Metabolic Brain Disease (2019) 34:675–679
https://doi.org/10.1007/s11011-019-0383-y

SHORT COMMUNICATION

# Peripheral viral challenge exacerbates experimental autoimmune encephalomyelitis

Tiffany J. Petrisko[1] · Gregory W. Konat[1]

Received: 18 December 2018 / Accepted: 3 January 2019 / Published online: 14 January 2019


## Abstract

Peripheral viral infections are potent triggers of exacerbation in multiple sclerosis (MS). Here, we used a preclinical model of MS, the experimental autoimmune encephalomyelitis (EAE) to corroborate this comorbidity in an experimental setting. EAE was induced by immunization of mice with MOG peptide, and paralysis was scored using a 5-point scale. At the onset of the chronic phase of the disease (Days 42–58 after MOG injection) the animals were divided into low responders (LR) and high responders (HR) with the mean score of 1.5 and 2.5, respectively. The acute phase response (APR) was induced by intraperitoneal injections of a viral mimetic, polyinosinic-polycytidylic acid (PIC). Two daily injections were performed on Days 42 and 44 ($PIC_{42,44}$ challenge) and on Days 54, 55 and 56 ($PIC_{54,55,56}$ challenge). $PIC_{42,44}$ challenge had no effect of EAE disease, whereas $PIC_{54,55,56}$ challenge rapidly increased paralysis but only in HR group. This exacerbation ultimately led to animal death by Day 58. These results demonstrate that antiviral APR is a potent exacerbator of EAE, and that this activity directly correlates with the severity of the disease. This in turn, indicates that antiviral APR might play a pivot role in linking peripheral viral infections with MS exacerbations.

**Keywords** Polyinosinic-polycytidylic acid · Experimental autoimmune encephalomyelitis · Inflammatory mediators · Viral challenge · Acute phase response · Multiple sclerosis

## Introduction

Multiple sclerosis (MS) is characterized by unpredicted occurrence of exacerbations that detrimentally affect the long term disability. Epidemiological studies strongly implicate viral infections in the periphery as triggers of the exacerbations (Sibley et al. 1985; Andersen et al. 1993; Panitch 1994; Edwards et al. 1998; Buljevac et al. 2002; Correale et al. 2006; Libbey and Fujinami 2010). Importantly, viral infection-instigated exacerbations result in more persistent neural deteriorations than other exacerbations. Although the mechanisms have not been defined, several pathways have been suggested. For example, the infections may decrease the ability of the CNS to protect itself against autoimmune attack by leukocytes (Matullo et al. 2011), enhance the autoimmune responsiveness of the host leukocytes (Correale et al. 2006), or induce re-activation of a CNS-resident virus (Borkosky et al. 2012).

Because MS exacerbations are induced by various, and often unrelated viruses (Libbey and Fujinami 2010; Kakalacheva et al. 2011), the triggering process is likely to involve a common viral signature. A good candidate is a double stranded RNA (dsRNA) that is generated by most viruses during their replication cycle (Jacobs and Langland 1996; Weber et al. 2006). dsRNA is recognized by the major antiviral receptors of the host cells, i.e., toll-like receptor 3 (TLR3), retinoic acid-inducible gene 1 (RIG-1), melanoma differentiation-associated protein 5 (MDA-5) and protein kinase R (PKR). The ligation of these receptors elicits the acute phase response (APR), the first line of antiviral defense characterized by the fulminant generation of interferons, cytokines, chemokines and other inflammatory mediators that curtail the spread of the infection. However, these inflammatory mediators are also conveyed via circulation to the CNS whereby they induce a "mirror inflammatory response" (Dantzer

✉ Gregory W. Konat
gkonat@wvu.edu

[1] Departments of Biochemistry and Neuroscience, Rockefeller Neuroscience Institute, West Virginia University School of Medicine, 4052 HSCN, P.O. Box 9128, Morgantown, WV 26506-9128, USA

and Kelley 2007). The inflammatory factors generated peripherally and/or centrally during antiviral APR are expected to exert a profound effect on the progression of MS.

A synthetic dsRNA, polyinosinic-polycytidylic acid (PIC), is a commonly used tool to induce antiviral APR [reviewed in (Konat 2016)]. APR induced by intraperitoneal PIC injection involves a robust but transient surge of blood cytokines, such as IL1β, IL6, TNFα, CXCL9, CXCL10, CXCL1, CXCL2, CCL2, CCL7, CCL12 and IFNβ (Michalovicz and Konat 2014; Petrisko and Konat 2017). The brain response to this surge entails the production of CXCL10, and to a lesser extent the production of CXCL1, CXCL2 and CXCL9. In addition, the cerebral response includes the production of complement proteins and the activation of anaphylatoxin cascades (Michalovicz and Konat 2014; Petrisko and Konat 2017). Most of these inflammatory factors have been implicated in the pathology of MS (Cheng and Chen 2014; Pranzatelli 2018).

We hypothesize that the inflammatory factors generated during antiviral APR augment the ongoing MS pathology resulting in the exacerbation of the disease. The present study was undertaken to test this hypothesis using the autoimmune model of MS, the experimental autoimmune encephalomyelitis (EAE) in mice. During the chronic phase of EAE, mice were challenged with PIC, and progression of the disease was monitored and compared to that in EAE animals without PIC challenge.

## Materials and methods

### Animals

Ten-week old female C57BL/6 mice obtained from Charles River Laboratories (Wilmington, MA) were housed on Diamond Soft bedding under 12-h light/dark cycle with ad libitum access to food and water. All procedures were approved by the West Virginia University Animal Care and Use Committee and conducted in compliance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

### EAE induction

The Hooke Kits™ for EAE Induction (Hooke Laboratories, Lawrence, MA) was used according to the manufacturer's protocol. Briefly, on Day 0, mice were lightly anesthetized with isoflurane and subcutaneously (s.c.) injected with the $MOG_{35–55}$ emulsion in CFA into four sites of the back (50 μL per site), followed by an intraperitoneal (i.p.) injection of 300 ng of pertussis toxin (PTx) in 100 μL of saline. The PTx injection was repeated the next day (Day 1). The animals were scored daily for signs of EAE using a 5 point scale: 0, unaffected; 1, loss of tail tone; 2, hind limb weakness; 3, complete paralysis of hind limbs; 4, quadriplegia; and 5, moribund. If an animal appeared to be between two scores, an average score was assigned.

### PIC challenge

On Day 42, mice were divided into high responders (HR) with scores >2 (mean score of 2.5 ± 0.1) and low responders (LR) with scores ≤2 (mean score of 1.5 ± 0.1). To induce antiviral APR, half of the animals in each group received two daily i.p. injections of 12 mg/kg of ultrapure PIC (Invivogen, San Diego, CA) in 100 μL of saline. These injections were spaced two hours apart. The first PIC challenge was performed on Days 42 and 44. The second PIC challenge was executed on Days 54, 55 and 56. The other half of the animals were injected with an equivolume saline in lieu of PIC. Thus, four groups were generated: LR-PIC, LR-Sal, HR-PIC and HR-Sal. The animals were scored and weighted daily. Severely affected mice were housed with animals of similar severity in cages containing HydroGel (Westbrook, ME). Animals that scored a 4.0 for two consecutive days or lost more than 30% of body weight were euthanized. A score of 5.0 was assigned to animals that were euthanized or died.

### Statistical analysis

Statistical comparisons were performed by two-way repeated measures ANOVA (RMANOVA) followed by the Holm-Sidak post hoc test. A Kaplan-Meir plot was generated and the logrank test was used to test differences in survival time. Differences between groups were considered significant at $P \leq 0.05$.

## Results

The initial wave of paralysis commenced on Day 10, peaked on Day 16 and partially resolved around Day 25 after MOG immunization (Fig. 1). This was followed by a second slower relapse that plateaued around Day 40 indicating a chronic phase of the disease. There was no mortality at any time point.

We tested the comorbidity of antiviral APR induced by PIC injection during the chronic EAE phase. Although the peak scores during the initial relapse (Fig. 1) were tightly clustered (e.g., 2.4 ± 0.1 at Day 16), the variability of sickness intensity increased substantially thereafter. Consequently, on Day 42, we divided the animals into two subpopulations based on the disease severity, i.e., high responder (HR) group with scores of 2.5 ± 0.1, and low responder (LR) group with scores of 1.5 ± 0.1.

PIC is rapidly deactivated within the peritoneal cavity, and consequently a single PIC injection results in a bolus

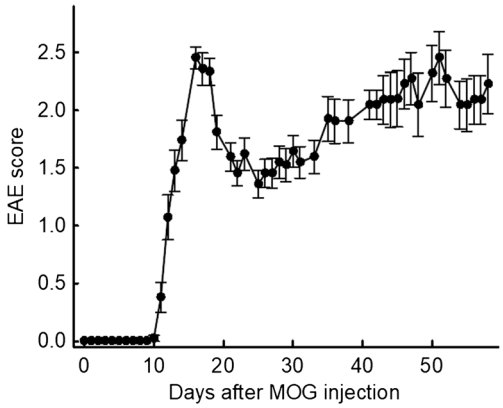


**Fig. 1** Time course of MOG-induced EAE. Ten-week old female C57BL/6 mice were immunized with $MOG_{35–55}$ peptide (Day 0) and the progression of paralysis was scored daily (for details see "Materials and methods" section). Points represent averages ± SE from 20 (Days 0–42) and 11 (Days 43–59) animals

stimulation of the innate immune system (Konat 2016). Because viral replication, and thus dsRNA generation in the host are protracted, we performed two consecutive PIC injections two hours apart to better simulate APR instigated by viral infection. To assess the effect of PIC challenge severity, we used two paradigms. Initially, the animals were challenged with PIC on Days 42 and 44 ($PIC_{42,44}$ challenge). After ten days of recovery, the animals were injected for three consecutive days, i.e., Days 54, 55 and 56 ($PIC_{54,55,56}$ challenge).

The response of animals during the chronic phase of EAE to PIC challenge is shown in Fig. 2. RMANOVA on Days 42–59 showed a significant effect of group [$F(3, 238) = 21.48$, $p < 0.001$], and time [$F(14, 238) = 4.38$, $p < 0.001$]. The interaction between groups and time was also significant [$F(42, 238) = 2.80$, $p < 0.001$]. In HR group, $PIC_{42,44}$ challenge tended to temporarily increase EAE scores as compared to EAE mice injected with saline, but the differences were not statistically significant. $PIC_{54,55,56}$ challenge profoundly exacerbated paralysis, and the difference in EAE scores between HR-PIC and HR-Sal groups reached high statistical significance ($p < 0.001$) on Days 56, 57 and 58. Moreover, the exacerbation of EAE symptoms resulted in animal death. The first animal in HR-PIC group died on Day 56, and no animal survived beyond Day 58.The difference from HR-Sal group assessed by the logrank test was highly significant ($p \leq 0.001$).

In contrast, no change in sickness scores was observed in LR-PIC group as compared to LR-Sal group after either $PIC_{42,44}$ or $PIC_{54,55,56}$ challenges (Fig. 2). Although $PIC_{42,44}$ challenge tended to ameliorate the disease in LR-PIC, the decrease of EAE scores was not statistically significant. Following $PIC_{54,55,56}$ challenge, one LR-PIC mouse died (20% mortality) in on Day 56, albeit the survival of this group did not significantly differ from LR-Sal group.

## Discussion

Previous preclinical studies addressed the comorbidity of peripheral viral infections and MS by focusing on their involvement in the induction phase of EAE (Peacock et al. 2003; Verbeek et al. 2007). These experiments have revealed that preceding peripheral viral infections increase the susceptibility of animals to develop EAE. However, the effect of viral insult during the effector phase of EAE has not been studied. In the present study, we demonstrated comorbid effect of viral

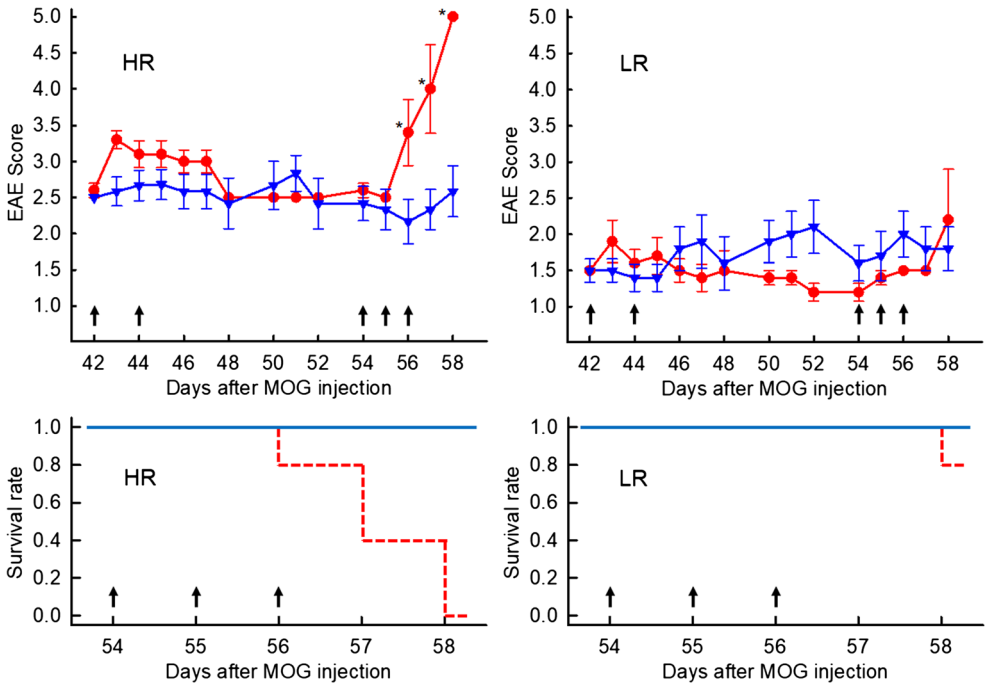


**Fig. 2** Comorbidity of PIC-induced APR during chronic phase of EAE. *Upper panels* show the effect of PIC challenges on neurological disability. Arrows indicate PIC injections during the first ($PIC_{42,44}$) and second ($PIC_{54,55,56}$) challenge (for details see text). Red circles; PIC-injected EAE mice. Blue triangles; saline-injected EAE mice. Symbols represent averages ± SE. LR-PIC ($n = 5$), LR-Sal ($n = 5$), HR-PIC ($n = 5$) and HR-Sal ($n = 6$). *$P \leq 0.001$. *Lower panels* show mortality after the second PIC challenge. Brocken red lines represent PIC-challenged EAE mice. Solid blue lines represent saline-injected controls

challenge in mice with established EAE. Consequently, our experimental paradigm models viral infection-induced exacerbations in MS patients (Sibley et al. 1985; Andersen et al. 1993; Panitch 1994; Edwards et al. 1998; Buljevac et al. 2002; Correale et al. 2006; Libbey and Fujinami 2010).

The effect of PIC-induced exacerbation of EAE disease depended on the strength of PIC challenge. Thus, no effect was evident in EAE mice following challenge on two alternate days ($PIC_{42,44}$). In contrast, PIC challenge for three consecutive days ($PIC_{54,55,56}$) induced a fulminant relapse of the disease as seen from the rapid exacerbation of paralysis and ultimate death. Although the animals were allowed to recover for ten days after the first PIC challenge, a possibility for a priming of the immune system for a heightened response to the second PIC challenge could also be considered.

The exacerbating effect of PIC challenge was also strongly dependent on the severity of EAE disease. Thus, the exacerbation of EAE symptoms occurred only in HR group, while no exacerbation was evident in LR group. It is expected that HR group with the initial score of 2.5 has more intense inflammation than LR group with the score of 1.5. These results might indicate the existence of a threshold in the inflammatory status required for the fulminant response to occur. Alternatively, the response might be more gradually dependent on disease severity. For example, although LR mice were evidently spared, a tendency to increase paralysis score and the death of one animals occurred on Day 58. Thus, the exacerbation in LR mice may be delayed beyond Day 58. This dependence on EAE severity is reminiscent of MS patient response to inflammatory mediators that is depend on the disease subtype (Nikfar et al. 2010). The effects of EAE severity as well as the strength of PIC challenge on EAE exacerbation will be delineated in future studies.

PIC is a potent inducer of generic APR in a manner independent of the viral type (Guha-Thakurta and Majde 1997; Traynor et al. 2004). The induction of antiviral APR is restricted to the peritoneal cavity, because PIC is swiftly degraded within this compartment, and does not reach the circulation (Konat 2016). PIC stimulates peritoneal macrophages and mesothelial cells, but does not cause tissue damage, and thus does not elicit the confounding effects of systemic response to tissue injury. Consequently, the present study provides the proof-of-concept that APR per se is a principal component of the innate immune response to viral challenge responsible for the exacerbation of EAE, and by extension, for MS exacerbations.

The inflammatory factors generated during PIC-induced APR are likely responsible for the augmentation of EAE pathology. For example, IL6 that is profoundly increased by PIC challenge in the blood (Michalovicz and Konat 2014) has been demonstrated to enhance the recruitment of patrolling leukocytes through the neuroendothelium (Richard et al. 2011). CXCL10 that is also robustly increased by PIC challenge in both the blood and CNS (Petrisko and Konat 2017) is a potent activator and chemoattractant of circulating leukocytes (Liu et al. 2001) and resident microglia (Rappert et al. 2004; Clarner et al. 2015). The increase of leukocytic infiltration promoted by IL6 and/or CXCL10 is likely to augment the neuroinflammatory milieu in the CNS parenchyma leading to exacerbation of the disease. Moreover, PIC challenge upregulates the generation and activation of the complement proteins in the CNS (Michalovicz et al. 2015; Petrisko and Konat 2017), and the complement system activation is an intrinsic feature of MS pathology (Storch et al. 1998). PIC-induced APR may also act at the periphery by enhancing the activity of lymphocytes. For example, PIC is a potent promoter of expansion and differentiation of CD8+ T cells (Ngoi et al. 2008), which are primarily responsible for CNS damage during MS relapses (Malmestrom et al. 2008). Finally, one should also be cognizant of the possibility that EAE animals with ongoing autoimmune pathology may generate different profiles of inflammatory factors in response to PIC challenge that differ from those induced in naïve animals used in the aforementioned studies. The identification of these factors would open the way for the development of therapeutic strategies to control the progression of MS.

In conclusion, this is the first study that translates the clinical correlation between peripheral viral infections and MS pathology to experimental exploration by focusing on the role of APR as the hub that links infections by unrelated viruses to MS exacerbations. We have developed a preclinical paradigm to examine this comorbidity, and demonstrated that antiviral APR exacerbates EAE in mice, an autoimmune model of MS. The PIC paradigm provides a unique model to identify the components of antiviral APR responsible for the disease exacerbation.

**Acknowledgements** This study was supported by a Pilot Grant from the National Multiple Sclerosis Society awarded to G.W.K.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest.



## References

Andersen O, Lygner PE, Bergstrom T, Andersson M, Vahlne A (1993) Viral infections trigger multiple sclerosis relapses: a prospective seroepidemiological study. J Neurol 240:417–422

Borkosky SS, Whitley C, Kopp-Schneider A, zur HH, de Villiers EM (2012) Epstein-Barr virus stimulates torque teno virus replication: a possible relationship to multiple sclerosis. PLoS One 7:e32160

Buljevac D, Flach HZ, Hop WC, Hijdra D, Laman JD, Savelkoul HF, van Der Meche FG, van Doorn PA, Hintzen RQ (2002) Prospective

study on the relationship between infections and multiple sclerosis exacerbations. Brain 125:952–960

Cheng W, Chen G (2014) Chemokines and chemokine receptors in multiple sclerosis. Mediat Inflamm 2014:659206

Clarner T, Janssen K, Nellessen L, Stangel M, Skripuletz T, Krauspe B, Hess FM, Denecke B, Beutner C, Linnartz-Gerlach B, Neumann H, Vallieres L, Amor S, Ohl K, Tenbrock K, Beyer C, Kipp M (2015) CXCL10 triggers early microglial activation in the cuprizone model. J Immunol 194:3400–3413

Correale J, Fiol M, Gilmore W (2006) The risk of relapses in multiple sclerosis during systemic infections. Neurology 67:652–659

Dantzer R, Kelley KW (2007) Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 21:153–160

Edwards S, Zvartau M, Clarke H, Irving W, Blumhardt LD (1998) Clinical relapses and disease activity on magnetic resonance imaging associated with viral upper respiratory tract infections in multiple sclerosis. J Neurol Neurosurg Psychiatry 64:736–741

Guha-Thakurta N, Majde JA (1997) Early induction of proinflammatory cytokine and type I interferon mRNAs following Newcastle disease virus, poly [rI:rC], or low-dose LPS challenge of the mouse. J Interf Cytokine Res 17:197–204

Jacobs BL, Langland JO (1996) When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 219:339–349

Kakalacheva K, Munz C, Lunemann JD (2011) Viral triggers of multiple sclerosis. Biochim Biophys Acta 1812:132–140

Konat G (2016) Cerebral response to peripheral challenge with a viral mimetic. Neurochem Res 41:144–155

Libbey JE, Fujinami RS (2010) Potential triggers of MS. Results Probl Cell Differ 51:21–42

Liu MT, Keirstead HS, Lane TE (2001) Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. J Immunol 167:4091–4097

Malmestrom C, Lycke J, Haghighi S, Andersen O, Carlsson L, Wadenvik H, Olsson B (2008) Relapses in multiple sclerosis are associated with increased CD8+ T-cell mediated cytotoxicity in CSF. J Neuroimmunol 196:159–165

Matullo CM, O'Regan KJ, Curtis M, Rall GF (2011) CNS recruitment of CD8+ T lymphocytes specific for a peripheral virus infection triggers neuropathogenesis during polymicrobial challenge. PLoS Pathog 7:e1002462

Michalovicz LT, Konat GW (2014) Peripherally restricted acute phase response to a viral mimic alters hippocampal gene expression. Metab Brain Dis 29:75–86

Michalovicz LT, Lally BE, Konat GW (2015) Peripheral challenge with a viral mimic upregulates expression of the complement genes in the hippocampus. J Neuroimmunol 285:137–142

Ngoi SM, Tovey MG, Vella AT (2008) Targeting poly(I:C) to the TLR3-independent pathway boosts effector CD8 T cell differentiation through IFN-alpha/beta. J Immunol 181:7670–7680

Nikfar S, Rahimi R, Abdollahi M (2010) A meta-analysis of the efficacy and tolerability of interferon-beta in multiple sclerosis, overall and by drug and disease type. Clin Ther 32:1871–1888

Panitch HS (1994) Influence of infection on exacerbations of multiple sclerosis. Ann Neurol 36(Suppl):S25–S28

Peacock JW, Elsawa SF, Petty CC, Hickey WF, Bost KL (2003) Exacerbation of experimental autoimmune encephalomyelitis in rodents infected with murine gammaherpesvirus-68. Eur J Immunol 33:1849–1858

Petrisko T, Konat G (2017) Peripheral viral challenge triggers hippocampal production of inflammatory proteins. Metab Brain Dis 32:1249–1254

Pranzatelli MR (2018) Advances in biomarker-guided therapy for pediatric- and adult-onset Neuroinflammatory disorders: targeting chemokines/cytokines. Front Immunol 9:557

Rappert A, Bechmann I, Pivneva T, Mahlo J, Biber K, Nolte C, Kovac AD, Gerard C, Boddeke HW, Nitsch R, Kettenmann H (2004) CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. J Neurosci 24:8500–8509

Richard JF, Roy M, Audoy-Remus J, Tremblay P, Vallieres L (2011) Crawling phagocytes recruited in the brain vasculature after pertussis toxin exposure through IL6, ICAM1 and ITGalphaM. Brain Pathol 21:661–671

Sibley WA, Bamford CR, Clark K (1985) Clinical viral infections and multiple sclerosis. Lancet 1:1313–1315

Storch MK, Piddlesden S, Haltia M, Iivanainen M, Morgan P, Lassmann H (1998) Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. Ann Neurol 43:465–471

Traynor TR, Majde JA, Bohnet SG, Krueger JM (2004) Intratracheal double-stranded RNA plus interferon-gamma: a model for analysis of the acute phase response to respiratory viral infections. Life Sci 74:2563–2576

Verbeek R, van Dongen H, Wawrousek EF, Amor S, van Noort JM (2007) Induction of EAE by T cells specific for alpha B-crystallin depends on prior viral infection in the CNS. Int Immunol 19:277–285

Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR (2006) Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J Virol 80:5059–5064