Neuron-intrinsic immune responses during viral encephalitis

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Background and preliminary data:

Current State of Research

Neurotropic viral infections of the central nervous system (CNS) present a significant worldwide health risk and a neglected medical problem. Viral encephalitis, mostly caused by herpes simplex virus (HSV), varicella–zoster virus, enteroviruses, or arboviruses, is igniting inflammation of the CNS parenchyma with associated neurological dysfunction and high mortality (1). Previous studies on CNS-specific immunity to viruses have primarily focused on roles of circulating leukocytes and resident microglial cells. Traditionally, neurons were viewed as passive victims of viral infection, dependent on external antiviral mechanisms for protection. However, over the past decade, there has been a growing recognition that neurons also have cell-intrinsic mechanisms to combat viruses (2).

The CNS has multi-layered defenses against viral infections, but once breached, damage is rapid and severe due to limited neuronal regenerative capacity. Pattern recognition receptors (PRRs) initiate the immune response by detecting viral molecules, triggering the synthesis of antiviral proteins and cytokines like type I (IFN-α/β) and II interferons (IFN-γ). If intrinsic defenses fail, sentinel cells are activated, producing more cytokines and triggering adaptive immunity. An overreaction, however, can cause immunopathology which are similar to conditions occurring in autoimmune CNS diseases (3).

Host-pathogen co-evolution has driven immune gene diversification (4), but extended innate immune responses can damage organs like the CNS. Interferon-stimulated genes (ISGs) upregulate antiviral activities but can disrupt host cell homeostasis. Recently, neuronal transcript profiling under sterile inflammatory conditions identified apolipoprotein L6 (APOL6), a member of the ISG family, as highly induced during inflammation in neurons (5). APOL6's function is largely unknown, but ISG screenings identified APOL6 as an inhibitor of coxsackie B virus and poliovirus, members of the picornavirus family, in cell culture (6). Furthermore, several studies associate the antiviral properties of APOL6 with additional types of viruses, including SARS-CoV-2 and HIV-1. Thus, APOL6 is a paradigmatic example of a neuron-intrinsic defense mechanism against neurotropic viruses.

Preliminary Work

In neuronal cells, CRISPRa-induced APOL6 expression provided significant protection against Theiler's murine encephalomyelitis virus (TMEV), comparable to IFN-γ pretreatment.

Moreover, subcellular localization and interactome studies showed APOL6 to be localized and interact with proteins at mitochondria and the endoplasmatic reticulum (ER), indicating association with mitochondria-associated ER membranes (MAMs), crucial for antiviral responses. We found overexpression of APOL6 to affect cell viability, ER calcium levels, and the mitochondrial membrane potential, suggesting interference in calcium homeostasis and mitochondrial function, which is crucial for certain virus life cycles. CRISPRa-mediated APOL6 upregulation revealed the induction of antiviral pathways, indicating APOL6's role in regulating interferon-signaling pathways.

To study APOL6 *in vivo*, we established *Apol6*-deficient mice and developed methods for neuron-specific overexpression of APOL6 using adeno-associated viruses. This approach, combined with our CRISPR technology, will allow us to further explore APOL6's role in CNS infections and inflammation, but also to expand our toolbox to other neuron-intrinsic ISGs.

Hypothesis:

We hypothesize that neuron-intrinsic immune responses to neurotropic viruses are in a delicate balance between virus containment and neuronal survival and self-damage, and that neurons have evolved specific defense mechanisms against viruses due to their unique properties.

Aims and Work Programme:

Viral encephalitis, with HSV-1 being the most common causative agent, is a major neglected medical issue. Additionally, emerging viral infections present a high risk for developing new types of encephalopathies, highlighting the need for targeted therapeutic strategies. Using APOL6 as a prime example, we aim to enhance our understanding of the antiviral, neuron-intrinsic immune response and to identify novel innate immune genes that could be leveraged for developing antiviral therapies for the CNS.

WP1: Deciphering the molecular mechanism of APOL6's antiviral function.

This work package builds upon our laboratory's research on the antiviral and neurotoxic properties of APOL6 within the SFB1648. We have already established a variety of methods in this context, including gene knockout and activation *in vitro* and *in vivo* using CRISPR-Cas technology, neuron-specific delivery *in vivo* using recombinant AAVs, mouse primary neuronal cultures, and human induced pluripotent stem cell (hiPSC)-derived neuronal cultures, coupled with longitudinal cell viability assays. These techniques provide an excellent foundation for the IDfellow to familiarize themselves with all relevant methodologies while simultaneously gaining new insights into this neuron-intrinsic antiviral protein. In this work package, we will investigate the molecular mechanisms by which APOL6 mediates its antiviral and neurotoxic activities. We will utilize mutational and CRISPR-KO screenings to identify the critical amino acids in the APOL6 protein and its downstream effector genes.

WP2: Identification of neuron-intrinsic antiviral immune response genes.

This work package aims to extend our knowledge about neuron-intrinsic ISGs to additional unknown candidates. Thus, here we want to identify novel factors involved in the neuron-intrinsic antiviral immune response. We will use murine and human neuronal cell lines, employing knockout and activation strategies. (i) Utilizing an ISG CRISPR-KO library, we will perform gene knockouts in combination with interferon treatment to render the cells resistant, followed by viral infection. Genes whose loss results in susceptibility will be identified through next-generation sequencing. (ii) Employing a CRISPRa library, we will activate genes followed by viral infection. Genes whose activation leads to resistance will be identified through next-generation sequencing. The identified candidate genes from both strategies will then be validated in murine and hiPSC-derived neuronal cultures and can be developed by the IDfellow into an independent mechanistic project.

Project-related publications:

1. A. Venkatesan, B. D. Michael, J. C. Probasco, R. G. Geocadin, T. Solomon, Acute encephalitis in immunocompetent adults. *The Lancet* **393**, 702–716 (2019).
2. S.-Y. Zhang, O. Harschnitz, L. Studer, J.-L. Casanova, Neuron-intrinsic immunity to viruses in mice and humans. *Curr. Opin. Immunol.* **72**, 309–317 (2021).
3. M. S. Woo, *et al.*, STING orchestrates the neuronal inflammatory stress response in multiple sclerosis. *Cell* **187**, 4043-4060 (2024).
4. E. E. Smith, H. S. Malik, The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. *Genome Res.* **19**, 850–858 (2009).
5. B. Schattling, *et al.*, Bassoon proteinopathy drives neurodegeneration in multiple sclerosis. *Nat. Neurosci.* **22**, 887–896 (2019).
6. J. W. Schoggins, *et al.*, Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature* **505**, 691–695 (2014).