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Differences in IPS-1-Mediated Innate Immune Responses between Neurotrophic Flavivirus Infection

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Introduction

The genus *Flavivirus* of the family *Flaviviridae* consists of more than 70 members and many of them are transmitted through arthropods [1]. These viruses can cause severe diseases in humans and animals [2]. This includes West Nile virus (WNV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Yellow fever virus (YFV), Tick-borne encephalitis virus (TBEV), Murray Valley encephalitis virus (MVEV) and St. Louis encephalitis virus (SLEV) [1]. In most cases no effective vaccine is available and thousands of new infections are registered annually [3,4]. To date there is only symptomatic treatment available for infected patients [3,4].

Innate Recognition of Flaviviruses

Evidence indicates that innate immune responses play a crucial role in the control of TBEV infection [2]. Nucleic acids are the main pathogen-associated molecular pattern (PAMPs) recognized by the innate immune system [5]. Sensing of PAMPs results in the control of the first wave of viral infection through the production of antiviral effector molecules and it contributes to the mobilization of the adaptive immune response [5]. Double-stranded RNA of TBEV is mainly sensed by Toll like receptors (TLRs) and Retinoic acid-inducible gene (RIG)-I like receptors (RLRs) [6,7]. RIG-I interacts with Interferon-β promotor stimulator-1 (IPS-1) upon activation and triggers the activation of Interferon regulatory factor-3 (IRF-3), IRF-7 and Nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), leading to the induction of type I interferons (IFNs) and proinflammatory cytokines [7].

Many, if not all viruses, developed strategies to evade recognition by the innate immune system [7,8]. RLR signaling is required to control viral spread in peripheral organs and to limit virus-mediated central nervous system (CNS) pathology in flavivirus infection [9]. WNV appears to delay pattern recognition receptor (PRR) activation, which gives the virus a replicative advantage within the cells during early stages of infection [10]. TBEV was shown to influence type I IFN responses by hiding its replication complexes inside replication vesicles [11]. This strategy protects viral dsRNA from early detection by the innate immune system [11]. Additionally, type I IFN responses can be inhibited [12]. DENV actively prevents type I IFN production by the viral NS2B3 protease which cleaves and degrades STING, whereas TBEV restricts Signal transducer and activator of transcription-1 (Stat-1) expression by the inhibition of the IFN-α/β receptor (IFNAR) [8,12,13]. Despite these evasion strategies, a clear correlation between TBEV RNA and IFN-β induction was detectable [9]. Higher amounts of viral RNA detected in the cell results in higher IFN-β production, independent of the viral strain that is used [11].

The role of IPS-1 in Flavivirus Infection

IPS-1 plays an important role in the regulation of type I IFNs [9,14]. Both, similarities and differences between mosquito and tick borne flaviviruses were seen in the dependency of type I IFN responses and the adaptor protein IPS-1 in the periphery [9,14]. In the absence of IPS-1, mice infected with WNV showed reduced specific antibody responses and an increase in spleen size due to decreased numbers of Tregs [14]. This indicates that IPS-1 is important for the correct mounting of adaptive immune responses in the periphery.

In contrast, lower systemic levels of IFN-α did not influence humoral or T cell responses in the periphery of IPS-1 deficient mice upon infection with Langat virus (LGTV), a tick borne flavivirus [9]. Type I IFN responses seem to mediate tissue tropism in the periphery of WNV infected mice due to expression of specific antiviral effector genes [15]. We detected a change in tissue tropism in the periphery of IPS-1 and IFNAR deficient mice compared to WT animals after LGTV infection [2,9]. Therefore, viral attachment and entry into cells via receptors might not be the only factor determining viral tropism of neurotropic flaviviruses. Instead, tissue tropism seems to be mediated also by the type I IFN system [2,9].

Viremia is a critical determinant in CNS entry [2]. In the absence of IPS-1, both WNV and LGTV infection led to increased viral titers and enhanced neuroinvasion [9,14]. One of the most important barriers to restrict entry into the CNS is the blood brain barrier (BBB) [16]. Some viruses like WNV mediate a breakdown of the BBB to achieve entry into the CNS [17]. Other viruses, e.g. TBEV, LGTV and JEV have been observed in the brain already before the BBB was affected independent of an intact type I IFN response [2,9,18,19]. Very little is known about pathways that TBEV uses to enter the CNS, but we assume that differences exist between neurotropic flaviviruses. Our results show that low pathogenic LGTV primarily infects the olfactory bulb (OB) of the CNS after peripheral administration [9]. The virus was completely restricted to the olfactory system and cleared by the innate immune system without involvement of the adaptive immune response [9]. TBEV targets the OB early in infection, however, similar to SLEV, more pathogenic virus strains are able to spread to other brain parts [20]. Interestingly, in contrast to WNV, where direct infection of the CNS leads to equal distribution of the virus to different brain regions [21], direct administration of LGTV and TBEV into the brain showed higher viral replication in the olfactory bulb and the cerebrum compared to brain stem and cerebellum [9]. This indicates site specific

viral replication in different parts of the brain [9]. For most viruses, IPS-1 dependent IFN-β induction was shown to be distinct between analyzed cell types [9,11]. We have previously demonstrated that upregulation of IFN-β in A549 cells strictly depends on virus replication and that enhanced level of IFN-β rely on IPS-1 in mouse embryonic fibroblasts (MEFs) [11,22]. In vivo, IPS-1 deficient mice displayed increased LGTV replication in all brain regions [9]. Normally, higher viral replication in the brain also results in higher IFN-β expression. Interestingly, higher viral replication in the OB resulted in lower IFN-β levels compared to WT mice [9]. This indicates that IFN-β upregulation in the OB is more dependent on IPS-1 compared to other brain parts [9].

Conclusion

It is becoming clear that different brain regions vary in their response to infection. This might have an impact on the severity of infections and therefore survival of the host. In this line, different brain regions depend on disparate PRRs for the upregulation of type I IFNs, which can lead to favored viral replication sites. However, which cell types within the CNS contribute to regional differences in the IFN response is currently under investigation.

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