

Antiviral Type I Interferon Pathway Activity Increases with Human Neuronal Differentiation, Promoting Enhanced Defense against Neurotropic Arboviruses

by

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LIST OF ABBREVIATIONS

Development

BDNF	brain-derived neurotrophic factor
BMP	bone morphogenic protein
CNS	central nervous system
FGF	fibroblast growth factor
GFAP	glial fibrillary acidic protein
hESC	human embryonic stem cell
iPSC	induced pluripotent stem cell
NPC	neural progenitor cell
RA	retinoic acid
SGZ	subgranular zone
SVZ	subventricular zone

Cell Signaling

IFN	interferon
IFNAR ^{-/-}	interferon α/β receptor knockout
IFNAR1	interferon α/β receptor 1 subunit
IFNAR2	interferon α/β receptor 2 subunit
IL	interleukin
IRF-9	interferon regulatory factor 9
ISG	interferon-stimulated gene
ISRE	interferon-stimulated response element
Jak1	Janus kinase 1

MHC	major histocompatibility complex
MxA	myxovirus resistance protein A
NFκB	nuclear factor κ B
PRR	pattern recognition receptor
STAT	signal transducer and activator of transcription
Tyk2	tyrosine kinase 2
TLR	Toll-like receptor

Viruses

BUNV	Bunyamwera virus
CEV	California encephalitis virus
CHIKV	Chikungunya virus
CMV	cytomegalovirus
CVB3	coxsackievirus B3
EEEV	eastern equine encephalitis virus
FMV	Fort Morgan virus
HIV	human immunodeficiency virus
JEV	Japanese encephalitis virus
LACV	La Crosse virus
RVFV	Rift Valley fever virus
SFV	Semliki Forest virus
SINV	Sindbis virus
SLEV	St. Louis encephalitis virus
VEEV	Venezuelan equine encephalitis
WEEV	western equine encephalitis
WNV	West Nile virus

ABSTRACT

Neurotropic arboviruses are leading causative agents of viral encephalitis worldwide. These pathogens specifically infect neurons to cause acute encephalitic disease and permanent neurological sequelae in humans, which are particularly severe in the pediatric population. Pathogenesis of neurotropic arboviruses correlates with the degree of neuronal maturity in the host, and populations of neural stem/progenitor cells demonstrate particular susceptibility to viral infection. However, the mechanism(s) by which defense against a neurotropic arbovirus increases with human neuronal development have yet to be fully dissected. To investigate changes in neuronal innate immune function over the course of development, we established a model for the *in vitro* derivation of enriched populations of human neural progenitor cells (NPCs) and mature human neurons from human embryonic stem cells (hESCs). Using the hESC model in conjunction with primary cortical rat neurons and human neuronal cells, we identified novel maturation-dependent changes in the neuronal type I interferon (IFN) signaling pathway, including upregulation of the IFN- α/β receptor 2 subunit (IFNAR2) on the cell surface of mature neurons. Furthermore, we determined that basal upregulation of IFNAR2 is sufficient for increased type I IFN-dependent inhibition of neurotropic arbovirus replication. Finally, we dissected a downstream mechanism by which type I

IFN mediates its antiviral activity by identifying MxA as a functional inhibitor of neurotropic arbovirus replication in human neuronal cells. Together our data demonstrate that the innate antiviral immune function of a human neuron increases with differentiation, resulting in enhanced defense against neurotropic arbovirus infection. Our careful dissection of maturation-dependent changes in the neuronal type I IFN signaling pathway *in vitro* paves the way for future investigations, which will determine the impact of neuron-specific innate immune function on global host defense against neurotropic arboviruses *in vivo*.