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Cytokine profiles in patients with toxoplasmic lymphadenitis in the setting of pregnancy

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ABSTRACT

Introduction: Majority of *Toxoplasma gondii* infections are benign and asymptomatic; however, some patients experience toxoplasmic lymphadenitis (TL). Factors associated as to whether infection will be symptomatic or not are unknown.
Methods: Dye test titers of patients with acute toxoplasmosis (pregnant and not pregnant) with TL (TL+) were compared with those in patients with asymptomatic acute infection (TL−). Additionally, mean levels of 62 serum cytokines were compared between TL+ and TL− pregnant women and between TL+ pregnant and non-pregnant women.
Results: During acute infection, mean dye test titer was higher in TL+ than in TL− patients ($p = 0.021$). In addition, out of 62 cytokines, CXCL9 and CXCL10 levels were higher ($p < 0.05$) and resistin mean levels were lower ($p < 0.05$) in pregnant women with TL+ compared to TL−. Among patients with TL+, levels of VCAM1 and CCL2 were lower ($p < 0.05$) in pregnant women than in non-pregnant women.
Conclusion: Here we report differences in dye test titers in patients with acute infection. Cytokine responses vary according to the presence of TL+ and to the pregnancy status. Factors underlying these differences are presently unknown and require further studies to define individual and combined roles of cytokines in TL+.

1. Introduction

In Europe and North America, *Toxoplasma gondii* primary acquired infection in immunocompetent patients appears to be asymptomatic in a vast majority of cases. Others may experience flu like symptoms, fever and/or lymphadenopathy. Lymphadenopathy due to *T. gondii* is commonly cervical and suboccipital rather than generalized [1]. Lymph nodes (LN) are non-tender and do not suppurate. In some patients with toxoplasmic lymphadenitis (TL), enlargement of the lymph nodes can persist and fluctuate for months, and accompanying systemic symptoms become severe. In these cases, clinicians often initiate anti-toxoplasma treatment and, at the same time, expand the differential diagnosis to include other infectious diseases, malignancy, and immunologic disorders. Serological profile of an acute infection is characterized by a positive dye test titers, positive IgM, low *T. gondii* IgG avidity and acute pattern in the differential agglutination test (AC/HS) (AC/HS is only performed at the Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory (PAMF-TSL, CA, USA; www.pamf.org)) [1]. In TL, serological findings often appear to be unique. Unfortunately, previous studies have not addressed serological difference in human patients with acute *T. gondii* infection between patients with TL (TL+) and those without TL (TL−).

Until now, the factors that determine whether acute *T. gondii* infection will be symptomatic or not are unclear. The clinical expression of the disease is likely linked to the host immune response, the host genetic background and the genotype of *T. gondii* strain. The host immune response against *T. gondii* involves both

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innate acute inflammatory responses and antigen-specific adaptive immunity including mobilization of a strong cell-mediated component [2]. Most of our knowledge on immune response against *T. gondii* has been derived from studies in animal models. In such models, the immune response against the parasite is a T_h1 response involving T_h1 effector cells and production of IFN-γ and IL-12 [2,3]. This response is counter-balanced by the anti-inflammatory cytokines such as IL-10, TGF-β and IL-27 that prevent immunopathology during toxoplasmosis [2,3]. Few studies have addressed the role of cytokine in human infected by *T. gondii*. Cytokines studies have revealed different cytokines profiles associated with presence of *T. gondii* infection, anatomical site and geographic area of infection. Indeed, in a study comparing pregnant women from the United States (USA) and Colombia infected with *T. gondii*, differences have been found in circulating cytokines between the two populations [4]. Likewise, the cytokine profile differed between chronically infected and uninfected patients [4]. Regarding the anatomical site of infection, in another study, patients with ocular toxoplasmosis had a high level of chemokine CXCL-8 and low level of IFN-γ and CCL2 compared to infected asymptomatic individuals [5–7]. In patients with cerebral toxoplasmosis, IFN-γ and IL-10 were found to be lower and TNF-α higher than in patients with asymptomatic chronic infection [7]. These studies highlight that the cytokine profile varies with the population studied, the geographical area of infection and the clinical manifestations of the disease.

Presently, there is a paucity of data on human immune responses including cytokine profiles in TL+ patients compared to TL− patients. Here, we report the serological and cytokine profiles of TL+ and TL− patients in the USA.

2. Materials and methods

We performed a retrospective cohort study using data collected by the PAMF-TSL; CA, USA, during 2004–2014. The study was approved by the Institutional Review Board at the Palo Alto Medical Foundation Research Institute.

2.1. Patients

Consecutive patients were selected for our study if they were older than 15 years old and deemed to be acutely infected by serological testing. Acute infection was defined according to the following very strict criteria: positive dye test, positive IgM ELISA ⩾ 3, low IgG avidity ⩽ 15 (VIDAS *Toxoplasma* IgG Avidity test, bioMérieux, Marcy l'Etoile, France) and differential agglutination (AC/HS) with an acute pattern [8–10]. The methods for serological testing at PAMF-TSL have been described elsewhere [8–10]. With these very strict criteria the selected patients were considered infected four months or less before the sample collection time.

2.1.1. Classifications of selected patients (Table 1)

After the selection based on the serological profiles, patients were classified into 2 categories: acute infection with TL (TL+) or acute infection without TL (TL−), according to the patient clinical history reported to the PAMF-TSL (Table 1). Whether the patient was pregnant or not was also recorded. In case of TL, we selected only the samples with date of onset of the lymphadenopathy within 4 months prior to the collection time. For dye test comparison, a total of 266 sera from 266 patients were analyzed: 97 TL+ patients and 169 TL− patients (Table 1 and Fig. 1). For measurement of cytokines, the selection and exclusion criteria are detailed in Fig. 1. A total of 99 pregnant women were studied separately with (12 TL+ patients) and without (87 TL− patients) TL. In addition a total of 35 TL+ women who were (12 patients) or were not (23 patients) pregnant were studied (Table 1 and Fig. 1).

Table 1
Summary of the 3 groups of patients studied. The dye test titers comparison was performed on the whole population of *T. gondii* acute infection. For the cytokines analysis, the first comparison was performed in TL+ and TL− pregnant women and the second comparison was performed in TL+ pregnant and non-pregnant women.

	Dye test comparison	Total
Acute infection	97 TL+ 169 TL−	266
	Cytokines profile comparison	
Pregnant women	12 TL+ 87 TL−	99
TL+	12 pregnant women 23 non pregnant women	35

2.2. Cytokine assays

Sera were stored at −20 °C before cytokine analysis. Serum samples were analyzed at the Human Immune Monitoring Core (Stanford, CA, USA) by Luminex Human 62-plex kits (Affymetrix, Santa Clara, CA, USA). The analysis encompassed 62 cytokines: BDNF, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CD40L, M-CSF, G-CSF, CXCL1, CXCL5, CXCL9, CXCL10, CXCL12, EGF, FASL, FGF-basic, GM-CSF, HGF, ICAM-1, IFN-α, IFN-β, IFN-γ, IL-1RA, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IL17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, LIF, Leptin, NGF, PAI-1, PDGF-BB, resistin, SCF, TGF-α, TGF-β, TNF-α, TNF-β, TRAIL, VCAM1, VEGF and VEGF-D. Plates were read using a Luminex LabMap200 instrument with a lower bound of 100 beads per sample per measured cytokine. Each sample was tested in duplicate according to the protocol already described [4]. Median fluorescence intensity (MFI) was averaged over duplicate wells for each cytokine per sample on each plate. Use of population marginal means, centered at mean age, allowed us to remove plate effects from these mean MFI values without removing effects of pregnancy or TL status [11]. For regression modeling, plate-detrended mean MFI values were centered and scaled through subtraction of the sample mean and division by the sample standard deviation, respectively.

2.3. Statistical analysis

2.3.1. Comparison of dye test titers between TL+ patients and TL− patients

Dye test titer data were transformed via log (titer + 128) and fit to a parametric accelerated failure time regression model for a log-logistic distribution [12]. Different transformations and parametric conditional distributions were examined and a final model was chosen based on the Akaike information criterion [13] and plot of fitted probability distribution. Regression model candidate predictor variables were TL, gender, and pregnancy for full sample (n = 266). The difference in geometric mean dye test titer between TL+ and TL− patients was estimated using population marginal means to correct for imbalances in gender and pregnancy between TL+ and TL− patients [14].

2.3.2. Analysis of cytokines in TL+ and TL− pregnant women and in pregnant and non- pregnant TL+ women

Analysis of association between TL status and cytokine responses was limited to pregnant women; and analysis of association between pregnancy and cytokine responses was limited to TL + women. The 3 groups of patients studied and are detailed in Table 1.