miR-155 and miR-146a expression in serum samples from patients with ocular toxoplasmosis

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Ocular toxoplasmosis (OT) is one of the most common manifestations of the toxoplasmosis and the main cause of retinochoroiditis, a disease that can cause serious sequels. The immune response might directly affect pathogenesis of OT and some cytokines have been shown to be fundamental to either control or block a protective response against *Toxoplasma gondii*. Currently, microRNAs (miRNAs) are small (18-24 nt) non-coding RNAs, which have important regulatory roles in cells by silencing messenger RNA (mRNA) expression by cleavage or translational repression. Over 100 different miRNAs have been shown to be expressed by cells of the immune system. This study evaluated the expression of two miRNAs on toxoplasmosis (miR-155 and miR-146a) in serum samples of OT patients. miRNAs levels were evaluated in 31 serum samples divided into two groups: I – 20 patients with OT and II- 11 individuals with chronic toxoplasmosis (CHR). miRNAs were extracted with a specific kit and, after cDNA synthesis, expression profile of each miRNA was determined by quantitative real-time PCR (qPCR). Differences in miRNAs expression were normalized in relation to an external spike-in control (a synthetic *C. elegans* miR-39) and the results are expressed in fold-change relative to the negative control (5 negative samples for toxoplasmosis), according to the comparative Ct method ($2^{-\Delta\Delta Ct}$). The statistical analyses were made by the non-parametric *Mann–Whitney* test and *p*-value <0.05 was considered to be statistically significant. Results showed that expression of both miRNAs was statistically up-regulated in OT patients compared CHR individuals. miR-155 was five times more express in OT patients than CHR individuals (mean of expression of 5.6 and 0.55, respectively). miR-146a was twenty-eight times more express in OT patients than CHR individuals (mean of expression of 31.7 and 3.7, respectively). miR-155 has been associated with regulation of different immune-related processes and was shown to be stimulated by TGF-β, to repress cytokines produced by Th2 cells and is implicated within a complex network regulating Th17 cytokines. Our previous study showed that OT patients had high levels of mRNA for TGF-β, IL-17 and IL-10, and decreased levels of mRNA for IL-4 compared to chronic subjects. miR-155 may be involved in the regulation of these cytokines. miR-146a was found to be highly expressed in Treg cells and to be vital for their ability to control IFN-γ-mediated Th1 immune response, since the loss of miR-146a in Treg cells resulted in an increased production of IFN-γ by $CD4^+$ and $CD8^+$ effector T cells. Still has been described that OT patients has low levels of mRNA for IFN-γ compared to chronic individuals as we are report in this study. The mechanisms behind this miRNAs under different phases of infection still need to be clarified. A proper balance between miRNA expression

patterns and cytokine profiles is required for T-cell homeostasis and self-tolerance. As such, miRNA-cytokine deregulation constitutes an informative disease biomarker and, most importantly, a promising target for therapeutic intervention.

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