

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

by

Jocelyn Farmer

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Microbiology and Immunology)
in the University of Michigan
2011

Doctoral Committee:

Associate Professor David Miller, Chair
Professor Michael J. Imperiale
Professor Nicholas W. Lukacs
Professor Sue O'Shea
Assistant Professor Yasmina Laouar

© Jocelyn Farmer

2011

To my AW and UC, who taught me what it means to love science. To Mom and Dad, who raised me to never give up. To my sister, who continually inspires me to be brave and seek out adventure. To MJ and Bev, who kept me laughing, especially in the hard times. To Mark, who never stopped believing that this was possible, even when I couldn't – thank you for teaching me, for listening to me, for putting up with me, but most of all, for loving me.

To all those who have ever had the thrill and the heartbreak of working with stem cells...
this one's for you.

ACKNOWLEDGEMENTS

I would like to acknowledge all of the past and present members of the Miller lab. Kenny and Spencer, I will forever feel like your little sister, thanks for all of the senior advice. Dan, thanks for the many fun scientific discussions. Josh, thanks for the shared excitement. Kate, thanks for all of the very hard work on the Mx story, you have a very bright future ahead of you.

I would also like to acknowledge all of the past and present members of the Michigan Stem Cell Core, Crystal in particular. Thanks for hosting me for the past three years and thank you so very much for the many troubleshooting sessions.

I am grateful for my funding from the University of Michigan Medical Scientist Training Program, the University of Michigan Rackham Graduate School, and the National Institute of Neurological Disorders and Stroke.

Additionally, I want to thank my thesis dissertation committee for the critical discussion of my work and for the many wonderful suggestions. Dr. Lukacs, I want to especially thank you for pushing me to entertain the larger implications of type I IFN production in the CNS. Dr. O'Shea, I want to especially thank you for giving me the space and the opportunity to play with stem cells, it truly has been a life-changing experience. Dr. Laouar, I want to especially thank you for your passionate critiques, you

have absolutely made me a better scientist. Dr. Imperiale, I want to especially say thanks for a great friendship over the past four years and for all of the wonderful advice, both scientific and otherwise. Finally, I must thank my advisor, Dr. Miller, for providing the framework that made scientific discovery possible.

This thesis also received substantial non-financial support of the Medical Scientist Training Program at the University of Michigan, Ron and Ellen in particular. The path to earning an M.D./Ph.D. is arduous, and one can frequently lose her way – thanks for keeping me on track!

Finally, I need to thank my Michigan, Maine, Vermont, Taiwan, North Carolina, Wake Forest, and Seattle families... this thesis was possible only with your unwavering support... thank you!

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGEMENTS	iii
LIST OF FIGURES	viii
LIST OF TABLES	x
LIST OF ABBREVIATIONS	xi
ABSTRACT	xiii
CHAPTER I: INTRODUCTION.....	1
The Neurotropic Arboviruses	1
Introduction.....	1
Pathogenesis.....	5
Immune Response.....	6
Maturation-Dependent Susceptibility	9
Mounting an Antiviral Response in the CNS	11
Introduction.....	11
Mechanisms of Regulation	12
Neurons as Immune-Competent Cells	14
The Pros and Cons of Type I IFN.....	16
Postnatal Development of the CNS	17
Introduction to Neural Progenitor Cells (NPCs).....	17
Modeling Human Neuronal Development <i>in vitro</i>	20
Viral Susceptibility of NPCs.....	21

The Type I Interferon (IFN) Pathway	24
Components of the Pathway	24
Component Expression Levels Impact Response to Type I IFN	29
Activation of the Pathway.....	31
The Mx GTPase Family of Antiviral Inhibitors	34
Conclusion	38
CHAPTER II: HUMAN NEURONAL DIFFERENTIATION MODULATES TYPE I INTERFERON PATHWAY ACTIVITY AND SUSCEPTIBILITY TO NEUROTROPIC ARBOVIRUS INFECTION	40
Abstract.....	40
Introduction.....	41
Experimental Procedures	44
Results.....	52
Discussion.....	72
CHAPTER III: MXA IS A FUNCTIONAL ANTIVIRAL EFFECTOR IN HUMAN NEURONAL CELLS WITH PREFERENTIAL ACTIVITY AGAINST BUNYAVIRUSES COMPARED TO NEW WORLD ALPHAVIRUSES	77
Abstract.....	77
Introduction.....	78
Experimental Procedures	81
Results.....	86
Discussion.....	97
CHAPTER IV: DISCUSSION	103
Overview.....	103
Human Neuronal Differentiation can be Modeled using Stem Cells	104
Antiviral Type I IFN Pathway Activity is Enhanced with Human Neuronal Differentiation.....	112
MxA is a Functional Antiviral Effector in Human Neuronal Cells.....	121
Conclusion	126

APPENDIX: STEM CELL CULTURE METHODS	127
Media Conditions.....	127
Plating Conditions.....	131
Methods for NPC Derivation	134
REFERENCES	139

LIST OF FIGURES

Figure I-1.	Immune response to a neurotropic arbovirus.....	8
Figure I-2.	Neural progenitor cells (NPCs).....	19
Figure I-3.	The canonical type I IFN signaling pathway	35
Figure II-1.	Enriched populations of NPCs and mature neurons can be derived from hESCs	53
Figure II-2.	Type I IFN pathway component expression and functions is enhanced with differentiation of human NPCs to mature neurons	56
Figure II-3.	Type I IFN pathway component expression and function is enhanced with differentiation of primary cortical rat neurons.....	58
Figure II-4.	Expression of type I IFN signaling pathway components increases with differentiation of human neuronal cells	60
Figure II-5.	Analysis of global and isoform-specific IFNAR2 transcript levels.....	62
Figure II-6.	Activation of the type I IFN pathway increases with differentiation of human neuronal cells	64
Figure II-7.	Kinetics of type I IFN pathway activation are unaltered with differentiation of human neuronal cells	66
Figure II-8.	Differentiation-dependent changes in neuronal type I IFN pathway function can be recapitulated with overexpression of IFNAR2 and STAT2.....	68

Figure II-9.	Overexpression of IFNAR2 in immature human neuronal cells is sufficient for increased inhibition of alphavirus replication	71
Figure II-10.	Stable shRNA-mediated knockdown of IRF-9 decreases type I IFN pathway activity in human neuronal cells.....	76
Figure III-1.	Mx expression and localization in human neuronal cells	87
Figure III-2.	MxA is antivirally effective against bunyaviruses in human neuronal cells	89
Figure III-3.	MxA can inhibit WEEV-mediated cytotoxicity, but not virion production in human neuronal cells.....	91
Figure III-4.	MxA is not antivirally effective against VEEV in human neuronal cells	94
Figure III-5.	Effect of MxA overexpression on subcellular localization of CEV antigen in human neuronal cells	96
Figure III-6.	Phylogram depicting relatedness of viral nucleocapsid proteins	102
Figure IV-1.	VEEV is less sensitive to type I IFN in the human neuroblastoma cell model	116
Figure IV-2.	SINV replication co-localizes with NPC marker expression in the SVZ.....	118
Appx Figure 1.	The effects of culture conditions on neuronal maturation, as measured by glutamate-mediated cytotoxicity	130
Appx Figure 2.	Extracellular matrices impact NPC morphology post-plating.....	133
Appx Figure 3.	NPC derivation protocols, comparison by immunocytochemistry.....	135
Appx Figure 4.	NPC derivation protocols, comparison by flow cytometry	137

LIST OF TABLES

Table I-1.	The neurotropic arboviruses	4
Appx Table 1.	Reagents for deriving NPCs and mature neurons from hESCs ...	128

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*.