THE CHOLINERGIC ANTI-INFLAMMATORY RESPONSE IN THE HIV CONTEXT

By

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LIST OF ABBREVIATIONS

CNS	central nervous system
α7-nAChR or α7	alpha7 nicotinic acetylcholine receptor
ACh	acetylcholine
NMJ	neuromuscular junction
ChAT	choline acetyltransferase
CAP	cholinergic anti-inflammatory pathway
[Ca(M)]	mecano-sensitive Ca^{2+} channel
[C(V)]	voltage-activated calcium channel
[Na(V)]	voltage-activated sodium channel
PNS	peripheral nervous system
LCP	lipidic cubic phase
α-BuTX	alpha bungarotoxin
RIC-3	resistance to inhibitors of cholinesterase 3
ER	endoplasmic reticulum
NMDARs	N-methyl-D-aspartate receptors
Ca^{2+}	calcium
PNS	peripheral nervous system
AChE	acetylcholinesterase
qRT-PCR	quantitative real time polymerase chain reaction

KO	knockout
Kin	knock in
PCR	polymerase chain reaction
LPS	lipopolysaccharide
TNF-α	tumor necrosis factor alpha
IL-1β	interleukin-1 beta
IL-6	interleukin-6
HMGB-1	high-mobility group box protein 1
IL-10	interleukin-10
TGF-β	transforming growth factor beta
TLR2	Toll Like Receptor 2
NTS	nucleus of the solitary tract
PCP	*Pneumocystis carinii* pneumonia
KS	Kaposi's sarcoma
CDC	Center for Disease Control and Prevention
HTLV	human T-lymphotropic virus
LAV	lymphadenopathy-associated virus
TAR element	Trans-activation response element
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
TNF-β	tumor necrosis factor beta

MIP-1α	macrophage inflammatory protein 1 alpha
Bcl-2	B-cell lymphoma 2
RRE	Rev response element
snRNA	small nuclear RNA
dUTP	deoxyuracil
dUTPase	deoxyuracil phosphatase
NF-κB	nuclear factor kappa beta
IL-1	interleukin 1
SP-1	specific protein 1
Lef	lymphoid enhancing factor
Ets	E-twenty six
NF-AT	nuclear factor of activated T cells
AP-1	activator protein 1
ORF	open reading frame
bp	base pair
MHC-1	major histocompatibility complex I
APOBEC3G	apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G
IFN-α	interferon alpha
IFN-γ	interferon gamma
IP-10	inducible protein 10
IL-15	interleukin 15

ELISA	enzyme-linked immunosorbent assay
TGF-β1	transforming growth factor beta 1
CXCR2	chemokine (C-X-C motif) receptor 2
HCl	hydrochloric acid
NaOH	sodium hydroxide
PNU-120596	1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea
CCK	cholecystokinin
CNI-1493	semapimod
SIV	simian immunodeficiency virus
DEPC	diethyl pyrocarbonate
GMF	geometric mean fluorescence
GRO-α	growth-related oncogene α
cART	combination antiretroviral therapy

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THESIS OUTLINE

Recent studies demonstrate that the central nervous system (CNS) and immune system interact with each other through a vagus nerve-dependent mechanism that involves afferent stimulation from peripheral inflammatory cytokines and the efferent release of the neurotransmitter acetylcholine (ACh). This circuit is called 'the cholinergic anti-inflammatory pathway' (CAP) because ACh binds to the alpha7 nicotinic acetylcholine receptor (α7-nAChR) in macrophages and inhibits the production of pro-inflammatory cytokines without altering the release of anti-inflammatory cytokines (1). The main objective of this thesis is to study the cholinergic anti-inflammatory response in the human immunodeficiency virus (HIV) scenario to better understand how the HIV affects the CAP and whether this understanding can help to comprehend and treat the chronic inflammation that affects HIV+ individuals.

In humans, during the early phase of HIV infection (acute), a dysregulation of cytokines and chemokines occurs causing an increase that correlates with plasma viremia (2). During this phase, pro-inflammatory waves occur prior to anti-inflammatory factors detection in HIV-infected donors, negatively affecting homeostasis. In fact, the long term consequences of this sustained dysregulation of pro- and anti-inflammatory mediators lead to chronic inflammation that lasts until death. Moreover, the inflammatory processes in these patients point toward HIV infection (3–8) and viral proteins, including gp120, (9) as causatives. For successful HIV infection, gp120 needs to bind CD4 and recruit CXCR4 or CCR5. Interestingly, gp120 has proven to bind to nAChRs-expressing cells from muscle and neuronal lineage (10) which could explain the infection of muscle and neuronal cells that are CD4 deficient (11,12). The lack of knowledge about the inflammatory role of the α7-nAChR in immune cells either recovered from

HIV+ individuals or *in vitro* cells exposed to viral proteins lead us to examine the cholinergic anti-inflammatory response in the HIV context.

Chapter 1 provides a general review on central and peripheral nAChRs. In addition, the available knowledge about CAP and the cholinergic anti-inflammatory reflex will be explained. Lastly, a review of inflammatory processes in HIV+ will be offered. **Chapter 2**, studies the *in vitro* effects of gp120 over α7-nAChR expression and discusses α7-nAChR studies in HIV-1 infected individuals. Moreover, it discusses inflammation assays to define the inflammatory phenotype of macrophages exposed to gp120. **Chapter 3**, examines the electrophysiological properties of α7-nAChR in human macrophages. **Chapter 4**, presents the α7-nAChR upregulation consequences regarding calcium mobilization and apoptosis. **Chapter 5**, addresses general conclusions. Finally, **Chapter 6** presents and discusses a number of future perspectives for the current thesis.

The effort contained in this thesis is dedicated to my family

who supported me each step of the way.

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ABOUT THE AUTHOR

Manuel Delgado Velez was born in Santurce, PR on July 15, 1978. His parents are Juan Delgado Peraza and Rosa Vélez Seguinot. Manuel was raised in Florida, PR and attended elementary school at Colegio Nuestra Señora del Rosario in Ciales. His high school was Colegio Nuestra Señora del Rosario in Vega Baja. After graduation he was accepted into the Universidad Interamericana de Puerto Rico, Arecibo Campus where his interest in science was cultivated and he entered the Biology Program. In 2003 he graduated Magna Cum Laude obtaining a bachelor's degree in biology, with a major in biomedical science. During his undergraduate days he identified scientific research as a potential way to fulfill his intellectual needs. Later, he worked at Abbott Biotechnology Ltd. (now Abbvie) in Barceloneta in the Microbial Environmental Control group (quality control lab) and later became a regular Abbott employee in the protein purification department. After two years in industry, he decided to move on to graduate school. During his first semester of graduate school, Dr. Fernando Renaud recruited him as research assistant, later he became a full graduate student. During his first year, he was awarded the Alliance for Minority Participation (AMP) fellowship. After Dr. Renaud's retirement he was accepted into Dr. José Lasalde's lab to start his doctoral thesis project. After two years of graduate school Manuel received the Research Initiative for Scientific Enhancement (RISE) fellowship. Manuel has been involved in several projects that include statin and alcohol studies in murine models of slow-channel congenital myasthenic syndrome (SCCMS) and studies on the effects of HIV-gp120 over the expression of α7-nAChR in immune cells, as well as the evaluation of this protein in HIV-infected subjects. Indeed, the latter is explained exhaustively in this thesis.