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perspectives on disease

Host and viral factors that influence viral neurotropism

II. Viral genes, host genes, site of entry and route of spread of virus

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In the previous article in this series, we focused on how the interaction between viral cell attachment proteins and receptor molecules on the surface of host target cells plays a major role in determining the cell and tissue tropism of many neurotropic viruses. In order to complete our review of viral factors that influence the tropism of viruses for the CNS, we will discuss the role of viral genes that function to specifically enhance the replication of viral proteins in certain cells or tissues (‘tissue-specific enhancers and promoters’). We will then examine the ways in which host factors, including specific host genes, can influence resistance or susceptibility to certain types of neurotropic viral infections. Finally, we will conclude by reviewing how factors that influence the interaction of the host and the virus, such as the site of viral entry and its route and method of spread, can influence the distribution of viral infection within the CNS.

Viral enhancers, promoters and transcriptional activators

Work with papovaviruses and murine retroviruses, as well as experiments with transgenic mice, has established the importance of elements in viral genomes that serve to enhance transcription of viral genes (promoters, enhancers or transcriptional activators) in the species-, tissue- and disease-specificity of certain viruses. The human papovavirus, JC, which is the agent responsible for progressive multifocal leucoencephalopathy (PML), has provided an important example of the significance of viral transcriptional regulatory elements as a determining factor in neurotropism. JCV contains an ‘enhancer’ region. Enhancers are short sets of nucleotides, often repeated in tandem, that operate in a position- and orientation-independent fashion to stimulate the transcription of associated genes. The JCV enhancer shows a strong tissue-type specificity for human fetal glial cells as compared to HeLa cells. This correlates well with the capacity of JCV to grow in the former but not in the latter cell type.

Information concerning the tissue specificity of viral enhancers has also come from studies of transgenic mice. Transgenic mice develop from fertilized eggs that have been microinjected with a foreign DNA molecule. Transgenic mice produced from microinjection with specific identified portions of SV40 viral DNA frequently develop choroid plexus epithelial cell tumours (Fig. 1). The SV40 genome contains an enhancer region, and if this region is deleted from the transfected DNA there is a striking...
The region of the SV40 genome that encodes the large T-antigen is also important in tumorigenesis\(^6\). Large T-antigen has been implicated in the transforming (oncogenic) potential of SV40, and may be responsible for the initial induction of tumours, with the enhancer region accounting for the choroid plexus specificity.

Transgenic mice with JCV early region genes (promoter/enhancer elements and T-antigens) have also been produced\(^6\). The offspring of these mice develop a severe and progressive action tremor and tonic seizures – a phenotype with certain similarities to the ‘quaking’ and ‘jumpy’ strains of myelin-deficient mice. The affected offspring of the original transgenic mice have hypomyelination and abnormal myelin in the CNS, due apparently to oligodendrocyte dysfunction. This dysfunction in turn correlates with the inheritance of JCV DNA sequences, and high levels of JCV DNA expression in the brain. It has been proposed that the cell-specificity of JCV DNA expression (i.e. in oligodendrocytes) might be due to the JCV enhancer elements, and that the subsequent expression of T-antigen in these cells could result in impairment of oligodendrocyte-induced myelination\(^8\). Thus, the results obtained from experiments involving transgenic mice have clearly indicated that enhancer sequences within the genomes of certain viruses may influence neurotropism.

Work with murine retroviruses has clearly documented the importance of enhancer sequences in or near the viral long terminal repeat (LTR) in determining the leukemogenic potential and disease specificity of Friend and Maloney murine leukemia viruses (see, for example, Ref. 7). The role of the LTR region in the induction of specific patterns of CNS disease has not been clearly established. Genetic studies with a paralytogenic strain of Maloney murine leukemia virus (ts1) indicate that gene sequences between nucleotides 1–567 and 6538–8264 are important in the neurotropism of this virus. This region encompasses the 3’ end of the viral envelope gene, the LTR, and the 5’ non-coding region. The relative contribution of each of these regions to neurotropism, and their mechanism of action, remain to be elucidated\(^8\).

HIV, and other lentiviruses, also contain gene sequences that serve as transcriptional activators (reviewed in Ref. 9) (Fig. 2). The products of both the tat and art/trs genes appear to be extremely important for high level expression of HIV genes in infected cells, and the production of cytopathic effect (CPE)\(^10\). It has been suggested that the protein product of the tat gene could act to increase transcription of specific HIV mRNAs by binding to target sequences within the HIV LTR. The LTR contains promoter and enhancer elements. The tat protein may also act at a post-transcriptional level; perhaps by binding to target sequences in the leader of HIV mRNAs and thereby either stabilizing them or enhancing their translation. Naturally, these two mechanisms are not mutually exclusive\(^9,13\).

The mechanism of action of art/trs is less clear than that of tat. The art/trs product appears to be necessary for expression of the HIV gag and env genes (which encode the capsid and envelope proteins)\(^12\). Two other novel HIV genes (sor and 3’ orf) do not appear necessary for either HIV replication or CPE\(^16\). There is no current evidence that either tat or art/trs has tissue- or cell-specific activity\(^18\). Current evidence from transfection experiments would seem to indicate, as reviewed earlier, that it is the interaction between the HIV cell attachment protein (gp120) and the cellular receptor (T4 antigen) that serves as the primary determinant of HIV tropism\(^19\).

The experiments reviewed above concerning the role of tissue-specific enhancers and other viral genetic elements lead to another principle of neurotropism: tissue-specific and cell-type-specific viral genetic elements are important determinants of neurotropism for certain groups of viruses.

In several cases, viral genes that do not encode either cell-attachment proteins or tissue-specific enhancers have been shown to be important determinants of neurovivulence or neurotropism, although in most cases their mechanism of action remains speculative. In the case of herpes simplex virus (HSV), the generation of intertype recombinant viruses containing parts of the genomes from an HSV type 1 and an HSV type 2 parent have produced interesting insights into regions of the HSV genome important in neurotropism. In a number of these studies, the region of the HSV genome between 0.71–0.83 map units (m.u.) has repeatedly been shown to be important in neurovivulence and neurotropism\(^21\). This region encodes a number of HSV proteins including the immediate early proteins (ICP0, ICP4, ICP27) as well as ICP34.5. In analogous studies, the region of the genome between 0.31–0.44 m.u. has been shown to contain determinants that are important in allowing HSV to spread to the CNS after peripheral inoculation\(^24\). This region also encodes a number of viral proteins including an envelope glycoprotein (gB), p40, ICP8, and the viral DNA polymerase. A great deal more work will be required before the

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**Fig. 1.** A choroid plexus papilloma in the brain of a transgenic mouse injected shortly after fertilization with SV40 early region DNA. The SV40 enhancer may be responsible for the tropism of the virus for choroid plexus epithelial cells. (Taken, with permission, from Ref. 3.)
regions of the HSV genome involved in neural spread, neurotropism, and neurovirulence can be defined at a more precise level. Nonetheless, these studies serve to emphasize the point that although a great deal of attention has focused on viral genes that encode cell attachment proteins or that act as tissue-specific enhancers, undoubtedly a large number of viral genes play critical roles at specific steps in viral pathogenesis and replication that are essential for infection of the CNS; alterations in many viral genes, by affecting a variety of steps in pathogenesis or replication, may consequently influence neurotropism. The principle that emerges from these studies is that neurotropism is influenced by viral genes acting at many steps in the viral replicative cycle and at different stages of pathogenesis.

**Host factors**

In a series of classic studies in the 1930s, Albert Sabin and Peter Olitsky established the importance of a variety of host constitutional factors including age, nutritional status, and genetic background in influencing the neurovirulence and neuroinvasiveness of neurotropic viruses. Sabin and Olitsky provided examples in which resistance to viral infection resulted from: (1) decreased replication of a virus at its site of entry; (2) the failure of a virus to spread from its site of entry to the CNS; and (3) inhibition of viral spread within the CNS itself.25,26

For many neurotropic viruses, young mice are more susceptible to infection than older mice, although poliovirus and viruses such as LCM, which produce immune-mediated pathology, represent exceptions to this rule. A variety of mechanisms may account for the development of age-related resistance to viral infection, including maturation of the immune response, changes in the susceptibility of macrophages to viral infection, increased interferon production, and changes in the susceptibility of critical target cell populations.27 Malnutrition and altered dietary states have been associated with increased susceptibility to infection with neurotropic viruses.27. A consistent sex-related difference (female resistance) in susceptibility to infection with toga viruses, picornaviruses, and rabies virus has also been seen.28

The importance of differences in the genetic background of the host in the resistance to neurotropic viral infections has been extensively investigated using inbred strains of mice.29 Examples of genetically related susceptibility have been described with rabies virus,29 neurotropic coronaviruses,31 certain neurotropic retroviruses,32 flaviruses, herpes simplex, and measles,33 and in diseases such as scrapie.34 In some cases, differences in the humoral and cell-mediated immune responses appear to be involved. For example, SJL and CBA mice are more resistant to intraperitoneal inoculation of street rabies virus than BALB/c or DBA mice. These differences correlate with the magnitude of the serum anti-rabies antibody response, which in turn seems to affect the degree of virus replication in the CNS.29-30 More commonly, genes determining susceptibility do not appear to be linked to immune responsiveness.33

One of the genes (Prn-1) that influences susceptibility to scrapie is closely linked to the gene encoding the major prion protein (PrP) and is located on mouse chromosome 2 (Ref. 35). The Prn-1 gene appears to be identical to the previously described scrapie incubation (Sin) gene. Host genes may also affect the sensitivity of specific viruses to the action of interferon, as illustrated by the capacity of the Mx gene in mice to protect carriers against lethal infection with influenza virus.36 Differences in susceptibility of inbred mouse strains may also reflect variation in the presence, nature, or distribution of viral receptors, as has recently been shown for mouse hepatitis virus (MHV), a coronavirus.37 Susceptibility to coronavirus-induced CNS disease has been mapped to mouse chromosome 7 (Ref. 38), and appears to be related to the presence or absence of receptors on susceptible cells.37

Another mechanism by which genes can control resistance to viral infection is illustrated by the ecotropic murine retroviruses. These viruses can produce paralytic disease in mice, associated with spongiform changes in the CNS. Mice carrying the Fv-4 resistance allele inherit a defective endogenous ecotropic provirus inserted in their genome. Expression of the envelope (env) gene of this defective provirus results in the production of the murine retrovirus cell attachment protein (gp70), which in turn binds to retrovirus receptors on the cell surface and thereby blocks the entry of exogenous ecotropic retroviruses into target cells, conferring resistance to the Fv-4+ inbred strains of mice.39

Host cells may also provide enzyme functions that are necessary for activation of viral infectivity. This phenomenon is well illustrated by the role of host cell
proteases in cleaving the fusion (F) protein of paramyxoviruses or the hemagglutinin (HA) protein of orthomyxoviruses.\textsuperscript{40} Paramyxoviruses, including neurotropic viruses such as measles and mumps, have a fusion (F) protein in the viral envelope that mediates fusion of the viral envelope with the membrane of target cells, and is necessary for the subsequent penetration of the viral genome into these cells. A host cell protease is required for the cleavage of F protein precursor into two disulfide-bonded subunits (F1, F2). The cleavage exposes a new N-terminus on F1. This part of F1 is highly conserved among the paramyxoviruses, and appears to be the critical component mediating their fusion function. In tissues or cells lacking the requisite host cell protease, the fusion precursor protein is not cleaved to its active form, and virus cannot replicate, spread or injure target cells.\textsuperscript{40,41}

In the case of myxoviruses, including influenza, the fusion function is mediated by the HA envelope protein. Activation of the HA requires both its cleavage by host-cell proteases and a pH-induced conformational change.\textsuperscript{42} The cleavage and pH-induced changes in the HA are necessary for the expression of viral virulence.\textsuperscript{40,43}

The results reviewed above can be summarized in the form of another basic principle of neurotropism: constitutional factors such as the age, sex, nutritional state, immune status, presence or absence of specific enzymes, and genetic background of the host can all profoundly influence the ultimate capacity of a virus to infect the CNS.

Site of entry and pathway of spread
In the 1920s and 1930s, a variety of investigators including E. W. Goodpasture, O. Teague, E. W. Hurst, A. B. Sabin and P. K. Olitsky, H. Howe and D. Bodian made histopathologic studies of CNS lesions produced by neurotropic viruses following different routes of inoculation into the host. These studies clearly demonstrated that the distribution of viral lesions in the CNS could be dramatically influenced by the site of entry of a virus. Since neurotropic viruses spread to the CNS primarily through the bloodstream (hematogenous spread), via nerves (neural spread), or by combination of these two routes, the effect of site of entry on the CNS tropism of viruses can conveniently be considered separately for viruses in each of these groups.

The neuroadapted Rockefeller MV strain of poliomyelitis virus serves as an example of a virus that spreads almost exclusively by neural routes. Howe and Bodian\textsuperscript{44} injected rhesus monkeys by various routes with this virus and found that the distribution of the subsequent lesions in the brain could be grouped into three major categories: (1) areas that were involved regardless of the route of entry of the virus (e.g. sensorimotor cortex, certain thalamic nuclei); (2) areas that were virtually never affected even after direct inoculation of virus (e.g. hippocampus, striate cortex); and (3) areas that were selectively involved only after inoculation of virus by specific routes (e.g. the olfactory bulbs and septal nuclei after intranasal inoculation). The dependence of the ultimate CNS tropism on site of entry has found ample confirmation in elegant neuroanatomical studies using herpesvirus suis\textsuperscript{45} and herpes simplex\textsuperscript{46} (Fig. 3).

The dependence of neurotropism on the site of inoculation is also illustrated by the distribution of virus in the spinal cord after a neurally spreading virus such as polio, herpes, rabies, or reovirus type 3 Dearing (T3) is inoculated into different peripheral sites (e.g. hindlimb versus forelimb footpad of an animal). For example, following inoculation of T3 into the forelimb footpad, virus appears first and in highest titre in the cervical segments of the spinal cord, and, conversely, following hindlimb footpad inoculation virus appears first and in highest titre in the thoracolumbar segments of the spinal cord.\textsuperscript{47} (Fig. 4).

The effect of route of entry on CNS tropism has not been as extensively investigated for hematogenously spreading viruses, as for neurally spreading ones. The recognition that there is an anatomical and physiological barrier to the free entry of macromolecules from the cerebral circulation into the brain parenchyma (the blood–brain barrier) led David Bodian and others to postulate that areas where the blood–brain barrier was permeable (e.g. due to the presence of fenestrated capillary endothelial cells, the lack of a dense basement membrane, and less densely applied astrocyte processes) could serve as loci minoris resistante for the establishment of infection by blood-borne viruses.
The choroid plexus does indeed seem to play this role for certain neurotropic viruses (e.g. mumps), although a similar role for other sites (e.g. area postrema) has never been consistently demonstrated.

In addition to the choroid plexus, routes of invasion into the CNS for hematogenously spreading viruses can include direct infection or passive transport across endothelial cells, and transport of virus across small vessels by transmigrating leukocytes (see Ref. 47 for review). Current understanding of the methods by which hematogenously spreading viruses invade the CNS makes it difficult to understand why the tropism of viruses spreading exclusively by this route should be affected by the site of entry into the host, providing that the generation and maintenance of an equivalent magnitude viremia occurs. Nonetheless, old studies on the mechanism of the 'provoking effect' in experimental poliomyelitis raise this possibility. Bodian observed that cynomolgus monkeys given intramuscular injections of a variety of different substances followed within a specified interval by intracardiac inoculation of poliomyelitis virus (Type 1, Mahoney), developed a disproportionate incidence of initial paralysis affecting the traumatized limb (the 'provoking effect'). He suggested that local trauma could increase the permeability of blood vessels in the region of the spinal cord innervating the injured limb and thereby result in preferential localization of blood-borne poliomyelitis virus in that region of the spinal cord. Persuasive evidence for this sequence of events has never been obtained, and the issue of the effect of route of entry on CNS tropism for hematogenously spreading viruses must be considered unresolved.

For some neurotropic viruses, the primary pathways of spread to the CNS, and the resulting CNS entry and route of spread of a virus can influence the distribution of viral infection within the CNS.

In this two-part series we have seen that the ability of a virus to infect specific cells and tissues within the CNS is influenced by a wide variety of host and viral factors. Viral genes may encode proteins that are capable of interacting with specific receptors on the surface of host cells. Viral genes can also determine whether transcription of viral genes and translation of viral mRNA occurs in specific types of cells. The susceptibility of the host to viral infections can be influenced by specific host genes that can determine the nature of the host response to viral infection (e.g. interferon production and antibody response) or the availability of receptors required for viral infectivity. Non-genetic factors such as the age and nutritional status of the host may also profoundly alter susceptibility to neurotropic viral infections. Finally, a number of interactions between the host and the virus such as the site of viral entry and the route of spread of virus can also influence the ultimate distribution of viral infection within the CNS.

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Quantifying receptor autoradiography

SIR: It is clear that a number of problems are associated with quantitative receptor autoradiography, many of which have been discussed recently. The main problem arises from the expression of receptor density in values traditionally used for homogenate receptor binding (i.e. fmol per mg protein). Since most of the popular radiolabelled ligands are still non-specific and many of which have been discussed recently, it is simple to carry out protein estimation in tissue homogenates in order to express receptor binding as fmol per mg protein, there are few cases in which protein estimation from discrete brain areas have been performed in order to be able to express receptor binding in tissue sections in an equivalent manner. Hence disparities will occur in the estimation of receptor densities between laboratories calculating receptor values from optical density. It is important that data are collected in order to establish 'normal' receptor values in both human and animal tissue before we can correlate receptor changes that occur in pathological and experimental tissue. Since the data collected image analysis systems allow accurate measurement of optical density, there is a real need to arrive at some acceptable means of quantifying these measurements of autoradiographic data and some agreement on the values to be used for the expression of receptor density. This should be a matter of discussion for an internationally agreed standard, perhaps at some forthcoming neuroscience meeting.

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