

Gene expression of cytokines in patients with symptomatic toxoplasmosis

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The aim of the present study was to evaluate the expression of genes encoding for the 9 cytokines in mononuclear cell (PBMC) samples from patients with ocular toxoplasmosis (OT) and formalin fixed paraffin embedded autopsies (FFPE) from patients with disseminated toxoplasmosis/AIDS (DT/AIDS). The analyzes were performed in a total of 58 clinical samples divided into 3 Groups. Group I, 22 FFPE autopsy samples (11 of lung and 11 of brain) from 11 patients who died with coinfection DT/AIDS. Group II, 27 PBMC samples from OT patients. Group III, 9 PBMC samples from individuals with chronic toxoplasmosis (CHR) (asymptomatic and immunocompetent). RNA extractions were done with specific kits. A volume of 100 ng/ μ L RNA was used for cDNA synthesis. The endogenous genes GAPDH and TBP were used as normalizers in FFPE and PBMC, respectively. The gene expression assays were done using the endogenous genes and genes encoding IFN- γ , TGF- β , IL-4, IL-6, IL-10, IL-12, IL-17, IL-27, TGF- β and TNF- α by real-time PCR in cDNA samples of the 3 Groups studied. The expression patterns of each gene were evaluated by the geNorm program. Values of mRNA expression were shown as Relative Quantification (RQ). Results showed a significant increase of mRNA levels for expressing IL-6 (RQ = 15.41), TGF- β (RQ = 28.04) and IL-10 (RQ = 7.8) in OT Group. CHR Group had RQ of 0.43, 7.54 and 0.67 for IL-6, TGF- β and IL-10 respectively (at $p < 0.0005$, $p < 0.005$ and $p < 0.05$). OT patients, also, had lower mRNA IFN- γ (RQ 4.1) and IL-4 (RQ 1.97) expression than those from CHR Group (IFN- γ , 24.49 and IL-4, 7.14 at $p < 0.05$). Levels of mRNA for TNF- α (RQ = 9.3 and 4.7), IL-12 (RQ = 515.3 and 219.9), IL-17 (RQ = 21.70 and 6.49) and IL-27 (RQ = 17.39 and 14.63) were also higher in OT patients compared to CHR, although no significant statistical difference. According to patients of DT/AIDS Group, despite the difficulty of isolating RNA from FFPE samples, it was possible to detect mRNA levels of two cytokines. In brain and lung samples, mRNA levels expressing IL-6 were significantly lower than those that expressed TGF- β . As IL-6 is able to antagonize the anti-microbial properties of IL-12 and IFN- γ inhibits TGF- β activity. Consistent with these findings, a few levels of mRNA expressing IL-6 in brain and lung of the patients were unable to inhibit TGF- β production. High levels of mRNA expression for TGF- β in lung and brain may elicit direct and indirect anti-inflammatory effects leading to immune suppression, which may result in a chronic course of toxoplasmosis with complications such as encephalitis, retinochoroiditis and others.

Keywords: disseminated toxoplasmosis, ocular toxoplasmosis, gene expression, cytokines.

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