

Cytokine Signatures Associated With Early Onset, Active Lesions and Late Cicatricial Events of Retinochoroidal Commitment in Infants With Congenital Toxoplasmosis

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Background. Ocular toxoplasmosis is a prominent and severe condition of high incidence in Brazil. The current study provides new insights into the immunological events that can be associated with retinochoroiditis in the setting of congenital toxoplasmosis in human infants.

Methods. Flow cytometry of intracytoplasmic cytokines in leukocyte subsets following in vitro short-term antigenic recall in infants with congenital *T. gondii* infection.

Results. Our data demonstrates that whereas neutrophils and monocytes from *T. gondii*-infected infants display a combination of proinflammatory and regulatory cytokine profiles, natural killer cells showed a predominantly proinflammatory profile upon in vitro *T. gondii* stimulation. The proinflammatory response of CD4⁺ and CD8⁺ T cells, characterized by the production of interferon γ (IFN- γ) and interleukin 17 in patients with an active retinochoroidal lesion, revealed the presence of IFN- γ and tumor necrosis factor α during early and late immunological events. This specific proinflammatory pattern is associated with early events and active retinochoroidal lesion, whereas a robust monocyte-derived interleukin 10-mediated profile is observed in children with cicatricial ocular lesions.

Conclusions. These findings support the existence of a progressive immunological environment concomitant with the initial, apical, and cicatricial phases in the process of retinochoroidal lesion formation in infants with congenital toxoplasmosis that may be relevant in the establishment of stage-specific clinical management.

Keywords. congenital toxoplasmosis; cytokine profile; retinochoroiditis; infants; active and cicatricial lesions.

Toxoplasma gondii infection is a leading cause of visual impairment globally, being responsible for 30%–50% of posterior uveitis cases in immunocompetent individuals. Ocular toxoplasmosis is a common complication in congenital and postnatally acquired toxoplasmosis [1]. The prevalence and severity of toxoplasmosis varies greatly worldwide and is believed to be under the influence of parasite genotype [2], host genetic background [3] and host immune system status [4].

Once the host is infected with *T. gondii*, a number of different host cells are mobilized to contain the spread of the parasite. The interaction between these cells is crucial to the hosts'

resistance against the infection. The immune response is initiated by the activation of cells of the innate immune system, which secrete proinflammatory cytokines to further polarize this response [5]. Interleukin 12 (IL-12), produced by innate immune cells, induces the recruitment and activation of adaptive immune cells, which are responsible for the neutralization and elimination of *T. gondii* by interferon γ (IFN- γ)-dependent mechanisms [6]. Ocular toxoplasmosis in humans has been associated with a proinflammatory cytokine profile orchestrated by a regulatory profile composed by cytokines such as interleukin 4 (IL-4) and interleukin 10 (IL-10) [7–8].

Very little is known about the systemic cytokine role in human congenital toxoplasmosis. Answers to questions about factors such as the important immunological events related to or even causative of eye injury remain elusive.

Here we evaluated the intracellular cytokine production associated with the type of eye lesion found in infants with congenital toxoplasmosis to understand the role of cytokines in the early stages of ocular toxoplasmosis. This is a unique opportunity to study the immune response elicited by *T. gondii*

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infection during the early stages of congenital ocular disease in an infant population.

METHODS

Study Population

This study was part of a broader investigation of neonatal screening for congenital toxoplasmosis, which was conducted by a multidisciplinary research group in the Brazilian state of Minas Gerais (UFMG Congenital Toxoplasmosis Brazilian Group). Air-dried whole-blood samples preserved on filter paper were collected from newborns from Minas Gerais ($n = 146\ 307$) and tested for anti-*T. gondii* immunoglobulin M (IgM) antibodies, using the Toxo IgM kit (Q-Preven, Symbiosis, Leme, Brazil). Confirmatory serological tests (for detection of immunoglobulin G [IgG], immunoglobulin A [IgA], and IgM, using the Vidas enzyme-linked fluorescent assay; bioMérieux, Lyon, France) were performed 31–86 days after birth (mean, 55.6 days), using serum samples collected from all infants with positive or inconclusive screening test results. Infants who displayed positive results of confirmatory serological tests and had persistent anti-*T. gondii* IgG reactivity 12 months after birth received a diagnosis of congenital toxoplasmosis. Infants who displayed negative results on confirmatory serological analysis and had negative anti-*T. gondii* IgG reactivity 12 months after birth were considered noninfected. Ophthalmologic examinations were performed on all children as described previously [9]. These infants were subdivided into 2 groups: (1) *T. gondii*-infected infants (ie, the TOXO group), comprising 58 infants with a diagnosis of congenital toxoplasmosis who had positive results of both confirmatory tests and persistent specific IgG antibodies, and (2) noninfected healthy control infants (ie, the NI group), which comprised 10 infants who tested negative for anti-*T. gondii* IgG. Among the 58 children from group TOXO, 15 had no retinochoroidal lesions (NRLs), 7 presented with active retinochoroidal lesions (ARLs), 16 had simultaneous active and cicatricial retinochoroidal lesions (ACRLs), and 20 had cicatricial retinochoroidal lesions (CRLs; [Supplementary Figure 1](#)). Infants from the NI group did not have any type of retinochoroidal lesions. The protocols used in this study were approved by the local ethics committee (Federal University of Minas Gerais, protocol 298/06), and mandatory written informed consent was obtained from the mothers of all infants included in this study.

Short-term Whole-Blood Culture In Vitro

Heparinized whole-blood samples (3 mL) were collected from 68 infants and used for short-term whole-blood culture in vitro as previously described [10], with the following modification: briefly, 1 mL of whole blood was distributed in polypropylene tubes (BD Bioscience, Bedford, Massachusetts) in the presence of 1 mL of Roswell Park Memorial Institute medium (control cultures [CCs]) or the presence of *T. gondii* tachyzoite

soluble antigen (STAg), produced as previously described [11], at final concentration of 5 $\mu\text{g}/\text{mL}$ (*T. gondii*-stimulated culture). Blood samples were incubated for 12 hours in a 5% CO_2 incubator at 37°C (Forma Scientific USA). Following incubation, Brefeldin A (Sigma, St Louis, Missouri) was added to each culture tube at a final concentration of 10 $\mu\text{g}/\text{mL}$. Blood samples were reincubated for 4 hours at 37°C in a 5% CO_2 humidified incubator. All cultures, including CCs and STAg cultures, were treated with 2 mM ethylenediaminetetraacetic acid (Sigma) and kept at room temperature for 15 minutes before immunostaining for intracellular cytokine analysis.

Intracellular Cytokine Staining

Following short-term stimulation in vitro, 500- μL aliquots of the whole-blood cultures were stained for surface molecules by incubation at room temperature for 30 minutes with anti-CD16-TC, anti-CD14-TC, anti-CD4-TC, anti-CD8-TC, or anti-CD19-TC. After surface staining, the red blood cells were lysed, and the leukocytes were fixed with 3.5 mL of lysing/fixing solution for 10 minutes at room temperature. The leukocytes were washed once with phosphate-buffered saline supplemented with 0.5% bovine serum albumin (PBS-W) and permeabilized with 1 mL of PBS-P (PBS-W supplemented with 0.5% saponin; Sigma) for 10 minutes at room temperature. Thirty-microliter aliquots of the fixed permeabilized cells were transferred to 96-well round-bottomed plates and stained separately with anti-tumor necrosis factor α (TNF- α)-PE, anti-IFN- γ -PE, anti-interleukin 1 (IL-1)-PE, anti-IL-4-PE, anti-interleukin 5 (IL-5)-PE, anti-interleukin 6 (IL-6)-PE, anti-interleukin 8 (IL-8)-PE, anti-IL-10-PE, anti-IL-12-PE, or anti-interleukin 17 (IL-17)-PE monoclonal antibodies for 30 minutes at room temperature. The cells were then washed once with PBS-P, followed by 1 wash with PBS-W, and were fixed with 300 μL of FACs fixing solution (10 g/L paraformaldehyde, 10.2 g/L sodium cacodylate, and 6.63 g/L sodium chloride; pH 7.2). Samples were stored in the dark for at least 30 minutes (and up to 24 hours) at 4°C before flow cytometry. At least 20 000 gated events were acquired using FACSCalibur (BD Biosciences), and FlowJo software (version 9.4.1; TreeStar, Ashland, Oregon) was used for data analysis. Lymphocytes were first selected on the basis of their size and granularity laser-scattering properties. Subsequently, the frequency of cytokine-producing cells within gated lymphocyte subsets or monocytes were calculated for CCs and STAg cultures. Further analysis was performed to estimate the *T. gondii*-specific cytokine production as the index of STAg-stimulated culture divided by the CC (hereafter, the STAg/CC index).

Statistical Analysis

This was an observational investigation of the cytokine profile associated with distinct clinical manifestations of congenital toxoplasmosis. Statistical analyses between TOXO and NI groups were performed by the Student *t* test. Comparative

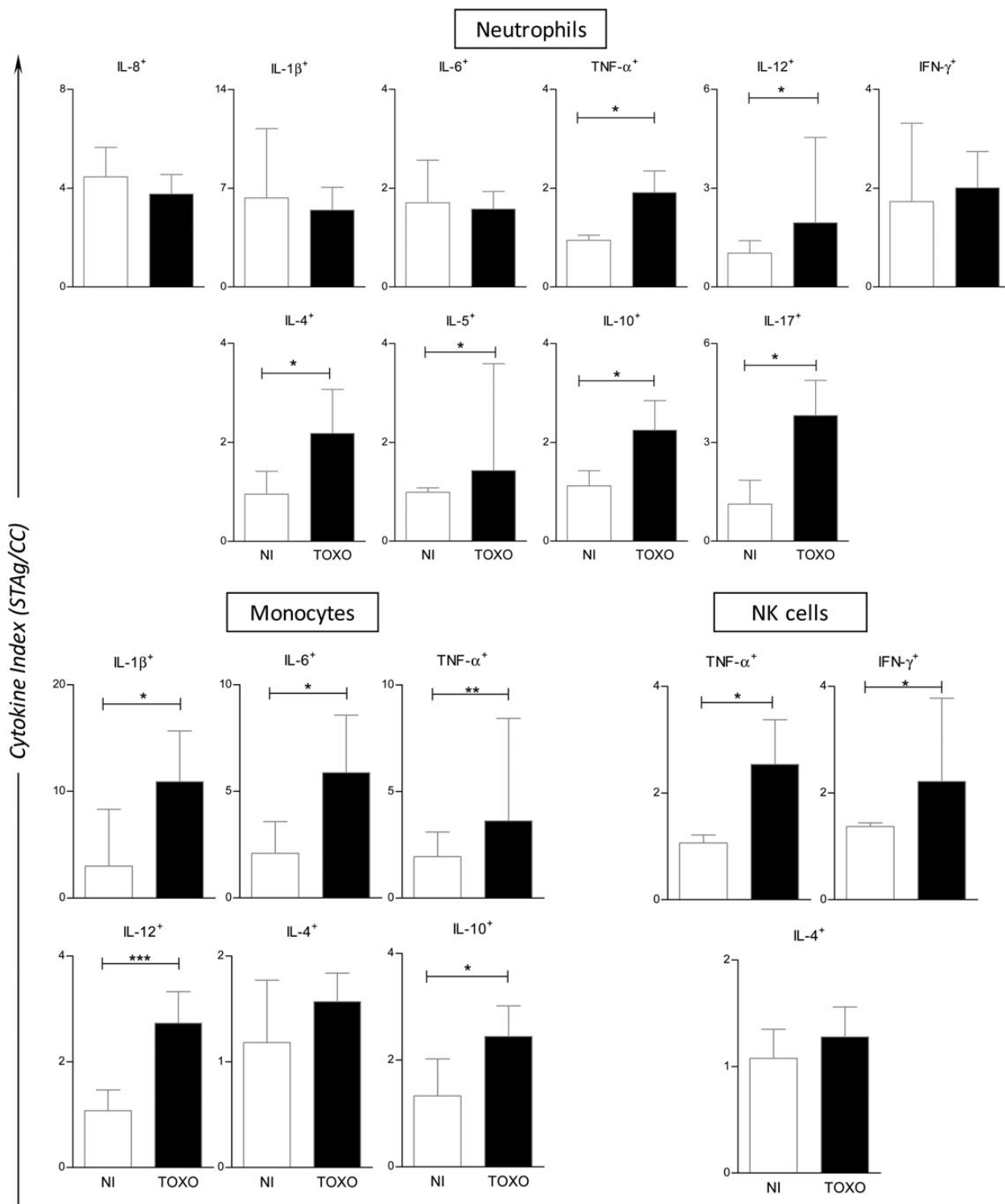


Figure 1. Innate cytokine profile in infants with congenital toxoplasmosis. Results are expressed as the cytokine index, calculated as the ratio of soluble *Toxoplasma* antigen over control culture (STAg/CC) for neutrophils, monocytes, and natural killer (NK) cells from 58 *Toxoplasma gondii*-infected infants (TOXO) and 10 noninfected infants (NI). Values are expressed as mean \pm standard error. * $P < .05$, ** $P < .01$, and *** $P < .001$. Abbreviations: IFN- γ , interferon γ ; IL-1 β , interleukin 1 β ; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; TNF- α , tumor necrosis factor α .

analyses among TOXO subgroups and the NI group were performed by 1-way analysis of variance, followed by the Tukey multiple comparison test. All data sets are presented as mean

values \pm standard error. In all cases, differences were assigned in the graphs by connecting lines considered statistically significant at a P value of $< .05$. The software GraphPad Prism 5.0

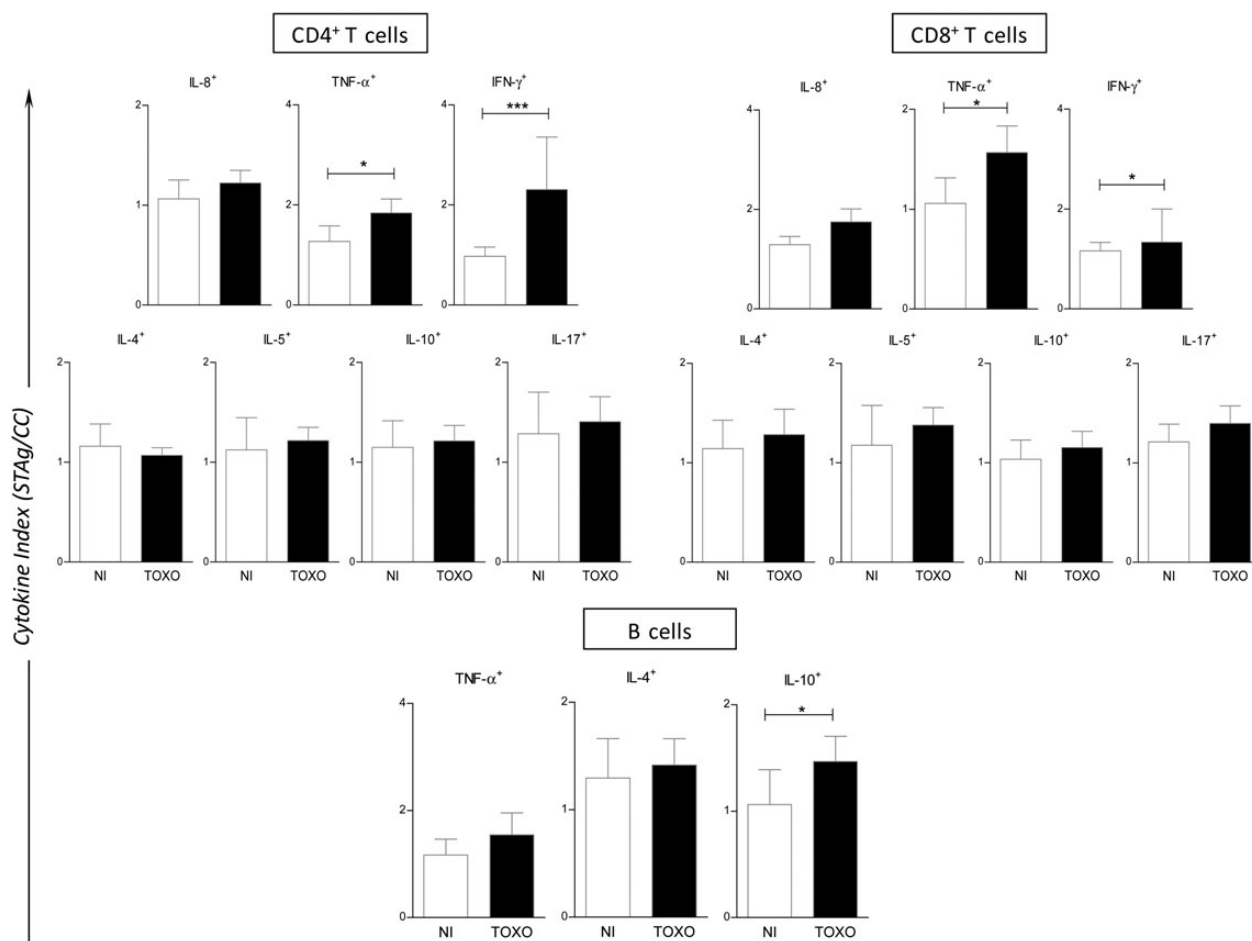


Figure 2. Cytokine profile produced by adaptive immune cells from infants with congenital toxoplasmosis. Results are expressed as the cytokine index, calculated as the ratio of soluble *Toxoplasma* antigen over control culture (STAg/CC) for CD4⁺ T cells, CD8⁺ T cells, and B cells for 58 *Toxoplasma gondii*-infected infants (TOXO) and 10 noninfected infants (NI). Values are expressed as mean \pm standard error. * $P < .05$, ** $P < .01$, and *** $P < .001$. Abbreviations: IFN- γ , interferon γ ; IL-4, interleukin 4; IL-5, interleukin 5; IL-8, interleukin 8; IL-10, interleukin 10; IL-17, interleukin 17; TNF- α , tumor necrosis factor α .

(GraphPad Software, San Diego, California) was used for statistical analyses and graphical representations.

RESULTS

Neutrophils and Monocytes Produce a Mixture of Proinflammatory/Regulatory Cytokines Whereas Natural Killer (NK) Cells Display a Predominantly Proinflammatory Response Upon Soluble *T. gondii* Stimuli

The cytokine profile of peripheral blood innate immunity cells from infants with congenital toxoplasmosis after in vitro *T. gondii* antigen recall is shown in Figure 1. The TOXO group presented a distinct pattern of intracellular cytokine production by neutrophils, monocytes, and NK cells when compared to NI controls. In fact, TOXO displayed a mixed cytokine pattern of proinflammatory cytokines (TNF- α and IL-12 expression) and an increased frequency of IL-4-, IL-5-, IL-10-, and IL-17-producing neutrophils. Moreover, the TOXO group also presented a mixed cytokine production profile, composed of the proinflammatory cytokines IL-1 β , IL-6, TNF- α , and IL-12

counterbalanced by an increased production of the modulatory cytokine IL-10 by monocytes. On the other hand, a clear proinflammatory response profile typified by increased IFN- γ - and TNF- α -producing NK cells was observed in the TOXO group.

Soluble *T. gondii* Antigen Recall Triggers a Proinflammatory Response in CD4⁺ and CD8⁺ T Cells Counterbalanced by B-Cell-Derived IL-10-Mediated Regulation

Analyses of the adaptive immune cells are presented in the Figure 2. The TOXO group showed a distinctive proinflammatory profile mediated by an increased frequency of TNF- α - and IFN- γ -producing CD4⁺ and CD8⁺ T cells with a regulatory role, mediated by enhanced levels of IL-10-producing B cells upon exposure to soluble *T. gondii* stimuli.

Intracellular Cytokine Production Profile in Both Innate and Adaptive Immunity Is Associated With the Clinical Status of Retinochoroidal Lesions

The NRL subgroup had a predominant proinflammatory profile characterized by an enhanced production of IL-1 β -, TNF- α -,

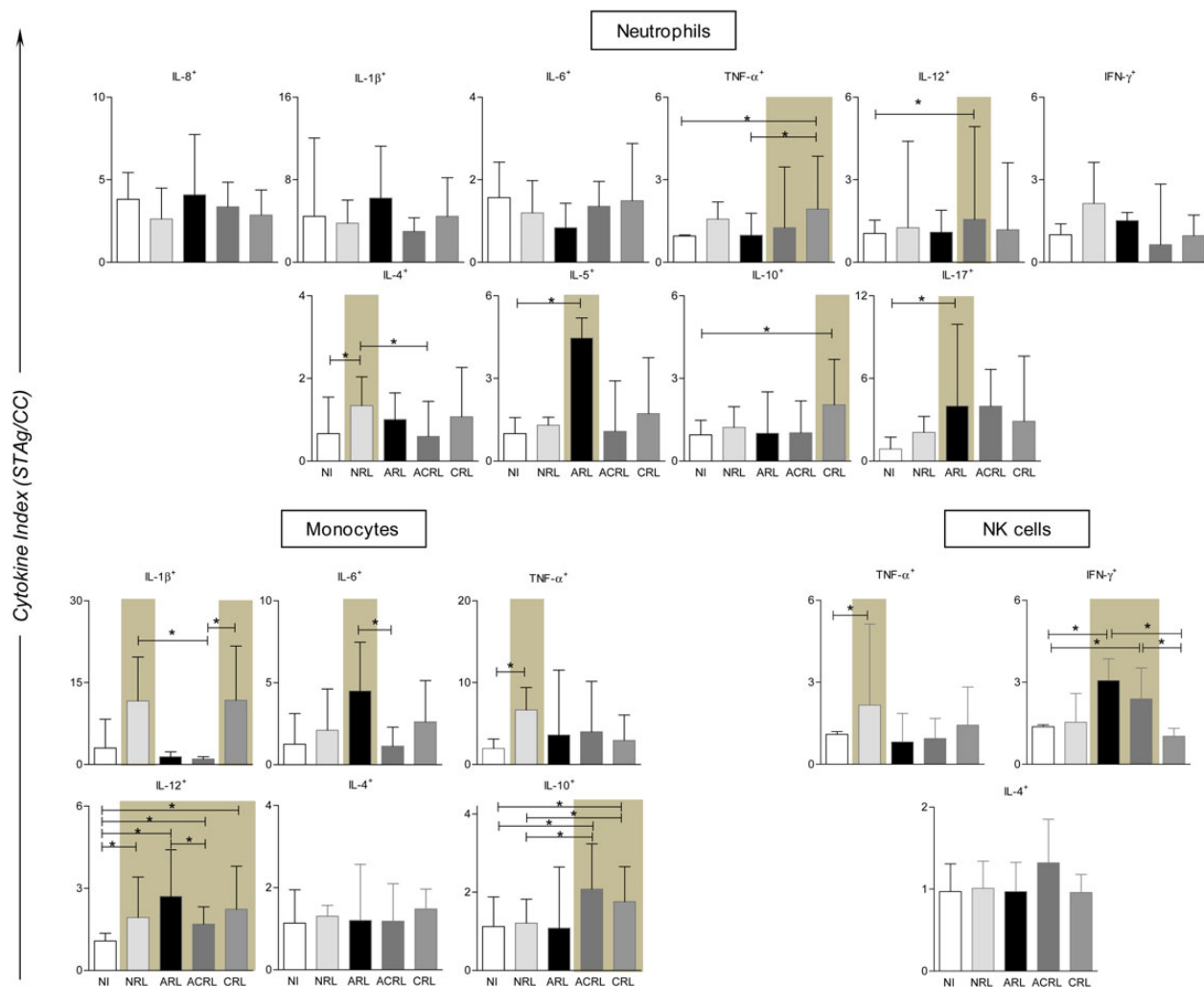


Figure 3. Innate cytokine profile in infants with congenital toxoplasmosis categorized according to status of retinochoroidal lesions. Results are expressed as the cytokine index, calculated as the ratio of soluble *Toxoplasma* antigen over control culture (STAg/CC) for neutrophils, monocytes, and natural killer (NK) cells for *Toxoplasma gondii*-infected infants (TOXO subgroups), classified according to findings of clinical ophthalmologic examination, as follows: no retinochoroidal lesions (NRL; n = 15), active retinochoroidal lesions (ARLs; n = 7), simultaneous active and cicatricial retinochoroidal lesions (ACRLs; n = 16), and cicatricial retinochoroidal lesions (CRL; n = 20). Data are also presented for 10 noninfected infants (NI). Values are expressed as mean \pm standard error. Gray rectangles are used to highlight the major significant differences. * $P < .05$, ** $P < .01$, and *** $P < .001$. Abbreviations: IFN- γ , interferon γ ; IL-1 β , interleukin 1 β ; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; TNF- α , tumor necrosis factor α .

and IL-12-producing monocytes along with TNF- α -producing NK cells and IL-4-producing neutrophils. The ARL subgroup also had an evident proinflammatory profile with a distinct cytokine signature: increased IL-6-producing and IL-12-producing monocytes, IFN- γ -producing NK cells, and the particularly unique presence of IL-5- and IL-17-producing neutrophils. The ACRL and CRL subgroups both had a cytokine profile composed of both proinflammatory and modulatory mediators. The profile of the ACRL subgroup was composed of proinflammatory TNF- α - and IL-12-producing neutrophils, IL-12-producing monocytes, and IFN- γ -producing NK cells, as well as the modulatory IL-10-producing monocytes. The profile of the CRL subgroup was composed of increased TNF- α -producing neutrophils, IL-1 β - and IL-12-producing

monocytes, and modulatory IL-10-producing neutrophils and monocytes (Figure 3).

The NRL subgroup displayed a clear TNF- α -mediated T-cell response (CD4 $^+$ and CD8 $^+$ T cells), even though infants from this subgroup did not have retinochoroidal lesions. They also showed IFN- γ production by CD4 $^+$ T cells. The ARL subgroup had an enhanced frequency of IFN- γ -producing CD4 $^+$ and CD8 $^+$ T cells and IL-17-producing CD4 $^+$ T cells. IFN- γ -producing CD4 $^+$ T cells were noted in both groups with cicatricial retinochoroidal lesions (ACRLs and CRLs). Infants with CRLs had enhanced levels of TNF- α -producing CD4 $^+$ T cells. We did not observe any significant difference in IL-10-producing B cells when the subgroups were compared (Figure 4).

A TNF- α -Mediated Environment Characterizes the Early Stage, a IFN- γ and IL-17 Environment Characterizes the Active Lesion Stage, and a IL-10 Environment Characterizes the Late Cicatricial Stage of Retinochoroiditis

The data described above allowed for a clear description of the overall intracellular cytokine profile of peripheral blood immune cells from infants with congenital toxoplasmosis categorized according to retinochoroidal lesion clinical status (Figure 5). Our findings include the presence of IL-1 β -producing monocytes, along with a range of TNF- α -producing cells (monocytes, NK cells, CD4⁺ T cells, and CD8⁺ T cells) in patients with NRLs. Moreover, IL-12-producing monocytes and CD4⁺ T-cell-derived IFN- γ were observed in all clinical stages of retinochoroiditis. A proinflammatory profile consisting of IFN- γ and IL-17 expression was characteristic of patients with active lesions. It is important to note the presence of IL-10-producing cells (neutrophils and monocytes), along with IL-1 β -producing monocytes and TNF- α -producing neutrophils and CD4⁺ T cells, in patients with CRLs.

DISCUSSION

In the present work, we report that the intracellular cytokine production profile is related to the ocular clinical status of infants with congenital toxoplasmosis and therefore can be used to identify the degree of progression of the condition. We show that a predominantly proinflammatory profile in innate immunity is clearly associated with early events of retinochoroidal lesions and ARLs, whereas a typical monocyte- and neutrophil-derived IL-10 profile is the most prominent feature of cicatricial ocular lesions. Moreover, the proinflammatory profile consisting of IFN- γ -producing CD8⁺ T cells and IFN- γ - and IL-17-producing CD4⁺ T cells is characteristic of patients with active retinochoroidal lesion. In addition, IL-1 β -producing monocytes are observed early on and then again, at the cicatricial stage. Similarly, TNF- α from different sources is observed early on in infants without retinochoroidal lesions and also at the cicatricial stage. IL-12-producing monocytes and IFN- γ -producing CD4⁺ T cells are observed in all stages of ocular toxoplasmosis.

The overall cytokine profile of infants with congenital toxoplasmosis showed that the intracellular production of a mixture of proinflammatory (TNF- α , IL-12, and IFN- γ) and regulatory (IL-4 and IL-10) cytokines in neutrophils and monocytes, while NK cells have an exclusive proinflammatory pattern. Furthermore, the adaptive response exhibited a robust proinflammatory profile in CD4⁺ T cells and CD8⁺ T cells, counterbalanced by clear B-cell-derived regulation of IL-10.

The production of proinflammatory cytokines, including TNF- α , IL-12, and IFN- γ , is crucial in the early stages of infection to control parasite proliferation [12]. This profile was only described in animal models and adult patients, but here we show similar results in human samples from infants with congenital toxoplasmosis. This finding is surprising considering previous reports, which have postulated that infants display

deficiencies in primary response against pathogenic microorganisms [13–14]. An exacerbated proinflammatory response is extremely harmful to the host, being one of the causes of toxoplasmosis-associated immunopathologic events. Thus, the increase in IL-10 production is essential to limit the inflammation resulting from the immune response against the parasite [7]. IL-10 specifically produced by B cells has been described in other parasitic diseases, and it was correlated with T-helper type 2 dominance [15]. Increased serum concentrations of IL-10 in infants with congenital toxoplasmosis have also been described, although the producing cell was not investigated [16]. Here, we show for the first time the presence of IL-10-producing B cells in infants with congenital toxoplasmosis.

Subsequently, we evaluated the overall cytokine synthesis profile of peripheral blood immune cells according to the clinical status of retinochoroidal lesions of members of the TOXO group. All stages of retinochoroiditis were associated with IL-12-producing monocytes and IFN- γ -producing CD4⁺ T cells. The presence of both inflammatory cytokines from the early stages of ocular lesions until late cicatricial stages suggests the importance of parasite control mechanisms mediated by the cytokines throughout the development of ocular toxoplasmosis. Indeed, IL-12 is necessary for parasite-induced IFN- γ synthesis by NK cells, which promote parasite control [7].

An interesting finding was the presence of IL-1 β -producing monocytes along with TNF- α -producing cells (monocytes, NK cells, CD4⁺ T cells, and CD8⁺ T cells) in patients with NRLs. Yamamoto et al [17] found higher levels of IL-1 β and TNF- α production in adults with postnatal ocular toxoplasmosis, compared with individuals in 3 other groups: persons without ocular symptoms, those with congenital ocular toxoplasmosis, and those who are healthy. Previous studies have shown that persistent production of TNF- α is associated with increased tissue damage, mainly due to the effects of reactive oxygen species, angiogenesis promotion, and breaking of the eye barrier [18–20]. In fact, it is thought that the increase in TNF- α levels may result in more-severe eye inflammation, with subsequent macular damage, leading to eyesight deterioration [20]. We speculate that the presence of IL-1 β and TNF- α in infants congenitally infected with *T. gondii* and without retinochoroiditis predicts lesion occurrence. Indeed, it is known that most of congenitally infected newborns are asymptomatic but that 85% develop retinochoroiditis before adulthood [21].

The ARL subgroups exhibited enhanced levels of cells with a proinflammatory profile that includes proinflammatory cytokines, including IFN- γ , IL-5, IL-6, IL-17, and IL-12. Similar results were described in aqueous humor specimens obtained from adult patients with ocular toxoplasmosis [22].

We also observed the presence of IL-17 exclusively in the ARL subgroups in an environment full of IFN- γ -producing NK cells, CD4⁺ T cells, and CD8⁺ T cells, suggesting that the

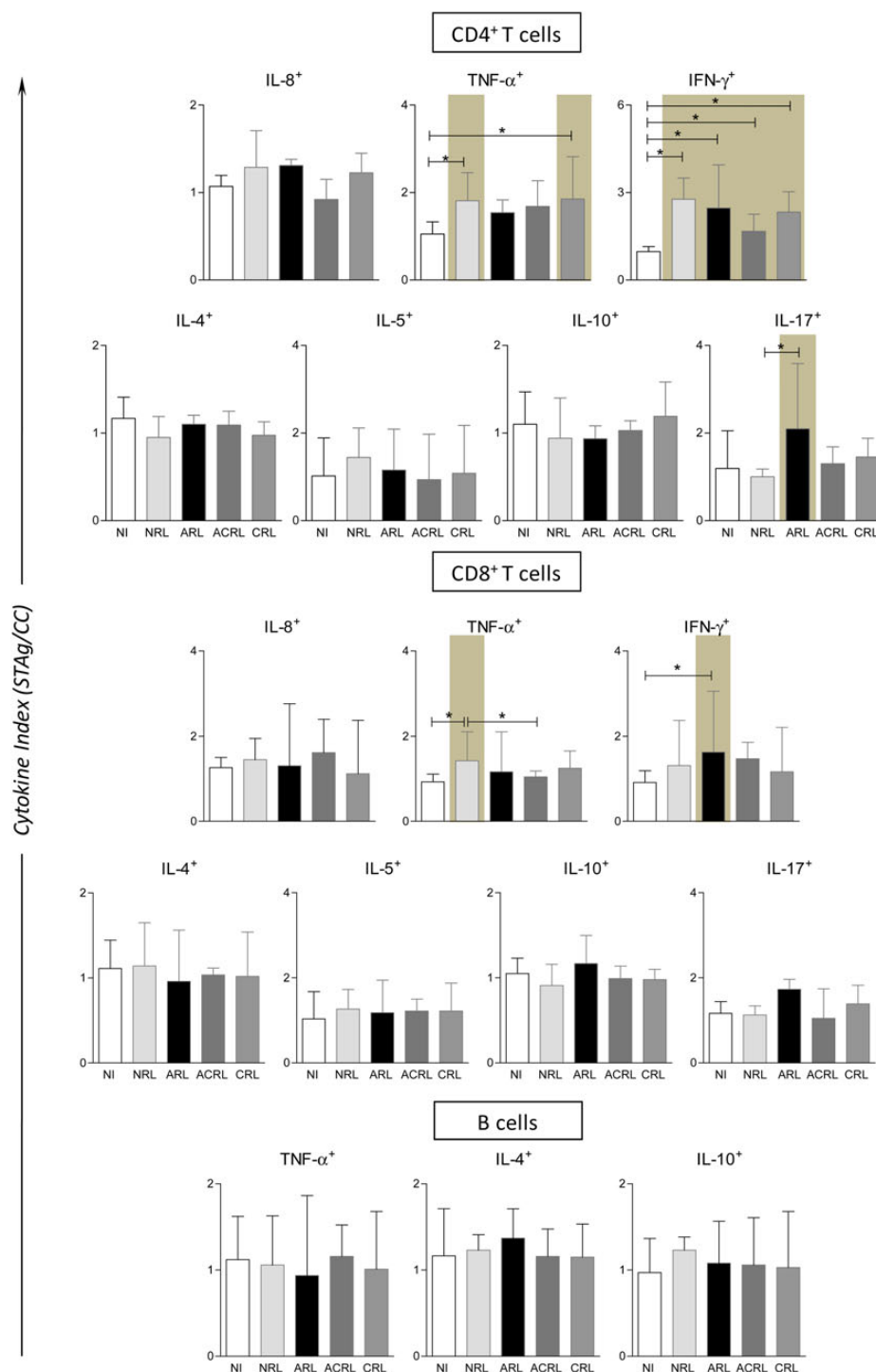


Figure 4. Adaptive immunity cytokine profile of infants with congenital toxoplasmosis categorized according to status of retinochoroidal lesions. Results are expressed as the cytokine index, calculated as the ratio of soluble *Toxoplasma* antigen to control culture (STAg/CC) for CD4⁺ T cells, CD8⁺ T cells, and B cells for *Toxoplasma gondii*-infected infants (TOXO subgroups), classified according to findings of clinical ophthalmologic examination, as follows: no retinochoroidal lesions (NRLs; n = 15), active retinochoroidal lesions (ARLs; n = 7), simultaneous active and cicatricial retinochoroidal lesions (ACRLs; n = 16), and cicatricial retinochoroidal lesions (CRLs; n = 20). Data are also presented for 10 noninfected infants (NI). Values are expressed as mean \pm standard error. Gray rectangles are used to highlight the major significant differences. * $P < .05$, ** $P < .01$, and *** $P < .001$. Abbreviations: IFN- γ , interferon γ ; IL-4, interleukin 4; IL-5, interleukin 5; IL-8, interleukin 8; IL-10, interleukin 10; IL-17, interleukin 17; TNF- α , tumor necrosis factor α .

IL-17 inflammatory role is associated with lesion activity. Our work is the first to link IL-17 with the active lesion stage in congenital toxoplasmosis. In contrast, de-la-Torre et al [23]

observed that the severity of ocular lesions caused by *T. gondii* Colombian strains in adult patients was associated with decreased production of IFN- γ and IL-17A. In a murine model

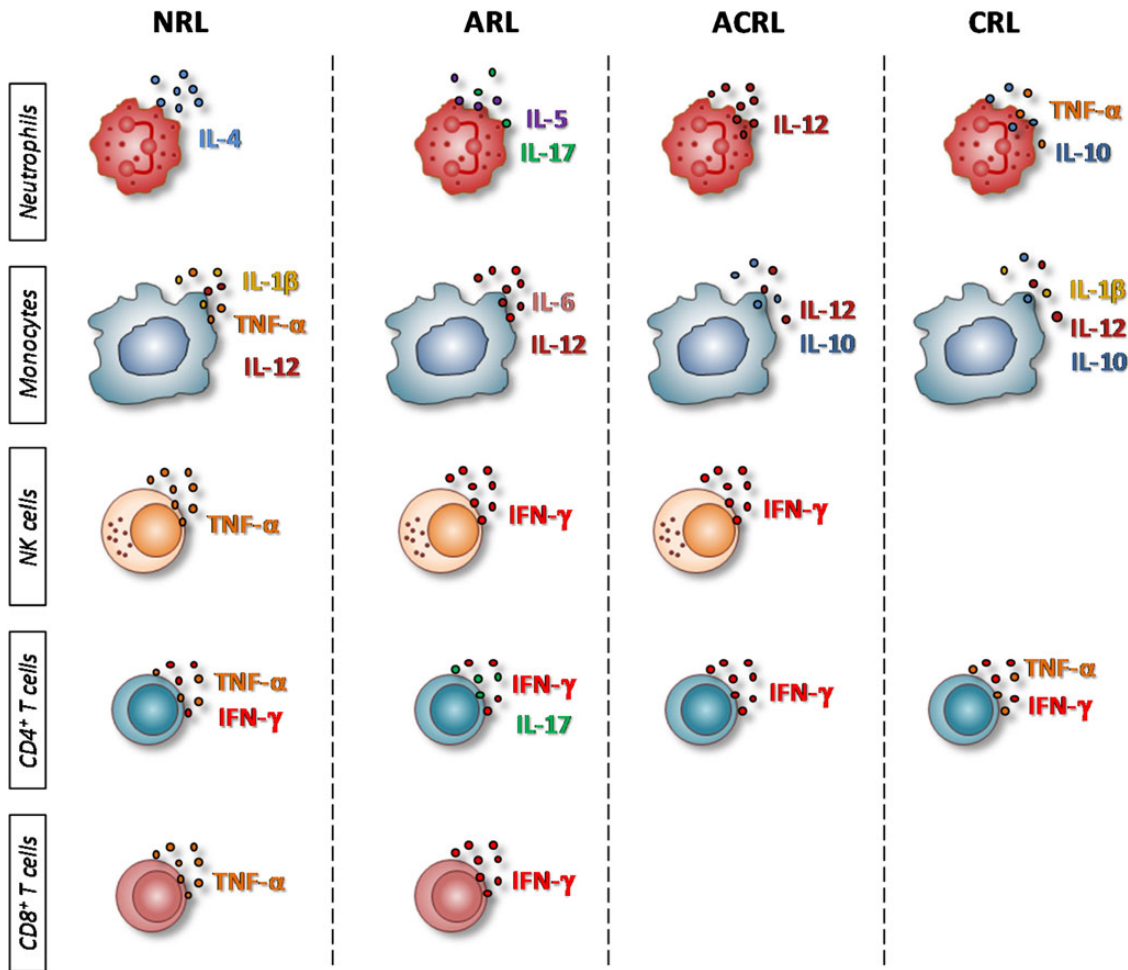


Figure 5. Immunological events during congenital toxoplasmosis in human infants, according to retinochoroidal lesion status. The significant features found in the cytokine index, calculated as the ratio of the soluble *Toxoplasma* antigen to control culture, from *Toxoplasma gondii*-infected infants (TOXO subgroups), were categorized according to findings of clinical ophthalmologic examination, as follows: no retinochoroidal lesions (NRLs), active retinochoroidal lesions (ARLs), simultaneous active and cicatricial retinochoroidal lesions (ACRLs), and cicatricial retinochoroidal lesions (CRLs). Abbreviations: IFN- γ , interferon γ ; IL-1 β , interleukin 1 β ; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; NK, natural killer; TNF- α , tumor necrosis factor α .

of toxoplasmosis, Afzali et al [24] showed that IL-17 stimulates IL-6 and nitric oxide production and amplifies the local inflammatory response in synergy with other mediators, such as IL-1 β , TNF- α , and IFN- γ . However, these studies did not elucidate the mechanism by which the IL-17 inflammatory role is associated with lesion activity in acquired and congenital toxoplasmosis.

The ACRL and CRL subgroups presented enhanced levels of IL-10-producing cells, suggesting an important role for this cytokine in the early and late modulation of the inflammatory process activated in response to *T. gondii*-induced ocular lesions in congenitally infected infants. Supporting our results, IL-10 gene polymorphism resulted in a low production of IL-10 and was linked to increased occurrence of ocular toxoplasmosis in adult patients [3]. Moreover, Lu et al [25] demonstrated that eye lesions are more severe in *Il10*^{-/-} mice than in wild-type mice. On the other hand, de-la-Torre et al [26] suggested a permissive state of IL-10 in the eyes of Colombian patients

with ocular toxoplasmosis. In the CRL subgroup, we found increased frequency of IL-10-producing neutrophils and monocytes, IL-1 β -producing monocytes, and TNF- α -producing neutrophils and CD4⁺ T cells. This pattern of proinflammatory cytokine production is similar to that found in the infants at the early stages of toxoplasmosis. In addition, IFN- γ -producing CD8⁺ T cells were observed only in infants with ARLs. The presence of IL-4-producing neutrophils in infants without retinochoroidal lesions and IL-10-producing monocytes in those with cicatricial lesions suggest the presence of regulatory events modulating the injury. Moreover, the absence of IFN- γ -producing CD8⁺ T cells in infants without retinochoroidal lesions or those presenting with a cicatricial status (ie, those with ACRLs and CRLs) also suggest the presence of regulatory events modulating the injury. The question that remains to be answered is whether congenitally infected children presenting with regulatory events somehow related to immune tolerance

may be predisposed to recurrent attacks of retinochoroiditis. Indeed, children included in the present study continued to be monitored and have elevated ocular reactivation events (unpublished oral communication with Daniel Vitor Vasconcelos-Santos). The next goal is to follow-up children with congenital toxoplasmosis for a longer period and evaluate the immune response over time.

The current study shows, for the first time, the cytokine profile of congenitally infected 2-month-old infants. A meticulous categorization of the ocular clinical status permitted the observation of the cytokine profile in the early and late stages of toxoplasmosis-associated retinochoroiditis. Our findings support the existence of a progressive immunological environment concomitant with the initial, apical, and cicatricial stages in the process of retinochoroidal lesion formation in infants with congenital toxoplasmosis that may be relevant to the establishment of stage-specific clinical management.

Supplementary Data

Supplementary materials are available at <http://jid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Vasconcelos-Santos DV. Ocular manifestations of systemic disease: toxoplasmosis. *Curr Opin Ophthalmol* **2012**; 23:543–50.
2. Carneiro AC, Andrade GM, Costa JG, et al. Genetic characterization of *Toxoplasma gondii* revealed highly diverse genotypes for isolates from newborns with congenital toxoplasmosis in southeastern Brazil. *J Clin Microbiol* **2013**; 51:901–7.
3. Cordeiro CA, Moreira PR, Costa GC, et al. Interleukin-1 gene polymorphisms and toxoplasmic retinochoroiditis. *Mol Vis* **2008**; 14:1845–9.
4. Garweg JG, Candolfi E. Immunopathology in ocular toxoplasmosis: facts and clues. *Mem Inst Oswaldo Cruz* **2009**; 104:211–20.
5. Gazzinelli RT, Denkers EY. Protozoan encounters with Toll-like receptor signalling pathways: implications for host parasitism. *Nat Rev Immunol* **2006**; 6:895–906.
6. Denkers EY, Gazzinelli RT. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin Microbiol Rev* **1998**; 11:569–88.
7. Gazzinelli RT, Wysocka M, Hieny S, et al. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. *J Immunol* **1996**; 157:798–805.
8. Hunter CA, Villarino A, Artis D, Scott P. The role of IL-27 in the development of T-cell responses during parasitic infections. *Immunol Rev* **2004**; 202:106–14.
9. Vasconcelos-Santos DV, Machado Azevedo DO, Campos WR, et al. Congenital toxoplasmosis in southeastern Brazil: results of early ophthalmologic examination of a large cohort of neonates. *Ophthalmology* **2009**; 116:2199–205.
10. Luiza-Silva M, Campi-Azevedo AC, Batista MA, et al. Cytokine signatures of innate and adaptive immunity in 17DD yellow fever vaccinated children and its association with the level of neutralizing antibody. *J Infect Dis* **2011**; 204:873–83.
11. Gazzinelli RT, Hakim FT, Hieny S, et al. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. *J Immunol* **1991**; 146:286–92.
12. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. *Semin Immunopathol* **2012**; 34:793–813.
13. Adkins B, Leclerc C, Marshall-Clarke S. Neonatal adaptive immunity comes of age. *Nat Rev Immunol* **2004**; 4:553–64.
14. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol* **2007**; 7:379–90.
15. Palanivel V, Posey C, Horauf AM, et al. B-cell outgrowth and ligand-specific production of IL-10 correlate with Th2 dominance in certain parasitic diseases. *Exp Parasitol* **1996**; 84:168–77.
16. Mayer JP, Biancardi M, Altcheh J, et al. Congenital infections with *Trypanosoma cruzi* or *Toxoplasma gondii* are associated with decreased serum concentrations of interferon-gamma and interleukin-18 but increased concentrations of interleukin-10. *Ann Trop Med Parasitol* **2010**; 104:485–92.
17. Yamamoto JH, Silveira C, Filho JK, et al. Discrimination between patients with acquired toxoplasmosis and congenital toxoplasmosis on the basis of the immune response to parasite antigens. *J Infect Dis* **2000**; 181:2018–22.
18. Santos Lacombe M, Marcos Martin C, Gallardo Galera JM, et al. Aqueous humor and serum tumor necrosis factor-alpha in clinical uveitis. *Ophthalmic Res* **2001**; 33:251–5.
19. Khera TK, Dick AD, Nicholson LB. Mechanisms of TNFalpha regulation in uveitis: focus on RNA-binding proteins. *Prog Retin Eye Res* **2010**; 29:610–21.
20. Rey A, Llorenç V, Pelegrín L, et al. Clinical pattern of toxoplasmic retinochoroiditis in a Spanish referral center. *Ophthalmologica* **2013**; 229:173–8.
21. Koppe JG, Loewer-Sieger DH, de Roeber-Bonnet H. Results of 20-year follow-up of congenital toxoplasmosis. *Lancet* **1986**; 1:254–6.
22. Lahmar I, Abou-Bacar A, Abdelrahman T, et al. Cytokine profiles in toxoplasmic and viral uveitis. *J Infect Dis* **2009**; 199:1239–49.
23. de-la-Torre A, Sauer A, Pfaff AW, et al. Severe South American ocular toxoplasmosis is associated with decreased IFN-gamma/IL-17a and increased IL-6/IL-13 intraocular levels. *PLoS Negl Trop Dis* **2013**; 7:e2541.
24. Afzali B, Lombardi G, Lechler RI, et al. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. *Clin Exp Immunol* **2007**; 148:32–46.
25. Lu F, Huang S, Kasper LH. Interleukin-10 and pathogenesis of murine ocular toxoplasmosis. *Infect Immun* **2003**; 71:7159–63.
26. de-la-Torre A, Pfaff AW, Grigg ME, et al. Ocular cytokinome is linked to clinical characteristics in ocular toxoplasmosis. *Cytokine* **2014**; 68:23–31.