Immunoregulation of cytokine signalling network in *Toxoplasma gondii* infected-women

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Abstract: The global risk of toxoplasmosis in human is dramatically rising up. Indeed, the disease is asymptomatic in human, but its risk can increase in pregnancy and immunodeficiency disorders. Cytokine profiling seems to be fundamental in determination of immunestatus of host during infection by pathogens, where the immunoregulation of host cytokine responses that occurs determinates the pathogenicity or maintenance and homeostasis of pathogen-infected organs and tissues. For investigating cytokine profile during toxoplasmosis, 50 of *Toxoplasma gondii*-infected women and 50 of *Toxoplasma gondii*-uninfected women (naive control group) were enrolled in this study. Interleukin 10 (IL-10), IL-11, IL-17, and tumour necrosis factor-beta (TNF-β) concentrations in the serum were monitored using enzyme-linked immunosorbent assay (ELISA) technique in both women infected and uninfected with *Toxoplasma gondii* parasites. In our study, the data indicated disruption in production of cytokines in *Toxoplasma gondii*-infected women in comparison with naive control group. Generally, our findings revealed an increase in the level of all cytokines that included in this study. Noticeably, this increasing was only significant in IL-10 and IL-11 concentrations comparing to *Toxoplasma gondii*-uninfected control group at p <0.0001 and p <0.05, respectively. Accordingly, the balance between productions of pro- and anti-inflammatory cytokines reflecting the responses of T helper 1 (Th1) and T helper 2 (Th2)-type CD4⁺ cells, respectively, which are essential for development and establishment of toxoplasmosis disease.

Key words: *Toxoplasma gondii*, Cytokines, Interleukins, T helper cells, Inflammation, ELISA


1. Introduction

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* parasites with worldwide prevalence. About 30% of whole world population are in toxoplasmosis risk [¹]. The disease is clinically subpatent under normal body conditions, but its risk can increase in cases of acquired immune deficiency syndrome (AIDS) and pregnancy. T-cells and Macrophages are the major cells that involve in immune responses against *Toxoplasma* parasite invasion by controlling cytokine signaling pathway [²,³]. Perturbation of interleukin levels such as IL-2 [⁴], IL-4 [⁵] and IL-10 [⁶], as well as, other cytokines including: IL-10, interferon-gamma (IFN-γ) and transforming growth factor-beta 1 (TGF-β1) were observed in *Toxoplasma gondii*-infected individuals and animals [⁷]. The immunoregulatory response of IL-10 includes immune suppression by inhibiting production of pro-inflammatory mediators from Th1-type CD4⁺ T cells [⁸].

IL-11 was initially isolated form bone marrow-derived fibrocytes as a major mediator that involves in haematopoiesis [⁹], before been highlighted as an immune regulator of inflammatory responses during cancer [¹⁰] and infectious diseases [¹¹]. Later, IL-11 is considered as an immune negative regulator that implicates in development of tumourigenesism and probably by inducing immunosuppressive molecule called arginase-1 [¹²]. Such mechanism of action of arginase-1 was suggested in murine toxoplasmosis by increasing the susceptibility of arginase-1-treated rats to *Toxoplasma* infection [¹³].

IL-17, a major pro-inflammatory interleukin, promotes synthesis and induction of antimicrobial peptides named β-defensin molecules that involves in elimination of extracellular bacteria and fungi, as well as this interleukin induces a chronic inflammation response during autoimmune diseases [¹⁴,¹⁵]. Similarly, β-defensin-mediated modulation was proposed in *Toxoplasma* -infected individuals [¹⁶]. Moreover, T helper 17 (Th17) cells that synthesize IL-17 implicate in anti-toxoplasmosis immune responses and this signaling pathway is mediated by TGF-β cytokine [¹⁷]. The role of TNF-α in toxoplasmosis and other infectious diseases has been highlighted as a positive regulator of immune responses [¹⁸], whilst TNF-β function during toxoplasmosis is still unknown properly. The objective of this study is to investigate the immunoregulation of cytokine profile in *Toxoplasma gondii*-infected-women, which highlighting the immune status of the host and/or parasite development.
2. Material & Methods

For cytokine profiling during *Toxoplasma* infection in women of childbearing age (20-45 years old), 50 samples of serum were collected from *Toxoplasma gondii*-infected women (20-45 years old) from Bent AL-Huda Teaching hospital in Nasiriya city / Thi-Qar Governorate in the South of Iraq, with 50 serum samples collected from healthy women as naive controls between November 2018 to April 2019. Toxoplasmosis disease was confirmed by rapid toxoplasmosis diagnosis technique using Point-of-Care strip test (MP Biomedicals, USA). The Point-of-Care strip test provides fast, reliable, and efficient diagnostic approach for toxoplasmosis diagnosis[19].

For determination serum cytokine concentration of IL-10, IL-11, IL-17 and TNF-β, the serum samples were undergone to enzyme linked immunosorbent assay (ELISA) (Sigma-Aldrich, UK) according to manufacturer’s instructions (Biosource, California, USA) using microplate spectrophotometric reader (Wellkang Ltd., London, UK). The ELISA microplates were prepared and measured spectrophotometrically at 450 nm. A standard curve of cytokine standards was obtained and cytokine concentrations of unknown samples calculated.

The serum concentration data of cytokines in *Toxoplasma gondii*-infected and uninfected-women were statistically analysed by t-test formulation using GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, California, USA). The probability value (p-value) of differences between variables were calculated and considered to be significant at *p*<0.05, *p*<0.01 and *p*<0.0001.

3. Results

To answer some frequent questions about toxoplasmosis-mediated inflammatory responses, monitoring of some pro- and anti-inflammatory cytokines in *Toxoplasma*-infected and uninfected women were investigated. In this study, the concentrations of anti-inflammatory interleukins including; IL-10 and IL-11 with pro-inflammatory mediators including; IL-17 and TNF-β were spectrophotometrically monitored using ELISA kits. Our data revealed overproduction in all of the 4 cytokines that enrolled in this study in *Toxoplasma*-infected women in comparison to uninfected-controls (Figure 1-A and 1-B). Noticeably, production of IL-10 and IL-11 was only significantly higher than uninfected controls at *p*<0.0001 and *p*<0.01, respectively (Figure 1-A and 1-B), whilst IL-17 and TNF-β concentrations were slightly higher (not significant) in *Toxoplasma*-infected women (Figure 1-A and 1-B). Taken together, our data highlighted disruption in cytokine signaling pathway during *Toxoplasma gondii* infection.

(A)  (B)

![Fig. (1): Serum concentration of cytokines including: IL-10, IL-17(A), with IL-11 and TNF-β (B) in *Toxoplasma*-infected women (brown bars) versus uninfected controls (blue bars). Increase in the cytokine levels was only significant in IL-10 (*p*<0.0001) and IL-11 (*p*<0.01) in *Toxoplasma*-infected women.](image-url)

4. Discussion

Cytokine signalling pathway thought to be essential in host antimicrobial defenses[20], but this pathway can also be exploited by some pathogens to avoid host immunity[21]. The cytokine network is controlled by set of effective immune cells especially T cells, macrophages, and dendritic cells which they release two types of cross-regulatory cytokines called pro-inflammatory and anti-inflammatory cytokines[22]. The cytokine signalling process also participates in development of immunological memory and tolerance against invading pathogens[23].

Overexpression of type 2 cytokines, in particular IL-10, as shown in our findings can induce anti-inflammation process during toxoplastic encephalitis[24]. It has been indicated that tachyzoite-derived extractsof *Toxoplasma gondii* can modulate the host immune responses by overproduction of B cell-produced IL-10 causing transition from acute
Toxoplasmosis to chronic infection which is characterized by cyst formation in different organs and tissues [29]. This may explain the significant increasing incirculating IL-10 and IL-11levels in our findings (Figure 1-A and 1-B), where both interleukins belong to anti-inflammatory cytokine group and are related to late stage of Toxoplasma infection. Inhibition of immune responses by increasing the action of IL-10 is introductory step toward homeostasis of eye tissues in ocularToxoplasma-infected murine was observed [26]. Overproduction of endogenous IL-10 can increase the susceptibility of mice to Toxoplasma infection by inducing tissue proliferation and responses of suppressor T cells [27]. IL-10, a multifunctional cytokine, protects host tissues from damage by inhibiting acute phase of immune responses leading to pathogen-induced tolerance in viral infections [28].

IL-11 is traditionally classified as a pro-inflammatory cytokine, but recently it has been reported to have anti-inflammatory activities as like as IL-6, where both interleukins belong to IL-6 cytokine family and initially sharing the same membrane bound-α receptors called transmembrane protein gp130 [39]. Thus, blockage of these two cytokines, IL-6 and IL-11, by interleukin neutralization using anti-interleukin monoclonal antibodies thought to be effective immunotherapy of inflammatory diseases [38]. In encephalic toxoplasmosis, triggering the signaling pathway of IL-6-dependent glycoprotein130 (gp130) demonstrated the inhibitory role of IL-6, IL-11 and IL-27 in inflammatory responses of central nervous system infection [31]. High expression of TNF-α, IFN-γ and IL-12 from splenocytes was observed in IL-10 gene knockout mice which subsequently led to rapid clearance of Toxoplasma parasites from host body [32]. In related study, circulating IL-10 level in aborted women during the course of Toxoplasma gondiiinfection was comparable to native control [33]. In contrast to our findings, inhibition of the IL-10 level may be due to resuming the acute stage of Toxoplasma infection in aborted women [34], which is characterized by production of Toxoplasma-related vascular lesions and placental thrombosis [35].

Although, IL-17 has complicated divergent effects within host immune system, but it is thought to be a major factor of microphages (neutrophils) recruitment in inflammatory reaction sites [36]. This role was confirmed by impairing of inflammatory response in IL-17-deficient mice and followed by infection with Toxoplasma gondiiparasites [37]. In the same way, the data achieved by Moroda et al. demonstrated that IL-17-deficient mice are more susceptible to Toxoplasma gondiiinfection than naive controls and this indicating the important role of IL-17 in anti-toxoplasmosis [38]. Induction of proliferation of IL-17-producing Th17 cells in ocular toxoplasmosis was reported to be as a hallmark of disease severity [39]. It has been reported that development and differentiation of naive CD4+ T cells to the Th17 cellss are associated with upregulation of IL-11 gene expression [40]. Similarly, triggering of inflammatory responses in the early stage of relapsing-remitting multiple sclerosis due to synergistic effect of IL-11 and IL-17 were proposed [41]. A similar serological data related to IL-17 level was obtained in study achieved by Wahaj et al., where they highlighted non-significant increasing in the level of IL-8 and IL-17 in Toxoplasma-infected women in the Sudan [42]. The capacity expansion of immune response via the action of IL-17 that is produced by natural killer (NK) cells implicates in resistance against development of Toxoplasma gondii during acute stage of parasite infection [43].

TNF-α, but not TNF-β, has been studied widely in toxoplasmosis [44]. In Toxoplasma-infected women, upregulation of Th1 CD4+ cells which is representing by increasing in TNF-α and IFN-γ concentrations reflecting protective inflammatory responses against Toxoplasma infection [45]. Chronic progressive toxoplasmosis was induced in ME49 mice, a low Toxoplasma parasitic mouse strain, by exposure to anti-TNF-α antibodies treatment prior to infection with Toxoplasma gondii parasites [37], due to increase of ME49 mice susceptibility to Toxoplasma infection which reflecting a protective role of TNF-α in Toxoplasma infection [41]. The role of TNF in limiting of Toxoplasma gondii infection and reduction of tissue cyst formation was determined by using anti-TNF agent, etanercept [46]. In neural toxoplasmosis, pro-inflammatory effect of TNF-α seems to be correlated with activities of IFN-γ that produced by astrocytes [47]. In the literatures, protective immunity of host against ocular Toxoplasma infection was highlighted. This protective immunity may be regulated via the interference between immune stimulation responses of TNF-α and IL-6, in addition toovereexpression of nitric oxide leading to inhibition of Toxoplasma parasites replication [48,49].

5. Conclusion

Our findings indicated that cytokine signalling responses as an inflammatory hallmark can be reeregulated in Toxoplasma gondii-infected women, which may be associated with modulation of immune responses of Th1 CD4+ T cells and Th2 CD4+ T cells by Toxoplasma gondii parasite to establish toxoplasmosis disease. Additionally, the significant expression of anti-inflammatory cytokines, IL-10 and IL-11, in Toxoplasma-infected women may reveal transmission to chronic stage of toxoplasmosis disease.

References


