is seen in a multinucleated giant cell from a case with HIV encephalitis (×1,071). This contrasts with (d) which shows absence of labelling on a consecutive section from this same case with an antibody against class I MHC antigens (×1,071) (From Ref. 49).

the monocytes mature into macrophages with the production of viral RNA and protein. This amplification process is accompanied by increased expression of Ia antigens in macrophages, leading to lymphoproliferation as suggested above in the case of the systemic lesions. Viral infection of oligodendrocytes resulting in alteration of host gene function could partly explain the demyelination observed, although the paucity of viral RNA-containing cells suggests that other mechanisms are almost certainly important. The latter could include local release of cytokines and interferon, causing membrane-mediated damage to neural cells, and inflammation [35].

Human immunodeficiency virus infection of humans

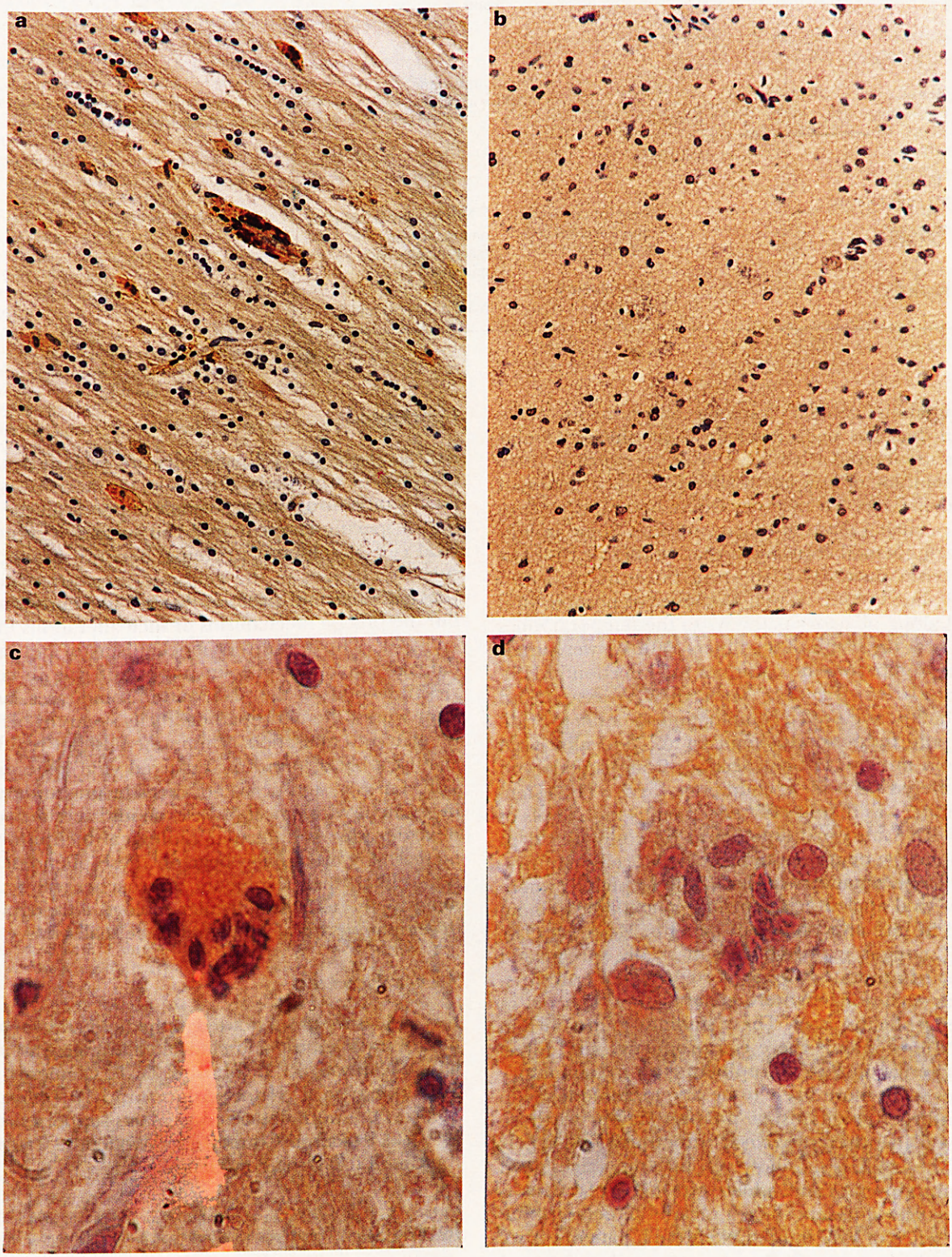
It has been apparent for some time that HIV is neurotropic as well as lymphotropic. The CNS is infected early in the disease, about 10% of all patients with HIV infection presenting with neurological features, [41,42]. Moreover, up to 90% of all patients with AIDS have pathological evidence of CNS involvement [43]. The neurological manifestations are protean and have been reviewed in detail elsewhere [43,44]. They can broadly be divided into infection, malignancies, vascular and neuromuscular disorders. The infectious diseases may be due either to the effect of HIV itself or to secondary infection with a variety of opportunistic agents including viruses, bacteria, fungi and protozoa [45]. One of the most important, frequent and enigmatic of the complications is HIV encephalitis which may be associated with a rapidly progressive dementia — indeed this term has also been known as the AIDS-dementia complex [12,44]. Patients with this condition generally have a degree of immunosuppression and it is a relatively late complication of HIV infection. Cognitive impairment of a subcortical nature in HIV encephalitis is progressive and followed by a variety of neurological problems including focal signs, seizures and eventually coma and death [12]. The pathological features include the presence of multinucleated giant cells, microglial nodules, diffuse white matter astrocytic proliferation, and perivascular and parenchymal macrophage and lymphocytic infiltration [12,44].

Although HIV has been demonstrated within the brains of such patients [44,46], viral nucleic acid and protein expression is limited and appears to be confined to macrophages, multinucleated giant cells and probably endothelial cells [44,47,48]. To date there is no really convincing evidence to show the presence of HIV within glial cells (oligodendrocytes and astrocytes) or neurons. In order to address the pathogenesis of HIV encephalitis we have recently carried out a study using immunocytochemical analysis and monoclonal antibodies to look for increased expression of MHC class II antigens in the brains from patients who had died with this condition [49]. Only a minority of the brain sections from cases of HIV encephalitis contained HIV antigens. However, about two-thirds of the cases showed increased and inappropriate expression of class II antigens compared with control sections (Fig. 5). Class I antigens were also detected in the HIV sections but at a much lower level. The interpretation of these findings is difficult at this stage of our knowledge of the disease, but Fig. 6 summarises a possible scenario for pathogenesis. In the peripheral blood both macrophages and T4 helper cells are infected, but the latter are killed while some infected macrophages cross the blood-brain barrier and infect other macrophages and tissue-fixed microglia within the brain. The infected microglia may secrete cytokines and/or other toxic substances which could directly damage the cell membranes of neural cells, thereby leading to neural disfunction with clinical features; and they may also increase class II antigen expression in macrophages and microglia. It is possible that upregulation of MHC class II molecules on macrophages within the brain may help set in train a more general autoimmune cascade, but it seems likely that, as in visna-maedi infection in sheep, indirect mechanisms of neurological disease are likely to be important in HIV infection of the nervous system.

Herpes virus infections in humans

Molecular techniques have helped to elucidate the pathogenesis of CNS and peripheral nervous system (PNS) disease caused by HSV and varicella-zoster virus (VZV) infections in man. HSV produces a number of neurological conditions, and by far the most important of these is an acute, often fatal, sporadic encephalitis [50,51]. The number of neurological syndromes associated with VZV is even greater and includes an encephalomyelitis, although this is generally a less serious disorder than that caused by HSV [50].

Reference has already been made to the ability of HSV to produce a latent infection in the spinal sensory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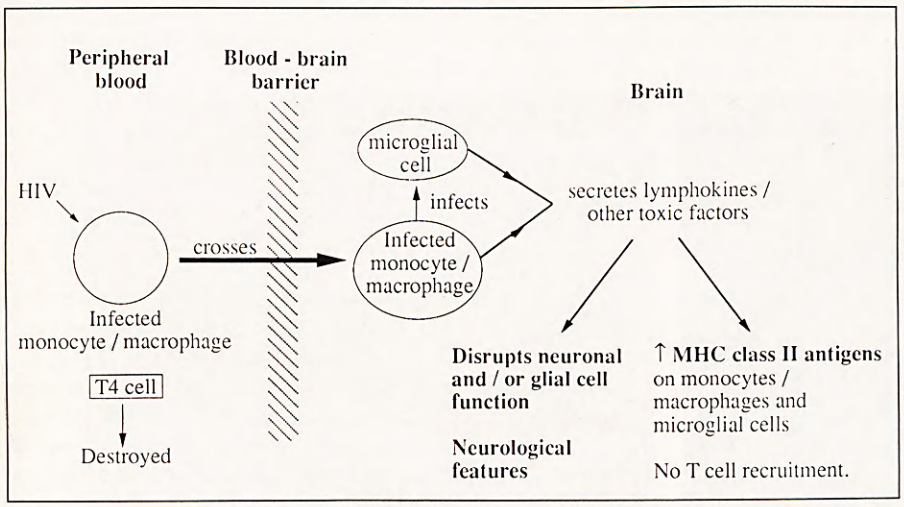

Fig. 6. *Chart showing suggested sequence of events leading to neurological damage in HIV encephalitis.* (From Ref. 49).

and trigeminal ganglia of animals and humans. While a good deal has been learned about the molecular events associated with HSV latency, there is little understanding of how latent HSV, which is so frequent in humans, can on rare occasions be reactivated to produce such a devastating encephalitis. Some insight has been gleaned from molecular studies of viral isolates from patients with simultaneous oral and encephalitic lesions, as well as studies of possible viral spread along olfactory pathways to the temporal lobes which bear the brunt of the illness [50]. We were interested in comparing the nature of the infection in HSV encephalitis with that seen in VZV-associated encephalitis, and used a combination of *in situ* hybridisation and immunocytochemistry to co-localise viral RNA and proteins in brain sections from patients who had suffered from these conditions [5,52]. The essential finding was that in HSV encephalitis high levels of HSV-1 RNA and proteins could be co-localised in macrophages, neurones and some glial cells, contrasting with the situation seen in VZV encephalitis where neither viral nucleic acid nor protein could be demonstrated in immunocompetent patients. However, class II MHC antigen expression was markedly upregulated in the inflammatory lesions in VZV encephalitis, whereas such antigens were generally not detected in HSV encephalitis cases. These findings indicated that in HSV encephalitis there is a productive viral infection, whereas in VZV encephalitis the inflammatory changes seem more likely to be due to immune-mediated indirect mechanisms, possibly similar to those observed in other retroviral infections [33,52]. It should be emphasised, however, that the situation in immunosuppressed individuals with VZV infections is probably very different since it is easier to demonstrate the presence of VZV in the CNS of such patients. Also, the far more sensitive PCR technique needs to be used in such tissues to examine these important questions in the future.

Conclusions

At least three general principles are evident from this overview of viral pathogenesis in the nervous system. First, viral neurotropism strongly depends on the binding of specific target neural cell surface receptors to viral cell attachment proteins. Second, limited and specific changes in the genomic structure of a neurotropic virus may profoundly alter its neurovirulence but the precise mechanisms of such alterations may be complex. Third, indirect mechanisms of neural cell damage appear to play an important role in certain viral infections of the CNS, and in some cases, such as lentiviruses, the neuropathogenicity seems to be closely related to alteration of immune antigen regulation against a background of restricted viral gene expression. Further significant advances in our understanding of neurovirological diseases should accrue over the next decade as molecular biological techniques are increasingly brought to bear on these phenomena. It is also likely that therapeutic approaches will be facilitated by the use of molecular biology, although these developments may take longer to be realised.

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