Molecular Pathogenesis of Neurotropic Viral Infections

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Classical virologists defined a number of viruses that affect the nervous system and identified tissue tropism, extraneural replication, and viremia as important parameters that determine whether viral infections will affect the central nervous system. Molecular techniques are expanding this knowledge by permitting us to relate specific genes and gene products to two defined phenotypes: neuroinvasion and neurovirulence. Two converging situations make this knowledge particularly useful: (1) the development of antiviral drugs and subunit vaccines, which mandate that pathogenesis be related to specific regions of the viral genome; and (2) the expanding problem of central nervous system infections in immunodeficient states.

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The recent revolution in the biological sciences has had a tremendous impact on virology, which benefited early from the technological advances in molecular biology. Viruses are now used to study gene expression and receptor-ligand interactions and to express cloned genes in eukaryotic systems. It has nevertheless remained easier to study viral infections in vitro rather than viral-host interactions in whole organisms. Recently a number of significant insights into the molecular and genetic basis for the pathogenesis of viral infections of the nervous system have been reported by different groups. In this review we will highlight some of the major findings in the field.

Viral pathogenesis is the process by which a virus causes disease in a susceptible host. Pathogenesis can be analyzed in terms of a series of stages (see [32] and [54] for reviews). To cause systemic illness, a virus must first enter the host animal, undergo primary replication at a site near its portal of entry, and then ultimately spread to distant target tissues, such as the central nervous system (CNS).

By definition, all animal viruses are intracellular pathogens, and the process of replication must commence with entry into a susceptible cell. An infecting animal virus faces two main blocks to penetration of the CNS or any other specific target organ: (1) a variety of barriers prevent the free access of viruses to target cells, and (2) even when these barriers are ineffective, only certain cell types will support the internalization and replication of a particular virus. It is useful to think of the capacity of a virus to establish a lethal infection within the CNS as the property of *neurovirulence* and the ability to penetrate the CNS after inoculation and growth at a peripheral site as the property of *neuroinvasiveness*. Experimentally, it is easy to bypass the barriers to CNS infection (by intracerebral inoculation, for example), and therefore each of these properties can be tested for independently. A major goal of much of the current work in virology has been the correlation of viral properties such as neurovirulence and neuroinvasiveness with specific viral genes or proteins or, when the systems have been more advanced, with specific regions of these genes and/or proteins.

Entry into the Host

Each of the potential entry routes into the organism—skin, mucosal membranes, gastrointestinal (GI) tract—is utilized by viruses capable of eventually infecting the CNS. The specific entry point will be determined by the biology and physicochemistry of the virus as well as by the need for vectors like mosquitoes, ticks (arboviruses), or mammals (rabies).

For a number of neurotropic viruses, the physical barrier provided by the skin is breached by the bite of an animal or arthropod vector, or through the use of contaminated needles or other foreign bodies. Many other neurotropic viruses enter the host via natural portals such as the respiratory and GI tracts. Entry via these routes may be direct (via contaminated saliva, for

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example) or through aerosols. The molecular mechanisms that influence the capacity of viruses to become aerosolized and their subsequent stability are unknown. Most nonenveloped viruses (poliovirus, reovirus) appear to lose infectivity in conditions of low (<50%) relative humidity. For example, aerosolized poliovirus shows a 10^3-fold drop in infectivity when kept for 1 hour at 15% relative humidity (when compared with 72% humidity) [58]. Conversely, the infectivity of aerosols of most enveloped viruses is not notably altered by changes in the relative humidity, although in general enveloped viruses are more sensitive to environmental inactivation.

The GI tract presents formidable chemical barriers to the entry of viruses. The mechanisms by which agents such as those present in the GI tract (e.g., acids, bile salts, proteolytic enzymes) affect viral structure, and hence determine the ability of a virus to survive transit through the local environments present at entry sites such as the GI tract, are gradually becoming understood. In the case of nonenveloped viruses, the outer capsid proteins appear to be the major determinants of viral stability to a wide variety of physicochemical agents. For example, rhinoviruses are rapidly inactivated at acidic pH levels, whereas other picornaviruses, such as polio, are not. One practical consequence of this is that rhinoviruses do not survive transit through the stomach and hence rarely produce disease outside the limited confines of the respiratory tract. Exposure of rhinoviruses to an acidic pH results in the loss of the viral capsid protein VP4. This in turn produces a "hole" in the virus particle through which the viral nucleic acid leaks out, leaving a noninfectious "empty" viral capsid. Interestingly, the same type of structural alteration can occur in polioviruses exposed to certain adverse physical conditions, which again emphasizes the role of the viral capsid in determining virion stability [45, 73].

Another example of the role of outer capsid proteins in determining viral stability can be seen in the reoviruses. These viruses, like the enteroviruses, are neurotropic viruses whose natural portal of entry is via the GI tract. The three serotypes of reovirus show profound variations in their in vitro sensitivity to a number of physicochemical agents, including temperature, pH, alcohol, and detergent solutions. Studies using reassortant viruses containing genes derived from "sensitive" and "resistant" serotypes of virus (see Fig. 2 for an analogous experiment) allowed specific genes to be identified and associated with particular phenotypes. Sensitivity to alkaline pH and guanidine mapped to the viral S1 gene, sensitivity to high temperature and the detergent sodium dodecyl sulfate mapped to the S4 gene, and sensitivity to phenol and ethanol mapped to the M2 gene. In each case these genes encode proteins that are components of the outer capsid of the virus, indicating again the importance of these proteins in determining susceptibility to environmental conditions [17, 18].

Just as they differ with respect to sensitivity to pH changes, viruses vary in their response to proteolytic enzymes. Some viruses are extremely sensitive to trypsin—foot and mouth disease virus demonstrates a 10^3 drop in infectious titer upon treatment with it—and others, like influenza, need the effects of a trypsin-like protease to become infectious [23, 41]. The human rotaviruses, which are enteric pathogens, show dramatic enhancement of infectivity in the presence of trypsin, which cleaves the VP3 outer capsid protein [69]. Reoviruses also differ in their sensitivity to proteolytic digestion with enzymes such as chymotrypsin [4, 34]. Genetic studies have shown that the viral M2 gene, which encodes an outer capsid polypeptide (M1c), is responsible for determining sensitivity or resistance to proteolysis by chymotrypsin [66].

The effects of proteolytic enzymes on the infectivity of ortho- and paramyxoviruses have been investigated extensively. In the case of influenza virus, a trypsin-like protease present in host cells cleaves the hemagglutinin protein into two smaller peptides, HA1 and HA2, held together by a disulfide bond. The cleavage and subsequent removal of an arginine residue exposes the hydrophobic NH2 terminal of HA2, which is essential for the fusion function of this virus (see below) [23]. If influenza virus is grown in cells that lack this peptidase activity, HA cleavage does not occur, and, though the virus still binds to cellular receptors, it is not infectious [20, 41, 67, 68].

Enveloped viruses—those that incorporate a lipid bilayer on their outer surface—are particularly sensitive to inactivation by bile salts, which dissociate the lipoprotein components of the viral envelope. This may explain why enveloped neurotropic viruses only rarely enter the host via the GI tract (the coronaviruses, which include the neurotropic mouse hepatitis virus, are a known exception to this rule). The neurotropic viruses that commonly use the GI portal of entry are all nonenveloped and therefore insensitive to the action of bile salts.

Dissemination and Penetration of the CNS

Many viruses, such as those that infect the GI and upper respiratory tracts, attack target cells that are near the point of entry. For those agents, infection of contiguous cells satisfies the requirement of their life cycle. Except in special circumstances [3], viruses that infect the CNS must reach it after entering the body at a distant site. In general, this means primary replication must occur in a target cell outside the CNS, and then the virus must reach the CNS by either the bloodstream (hematogenous spread) or via nerves (neural spread). It is (1) the availability of an appropriate extra-

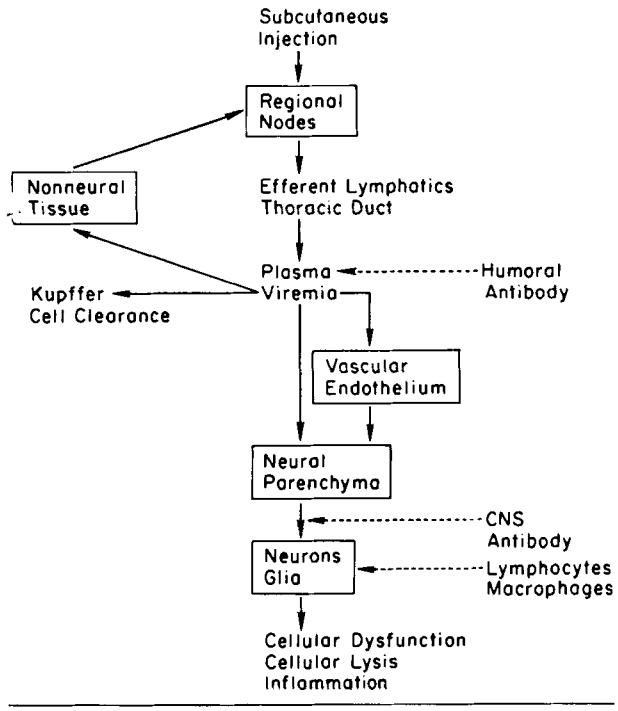

Fig 1. Pathogenesis of viral infections of the central nervous system (CNS). Typical stages in the pathogenesis of viral CNS infections are exemplified in this schema of the sequential stages of infection with the St Louis encephalitis virus. Virus enters the host after inoculation by the bite of an arthropod vector, replicates in regional lymph nodes and other nonneural tissues, spreads through the bloodstream, and then enters specific populations of cells within the CNS. (Reprinted from Nathanson {52} with permission from the American Public Health Association.)

CNS target cell, (2) the efficiency of spread after the primary infection of these target cells, and (3) the success of the host's immune response that determine whether a virus will be neuroinvasive. Incidentally, under most circumstances this combination of factors reduces the incidence of successful CNS infections, making these a small proportion of the total number of infections caused by viruses, even those that are traditionally considered neurotropic.

Hematogenous Spread

Most neurotropic viruses reach their target tissue through the bloodstream. Arboviruses, for example, are inoculated directly into the subcutaneous tissue or bloodstream, then replicate in extraneural tissues such as muscle or regional lymph nodes [48, 52]. Figure 1 is a schematic diagram of the spread of an arbovirus from the blood to the nervous system. Following primary replication, the virus is released into the bloodstream, where it is transported in the plasma. For a viremia to serve as a source of dissemination of virus to target organs, it must be of adequate magnitude and duration. Studies with a variety of arboviruses have shown

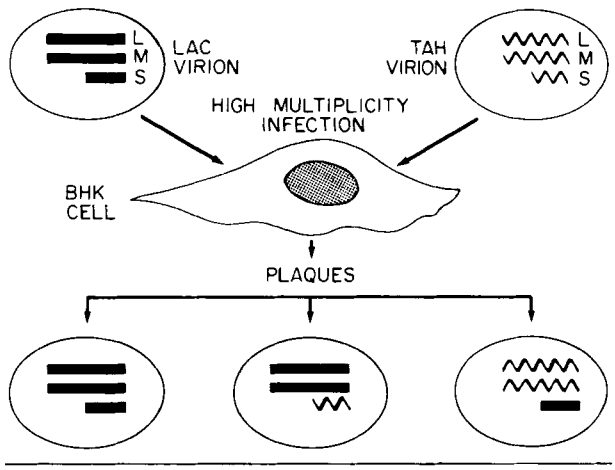

Fig 2. Preparation of reassortant viruses. Related viruses with segmented genomes can be recombined in the laboratory to generate hybrid virions. Here two strains of the California serogroup are used to coinfect a tissue culture cell line. Individual clones containing gene segments from the two strains can be typed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and/or RNA-RNA hybridization (for details, see text and {31}). All of the viruses with segmented genomes, including reoviruses, can be recombined in this fashion. (L = large, M = medium, S = small RNA segments; BHK = baby hamster kidney.)

that the degree and persistence of viremia is directly related to the ability to penetrate the CNS [30, 52].

The viruses of the California encephalitis group, which are transmitted by mosquito species endemic to the midwestern United States and to upper New York State, are responsible for the majority of the cases of arboviral encephalitis in this country. Their RNA genome is segmented (a quality they share with the influenza viruses, arenaviruses, and rotaviruses and reoviruses), and different "pieces" can be recombined artificially in the laboratory. By taking genomic segments from neuroinvasive and noninvasive strains, one can ask the question: which genome segment is responsible for the ability to penetrate the CNS? Figure 2 summarizes such an experiment, in which viruses representing neuroinvasive and noninvasive strains were used to coinfect cells. "Reassortant" viruses, containing genomic elements from two different strains, were thus generated and then tested for their ability to penetrate the murine CNS. Using this approach, it has been determined that the gene coding for the envelope glycoproteins cosegregates with the phenotypic property of neuroinvasiveness, although the genes coding for the other structural proteins of the virus can modulate this effect [31].

The same line of reasoning can be extended by using viral mutants that have changes in a single protein product. One frequently used approach, primarily with RNA viruses, is the selection of antigenic variants with monoclonal antibodies [26, 39, 71, 72]. In this ap-

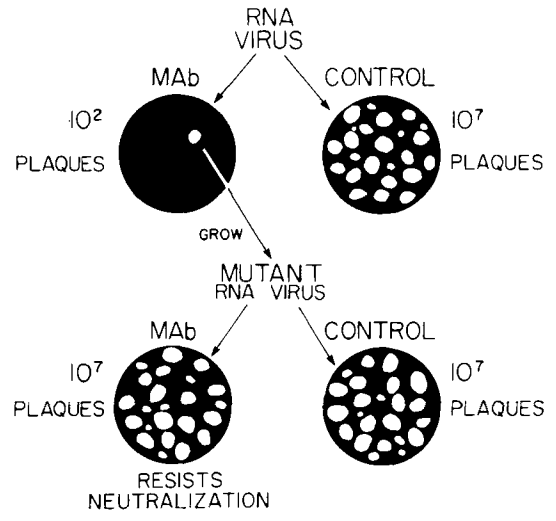

Fig 3. Selection of monoclonal antibody variants. A wild-type virus stock is combined with a neutralizing monoclonal antibody. Antibody escape plaques are present at a frequency of approximately 1:100,000. The single plaques initially isolated can be expanded such that a new stock of virus, resistant to the monoclonal antibody, is now available. This approach has been used with a large number of virus families (see text for details).

proach, a virus mutant present in the initial stock at low concentration is amplified by neutralizing the wild-type virus with a monoclonal antibody (Fig 3). The resultant virus variant contains a mutation in the protein against which the neutralizing antibody is directed, which in most instances is the protein responsible for attachment to cellular receptors. That mutation can usually be mapped to one or two amino acids which, being altered, prevent binding of the mutant virus by the monoclonal antibody. Monoclonal antibody variants have been used to map the antigenic sites of the influenza hemagglutinin [22, 76, 77] and have been used successfully to define important regions of the cellular binding proteins of rabies virus, reovirus, coronaviruses, and the California serogroup—all CNS pathogens.

Antigenic variants of the California serogroup obtained with a single monoclonal antibody have altered neuroinvasiveness; if injected directly into the CNS their pathogenesis is not altered. Thus, the property of neuroinvasiveness can be specifically associated with a single protein in this system [26]. By sequencing the genome of the variant virus and comparing this sequence with that of the wild-type parent, it may be possible to show that the property of neuroinvasiveness is associated with specific regions within the glycoprotein molecule, as has been done in other systems [34].

Besides arboviruses, other agents, like poliovirus, also use the bloodstream as their primary route to the nervous system. In polio the organ of primary replication is the gut, yet it is release into the bloodstream that determines whether the characteristic myelitis will occur. In addition to plasma viremia, some viruses, including human immunodeficiency virus (HIV), are transported in the bloodstream in association with lymphocytes or macrophages [2, 37].

Following hematogenous spread, neuroinvasive agents penetrate the CNS through the choroid plexus or through the endothelial cells. The lack of endothelial or choroid plexus tissue culture systems has made it difficult to analyze in detail the molecular mechanisms of penetration of these tissues.

Neural Spread

Classic investigations by Pasteur and colleagues on rabies virus, and by Goodpasture and colleagues on herpesviruses clearly established that neurotropic viruses could spread to the CNS via nerves [32]. More recent investigations with a number of viruses including rabies, polio, and herpes have provided abundant confirmation and more detailed insights into the process of neural spread [12, 48, 50, 51, 75]. However, until recently it was not possible to identify the role of specific viral genes in influencing the capacity of viruses to spread through nerves. In the case of rabies virus, after an initial period of replication in skeletal muscle, the virus concentrates at the neuromuscular junction in close proximity to neuromuscular and neurotendinous spindles. The virus then enters nerve terminals in proximity to these sites and is transported to the dorsal root ganglia and spinal cord [50, 51]. Rabies virus mutants with alterations in the virion glycoprotein (selected with monoclonal antibodies, as described for California encephalitis virus) appear to have altered capacity to enter certain types of nerve fibers [13–16]. These findings suggest that the rabies glycoprotein may play an important role in viral entry into and transport within cells.

Reovirus type 3 has also been shown to spread from peripheral tissue to the CNS via nerve cells. Furthermore, the microtubule-associated system of fast axonal transport has been implicated in this spread, because experimental infection of the CNS following footpad inoculation of mice is inhibited by low concentrations of colchicine. Selective inhibitors of slow axonal transport had no effect on spread to the CNS [75].

Like the California encephalitis viruses, reoviruses have a segmented genome (double-stranded RNA in this case). Experiments similar to the one outlined in Figure 2 can be used to generate reassortant viruses. Unlike type 3 reovirus, type 1 reoviruses spread to the CNS via a hematogenous route. Tyler and collaborators exploited this fact to test reassortants of type 1 and type 3 viruses and again ask the question: which gene segment accounts for the different patterns of spread in these two strains? These reassortant vi-

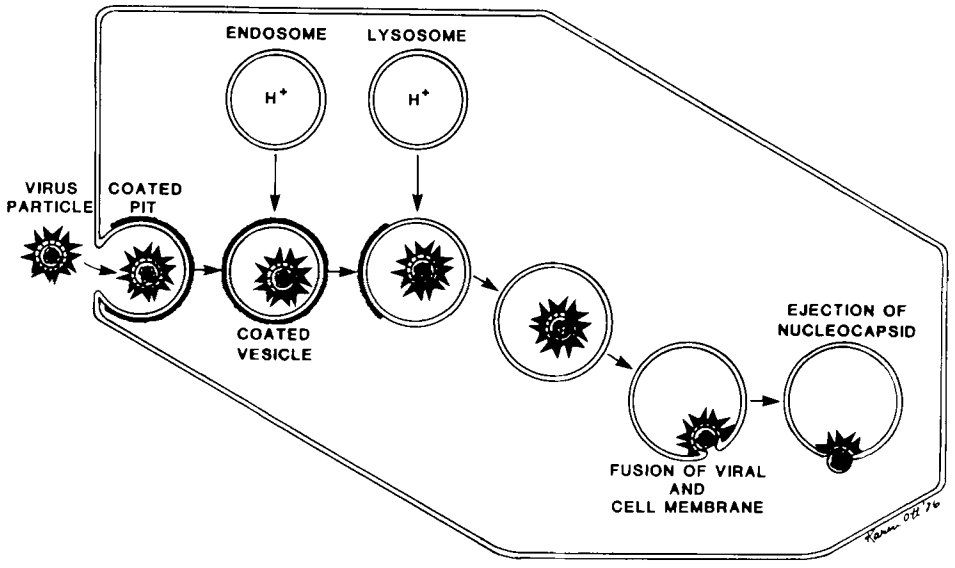

Fig 4. Entry of viruses into susceptible cells. Enveloped viruses are capable of entering cells by either fusion at the plasma membrane or by receptor-mediated endocytosis involving acidic vesicles. An enveloped virus binds to receptors in coated pits and is endocytosed into a vesicle that is capable of generating an acidic pH. Exposure of the hydrophobic regions present on the viral proteins on exposure to acid leads to fusion of the viral envelope with the membrane of the vesicle, and the viral contents (nucleic acid and proteins necessary for replication) are discharged into the cytosol. The process of viral replication can then start.

ruses, containing gene segments from both type 1 and type 3 viruses, have clearly shown that the property of spread can be associated with the gene that codes for the sigma 1 polypeptide—an outer protein that functions as the viral hemagglutinin and cell attachment protein.

Additional insights into the molecular basis for neural spread have recently come from the study of herpes simplex virus recombinants. For these experiments, two strains (HSV-1, 17; HSV-2, 186), which differ in their ability to spread to the mouse CNS after corneal inoculation, were utilized to generate recombinant viruses [55]. The region of the HSV-1 genome which codes for a number of proteins including the gB glycoprotein, a nucleocapsid protein (p40), and a DNA-binding protein (ICP-8), as well as the DNA polymerase, was found to be important in the spread of viruses from cornea to CNS.

In summary, the available evidence all points to the conclusion that, in several different viral systems, the envelope proteins (in the case of enveloped viruses) or the capsid proteins (in the nonenveloped agents) are the major determinants of spread to the nervous system. The outside proteins naturally play a very important part in other aspects of penetration into the organism, because they confer resistance to proteolysis, dehydration, and pH changes. In most instances, this has been determined through a combination of genetic methods, usually involving either reassortant viruses or selected antigenic variants. In the next section we discuss the role of these proteins in determining tropism at the cellular level.

Cell Tropism

Binding to Receptors

The process of entry of viruses into typical mammalian cells is outlined in Figure 4. Experimentally and biologically it can be divided into two main steps: (1) binding to the plasma membrane, and (2) penetration and uncoating. Many of the concepts regarding virus binding to plasma membranes have developed from the pioneering work of Brown and Goldstein on the low-density lipoprotein receptor [25]. In this model, macromolecules destined for the cytoplasm first interact with cellular receptors. The interaction with these receptors is quite specific and accounts for the restriction of unwanted macromolecules from the cells.

Viruses bind to the plasma membrane of susceptible target cells through specific receptors which may be proteins (HIV), lipids (vesicular stomatitis virus), or contain sialic acid (reovirus, influenza) [21, 64]. In some instances, neurotropic viruses have been thought to bind to pharmacological receptors. This has been best demonstrated for reovirus type 3, which in some tissues appears to bind to the beta-adrenergic receptor [9, 10], but it has also been suggested for rabies virus, which may use the acetylcholine receptor as its entry point [43, 51]. To a large extent, the specificity of this virus-receptor interaction can dictate the pathogenesis of a particular agent. A topical example is the virus

that causes the acquired immunodeficiency syndrome (AIDS), now termed HIV [2]. This virus binds to the T4 molecule, a membrane protein of unknown function which defines a particular subclass of lymphocytes [28, 29, 60].

Many viruses can infect cells if their nucleic acid genome is introduced directly into them—in the absence of any viral polypeptides. Introduction of infectious DNA clones has been used to bypass cellular receptors in infections with HIV. In such an experiment a variety of cell types can be infected, not necessarily only those containing the appropriate membrane receptor [1].

Another well-known receptor–virus interaction involves the influenza hemagglutinin, which binds to receptors containing sialic acid. Unlike the HIV system, where knowlege of the interaction between the receptor and the virus is still at a descriptive stage, the relationship of the influenza hemagglutinin to its receptor has been characterized to the single amino acid level, because the molecular structure of the influenza hemagglutinin has been determined through x-ray crystallography [76, 77]. The hemagglutinin molecule is present as a trimer on the virion surfaces. This trimer contains a "pocket" that appears to function as the site of attachment of the receptors present on susceptible cells. Viruses selected from different hosts (avian and equine, for example), which have naturally different enzymatic activities and slightly different sugar residues on their sialic acid molecules, have variations in the amino acids surrounding this pocket [6, 64]. Thus, even though the target cells are presumably the same, small changes in the receptor-binding site of influenza virus may account for species specificity.

Different technology has been used to identify the receptor-binding site of rhinoviruses. The large number of rhinovirus serotypes has made production of a general vaccine impractical. The majority of serotypes use a single specific receptor present on the surface of infectable cells. This receptor was initially identified through the use of a polyclonal antibody, then by monoclonal antibodies [11]. The virus itself has subsequently been crystallized [65], and a large cleft or "canyon" present on each icosahedral surface is thought to be the receptor-binding site, by virtue of its relationship to antibodies capable of neutralizing the virus. Drugs are now being designed to block this cleft. Although rhinoviruses are not neurotropic, they form part of the same family (Picornaviridae) as poliovirus, and many of the features of the viral topology are being extended to that group [47] as well as to foot-and-mouth disease [7] and Theiler's virus, other members of the Picornaviridae.

In summary, a necessary condition for the entry of viruses into all cells, and nerve cells in particular, is the availability of specific receptor molecules on the surface of such cells. Some receptors are undoubtedly present in all CNS cells, and viruses that utilize such may cause a generalized infection, or panencephalitis.

In the CNS, the pattern of illness a virus produces is determined in large part by the specific regions of the brain that are infected and by the population of cells in the affected regions that are injured or destroyed. Some viruses, like JC papovavirus, which is responsible for progressive multifocal leukoencephalopathy, infect astrocytes and oligodendroglia while largely sparing neurons, whereas herpes simplex infects all cell populations. Striking differences also occur in the topographical distribution of viral injury to the CNS. Rabies virus causes pathology in the cerebellum, hippocampus, and limbic areas, whereas polio affects the motor nuclei in the cortex, brainstem, and anterior horns of the spinal cord [32, 50]. Many of these specific topographic associations have been postulated to be the result of specific receptor-binding interactions.

Entry of Virus into Cells

After a virus has bound the appropriate receptors, the process of internalization can proceed in one of two ways: (1) viruses can fuse with the plasma membrane, discharging their contents directly into the cytosol, or (2) viruses may be internalized through the endocytotic pathway. Fusion at the plasma membrane has been observed for viruses like herpes simplex and coronaviruses, both of which are capable of forming giant syncytia during routine infection. Presumably there are no special requirements for internalization under these circumstances, and if the appropriate receptors are present the virus will appear within the cell. Other viruses are not capable of fusing directly with the cell membrane, and for those the endocytotic pathway (outlined in Figure 4) is the method of internalization. This pathway, which again resembles the mechanism of internalization of low-density lipoproteins [25], involves the collection of receptor–ligand complexes at regions of the membrane characterized by electron-dense material at their cytoplasmic side. The viruses are then transported within endocytotic vesicles, which have been determined to have a mildly acidic pH [42, 46]. For many enveloped viruses—those viruses that incorporate a lipid bilayer into their structure—it has been shown that exposure to such a low pH leads to alterations in the conformation of the envelope proteins [68]. Again using the influenza hemagglutinin as a model, such a change probably brings to the surface stretches of hydrophobic amino acids normally buried within the viral protein. The hydrophobic residues interact with the membrane of the endosome, fusing the viral envelope to it and releasing the contents of the virus into the cytoplasm, where the process of replication can then begin. In influenza and

related viruses, a host protease is responsible for the cleavage step that prepares this portion of the hemagglutin for this exposure (from a precursor protein). This proteolysis is essential for replication and accounts for some of the host specificity of the viruses, as noted above. In fact, epidemics of influenza in avian species have been ascribed to the ease of proteolysis in different strains.

For nonenveloped viruses (poliovirus, rhinovirus), there is also evidence that a pH-dependent step may be important in infection, but the process has not been observed clearly, as it has been in the enveloped agents. The degree of acidity that can elicit the changes in the viral proteins necessary for fusion to occur varies for different viruses. Because of the inherent difficulties of such measurements, the endosomal pH has not been determined for a large number of cell lines (and for none of the CNS cell lines). However, one can easily theorize that the relationship between the viral requirements and the acidity of these cellular organelles must influence the potential for infection of a particular cell type. In fact, the ability to fuse has been correlated with the encephalitogenic potential of bunyaviruses and mumps virus strains [26, 78].

Replication

Once the contents of the viral envelope (genome and proteins necessary for replication) have been discharged into the cytosol, the process of viral replication can begin. It is likely that for most nononcogenic RNA viruses the specificity of the virus-target interaction is determined prior to this step. An efficiently replicating virus, like vesicular stomatitis virus, for example, is probably capable of initiating replication of its genome in any cell line that it can penetrate. However, not all proteins or genome segments may be reproducible in any given system, and for lymphocytic choriomeningitis virus it appears that restriction at the segment level may be responsible for the outcome of infection [62]. For many DNA viruses like herpes simplex and varicella-zoster, persistence is clearly associated with the arrest of replication. Such mechanisms are highly specific for different families.

Recently the role of regulatory signals in the enhancement of replication has become a prominent area of investigation. Signals within the viral genome that stimulate gene transcription or translation appear to play an important role in determining viral host range and tissue tropism in certain systems [19]. These signals, variously termed enhancers, promoters, and transactivators depending on their position and orientation, are capable of stimulating the transcription of genes. Some of these enhancers determine tissue tropism (for example, in polyoma virus), whereas others are active in all tissues (simian virus [SV]-40, herpes simplex) [38, 40, 61, 70].

Transcriptional activators are genes whose products can activate the transcription of other genes. The viruses associated with AIDS have prominent transactivating activity, and they may also have regulatory activities at posttranscriptional events. In any case this kind of "tat" activity can greatly amplify the production of viral gene products and may be responsible for increased cytopathological changes. In some instances it may be responsible for host specificity. Tat activity had previously been shown to be an important feature of SV-40 [35], an experimentally important virus that is related to JC virus, the etiological agent for progressive multifocal leukoencephalopathy. This kind of activity may be important in the selectivity that this virus shows for glial cells and could play a role in a relationship between HIV and JC. In several experimental models, the relative contribution of JC virus regulatory sequences in conferring tissue specificity has been studied [8, 36]. It has been argued that the JC virus promoter may contribute substantially to neurovirulence.

Outcome of Infection

The final determinant of pathogenesis is the outcome of infection of the host cell. Four major pathways exist: (1) death of the cell; (2) alteration of its growth pattern and change into a cancerous phenotype (transformation); (3) persistence of infection without obvious cellular change; and (4) persistence of infection with alteration of specialized cellular functions (luxury functions). Neurotropic viruses can be responsible for any of these final results. Transformation is a highly intricate subject and will not be discussed further. Interested readers should refer to general reviews [27, 44].

Cell Death

Cytopathic effect significant enough to stop cellular metabolic activity is obviously the most common outcome of viral infection in all acute and some subacute viral infections. For the most part, morphological evidence has been utilized as the primary way of determining cell death. At a molecular level, mechanisms of viral cytopathology include the inhibition of production of host cell DNA, RNA, or protein. For some RNA viruses it is known that interference with the transfer of a methylguanosine "cap"—m^7GpppN—to host messenger RNA (mRNA) occurs in order to direct the host machinery to translate viral proteins preferentially. For influenza viruses and bunyaviruses (California encephalitis), the stolen cap is essential for transcription of the viral RNA to proceed efficiently [57, 59]. Except for these and a few other examples, the specific virally mediated mechanism for inhibition of cellular metabolism is unknown.

Much has been written about the role of the host immune response in mediating cell death. The immune response is frequently capable of harming the host instead of protecting it from viral infection as has been clearly demonstrated for a variety of neurotropic agents, including rabies, lymphocytic choriomeningitis virus, coronaviruses, and others (for review, see [53]). There has been a recent surge of interest in the role of viruses in inducing cell membrane molecules involved in the recognition of foreign antigens. These H-2 antigens are generally expressed at a low level in neural cells, but they increase in the presence of certain viral infections and interferons [74]. It has been postulated that the presence of these antigens on neural cells may make them a better target for cellular immunity directed at viral components and thus increase the possibility of virally triggered immunopathology. Although this work is clearly just beginning, the availability of nucleic acid probes for the mRNA's coding for these antigens should make it possible to study potential immunopathological mechanisms at a molecular level. Similarly, the wealth of information now available about the T-cell receptor molecules [29] should allow exploration of the genetic correlation with immunopathological diseases. Because of the complexity of the work involving virally induced immunopathology, we will not discuss it further here.

Persistence

PERSISTENT INFECTION WITH NO ALTERATION IN CELLULAR FUNCTION. A variety of viruses are capable of persistently infecting cells of the CNS with either no detectable abnormalities at the cellular level or minimal cellular changes. The herpesviruses—herpes simplex virus and varicella-zoster virus—are probably the best known and most studied examples of persistence. A variety of animal models have been developed with RNA viruses as well, including coronaviruses and Theiler's murine encephalomyelitis virus.

Infectious herpes simplex virus can be recovered from the trigeminal ganglia of many human cadavers and from some animals that have been experimentally infected. For in situ hybridization, a labeled genomic probe is base-paired to a cell genome immobilized on a glass slide. Enzymatic treatment of the cellular elements can distinguish between base pairing to DNA and RNA (suggesting, in some examples, that a DNA virus is being transcribed, or that an RNA virus has integrated itself in the host genome). Such techniques have demonstrated that herpes simplex virus DNA is present in most mice that have been infected and whose infection has become latent [63], in spite of the inability to recover infectious virus from explanted cultures. Hybridization can also be performed on whole-tissue DNA. Although this method is less sensitive, particularly when dealing with clinical specimens, it can be very useful in terms of analyzing the state of the latent viral DNA in experimental infections. Although considerable effort has been spent on this question, to date there is no clear indication whether herpes simplex virus is integrated into host DNA or is present as an "episome" in latently infected ganglia [5]. Work by Fraser and co-workers indicates that the DNA is present in a form distinct from that of the isolated virions [49, and personal communication].

Similar techniques can also be used to demonstrate that varicella-zoster virus is present latently in human dorsal root ganglia, and probably reactivated to cause zoster dermatitis [24]. This particular herpesvirus has not been cultured in explants of such ganglia. When the histological features so allow, the particular cell type that is latently infected may be identifiable—in varicella-zoster virus the genome is thought to be harbored exclusively by neurons of the dorsal root ganglia. Because of the lack of a suitable experimental animal, the state of the varicella-zoster virus latent genome is even less known than that of herpes simplex virus.

PERSISTENT INFECTION WITH ALTERATION OF LUXURY FUNCTIONS. Oldstone and co-workers have recently introduced the concept that some persistent viral infections may lead to the alteration of cellular function without any morphological change. In young mice infected with lymphocytic choriomeningitis virus, changes of growth and glucose metabolism are associated with persistent infection of the pituitary [56]. Such effects are subject to significant variation, depending on the age and strain of the mice as well as the viral strain. However, when present, this persistent infection of the mouse CNS is associated with the absent expression of the viral glycoproteins. The synthesis of these glycoproteins may be decreased, allowing the virus to escape detection by the cellular immune mechanisms.

Summary

A variety of factors affect the ability of viruses to infect cells within the CNS. Neurotropic viruses must penetrate the external barriers that protect the organism, replicate in an appropriate peripheral site, and then spread through either the bloodstream or through nerves to the CNS itself. Genetic analysis in several systems has pointed to the external proteins as the main determinants of neuroinvasiveness and neurovirulence. However, other factors, including host enzymes, activating factors, and the replicative machinery of the virus itself, must come into play. Ultimately, the balance between the viral machinery and the host immune system will determine the outcome of any infection.

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