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Pattern recognition receptor mRNA expression and cytokine and granzyme levels in HIV infected individuals with neurotuberculosis

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ABSTRACT

Neurotuberculosis is one of the commonest HIV-associated opportunistic infections (OI) of the CNS. Cross-talk between HIV, *Mycobacterium tuberculosis* and host immune responses may alter expression of pattern recognition receptors (PRRs), thereby affecting cytokine profiles and functional responses. We examined PRR mRNA expression and cytokine and granzyme levels in HIV infected individuals with neurotuberculosis and found significant downregulation of TLR9 and increased MDA5 expression compared to healthy subjects. Significantly higher Granzyme A and IFN-γ levels were also observed in the CSF of this group compared to CSF from non-infectious controls. These alterations may lead to inappropriate recruitment of immune cells to the CNS, leading to disease severity.

1. Introduction

Chronic disease conditions such as co-infection with the HIV and *Mycobacterium tuberculosis* (MTB) present challenges in understanding the complexities, mechanisms and consequences of host-pathogen interactions. Innate immune responses along with the involvement of inflammatory mediators such as cytokines and cells play an important role in the defense against these pathogens.

Recognition of the conserved pathogen-associated molecular patterns (PAMPs) is important in initiating host defense. Receptors of the innate immune system called pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs), the cytosolic RIG-I-like receptors (RLRs) and Nod-like receptors (NLRs) recognize PAMPs, induce immune response and modulate cytokine production (Medzhitov and Janeway, 2002), thus shaping the outcome of the infection. Abnormalities in PRR signaling pathways regulated by HIV and MTB may affect disease pathogenesis and hence the mechanisms through which immune cells recognize them need to be elucidated. Literature regarding PRR expression profiles in HIV-TB co-infected individuals is very limited.

Abnormal PRR signaling is likely to result in dysregulation of cytokines, thereby leading to progression of disease in HIV-TB co-infected patients. Although much remains to be understood about disease pathogenesis in HIV-TB co-infection, it is known that alterations in cytokine and chemokine levels results in increased T cell activation, enhanced HIV replication and defects in the immune response (Goletti et al., 1996; Hertoghe et al., 2000; Toossi et al., 2001a,b). Alterations in cytokine profiles in HIV infected individuals have been documented by several studies (Christo et al., 2009; Graziosi et al., 1996; Hittinger et al., 1998; Van der Watt et al., 2014; Yuan et al., 2015). Reports detailing the cytokine levels in HIV-TB co-infected individuals have also been published (Bal et al., 2005; da Silva et al., 2013; Mihret et al., 2014; Rai et al., 2014; Subramanyam et al., 2004). However, there are very few reports that describe the profile of cytokine alterations in HIV infected individuals with TB of the central nervous system (HIVNTB).

This study was therefore undertaken to compare PRR mRNA expression profiles in HIVNTB cases as compared to individuals belonging to the other groups included in the study. Additionally, the levels of cytokines and chemokines in plasma and cerebrospinal fluid (CSF) specimens from cases of neurotuberculosis in HIV positive individuals in comparison to HIV negative neurotuberculosis cases and controls were also measured.

2. Materials and methods

2.1. Study population

This case-control, prospective study was conducted at the National

Abbreviations: CNS, central nervous system; MTB, *Mycobacterium tuberculosis*; OI, opportunistic infections; PRR, pattern recognition receptor; TLR, toll-like receptor; CSF, cerebrospinal fluid; IFN-γ, interferon gamma; TNF, tumor necrosis factor; ART, antiretroviral therapy; IL, interleukin; MIP-1β, macrophage inflammatory protein-1β

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Table 1
Subjects of the study categorized into different groups.

Group 1 HIV positive with neuro TB (HIVNTB) (n = 20)	HIV positive individuals presenting with clinical symptoms of neurotuberculosis admitted to the casualty and emergency services, neurology and neurosurgery wards of NIMHANS.
Group 2 HIV positive with systemic TB (HIVSTB) (n = 20)	HIV positive individuals with no neurological involvement but presenting with clinical features of systemic tuberculosis attending the integrated counseling and testing centre (ICTC), NIMHANS for CD4 enumeration.
Group 3 Asymptomatic HIV positive (HIVAsym) (n = 20)	Asymptomatic HIV positive individuals exhibiting no symptoms and signs (on clinical examination) of TB or any other HIV-associated infections attending the ICTC, NIMHANS for HIV testing/CD4 enumeration.
Group 4 HIV negative with neuro TB (NTB) (n = 20)	HIV negative individuals presenting with clinical features of neuro-tuberculosis admitted to the casualty and emergency services, neurology and neurosurgery wards of NIMHANS.
Group 5 HIV negative with systemic TB (STB) (n = 20)	HIV negative individuals with no neurological involvement but presenting with clinical features of systemic tuberculosis attending the ICTC, NIMHANS for HIV testing.
Group 6 Healthy controls (HC) (n = 20)	Healthy controls (negative for both HIV and TB) age matched with HIVNTB (Group 1).

Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India from June 2011 to August 2015. The subjects included in the study were categorized into six different groups. The details of the inclusion criteria as well as the various clinical groups are described in Table 1.

Both treatment-naïve individuals and individuals on antiretroviral therapy (ART) were included in the HIV-infected groups in the study. ART treatment undertaken was according to the guidelines issued by the National AIDS Control Organization (NACO).

The demographic, clinical and laboratory data of subjects in the different groups in the study are shown in Table 2.

2.2. Laboratory diagnosis

Confirmation of neurotuberculosis and systemic TB was achieved through smear examination (Zeihl-Neelsen stain) or culture (definitive) or through clinical, CSF biochemical and cellular and/or imaging findings (probable). Confirmation of HIV infection was achieved by rapid tests according to guidelines issued by NACO.

2.3. Clinical samples

Whole blood samples (10 ml) in EDTA vacutainers (BD Biosciences, USA) were collected from all individuals categorized into the six groups detailed in Table 1. CSF samples from patients in HIVNTB and NTB groups, collected for biochemical/microbiological laboratory analysis, if available, were also stored. These included 5 samples from HIVNTB ART naïve patients, 6 samples from HIVNTB patients on ART and 15 samples from HIV negative NTB cases.

The study was approved by the Institutional Ethics Committee of NIMHANS (Ref no: NIMH/DO/SUB-COMMITTEE/2012). Samples were collected after obtaining signed informed consent from all individuals.

2.4. Enumeration of CD4 T cells

CD4 cell counts in whole blood were enumerated in the Groups 1, 2 and 3 using BD TriTEST CD3/CD4/CD45 reagent and the TruCOUNT tube (BD Biosciences, USA) as per manufacturer's instructions and run on the BD FACS Calibur flow cytometer. Data was analyzed using the BD MultiSET™ software.

2.5. Isolation, cryopreservation and revival of peripheral blood mononuclear cells (PBMC) from whole blood specimens

PBMC from venous blood (10 ml in EDTA) collected from each individual was isolated by density gradient centrifugation (Histopaque 1077, Sigma, USA) and preserved in liquid nitrogen. Plasma was stored at − 70 °C. Stored PBMC were revived and the pellet re-suspended in RPMI-1640 (Sigma, USA) medium with 10% Fetal Calf Serum (FCS; Sigma, USA) (R10). Cell count was performed by Trypan Blue (Sigma, USA) method for viability.

2.6. HIV load

Viral load in plasma of samples collected from HIV infected individuals was estimated by an in-house real time TaqMan PCR previously designed and standardized in the Department of Neurovirology, NIMHANS for the quantitation of HIV-1 viral load (Kamat et al., 2007).

Table 2
Summary of demographics and laboratory data of individuals included in the group under study.

Characteristics		Study groups					
		HIVNTB	HIVSTB	HIVAsym	NTB	STB	HC
Age in years		35 (19–66)	38 (23–68)	37 (20–55)	25 (18–68)	38 (19–50)	36 (18–67)
Gender – no. (%)							
Male		10 (50%)	13 (65%)	8 (40%)	12 (60%)	14 (70%)	11 (55%)
Female		10 (50%)	7 (35%)	12 (60%)	8 (40%)	6 (30%)	9 (45%)
No. of ART naïve cases		09	13	12	NA	NA	NA
No. of cases on ART		11	7	8			
CD4 T cells (cells/μl)	ART Naïve	101 (25–303)	167 (6–846)	372 (276–1165)	NA	NA	NA
	On ART	291*$^{\delta}$ (28–896)	412.5 (56–1305)	469.5$^{\theta}$ (71–1447)			
Viral load (log_{10} copies/ml)	ART Naïve	5.27 (1.95–6.27)	5.00 (1.95–7.05)	4.58 (1.95–5.97)	NA	NA	NA
	On ART	1.95* (1.23–5.21)	2.46 (1.95–6.48)	1.95* (1.95–4.41)			

Data of age, CD4 T cell counts, viral load and CD4/CD8 T cell ratio presented as median values with the range in parenthesis.
A p-value of < 0.02, indicated by *, was considered significant for comparison between treated and untreated in each HIV infected group.
A p-value of < 0.006 was considered significant for comparison between different groups. δ indicates significant difference in comparison to HIVAsym group, α indicates significant difference in comparison to NTB group, β indicates significant difference in comparison to STB group and σ indicates significant difference in comparison to HC group. θ indicates significant negative correlation with HIV load.