The Role of Cytokines in the Immunopathogenesis of Toxoplasmosis

Toksoplazmozun İmmünopatogenezinde Sitokinlerin Rolü

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ABSTRACT Objective: Infection caused by the obligatory intracellular parasite *Toxoplasma gondii* is generally asymptomatic in immunocompetent individuals but may be life-threatening in immunosuppressed patients and congenitally infected fetus. Scientists believed that the pathogenesis of the infection could be well established if the relation between cellular and humoral immune response was documented. **Material and Methods:** Starting from this point we tried to establish this relation, by measuring the production of Interferon-gamma (IFN-γ) (T_h1 cytokine) and interleucine (IL)-5 (T_h2 cytokine) in supernatants of peripheral blood mononuclear cells (PBMC) after in vitro specific *T. gondii* antigen stimulation in 19 patients that were grouped according to their anti-Toxoplasma IgM and IgG levels. **Results:** In our study, we observed that the level of IL-5 synthesis was higher than IFN-γ in the initial phase of the infection; as the specific IgG titers started to rise, IFN-γ synthesis increased and suppressed the synthesis of IL-5. As the infection became chronic, a decrease in the IFN-γ synthesis and a slight increase in IL-5 synthesis were noted. In our seronegative patient group, cytokine production pattern showed mainly T_h0 subgroup profile. **Conclusion:** As a result, we suggest that evaluation of antigen specific cytokine synthesis parallel to humoral response in the different stages of toxoplasmosis would be beneficial both in the diagnosis and in follow-up.

Key Words: *Toxoplasma gondii*, cytokines

ÖZET Amaç: İntraselüler, fırsatçı bir parazit olan *Toxoplasma gondii*'nin insanlarda oluşturduğu enfeksiyon, genellikle asemptomatik geçirilirken, immün sistemi baskılanmış hastalarda ve konjenital olarak enfekte fetusta ciddi mortalite ve morbiditeye sebep olmaktadır. *T. gondii* enfeksiyonunun oluşturduğu humoral ve hücresel immün cevap arasındaki bağlantının ortaya konulmasının, enfeksiyonun patogenezinin anlaşılmasını kolaylaştıracağı düşünülmektedir. **Gereç ve Yöntemler:** Bu amaçla çalışmamızda IgG ve IgM antikorları göz önüne alınarak 4 gruba ayrılmış 19 hastanın periferik kan mononükleer hücreleri (PKMH)'nin *T. gondii* eriyik antijenine verdikleri, T_h1 sitokinlerinden interferon-gamma (IFN-γ) ve T_h2 sitokinlerinden interlökin (IL)-5 cevabı incelenmiştir. **Bulgular:** Çalışmamızda, enfeksiyonun başlangıcında IgM antikorlarının yükselmesiyle birlikte IL-5 sentezinin IFN-γ sentezinden daha fazla olduğu, IgG antikorlarının artışıyla birlikte IFN-γ sentezinin ön plana çıkıp, IL-5 sentezini baskıladığı saptanmıştır. T_h1 lenfositlerinden salgılanan IFN-γ'nın, T_h2 lenfosit kökenli sitokinlerden olan IL-5 sentezini inhibe ettiği gözlenmiştir. Enfeksiyonun kronikleşmesiyle birlikte IFN-γ sentezi azalmış, buna bağlı olarak IL-5 sentezinde hafif bir artış tespit edilmiştir. Seronegatif hastaların sitokin sentez sonuçlarına bakıldığında, bu hastaların T lenfositlerinin daha çok T_h0 alt-grubunu taşıdıkları görülmüştür. **Sonuç:** Çalışmamızın sonucunda, *T. gondii* enfeksiyonunun farklı evrelerinde antijene özgün sitokin sentezi takibinin, humoral cevaba paralel olarak yapılmasının enfeksiyonun tanı ve takibinde önemli olabileceği kanısına varılmıştır.

Anahtar Kelimeler: *Toxoplasma gondii*, sitokinler

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Toxoplasma gondii is one of the most common parasites infecting humans and animals throughout the world. It is estimated that toxoplasmosis exists in a chronic asymptomatic form in 5 hundred million

to 1 billion of the world human population. Although human infection with *T. gondii* is usually asymptomatic in most individuals, it may cause serious morbidity and mortality in immunocompromised individuals and congenitally infected infants.[1,2] Infection with *T. gondii* induces both humoral and cell-mediated immune responses. Effective systemic immune response is responsible for the early clearance of the *T. gondii* tachyzoites from the peripheral blood soon after the onset of the infection and limits the parasitemia in the circulation. Scientists believed that, the pathogenesis of the infection could be well-established if the relation between cellular and humoral immune response was clearly documented.[3,4] Starting from this point, we tried to establish this relation by measuring the production of IFN-γ (T_h1 cytokine) and IL-5 (T_h2 cytokine) in the supernatants of PBMCs following *in vitro* specific *T. gondii* antigen stimulation.

MATERIAL AND METHODS

PATIENTS

Blood samples were collected from 19 subjects aged 20-36 years; participants were allocated to four groups formed according to their anti-Toxoplasma IgM and IgG levels. Antibody titers were evaluated by in-house indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) methods and positive IgM titers were confirmed by immune capture ELISA test (Organon Technica, Netherlands). This study was approved by the ethical committee of the Ege University Hospital. All patients were informed about the study and signed consents were obtained. Details for the patients were shown on Table 1.

PROLIFERATION ASSAY

PBMCs obtained by gradient centrifugation, were diluted to 10^6 cells/mL and were added to 96-well

TABLE 1: Details about the patients included to the study.

			Serology		
Group / Patient No	Age / Gender	Complaint	IFA IgG	ELISA IgM	ELISA IgG
1 / 1	30 / M	None	NEG	NEG	NEG
1 / 2	33 / F	None	NEG	NEG	NEG
1 / 3	27 / F	None	NEG	NEG	NEG
1 / 4	35 / M	None	NEG	NEG	NEG
1 / 5	34 / F	None	NEG	NEG	NEG
2 / 6 *	19 / F	LAP(cervical)	NEG	POS	NEG
2 / 7 *	28 / M	LAP(cervical)	1/64	POS	1/128
2 / 8 *	21 / F	LAP(cervical)	NEG	POS	NEG
2 / 9 *	33 / F	Abortus history (2 months ago)	NEG	POS	NEG
3 / 10	26 / F	None	1/256	NEG	1/2048
3 / 11	22 / F	Abortus history (13 months ago)	1/512	NEG	1/2048
3 / 12	31 / F	None	1/512	NEG	1/4096
3 / 13	35 / F	None	1/1024	NEG	1/16000
4 / 14	26 / M	None	1/128	NEG	1/512
4 / 15	21 / M	None	1/64	NEG	1/256
4 / 16	20 / F	None	1/128	NEG	1/1024
4 / 17	30 / F	None	1/64	NEG	1/128
4 / 18	33 / F	None	1/64	NEG	1/256
4 / 19	29 / F	None	1/128	NEG	1/512

* Immune capture ELISA (+), LAP: Lymphadenopathy, NEG: Negative, POS: Positive.

flat bottom microtiter plates (TPP). Cultures were stimulated with either 2.5 mg/mL phytohemagglutinin (PHA) as positive control or 5 mg/mL soluble *Toxoplasma* tachyzoite antigen (STAg).[5,6] Cell culture medium (RPMI 1640) containing 10% fetal calf serum was used as negative control in a final volume of 200 ml per well. PBMCs were cultured for 72 h and supernatants were collected and stored at -50°C.

PREPARATION OF SOLUBLE TOXOPLASMA ANTIGEN (STAG)

T. gondii tachyzoites of the TRRH strain were maintained by in vivo passaging. For antigen preparation, tachyzoites were harvested from the peritoneal cavity of mice and were washed three times with phosphate buffer saline (PBS). Tachyzoites were then lysed with 1% sodium dodecylsulfate and were centrifuged at 10.000 rpm at +4°C for 30 min. The supernatant was filtered through 0.2 mm membranes and after measuring the protein concentration, it was used as soluble antigen in the ELISA test.

CYTOKINE DETECTION IN CULTURE SUPERNATANTS

The elicitations of IFN-γ and IL-5 were measured by double sandwich ELISA using antibody pairs at predetermined concentrations. Microtiter plates (Nunc Maxisorp) were coated with capture antibody (Mouse anti-human IFN-γ or rat anti-mouse/human IL-5) (Pharmingen, USA) in bicarbonate buffer (pH 9.6) and were kept at 4°C overnight. Following 6 washes with PBS (pH 7) containing 0.1% tween 20, plates were blocked with PBS containing 1% bovine serum albumin and were kept at room temperature for four hours. Samples were applied in duplicate at ½ dilutions with blocking buffer solution, along with standards, consisting of recombinant cytokine IFN-γ or IL-5 (Pharmingen, USA) and were incubated at room temperature for 2 h; after washing 6 times, biotinylated detecting antibody (mouse anti-human IFN-γ or rat anti-human IL-5) (Pharmingen, USA) was added to each well. The plates were incubated at room temperature for an hour and horseradish peroxidase -conjugated streptavidin (Zymed, USA) was added to each well and plates were kept for an additional hour. Plates were washed 6 times again with washing buffer. Binding was visualized with tetramethylbenzidine substrate (Organon Technica, Netherlands). Following incubation for one hour, absorbance values were measured with a Titertek ELISA reader at 450 nm. Cytokine concentrations were determined by using computer program Table Curve 2d v.5.

STATISTICAL ANALYSIS

Distribution of data was evaluated with one sample Kolmogorov-Smirnov test and normally distributed cytokine levels were analyzed using ANOVA with post-hoc LSD test.

RESULTS

Considering the production of IFN-γ, supernatants of STAg stimulated cultures from group *1* (167.9 ± 141.7 pg/mL) and group *4* (147.3 ± 54.5 pg/mL) showed similar levels; group *3* (1601.6 ± 1585.4 pg/mL) revealed significantly higher IFN-γ production than the other groups (p= 0.001). The production of IFN-γ, detected in group *2* (339.0 ± 310.1 pg/mL) was higher than in group *1* and group *4* but not as high as in group *3* (Figure 1).

When the production of IL-5 was considered, group *2* (290.1 ± 171.4 pg/mL) had higher levels of IL-5 production than the others (p= 0.611). Low levels of IL-5 were detected in group *3* (140.7 ± 114.7 pg/mL) compared to the other groups. Mean IL-5 productions were 192.3 ± 190.8 pg/mL and 174.9 ± 79.9 pg/mL in group *1* and group *4* (Figure 2).

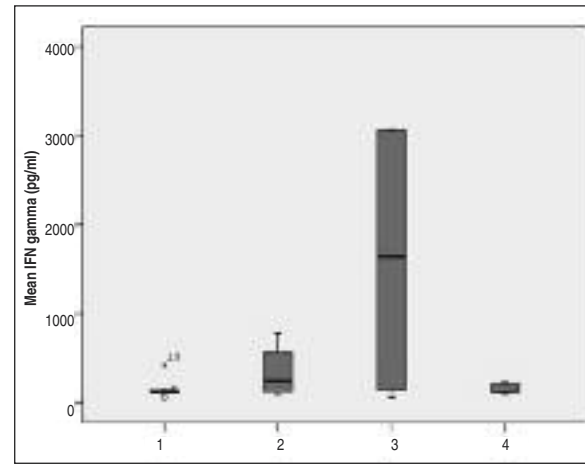

FIGURE 1: Mean IFN-γ production of groups.

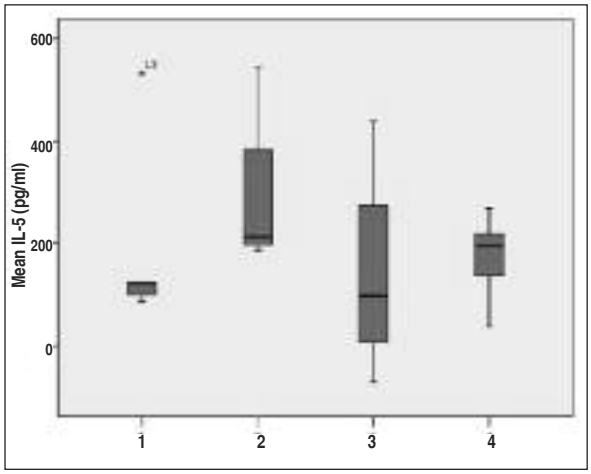

FIGURE 2: Mean IL-5 production of groups.

As shown in Figure 3, the low levels of IL-5 and IFN-γ in group *1* (seronegative group) increased with the rise of *T. gondii* specific IgM antibody in group *2* (IgM positive group); in group *3* (High IgG positive group), when the IgG antibody increased IFN-γ production was still increasing but IL-5 production was decreasing. In group *4,* (low IgG positive group) we noted a slight increase in IL-5 production while there was a decrease in IFN-g production.

DISCUSSION

It is well recognized that cell-mediated immunity plays a major role in the host resistance to *T. gondii* infection, although recent studies have highlighted the importance of the humoral response on this intracellular pathogen.[7,8] Various studies have demonstrated that some proteins of *T. gondii* antigen can induce T cell proliferation and cytokine production.[9-14] However, very few studies addressed the relation between cellular and humoral immunity responses induced by *T. gondii* antigen.[7,12,13] The protective role of Th1 type immune responses, especially the production of IFN-γ is well established in toxoplasmosis. However, limited data have so far been obtained for IL-5 from clinical studies.[15]In the present study; we showed the changes in IFN-γ and IL-5 productions, related with antibody response in people with different stages of toxoplasmosis.

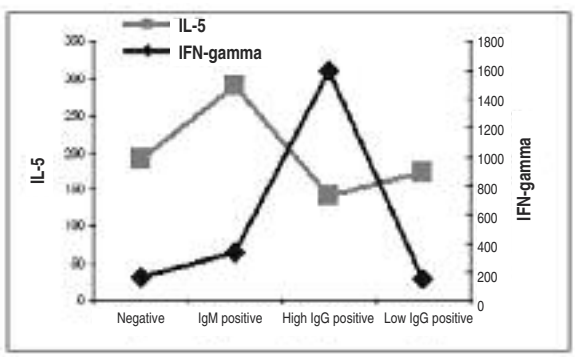

FIGURE 3: The changes in IL-5 and IFN-γ production (pg/mL) in relation to antibody production of groups.

We showed that when specific anti- *T. gondii* IgM antibody was formed, increased levels of IL-5 production was accompanied by low levels of IFN-γ production, which indicated the dominance of T_h2 profile of T cells in the initial phase of *T. gondii* infection. In the following period, as the anti- *T. gondii* IgG antibody started to increase, the noted increase in IFN-γ indicated T_h1 profile of T cells, favoring protection and control of the *T. gondii* infection.[16] In the same period IL-5 decreased because of the inhibitory effect of T_h1 cytokines on T_h2 cytokines.[17] Lymphocytes from immunocompetent adults with acquired toxoplasmosis probably display a dominant T_h1 profile favoring protection and control of the *T. gondii* infection.[13] As the infection became chronic, a decrease in the synthesis of IFN-γ and a slight increase in the synthesis of IL-5 were noted. Experimental models of murine toxoplasmosis suggest that a T_h1 and T_h2 balance is implicated in the regulation of *T. gondii* infection.[18] Also in our seronegative patient group, cytokine production pattern showed mainly T_h0 subgroup profile. [12] In this study, we showed that in all stages of toxoplasmosis overproduction of IFN-γ with respect to IL-5 was present.

In conclusion, our data showed that evaluation of antigen specific cytokine synthesis parallel to humoral response in different stages of toxoplasmosis could be beneficial both in the diagnosis and in the follow-up of toxoplasmosis. However, further studies are necessary to verify the host factors, such as genetic background, immune status or drugs used for treatment that could alter the T_h1 - T_h2 cytokine balance.

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