

Novel Insights into the Role of Defensins in Virus-Induced Autoimmunity in the Central Nervous System

Evangelos I Kazakos^{1*}, Jannis Kountouras¹ and Georgia Deretzi²

¹Department of Medicine, Second Medical Clinic, Aristotle University of Thessaloniki, Ippokraton Hospital, Thessaloniki, Greece

²Department of Neurology, Multiple Sclerosis Unit, Papageorgiou Hospital, Thessaloniki, Greece

*Corresponding author: Evangelos I Kazakos, 2 El. Venizelou street, 50100, Kozani, Greece, Tel: 00306938114741; E-mail: ekazakos@gmail.com

Rec Date: May 06, 2016, Acc Date: May 31, 2016, Pub Date: Jun 03, 2016

Copyright: © 2016 Kazakos EI, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Introduction

Virus-induced autoimmunity in the brain, an immune-privileged site, occurs primarily from a breach in thymic selection mechanisms that constitute peripheral tolerance, culminating in a self-directed, T-cell-mediated immune response traditionally thought to occur via molecular or epitope mimicry; immune recognition of novel self-antigens; bystander activation of autoreactive defensin-specific, T-lymphocytes (ATLs); and aberrant expression of cytokines in polyclonally expanded T-cell subsets [1]. Despite robust scientific evidence calling for an approach regarding the immune components of virus-related neurodegenerative diseases [2], the relative impact of defensins, as antiviral and immunomodulatory peptides, remains ill-defined. A growing body of evidence highlighted crucial, context-dependent roles of defensins in viral infections [3] and we have recently proposed several mechanisms by which dysregulation of defensin expression may trigger neuroinflammatory events in response to certain bacterial/viral stimuli [4].

Recently, key discoveries describing the presence of a brain-wide perivascular pathway—designated as the glymphatic system [5]—that supports, via astroglial intermediaries, cerebrospinal fluid (CSF) recirculation and drainage of interstitial solutes. Moreover the existence of a dedicated dural lymphatic vasculature allows bilateral movement of immune cells and immunoregulatory molecules between the brain parenchyma and the periphery [6,7], thereby challenging basic dogmas of neuroscience. These findings may call for a critical appraisal of basic concepts in central nervous system (CNS) immune surveillance and disease.

Herein, we consider recent defensin-related evidence and proposed novel pathways with a view to further address their involvement in brain inflammatory cascades that govern virus-induced autoimmune neuropathology.

Defensin Impact on Blood-Brain Barrier Integrity

Defensins, secreted by activated granulocytes, penetrate the blood-brain barrier (BBB), gaining access into brain, thereby possibly contributing to neurodegeneration; host defence peptides induce recruitment of mast cells involved in the production and release of inflammatory mediators that, in turn, induce BBB disruption and neuropathological changes associated with chronic disease conditions of the CNS involve abnormal expression and regulatory function of specific antimicrobial peptides (AMPs), including defensins [4]. In line with perceived vascular contributions to neurodegeneration [8], impaired expression of defensins by microglia, astrocytes, choroid plexus and possibly pericytes that create a distinct perivascular space may impede glymphatic fluid fluxes retaining blood-derived

neurotoxic metabolites and activated T-cells in the brain that impair BBB integrity and hamper viral clearance; development and maintenance of the BBB depends on the interaction of cerebral endothelial cells with pericytes and astrocytes (key components of the BBB) [9] and alterations of these components result in BBB dysfunction. In this regard, binding of the endothelial-secreted platelet-derived growth factor BB (PDGF-BB) to heparin sulfate proteoglycans (HSPGs) on the BBB basement membrane regulates pericyte proliferation and recruitment ensuring CNS microvascular stability [10]. Despite the fact that HSPGs facilitate simultaneous binding of human β -defensins and viral (HIV) gp120 to epithelial cells that favors the formation of immunogenic oligomers inside endosomes thereby reducing virion infectivity [11], cross-competition for shared receptors may disrupt PDGF-BB/PDGFR- β signalling leading to BBB vascular mural dysfunction.

Defensin-Related Autoreactivity and Autoimmunity

Blood-brain barrier breakdown results in loss of immune privilege and the development and/or adoptive transfer of anti-brain antibodies specifically those directed against non-myelin antigens that are known to play a significant role in multiple sclerosis-related early events. Autoimmune regulator (AIRE), a gene expressed in medullary thymic epithelial cells, is crucial for the induction of central tolerance to encephalitogenic myelin antigens [12] whereas its absence or dysfunction results in loss of T-cell negative selection and the development of polyclonal ATLs [13] that enter circulation and possibly brain, thereby initiating a dose-dependent CNS inflammatory response. As a result of further loss of the immunomodulatory effect of defensins, down-regulation of CD91 and CD91/LRP1 expression impairs peptide autocrine amplification and receptor-mediated clearance of amyloid β (A β) that ultimately favours the formation of aggregated protein deposits, a hallmark of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Recent dissection of the humoral repertoire elicited during infection by mainly enveloped viruses has revealed the presence of autoreactivity/autoimmune phenomena as a collateral effect of anti-viral immune response [14]. More specifically, preferential over-production of broadly neutralizing antibodies such as VH1-69 in HIV infection binding the membrane proximal external region (MPER) of gp41 is strongly associated with autoimmune patterns [15]. Defensins have recently been shown to augment mucosal immunogenicity and exposure of HIV gp41 MPER, possibly due to its rich hydrophobic residues, in vaccine preparations thereby enhancing autoreactivity [16]. VH1-69 germline antibodies are being over presented in HCV-chronically-infected individuals where they act by inhibiting CD81

receptor binding to HCV E2 glycoprotein-a crucial step in virus attachment and entry [17]. Nonetheless, as in the case of HIV infection, VH1-69-related neutralizing potential is often burdened by the cross-recognition of host cell membrane antigens driving the formation of cryoglobulins peculiar for type II mixed cryobulinemia-a HCV-related clinical syndrome with variable neurologic involvement [18]. Defensin affinity towards HCV surface glycoprotein epitopes may create a protective barricade that blocks viral fusion [19], thus displaying adjuvant properties to neutralizing antibodies, albeit at the expense of autoimmunity.

Implications of Bacteria-Driven Defensin Expression

Recent focus on symbiotic events between microbiota and viruses able to establish persistent infections is beginning to unveil highly complex synergies that modulate the thresholds of ATL activation and initiate a pro-inflammatory milieu able to induce distal signalling events in the CNS [20].

In this context, co-stimulated defensin expression by specific T-cell subsets, epithelial cells and perivascular macrophages provides a mechanistic mode that may induce differentiation and trafficking of immature, cytokine-expressing dendritic cells, CD4⁺ and CD8⁺ T-cells and inhibit apoptotic deletion that further fuels mucosal and systemic inflammation and contributes to the breakdown of self-tolerance. Contrastively, human β -defensin-3, induces *in vitro* regulatory T-cell-specific markers in CD4 (+) CD25 (-) T-cells, shifting those into a regulatory phenotype with suppressive features [21].

A Potential Impact of Defensins in HCV-Induced Autoimmunity in the CNS

Only a few evidence supports the hypothesis that neuropathological changes associated with chronic disease conditions of the CNS involve abnormal expression and regulatory function of specific AMPs including defensins. It is proposed that alterations of AMPs exacerbate proinflammatory conditions within the brain that ultimately potentiate the neurodegenerative process; for instance, the proposal that AMPs function within the brain is supported by the cytokine-induced expression of AMPs by human astrocytes and microglial. On the other hand, some research studies indicated that HCV RNA has been associated with CNS tissue, and reports of viral sequence diversity between brain and liver tissue suggest independent viral evolution in the CNS and liver. For instance, analysis of human HCV seropositive cases demonstrated widespread damage to neuronal dendritic processes and sustained activation of extracellular signal-related kinase (ERK); HCV core protein neurotoxicity may be mediated by the sustained activation of ERK/ Signal Transducer and Activator of Transcription 3-dependent manner dependent on toll-like receptor 2 signaling pathway, thereby providing novel targets for development of neuroprotective treatments for HCV involvement of the CNS [22]. Moreover, HCV NS3 protein was capable of activating microglia and the inflammatory response could be controlled by blocking the transcription nuclear factor- κ B, or by treating the microglia with TLR ligands which likely function via secreting anti-inflammatory cytokines such as interleukin-10, thereby having therapeutic potential in controlling HCV mediated neuroinflammation [23].

Viewing such aforementioned data we proposed that inappropriate expression of defensins may be involved in HCV-induced autoimmunity in the CNS. Establishing *in vivo* evidence for the presence of aforementioned pathways represents a challenging issue

with substantial repercussions in current virus-induced autoimmunity therapeutic targets.

References

1. Getts DR, Chastain EML, Terry RL, Miller SD (2013) Virus infection, antiviral immunity and autoimmunity. *Immunol Rev* 255: 197-209.
2. Itzhaki RF, Lathe R, Balin B, Ball M, Bearer EL, et al. (2016) Microbes and Alzheimer's disease. *J Alzheimers Dis* 51: 979-984.
3. Wiens ME, Wilson SS, Lucero CM, Smith JG (2014) Defensins and viral infection: dispelling common misconceptions. *PLoS Pathog* 10: e1004186.
4. Kountouras J, Deretzi G, Gavalas E, Zavos C, Polyzos SA, et al. (2014) A proposed role of human defensins in *Helicobacter pylori*-related neurodegenerative disorders. *Med Hypotheses* 82: 368-373.
5. Iliff JJ, Goldman SA, Nedergaard M (2015) Implications of the discovery of brain lymphatic pathways. *Lancet Neurol* 14: 977-979.
6. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, et al. (2015) Structural and functional features of central nervous system lymphatic vessels. *Nature* 523: 337-341.
7. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, et al. (2015) A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med* 212: 991-999.
8. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV (2015) Establishment and dysfunction of the blood-brain barrier. *Cell* 163: 1064-1078.
9. Wilhelm I, Nyúl-Tóth Á, Suciú M, Hermenean A, Krizbai IA (2016) Heterogeneity of the blood-brain barrier. *Tissue Barriers* 4: e1143544.
10. Armulik A, Genové G, Betsholtz C (2011) Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell* 21: 193-215.
11. Herrera R, Morris M, Rosbe K, Feng Z, Weinberg A, et al. (2016) Human beta-defensins 2 and -3 coinfect with human immunodeficiency virus via heparan sulfate proteoglycans and reduce infectivity of intracellular virions in tonsil epithelial cells. *Virology* 487: 172-187.
12. Tagawa A, Aranami T, Matsumoto M, Yamamura T (2014) Autoimmune regulator gene, Aire, is involved in central tolerance to DM20 isoform of proteolipid protein and the prevention of autoimmune inflammation. *Clin Exp Neuroimmunol* 5: 304-314.
13. Dobes J, Neuwirth A, Dobesova M, Voboril M, Balounova J, et al. (2015) Gastrointestinal autoimmunity associated with loss of central tolerance to enteric α -defensins. *Gastroenterol* 149: 139-150.
14. Cappelletti F, Clementi N, Mancini N, Clementi M, Burioni R (2015) Virus-induced preferential antibody gene-usage and its importance in humoral autoimmunity. *Semin Immunol* 27: 138-143.
15. Breden F, Lepik C, Longo NS, Montero M, Lipsky PE, et al. (2011) Comparison of antibody repertoires produced by HIV-1 infection other chronic and acute infections, and systemic autoimmune disease. *PLoS One* 6: e16857.
16. Mohan T, Verma P, Rao DN (2014) Comparative mucosal immunogenicity of HIV gp41 membrane-proximal external region (MPER) containing single and multiple repeats of ELDKWA sequence with defensin peptides. *Immunobiol* 219: 292-301.
17. Mancini N, Diotti RA, Perotti M, Sautto G, Clementi N, et al. (2009) Hepatitis C virus (HCV) infection may elicit neutralizing antibodies targeting epitopes conserved in all viral genotypes. *PLoS One* 4: e8254.
18. Sautto G, Mancini N, Clementi M, Burioni R (2012) Molecular signatures of hepatitis C virus (HCV)-induced type II mixed cryoglobulinemia (MCII). *Viruses* 4: 2924-2944.
19. Tarr AW, Urbanowicz RA, Ball JK (2012) The role of humoral innate immunity in hepatitis C virus infection. *Viruses* 4: 1-27.
20. Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. *Nat Rev Immunol* 15: 545-558.
21. Bruhs A, Schwarz T, Schwarz A (2016) Prevention and Mitigation of Experimental Autoimmune Encephalomyelitis by Murine β -Defensins via Induction of Regulatory T Cells. *J Invest Dermatol* 136: 173-181.

-
22. Paulino AD, Ubhi K, Rockenstein E, Adame A, Crews L, et al. (2011) Neurotoxic effects of the HCV core protein are mediated by sustained activation of ERK via TLR2 signaling. *J Neurovirol* 17: 327-340.
23. Rajalakshmy AR, Malathi J, Madhavan HN (2015) Hepatitis C Virus NS3 Mediated Microglial Inflammation via TLR2/TLR6 MyD88/NF- κ B Pathway and Toll Like Receptor Ligand Treatment Furnished Immune Tolerance. *PLoS One* 10: e0125419.