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TITLE:
Novel approaches and challenges to treatment of central nervous system viral infections

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.

Broad-Spectrum Antiviral Agents ::: Pharmacological Therapies ::: Therapeutic Pipeline in 2013:
Nitazoxanide (NTZ) is a thiazolide anti-infective agent with activity against anaerobic bacteria, protozoa, and viruses (Table 1).15, 16, 17, 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. Recent randomized double-blind clinical trials have demonstrated effectiveness of NTZ against treating rotavirus and norovirus,18 and it 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 (WNV), 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 ::: Pharmacological Therapies ::: Therapeutic Pipeline in 2013:
Unique enzymes and regulatory proteins encoded by the genome of specific neurotropic viruses are excellent targets for specific antiviral therapy (Table 2). This approach has worked well, as exemplified by drugs targeting DNA polymerase 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 regard 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 telaprevir) development against hepatitis C virus.24

Anti-HIV Therapies for HIV-Associated Neurocognitive Disorders ::: Pharmacological Therapies ::: Therapeutic Pipeline in 2013:

It has become clear that current antiretroviral therapies (ARTs) are not able to eliminate HIV-associated neurocognitive deficits,[25] and despite intensification of 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 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 ::: Pharmacological Therapies ::: Therapeutic Pipeline in 2013:

Although acyclovir remains the most widely used drug for HSV, in recent years several drugs have seen clinical use. These include valaciclovir, valganciclovir, famciclovir, and foscarnet. Other drugs in clinical trials include 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. In a particular subtype of antisense molecules, 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 WNV[38] in mice. Related strategies using RNA interference and small-interfering RNAs 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 by a persistent measles infection.[41] The success of a preclinical 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 are available.

Innate Immune Responses ::: Biologic Therapies ::: Therapeutic Pipeline in 2013:

RNA and DNA produced during viral replication can be recognized by host pathogen recognition receptors, which include the family of toll-like receptors (TLRs) and the RIG-I–like receptors (RLRs). Signaling through TLRs and RLRs occurs via intermediary proteins (eg, 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 vs nonsymptomatic disease, neuroinvasive disease) with interferon-associated genes, including members of the oligoadenylate synthetase (OAS) family,[42, 43, 44, 45] interferon regulatory factor-3, and 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 tumor necrosis factor-α (TNFα) 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 TNFα may increase the risk of developing West Nile neuroinvasive disease by impairing endothelial cell integrity and facilitating viral entry across the blood–brain barrier, mice lacking TNF

receptor 1 or treated with neutralizing TNFα monoclonal antibodies (MAbs) show increased mortality after WNV challenge and increased viral loads in the CNS.48 This suggests that the role of TNFα 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 TNFα promoter region that are associated with alterations in the transcription of TNFα 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 lead to activation of type I interferon (IFN) responses and transcriptional responses mediated through IFN regulatory factors 3 and 7. Mice lacking interferon α or β receptors,50 TLR3 or TLR7,51, 52 or the RLR MyD8852, 53 all show enhanced severity of WNV infection and enhanced viral replication compared to their wild-type counterparts. In the case of TLR7 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.53

The majority of cases of HSVE are sporadic in nature and lack defined immunological risk factors. However, children with inborn errors in TLR3 signaling54, 55, 56 or defects resulting in abnormal signaling through several antiviral TLRs including 3, 7, 8, and 9 (eg, UNC-93B deficiency)57 are susceptible to HSVE. A common component associated with these defects is impaired interferon α/β signaling. In many cases, cells derived from affected individuals show enhanced HSV replication and cytopathicity, phenotypes that can be rescued by treatment with IFNα or β.56, 58 The human genetic data are consistent with experimental studies showing that mice lacking IFN receptor 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 ::: Biologic Therapies ::: Therapeutic Pipeline in 2013:

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 IFNα show an ~30% reduction in mortality after HSV challenge compared to mice treated with acyclovir alone.61 Agonists of TLR3 and TLR9 have also been studied in murine models of HSVE. TLR9 agonists are typically CpG oligodeoxynucleotides that activate production of IFNα/β. In a 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 had already been established increased mortality and disease severity.62 In a similar experimental model, TLR9 agonist pretreatment 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 (15–30%). 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 TLR3 agonist rintatolimod (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 +24 hours 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 hours, treated mice showed decreased CNS viral invasion and minimal disease.64

Another strategy to reduce production of proinflammatory 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 proinflammatory 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 ::: Biologic Therapies ::: Therapeutic Pipeline in 2013:

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 the treatment of several human neurotropic viral infections involves the direct administration of IFN preparations. In a nonrandomized trial involving Saint Louis encephalitis, patients receiving IFNα2b (3 million units intravenously then subcutaneously after 12 hours, then daily × 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, IFNα2a (10 million units/m2 intramuscularly × 7 days) had no effect on mortality or incidence of severe sequelae.[74] Furthermore, IFNα is unlikely to be of benefit in progressive multifocal leukoencephalopathy due to JCV infection.[75] In the case of HSVE, almost no data are available. In a small study of 14 children with acute focal encephalitis who received either acyclovir alone or acyclovir plus recombinant IFNβ, there was no appreciable difference in outcomes between the 2 treatment groups.[76]

There are now 5 licensed TNFα inhibitors utilized in the treatment of psoriasis, inflammatory bowel disease, and rheumatoid arthritis (adalimumab, etanercept, infliximab, golimumab, certolizumab), but there are no data concerning possible utility in CNS viral infection. TLR agonists, including Ampligen, are now in human clinical trials in settings including HIV infection (eg, NCT00002269) and chronic fatigue syndrome (NCT00215813), but have not yet been evaluated in CNS infection. A potential cautionary note to the use of TNFα antagonists in antiviral therapy is that at least 3 cases of HSV encephalitis have 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 TNFα 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 TNFα antibodies (adalimumab, infliximab) and 23 with TNFα antagonist (etanercept).[78] The estimated hazard ratio (HR; corrected for epidemiological factors) was 1.82 (95% confidence interval [CI] = 1.05–3.15) for the antibodies, but nonsignificant (HR = 1.36, 95% CI = 0.73–2.55) for etanercept. A more recent US study failed to find an increased risk for herpes zoster in rheumatoid arthritis patients treated with anti-TNF therapy,[79] so the area remains unsettled. These studies at least raise the cautionary possibility that, for herpesviruses, TNFα may play a role in controlling reactivation from latency. Latent states are not seen in arboviral infections, and the potential risks and benefits of TNFα inhibition in these infections may be different than those 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 US Food and Drug Administration's Adverse Event Reporting System database that identified 6 such cases in patients with rheumatic diseases concluded that a causal relationship between PML and anti-TNF therapy was unlikely.[83]

Cytokines such as interleukin (IL) 2 and IL7 have also been used to simulate the immune system so as to enhance the antiviral response. However, IL2 failed to show any clinical benefit in 2 large clinical trials,[84] and supplemental IL7 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 proinflammatory cytokine production. In a clinical study on infection by the flavivirus dengue, doxycycline treatment (200mg load then 100mg every 12 hours × 10 days) was shown to reduce serum levels

of a variety of proinflammatory cytokines (IL6, IL1β, TNF) whose expression has been linked to increasingly severe disease at days 3 and 7 post-treatment compared to controls.[86]

Manipulating Cellular Immunity in Human CNS Viral Infection ::: Cellular Immunity ::: Therapeutic Pipeline in 2013:

Traditionally, vaccinations have been used as a means to boost immune responses against viral pathogens. This approach has been successful with varicella zoster virus for prevention of zoster, and a similar approach is being considered for JCV. 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, JCV antigen-specific CTLs were generated after in vitro stimulation of these cells with peptides derived from the JCV for 1 month, and the patient received 2 infusions. He developed measurable CTL activity against the viral protein that had not been detectable preinfusion. He showed remarkable signs of clinical (ambulation, motor function, cognition) and magnetic resonance imaging improvement, and virus was cleared from the cerebrospinal fluid (CSF). Because this patient also received antiviral therapy and because of 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 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 IFNγ-secreting cells and transferring them back into the host. All of this can be accomplished in <24 hours.[100, 101] Transfer typically results in development of measurable viral-specific CTL activity in recipients in whom this was often absent pretransfer. Cell transfer has been used successfully both in patients with disease refractory to antiviral therapy[100, 102] and in models of prophylaxis.[101] In a study involving 18 patients, 2 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 (eg, CMV pp65 or EBV nuclear antigen peptide) coupled to the appropriate major histocompatibility complex human leukocyte antigen.[102]

Humoral Immunity ::: Therapeutic Pipeline in 2013:

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 MAb technology, it became possible to map out protective epitopes on specific viral proteins (reviewed in Griffin et al[103]). It was shown that for some viruses, 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, including Venezuelan equine encephalitis, JEV, and WNV, as well as in postexposure prophylaxis (PEP) against rabies.[106, 107, 108, 109, 110] Humanized MAbs 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 ::: Therapeutic Pipeline in 2013:

A humanized MAb (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 30mg/kg by intravenous infusion and was well tolerated. Pharmacokinetic studies indicated that the half-life was 27 days and that levels achieved substantially exceeded those required for protection in hamster models.[115]

Another promising area for use of humanized MAbs is in PEP for rabies virus infection. It has been estimated that 10 million to 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 immunoglobulin coadministered with rabies vaccine. Phase I trials indicated that a cocktail of humanized anti–rabies virus MAbs (CL184 manufactured by Crucell Holland, Leiden, the Netherlands) administered intramuscularly was 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 United States (NCT00656097), followed by a third trial in adults in India that was just completed in late 2012 (NCT01228383). The data available suggest that humanized MAbs will be a viable alternative to polyclonal antirabies immunoglobulin for PEP.

New technological approaches may enhance the utility of humanized MAb approaches to CNS viral infection. Almost all trials of humanized MAbs have utilized passive transfer techniques in which antibody is directly administered either intravenously (eg, WNV MGAWN1) or intramuscularly (rabies CL84). A novel strategy referred to as vectored immunoprophylaxis 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 antibodies are also protective when expressed after a single intramuscular injection in mice using an adeno-associated vector.[119] In this system, antibody was detected within a week of injection, peaked at 12 to 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 2 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 MAb, 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 preclinical 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 in establishing an economically viable pathway for new drug development. Nonetheless, the economic and public health burdens of both existing and emerging CNS viral threats make progress in identifying both specific and broad-spectrum antiviral therapies imperative.