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The chitinases expression is related to Simian Immunodeficiency Virus Encephalitis (SIVE) and in HIV encephalitis (HIVE)

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ABSTRACT

Objectives: Human Immunodeficiency Virus (HIV) infection can induce neurocognitive complications classified as HIV-associated neurocognitive disorder (HAND). The chitinase family is associated with innate immunity cells and many infectious diseases.
Methods: We analyzed microarray datasets obtained from NCBI in order to verify the expression of chitinase family genes in hippocampus of uninfected rhesus macaques versus those with histopathologic evidence of Simian Immunodeficiency Virus Encephalitis (SIVE). Moreover, we have analysed two human microarray datasets to verify the results obtained in macaques hippocampus affected by SIVE. For these studies, we have also used the open source tools Genome-scale Integrated Analysis of gene Networks in Tissues (GIANT) to identify the chitinase genes network.
Results: CHIT1, CHI3L1 and CHI3L2 levels were significantly increased in SIVE hippocampus as compared to non-infected control specimens. Furthermore, we found a negative correlation between CHIA *vs.* Brain Viral Load (BVL). These data was confirmed partially in human brain section of HAD/HIVE subjects. Also, we showed that HIV-1 was able to modulate the expression of CHIT1, CHI3L1, CHI3L2 and CHID1 in human macrophages.
Conclusions: These results suggest that chitinase gene expression is altered in SIVE and in HAD/HIVE brain sections and call for more studies examining whether this is a protective immunological reaction or a destructive tissue response to encephalitis.

Abbreviations: HIV-1, human immunodeficiency virus 1; SIV, simian immunodeficiency virus; SIVE, SIV encephalitis; AIDS, acquired immune deficiency syndrome; CHIT1, Chitotriosidase; CHIA, acidic mammalian chitinase; CHI3L1, BRP-39, chitinase 3-like-1; CHI3L2, chitinase 3-like-2; CHID1, chitinase domain–containing 1; CNS, central nervous system; CSF, cerebrospinal fluid; SMOX, spermine oxidase; DCBLD2, discoidin, CUB and LCCL domain containing 2; LDLRAD4, low density lipoprotein receptor class A domain containing 4; ADAMTS2, ADAM metallopeptidase with thrombospondin type 1 motif, 2; RRAD, Ras-related associated with diabetes; C1s, complement component 1, subcomponent; PMP22, peripheral myelin protein 22; MT1B, metallothionein 1 B; HOMER3, homer homolog 3 (Drosophila); PTK2, protein tyrosine kinase 2; SLC11A1, solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1; TRIM10, tripartite motif containing 10; P2RX6, purinergic receptor P2X, ligand-gated ion channel, 6; APOC4, apolipoprotein C-IV; ARHGEF15, Rho guanine nucleotide exchange factor (GEF) 15; GIANT, Genome-scale Integrated Analysis of gene Networks in Tissues; MeV, MultiExperiment Viewer; ES, edge score; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; HIVE, HIV encephalitis; HAD, HIV-associated dementia; HAND, HIV-associated neurocognitive disorders; MDM, monocyte-derived macrophages.

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1. Introduction

Human Immunodeficiency Virus (HIV) infection can induce neurocognitive complications classified as HIV-associated neurocognitive disorder (HAND) (Antinori et al., 2007). According to standardized measures of cognitive dysfunction, it is possible to define three stages of HAND: *asymptomatic neurocognitive impairment* (ANI), *mild neurocognitive disorder* (MND) and *HIV-associated dementia* (HAD) (Elbirt et al., 2015). The most severe form of HAND is HAD (Etherton et al., 2015). This condition, characterized by cognitive dysfunction, motor disorders and deficits in learning and memory, occurs in less than 5% of people who have access to antiretroviral therapy (McArthur et al., 1993). Highly active antiretroviral therapy (HAART), a major advance in the treatment of HIV infection, has improved the survival of HIV patients (Fortunak et al., 2014; Nunnari et al., 2012). As HIV patients now live longer, the prevalence of HAND has increased (Ellis et al., 1997). HAD correlates better with activation of brain mononuclear phagocytes (macrophages and microglia) than with quantitative measures of brain infection (Kaul et al., 2005). It is very likely that HAD is a secondary response to innate immunity activation induced by HIV infection (Diesing et al., 2002; Kaul et al., 2005). In fact, HIV-1 can infect macrophages and microglia directly via gp120 binding, which in turn may induce the release of pro-inflammatory factors and proteins that can be recognized as markers of microglial activation (Pinzone et al., 2013a). Macrophages/microglial express CCR5 and CXCR4 (He et al., 1997) that are chemokine receptors on their surface in addition to CD4 (Jordan et al., 1991) and viral gp120 binds via these receptors (Pinzone et al., 2013b). The neurons and astrocytes have been reported to also possess CXCR4 and CCR5 receptors on their surface (Hesselgesser et al., 1997). During the HIV-1 infection the macrophage/microglial enhances their production of lysosomal vesicles and here the virus is assembled and acquires antigens characteristic of this compartment (Pelchen-Matthews et al., 2003).

The chitinase family is an ancient gene family, which has evolved to hydrolyze chitin and its derivative (Eide et al., 2016; Henrissat and Davies, 1997). The chitinase family includes proteins both with and without glycohydrolase enzymatic activity against chitin, which are expressed in cells of the innate immune system (Di Rosa et al., 2009). Chitotriosidase (CHIT1) and acidic mammalian chitinase (CHIA or AMCAse) are the only two true chitinases possessing chitinolytic activities (Di Rosa and Malaguarnera, 2012). The other members, chitinase-like-lectins (Chi-lectins) or chitinase-like proteins (C/CLPs), including chitinase 3-like-1 (CHI3L1, also called YKL40 or HC-gp39), chitinase 3-like-2 (CHI3L2, CHIL2, YKL-39), chitinase domain containing 1 (CHID1), are structurally homologous to CHIT1 and CHIA but lack the essential catalytic residues with the preservation of the substrate-binding cleft of the chitinases (Lee et al., 2011).

Chitinase expression is greatly amplified during many infections, highlighting their potential biological roles in inflammatory diseases and in innate immuno-activation (Di Rosa et al., 2016a,b). Little is known about the physiological role of chitinases in the central nervous system (CNS). It has been shows that many members of the mammalian chitinase-like gene family may be involved in limiting the inflammatory response to cerebral insults (Wiley et al., 2015a). Canto and colleagues found a compensatory increase in chitinase-like mRNA in the CNS (namely chi3l3 and chi3l4) during experimental autoimmune encephalomyelitis (EAE) in BRP-39 KO mice at average clinical severity score 10 and 14 dpi (Canto et al., 2015). C.A. Wiley et al. in 2014 showed that astrocytic expression of CHI3L1 limits the extent of both astrocytic and microglial/macrophage facets of neuroinflammation in Traumatic brain injury (TBI) (Wiley et al., 2015b). CHIT1 and CHI3L1 have been reported to be upregulated in a variety of neurological degenerative disorders (Di Rosa et al., 2006; Harris and Sadiq, 2014; Varghese et al., 2013). In cerebrospinal fluid (CSF) from AD patients, CHIT1 and CHI3L1 levels were higher compared to those found in neurologically normal control individuals (Choi et al., 2011; Olsson et al., 2012; Rosen et al., 2014). Furthermore, high CHIT1 levels in CSF from ALS patients suggested a possible role in disease progression (Pagliardini et al., 2015; Varghese et al., 2013). Most of these studies suggest that increase CSF chitinase activity reflects microglial activation as a response to or contributing factor in neurodegeneration. Recently, CHI3L1 was associated to the dendritic cell activation and differentiation (Di Rosa et al., 2016b). High CSF CHI3L1 levels were recently described in macaques and humans with lentiviral encephalitis (Bonneh-Barkay et al., 2008). In addition, it has been shown that CHI3L1 expression may be induced in astrocytes in traumatic brain injury (Bonneh-Barkay et al., 2010). Some (Craig-Schapiro et al., 2010; Olsson et al., 2013), but not all (Lee et al., 2011), studies have found increased levels of CHI3L1 in AD patients.

CHI3L2, another chitinase family member, has been reported to be overexpressed in AD brains (Colton et al., 2006). Recently, increased CSF levels of CHI3L2 were measured in multiple sclerosis (Hinsinger et al., 2015). It is also one of the most expressed genes in glioblastomas but its function remains still obscure (Areshkov and Kavsan, 2010; Saidi et al., 2008).

CHIA is one of the enzymes with true chitinase activity (Di Rosa et al., 2013a). The relatively abundant expression of CHIA in the gastrointestinal tract and lung supports a possible role in the mucosal immune system and potentially also as a digestive enzyme (Ohno et al., 2012). The precise role of CHIA in immune-mediated diseases is not clear but many reports suggesting that chitinase activity exerts a beneficial effect by negatively regulating chitin-induced tissue infiltration of innate immune cells associated with allergy (Reese et al., 2007). Some studies suggest that CHIA activity may be needed in the brain to protect from protozoan infections (Nance et al., 2012).

Scarce informations are available on CHID1. It can be used as a marker of alternative macrophage activation (Riabov et al., 2014) and is expressed in some brain tumours (Kzhyshkowska et al., 2016). Furthermore, CHID1 is expressed by specialized tissue macrophages (placenta, skin, gut and pancreas) and in cardiac and skeletal muscle by sinusoidal endothelial cells in liver, spleen, bone marrow and lymph nodes (Kzhyshkowska, 2010).

In this study, we test the hypothesis that chitinase expression may be regulated during macrophage and microglial activation in a macaque model of HAD.

2. Methods

2.1. Selection of a microarray expression dataset and bioinformatics analysis

For this study, we analyzed microarray datasets obtained from NCBI (http://www.ncbi.nlm.nih.gov/) under accession number GDS4214 in order to examine mRNA levels of chitinase family genes in hippocampus specimens of uninfected rhesus macaques (animal control) versus those with histopathologic evidence of *Simian Immunodeficiency Virus Encephalitis* (SIVE). We decided to select an animal model because it was extremely stable and evolutionarily close to the chitinase. It has been showed that people who have lost brain cells in the hippocampus area of the brain are more likely to develop dementia (den Heijer et al., 2010; La Joie et al., 2013).

The MultiExperiment Viewer (MeV) software was used to identify different expression of chitinase genes and to generate expression heatmaps. In cases where multiple probes insisted on the same NCBI GeneID, we used those with the *highest variance*.