Journal of Medical Virology 88:1297–1302 (2016)

Demethylation Profile of the TNF-α Promoter Gene Is Associated With High Expression of This Cytokine in *Dengue virus* Patients

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Dengue is the most prevalent arthropod-borne viral illness in humans. The overexpression of cytokines by *Dengue virus* (DENV) infected cells is associated with the most severe forms of the disease. Unmethylated CpG islands are related to a transcriptionally active structure, whereas methylated DNA recruits methyl-binding proteins that inhibit gene expression. Several studies have described the importance of epigenetic events in the regulation and expression of many cytokines. The purpose of the present study was to evaluate the methylation status of the IFN-γ and TNF-α promoters in DNA extracted from dengue infected patients using methylation-specific polymerase chain reaction. A high frequency of demethylation was observed in the TNF-α promoter of DENV infected patients when compared to non-infected controls. The patients with an unmethylated profile showed higher expression of TNF-α mRNA than patients with the methylated status. No difference was found in the methylation frequency between the two analyzed groups regarding the IFN-γ promoter or in the expression of IFN-γ transcripts. The present study provides the first association of TNF-α promoter demethylation in DENV infected individuals and demonstrates a correlation between the methylation status of the region analyzed and the expression of TNF-α transcripts in DENV infected patients. ***J. Med. Virol. 88: 1297–1302, 2016***.

KEY WORDS: *Dengue virus*; DNA methylation; promoter; IFN-γ; TNF-α

INTRODUCTION

Dengue virus (DENV) is an arbovirus transmitted to humans through the bite of infected *Aedes aegypti* female mosquitoes. Dengue is endemic in several countries and almost half of the world's population lives in risk areas of dengue [Guzman and Harris, 2015]. DENV is a positive single strand RNA virus that belongs to the family Flaviviridae, genus *Flavivirus*. After infection, the patient may develop a mild, self-limiting febrile illness called Dengue Fever or a more severe clinical manifestation, known as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [Halstead, 2007; Ranjit and Kissoon, 2011].

These forms of the disease are characterized by spontaneous bleeding and plasma leakage after excessive immune activation of T cells and macrophages, which produce several cytokines in large quantities, which in turn can increase vascular permeability and cause endothelial dysfunction [Friberg et al., 2011; Malavige and Ogg, 2013; Guzman and Harris, 2015]. The cytokines involved in dengue pathogenesis are pro-inflammatory cytokines, such as tumor necrosis alpha (TNF-α) and interferon gamma (IFN-γ). The high reactivity to DENV infection could be attributed to cross-reactive T cells that produce high concentrations of pro-inflammatory cytokines, such as TNF-α and IFN-γ, in a secondary infection caused by a heterologous serotype [Friberg et al., 2011; Malavige and Ogg, 2013].

DNA methylation is an epigenetic control mechanism of gene expression characterized by the addition of a methyl group to a cytosine within cytosine-phosphate-guanine islands. Unmethylated

Grant sponsor: CNPq; Grant numbers: 475940/2011-3; 404005/2012-8

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Accepted 19 January 2016

DOI 10.1002/jmv.24478
Published online 2 February 2016 in Wiley Online Library (wileyonlinelibrary.com).