Neurovirological methods and their applications

P G E Kennedy

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Over the last 30 years neurovirology has emerged as a major discipline which has much relevance to both human disease and many aspects of neuroscience. This overview of the field aims to define briefly most of the major neurovirological techniques, both "classical" and more recent, and to indicate how these have been used to gain knowledge about the pathogenesis, clinical investigation, and treatment of viral infections of the central nervous system.

Neurovirology has emerged over the last 30 years as a major discipline that embraces a range of subject areas including virology, neurosciences and clinical neurology, molecular biology, and immunology.¹ As Richard T Johnson, one of the pioneers in this field, has explained, the term neurovirology is a broad one and is used to cover studies of the pathogenesis of viral infections of the nervous system (both in humans and experimental animal models), in vitro studies of the effects of viruses on defined neural cell types, the use of viruses as tools in neuroanatomical and developmental studies, and the use of genetically engineered viruses as vectors to deliver therapeutic genes into the human central nervous system (CNS).¹ It also includes the diagnosis and epidemiology of viral infections of the CNS, as well as the development of specific antiviral agents to treat disease. The use of acyclovir for the effective treatment of herpes simplex encephalitis is a good example of the latter.² Thus it is a discipline that has much relevance to both human disease and many aspects of neuroscience. Clinical and basic neurovirological studies sometimes yield complementary information.

Historically much of neurovirological research has focused on herpes simplex virus types 1 and 2 (HSV-1, HSV-2) (especially herpes simplex encephalitis and herpes virus latency), varicella-zoster virus, HIV (both basic and clinical aspects), human T cell leukaemia virus (HTLV-1), JC virus (the cause of progressive multifocal leucoencephalopathy in humans), measles virus, poliovirus, rabies virus, Borna disease virus, and animal

models of demyelinating disease such as Theiler's virus, Semliki Forest virus, coronaviruses, and visna-maedi virus.³ More recently there has been much interest in "emerging" viral infections of the nervous system such as Nipah virus and West Nile virus.^{4,5} Research has also focused on the host immune response to CNS virus infections, and work on virus associated apoptosis, cytokines, and chemokines is also flourishing.^{3,6}

The burden of neurovirological disease worldwide is considerable. For example, nervous system involvement in HIV infection is very common, with 10% of AIDS patients presenting with neurological features and over 80% being neurologically affected at necropsy.⁷ With the introduction of highly active retroviral therapy (HAART), clinicians have had to deal with the challenge of a changing neurological disease profile of HIV infection. There are also various common viruses that affect the CNS, such as influenza, where the neuropathogenesis is not well understood. In addition, the immunosuppression that accompanies more effective antibacterial treatment has in turn led to increased interest in the better treatment of neurovirological disease, while more widespread international air travel has raised the profile of viral infections of the nervous system arising in African and Asia, such as Japanese encephalitis.⁸ Thus both host and viral factors may be contributing to the changing pattern of neurovirological disease.

Fortunately these changes in disease patterns have been accompanied by increasingly sophisticated investigative tools, including the molecular analysis of both the virus and the host response to viral infection. In this brief overview of the field my approach will be to define briefly most of the major neurovirological techniques, both "classical" and more recent (table 1), and then indicate how these have been used to gain knowledge about the pathogenesis, clinical investigation, and in some cases treatment of viral infections of the CNS. The coverage is not exhaustive, and only selected examples of these methods and their uses will be given. I will not specifically cover the important area of viral immunology, which involves host immune responses to viruses and immune mediated neurological disease. Animal

Correspondence to:
Professor P G E Kennedy,
Division of Clinical
Neurosciences, Department
of Neurology, Institute of
Neurological Sciences,
Southern General Hospital,
Glasgow G51 4TF, UK;
p.g.kennedy@clinmed.gla.
ac.uk

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Table 1 Summary of the techniques described

Classical	Modern
Tissue culture of the nervous system	Polymerase chain reaction (PCR)
Virus isolation	Molecular hybridisation methods including in situ hybridisation
Electron microscopy	Gene microarrays
Serological studies	Molecular analysis of viral isolates
Viral antigen detection	Use of viral and animal mutants in neuropathogenesis studies

models—which have been used extensively in neurovirology—will be alluded to briefly in the context of the specific topics. I will place emphasis on the newer approaches such as advanced molecular hybridisation techniques, gene microarrays, and viral vectors to deliver therapeutic genes to the CNS.

SPECIFIC LABORATORY METHODS

Classical techniques

For many years what I refer to as “classical” techniques have been used in neurovirology, and I shall mention these briefly here.

Tissue culture of the nervous system

This is included here because of its longstanding use. Cultures of dissociated or explanted CNS or peripheral nervous system tissues containing identifiable neural cells provide a useful *in vitro* tool for investigating virus–neural cell interactions where the environmental conditions can be precisely controlled. While such a system can never mimic the complexities of the *in vivo* situation, such studies have provided a wealth of useful information. For example, the differential susceptibility of human glial cells and neurones to HSV-1 infection has been defined in this way,^{9–10} infection of dissociated neural cells by HIV has been investigated,¹¹ *in vitro* models of HSV latency have been devised,¹² and the effects of either toxic viral products or drugs on neural cells can be assessed.

Virus isolation

Isolation of viruses from human tissues and body fluids is a classical method of establishing the viral aetiology of a neurological condition. Viruses can be isolated through their production of a characteristic cytopathic effect on susceptible indicator cells. More recently molecular analysis of viral isolates has been important, but this will be discussed below. Potential problems with interpretation of virus isolation results include the possibility that they may represent an irrelevant or coexisting virus infection, the presence of asymptomatic virus secretion by the patient, accidental viral contamination, or the presence of a viral reactivation rather than a primary infection.¹³ Numerous different viruses have been isolated over the course of many years from the tissues of patients with multiple sclerosis.¹⁴ However, while this disease may well have a viral aetiology, such reports have generally been viewed with scepticism and there is no hard evidence to date for a direct viral cause.¹⁵ It is quite possible, however, that this question may be clarified in the future by the use of recently developed molecular technology, and the current interest in the possible role of human Herpesvirus-6 (HHV-6) in multiple sclerosis¹⁶ is intriguing and worthy of further detailed study.

Electron microscopy

This technique has been useful in identifying various viruses in neurological conditions. Electron microscopy was used, for example, to show that the papovavirus virus was the cause of progressive multifocal leucoencephalopathy,¹⁷ and such methods have also been employed to demonstrate herpes simplex virus in brain biopsies of patients with herpes simplex encephalitis.^{3–18}

Serological analyses

A significantly rising viral antibody titre in paired serum or cerebrospinal fluid (CSF) samples provides convincing evidence of a recent virus infection as the cause of a neurological illness, and it has also been a useful tool in epidemiological studies. While the polymerase chain reaction (PCR) (see below) has to a large extent superseded serology for CNS viral diagnosis, nevertheless the latter is still widely employed.

Potential problems with interpretation of antibody measurements include non-specific polyclonal activation of virus resulting from a generalised immune response to infection, persisting viral antibody levels from a previous infection, inadequate test sensitivity and specificity, and the practical problems of obtaining the specimens and making important decisions in an acutely ill patient many days before the antibody results are available.¹³

Viral antigen detection

Identification of viral antigens in tissues and body fluids of patients provides strong evidence of the involvement of a virus in an illness, although some of the caveats mentioned above also pertain here, especially the potential problems of viral contamination and of a coexisting but aetiologically irrelevant viral infection. The techniques used include enzyme linked immunosorbent assays (ELISA), western blotting for viral proteins, immunofluorescence, and immunocytochemistry.¹³ Some of these techniques have been used, for example, for the diagnosis of HIV and herpes simplex virus infections, and also in viral pathogenic studies.^{13–19–20} Double label immunofluorescence or immunocytochemistry are techniques whereby two viral antigens can be colocalised within the same tissue region or cell, or where a viral antigen can be visualised within a neural cell type identified by an antigenic marker—for example, a herpes simplex virus antigen within a human astrocyte during natural or experimental viral infection.⁹ Evidence of viral protein expression is critical in assessing the nature of the infection, such as whether it is a latent infection (with no or restricted protein expression) or a productive infection.²¹ Also, a knowledge of which viral proteins are expressed during infection may allow the development of non-live or subunit vaccines directed against those proteins in order to generate antiviral immunity.

Polymerase chain reaction

PCR has revolutionised both CNS viral diagnosis and pathogenic studies (see Morrison for a detailed description²²). PCR uses oligonucleotide primers, an ingenious thermal cycling method, and a specific thermostable DNA polymerase to provide rapid and massive amplification of target nucleic acid (DNA or cDNA). PCR can identify a single viral genome in a tissue specimen containing many thousands of cells, and the specificity and sensitivity can be even further increased by various modifications.²² A significant problem with PCR, however, is potential contamination during the procedure, and there are also various technical problems related to the choice of the most appropriate primer sequences.²² A further advantage of this technique is that reverse transcription PCR (RT-PCR) can be used to detect viral RNA, which can be quantitated; this allows the measurement of viral load—for example, in patients with HIV infection.²³

A recently developed and extremely sensitive technique known as real time PCR uses a special PCR system to quantitate viral DNA or RNA in terms of viral copies.²⁴ High throughput real time RT-PCR is proving of value in diagnostic studies where a range of viruses can be looked for in patient samples. PCR is also used in other molecular techniques relevant to neurovirology, such as DNA sequencing and cloning of genomic DNA.²²

PCR has proved very valuable in the rapid and specific diagnosis of CNS viral infections such as HSV-1, cytomegalovirus, varicella-zoster virus, and enteroviruses, and is now the viral diagnostic method of choice in such diseases. The value of CSF PCR in the diagnosis of herpes simplex encephalitis has been particularly well studied, and its specificity in experienced laboratories is over 95%.^{19–25} False negative results are most likely to occur if the CSF sample is obtained within the first 24 to 48 hours or after 10 to 14 days from the onset of the illness, but the sensitivity of the test is otherwise about 95%.^{25–26} PCR

has also been frequently used to examine tissue samples for the possible presence of viruses.^{3–20} While such an approach can yield potentially valuable information about a possible viral component in the pathogenesis of a disease, care must be taken to avoid contamination and caution is needed in the interpretation of either negative or positive results.

Molecular hybridisation studies

Several techniques that are used in conventional molecular biology have also been of value in neurovirological studies, and I will summarise these briefly.

Southern and northern blots

These techniques detect DNA and RNA, respectively. In both cases target nucleic acids are prepared from the relevant tissues under test, then immobilised on a hybridisation membrane—for example, a nylon filter—and the nucleic acid fragments hybridised with a radiolabelled molecular probe. The result is visualised autoradiographically.^{20–27} Quantitation of nucleic acids is possible with the use of careful controls; however, northern blots require relatively large quantities of RNA, and both techniques are more useful in research than as clinical tools.

In situ hybridisation

In situ hybridisation (ISH) is one of the most useful techniques currently available in studies of viral pathogenesis. Nucleic acid probes, either radiolabelled (for example with ³⁵S) or chemically labelled (as with digoxigenin), are pretreated and hybridised in situ with sections of relevant tissues or cell cultures fixed onto glass slides.^{27–28} After rigorous washing, the hybridised tissues or cells are processed autoradiographically so that the presence, cellular distribution, and location of viral DNA or RNA within the specimen (depending on the particular ISH methodology) can be determined. ISH is more sensitive than Southern and northern blotting, being able to detect a few copies (typically 10 or less) of the viral genome per cell.²⁷ However, the technique is labour intensive, difficult, and prone to sampling error and artefacts, so that both care and experience are necessary when interpreting the results. ISH has been used extensively in attempts to identify particular viral nucleic acid sequences (for example, for varicella-zoster and measles viruses^{28–29}) within nervous tissues from both normal individuals and patients with neurological conditions. It has also been particularly useful in studies of varicella-zoster virus latency in both humans^{30–31} and animals.³²

PCR in situ amplification

This is a recently developed technique that combines the exquisite sensitivity of PCR with the cell localising ability of ISH. The technique uses a specially modified PCR machine which carries out thermocycling on glass slides. Owing to its increased sensitivity, tissues which are negative for the DNA of a particular virus using conventional ISH may test positive when analysed by PCR in situ, as has recently been shown in the case of latent varicella-zoster virus in human trigeminal ganglia.²⁸ Although the technique is far more sensitive than ISH alone, a major drawback with PCR in situ is that it is very prone to artefacts, so great caution must be taken to include rigorous control procedures. RT-PCR in situ amplification to detect RNA in specific tissue areas has also been described. These techniques may be capricious and difficult, but when rigorously controlled can yield useful information.

Combined ISH and immunocytochemistry

This is another useful but technically difficult technique in which tissues or cells under test are first labelled immunocytochemically with specific antibodies against, for example, viral proteins or cellular constituents and are then processed

for ISH as described above.³³ Using this technique it is possible to colocalise viral nucleic acids and proteins in the same cell, and also to identify nucleic acids in marker identified neural cell types. Both types of information can contribute to our understanding of the possible ways in which the virus produces neurological damage.

Gene microarrays

The advent of gene microarrays for analysing thousands of different genes simultaneously represents one of the most exciting and promising advances in basic and applied molecular biology in recent years. The detailed technology and potential applications of microarrays will be covered in another article in this series, and has been the subject of extensive recent reviews (see, for example, Aitman³⁴ and Kellam³⁵), so I will only give a brief outline here, concentrating on its application to neurovirology. Whereas the molecular hybridisation techniques described above are designed to use a single probe to detect one target gene at a time, the microarray system contains thousands of different test genes, consisting of virus or host specific nucleic acid sequences, contained on a single and very small silica or glass slide.^{34–35}

The two main types of microarray are *oligonucleotide arrays*, consisting of thousands of viral or other oligonucleotides corresponding to specific open reading frames (ORFs), representing different genes, which are printed onto a solid, for example silica, slide; and *DNA microarrays* in which DNA in the form of cDNA, PCR products, or oligonucleotides is spotted onto either glass slides or nylon membranes.^{34–35} During this procedure, RNA is extracted from the tissue or other test sample, labelled with a fluorescent dye, and then exposed to the microarrays to allow hybridisation with the various target spots. The method includes a sophisticated computer assisted analysis of the intensity of each array spot after hybridisation so that a quantitative readout is obtained. Significant increases in array spot intensity are then computed, allowing an analysis of the expression of thousands of different genes simultaneously.

The potential applications of this methodology are numerous, and array technology is rapidly becoming more sophisticated in its efficiency and scope.³⁵ In neurovirology, there are several important applications of microarrays. As the entire genome of a virus can be represented by hundreds or thousands of probe spots on a microarray, it is possible to study the function of multiple genes during various types of viral infection. For example, in the case of HSV-1, the expression of all the viral genes can be studied during different stages of an acute lytic infection of neural or other susceptible cells, allowing detailed transcriptional analysis leading to advances in our understanding of viral gene function. During a latent viral infection caused by HSV-1 or varicella-zoster, for example, it should be possible to determine the full extent of viral gene expression using microarrays. Until the present time this had only been possible using conventional molecular hybridisation techniques to detect single viral genes at a time.^{28–30} Knowledge of which viral genes are expressed during a latent infection should facilitate the development of anti-viral strategies against specific viral target genes and their products.³⁶

Microarrays can also be used to study multiple host cell functions during lytic and latent viral infections. The expression of many thousands of different host genes subserving a myriad of functions (for example, genes encoding cell replication and degradation, various neural functions, signal transduction, stress responses, and immune responses) can be studied simultaneously on the same specimen using microarrays. Thus it has recently been possible to employ arrays to analyse in detail host cell gene transcription during acute varicella-zoster virus infection of human T cells and fibroblasts using a 40 000 spot human cDNA microarray

system.³⁷ Similar experiments should be possible in lytic infection of neural cells and latent viral infections.

Microarrays also have promise in the diagnosis of infectious diseases, including those caused in the CNS by viruses. Several pathogen arrays have already been constructed, including those for bacteria and viruses.³⁵ Using virus pathogen “chips” it should be possible to screen patients’ sera and CSF for many different viruses simultaneously and quickly. At present array technology is extremely expensive, but over time it is to be hoped that the cost-effectiveness of this technology will increase and the basic costs decrease. Many advances in our understanding of the host and viral responses during viral infection of the CNS can be expected over the next decade (see below). In addition, rapid advances in “proteomics,” including the development of protein microarrays, are likely to clarify many host-virus interactions and will also provide valuable information on a variety of protein-protein interactions,³⁵ which will help us to understand in more detail how viruses affect neural cells. In a wider sense this technology should allow the detection of genetic and environmental factors which influence human susceptibility to viral infections of the nervous system. Further, in the future, microarray based PCR analysis of samples obtained from, for example, encephalitis of unknown origin, may prove a previously unsuspected virus link, or confirm a suspected one, to that condition—a form of “virus discovery” that can be applied to many different neurological conditions.

Molecular analysis of viral isolates

The molecular analysis of viral isolates from patient’s tissues has for many years been a useful tool in both viral diagnosis and pathogenesis. A seminal example was provided by Whitley and colleagues,³⁸ who used restriction enzyme analysis to characterise paired HSV-1 isolates from oral-labial and brain sites in patients with herpes simplex encephalitis. By comparing the molecular profiles of isolates from the two sites they were able to conclude that this disorder can result from a primary HSV-1 infection, a reinfection with a second HSV-1, or a reactivation of latent HSV-1. Another important study in this area was reported by Evans *et al.*,³⁹ who sequenced the genomes of daily faecal poliovirus isolates from infants who had received oral Sabin type 3 vaccine virus. As the vaccine strain passed through the infant’s gut it was found to be rapidly mutated to a neurovirulent strain which was attributed to a single nucleotide change of uridine to cytidine in the 5′ non-coding region of the genome, which presumably altered the secondary structure of the virus. Thus a very small change in the viral genome had a profound effect on the neurovirulence of the virus.²⁰

This type of approach has also been used to study the “molecular epidemiology” of virus infections. A recent and elegant example of this was provided by the study by Quinlivan *et al.*,⁴⁰ in which restriction enzyme analysis (using the BglII site of gene 54) of varicella-zoster virus isolates was carried out in patients from the United Kingdom, North and South America, Africa, Asia, and the Far East. Significant differences were found in positivity for this particular site in the various patient populations, with, for example, less than 22% of varicella-zoster strains from British and American patients being positive, compared with a positivity rate of 98.6% for patients from the other areas. Thus this analysis provided strong evidence for geographical segregation of different varicella-zoster strains. Clearly this might account for differences in the clinical presentations of varicella and herpes zoster in different parts of the world. Restriction enzyme analysis has also been used to characterise viral isolates in the trigeminal and autonomic ganglia in individuals latently infected with HSV-1⁴¹ allowing conclusions to be drawn regarding viral neuropathogenesis.

USE OF VIRAL AND ANIMAL MODELS IN NEUROPATHOGENESIS STUDIES

The use of specially constructed or naturally occurring viral mutants has been a highly productive method of studying viral determinants of neuropathogenesis. Such mutants have included temperature sensitive mutants that replicate at 31°C but not at 38.5°C, deletion mutants with larger changes in their genomic structure, and viral antigenic variants produced by exposure to monoclonal antibodies of varying specificities.^{20–27} I will give a few examples briefly here to indicate their value. In all cases a general principle is that a specific molecularly characterised viral mutation is correlated with a loss or alteration of a neurological disease phenotype such as neurovirulence or latency, thereby allowing the attribution of the particular impaired or absent function to the deleted gene sequence.²⁰ It should then be possible to reinsert the missing viral gene to restore the specific biological function and prove a cause and effect relation.

Such mutants have been very useful in studying HSV-1 latency. For example, HSV-1 mutants which have had the LAT (latency associated transcripts) gene deleted show an altered phenotype in which the virus takes longer than usual to reactivate from latency in experimental mouse ganglia, thus indicating the importance of the LAT gene in viral reactivation from latency.⁴² In human latency, Brown *et al.* used various temperature sensitive herpes simplex virus mutants to “superinfect” explanted human ganglionic tissues in order to detect genetic information from latent herpes simplex virus in the ganglia.⁴³ This was made possible because of interactions between the input superinfecting virus and the genome of the latent virus within the ganglia.

Deletion mutants of certain strains of polioviruses have been used to show that the 5′ non-coding region of the viral genome is important in producing poliovirus neurovirulence in experimental monkeys.^{27–44} Monoclonal antibody derived viral variants of rabies virus have been used to show that the presence of a positively charged amino acid (arginine or lysine) in position 333 of the rabies virus glycoprotein is necessary for viral neurovirulence in mice.^{27–45} It is remarkable how such small alterations in the viral genome can result in such a disrupted neurological phenotype. A recent study has shown that the rabies viral glycoprotein determines the distribution of rabies virus strains within the brains of experimental rats stereotactically inoculated with different viruses.⁴⁶

Sharpe and Fields⁴⁷ carried out extensive studies in the nervous system using reassortant mutants containing segmented gene regions from reovirus types 1 and 3. The generation of these mutants with reassorted genomes allowed the correlation of defined genomic regions with particular neuropathogenic effects. For example, the reovirus S1 gene—which encodes the viral cell attachment protein known as sigma-1—is the key determinant of whether the virus spreads by neural or haematogenous pathways, and whether the virus infects neurones or ependymal cells in mouse brain.^{47–48} A general principle that has emerged from this type of study, and others, is that the basis of viral neurotropism is the interaction of viral attachment proteins to specific cell surface receptors on the target neural cell.²⁷

I have already alluded to the use of animal models in attempts to understand the mechanisms by which viruses cause neurological disease. While a detailed discussion of this subject is beyond the scope of the present article, the value of such models has been considerable. Neurotropic viruses can be inoculated into different disease susceptible animal strains (for example, mouse, rat, or rabbit) and a useful and reproducible animal model of a CNS viral disease may thereby be produced. Genetic determinants of the host response to virus infection can be studied in different inbred mouse strains.⁴⁹ In addition, a particular host mutation can be introduced into the animal itself. Examples of the latter include the

Table 2 Strategies for gene therapy in the CNS using viral vectors

(1)	Replace missing or defective gene
(2)	Replace or enhance local growth factors or enzyme production
(3)	Virus directed enzyme pro-drug treatment
(4)	Direct cell killing of CNS tumours
(5)	Delivery of anti-sense sequences to particular genes
(6)	Use of inserted DNA to increase cellular antigen expression, to boost immune response

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Table 3 Ideal properties of viral vectors for gene therapy

(1)	Enough capacity of virus to package foreign gene
(2)	Vector must be effectively delivered to target cells
(i)	Appropriate route of delivery
(ii)	No vector induced damage along route or at target
(iii)	Vectors must remain intact during delivery
(iv)	Vector must target appropriate neural cells
(3)	No viral replication and/or reactivation at target tissue site
(4)	Foreign gene must be stably expressed in target tissue
(5)	Must have ability to control level of expression of the gene products
(6)	Vector should not elicit immune responses in the host
(7)	Appropriate in vitro and in vivo models should be available before human treatment

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extensive use of gene “knockout” mice, in which specific genes (such as those for immune function) have been deleted using genetic technology, or “transgenic” mice in which a foreign gene has been inserted into the germ cell line so that it is expressed or overexpressed in particular cells and tissues in the developed mouse. In both cases the phenotype of the genetically altered mice is studied following specific viral infections and may reveal the importance of particular genes in determining, for example, neurovirulence.

Theiler’s murine encephalomyelitis virus (TMEV) infection of the mouse has proved to be a highly effective model of CNS demyelination.⁴⁹ Using knockout mice which lack the gene coding for the intercellular adhesion molecule-1 (ICAM-1), it was found that there was an increase in the levels of pathology in the grey matter regions and meningeal inflammation in virally infected knockout mice compared with the infected control mice.⁵⁰ However, there were no differences in the degree of demyelination. This showed that ICAM-1 is crucial for protection from TMEV induced neuronal damage but not from demyelination. In the case of mouse hepatitis virus infection, it was found that virally infected knockout mice lacking the gene coding for nitric oxide synthase type 2 (NOS2) showed a marked decrease in mortality and apoptotic neurones in the brain compared with wild type mice, although the degree of demyelination was comparable in the two groups.⁵¹ This showed that NOS2 generated nitric oxide contributes to apoptosis of neurones but not demyelination after mouse hepatitis virus infection. The transgenic approach has been used to demonstrate the important role of the proinflammatory cytokine interleukin 6 (IL-6) in HSV-1 infection of mice.⁵² Ocular HSV-1 infection was studied in transgenic mice which expressed IL-6 homozygously or heterozygously in astrocytes. The accumulated results observed in this experimental system showed that there was a dose dependent IL-6 antagonism of acute HSV-1 infection in vivo.

VIRAL VECTORS FOR GENE THERAPY OF NEUROLOGICAL DISEASES

This is one of the most important and rapidly developing areas in neurovirology, and I will give some general principles and examples (but see elsewhere for a detailed discussion⁵³). The striking advances in molecular genetics and biochemistry, virology, and our understanding of the molecular basis of

neurological diseases that have occurred over the last two decades underlie the current interest and feasibility of gene therapy. Viral vectors represent one of the most prevalent methods of delivering exogenous genes into the nervous system because of the ability of certain viruses to spread along neural pathways to infect particular regions of the CNS. The general principle is to disable the virus using targeted gene deletions, so that the virus retains its “useful” functions, such as the ability to spread along neurones and attach to and enter target neural tissues, but is sufficiently “disabled” by the gene deletion to lose its ability to replicate in those cells, elicit an immunological response, or produce disease. The viral vector, as it is called, is able to cross the cell membrane of the target cell to which the delivered foreign gene is inserted, something which enzymes by themselves cannot typically do. The foreign gene must then be transiently or stably expressed, with both transcription and translation of the genome occurring—producing RNA and protein, respectively. In this way the hope is that a therapeutic effect can be produced.

There are various potential strategies for gene therapy which are summarised in table 2, and some are probably more feasible than others. It is important to mention that some forms of what is termed “gene therapy” consist of direct cell killing of a tumour by a disabled virus which only grows in rapidly dividing tumour cells (see below). In some cases a viral vector can be used to insert a drug susceptibility gene into a tumour which is then killed when the drug is given systemically. A good example of this is the insertion of the HSV-1 thymidine kinase gene into a tumour, which is then destroyed after treatment with the anti-HSV drug acyclovir.⁵³ While numerous studies of viral vector mediated gene therapy have been undertaken in experimental animals, there is now an increasing number of clinical trials of such treatment in patients.

There are many technical aspects of gene therapy which are becoming increasingly sophisticated and will not be discussed here. However, it should be emphasised that the problems are formidable, and it is still not known just how successful this new form of treatment is ultimately going to be, or how many neurological diseases will prove to be amenable to gene therapy. If one were to design the ideal viral vector for gene therapy then it would possess all the properties listed in table 3. Two of the most problematic issues appear to be maintaining long term expression of the delivered gene and controlling

Table 4 Possible major applications of techniques in neurovirology over the next 10 years

1. Greatly improved viral diagnostic advances, especially from high throughput gene microarrays
2. Effective gene therapy for some neurological conditions using viral vectors
3. Effective use of gene microarrays and proteomics to identify and utilise expressed viral genes as targets for antiviral treatment
4. Greatly increased understanding of neuropathogenesis of viral infections of the nervous system
5. Greatly increased understanding of the alterations in host gene function in some neurovirological diseases
6. Increased understanding of the molecular evolution of viruses affecting the CNS
7. Major advances in virus discovery in nervous system diseases currently of unknown aetiology
8. Discovery of host genetic susceptibility to virus infection by array analysis

the level of expression of the gene in the target tissues. The main viruses that have been used to date for such treatment include HSV-1, adenovirus, adeno-associated virus, and retroviruses, the relative merits of which have been described in detail elsewhere.⁵³ HSV-1 has the particular advantages of having a very large genome which can be easily engineered to remove relevant "harmful" regions, and the ability to spread along neural pathways and then produce a non-pathogenic latent infection in neurones.^{21 53 54} Viral vectors need to contain molecular elements which drive transcription of the exogenous gene, and in practice the vectors contain both viral and cellular promoters, the former controlling transcription of the inserted gene and the latter, in principle, controlling its cell type specificity,^{53 54} although in practice these promoters do not necessarily function in this way as well as might be predicted.

There are several human neurological diseases that could benefit from the gene therapy approach using viral vectors, including Parkinson's disease, malignant brain tumours, motor neurone disease, inherited conditions caused by single gene defects, demyelinating diseases, cerebrovascular disease, and acute CNS trauma.⁵³ Two conditions for which gene therapy is likely to be successful are Parkinson's disease and malignant gliomas. In the former, for example, the gene coding for tyrosine hydroxylase can be delivered to the affected basal ganglion region with a resultant increase in brain dopamine levels to produce clinical improvement.^{53 55 56} With respect to brain tumours, clinical trials are now well under way to investigate the safety and efficacy of direct tumour killing by mutant HSV-1. In Glasgow, for instance, the herpes simplex virus mutant, HSV-1716—which is cell state and cell type specific, replicating in rapidly dividing tumour cells but not normal cells⁵⁷—has been used in clinical trials in patients with grade IV glioma since 1997.⁵⁸ The treatment was found to be safe, and three patients are surviving after three to four years, which is very promising, although further ongoing studies will be required to determine the future role of this treatment in such patients. It has already been established that HSV-1716 replicates in high grade glioma without causing toxicity in patients, whether seropositive or seronegative for the herpes simplex virus,^{58 59} and this approach may well lead to important adjunctive treatment.

POTENTIAL APPLICATIONS OF THESE TECHNOLOGIES OVER THE NEXT 10 YEARS

Table 4 gives a highly speculative list of the most likely applications of these various techniques in neurovirology in both clinical and neuropathogenetic aspects. As can be seen, these are somewhat interconnected and certainly not mutually exclusive.

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Glossary

Gene microarray: A method of detecting thousands of viral or host genes simultaneously on a chip or slide, of great use in analysing gene expression.

Gene therapy: The delivery of an exogenous gene to an organ resulting in expression of the introduced gene leading to a therapeutic effect.

ISH: In situ hybridisation. A sensitive molecular technique that can detect small amounts of DNA or RNA in a localised region of tissue.

Neuropathogenesis: The mechanism(s) by which a virus is able to cause disease in the nervous system.

Neurotropism: The ability of a virus to bind to a particular neural cell type.

Neurovirulence: The ability of the virus to produce actual neurological disease

PCR: Polymerase chain reaction—an extremely sensitive molecular technique for amplifying small amounts of specific DNA.

PCR in situ amplification: A technique in which DNA is amplified by PCR within a localised region of tissue, giving greater sensitivity than ISH alone.

RT-PCR: Reverse transcription polymerase chain reaction. A type of PCR that amplifies RNA rather than DNA.

Viral mutant: A virus which has had a part of its genetic structure altered leading to a detectable phenotype.

Viral vector: A virus which has been genetically modified so that it can deliver an exogenous therapeutic gene to a target organ without producing deleterious effects.

Useful website: www.isnv.org (official website of the International Society for Neurovirology, with details of current activities and the *Journal of NeuroVirology*).

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