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The case for immunomodulatory approaches in treating HSV encephalitis

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Abstract

HSV encephalitis (HSE) is the most prevalent sporadic viral encephalitis. Although safe and effective antiviral therapies and greatly improved noninvasive diagnostic procedures have significantly improved outcomes, mortality (~20%) and debilitating neurological sequelae in survivors remain unacceptably high. An encouraging new development is that the focus is now shifting away from the virus exclusively, to include consideration of the host immune response to infection in the pathology underlying development of HSE. In this article, the authors discuss results from recent studies in experimental mouse models, as well as clinical reports that demonstrate a role for exaggerated host inflammatory responses in the brain in the development of HSE that is motivating researchers and clinicians to consider new therapeutic approaches for treating HSE. The authors also discuss results from a few studies that have shown that immunomodulatory drugs can be highly protective against HSE, which supports a role for deleterious host inflammatory responses in HSE. The impressive outcomes of some immunomodulatory approaches in mouse models of HSE emphasize the urgent need for clinical trials to rigorously evaluate combination antiviral and immunomodulatory therapy in comparison with standard antiviral therapy for treatment of HSE, and support for such an initiative is gaining momentum.

Keywords

acyclovir; encephalitis; herpesvirus; immune pathology; immunomodulation; inflammation; innate immunity; intravenous immunoglobulin; reactive oxygen species

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HSV-1 is a neurotropic α -herpesvirus that is pervasive in the human population, with seropositivity rates approaching 90% in the USA. Following primary infection at mucosal sites, the virus spreads rapidly via innervating sensory neurons to replicate in sensory ganglia and the CNS. Although infections in the nervous system are cleared rapidly in

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immunocompetent individuals, nonreplicating viral genomes persist in latently infected neurons of sensory ganglia of the peripheral nervous system (PNS) and also in brainstem (BS) and brain neurons for the life of the host [1,2]. Exposure to various stressors causes virus reactivation resulting in further spread and recurrent disease, most often including mucosal lesions and less frequently, but more seriously, diseases such as herpetic stromal keratitis and HSV encephalitis (HSE). Failure to achieve efficient *in vitro* reactivation of latent HSV-1 from brain tissues cemented the idea that *in vivo* HSV-1 reactivation occurs exclusively in PNS neurons. However, efficient *in vitro* reactivation of HSV-1 from latently infected brain tissues has recently been reported, which raises the possibility that HSE may result from reactivation in the brain rather than exclusively in PNS neurons [3,4]. Primary or reactivated HSV infection in immunocompromised individuals is often more severe, and in neonates, whose immune systems are underdeveloped, often results in disseminated infection involving the skin, eye, mouth, liver, lungs, adrenal glands and CNS [5,6].

Clinical introduction

HSE runs an acute course. Patients are typically comatose within 6 days of the first neurological symptom. Since classical HSE is a focal encephalitis involving the medial temporal lobe, the presenting symptoms often involve behavioral changes, mimicking drug ingestion or an acute psychiatric disorder. Headache and fever occur early, seizures and language disturbance are common, and without treatment the patient lapses into coma. Brain MRI typically has increased signal on the fluid-attenuated inversion recovery sequence in the hippocampus, and the EEG may show periodic lateralized epileptiform discharges (Figure 1) [7]. Early in the course, the MRI abnormality may be so subtle that it is overlooked. Unlike HSV-2 meningitis and the generalized meningoencephalitis in neonates, HSE almost always results from reactivation of a latent HSV-1 infection. It can occur in immunocompromised patients as illustrated in Figure 1; however, it is most often a sporadic infection in previously healthy individuals. The incidence is 2.2 per million population [8], accounting for approximately 20% of cases of sporadic encephalitides.

The reason for the limbic system localization of HSE remains obscure. Speculation includes: virus spread via the olfactory bulb; viral reactivation in trigeminal ganglion nerve cell bodies that provide sensory distribution to meninges of the middle cranial fossa; and special affinity or permissiveness of limbic cortices to HSV-1 [9,10]. HHV6 encephalitis can produce identical clinical and MRI features in immunosuppressed patients [11]. There is also similar medial temporal localization in autoimmune limbic encephalitis, a subacute immune-mediated process sometimes seen, for example, in connection with small-cell lung cancer [12]. Both autoimmune encephalitis and HSE can involve other brain areas as well as the medial temporal lobes, but it is surprisingly rare for HSE to involve the brainstem [13].

Laboratory diagnosis

In the 1990s, cerebrospinal fluid (CSF) PCR assays became the diagnostic standard of HSE [14]. Prior studies required brain biopsy for definitive diagnosis; virus is very seldom cultured from the CSF. Almost half of brain biopsies either gave diagnoses other than HSE or were nondiagnostic [15]. Since false negatives were documented in almost 10% of biopsies, the early practice was to start antiviral drugs as soon as HSE was suspected, often eschewing brain biopsy altogether, or to continue a full course of antiviral drug regardless of biopsy results. Similar false negative results can occur with PCR, particularly in early lumbar punctures, and prudent practice is to maintain treatment in typical cases even in the face of a negative PCR test. Serological tests are not helpful in HSE where prompt diagnosis is important; however, ratios of CSF and serum antibodies can be used late in the course for retrospective diagnosis.

Antiviral treatment

Early studies with vidarabine in the 1970s showed correlation of mortality and morbidity with age and the degree of consciousness: no patients over the age of 30 years who were comatose at the start of treatment survived without neurological disability. In a comparison trial, acyclovir (ACV) proved superior to vidarabine [16]. The dose of ACV sodium now used (10 mg/kg intravenously three times a day) is twice the intravenous dose used for mucocutaneous HSV infection in immunocompromised patients. Despite optimal ACV therapy, HSE has remained a devastating disease with mortality rates of 15–20%; virtually all surviving patients have persistent neurological signs or symptoms, including memory and behavior disturbances, dysphasia and seizures. Only 50–65% of surviving patients return to independent living [17,18]. A delay in initiating ACV past the second hospital day is associated with a poor outcome. Prognosis does not appear to correlate with intrathecal antibody production or viral load at presentation [19]. High CSF level of IFN- γ early in the course of HSE portends a worse outcome [20].

Intrathecal immune activation & recurrent disease

Several lines of evidence suggest ongoing intrathecal immune stimulation after the acute bout of HSE. Macrophage/monocyte-derived neopterin is present in CSF, at particularly high levels in fatal cases [21]. Unlike other encephalitides, CSF neopterin remains elevated for years afterwards. It is likely that HSV reactivates in the CNS, producing intermittent antigen expression, which drives immune activation and cytokine expression. Approximately 10% of patients develop relapsed neurological disease localized to the original limbic site of HSE some 1–4 months after completing a course of ACV [22]. There are only a limited number of histopathological reports on relapsed or recurrent HSE. The neuropathology – at least in adults – is not suggestive of post-viral demyelination. Neuropathological study of one patient who developed fatal status epilepticus 5 years after HSE showed HSV nucleocapsids by electron microscopy and other changes consistent with acute viral cytopathology [23]. However, CSF HSV PCR assays are typically negative in patients with more typical relapsed disease within the first 4 months of HSE. Treatment usually is with repeat course of ACV along with corticosteroids, and generally there is recovery to prerelapse disability state. It is uncertain to what extent the neurological damage in relapsed disease comes from direct viral cytolysis and to what extent from inflammatory cell mediators.

To answer the question of whether recurrent disease could be prevented by a longer term shutdown of HSV expression, the National Institute of Allergy and Infectious Diseases sponsored a multicenter trial titled Long Term Treatment of HSV with Valacyclovir. This Phase III double-blind study design involved valacyclovir at 2 g three times daily orally for 90 days (or placebo) with the primary end point being survival with no or minimal neuropsychological impairment at 90 days after study drug initiation. To be eligible, patients were required to have a laboratory-confirmed diagnosis of HSE, 14–21 days of treatment of ACV at 30–60 mg/kg/day and initiation of study drug within 3 days of completion of ACV. The study was completed in February 2011, but not yet reported [201].

Corticosteroids in HSE

Although most investigators consider immunopathogenesis likely in recurrent disease, it is controversial whether, or to what degree, immune mechanisms contribute to the CNS damage of acute, sporadic HSE. Arguing in favor of an immune role is a Japanese retrospective study that found corticosteroid use with ACV was a significant positive predictor of HSE outcome [24]. Also in support of such combination therapy is a report in a laboratory HSE model with MRI monitoring [25].

In an attempt to settle the question, a German-based group designed a randomized double-blind, placebo-controlled study (named GACHE) [26]. Dexamethasone (DEX) at 40 mg/d for 4 days (or placebo) was added to ACV. Unlike the Japanese retrospective study where corticosteroid dose and schedule varied but corticosteroids were started concurrently with ACV, in the German study, ACV was started when clinically indicated and DEX or placebo was delayed until PCR confirmation of HSE. The primary end point was a favorable score at 6 months on the modified Rankin scale (i.e., able to look after one's own affairs without assistance).

There is a moderate amount of anecdotal experience with DEX therapy in HSE, including what was considered a beneficial effect as sole therapy prior to the antiviral drug era. The effect of DEX then was attributed to a decrease in brain swelling. Clinical practice has subsequently varied. In published antiviral trials, supportive-care corticosteroid use frequency has been as high as 90% of patients [21]. If GACHE confirms a corticosteroid effect, the anticipation would be for a modest benefit at best; however, such a benefit would encourage the exploration of other anti-inflammatory and immunomodulatory approaches in HSE. This review considers experimental studies that establish the rationale for immunomodulation in HSE, particularly with intravenous immunoglobulin (IVIG).

Experimental studies in murine models supporting a role for immune mechanisms in HSE

Experimental studies of HSE have been performed primarily in the mouse model because of the availability of a wealth of genetic and immunological resources, including inbred mouse strains that are variably susceptible to HSE. Early genetic studies involving the analysis of crosses between susceptible and resistant mouse strains suggested that one or two dominantly inherited genes determined resistance to HSE, and in more recent studies the authors and others reached a similar conclusion [27–30]. In further studies to define the genetic basis of susceptibility to HSE, the authors reported that resistance and susceptibility to HSE could be transferred to B6 immunodeficient mice lacking B and T cells by bone marrow (BM) from resistant C57BL/6/J (B6) or susceptible 129S6 (129) mice, respectively, which implicated immune responses from 129 BM mice in the etiology of HSE [30]. Likewise, Figure 2 shows that transfer of B6 BM or spleen cells into 129-Rag mice prevented the development of fatal HSE, whereas most recipients of 129-spleen cells succumbed, which confirms the role of 129-mediated immune responses in fatal HSE. Here the authors review studies that support a role for immune-mediated mechanisms in the pathogenesis of HSE.

Inflammation & HSE

It has been shown, using a variety of approaches, that mortality in susceptible 129 mice is primarily due to immune pathology rather than virus-mediated cytopathology [31]. Mortality in 129 and BALB/c mice was associated with focal BS lesions densely populated by infiltrating Ly6Chigh monocytes and neutrophils [31,32], whereas in contrast, resistant B6 mice had mild BS inflammation and no lesions. Moreover, as discussed in detail later, delayed treatment with the antiviral drug ACV starting on day 4 postinfection (pi) allowed us to effectively separate viral replication and inflammation, and this provided irrefutable evidence for exaggerated CNS inflammatory responses rather than viral replication being the cause of fatal HSE [31,32]. A key question is why susceptible and resistant mouse strains mount such divergent CNS inflammatory responses to infection with virulent strains such as HSV-1 17+. The authors speculate that this relates to how the infection is first perceived in these strains.

Detection of HSV-1 infection by pattern recognition receptors

Cells expressing pattern recognition receptors (PRRs) that bind pathogen-associated molecular patterns (PAMPs) respond to HSV infection by initiating inflammatory responses, which include prominent production of IFN-I and coordinated activation of innate immune response genes [33]. A diverse array of germline-encoded PRRs including Toll-like receptors (TLRs), NOD-like receptors, RIG-like receptors, RNA polymerase III, IL-1 receptors and AIM2-like receptors function as sentinels to detect invading pathogens in virtually all cellular compartments [33–35]. Upon binding pathogen components, these PRRs initiate signaling cascades that converge on the activation of transcription factors including NF- κ B, AP-1 and interferon regulatory factors to promote the expression of genes encoding proteins with proinflammatory and microbicidal activities and IFN-I [36]. TLRs are membrane-embedded PRRs that survey the endosomal compartment (TLR3, 7–9) and the extracellular environment (all other TLRs) for PAMPs comprising nucleic acids and lipids and proteins, respectively. Numerous studies have shown that recognition of HSV PAMPs by TLRs leads to the production of proinflammatory cytokines including IFN-I in various cell types and the induction of innate immune responses [35].

Recognition of HSV proteins by TLR2, which is expressed mainly by myeloid cells, microglia, astrocytes and neutrophils, activates signaling pathways that stimulate production of proinflammatory cytokines and the production of IFN-I. Activation of TLR2 by HSV has been shown to have both beneficial and deleterious effects in host protection, and a notable study showed that neonatal TLR2-knockout (KO) mice were protected from lethal encephalitis. Importantly, the absence of TLR2 signaling did not affect viral loads in the CNS of infected mice, but rather the enhanced survival of TLR2 KO mice compared with wild-type mice correlated with decreased levels of MCP-1 and dramatically reduced monocyte infiltration into the CNS, which implicated inflammatory monocytes in the development of fatal HSE [37,38]. Following ocular HSV-1 inoculation, TLR2 signaling was also shown to contribute to development of inflammatory lesions in the cornea that are a major cause of blindness in humans [39]. The HSV ligand(s) responsible for activation of TLR2 are unknown but interestingly, most HSV-1 laboratory strains and clinical isolates failed to activate TLR2 [40]. Further studies have revealed that TLR2 sensing of HSV *in vivo* depends on the combination of mouse and HSV strains, route of inoculation and specific target tissues involved and significant differences have been observed for infection with HSV-2 either by intraperitoneal or vaginal inoculation [40,41]. Since the virus ligand for TLR2 has not been identified, it is possible that this could be cellular constituents contaminating the virus preparation or a cell-associated protein; this is an important issue, as TLR2 is the only PRR that has been shown to promote deleterious inflammatory responses in the context of HSV infection.

The HSV genome is G + C rich, lacks CpG methylation and is enriched for immunostimulatory CpG motifs typical of microbial DNAs [42] that, when recognized by TLR9, trigger production of IFN-I and inflammatory cytokines by a MyD88-dependent mechanism resulting in induction of innate host responses [43]. The absence of TLR9 or the TIR-domain- containing adapter molecule, MyD88, did not increase susceptibility to HSV infection initiated by intraperitoneal or corneal inoculation of mice [44,45], whereas inoculation via the intranasal route significantly enhanced susceptibility to HSE. The incidence of fatal HSE was significantly increased in TLR9-deficient mice and TLR2/9 double KO mice succumbed, although all paradoxically, TLR9 deficiency did not affect survival following intracranial inoculation of mice [46]. By contrast, absence of TLR2 did not impact virus clearance or survival following intranasal inoculation of resistant B6 mice with HSV-1. This suggests that protection against fatal HSE is mediated by synergistic interactions between TLR2 and TLR9 [41,47]. These differences in TLR protection against

HSE may reflect tissue-specific MyD88-dependent and -independent pathways for IFN-I production or, alternatively, result from differences in the combination of mouse and HSV strains used in different studies [35]. Several other intracellular and extracellular sensors, including TLR3, RIG-1, DAI, IFI16, STING and C-type lectin receptors are involved in detecting HSV nucleic acids [48–50] and triggering signaling pathways that also mostly converge on IFN-I production and induction of innate host defenses. It should be noted that some molecules, such as Pol III and STING, can function both as a PRR or as an adaptor or downstream effector molecule depending on the particular microbe involved [34,51,52]. Only some HSV-1 strains such as 17+ and McKrae trigger excessive CNS inflammatory responses compared with less virulent strains such as F and KOS in susceptible mouse strains such as BALB/c and 129, which suggests that these strains are recognized differently by PRRs. Understanding the molecular basis for this differential recognition may provide important information linking the activation of PRRs to initiation of deleterious inflammatory responses resulting in HSE. A thorough review of the beneficial and potentially deleterious host inflammatory responses initiated by recognition of HSV-1 infection by various PRRs in the context of development of HSE by Conrady *et al.* is recommended reading, given the cursory coverage of this topic here [53].

Role of proinflammatory cytokines & chemokines involved in HSE

Prominent induction of IFN- α/β , Type I IFN, in response to the activation of various PRRs by HSV and corresponding rapid deployment of HSV-encoded countermeasures to evade IFN's antiviral effects indicate an important role for IFN-I in defense against HSV infection and indeed, numerous published studies support this view [35,45,54–56]. Although IFN-I is renowned for its antiviral effects, over the last decade it has gained prominence as an important immune regulator in common with IFN- γ (IFN-II) that is involved in orchestrating innate and adaptive responses to HSV-1. However, IFN is not always protective and its effects depend on virus strain and host genetic background, the immune cells mediating innate and adaptive responses and other downstream effector cytokines and chemokines. Thus, antibody-mediated *in vivo* neutralization of downstream chemokines induced by IFN- γ such as the CXCR3 ligands monokine induced by IFN- γ (MIG, CXCL9) and interferon inducible protein-10 (IP-10, CXCL10) or genetic ablation of CXCR3 during HSV infection of susceptible BALB/c mice significantly enhanced survival, whereas survival of resistant B6 mice was not affected by the absence of CXCR3 signaling [57]. Similarly, IFN- γ did not affect CNS viral titers in susceptible 129 and BALB/c mice or resistant B6 mice infected with avirulent strains of HSV-1-like KOS that do not cause HSE [58,59], while it enhanced protection against HSE in susceptible 129 and BALB/c mice inoculated with the virulent HSV-17+ strain without affecting viral replication [60,61]. Enhanced survival could be attributed to IFN- γ regulating myeloid cell infiltration into the CNS [61]. Several studies by Lokensgard and colleagues have demonstrated vigorous microglial responses with the production of chemokines, cytokines and NO during HSV infection that nonetheless did not appear to contribute to protection. However, as these results derive primarily from *in vitro* studies, the precise role of microglia in the pathogenesis of HSE remains to be elucidated [62–64].

B6 mice are resistant to fatal HSE compared with most other inbred mouse strains, particularly A/J, BALB/c and 129 [27,28,30,65]. The idea that this differential resistance could stem from intrinsic differences in the ability of the different mouse strains to induce rapid IFN-I responses has gained much support [66–68]. However, it was observed that despite producing higher levels of IFN-I in the BS compared with resistant B6 mice, only susceptible 129 mice infected with the virulent HSV-17+ succumbed to HSE (Figure 3). Studies using IFN- α/β and IFN- $\alpha/\beta/\gamma$ receptor (IFN- α/β R and IFN- $\alpha/\beta/\gamma$ R) KO mice on the susceptible 129 strain background [59,69] have confirmed the importance of IFN-I for

controlling HSV replication and spread. However, since all wild-type and IFN- α/β KO mice infected with the avirulent HSV-1 KOS strain survived, it is evident that the absence of IFN- α/β signaling does not necessarily predispose to developing fatal HSE, at least in the 129 mouse strain [59]. Moreover, infection of wild-type or IFN- α/β KO 129-mice with the virulent HSV-1 strain 17+ was uniformly fatal [30,59], which shows that IFN- α/β signaling cannot prevent fatal HSE in genetically susceptible mice. An often cited study that purportedly demonstrated the protective effect of IFN- α/β is seriously flawed because it compared HSV-1 infection in IFN- α/β and IFN- $\alpha/\beta/\gamma$ deficient mice on the susceptible 129 strain background using resistant B6 as wild-type controls instead of 129 mice [70]. Support for the importance of IFN-I in control of HSV infection comes from several recent reports that patients with mutations in *UNC93B*, *TLR3* and *STAT1* that impair the IFN-I pathway are more susceptible to HSE [71–73]. Aside from induction of IFN-I, *TLR3* and *UNC93B* mediate the response of other genes. For example, *UNC93B* is required for translocation of *TLR3*, *TLR7* and *TLR9* from the endoplasmic reticulum to endosomes hence its absence would impact signaling via all three genes [74]. However, an important caveat in these studies is that the defective IFN-I responses were determined by *in vitro* studies of patient-derived cells, but no clear pathway(s) linking these cellular genetic defects to increased susceptibility to HSE has been presented.

Studies in mice and patients aimed at defining the role of IFN-I in HSV disease have focused primarily on local antiviral effects. However, IFN-I can exert effects well beyond sites of infection that include priming of T cells, conditioning naive monocytes in the BM and inducing BM hematopoietic stem cells to enter the cell cycle [75]. Viruses that replicate in specific target tissues, for example the lung, trigger vigorous local inflammatory responses that include production of cytokines, chemokines and growth factors that promote infiltration of effector leukocytes [75,76]. An important recent study showed that IFN-I produced in the lung in response to influenza or Sendai virus infection was transported via blood to the BM, where it conditioned monocytes to be more responsive and resistant to infection by a broad array of viruses [77]. Communication between the lung and the BM, the primary site of hematopoiesis, via IFN-I was essential for recruitment of monocytes with an acquired antiviral activity that led to efficient clearance of virus from the lung [75,77,78]. An emerging theme is that proinflammatory cytokines, including IFN-I, are crucially involved in regulating the differentiation of hematopoietic stem cells in BM that are now recognized as ‘first responders’ in microbial infections. Consequently, dysregulated cytokine responses can jeopardize initiation of appropriately controlled inflammatory responses to clear viral infections without concomitant bystander immune pathology [75,79–81]. Thus, the significantly elevated levels of IFN-I produced by infected susceptible 129 mice (Figure 3) and the ensuing hyperinflammatory environment likely contributes to the development of HSE by promoting emigration of highly activated Ly6Chigh inflammatory monocytes from the BM to the BS and other sites of infection, resulting in bystander tissue damage that we reported underlies the pathology associated with HSE [32]. In addition to effects on virus replication and spread, deficient IFN-I signaling can abrogate resistance to infection by impairing control of monocytes and neutrophils, as reported recently for influenza infection [78]. Thus, attributing the resistance of B6 mice relative to susceptible 129, A/J and BALB/cJ mice to an enhanced intrinsic ability for B6 mice to mount rapid IFN-I responses is probably an oversimplification [66,82,83].

A role for immunomodulators in treatment of HSE

HSE can be prevented by treating mice with a 7-day course of ACV starting on day 2 pi, which effectively blocks HSV entry into the CNS and consequently induction of inflammatory responses. Although HSV replication was blocked by day 6 pi in the BS when ACV treatment was started on day 4 pi, infiltration of inflammatory monocytes continued

unabated resulting in death of both ACV and phosphate-buffered saline (PBS)-treated mice with the same kinetics. Interestingly, starting ACV treatment on day 3 pi when HSV first reaches the CNS prolonged survival (Figure 4). Notably, the mice that died beginning day 16 pi had no infectious virus in the BS; instead, an exuberant T-cell infiltrate, rather the usual monocytic infiltrate, was evident in the BS (Table 1), which is reminiscent of clinical descriptions of relapsing HSE in the absence of detectable virus replication [22,84,85]. Figure 5 presents a schematic illustration of the progression of HSV infection from the periphery to the nervous system, depicting the ensuing inflammatory response that culminates in fatal HSE in susceptible mice.

IVIG is a preparation of human polyclonal IgG obtained from the pooled plasma of large numbers of healthy donors. Initially, it was used as replacement therapy for treating primary and secondary immune deficiencies. The use of IVIG has expanded to include treatment of various autoimmune and inflammatory diseases, and it is now US FDA licensed for treating immune thrombocytopenia, Kawasaki's disease, Guillain-Barre syndrome, pediatric HIV infection, allogeneic bone marrow transplantation and chronic inflammatory demyelinating polyneuropathy. IVIG's benefit in these diseases derives from its potent anti-inflammatory and immunomodulatory activities, which also account for the dramatic increase in off-label uses to include multiple sclerosis, systemic lupus erythematosus, recurrent pregnancy loss and Alzheimer's disease [86,87]. However, as discussed in several recent reviews [88–91], IVIG appears to have multiple mechanism(s) of action that remain ill defined despite decades of intense study. The recent report by Ravetch and colleagues demonstrating that the protective effects of IVIG in immune thrombocytopenia, arthritis and nephrotoxic nephritis mouse models could be recapitulated by a subset of IgG molecules sialylated on Asp297 in the Fc domain represented a significant advance. Furthermore, this finding explained the puzzling requirement for high-dose IVIG (1–2 g/kg), as sialylated IgGs (sIgG) comprising 1–3% of IVIG were shown to be effective at an approximately tenfold lower concentration [88,92]. A model to explain the antiinflammatory effects of IVIG mediated by sIgGs and shown to involve a Th2-dependent pathway was published recently [93]. According to this model, sIgGs bind DC-SIGN receptor or the homologous receptor, SIGN-R1, on human and mouse myeloid cells, respectively, to drive IL-33 expression, which in turn activates FcεRI⁺ innate leukocytes (e.g., basophils and mast cells) to produce IL-4. As a result, expression of the inhibitory FcγRIIB receptor on macrophages is dramatically upregulated concomitant with downregulation of the activating FcγRIV receptor, which effectively increases the activation threshold for initiation of inflammation [88,93].

Consistent with HSE being an inflammatory disease, we reported that IVIG protected all mice from developing HSE by exerting potent IL-10-mediated immunomodulatory effects that included modulating production and infiltrating of Ly6Chigh inflammatory monocytes into the CNS and induction of regulatory T-cell populations [32]. Although first reported by Erlich and colleagues more than two decades ago, IVIG protection against HSE gained scant attention [94]. It is important to stress that IVIG protection against HSE is independent of HSV-1-neutralizing antibodies and sIgGs, and that protection can be achieved with low-dose IVIG (160 mg/kg) [32]. We have extended these studies to demonstrate low-dose IVIG protection against West Nile virus encephalitis [Srivastave R, Ramkrishna C, Cantin E. IVIG protects against West Nile virus encephalitis independently of neutralizing antibodies (2013), Manuscript in Preparation], in which Ly6Chigh inflammatory monocytes also contribute to CNS pathology [95]. The sIgG-independent anti-inflammatory and immunomodulatory mechanism(s) of low-dose IVIG are under investigation.

We and others have documented prolonged inflammation involving macrophages, neutrophils and CD4⁺ and CD8⁺ T cells in both sensory ganglia and the CNS of mice inoculated peripherally, with HSV-1 persisting for up to 6 months after infection in the

absence of infectious virus or detectable virus antigen [96–101]. An unexpected finding first reported by the authors was that IFN- γ and TNF expression in sensory ganglia and BS of ocularly inoculated mice were similarly prolonged, and recent studies have confirmed this observation in intranasally inoculated mice [96,100,101]. IFN- γ was not involved in controlling viral replication [60,96] and as there is no clear evidence for its involvement in the observed neuropathology in mice surviving HSE, its precise role is of significant interest. Although high levels of IFN- γ have been linked with clinically deteriorating HSE [20], complete absence of IFN- γ can also exacerbate HSE in the mouse model. Thus, we determined an important role for IFN- γ in regulating inflammatory monocytes and neutrophils, and in its absence, IVIG's anti-inflammatory effects were severely impaired, which suggests that IFN- γ has an important immunomodulatory role, at least in the mouse HSE model [61].

Despite clinical recovery and low HSV-1 loads, chronic progressive cerebral MRI abnormalities have been observed in both patients and experimentally inoculated mice [102,103], which strongly implicates the aforementioned persistent inflammatory responses localized to lesion sites in the neuropathology of HSE. Serial cranial MRI in intranasally infected mice revealed a striking reduction in the severity of long-term MRI abnormalities in mice treated with a combination of ACV and corticosteroids compared with mice receiving either ACV or PBS alone. Since virus loads were dramatically reduced in brains of both groups of ACV-treated mice, the decrease in abnormal MRI findings can be attributed to corticosteroid treatment [25]. Importantly, corticosteroid treatment did not increase HSV-1 replication and dissemination in a rat model of focal encephalitis [104]. A recent study linking persistent CNS inflammatory responses in mice surviving HSE to memory impairment [101], which is the most common neurological deficit in HSE patients treated with ACV, emphasizes the urgent need for new treatment modalities to ameliorate such devastating neurological sequelae, especially in neonates surviving HSE. Significantly improved neurodevelopmental outcomes were recently reported for infants surviving neonatal HSV disease with CNS involvement after suppressive oral ACV for 6 months [105].

As discussed, clinical observations and experimental studies in rodents have provided compelling evidence for immune mechanisms in the etiology of HSE [31,32,106]. The recent revelation that ACV is ineffective in curtailing deleterious CNS inflammatory responses makes clinical evaluation of combined immunotherapeutic and antiviral approaches for treatment of HSE a matter of urgency. The significant differences for HSE in humans and mice that complicates transferring mouse data to humans provide the impetus for a clinical trial. We propose IVIG as an ideal candidate for testing in combination with ACV because it exerts potent immunomodulatory rather than just immunosuppressive or anti-inflammatory activity, which is the case for glucocorticoids such as DEX [107,108]. As an isolated example, an immunocompromised child with disseminated varicella causing multiple organ failure was rescued with concomitant treatment with IVIG and ACV [109]. A significant advantage of IVIG use is that its safety is well established by decades of use as an FDA approved biological for treating a variety of autoimmune and neuroinflammatory diseases, and bacterial and viral diseases [93,110–112].

An important issue is that patients who are suspect for HSE will already be infected and likely have ongoing CNS inflammation. This complicates immunotherapeutic approaches involving dexamethasone, corticosterone (Cort) or IVIG treatment, as in animal studies efficacy is critically dependent on the time of drug administration after infection. Only 7% of mice treated with Cort prior to infection survived compared with 56% of mice treated from day 3 pi; this is attributable to early Cort abrogating innate inflammatory responses required for control of virus replication and appropriate instruction of ensuing adaptive

responses. By contrast, IVIG protection declined steadily with time after infection and was absent by 72 h pi [113], which, as discussed [32], reflects cells not subject to peripheral immune modulation that infiltrated the CNS prior to IVIG treatment. Early elimination of HSV-1 from the CNS is accomplished by the rapid and vigorous production of reactive oxygen species (ROS) by infiltrating monocytes and macrophages that, if not carefully controlled, can cause severe oxidative damage to tissues as in HSE [114–116]. However, recent studies also indicate an important role for ROS in regulating innate responses to HSV-1 [117,118]. Thus, to ameliorate neuropathology arising from inflammatory cells already in the CNS, IVIG treatment could be combined with either ROS inhibitors or stimulators of endogenous antioxidant responses, as these approaches have been beneficial in treating influenza pneumonia [119] and HSE [120], respectively. Although IVIG treatment alone is sufficient to protect all wild-type 129 mice, as it exerts potent antiviral activity in addition to its immunomodulatory activity [32], ACV treatment would need to be included in any clinical trial evaluating IVIG combined with a ROS inhibitor or an inducer of antioxidant activity.

Future perspective

Over the next several years, a report on the German clinical trial GACHE, a controlled study evaluating the corticosteroid dexamethasone added to acyclovir in HSE, is expected [26]. Regardless of the results of this study, we anticipate that there will continue to be reports linking dysregulated host inflammatory responses to the development of HSE and that these reports, along with the results reviewed here, will provide a strong incentive for clinical trials combining antiviral and immunomodulatory drug therapy.

The increasingly broad off-label application of IVIG for treating a variety of neuroinflammatory diseases is expected to increase studies on deciphering its immunomodulatory mechanism(s) of action, which will hopefully yield alternative, less complex immunomodulatory drugs, thus alleviating demand for IVIG, which is costly and in short supply.

Viruses are masters of immune evasion and we are learning to exploit their talents. Poxviruses are notable for encoding a plethora of secreted molecules that antagonize host immune responses. The realization that Serp-1, a viral serpin secreted by myxoma virus-infected cells, acts as an anti-inflammatory agent by blocking the migration of innate immune cells to sites of infection has led to its development as an immunomodulatory drug that was successfully tested in a recent clinical trial of patients with unstable coronary inflammatory disease. Serp-1 and other viral immunomodulatory proteins may prove efficacious in preventing HSE when used in combination with standard antiviral therapy.

If, as predicted by studies in mice, clinical efficacy of combination therapy proves superior to standard antiviral therapy, particularly in terms of improved neurological outcomes for survivors, it will radically change treatment for HSE and likely other viral encephalitides, including West Nile and Japanese encephalitis virus, which also involve inappropriate CNS inflammatory responses.

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References

Papers of special note have been highlighted as:

■ of interest

■ ■ of considerable interest

1. Cabrera CV, Wohlenberg C, Openshaw H, Rey-Mendez M, Puga A, Notkins AL. Herpes simplex virus DNA sequences in the CNS of latently infected mice. *Nature*. 1980; 288(5788):288–290. [PubMed: 6253827]
2. Deatly AM, Spivack JG, Lavi E, O'Boyle DRD, Fraser NW. Latent herpes simplex virus type 1 transcripts in peripheral and central nervous system tissues of mice map to similar regions of the viral genome. *J. Virol.* 1988; 62(3):749–756. [PubMed: 2828670]
3. Chen S-H, Yao H-W, Huang W-Y, et al. Efficient reactivation of latent herpes simplex virus from mouse central nervous system tissues. *J. Virol.* 2006; 80(24):12387–12392. [PubMed: 17005636]
4. Yao H-W, Ling P, Chen S-H, Tung Y-Y, Chen S-H. Factors affecting herpes simplex virus reactivation from the explanted mouse brain. *Virology*. 2012; 433(1):116–123. [PubMed: 22884293] ■ This study provides convincing evidence for HSV-1 reactivation in brainstem.
5. Whitley R. Herpes virus infections of the central nervous system. *Herpes*. 2003; 11(Suppl. 2):1–84.
6. Whitley R, Arvin A, Prober C, et al. Predictors of morbidity and mortality in neonates with herpes simplex virus infections. The National Institute of Allergy and Infectious Diseases collaborative antiviral study group. *N. Engl. J. Med.* 1991; 324(7):450–454. [PubMed: 1988830]
7. Openshaw H. Neurological complications of hematopoietic cell transplantation. In: Blume, KG.; Forman, SJ.; Appelbaum, FR., editors. *Thomas' Hematopoietic Cell Transplantation*. NJ, USA: Wiley-Blackwell; 2004. p. 1653p. 1663
8. Hjalmarsson A, Blomqvist P, Skoldenberg B. Herpes simplex encephalitis in Sweden, 1990–2001. Incidence, morbidity, and mortality. *Clin. Infect. Dis.* 2007; 45(7):875–880. [PubMed: 17806053]
9. Davis LE, Johnson RT. An explanation for the localization of herpes simplex encephalitis? *Ann. Neurol.* 1979; 5(1):2–5. [PubMed: 426465]
10. Damasio AR, Van Hoesen GW. The limbic system and the localisation of herpes simplex encephalitis. *J. Neurol. Neurosurg. Psychiatry*. 1985; 48(4):297–301. [PubMed: 3998736]
11. Wainwright MS, Martin PL, Morse RP, et al. Human herpesvirus 6 limbic encephalitis after stem cell transplantation. *Ann. Neurol.* 2001; 50(5):612–619. [PubMed: 11706967]
12. Tuzun E, Dalmau J. Limbic encephalitis and variants: classification, diagnosis and treatment. *Neurologist*. 2007; 13(5):261–271. [PubMed: 17848866]
13. Livorsi D, Anderson E, Qureshi S, Howard M, Wang Y, Franco-Paredes C. Brainstem encephalitis: an unusual presentation of herpes simplex virus infection. *J. Neurol.* 2010; 257(9): 1432–1437. [PubMed: 20495814]
14. Lakeman FD, Whitley RJ. National Institute of Allergy and Infectious Diseases collaborative antiviral study group. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. *J. Infect. Dis.* 1995; 171(4):857–863. [PubMed: 7706811]
15. Whitley R. Diagnosis and treatment of herpes simplex encephalitis. *Annu. Rev. Med.* 1981; 32:335–340. [PubMed: 7013672]
16. Skoldenberg B, Forsgren M, Alestig K, et al. Acyclovir versus vidarabine in herpes simplex encephalitis. Randomised multicentre study in consecutive Swedish patients. *Lancet*. 1984; 2(8405):707–711. [PubMed: 6148470]
17. Mcgrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome. *J. Neurol. Neurosurg. Psychiatry*. 1997; 63(3):321–326. [PubMed: 9328248]
18. Raschilas F, Wolff M, Delatour FDR, et al. Outcome of and prognostic factors for Herpes simplex encephalitis in adult patients. Results of a multicenter study. *Clin. Infect. Dis.* 2002; 35(3):254–260. [PubMed: 12115090]

19. Hjalmarsson A, Granath F, Forsgren M, Brytting M, Blomqvist P, Skoldenberg B. Prognostic value of intrathecal antibody production and DNA viral load in cerebrospinal fluid of patients with herpes simplex encephalitis. *J. Neurol.* 2009; 256(8):1243–1251. [PubMed: 19353228]
20. Kamei S, Taira N, Ishihara M, et al. Prognostic value of cerebrospinal fluid cytokine changes in herpes simplex virus encephalitis. *Cytokine.* 2009; 46(2):187–193. [PubMed: 19261488]
21. Aurelius E, Forsgren M, Skoldenberg B, Strannegard O. Persistent intrathecal immune activation in patients with herpes simplex encephalitis. *J. Infect. Dis.* 1993; 168(5):1248–1252. [PubMed: 8228358]
22. Sköldenberg B, Aurelius E, Hjalmarsson A, et al. Incidence and pathogenesis of clinical relapse after herpes simplex encephalitis in adults. *J. Neurol.* 2006; 253(2):163–170. [PubMed: 16222428]
23. Yamada S, Kameyama T, Nagaya S, Hashizume Y, Yoshida M. Relapsing herpes simplex encephalitis: pathological confirmation of viral reactivation. *J. Neurol. Neurosurg. Psychiatry.* 2003; 74(2):262–264. [PubMed: 12531966]
24. Kamei S, Sekizawa T, Shiota H, et al. Evaluation of combination therapy using aciclovir and corticosteroid in adult patients with herpes simplex virus encephalitis. *J. Neurol. Neurosurg. Psychiatry.* 2005; 76(11):1544–1549. [PubMed: 16227548]
25. Meyding-Lamade UK, Oberlinner C, Rau PR, et al. Experimental herpes simplex virus encephalitis. a combination therapy of acyclovir and glucocorticoids reduces long-term magnetic resonance imaging abnormalities. *J. Neurovirol.* 2003; 9(1):118–125. [PubMed: 12587075] ■ Demonstrates that combination treatment with acyclovir (ACV) + glucocorticoids suppressed HSV-induced inflammatory CNS lesions, whereas ACV alone was ineffective despite inhibition of virus replication.
26. Martinez-Torres F, Menon S, Pritsch M, et al. Protocol for German trial of Acyclovir and Corticosteroids in Herpes-simplex-virus-Encephalitis (GACHE): a multicenter, multinational, randomized, double-blind, placebo-controlled German, Austrian and Dutch trial [ISRCTN45122933]. *BMC Neurol.* 2008; 8(1):40. [PubMed: 18959773]
27. Lopez C. Resistance to herpes simplex virus – type 1 (HSV-1). *Curr. Top. Microbiol. Immunol.* 1981; 92:15–24. [PubMed: 6171384]
28. Kastrukoff LF, Lau AS, Puterman ML. Genetics of natural resistance to herpes simplex virus type 1 latent infection of the peripheral nervous system in mice. *J. Gen. Virol.* 1986; 67(Pt 4):613–621. [PubMed: 3007658]
29. Simmons A. H-2-linked genes influence the severity of herpes simplex virus infection of the peripheral nervous system. *J. Exp. Med.* 1989; 169(4):1503–1507. [PubMed: 2538554]
30. Lundberg P, Welander P, Openshaw H, et al. A locus on mouse chromosome 6 that determines resistance to herpes simplex virus also influences reactivation, while an unlinked locus augments resistance of female mice. *J. Virol.* 2003; 77(21):11661–11673. [PubMed: 14557652]
31. Lundberg P, Ramakrishna C, Brown J, et al. The immune response to herpes simplex virus type 1 infection in susceptible mice is a major cause of CNS pathology resulting in fatal encephalitis. *J. Virol.* 2008; 82(14):7078–7088. [PubMed: 18480436] ■■ This study used ACV to effectively separate virus replication from inflammation to demonstrate unequivocally that immune pathology was primarily responsible for fatal HSV encephalitis (HSE).
32. Ramakrishna C, Newo ANS, Shen Y-W, Cantin E. Passively administered pooled human immunoglobulins exert IL-10 dependent anti-inflammatory effects that protect against fatal HSV encephalitis. *PLoS Pathog.* 2011; 7(6):e1002071. [PubMed: 21655109] ■■ This study shows that intravenous immunoglobulin (IVIG) protects against HSE independently of neutralizing antibodies by a mechanism involving IL-10 suppression of Ly6C^{hi} inflammatory monocytes in the CNS.
33. Rathinam VA, Fitzgerald KA. Innate immune sensing of DNA viruses. *Virology.* 2011; 411(2): 153–162. [PubMed: 21334037]
34. Chiu YH, Macmillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell.* 2009; 138(3):576–591. [PubMed: 19631370]
35. Chew T, Taylor K, Mossman K. Innate and adaptive immune responses to herpes simplex virus. *Viruses.* 2009; 1(3):979–1002. [PubMed: 21994578]
36. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell.* 2010; 140(6):805–820. [PubMed: 20303872]

37. Kurt-Jones EA, Chan M, Zhou S, et al. Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. *Proc. Natl. Acad. Sci. USA.* 2004; 101(5):1315–1320. [PubMed: 14739339] ■ This study showed that signaling via TLR2 contributed to the development of lethal HSE without affecting virus replication in the CNS.
38. Kurt-Jones EA, Belko J, Yu C, et al. The role of Toll-like receptors in herpes simplex infection in neonates. *J. Infect. Dis.* 2005; 191(5):746–748. [PubMed: 15688290]
39. Sarangi PP, Kim B, Kurt-Jones E, Rouse BT. Innate recognition network driving herpes simplex virus-induced corneal immunopathology: role of the Toll pathway in early inflammatory events in stromal keratitis. *J. Virol.* 2007; 81(20):11128–11138. [PubMed: 17686871]
40. Sato A, Linehan MM, Iwasaki A. Dual recognition of herpes simplex viruses by TLR2 and TLR9 in dendritic cells. *Proc. Natl. Acad. Sci. USA.* 2006; 103(46):17343–17348. [PubMed: 17085599]
41. Sorensen LN, Reinert LS, Malmgaard L, Bartholdy C, Thomsen AR, Paludan SR. TLR2 and TLR9 synergistically control herpes simplex virus infection in the brain. *J. Immunol.* 2008; 181(12): 8604–8612. [PubMed: 19050280]
42. Lundberg P, Welander P, Han X, Cantin E. Herpes simplex virus type 1 DNA is immunostimulatory *in vitro* and *in vivo*. *J. Virol.* 2003; 77(20):11158–11169. [PubMed: 14512563]
43. Haas T, Metzger J, Schmitz F, et al. The DNA sugar backbone 2' deoxyribose determines Toll-like receptor 9 activation. *Immunity.* 2008; 28(3):315–323. [PubMed: 18342006]
44. Hochrein H, Schlatter B, O'Keefe M, et al. Herpes simplex virus type-1 induces IFN- α production via Toll-like receptor 9-dependent and -independent pathways. *Proc. Natl Acad. Sci. USA.* 2004; 101(31):11416–11421. [PubMed: 15272082]
45. Krug A, Luker GD, Barchet W, Leib DA, Akira S, Colonna M. Herpes simplex virus type 1 (HSV-1) activates murine natural interferon-producing cells (IPC) through Toll-like receptor 9. *Blood.* 2003; 103(4):1433–1437. [PubMed: 14563635]
46. Wang JP, Bowen GN, Zhou S, et al. Role of specific innate immune responses in herpes simplex virus infection of the central nervous system. *J. Virol.* 2011; 86(4):2273–2281. [PubMed: 22171256]
47. Lima GK, Zolini GP, Mansur DS, et al. Toll-like receptor (TLR) 2 and TLR9 expressed in trigeminal ganglia are critical to viral control during herpes simplex virus 1 infection. *Am. J. Pathol.* 2010; 177(5):2433–2445. [PubMed: 20864677]
48. Melchjorsen J. Sensing herpes: more than Toll. *Rev. Med. Virol.* 2012; 22(2):106–121. [PubMed: 22020814]
49. Paludan SRR, Bowie AG, Horan KA, Fitzgerald KA. Recognition of herpesviruses by the innate immune system. *Nat. Rev. Immunol.* 2011; 11(2):143–154. [PubMed: 21267015]
50. Holm CK, Jensen SB, Jakobsen MR, et al. Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. *Nat. Immunol.* 2012; 13(8):737–743. [PubMed: 22706339]
51. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat. Immunol.* 2009; 10(10):1065–1072. [PubMed: 19609254]
52. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. *Proc. Ntl Acad. Sci. USA.* 2012; 109(47):19386–19391.
53. Conrady CD, Drevets DA, Carr DJ. Herpes simplex type I (HSV-1) infection of the nervous system: is an immune response a good thing? *J. Neuroimmunol.* 2010; 220(1–2):1–9. [PubMed: 19819030]
54. Ellermann-Eriksen S. Macrophages and cytokines in the early defence against herpes simplex virus. *Virol. J.* 2005; 2(1):59. [PubMed: 16076403]
55. Paladino P, Mossman KL. Mechanisms employed by herpes simplex virus 1 to inhibit the interferon response. *J. Interferon Cytokine Res.* 2009; 29(9):599–607. [PubMed: 19694546]
56. Rasmussen SB, Sorensen LN, Malmgaard L, et al. Type I interferon production during herpes simplex virus infection is controlled by cell-type-specific viral recognition through Toll-like receptor 9, the mitochondrial antiviral signaling protein pathway, and novel recognition systems. *J. Virol.* 2007; 81(24):13315–13324. [PubMed: 17913820]

57. Lundberg P, Openshaw H, Wang M, Yang HJ, Cantin E. Effects of CXCR3 signaling on development of fatal encephalitis and corneal and periocular skin disease in HSV-infected mice are mouse-strain dependent. *Invest. Ophthalmol. Vis. Sci.* 2007; 48(9):4162–4170. [PubMed: 17724202]
58. Cantin E, Tanamachi B, Openshaw H, Mann J, Clarke K. Gamma interferon (IFN-gamma) receptor null-mutant mice are more susceptible to herpes simplex virus type 1 infection than IFN-gamma ligand null-mutant mice. *J. Virol.* 1999; 73(6):5196–5200. [PubMed: 10233988]
59. Luker GD, Prior JL, Song J, Pica CM, Leib DA. Bioluminescence imaging reveals systemic dissemination of herpes simplex virus type 1 in the absence of interferon receptors. *J. Virol.* 2003; 77(20):11082–11093. [PubMed: 14512556]
60. Lekstrom-Himes JA, Leblanc RA, Pesnicak L, Godleski M, Straus SE. Gamma interferon impedes the establishment of herpes simplex virus type 1 latent infection but has no impact on its maintenance or reactivation in mice. *J. Virol.* 2000; 74(14):6680–6683. [PubMed: 10864685]
61. Ramakrishna C, Shen Y, Cantin E. Anti inflammatory role of IFN gamma in a viral model of encephalitis. *J. Immunol.* 2011; 186:115–121.
62. Aravalli RN, Hu S, Rowen TN, Palmquist JM, Lokensgard JR. Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. *J. Immunol.* 2005; 175(7):4189–4193. [PubMed: 16177057]
63. Hu S, Sheng WS, Schachtele SJ, Lokensgard JR. Reactive oxygen species drive herpes simplex virus (HSV)-1-induced proinflammatory cytokine production by murine microglia. *J. Neuroinflammation.* 2011; 8:123. [PubMed: 21943001]
64. Marques CP, Hu S, Sheng W, Lokensgard JR. Microglial cells initiate vigorous yet non-protective immune responses during HSV-1 brain infection. *Virus Res.* 2006; 121(1):1–10. [PubMed: 16621100]
65. Simmons A, La Vista AB. Neural infection in mice after cutaneous inoculation with HSV-1 is under complex host genetic control. *Virus Res.* 1989; 13(3):263–270. [PubMed: 2549748]
66. Pedersen EB, Haahr S, Mogensen SC. X-linked resistance of mice to high doses of herpes simplex virus type 2 correlates with early interferon production. *Infect. Immun.* 1983; 42(2):740–746. [PubMed: 6196293]
67. Ellermann-Eriksen S, Liberto MC, Iannello D, Mogensen SC. X-linkage of the early *in vitro* alpha/beta interferon response of mouse peritoneal macrophages to herpes simplex virus type 2. *J. Gen. Virol.* 1986; 67(Pt 6):1025–1033. [PubMed: 3011968]
68. Ellermann-Eriksen S, Sommerlund M, Mogensen SC. Differential sensitivity of macrophages from herpes simplex virus-resistant and -susceptible mice to respiratory burst priming by interferon-alpha/beta. *J. Gen. Virol.* 1989; 70(Pt 8):2139–2147. [PubMed: 2769233]
69. Leib DA, Harrison TE, Laslo KM, Machalek MA, Moorman NJ, Virgin HW. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses *in vivo*. *J. Exp. Med.* 1999; 189(4):663–672. [PubMed: 9989981] ■■ This study demonstrated the importance of type I IFN for controlling HSV and additionally revealed the importance of host factors in determining the phenotype of HSV mutants.
70. Vollstedt S, Arnold S, Schwerdel C, et al. Interplay between alpha/beta and gamma interferons with B, T, and natural killer cells in the defense against herpes simplex virus type 1. *J. Virol.* 2004; 78(8):3846–3850. [PubMed: 15047800]
71. Zhang S-Y, Jouanguy E, Ugolini S, et al. TLR3 deficiency in patients with herpes simplex encephalitis. *Science.* 2007; 317(5844):1522–1527. [PubMed: 17872438]
72. Casrouge A, Zhang S-Y, Eidenschenk C, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science.* 2006; 314(5797):308–312. [PubMed: 16973841]
73. Guo Y, Audry M, Ciancanelli M, et al. Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency. TLR3 is otherwise redundant in protective immunity. *J. Exp. Med.* 2011; 208(10):2083–2098. [PubMed: 21911422]
74. Fukui R, Miyake K. Controlling systems of nucleic acid sensing-TLRs restrict homeostatic inflammation. *Exp. Cell Res.* 2012; 318(13):1461–1466. [PubMed: 22507273]
75. Lopez CB, Hermesh T. Systemic responses during local viral infections: type I IFNs sound the alarm. *Curr. Opin. Immunol.* 2010; 23(4):495–499. [PubMed: 21752617]

76. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.* 2011; 11(11):762–774. [PubMed: 21984070]
77. Hermesh T, Moltedo B, Moran TM, Lúpez CB. Antiviral instruction of bone marrow leukocytes during respiratory viral infections. *Cell Host Microbe.* 2010; 7(5):343–353. [PubMed: 20478536]
78. Seo S-U, Kwon H-J, Ko H-J, et al. Type I interferon signaling regulates Ly6C monocytes and neutrophils during acute viral pneumonia in mice. *PLoS Pathog.* 2011; 7(2):e1001304. [PubMed: 21383977] ■ Along with [77], these papers show the importance of type I IFN in shaping antiviral immune responses by modulating bone marrow hematopoietic cells.
79. Boiko JR, Borghesi L. Hematopoiesis sculpted by pathogens: Toll-like receptors and inflammatory mediators directly activate stem cells. *Cytokine.* 2012; 57(1):1–8. [PubMed: 22079335]
80. Takizawa H, Boettcher S, Manz MG. Demand-adapted regulation of early hematopoiesis in infection and inflammation. *Blood.* 2012; 119(13):2991–3002. [PubMed: 22246037]
81. King KY, Goodell MA. Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response. *Nat. Rev. Immunol.* 2011; 11(10):685–692. [PubMed: 21904387]
82. Halford WP, Balliet JW, Gebhardt BM. Re-evaluating natural resistance to herpes simplex virus type 1. *J. Virol.* 2004; 78(18):10086–10095. [PubMed: 15331741]
83. Ellermann-Eriksen S, Justesen J, Mogensen SC. Genetically determined difference in the antiviral action of alpha/beta interferon in cells from mice resistant or susceptible to herpes simplex virus type 2. *J. Gen. Virol.* 1986; 67(Pt 9):1859–1866. [PubMed: 3018128]
84. Peters N, Fesl G, Theil D, et al. Subacute herpes simplex encephalitis presenting as relapsing encephalitis. *J. Neurol.* 2010; 257(5):843–845. [PubMed: 20099066]
85. Brown WD, Bearer EL, Donahue JE. Chronic active herpes simplex type 2 encephalitis in an asymptomatic immunocompetent child. *J. Child Neurol.* 2010; 25(7):901–908. [PubMed: 20179002]
86. Looney JR, Huggins J. Use of intravenous immunoglobulin G (IVIG). *Best Pract. Res. Clin. Hematol.* 2006; 19(1):3–25.
87. Kotlan B, Stroncek DF, Marincola FM. Intravenous immunoglobulin-based immunotherapy: an arsenal of possibilities for patients and science. *Immunotherapy.* 2009; 1(6):995–1015. [PubMed: 20635915]
88. Clynes R. Protective mechanisms of IVIG. *Curr. Opin. Immunol.* 2007; 19(6):646–651. [PubMed: 18032008]
89. Durandy A, Kaveri SV, Kuijpers TW, et al. Intravenous immunoglobulins – understanding properties and mechanisms. *Clin. Exp. Immunol.* 2009; 158(Suppl. 1):2–13. [PubMed: 19883419]
90. Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. *Immunol. Rev.* 2010; 236(1):265–275. [PubMed: 20636822]
91. Tha-In T, Bayry J, Metselaar HJ, Kaveri SV, Kwekkeboom J. Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immunol.* 2008; 29(12):608–615. [PubMed: 18926775]
92. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science.* 2006; 313(5787):670–673. [PubMed: 16888140]
93. Anthony RM, Kobayashi T, Wermeling F, Ravetch JV. Intravenous gammaglobulin suppresses inflammation through a novel Th2 pathway. *Nature.* 2011; 475(7354):110–113. [PubMed: 21685887] ■■ This study documents a novel IL-33-dependent anti-inflammatory pathway mediated by sialylated IgGs present in limiting amounts in IVIG.
94. Erlich KS, Dix RD, Mills J. Prevention and treatment of experimental herpes simplex virus encephalitis with human immune serum globulin. *Antimicrob. Agents Chemother.* 1987; 31(7):1006–1009. [PubMed: 2821882] ■ First study to document IVIG protection against HSE and to additionally show that protection was independent of neutralizing antibodies.
95. Getts DR, Terry RL, Getts MT, et al. Ly6c⁺ “inflammatory monocytes” are microglial precursors recruited in a pathogenic manner in West Nile virus encephalitis. *J. Exp. Med.* 2008; 205(10):2319–2337. [PubMed: 18779347]
96. Cantin EM, Hinton DR, Chen J, Openshaw H. Gamma interferon expression during acute and latent nervous system infection by herpes simplex virus type 1. *J. Virol.* 1995; 69(8):4898–4905. [PubMed: 7609058]

97. Cheng H, Tumpey TM, Staats HF, Van Rooijen N, Oakes JE, Lausch RN. Role of macrophages in restricting herpes simplex virus type 1 growth after ocular infection. *Invest. Ophthalmol. Vis. Sci.* 2000; 41(6):1402–1409. [PubMed: 10798656]
98. Liu T, Tang Q, Hendricks RL. Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. *J. Virol.* 1996; 70(1):264–271. [PubMed: 8523535]
99. Decman V, Freeman ML, Kinchington PR, Hendricks RL. Immune control of HSV-1 latency. *Viral Immunol.* 2005; 18(3):466–473. [PubMed: 16212525]
100. Marques CP, Cheeran MCJ, Palmquist JM, Hu S, Urban SL, Lokensgard JR. Prolonged microglial cell activation and lymphocyte infiltration following experimental herpes encephalitis. *J. Immunol.* 2008; 181(9):6417–6426. [PubMed: 18941232]
101. Armien AG, Hu S, Little MR, et al. Chronic cortical and subcortical pathology with associated neurological deficits ensuing experimental herpes encephalitis. *Brain Pathol.* 2010; 20(4):738–750. [PubMed: 20002440]
102. Meyding-Lamade U, Lamade W, Kehm R, et al. Herpes simplex virus encephalitis. chronic progressive cerebral MRI changes despite good clinical recovery and low viral load – an experimental mouse study. *Eur. J. Neurol.* 1999; 6(5):531–538. [PubMed: 10457385]
103. Meyding-Lamade UK, Lamade WR, Wildemann BT, Sartor K, Hacke W. Herpes simplex virus encephalitis: chronic progressive cerebral magnetic resonance imaging abnormalities in patients despite good clinical recovery. *Clin. Infect. Dis.* 1999; 28(1):148–149. [PubMed: 10028092]
104. Thompson KA, Blessing WW, Wesselingh SL. Herpes simplex replication and dissemination is not increased by corticosteroid treatment in a rat model of focal herpes encephalitis. *J. Neurovirol.* 2000; 6(1):25–32. [PubMed: 10786994]
105. Kimberlin DW, Whitley RJ, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N. Engl. J. Med.* 2011; 365(14):1284–1292. [PubMed: 21991950]
106. Openshaw H, Cantin EM. Corticosteroids in herpes simplex virus encephalitis. *J. Neurol. Neurosurg. Psychiatry.* 2005; 76(11):1469. [PubMed: 16227530]
107. Sergerie Y, Boivin G, Gosselin D, Rivest S. Delayed but not early glucocorticoid treatment protects the host during experimental herpes simplex virus encephalitis in mice. *J. Infect. Dis.* 2007; 195(6):817–825. [PubMed: 17299711]
108. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin. Sci.* 1998; 94(6):557–572. [PubMed: 9854452]
109. Lu YC, Fan HC, Wang CC, Cheng SN. Concomitant use of acyclovir and intravenous immunoglobulin rescues an immunocompromised child with disseminated varicella caused multiple organ failure. *J. Pediatr. Hematol. Oncol.* 2011; 33(8):e350–e351. [PubMed: 21127434]
110. Zandman-Goddard G, Krauthammer A, Levy Y, Langevitz P, Shoenfeld Y. Long-term therapy with intravenous immunoglobulin is beneficial in patients with autoimmune diseases. *Clin. Rev. Allergy Immunol.* 2012; 42(2):247–255. [PubMed: 21732045]
111. Niimi N, Kohyama K, Kamei S, Matsumoto Y. Intravenous immunoglobulin therapy prevents development of autoimmune encephalomyelitis and suppresses activation of matrix metalloproteinases. *Neuropathology.* 2011; 31(4):392–400. [PubMed: 21175863]
112. Lehmann HC, Hartung H-P. Plasma exchange and intravenous immunoglobulins: mechanism of action in immune-mediated neuropathies. *J. Neuroimmunol.* 2011; 231(1–2):61–69. [PubMed: 21056913]
113. Dalai SK, Pesnicak L, Miller GF, Straus SE. Prophylactic and therapeutic effects of human immunoglobulin on the pathobiology of HSV-1 infection, latency, and reactivation in mice. *J. Neurovirol.* 2002; 8(1):35–44. [PubMed: 11847590]
114. Schachtele SJ, Hu S, Little MR, Lokensgard JR. Herpes simplex virus induces neural oxidative damage via microglial cell Toll-like receptor-2. *J. Neuroinflammation.* 2010; 7:35. [PubMed: 20584314]
115. Milatovic D, Zhang Y, Olson SJ, et al. Herpes simplex virus type 1 encephalitis is associated with elevated levels of F2-isoprostanes and F4-neuroprostanes. *J. Neurovirol.* 2002; 8(4):295–305. [PubMed: 12161814]

116. Valyi-Nagy T, Olson SJ, Valyi-Nagy K, Montine TJ, Dermody TS. Herpes simplex virus type 1 latency in the murine nervous system is associated with oxidative damage to neurons. *Virology*. 2000; 278(2):309–321. [PubMed: 11118355]
117. Gonzalez-Dosal R, Horan KA, Paludan SRR. Mitochondria-derived reactive oxygen species negatively regulates immune innate signaling pathways triggered by a DNA virus, but not by an RNA virus. *Biochem. Biophys. Res. Commun.* 2012; 418(4):806–810. [PubMed: 22310710]
118. Gonzalez-Dosal R, Horan KA, Rahbek SH, et al. HSV infection induces production of ROS, which potentiate signaling from pattern recognition receptors: role for S-glutathionylation of TRAF3 and 6. *PLoS Pathog.* 2011; 7(9):e1002250. [PubMed: 21949653]
119. Vlahos R, Stambas J, Bozinovski S, Broughton BRS, Drummond GR, Selemidis S. Inhibition of Nox2 oxidase activity ameliorates influenza A virus-induced lung inflammation. *PLoS Pathog.* 2011; 7(2):e1001271. [PubMed: 21304882]
120. Schachtele SJ, Hu S, Lokensgard JR. Modulation of experimental herpes encephalitis-associated neurotoxicity through sulforaphane treatment. *PLoS One*. 2012; 7(4):e36216. [PubMed: 22558388]

Website

201. ClinicalTrials.gov. www.clinicaltrials.gov

Executive summary

Evidence in HSV encephalitis patients of ongoing intrathecal immune stimulation & possible adverse effects of inflammation

- There is persistence of monocyte–macrophage-derived neopterin in cerebrospinal fluid.
- A higher level of IFN- γ is present in more severe HSV encephalitis (HSE) cases.
- Cerebrospinal fluid PCR is often negative for HSV-1 in patients with neurological relapses of clinical HSE.
- There are uncontrolled observations of beneficial effects of corticosteroids added to antiviral therapy in HSE.

Mortality in experimental HSE results from immunopathology

- HSE-susceptible mouse strains (129S6 and BALB/c) have extensive brainstem inflammatory lesions, which are not seen in resistant strains (C57BL/6) despite the presence of infectious virus in the brainstem of both susceptible and resistant strains.
- HSE susceptibility can be transferred by bone marrow cells from a susceptible (129S6) to a resistant (C57BL/6) mouse.
- Similarly, an HSE-susceptible mouse (129S6) can be made resistant by infusion of bone marrow from a resistant-strain mouse (C57BL/6).
- The antiviral drug acyclovir decreases mortality from HSE when given early enough to abrogate brainstem inflammation (day 3 after HSV-1 inoculation), but acyclovir started later (day 4 after HSV-1 inoculation) does not prevent mortality, despite the elimination of brainstem infectious virus.
- Intravenous immunoglobulin (IVIG) decreases HSE mortality through an immunomodulatory effect.

Protective mechanism of IVIG in experimental HSE

- The immunomodulatory effects of IVIG are independent of HSV-neutralizing antibodies.
- These immunomodulatory effects are independent of sialylated IgGs in IVIG.

Unanswered questions concerning inflammatory response in HSE

- Why does a virulent HSV-1 strain elicit exaggerated CNS inflammatory responses in a susceptible (129S6), but not a resistant (C57BL/6) mouse strain?
- What is the contribution of neural cells to the inflammatory response?

Proposed triple therapy in patients with HSE: antiviral, immunomodulatory & neuroprotective

- Acyclovir or alternative antiviral drugs started as early as possible in the clinical course is required to eradicate viral replication and minimize the initial brain inflammation.
- IVIG or alternative immunomodulatory drugs also started as early as possible are required to attenuate the inflammatory response.

- Reactive oxygen species inhibitors or a therapeutic means to stimulate brain endogenous antioxidant responses are added to decrease the neurotoxic effect of inflammatory mediators.

Figure 1. EEG (left) and brain MRI (right) fluid-attenuated inversion recovery in a 46-year-old man who developed abrupt confusion 8 months after allogeneic hematopoietic cell transplantation for acute myelogenous leukemia

EEG showed right-sided periodic sharp waves and MRI showed increased signal in the right hippocampus. Cerebrospinal fluid had 44 white blood cells/ μ l (87% lymphocytes) and 11 red blood cells/ μ l. HSV-1 was cultured from the cerebrospinal fluid. Despite treatment with acyclovir and then foscarnet, the patient became amnesic, unable to form new memory traces.

L: Left; R: Right.

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Figure 2. Hematopoietic cells control decisions on survival

Irradiated 129 Rag-knockout mice received B6 BM while nonirradiated 129 Rag-knockout recipients were transferred with splenocytes isolated from either C57BL/6 (B6) or 129 WT mice. Mice were infected with 3200 PFU HSV 17+ and observed for mortality.

n = 6–10; ***p < 0.0005 for B6 versus 129.

BM: Bone marrow; pi: Postinfection; WT: Wild-type.

Figure 3. Increased inflammation in susceptible 129 mice

(A) 129 mice are highly susceptible to HSV 17+ infection compared with B6 mice. (B) Type I IFN transcripts were measured by RT-PCR in BS (BS IFN- α) or protein in sera by ELISA (sera IFN- α) at day 4 pi. CD45^{hi} inflammatory cells in the BS were detected by flow cytometry (BS CD45^{hi} cells) at days 4 and 8 pi.

**** $p < 0.0001$.

BS: Brainstem; FACS: Fluorescence-activated cell sorting; pi: Postinfection; RT-PCR: Real-time PCR; WT: Wild-type.

Figure 4. Acyclovir administered early but not late is protective

A 7-day ACV course of treatment was started 48 h (D2), 72–80 h (D3) or 96 h (D4) pi after inoculation of mice with HSV 17+; mice were monitored daily for mortality and symptoms of HSV-1 encephalitis necessitating euthanasia. CTRLs: phosphate-buffered saline; n = 12–20 129S6 mice. ACV-D4 versus CTRL = NS.

*p = 0.032; **p = 0.002; ****p < 0.00001.

ACV: Acyclovir; CTRL: Control; D: Day; NS: Nonsignificant; pi: Postinfection.

Figure 5. HSV pathogenesis in mice

Susceptible mice: increased inflammation in BS (lesion hyperintensity in MRI – yellow arrow) and virus spread to cerebrum and spinal cord. Resistant mice: reduced inflammation in BS (no lesion hyperintensity in MRI – yellow box) and limited spread to cerebrum and spinal cord.

BS: Brainstem.

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Table 1

Brainstem infiltrates in acyclovir-treated 129 mice.

Day pi	CD45 ^{hi} (%)	MACs (%)	T cells (%)
D6 control	35	80	<10
D6 ACV	14	65	15
D21 ACV	28	25	60

HSV-infected mice were given phosphate-buffered saline or ACV (50 mg/kg) beginning D3 pi for 1 week. All phosphate-buffered saline-treated control mice died between days 6 and 12 pi. CD45^{hi} infiltrating leukocytes detected by flow cytometry are expressed as the percentage of cells within total brainstem mononuclear cells. The MACs and T cells columns represent the percentage of each subset within the CD45^{hi} leukocyte population.

ACV: Acyclovir; D: Day; MAC: Macrophage; pi: Postinfection.