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Neuroimmunology of central nervous system viral infections: the cells, molecules and mechanisms involved

Carine Savarin and Cornelia C. Bergmann

Address: Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue NC-30, Cleveland, OH 44195 (bergmac@ccf.org; savaric@ccf.org)

Summary

Viral infections of the central nervous system (CNS) necessitate rapid, yet tightly controlled responses to contain viral spread while limiting tissue damage. All CNS resident cell types are equipped with pattern recognition receptors (PRRs) to respond to viruses. The resulting activation of IFN- α/β , proinflammatory cytokines and chemokines is dependent on the virus replication strategy, tropism and PRR distribution. Although IFN- α/β induced antiviral mediators are essential to restrict initial viral spread, adaptive immunity promoted by chemokines, cytokines and metalloproteinases is equally critical in lowering viral burden. Recognition of viral antigen presented by MHC molecules is critical for T cell retention and function. Non lytic clearance mechanisms mediated by IFN- γ and antibodies prevail in providing protection. Targeted intervention can be achieved by PRR stimulation, chemokine-receptor blockade and immune modulation of T cell function. However, due to the extensive positive and negative feedback signaling cascades linking innate and adaptive immune responses, enhanced anti-viral functions will have to be counterbalanced to avoid pathology.

Introduction

Viruses invading the CNS and the consequences

Viruses invading the human central nervous system (CNS) include herpesviruses, JC virus, retroviruses (HIV and HTLV-1), poliovirus, Rabies virus, and insect borne RNA viruses (West Nile Virus (WNV), St Louis encephalitis virus). Whereas infections limited to the leptomeningis cause meningitis generally without consequences, infections targeting glia and neurons commonly cause encephalomyelitis and can be fatal. Long term neurological dysfunction resulting from viral cell destruction or immune pathology are common.

Immune responses to CNS infections and their problems

Control of CNS infections pose a challenge due to the immunologically unique environment and detrimental consequences of damage to specialized, non renewable cells essential for host cognition and mobility [1,2]. Activation of various pattern recognition receptors (PRRs) provides a first line of defense by inducing IFN- α/β , proinflammatory cytokines and chemokines [3–5]. These early responses are protective by activating and amplifying antiviral mediators, as well as recruiting leukocytes expressing antiviral function. However, the same mediators promote tissue damage if not dampened in a timely manner. This review focuses on

Correspondence to: Cornelia C. Bergmann.

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the interdependent mechanisms utilized by CNS resident and infiltrating cells to contain viruses, while minimizing immune pathology. The discussion is based on conceptual insights gained from experimental rodent models due to inherent difficulties in sampling during human CNS infections.

IFN- α/β mediated functions triggered by PRRs: essential, yet unresolved

A first line of defense against viral spread to and within the CNS is provided by IFN- α/β (Fig. 1A), as demonstrated by high virus loads and mortality of mice deficient in IFN- α/β receptor [5–8]. By restricting virus tropism at both extraneural and intraneural sites IFN- α/β is a potent determinant of tropism in addition to viral receptors. Nevertheless, IFN- α/β induction, IFN- α/β receptor signaling, activation of IFN- α/β target genes, as well as their respective mode of action is cell type and virus specific [3–5,9]. Viral nucleic acids trigger IFN- α/β production through membrane associated toll-like receptors (TLRs) and the cytoplasmic-localized retinoic acid-inducible gene I (RIG-I)-like helicases (RLHs) in a ligand dependent manner [3–5]. DNA rich in CpG motifs and herpes virus infection activate TLR-9. RNA viruses and ssRNA activate TLR-7 and TLR-8. TLR-3 is less selective and recognizes dsRNA formed during replication of both RNA and DNA viruses, but can also recognize ssRNA. The RLHs RIG-I and MDA-5 also recognize RNA viruses, but have distinct fine-specificities for RNA structures [4]. PRRs use diverse adaptor proteins and signaling pathways resulting in activation of a distinct set of transcription factors; nevertheless these invariably include NF κ B and IFN-regulatory factors (IRFs) [3,4,9]. TLR-3 and RHLs prominently trigger IRF-3 and -7, which are strong inducers of IFN- α and - β transcription. NF κ B primarily regulates transcription of proinflammatory mediators, including IL-6, TNF- α , IL-12, CXCL10, and CCL5, and has limited ability to induce IFN- β . IFN- α/β signaling triggers transcription of IFN- α genes and IFN stimulated genes (ISGs), resulting in autocrine and paracrine amplification of the original stimulus.

The involvement of PRRs in viral CNS infections is just emerging. TLR-3, -7 and -9 are among the broad array of TLR mRNAs basally expressed in mouse brains and thus implicated in initiating antiviral responses [5,10]. Constitutive TLR expression is most extensive in microglia and astrocytes [5]. Astrocytes stand out in expressing especially high levels of TLR3 mRNA as well as protein at their surface. Neurons, oligodendrocytes and ependymal cells are more limited in their basal, as well as inducible expression patterns. Semliki Forest and rabies virus infections upregulate a significantly broad, yet distinct pattern of TLRs [10]. Interestingly, coordinated PRR amplification involves IFN- α/β dependent and independent pathways. This broad amplification of sensory awareness provides a mechanisms to respond to diverse nucleic acid structures released from apoptotic cells or to viral glycoproteins recognized by surface localized TLR-2 and TLR-4 [3].

Although TLR-3 signaling can induce IFN- α/β in neurons, astrocytes and microglia [11–13], several observations implicate alternate PRRs in viral recognition. Theiler's murine encephalomyelitis virus (TMEV) induced antiviral pathways are TLR-3 independent in astrocytes [12]. TLR-3^{-/-} mice controlled various virus infections, including neurotropic viruses, as effectively as wildtype (wt) mice [14]. Furthermore, survival rates of TLR-3^{-/-} and wt mice infected with WNV intracranially were similar [15]. Nevertheless, increased resistance of TLR-3^{-/-} mice to virus CNS entry following peripheral infection revealed a critical distinction between systemic versus CNS TLR-3 signaling [15]. The detrimental peripheral TLR-3 affect was attributed to increased TNF- α and IL-6 mediated BBB permeability, thus enhancing inflammation, virus CNS entry, and pathology. The role of RHLs in viral CNS replication remains to be investigated. RIG-I is not detected in astrocytes or cortical neurons, but is inducible in astrocytes [16]. Similarly, RIG-I and MDA-5 transcription is induced by WNV infection of primary cortical neurons [17].

The regulation of IFN- α/β and ISGs in diverse CNS cell types during CNS infections in vivo has not been explored extensively. Factors directly affecting viral replication are PKR, OAS, RNaseL, and ISG54 and ISG56, which all modulate translation; Mx, which inhibits viral transcription; and the RNA deaminases ADAR-1 and APOBEC3G, leading to mutations in viral genomes [9]. IFN- α/β responsiveness in CNS cells is indicated by induction of various ISGs, including RNaseL, ISG54 and ISG56, Mx, IRF-7 and -9 following distinct infections [7,17–20]. However, basal PRR and ISG expression in individual cell types strongly influences virus permissiveness. For example, higher WNV susceptibility of neurons compared to macrophages is attributed to undetectable basal levels of RIG-I/MDA5 as well as ISG54 and ISG56 [17]. Tight regulation of the IFN pathway in vivo is supported by IFN- α/β transcription in only a minority of neurons infected with a neurotropic RNA virus [18].

IFN- α/β exerts anti-viral effects at multiple levels including induction of anti-viral genes, augmentation of antigen presentation, and regulation of lymphocyte function and survival [7, 9,21] (Fig. 1A). However, most viruses have evolved to antagonize IFN- α/β pathways at multiple levels from suppressing initial IFN- α/β induction to blocking IFN- α/β signal transduction and inhibiting IFN target proteins [22]. Triggering of protective IFN thus appears a simple preventive strategy to reduce viral spread. Furthermore, exuberant responses may be counteracted by ISGs encoding the suppressor of cytokine signaling (SOCS) proteins, known to downregulate CNS inflammation [23]. A variety of PRR agonists are evaluated to enhance antimicrobial responses in place of direct IFN- β treatment. Synthetic CpG oligonucleotides activate TLR9, synthetic imidazoquinoline components, such as Imiquimod and R848 activate TLR7/8 and poly I:C is used to activate TLR3 as well as RLHs [9]. Nevertheless, TLR-3, RIG-I and MDA5 have distinct, yet overlapping substrate specificities when analyzed for responsiveness to viral infections [4]. Development of agonists selectively targeting PRRs for enhanced IFN- α/β , but not proinflammatory responses, may provide future strategies to stall CNS infection. Such approaches have proven effective in controlling HSV infection [24]. A natural example is the protective role of TLR-7 dependent IFN- α/β initiated by plasmacytoid DC during peripheral MHV infection [8].

Pro inflammatory mediators during CNS infections: good early, bad late

PRR and IFN- α/β signaling induce glial cell activation and alterations in BBB integrity (Fig. 1A). Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) are upregulated coincident with chemokines and pro-inflammatory cytokines [25]. Many of the tyrosine kinases and transcription factors involved in the IFN- α/β signaling cascade also participate in cytokine induced signaling [26]. Furthermore, cell type dependent recruitment of additional protein kinases into the IFN- α/β receptor response pathway fine tune transcriptional regulation of chemokines [9]. Nevertheless, many CNS infections induce common proinflammatory cytokines, e.g. IL-1 α , IL-1 β , TNF α , IL-6 and IL-12, predominantly in astrocytes and microglia [25]. Specifically IL-6, in conjunction with upregulation of adhesion molecules on CNS endothelium and MMP-9 activity, enhances inflammatory cell migration across the BBB [27]. Prolonged or enhanced viral replication is generally associated with increased levels of IL-6 and TNF- α [7,25]. TNF- α appears to have no direct antiviral or disease exacerbating role [1,25], although it may be involved in BBB disruption [15].

MMP expression is a hallmark of CNS damage following infections as well as other inflammatory diseases [28]. MMPs remodel the extracellular matrix, contribute to loss of BBB integrity, promote leukocyte inflammation and disrupt cellular communication. The induction pattern of MMPs and TIMPs is unique to individual viruses, as well as the anatomical site of infection. MHV infection induces MMP-3 and -12 in astrocytes and oligodendroglia, whereas MMP-9 activity is attributed to neutrophils [25]. Neutrophil depletion inhibits lymphocyte CNS infiltration, demonstrating a beneficial role of MMP-9 in BBB breakdown by facilitating

T cell entry [29]. By contrast, neurotropic canine distemper virus infection, a morbillivirus related to human measles virus, induces expression of MMP-2 in astrocytes and MMP-9 predominantly in neurons [30]. Elevated MMPs are also implicated in HIV-associated encephalitis [28]. The degree to which MMPs perturb the BBB and/or local neural communication networks [28] thus varies depending on the type, site and stage of infection. Synthetic MMP inhibitors are available as candidate therapeutics to target virus associated CNS pathologies [31]. MMP inhibitors indeed ameliorate murine CNS autoimmune disease [32]. Nevertheless, the application of inhibitors is premature based on specificity issues and lack of in vivo efficacy in other diseases [31]. Furthermore, the beneficial role of MMPs in migration and repair will require extremely careful evaluation of timing and treatment efficacy [28].

Dual roles of chemokines and their receptors in regulating CNS infection

Neurotropic MHV infection has provided important insights into the functions of chemokines during CNS infections (Fig. 2) [33]. The upregulation of a wide range of chemokines, primarily by astrocytes, is associated with detection of their respective receptors. The regulated expression of these chemokines orchestrates leukocyte recruitment and thereby adaptive antiviral mediators. CXCL10-CXCR3 and CCR5-CCL5 signaling are essential in the antiviral response to MHV and WNV infections by recruiting virus-specific T cells [33–35]. Preferential induction of CXCL10 in the cerebellum by WNV infection promotes a region-specific antiviral T cell response [36]. Abrogation of CXCL10 leads to decreased T cell CNS infiltration, increased viral burden and increased mortality [33]. Interestingly, although both CD4 and CD8 T cells express CXCR3, CNS migration of CD4 T cells appears more stringently dependent on CXCR3-ligand interactions [37]. Similarly, CCR5^{-/-} CD4 T cells transferred into Rag^{-/-} mice are unable to traffic into the CNS, while CCR5 is not required for CD8 T cell recruitment [33]. However, increased antiviral function of CCR5^{-/-} CD8 T cells indicates CCR5 modulates antiviral activity [33]. MHV infected CCR2^{-/-} mice also implicated CCR2 in T cell recruitment to the CNS, yet independent of CCL2 [33]. Therefore, multiple chemokines induced during acute infection enhance CNS recruitment of antiviral lymphocytes. However, if not shut down, chemokine signaling contributes to ongoing pathology during viral persistence. For example, blocking experiments implicate ongoing CXCL10 expression in promoting CD4 T cell recruitment and demyelination [33]. Moreover, reduced CCL5 production, as a result of decreased CD4 T cells, diminishes macrophage infiltration, also resulting in reduced demyelination. CCL2 and CCR2 are associated with tissue damage following both MHV and TMEV infection [38,39], most likely by enhancing CNS macrophage infiltration [33]. Finally, a neuroprotective role for chemokines is suggested by increased mortality of MHV infected CCR1^{-/-} deficient mice, without an apparent defect in virus clearance, CNS leukocyte recruitment or increased demyelination [40].

The regulation of CNS virus infections by chemokines and chemokine receptors makes these molecules prime therapeutic targets to manipulate distinct leukocyte functions. Induction of specific chemokines is a potent strategy to combat viral infection, as exemplified by CXCL10 mediated lymphocyte recruitment and enhanced virus clearance [41]. Paradoxically, prevention of chemokine function limits ongoing inflammation and tissue damage. This dual role of chemokines during viral CNS infections makes their use as a therapeutic targets challenging: Ideally, selective, early activation of chemokines such as CXCL-10 reduces viral burden, while select blocking of macrophage recruiting chemokines ameliorates demyelination. In designing such treatments, cell or tissue specific homeostatic regulation of chemokines, as well as time dependant chemokine functions need to be considered [42]. The lack of one chemokine receptor leads to an increase of related ligand(s). As a single chemokine can bind different receptors, high chemokine expression can lead to unanticipated results. Thus,

targeting the chemokine system to treat virus induced CNS pathologies will require evaluation of redundant and compensating signals.

Antiviral adaptive immunity: strengths and weaknesses

Despite their crucial role in alerting the immune system and stalling viral spread, innate responses rarely suffice to control CNS infections in the absence of adaptive immunity. Natural CNS infections are initiated at extraneural sites. Virus specific immune cells are thus first activated in regional lymph nodes. This is also true for experimental infections [1,25]. Peripheral lymphocyte activation further upregulates adhesion molecules and chemokines receptors, which both facilitate entry of circulating lymphocytes into the CNS. It is critical to note that while trafficking of T cells into the CNS is antigen independent, retention and antiviral function is dependent on MHC-restricted antigen recognition [1,25,43].

The predominant mechanism of T cell mediated viral control in the CNS is IFN- γ dependent, although distinct, yet interdependent functions of perforin and IFN- γ are evident in many models [1,25,44–47]. CD8 T cells are primary mediators reducing viral replication, even in neurons [1,47]. The absence of detectable class II expression on neurons, oligodendrocytes and astrocytes during most viral infections, has implicated CD4 T cells in providing helper function rather than direct antiviral activity [25]. Nevertheless, class II is readily induced on microglia and is present on infiltrating monocytes and dendritic cells during encephalitis. Uptake of viral Ag by these cell types may contribute to both CD8 and CD4 T cell responsiveness via crosspresentation, even if they are not infected [48]. The collaborative and cell type dependent roles of IFN- γ and perforin are well documented in the non lethal MHV model [25] (Fig. 1B). IFN- γ is more critical than perforin in viral control and protection from disease. Furthermore, while perforin suffices to control viral replication in astrocytes and microglia, IFN- γ is necessary to affect replication in oligodendrocytes. The selective inability of transgenic mice with an IFN- γ signaling defect in oligodendroglia to clear virus substantiates direct IFN- γ signaling in these cells [4]. Cell type dependent susceptibilities to T cell mediated antiviral functions likely contribute to viral persistence.

The inability of T cells to effectively eliminate viruses from the CNS requires further mechanistic exploration, but several concepts are emerging. One is the apparent loss of CD8 T cell function during persistent infection [25,50]. Another is the vastly distinct regulation of MHC molecule as well as inhibitory and activating ligands expression on CNS resident cells. Distinct basal levels of mRNAs encoding class I antigen processing components in glia subsets, as well as responsiveness to IFN- α/β and IFN- γ is associated with delayed MHC class I expression on astrocytes and oligodendrocytes compared to microglia during MHV infection [51,52]. Neurons may evade T cell function by their paucity to upregulate MHC class I molecules, their expression of anti-apoptotic molecules, as well as distinct susceptibilities to IFN- γ [1,44,47]. Furthermore, inhibitory interactions between the CD94-NKG2a receptor on CD8 T cells and its Qa-1b ligand on neurons may temper T cell effector function during HSV infection [53]. By contrast, upregulation of the NKG2D ligands RAE-1, MULT1 and HS60 during MHV infection enhance CD8 T cell cytolysis without affecting IFN- γ production or demyelination [54]. In this context it is also noted that similar to viral encoded proteins antagonizing IFN- α/β function, members of the herpesvirus and retrovirus families express proteins downregulating MHC antigen presentation to evade T cell effector function [55,56]. Technologies aimed at evaluating the cell biology of T cell interactions with infected glia and neurons *in vivo* will no doubt be instrumental in understanding the limits of T cell effector function at the single cell level [47,57,58]. In lieu of vaccination strategies to enhance T cell memory, CD8 T cell function may thus be enhanced at the level of selective blockade or activation of modulatory molecules. Efficacy for this approach is demonstrated by blocking inhibitory PD-L1-PD-1 interactions during persisting peripheral infections [59]. Application

of related strategies to combat persisting CNS infections will have to be evaluated for T cell mediated pathologies.

Humoral immunity circumvents a necessity for MHC dependent T cell function, which may not be maintained at localized sites of persistence [49]. Sustained intrathecal antibody (Ab) production by virus-specific Ab secreting cells (ASC) is associated with control but not clearance of virus during persistent CNS infections [1,60] (Fig. 1C). However, little is known on ASC recruitment, regulation or their mechanism of action in the CNS. Ongoing differentiation and prolonged expression of chemokines and the survival factor BAFF suggests virus induced CNS inflammation maintains an environment that sustains local protective ASC.

Conclusion and prospective

Control of viral infections in the CNS is determined by rate of viral spread relative to the strength of innate and adaptive immune responses. Based on the protective, yet mostly insufficient role of endogenously induced IFN- α/β in limiting CNS virus replication, accelerated induction of IFN- α/β is a promising therapeutic strategy. Although this is readily achieved by poly I:C activation of TLR-3 in astrocytes and microglia, the antiviral efficacy of PRR activation by synthetic agonists in vivo has not been explored. Based on unique PRR signaling pathways, the design of agonists allowing manipulation of the relative levels of IFN- α/β versus pro-inflammatory signals in the CNS may be particularly useful in activating antiviral mechanisms, while minimizing pathology attributed to excessive chemokine and cytokine function. By contrast, targeted chemokine induction may be a strategy to enhance anti-viral adaptive immune responses. Tailored PRR stimulation will require more in depth knowledge of cell type specific expression of basal levels of PRRs, antiviral genes, as well as factors involved in signaling cascades. Furthermore, exciting new insights into cell type specific MHC as well as co-stimulatory and inhibitory molecule expression provide avenues to modulate T cell effector functions. Lastly, although immune mediated pathologies may be effectively treated by chemokine blocking strategies to limit monocyte or T cell recruitment, a potential danger is the abrogation of immune protection during viral persistence. Treatment of CNS viral infections will thus require a thorough investigation of the beneficial versus destructive consequences of the immune response.

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Figure 1. Regulation of innate and adaptive immune components during viral CNS infection

(A) Select activation of constitutively expressed PRRs by viral nucleic acids leads to cell type dependent induction of IFN- α/β , proinflammatory chemokines and cytokines. For simplicity, nuclear translocation of transcription factors activated by PRR signal transduction is magnified as a blue rectangle representative of all CNS cells. IFN- α/β amplifies PRR signaling and induces antiviral ISGs and class I expression, while chemokines and cytokines enhance leukocyte recruitment. MMP expression by CNS cells as well PMNs facilitate lymphocyte CNS entry, but also contribute to neurotoxicity. (B) CNS infiltrating T cells are attracted to infected cells. Their antiviral function depends on the ability of infected cells to present viral antigen. Target cell recognition results in viral control via targeted release of perforin and granzymes and IFN- γ production, which in turn amplifies MHC expression. However, cell type dependent regulation of T cell effector functions by immunomodulatory molecules may contribute to persistence. (C) Persistent, low level infections are associated with ongoing chemokine and cytokine expression, potentially mediated by PRRs. Their control by persisting T cells and/or local antibodies depends on continued antigen presentation and maintenance of ASC.

Figure 2. Diverse roles of chemokine-chemokine receptor expression during viral CNS infections
The dual roles of individual chemokines and chemokine receptors associated with viral infection in enhancing antiviral responses early, but sustaining pathogenic T cells and macrophages is outlined.