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7
Host and viral genetic factors which influence viral neurotropism

Kenneth L. Tyler

INTRODUCTION

The capacity of viruses selectively to infect and injure specific regions of the central nervous system (CNS), and even specific populations of cells within these regions ('tropism'), depends on a series of complex interactions between viral genes and proteins and a number of host factors. In this chapter recent insights into the molecular and genetic basis for viral neurotropism are reviewed.

HOST FACTORS INFLUENCING SUSCEPTIBILITY TO VIRAL INFECTION

Studies using inbred strains of mice with well-defined genetic backgrounds have led to a number of important discoveries concerning the many mechanisms by which genes in the host can influence susceptibility to viral infection. The list of host genes which affect susceptibility to neurotropic viruses is extensive, and the following examples are meant to illustrate several mechanisms by which these genes appear to act rather than to serve as an exhaustive catalogue (*see* Brinton and Nathanson (1981); Brinton, Blank and Nathanson (1984); Rosenstreich, Weinblatt and O'Brien (1982) for review). In the case of some neurotropic viral diseases, such as that caused by lymphocytic choriomeningitis virus (LCM), and the chronic paralysis and demyelinating disease induced by Theiler's virus, immune-mediated tissue injury is a cardinal feature of the infection. In both these cases, differences in susceptibility to clinically apparent disease have been mapped to genes within the major histocompatibility complex (H2 in the mouse) (Buchmeier *et al.*, 1980; Clatch *et al.*, 1985). A discussion of the genetic factors that influence this type of immune-mediated tissue injury is beyond the scope of this chapter, and interested readers are referred elsewhere for more information (Notkins and Oldstone, 1985; 1986).

There are several examples where differential susceptibility to infection with viruses in different strains of inbred mice correlates with variations in the magnitude or nature of the immune response to the inciting virus. Interestingly, the host genes involved are often not linked to immune response (*Ir*) genes within the mouse major histocompatibility complex (H2). For example, A/WySnJ mice are

extremely susceptible to intraperitoneal inoculations of street rabies virus, whereas SJL/J and CBA/J strain mice are relatively resistant. These differences correlate with the magnitude of the antirabies virus antibody titre, with the resistant mice having higher titres then the susceptible ones. High serum antibody titres, in turn, seem to result in decreased viral replication in the CNS (Lodmell and Ewalt, 1985). The magnitude of the serum neutralizing antibody response in inbred mice immunized with a vaccine strain of rabies virus also seems to be genetically determined. For example, C3H/J mice are 'high responders' and C57BL/6 mice are 'low responders' in terms of serum antibody levels following vaccination. Studies of the F1 hybrid cross between these two strains indicates that the 'high response' is dominant, and that it is not linked to H2 genes (Templeton *et al.*, 1986).

Another mechanism of genetically controlled resistance to viral infection is seen in A2G strain mice infected with influenza virus. Although influenza is not, strictly speaking, usually considered a neurotropic virus, the general principles involved may turn out to have broader applicability to paramyxoviruses in general and possibly other unrelated viral groups. A2G mice have a dominant allele ('Mx+') located on chromosome 16 which confers resistance to influenza virus infection. This effect appears to be mediated via α and β (not γ) interferon. Following infection with influenza virus, but not other unrelated viruses, Mx+ mice produce an interferon-induced 75 kD protein that appears to be essential for the specific anti-influenza viral resistant state. This protein alone, even in the absence of other interferon-induced proteins, is sufficient to mediate the antiviral state in these mice, although its mechanism of action remains obscure (Staehli *et al.*, 1986).

An obvious method by which host genes may affect susceptibility to viral infection is by encoding viral receptors. Mice lacking or carrying an altered form of the receptor gene would presumably be resistant to receptor-mediated infection by the homologous virus. This mechanism may very well explain differences in the susceptibility of some strains of mice to infection with mouse hepatitis virus (MHV), a coronavirus. BALB/c mice are susceptible to MHV infection, whereas SJL/J mice are resistant. The resistant locus ('Mhv-1') has been mapped to chromosome 7 (Knobler *et al.*, 1984). Recent studies have indicated that the resistant BALB/c mice lack receptor for MHV on intestinal epithelial cells and hepatocytes (Boyle, Weismiller and Holmes, 1987). It appears that the Mhv-1 locus actually encodes the gene for the MHV receptor (J. Boyle, personal communication).

A novel mechanism of receptor-related genetically controlled resistance to viral infection is exemplified by resistance of some mouse strains to infection with ecotropic murine retroviruses. Some of these viruses can produce paralytic disease in mice associated with spongiform neuropathological changes in the CNS. Retroviruses are RNA viruses which transcribe their genomic RNA into DNA with the aid of the enzyme RNA-dependent DNA polymerase ('reverse transcriptase'). The viral DNA can then become integrated into the genomic DNA of the host target cells. At this stage in its life cycle the retrovirus is often referred to as a 'provirus'. In some cases the integrated proviral DNA does not contain a full complement of all the retroviral genes required for encoding all the viral proteins ('defective provirus'). A defective provirus is unable to activate from its integrated state to produce infectious virion particles. Mice carrying the Fv-4 allele in their genome inherit a defective endogenous ecotropic retrovirus. The envelope (*env*) gene of the provirus, which is the viral cell attachment protein, is transcribed in host cells and viral envelope protein is produced. This envelope protein then binds

to the receptor on the host cell surface used by ecotropic retroviruses, and prevents infection (due to receptor blockade) by exogenous ecotropic retroviruses (Yuen *et al.*, 1986).

Host genes are also related to susceptibility of mice to infection with the still unidentified agent responsible for scrapie. Dr Stanley Prusiner and his colleagues have suggested that scrapie is caused by an infectious protein ('prion') devoid of detectable nucleic acid. The major component of scrapie prions is a 27–30 kD protein designated PrP 27–30. This protein is encoded by a cellular gene, located on mouse chromosome 2, that is found in both scrapie-infected and uninfected animals. mRNA transcribed from this gene is also found in both healthy and infected animals, although the ultimate protein product appears to differ in terms of several biological properties in the two instances. One of the genes that influences susceptibility to scrapie infection in the mouse ('Prn-1') is closely linked to the gene encoding PrP 27–30 (Carlson *et al.*, 1986). It remains to be established whether other genes known to influence scrapie susceptibility or incubation time (for example, the scrapie incubation or *Sinc* gene) are identical to Prn-1, allelic, or unrelated but also linked to the PrP 27–30 gene (*see* Hope and Kimberlin, 1987, for review).

Host genes also appear to play a role in determining susceptibility to latent as well as acute viral infections. Following inoculation of herpes simplex virus type 1 (HSV-1) into the lip of an adult mouse, virus replicates locally and then spreads retrogradely through the peripheral branches of the trigeminal nerve to reach the trigeminal ganglion. During the acute phase of viral infection (which lasts for about 2 weeks) HSV-1 can be isolated from tissue homogenates of the trigeminal ganglion. Following this time period virus is no longer detectable in trigeminal tissue homogenates. However, virus can be recovered if the trigeminal ganglion is explanted, minced into small pieces, and co-cultivated with an indicator cell line (for example, CV-1 cells). When a large number of inbred strains of mice are tested for their susceptibility to latent HSV-1 infection, some strains (for example, C57BL, BALB/cByJ) are resistant to the development of latent infections whereas others are extremely susceptible (for example, PL/J, LP/J, CBA/J). Resistance appears to be a dominant trait, which is not linked to H2 genes (Kastrukoff, Lau and Puterman, 1986). Interestingly, resistance to development of latent HSV-1 infection, and resistance to acute infection with HSV-1, do not always cosegregate in inbred strains of mice (Lopez, 1975; Kastrukoff, Lau and Puterman, 1986). At least two independently segregating, non-H2-linked, genes govern resistance to acute HSV-1 infection in the mouse (Lopez, 1980).

The mechanism(s) responsible for the resistance of mice to either acute or latent infections with HSV remain unknown. Interferon may play a role in some types of resistance. Evidence supporting this view comes from studies in which administration of anti-interferon (α/β) antibodies converted resistant strains of mice to susceptibility to HSV-1 infection (Gresser *et al.*, 1976). The specific pathway and cellular targets through which endogenous interferon acts to induce HSV-1 resistance are not known. Macrophages would appear to be a likely candidate cell, as macrophages from resistant mouse strains do not appear to support HSV-1 replication to nearly the same degree as cells from susceptible strains. Anti-interferon (α/β) antibody treatment of the macrophages from resistant mice renders them susceptible to HSV-1 infection. Support for the idea that it is the macrophages that are the target cell for endogenous interferon action comes from studies with C3H/HeJ mice. These mice carry an autosomal gene defect (Lps^{d}) on

chromosome 4 that makes them resistant to the effects of endotoxin (lipopolysaccharide). Resistance to endotoxin appears to be due to abnormalities in macrophage function. It has been suggested that one abnormality in macrophage function in these mice is the deficient production of endogenous interferon, and that this accounts for the heightened susceptibility of these mice to infection by a wide variety of pathogens including viruses (Vogel and Fertsch, 1987). So in this case, susceptibility to viral infection appears to be linked to a gene that regulates specific aspects of macrophage function, a similar mechanism could theoretically occur to account for some cases of HSV-1 susceptibility as well. It is important to recognize, however that other arms of the immune response including T-lymphocytes, macrophages, and natural-killer cells have all been shown to play a role in resistance to HSV infections (*see* Rosenstreich, Weinblatt and O'Brien, 1982 for review).

The examples reviewed above illustrate the fact that genetically controlled resistance to viral infections may operate through a wide variety of mechanisms. Interestingly, in virtually every case identified to date the genes mediating resistance and susceptibility appear to be unique for different viruses, and seem to segregate independently. This suggests that the mechanisms by which host cell genes influence susceptibility to viral infections may very well be as diverse as viruses themselves.

VIRAL FACTORS

Site of entry and route of spread

It has been known for many years that neurotropic viruses depend primarily on spread through the bloodstream or via nerves to reach the CNS from their diverse sites of entry into the host (*see* Johnson, 1982, for review). Viruses which are capable of spreading via nerves include rabies, herpes simplex, herpes simiae, polioviruses and reovirus type 3. Since nerves provide specific pathways of connectivity between sites in the periphery and discrete, defined regions of the CNS, it is not surprising that for virtually all neurally spreading viruses that the ultimate distribution within the CNS can be profoundly influenced by their initial site of entry into the host (*see*, for example, Sabin and Olitsky, 1937, 1938; Howe and Bodian, 1942; and, more recently, Martin and Dolivo, 1983; Anderson and Field, 1983). The effect of site of entry on CNS tropism has been less clearly defined for blood-borne viruses. Since the pathway of spread of a virus may affect its CNS tropism, it is appropriate to review briefly the current state of our understanding of the role played by viral genes in determining specific patterns of viral spread within an infected host.

Recent studies with reovirus reassortants and intertypic recombinants of HSV have provided some insights into the molecular and genetic basis for viral spread. Although reoviruses are not important human pathogens, they have proved to be valuable experimental models for identifying the role of individual viral genes and the proteins they encode at specific stages in viral pathogenesis (*see* Tyler and Fields, 1985; Sharpe and Fields, 1983, 1985, for review). Reoviruses have a genome consisting of ten discrete segments of double-stranded RNA ('genes'), each segment encoding a unique mRNA, that is translated into at least one protein product. If susceptible cells are simultaneously coinfected with reoviruses of two different serotypes, intertypic reassortants (in other words, viruses containing gene segments derived from each of the two parental serotypes) can be generated. By

comparing the behaviour of these reassortant viruses to that of the two parental viruses, the role of specific viral genes and proteins in determining specific biological properties of the virus can often be determined. This approach has been used to identify the importance of the S1 viral gene in determining the capacity of reoviruses to utilize either neural or haematogenous routes to spread to the CNS in infected animals (Tyler, McPhee and Fields, 1986).

After inoculation into either the hindlimb or forelimb footpad of neonatal mice reovirus type 3 spreads to the CNS via nerves, whereas type 1 spreads via the bloodstream. Studies with intertypic (T1 × T3) reassortants, which contain various combinations of genes derived from reoviruses types 1 and 3, indicate that viruses containing an S1 gene segment derived from reovirus type 3 spread to the CNS via nerves, whereas viruses with an S1 gene segment derived from reovirus type 1 spread haematogenously (Tyler, McPhee and Fields, 1986). The S1 gene encodes a viral outer capsid protein, σ 1, that also serves as the viral cell attachment protein. Data derived from kinetic and pharmacological experiments strongly suggest that the neural spread of reovirus type 3 is via the microtubule-associated pathway of fast axonal transport, although the mechanism by which the σ 1 protein mediates this transport remains unknown.

Information is also rapidly accumulating about genes that appear to be important in determining both the capacity of HSV to spread to the CNS from peripheral sites, and its virulence after this type of inoculation. For example, by studying the spread of herpes virus from the cornea to the CNS in mice, Oakes, Gray and Lausch (1986) established that HSV-1 DNA sequences that lie between 0.31 and 0.44 map units (mu) are important in determining the capacity of the virus to spread to the CNS. When HSV-1 (strain 17) is inoculated onto the cornea of mice, it replicates in corneal epithelial cells and then spreads rapidly to the trigeminal ganglia and brain. By contrast, although HSV-2 (strain 186) replicates in the cornea, it reaches the trigeminal ganglia only in small quantities and is not detectable in the brain following corneal inoculation. Both strains of virus are equally lethal after direct intracerebral inoculation into mice. In order to determine which HSV-1 genes were responsible for these differences, an intertypic recombinant virus was generated which contained a complete HSV-2 genome except for the portion between 0.31 and 0.44 mu which was derived from HSV-1. This recombinant virus spread to the CNS in a fashion indistinguishable from HSV-1 indicating that the transferred HSV-1 DNA sequences are important in directing the capacity of herpes virus to spread to the CNS. The region in question contains genes coding for a large number of viral proteins including the nucleocapsid protein p40, glycoprotein gB, the DNA-binding protein ICP8, the viral DNA polymerase, and an origin of DNA replication. It is unclear which of these proteins play the critical role in viral spread.

A sequence in the herpesvirus genome between mu 0.762 and 0.787 appears to be important in determining the virulence of HSV-1 isolates for mice after intraperitoneal inoculation (Becker *et al.*, 1986). An overlapping portion of the genome (0.71–0.83 mu) has been shown to be a determinant of HSV-1 virulence after intracerebral inoculation into mice (Thompson *et al.*, 1985).

Cell receptors and viral cell attachment proteins

A series of classic studies performed by J. J. Holland and L. C. McLaren in the late 1950s and early 1960s using poliovirus helped to establish the principle that one of

the major determinants of the tropism of viruses was the availability of receptors on target cells. In the subsequent quarter century there has been tremendous growth in understanding of the nature of viral cell attachment proteins, and in the identification of putative host cell receptors for virus. In this section selected examples designed to highlight current knowledge in this area as it pertains to neurotropic viruses are reviewed.

All three serotypes of poliovirus compete for attachment to a common receptor which differs from the receptors utilized by other enteroviruses (such as coxsackie, echovirus) and picornaviruses (such as rhinovirus, Theiler's virus, mengovirus). The poliovirus receptor is an integral membrane protein, present in several thousand copies per cell (Krah and Crowell, 1985). The gene encoding the receptor is located on human chromosome 19 (Miller *et al.*, 1974). It has recently been demonstrated that previously poliovirus-resistant cells, such as mouse L-cell fibroblasts, can be made susceptible to poliovirus infection if they are transfected with specific DNA fragments derived from a poliovirus susceptible human (HeLa) cell line (Mendelsohn *et al.*, 1986). The transfected DNA presumably contains the gene coding for the poliovirus receptor, and further characterization of the transfected DNA may lead the way toward isolation and characterization of the mammalian poliovirus receptor.

The application of the techniques of X-ray crystallography, nucleotide sequence analysis of viral genomic RNA, and the use of monoclonal antibodies has resulted in an extraordinary synergistic growth of knowledge concerning structure–function relationships for poliovirus and other related picornaviruses. This is well illustrated in the case of polioviruses.

(1) The complete nucleotide sequence of the entire genome of all three serotypes of poliovirus has now been established (*see*, for example, Stanway *et al.*, 1984).
(2) The three-dimensional crystal structure of poliovirus has recently been solved at extremely high resolution (< 3 Å) (Hogle, Chow and Filman, 1985). This has allowed the accurate determination of the three-dimensional position of virtually all the amino acids composing the viral structural proteins.
(3) Neutralizing monoclonal antibodies (N-MAbs) have been generated against the major antigenic epitopes on the poliovirus surface, and variant viruses have been generated which resist neutralization by these N-MAbs (Diamond *et al.*, 1985).

These variants typically contain only single amino acid substitutions in individual viral proteins. Transposing information gained from studies with MAbs and MAb-resistant variant viruses to the three-dimensional crystal structure of the virus has allowed the identification of specific regions on the poliovirus surface that serve as the major antigenic domains for neutralizing antibody production in the host, as well as the identification of a candidate region for the viral cell attachment protein.

The poliovirus crystal structure shows that the viral outer surface is studded with 'peaks' composed of the outer capsid protein VP1. These peaks are surrounded by broad 'valleys', the floors of which are composed of portions of proteins VP1 and VP3 (Hogle, Chow and Filman, 1985). Structural features analogous to the poliovirus valley have been seen in the three-dimensional crystal structures of rhinovirus ('canyon') (Rossman *et al.*, 1985) and mengovirus ('pit') (Luo *et al.*, 1987). It has been suggested that it is these regions of these related viruses which serve as the viral attachment site to host cell receptors.

Studies with rabies virus have provided additional insights concerning the nature of viral cell attachment proteins and their host-cell receptors. Following intramuscular inoculation into experimental animals, rabies virus localizes at the neuromuscular spindles and in proximity to the neuromuscular junction (NMJ), and is then subsequently transported via nerves to reach the CNS (Murphy, 1977). It has been suggested that the acetylcholine receptor (AChR) serves as a rabies virus receptor at the NMJ. Pretreatment of cultured chick myotubes with α-bungarotoxin, which binds to the AChR, or with antibodies to the α-subunit of the AChR, blocks binding of rabies virus to these cells and inhibits infection (Lentz *et al.*, 1982; Burrage, Tignor and Smith, 1985).

The suggestion that the AChR serves as a rabies virus receptor has generated considerable controversy. Cells lacking AChR can still be infected with rabies virus *in vitro* clearly indicating that the AChR is not the exclusive receptor for rabies virus. A considerable body of experimental evidence has been accumulated that indicates that gangliosides, including phosphatidyl serine, may be important components of a rabies virus receptor distinct from the AChR (Wunner, Reagan and Koprowski, 1984).

The rabies virus genomic RNA is enclosed in a protein shell (nucleocapsid) that is surrounded by a lipoprotein envelope that is derived as the virus buds from the plasma membrane of infected cells. A virally encoded glycoprotein ('G') is inserted into this envelope. The G glycoprotein appears to function as the viral cell attachment protein and specific regions of this protein have been identified that appear to be major determinants of viral virulence *in vivo*. N-MAbs directed against the G glycoprotein have been used to generate rabies virus variants with alterations in the amino acid sequence of the G glycoprotein. Comparison of the amino acid sequences of the glycoprotein of avirulent or attenuated variants with that of fully pathogenic variants and the wild-type virus has indicated that an arginine residue at amino acid position 333 is an important marker of viral virulence (Dietzschold *et al.*, 1983; Seif *et al.*, 1985). Variants with any one of a number of amino acid substitutions at this position (for example, glutamine, glycine, isoleucine) are avirulent or have attenuated virulence, whereas variants which preserve ARG 333 (but have substitutions at nearby positions such as 330, 336, 338) remain virulent. The mechanism(s) by which changes in the rabies glycoprotein at position 333 result in altered neurovirulence are being actively investigated. Studies of several of the avirulent variants indicate that they compete for the same cellular receptor as the wild-type virus on cultured cells, and that they do not have altered CNS tropism *in vivo*. The variants appear to spread less quickly within the CNS than the wild-type virus and appear to be restricted in their capacity to spread via certain specific neural pathways, when compared to the wild-type virus (Dietzschold *et al.*, 1985; Kucera *et al.*, 1985). Thus, alterations in the rabies glycoprotein may result in changes in the capacity of certain variant viruses to spread both to and within the CNS.

Reoviruses have also provided an important experimental system for studying the nature of viral and host factors that determine neurotropism. Reoviruses are non-enveloped viruses whose genetic information is contained in the form of ten discrete segments of double-stranded (ds) RNA. As discussed earlier, it is possible to generate reassortment viruses containing combinations of genes derived from different serotypes of virus, or even from different strains of the same serotype. After intracerebral inoculation into neonatal mice reovirus serotype 1 infects the ependymal cells lining the ventricles, but does not infect neurons. Conversely,

reovirus serotype 3 infects neurons and produces acute lethal encephalitis, but does not infect ependymal cells. Using reassortants derived from types 1 and 3 it was shown that the viral S1 dsRNA segment, which encodes the outer capsid protein σ1, was the major determinant of neuronal or ependymal tropism of reoviruses (Weiner, Powers and Fields, 1980). Subsequent studies have extended this observation by documenting the importance of the same viral gene for reovirus tropism within the pituitary (Onodera *et al.*, 1981), and the retina (Tyler *et al.*, 1985).

In order to define the portions of the σ 1 protein involved in biological functions mapped to the S1 gene, variants of reovirus T3 Dearing with altered σ 1 proteins have been generated using anti-σ 1 N-MAbs (Spriggs and Fields, 1982). Comparison of the predicted amino acid sequence of the σ 1 protein of five of the variant viruses with that of their wild-type parent revealed that in each case a single amino acid substitution was present at one of two locations (amino acid 340 or 419) (Bassel-Duby *et al.*, 1986). The T3 σ 1 variants have attenuated neurovirulence and altered CNS tropism compared to the wild-type virus. For example, although the variants still infect and injure the hippocampus, septal nuclei, and mamillary bodies, they no longer injure the cortex (Spriggs, Bronson and Fields, 1983; Bassel-Duby *et al.*, 1986). A reassortant virus containing only the S1 dsRNA segment derived from a variant virus shows the same pattern of restricted neurotropism and attenuated neurovirulence as the original variants, indicating that the single amino acid substitution found in the σ 1 protein is indeed responsible for the altered phenotype of these viruses (Kaye *et al.*, 1986).

A receptor for reovirus type 3 has been identified on cultured neuronal and lymphoid cells (Co *et al.*, 1985a, 1985b). The receptor is a glycoprotein of about 67 kD. A variety of structural and pharmacological criteria have been used to demonstrate that the reovirus receptor is structurally similar to the mammalian β-adrenergic receptor. It remains to be seen whether other cellular structures can also be utilized by reovirus type 3 as cellular receptors.

An increasing body of evidence has also accumulated concerning the cell attachment proteins of both mouse hepatitis virus (MHV), a coronavirus, and lymphocytic choriomeningitis (LCM) virus. The coronaviruses are enveloped RNA viruses which have two proteins (E1, E2) inserted into their envelope. Variants of MHV have been selected with N-MAbs directed against the E2 glycoprotein (Fleming *et al.*, 1986; Dalziel *et al.*, 1986). Many of the E2 variants have attenuated neurovirulence and an altered pattern of neuropathogenicity compared to their wild-type parents. The wild-type viruses typically produce a severe acute encephalitis, although some of the survivors go on to develop a chronic demyelinating disease. Many of the E2 variant viruses produce a high incidence of demyelinating disease with little or no evidence of preceding encephalitis. Passive immunization of mice with certain anti-E2 MAbs can also convert the pattern of illness from encephalitis to demyelinating disease (Buchmeier *et al.*, 1984). These findings strongly suggest that the E2 glycoprotein of coronaviruses is a major determinant of viral pathogenicity, and that it may be involved in the tropism of these virus for neurons as variation in this protein can result in diminished neuronotropism and enhanced tropism for oligodendroglia.

The receptor for neurotropic coronaviruses such as MHV on neuronal cells has not yet been identified. A 100–110 kD protein has been found on hepatocytes and intestinal epithelial cells that appears to serve as the viral receptor in these tissues (Boyle, Weismiller and Holmes, 1987). This receptor appears to be encoded on

mouse chromosome 7 (J. Boyle, personal communication), at the same locus that has previously been shown to encode a gene that mediates susceptibility to MHV infection within certain strains of inbred mice (Knobler *et al.*, 1984).

Lymphocytic choriomeningitis virus (LCMV) is an enveloped virus whose genetic information is contained in two segments of RNA. Some strains of mice infected with specific strains of LCMV develop a runting syndrome. Mice with this syndrome have high titres of LCMV in the growth hormone producing cells of the anterior pituitary. Infection of these cells results in markedly reduced levels of circulating growth hormone and this in turn accounts for the runting syndrome (Oldstone *et al.*, 1985). Reassortant viruses have been generated between strains of LCMV that produce runting syndrome in susceptible mice (such as Armstrong) and strains of LCMV that do not (such as WE). It appears that the LCMV small (S) RNA segment, which encodes the two viral envelope glycoproteins, determines the capacity of these viruses to infect growth-hormone producing pituitary cells and produce runting syndrome (Riviere *et al.*, 1985). LCMV thus provides another example of the importance of viral surface proteins in determining specific patterns of neurotropism. Interestingly, the infected growth-hormone producing cells typically do not show obvious evidence of injury at either the light or electron-microscopic level. This exemplifies a point frequently stressed by Dr Oldstone and his colleagues that the so-called 'luxury functions' of cells such as neurons can be dramatically affected by viral infection, in the absence of either alterations in more basic 'housekeeping' functions or detectable morphological changes.

Mumps virus provides another illustration of the importance of envelope glycoproteins in determining viral neurotropism. Variants of a neuroadapted strain of mumps virus (Kilham) have been selected by their resistance to N-MAbs directed against the haemagglutinin–neuraminidase (HN) glycoprotein of the viral envelope. Suckling hamsters inoculated intracerebrally with mumps virus develop lethargy, muscular wasting and incoordination followed later by hydrocephalus. The mumps virus infects the ependymal cells lining the ventricles and the aqueduct of Sylvius. Hydrocephalus develops as a result of the obstruction to CSF flow that follows aqueduct stenosis. The virus also infects neurons and glial cells throughout the cerebrum and brainstem producing a severe meningoencephalitis. By contrast, one of the mumps HN variants (M13) does not produce the early illness described above, but still causes the later developing hydrocephalus. Examination of the brains of M13 infected hamsters shows evidence of ependymal cell infection identical to that seen with wild-type virus, but only minimal signs of neuronal injury (Love *et al.*, 1985). When the structure of individual polypeptides from strains of mumps virus that differ in their neuropathogenicity are compared, the HN protein appears to show the greatest degree of variation, although no one particular HN glycoprotein peptide map correlates exactly with viral virulence (Wolinsky and Server, 1985). These results support the hypothesis that the mumps virus HN protein plays an important role in determining viral neurotropism and virally induced neuronal injury.

Some of the most dramatic advances in understanding the nature of viral–host interactions have come from investigations of the human immunodeficiency virus (HIV), the agent responsible for the acquired immunodeficiency syndrome (AIDS). Studies using a variety of techniques including culture, electron microscopy, immunocytochemistry, and *in situ* hybridization, have provided clear evidence that HIV can be found in the CNS, CSF, peripheral nervous system and

muscle. The evidence accumulated to date indicates that the brunt of detectable virus, viral antigen, or viral nucleic acid is found is macrophages, endothelial cells, and to a much lesser extent, glial cells. There is, as yet, no unequivocal evidence for neuronal infection (Ho *et al.*, 1985; Shaw *et al.*, 1985; Gabuzda *et al.*, 1986; Koenig *et al.*, 1986; Wiley *et al.*, 1986).

The nature of the cell attachment protein and the cellular receptor for HIV have been extensively investigated. The HIV envelope glycoprotein (gp120) serves as the viral cell attachment protein. Comparison of the amino acid sequence of this protein from a variety of HIV isolates indicates that there are both 'constant' and 'variable' regions within the protein (Hahn *et al.*, 1986; Starcich *et al.*, 1986). To date there has been no convincing correlation between variations in the amino acid sequence of the HIV glycoprotein and specific biological properties of HIV such as virulence, neurotropism, etc. Another intriguing area of investigation involves the search for amino acid sequence homologies between the HIV glycoprotein and other biologically important molecules. A number of these have been reported, but their significance remains speculative. Some of the tantalizing homologies involve regions of immunoglobulin heavy chains (Maddon *et al.*, 1986), neuroleukin (Gurney *et al.*, 1986), and vasoactive intestinal peptide (Pert *et al.*, 1986).

The CD4 antigen has been identified as the cellular receptor for HIV on human lymphoid cells (Dalgleish *et al.*, 1984; Klatzman *et al.*, 1984). The introduction of cDNA encoding this antigen into a variety of previously HIV-resistant ($CD4^-$) human lymphoid and epithelial cell lines renders them both $CD4^+$ and susceptible to HIV infection, although the same does not appear to be true for non-human (for example, mouse) cells (Maddon *et al.*, 1986). Competition binding experiments using a large number of anti-CD4 MAbs suggest that the viral binding site is relatively large as MAbs binding to at least four distinct (non-competing) epitopes can interfere with viral infection (Sattentau *et al.*, 1986).

The observations that the HIV is neurotropic and that the CD4 antigen serves as a viral receptor have prompted an extensive search for the presence of this antigen on cells within the human CNS. Homogenates of brain tissue contain detectable CD4 mRNA sequences, indicating that this antigen is present in the CNS, assuming the mRNA is translated (Maddon *et al.*, 1986). However, knowledge concerning the precise anatomical regions within which this antigen is located and the cell types on which it is expressed remains rudimentary.

Viral enhancers, promoters, and transcriptional activators

The studies described in the preceding section provide ample evidence for the importance of viral cell attachment proteins and host cell receptors in determining the tissue tropism of a number of viruses. However, recent investigations with papovaviruses and retroviruses have provided new insights into the importance of specific elements within viral genomes, which serve to enhance transcription of viral genes or translation of viral proteins in determining both the tissue and species specificity of viruses. Binding of a virus via a specific cell attachment protein to a specific host–cell receptor may be a necessary step in the infectious cycle but should not be seen, *de facto*, as either the exclusive or even principal determinant of tropism for all viruses.

The human papovavirus, JCV, is one of the agents responsible for the disease progressive multifocal leukoencephalopathy (PML). In this disease virus infects and kills oligodendroglia, resulting in extensive demyelination throughout the CNS. JCV infection also results in dramatic and often bizarre alterations in astrocyte morphology, although infection of these cells appears to be non-permissive (that is, does not result in the production of infectious virus). Neurons are not infected by JCV. Recent studies have suggested that an 'enhancer' region present in the JCV genome may be the major determinant of this pattern of viral tropism within the CNS. Enhancers are short nucleotide sequences, often multiply repeated in tandem, that act to stimulate the transcription of associated genes (*see* Brady, Feigenbaum and Khoury, 1986, for review). In order to study the tissue specificity of the JCV enhancer region, this portion of the JCV genome was attached to an indicator gene, chloramphenicol acetyltransferase (CAT), and this complex was transfected into a variety of cell lines (Kenney *et al.*, 1984). The experimental design was based on the assumption that if the JCV enhancer was active in a particular cell line then transcription of the CAT gene would occur, and following translation of the resulting mRNA into protein, an increase in CAT enzymatic activity would be detectable. Using this assay system it was shown that the JCV enhancer is specifically active in human fetal glial cells and relatively inactive in CV-1 and HeLa cells, an *in vitro* analogue to the previously discussed *in vivo* tropism of this virus. By contrast, the enhancer region of another papovavirus, SV40, is 'promiscuous', that is, active in a wide variety of cell lines. These experiments indicate that enhancers can potentially, but do not invariably, serve as determinants of cell tropism.

Fertilized mouse eggs can be microinjected with foreign DNA. Mice which develop from these eggs contain both native and foreign DNA and have been referred to as 'transgenic' mice (*see* Messing, Chapter 9 of this volume). Transgenic mice containing the 'early region' of the SV40 genome frequently develop choroid plexus epithelial cell tumours (Brinster *et al.*, 1984). The early region of the SV40 genome contains an enhancer region as well as regions encoding a protein implicated in transformation and oncogenesis (T-antigen). If the enhancer region is omitted from the microinjected foreign DNA constructs, the frequency of choroid plexus tumours drops significantly. These results have led to the suggestion that the SV40 enhancer may determine the targeting of the T-antigen to choroid plexus epithelial cells, and that the expression of T-antigen in these cells leads to tumour formation (Palmiter *et al.*, 1985).

Transgenic mice have also been produced following microinjection of the early region of JCV (Small *et al.*, 1986). Offspring of these mice inherit JCV genomic sequences, and develop a syndrome characterized pathologically by dysmyelination within the CNS, and clinically by tremor, incoordination and seizures. The development of CNS dysmyelination correlates with the inheritance of JCV DNA sequences and the expression of high levels of JCV mRNA within the brain. It has been postulated that the JCV enhancer sequences are responsible for targeting the associated T-antigen sequences to oligodendroglia, where their expression results in cellular dysfunction leading to disordered myelination.

At least four genes that are involved in the regulation of gene transcription and in some cases mRNA translation have been identified within the HIV genome. Two of these genes (*sor* and 3′ *orf*) do not appear to be essential for HIV replication or cytopathogenicity (CPE). The remaining two genes (*tat* and *art/trs*) are required for

both viral replication and CPE. Despite accumulating evidence of the mechanism(s) by which these genes and their products act (*see* Chen, 1986, for review), there is no evidence to date that they function in either a cell, tissue, or species-specific fashion. It would thus appear that the interaction of the HIV cell glycoprotein and host–cell receptors (for example, CD4 antigen) serves as the major determinant of HIV tropism, but the issue must be considered a potentially open one.

CONCLUSIONS

In this brief review the author has attempted to highlight some of the recent insights that have been gained into the molecular and genetic basis for viral tropism, with specific emphasis on those factors which appear particularly relevant to understanding the basis for the tropism of viruses for the nervous system. We have seen how host genes, acting through a variety of mechanisms, can influence the susceptibility or resistance of animals to neurotropic viruses. We have also reviewed investigations concerning the role played by individual viral genes and the proteins they encode in determining specific pathways of viral spread to the CNS in the infected host. Several examples illustrating the current state of knowledge concerning the nature of viral cell attachment proteins and host cell receptors for neurotropic viruses have been reviewed. Finally, we have seen examples of how specific viral genetic elements, such as enhancers, can act to determine the cell-specificity of certain neurotropic viruses.

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