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Novel approaches and challenges to treatment of CNS viral infections

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Abstract

Existing and emerging viral CNS infections are major sources of human morbidity and mortality. Treatments of proven efficacy are currently limited predominantly to herpesviruses and human immunodeficiency virus. Development of new therapies has been hampered by the lack of appropriate animal model systems for some important viruses and by the difficulty in conducting human clinical trials for diseases that may be rare, or in the case of arboviral infections, often have variable seasonal and geographic incidence. Nonetheless, many novel approaches to antiviral therapy are available including candidate thiazolide and purazinecarboxamide derivatives with potential broad-spectrum antiviral efficacy. New herpesvirus drugs include viral helicase-primase and terminase inhibitors. The use of antisense oligonucleotides and other strategies to interfere with viral RNA translation has shown efficacy in experimental models of CNS viral disease. Identifying specific molecular targets within viral replication cycles has led to many existing antivirals and will undoubtedly continue to be the basis of future drug design. A promising new area of research involves therapies based on enhanced understanding of host antiviral immune responses. Toll-like receptor agonists, and drugs that inhibit specific cytokines as well as interferon preparations have all shown potential therapeutic efficacy. Passive transfer of virus-specific cytotoxic T-lymphocytes have been used in humans and may provide an effective therapies for some herpesvirus infections and potentially for progressive multifocal leukoencephalopathy. Humanized monoclonal antibodies directed against specific viral proteins have been developed and in several cases evaluated in humans in settings including West Nile virus and HIV infection and in pre-exposure prophylaxis for rabies.

Global Importance

The national and worldwide burden of neurological infections continues to grow. New infections continue to emerge at a rapid pace as humans explore every remote corner of the planet and use animal and human products for treatment and transplantation. Once an infection enters the population the globalization of human travel helps spread infections quickly. Recent emerging viral outbreaks include those caused by Hanta virus, Marburg virus, influenza strains, SARS coronavirus, enteroviral encephalitis and West Nile

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encephalitis. These viral infections frequently involve the central nervous system (1–3). As better treatments are becoming available for treatment of cancer and immune mediated diseases, opportunistic infections are also on the rise. Several herpesvirus infections and progressive multifocal leukoencephalopathy (PML) due to JC virus are commonly seen in immune suppressed individuals (4). Additionally, there are many patients with undiagnosed meningoencephalitis where an infection is suspected but not confirmed. In one study nearly one third of patients with suspected infections of the nervous system in a tertiary care facility remained undiagnosed (5).

Currently, except for some of the herpesviruses and human immunodeficiency virus (HIV), there are no treatments of proven efficacy available for CNS viral infections. The absence of treatment contributes to high associated morbidity and mortality, leading to large health care costs with major socio-economic consequences. There is great need for development of antiviral therapeutics that would be effective in brain infections. However, development of therapeutics for infections of the central nervous system poses unique challenges. Delivery of drugs to the brain requires either the use of small molecules that follow Lipinski's rules for predicting activity based on pharmacokinetic principles and "likeness" to known active drugs (6) or requires direct delivery to the brain by invasive procedures such as a lumbar puncture, a reservoir placed in the lateral ventricle or by convection enhanced delivery. However if there is sufficient inflammation associated with the infection it may aid the delivery of the therapeutic agent to the site of infection through the cerebral vasculature.

The lack of animal models for CNS infections (e.g. JCV-induced PML) that replicate human disease means that human studies may need to be conducted following *in vitro* efficacy studies and in the absence of pre-clinical animal safety and efficacy testing, enhancing the risk of failure or unexpected side effects. For example a recent multicenter study on the use of mefloquine for PML was stopped prematurely due to lack of efficacy in humans despite promising *in vitro* studies (7). It is possible that humanized rodent models could be developed for some pathogenic human viruses, but the process is technically challenging and there are potential ethical limitations related to introducing human brain cells into rodent brain (8). Conducting clinical trials for viral infections of the nervous system also pose unique challenges. The infections maybe seasonal, outbreaks may occur in regions where imaging and monitoring facilities may not be available. The acute nature of the illness demands quick action and set up. For some viruses, reactivation may not always be pathogenic, which is the case for example with human herpes virus-6 (HHV-6) and Epstein Barr virus (EBV) (9,10). The rarity of many CNS viral infections means that multicenter studies are essential even for phase 2 studies to achieve the targeted sample size. Despite these challenges several multicenter studies have been conducted for PML, herpes simplex virus encephalitis (HSVE) and neurological complications of HIV infection (11–14). Companies interested in development of therapeutics for neurovirology can access clinical expertise through the section of CNS infections of the American Academy of Neurology (www.aan.com), and basic science expertise through the International Society of Neurovirology (www.isnv.org).

Traditionally, drug development has been the purview of pharmaceutical companies and they have limited interest in rare diseases including many CNS viral infections due to

limitations in the ultimate size of the potential market. Recently, pharmaceutical companies has shown an interest in rare diseases only if the drugs can be priced so as to make a profit. The high cost of drug development is largely driven by the large failure rate and the inability to predict efficacy in humans. Most drug trials for CNS viral infections have been conducted with drugs approved for systemic indications rather than specifically developed for use in the CNS. It is possible that if broad-spectrum antivirals were to be developed which penetrated the blood brain barrier, new therapeutics would become available.

Drug development for CNS viral diseases has one distinct advantage over that for chronic diseases which is, the availability of measurements of viral load as a dependable surrogate marker of disease. It is expected that if the viral load decreases clinical improvement should follow. Hence clinical trials could potentially be conducted in smaller sample sizes over shorter periods of time. However, resources for medicinal chemistry, toxicology, pharmacodynamics and pharmacokinetic studies are limited in academic institutions and unless these aspects are addressed, the challenge in treating these illnesses may continue into the foreseeable future.

In the following sections some of the new pharmacological, biological and immunomodulatory approaches to treatment of CNS viral diseases are briefly reviewed.

Therapeutic Pipeline in 2013 Pharmacological therapies

Broad-spectrum antivirals (Table 1)

Nitazoxanide (NTZ) is a thiazolide anti-infective with activity against anaerobic bacteria, protozoa and viruses (15–18). Originally developed as a treatment for intestinal protozoan infections, the antiviral properties of NTZ were discovered during the course of its development for treating cryptosporidiosis in patients with acquired immune deficiency syndrome (AIDS). Recent randomized double blind clinical trials have demonstrated effectiveness of NTZ against treating rotavirus and norovirus (18) and may be effective against hepatitis virus as well (19). These broad-spectrum effects suggest that this drug and its derivatives may be candidates for testing against neurotropic viruses if they can be delivered across the blood brain barrier.

A series of pyrazinecarboxamide derivatives, T-705 (favipiravir), T-1105 and T-1106, are broad-spectrum antiviral drugs that target RNA viruses such as influenza virus, arenaviruses, bunyaviruses, West Nile virus, yellow fever virus, and foot-and-mouth disease virus. These compounds do not inhibit host DNA and RNA synthesis. These compounds were effective in protecting animals even when treatment was initiated after virus inoculation. Importantly, T-705 imparts its beneficial antiviral effects without significant toxicity to the host (20). Two structurally unrelated compounds (LJ-001 and dUY11) have broad-spectrum activity against virtually all enveloped RNA and DNA viruses (21,22).

Targeted treatments for specific viruses (Table 2)

Unique enzymes and regulatory proteins encoded by the genome of specific neurotropic viruses are excellent targets for specific antiviral therapy. This approach has worked well as exemplified by drugs targeting thymidine kinase for herpes simplex virus (HSV) and reverse

transcriptase, integrase and protease for HIV. Understanding the steps in the viral replicative cycle should allow a similar approach for other neurotropic viruses. For example, with regards to flaviviruses, the envelope glycoprotein, and several enzymes (NS3 protease, NS3 helicase, NS5 methyltransferase and NS5 RNA-dependent RNA polymerase) are all potential drug targets (23). Similarly, targeting viral protease has been successful in drug (boceprevir and telaprivir) development against hepatitis C virus (24).

Anti-HIV therapies for HIV associated Neurocognitive Disorders

It has become clear that current antiretroviral therapies (ART) are not able to eliminate HIV-associated neurocognitive deficits (HAND) (25) and despite intensification of antiretroviral therapy (ART), CNS dysfunction persists in many patients (26). The underlying reasons for this failure and persistent immune activation are unclear. However several factors have been considered. This includes microbial translocation and persistent production of early viral products such as Tat protein (27). Currently, available drugs do not impact Tat transcription from the proviral DNA Novel approaches for treatment of HIV infection include targeting host proteins involved in viral assembly and maturation (28). However drugs that target the Tat are needed. A major shift in HIV drug development occurred recently with the realization that in an isolated case, HIV could be eliminated from reservoirs allowing a true “cure” of the disease (29). There are now several clinical trials underway that use a variety of approaches to develop HIV resistant lymphocytes or activate the latent reservoirs and the immune system in the presence of ART with the hope that the cytotoxic lymphocytes would then eliminate the viral reservoirs. Thus far these approaches have failed to produce a cure and concerns have been raised about the activation of HIV in the brain (30).

Anti-herpes virus therapies

Although acyclovir remains the most widely used drug for HSV, in recent years several drugs have been used for clinical use. These include valaciclovir, valganciclovir, famciclovir and foscarnet. Other drugs in clinical trials are CMX001 which is a prodrug of cidofovir, a helicase-primase inhibitor AIC316, FV-100, the valine ester of Cf 1743), and the terminase inhibitor letermovir (21,22). CMX001 can be given orally and has antiviral activity against most DNA viruses. However, toxicity, CNS delivery and development of viral resistance are potential limiting factors for generalized use for these newer agents.

A variation on the theme of targeted therapies is the use of antisense oligonucleotide analogs that are engineered to inhibit translation of viral proteins by specifically binding RNA sequences in the viral genome. A particular subtype of antisense molecules is one in which the DNA ribose ring is replaced with a morpholine ring and the phosphodiester linkages are replaced with phosphorodiamidate (“phosphorodiamidate morpholino oligomers”, PMOs) (31). These PMOs successfully inhibit a wide variety of neurotropic viruses including Japanese encephalitis virus (JEV) (32), arenaviruses (33) and filoviruses (34) in cell culture and more significantly influenza A (35), the alphavirus Sindbis (36), JEV (37), lymphocytic choriomeningitis virus (33), and West Nile virus (WNV) (38) in mice. Related strategies using RNA interference and small-interfering RNAs (siRNA) have also proven successful in animal models of both JEV (39) and rabies (40) and are being developed for measles as well and have the potential for treating subacute sclerosing panencephalitis which is caused a

persistent measles infection (41). The success of a pre-clinical study led to a small scale trial by AVI BioPharma (now Sarepta Therapeutics) of interference of WNV mRNA translation using a PMO in humans (NCT00091845). This trial and related studies suggested that the PMO tested, AVI-4020, crossed the blood-brain-barrier and was safe to administer, although no efficacy data is available.

Biologic Therapies

One of the most effective mechanisms for controlling and preventing neurotropic viral infections are the host's own array of innate and acquired immune defenses. One illustration of the general effectiveness of these defenses is that for the most common causes of viral encephalitis (e.g. arboviruses and herpesviruses), neuroinvasive disease is in fact an exceedingly rare outcome of infection. For example, millions of individuals are latently infected with herpesviruses yet there are likely only a few thousand cases a year of HSVE and other herpesvirus encephalitides in the U.S. each year. Similarly, for virtually all of the common arbovirus infections (e.g. WNV and JEV) neuroinvasive disease occurs in <1% of those infected. Data concerning the potential role of innate immunity, cell-mediated immunity, and antibody responses in important CNS viral infections is briefly reviewed below along with potential opportunities for manipulating these systems in antiviral therapy.

Innate immune responses

RNA and DNA produced during viral replication can be recognized by host "pathogen recognition receptors" that include the family of toll-like receptors (TLRs) and the RIG-I like receptors (RLRs). Signaling through TLRs and RLRs act via intermediary proteins (e.g. Interferon Regulatory Factors) to induce new gene transcription and activation of antiviral programs best exemplified by type I interferon responses. These gene products have been implicated in the pathophysiology of most viral infections. For example, several studies in humans and animals have linked aspects of WNV infection (acquisition, symptomatic versus non-symptomatic disease, neuroinvasive disease) with interferon-associated genes including members of the oligoadenylate synthetase (OAS) family (42–45), interferon regulatory factor-3 , or the myxovirus-resistance family gene (44). OAS1 genes have also been linked to resistance to flavivirus infection in mice (46), and to susceptibility to develop WNV encephalitis in horses (47).

The cytokine TNF-alpha also plays a role in innate immune responses against most viral infections. For example, in WNV infection although it has been suggested that elevated levels of TNFalpha may increase the risk of developing West Nile neuroinvasive disease (WNND) by impairing endothelial cell integrity and facilitating viral entry across the blood-brain-barrier, mice lacking TNF-receptor1 or treated with neutralizing TNF-alpha monoclonal antibodies show increased mortality after WNV challenge, and increased viral loads in the CNS (48). This suggests that the role of TNF-alpha may be complex with some aspects facilitating neuroinvasion and others facilitating antiviral roles including accumulation of CD8+ T-cells and macrophages (48). Interestingly, polymorphisms in the TNF-alpha promoter region that are associated with alterations in the transcription of TNF-alpha and its plasma levels have been linked to the risk of developing encephalitis, as

opposed to milder febrile illness, following JEV infection. For example patients with the 308A allele have an odds ratio (OR) of 0.09 for developing JEV fever as opposed to encephalitis whereas those with the 308G allele have an OR of 11.6 a >100-fold risk difference (49).

Multiple studies in mice indicate that TLRs and RLRs recognize WNV-associated nucleic acids generated during viral replication and subsequently led to activation of type I Interferon (IFN) responses and transcriptional responses mediated through interferon regulatory factors-3 and 7 . Mice lacking interferon alpha or beta receptors (50), TLRs 3 or 7 (51,52), or the RLR MyD88 (52,53), all show enhanced severity of WNV infection and enhanced viral replication compared to their wild-type counterparts. In the case of TLR 7 deficiency, at least some of the enhanced susceptibility is likely due to the failure of CD45 leukocytes and CD11b macrophages to home to WNV-infected cells or infiltrate infected target organs. Similarly, at least part of the effect of MyD88 deletion in enhancing WNV mortality and spread can be linked to its role in inducing cytokines that in turn facilitate recruitment of macrophages and T-cells into the brain (54).

The majority of cases of HSVE are sporadic in nature and lack defined immunological risk factors. However, children with inborn errors in TLR3 signaling (54–56) or defects resulting in abnormal signaling through several antiviral TLRs including 3, 7, 8, 9 (e.g. UNC-93B deficiency)(57) are susceptible to HSVE. A common component associated with these defects is impaired interferon alpha/beta signaling. In many cases cells derived from affected individuals show enhanced HSV replication and cytopathicity, phenotypes which can be ‘rescued’ by treatment with IFN alpha or beta (56,58). The human genetic data is consistent with experimental studies showing that mice lacking IFNR-1 have enhanced growth of HSV in CNS and increased mortality after intracerebral viral challenge. These studies indicate that an effective type I IFN response is critical for murine survival from HSVE (59). Murine studies also point to the importance of TLRs in controlling HSV infection. For example, mice lacking TLR9, showed enhanced mortality after HSV challenge (60).

Manipulating innate immune responses as antiviral therapy

The importance of innate immunity in natural control of CNS viral infections suggests that augmentation of these pathways could provide a novel strategy for antiviral therapy. Some experimental evidence is available to support this approach. For example, mice treated with a combination of acyclovir and IFN alpha show an ~30% reduction in mortality after HSV challenge compared to mice treated with acyclovir alone (61). Agonists of TLRs 3, 9 have also been studied in murine models of HSVE. TLR9 agonists are typically CpG oligodeoxynucleotides that activate production of IFNalpha/beta. In one study in a mouse model of HSVE intranasal administration of the TLR3 agonist polyinosinic:polycytidylic acid before intranasal HSV challenge reduced severity of disease, however administration after infection was already established increased mortality and disease severity (62). In a similar experimental model, TLR9 agonist pre-treatment increased survival from 15% to 70% (62,63). Interestingly, a TLR9 *antagonist* also had a modest effect in improving survival when given either before or following viral challenge (15% to 30%). These results suggest that in some cases the very same cytokine responses that may reduce risk of

infection play detrimental roles during established infection and suggest the potential complexity of targeting these pathways for antiviral therapy. The TLR-3 agonist Ampligen has also been evaluated in a murine model of Venezuelan equine encephalitis virus CNS infection. Intranasal and intraperitoneal administration of Ampligen at −4 and +24hrs in relation to intranasal Venezuelan equine encephalitis virus challenge prevented neuroinvasion and the development of symptomatic disease. Even when Ampligen was administered only at +24 hrs, treated mice showed decreased CNS viral invasion and minimal disease (64).

Another strategy to reduce production of pro-inflammatory cytokines that may have a detrimental role in infection involves the use of tetracycline class antibiotics including minocycline and doxycycline. These agents may exert neuroprotective effects by reducing microglial activation and subsequent production of pro-inflammatory cytokines. Beneficial effects of these drugs on disease severity and CNS injury have been reported in murine models of alphavirus (Sindbis) (65), flavivirus (JEV)(66) and reovirus encephalitis (67) and in a macaque model of SIV encephalitis (68). One notable exception to these mostly positive results has been in rabies encephalitis in which therapy actually enhances disease severity (69).

Manipulating innate immunity in human CNS viral infection

Experimental studies suggest that manipulation of host innate immune responses might produce novel strategies for treating CNS viral infection. One obvious approach that has been tried in treatment of several human neurotropic viral infections involves the direct administration of Interferon preparations. In one non-randomized trial involving St. Louis encephalitis, patients receiving IFNalpha2b (3 million units iv then sq after 12 hrs, then daily x 14 days) seemed to have less persistent quadriparesis, quadriplegia and respiratory insufficiency than untreated controls (70). Isolated case reports of IFN therapy in WNV have shown examples of both apparent benefit (71,72) and no effect (73). In a randomized placebo-controlled trial in 112 children in Vietnam with JEV, IFN alpha2a (10 million units/m^2 IM x 7d) had no effect on mortality or incidence of severe sequelae (74). Furthermore, IFN alpha failed to show any benefit in progressive multifocal leukoencephalopathy due to JC virus infection (75). In the case of HSVE, almost no data is available. In one small study of 14 children with acute focal encephalitis who received either acyclovir alone or acyclovir plus recombinant IFNbeta, there was no appreciable difference in outcomes between the two treatment groups (76).

There are now five licensed TNFalpha inhibitors utilized in the treatment of psoriasis, inflammatory bowel disease and rheumatoid arthritis (adalimumab, etanercept, infliximab, golimumab, certolizumab) but no data concerning possible utility in CNS viral infection. TLR agonists, including Ampligen are now in human clinical trials in settings including HIV infection (e.g. NCT00002269) and chronic fatigue syndrome (NCT00215813) but have not yet been evaluated in CNS infection. A potential cautionary note to the use of TNF-alpha antagonists in antiviral therapy comes from the fact that at least three cases of HSV encephalitis has been reported in patients being treated with infliximab (n=1) and adalimumab (n=2) for rheumatologic disorders including psoriatic arthritis, rheumatoid

arthritis, and inflammatory polyarthritis. (77). Similarly, treatment with TNF-alpha inhibitors may increase the risk of herpes zoster. A review of a German registry for patients being treated with biologics found 23 cases of zoster associated with treatment with TNF-alpha antibodies (adalimumab, infliximab) and 23 with TNFalpha antagonist (etanercept) (78). The estimated hazard ratios (corrected for epidemiological factors) were 1.82 (95% CI 1.05–3.15) for the antibodies, but nonsignificant (HR of 1.36, 95% CI 0.73–2.55) for etanercept. A more recent U.S. study failed to find an increase risk for herpes zoster in rheumatoid arthritis patients treated with anti-TNF therapy (79), so the area remains unsettled. These studies at least raise cautionary possibility that, for herpesviruses, TNFalpha may play a role in controlling reactivation from latency. Latent states are not seen in arboviral infections, and the potential risks of benefits of TNF-alpha inhibition in these infections may be different than that seen in herpes viral infections. The use of TNF blockers may also increase the risk of reactivation of mycobacterial infections (80) which may further limit their use in populations where mycobacterial infections are endemic. Although there have been reports of PML in patients receiving anti-TNF therapies including infliximab (81) and etanercept (82), all patients reported to date have had other diseases or therapies associated with risk of PML. A recent review of the FDA's Adverse Event Reporting System database which identified six such cases in patients with rheumatic diseases concluded that a causal relationship between PML and anti-TNF therapy was unlikely (83).

Cytokines such as IL-2 and IL-7 have also been used to simulate the immune system so as to enhance the anti-viral response. However IL-2 failed to show any clinical benefit in two large clinical trials (84) and supplemental Il-7 treatment actually enhanced HIV persistence in patients receiving antiretroviral therapy (85). Cytokine therapy has yet to be specifically applied in patients with CNS viral infections.

Treatment with tetracycline class antibiotics may have broad-spectrum effects on pro-inflammatory cytokine production. In one clinical study on infection by the flavivirus dengue, doxycycline treatment (200 mg load then 100 mg q12h x 10d) was shown to reduce serum levels of a variety of proinflammatory cytokines (IL6, IL1beta, TNF) whose expression has been linked to increasingly severe disease at days 3 and 7 post-treatment compared to controls (86)

Cellular Immunity

A variety of studies suggest that cellular immunity plays a critical role in the control of viral infections, and in particular emphasize a critical role for both CD4+ and CD8+ -mediated T-cell immunity. In murine models of WNV, age-related declines in CD4/CD8 T-cell responses may also explain at least some of the age-related susceptibility to CNS infection (87), whether similar defects occur in humans remains uncertain (88). Mice lacking CD8+ T-cells show decreased CNS viral clearance and increased mortality after challenge with virulent WNV strains (89), although this depletion may actually reduce severity of disease after challenge with some attenuated WNV strains. This suggests that CD8+ T-cells may have beneficial effects through accelerated viral clearance and potentially deleterious effects via immunopathology. The importance of CD8+ T-cells in viral clearance may vary even for

viruses in the same family. For example, in contrast to their relative importance in WNV pathogenesis, CD8+ T-cells apparently play a much more subsidiary role in CNS clearance of another neurotropic flavivirus, JEV as compared to virus-specific antibody (90).

Failure of WNV-specific T-cells to migrate to the CNS even when present has essentially the same effect as CD8 deficiency (91). Infection of neurons and other cells can result in production of Cxcl10 which binds to the Cxcr3 receptor and promotes trafficking of virus-specific CD8+ T-cells into the CNS. Trafficking of both CD4+ and CD8+ T-cells into the CNS is also dependent on the chemokine receptor Ccr5, and mice lacking Ccr5 have increased mortality, higher WNV viral titers in brain, and a paucity of infiltrating CD3+ inflammatory cells (92). CCR5 deficiency may also exacerbate experimental HSVE (93). Approximately 1% of the U.S. Caucasian population is homozygous for a deletion (delta32) in Ccr5, resulting in its complete loss of function. The presence of the Ccr5delta32 homozygosity does not increase susceptibility to WNV infection but does increases the risk of severe WNV disease by approximately 4-fold (92,94,95). CCR5 is also a well known co-receptor for HIV infection and in contrast, individuals with the Ccrdelta32 mutations have milder form of the disease with slower progression (96).

Manipulating Cellular Immunity in human CNS viral infection

Traditionally vaccinations have been used as a means to boost immune responses against viral pathogens. This approach has been successful with VZV for prevention of zoster and a similar approach is being considered for JC virus. However this approach may have limitations in patients with significant immune suppression. Hence other approaches are being considered. Passive transfer of virus-specific cytotoxic T cells (CTLs) can protect against disease (97,98). These studies suggest the possibility that transfer of virus-specific immune cells may be useful in the treatment of human viral CNS infections. A proof of principle in human CNS disease comes from treatment of a patient with PML following hematopoietic stem cell transplantation and immunosuppression for graft versus host disease (99). Peripheral blood mononuclear cells (PBMCs) were obtained from the stem cell donor and JC virus antigen-specific CTLs were generated after in vitro stimulation of these cells with peptides derived from the JC virus for one month and the patient received two infusions . He developed measurable CTL activity against the viral protein that had not been detectable pre-infusion. He showed remarkable signs of clinical (ambulation, motor function, cognition) and MRI improvement and cleared virus from CSF. Because this patient also received antiviral therapy and alterations in his immunosuppressive therapy it is not possible to unequivocally attribute his response to his immunotherapy. Nonetheless this case shows that it is potentially feasible to generate and infuse virus-specific CTLs that impart measurable antiviral CTL activity to the recipient, and that this can be done safely.

A number of studies of passive transfer of donor-derived cytomegalovirus (CMV) and Epstein Barr virus (EBV)-specific CTLs have been reported. As the technique has matured efforts have been focused on developing more rapid culture and in vitro stimulation with viral peptides followed by selectively purifying IFNgamma secreting cells and transferring them back into the host. All of this can be accomplished in less than 24 hrs (see 100,101). Transfer typically results in development of measurable viral-specific CTL activity in

recipients in whom this was often absent pre-transfer. Cell transfer has been used successful both in patients with disease refractory to antiviral therapy (100,102) and in models of prophylaxis (101). In one study involving 18 patients, two had CMV encephalitis and both responded clinically with clearance of virus from blood and or CSF after immunotherapy (100). Virus-specific CTLs from donor PBMCs can also be isolated by binding of their T-cell receptor to a specifically constructed multimer containing the target of interest (e.g. CMV pp65 or EBV EBNA peptide) coupled to the appropriate MHC HLA. (102).

Humoral Immunity

Perhaps the best-studied component of the host's defense against viral infection is the generation of a virus-specific antibody response. With the advent of monoclonal antibody (MAb) technology it became possible to map out protective epitopes on specific viral proteins (reviewed in 103). It was shown that for some viruses that passive transfer of MAbs directed against these proteins could clear virus and viral nucleic acid from the CNS and from neurons even in mice with deficient cellular immunity (104) and that such clearance could occur after infection in the CNS was established (105). Humanized forms of these antibodies have been developed with similar protective capacity in experimental models of encephalitis, such as those involving arboviruses such as Venezuelan equine encephalitis, JEV, and WNV as well as in post-exposure prophylaxis (PEP) against rabies (106–110). Humanized monoclonal antibodies have also been tested in animal models of other neurotropic viruses including Enterovirus 71 (111) and in models of infection with influenza viruses including H5N1 (112) and pandemic H1N1 (113). Studies using humanized MAbs for treatment of herpesvirus infections are more limited, although there is one report of the use of a humanized anti-HSV MAb in a mouse model of HSV ocular disease (114) in which antibody-treated animals had significantly reduced ocular disease.

Novel approaches to antibody therapy in human CNS viral infection

A humanized monoclonal antibody ("MGAWN1") directed against an epitope on the WNV envelope glycoprotein that was protective in mouse and hamster models of WNV encephalitis has been tested for safety in humans. This antibody was administered to normal volunteers at doses up to 30 mg/kg by intravenous infusion and was well tolerated. Pharmacokinetic studies indicated that the half-life was ~27days and that levels achieved substantially exceeded those required for protection in hamster models (115). However, enrolling patients with acute encephalitis to a clinical trial can be challenging.

Another promising area for use of humanized monoclonal antibodies is in PEP for rabies virus infection. It has been estimated that 10–16 million people worldwide receive rabies PEP every year and that a minimum of 55,000 rabies deaths occur (116). Current regimens rely on use of either human or equine rabies immune globulin co-administered with rabies vaccine. Phase I trials indicated that a cocktail of humanized anti-rabies virus monoclonal antibodies (CL184 manufactured by Crucell Holland BV) administered intramuscularly were safe in Phase I human trials and did not interfere with the development of subsequent vaccine-induced rabies virus neutralizing antibodies (117). Two phase II trials were completed in 2008 in children and adolescents in the Philippines (NCT00708084) and in

adults in the U.S. (NCT00656097), followed by a third trial in adults in India that was just completed in late 2012 (NCT01228383). The data available suggests that humanized MAbs will be a viable alternative to polyclonal anti-rabies immunoglobulin for PEP.

New technological approaches may enhance the utility of humanized monoclonal antibody approaches to CNS viral infection. Almost all trials of humanized monoclonal antibodies have utilized passive transfer techniques in which antibody is directly administered either intravenously (e.g. WNV MGAWN1) or intramuscularly (rabies CL84). A novel strategy referred to as 'vectored immunoprophylaxis' (VIP) utilizes gene transfer to express high levels of antibodies for prolonged periods. Humanized MAbs transferred by conventional passive methods can protect mice with humanized immune systems from HIV challenge (118). Human neutralizing anti-HIV Abs are also protective when expressed after a single intramuscular injection in mice using an Adeno Associated Vector (AAV) (119). In this system antibody was detected within a week of injection and peaked at 12–16 weeks and persisted for >64 weeks. This type of model may be a promising approach to avoiding repeated administration of antibodies in situations such as chronic disease or continued risk exposure where treatment must ideally be maintained for periods exceeding the half-life of individual antibody administration.

As noted the typical target for humanized antiviral antibodies is against specific viral proteins, and the use of cocktails or pools of several antibodies may enhance efficacy and reduce the likelihood of the selection of viral escape mutants. Another strategy for use of these antibodies is to target key host cell components involved in viral binding or entry rather than viral proteins per se. This strategy has been successfully utilized in HIV infection in which two different humanized MAbs (PRO 140 and HGS004) directed against domains on the amino-terminal and extracellular loops of the HIV receptor CCR5 have been shown to be safe and well tolerated and to reduce plasma HIV viral load in Phase I/II trials (120,121). Phase I trials have also shown similar safety and efficacy for a humanized monoclonal antibody, ibalizumab (TNX-355), directed against CD4 (122).

Conclusions

New therapies for viral CNS infections are in development based both on understanding and targeting specific steps in the virus replication cycle and utilizing knowledge gained from increasingly more sophisticated understanding of host antiviral immune responses including innate, humoral and cell-mediated immunity. A host of potentially novel approaches to CNS antiviral therapy are available at the experimental and pre-clinical level. The rarity of many CNS infections and their unpredictability, especially for vector-borne diseases, pose formidable challenges in designing clinical trials and equally daunting challenges to establishing an economically viable pathway for new drug development. Nonetheless, the economic and public health burdens of both existing and ever emerging CNS viral threats makes progress in identifying both specific and broad-spectrum antiviral therapies imperative.

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Table 1

Broad spectrum antiviral drugs*

Drug	Viral Targets
Nitazoxamide	RNA viruses
Pyrazinecorboxamide	RNA viruses
LJ-001	Anti-enveloped RNA and DNA viruses
dUY11	Anti-enveloped RNA and DNA viruses
CMX-001	Anti-DNA viruses

* efficacy in CNS infections remains to be established

Table 2

Novel targets in viral gene products for drug development

Virus	Target	Function
Flaviviruses	protease	Processing of precursor polyprotein
	Helicase	Initiation of viral replication
	Methyltransferase	Needed to form mature RNA cap structure
	RNA-dependent RNA polymerase	Synthesis of minus strand RNA
	unknown	
HIV	Tat	transactivation of HIV genome
Herpes	helicase	Unwind viral DNA to initiate replication
	Terminase	Helps package DNA into the capsid
JC virus	T antigen	Regulatory protein
	Agnoprotein	Regulatory protein
	VP-1	Core protein