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ORIGINAL ARTICLE

Immune Mediators against *Toxoplasma Gondii* during Reactivation of Toxoplasmic Retinochoroiditis

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ABSTRACT

Purpose: The purpose of this article is to analyze possible associations between systemic and ocular cytokine levels and specific clinical ophthalmologic signs from patients with a reactivation of toxoplasmic retinochoroiditis (RTR).

Methods: A total of 18 patients with an active RTR episode, 8 patients with inactive scars, and 14 control patients were included in the study. Serum samples and aqueous humor (AH) samples were analyzed for IFN (interferon)-γ, interleukin (IL)-10, and IL-6 levels by ELISA. Inflammation grade, location, and size of the retinochoroidal active lesion, sampling time, and time to resolution were recorded.

Results: A significantly negative correlation between AH and serum levels of IFN-γ was detected ($p < 0.05$). Patients with an AH IFN-γ/IL-10 ratio lower than 1 were associated with the longest time to resolution and/or severe complications.

Conclusion: Serum IFN-γ levels may be used as a prognostic marker for both time to resolution and the development of possible severe complications during a given RTR episode.

Keywords: Argentina, IFN-γ, immune response, retinochoroiditis, Toxoplasmosis

Toxoplasma gondii (*T. gondii*) infects approximately one-third of the population worldwide. In most cases, the infection course is asymptomatic. The retina, neurons, and muscle fibers are target cells in humans. Symptoms arise when the infected tissue becomes inflamed. The initial recognition of *T. gondii* by dendritic cells and cells of the mononuclear phagocytic system stimulates the release of interleukin-12 (IL-12) by both cell types and also tumor necrosis factor-α (TNF-α) by the dendritic cells.[1] IL-12 stimulates NK cells as well as CD4+ and CD8+ T cells to release interferon-γ (IFN-γ). This is the main mediator of the immune system against *T. gondii*.[2] Its release may be inhibited by IL-10. IFN-γ decreases the number of parasites inside mononuclear phagocytic cells by increasing the intracellular levels of nitric oxide and reactive oxygen species. In mice, IFN-γ increases the transcription of Immune-Related GTPases(IRGs) and guanine-binding proteins, both of which are localized at the parasitophorous vesicle, mediating its destruction. Although many studies have investigated the role of the immune response in several mouse models, it should be taken into account that they do not necessarily reflect the human infection, since significant differences have been observed between them. In this regard, the dramatically enhanced susceptibility of AIDS patients and those on immunodepletion therapy indicates that the adaptive immune system plays a key role in immunity to human toxoplasmosis. In contrast, it is much more difficult to define the role of the

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innate response in this host. As an example, both TLR-11 and -12 play a crucial role in mouse resistance against *T. gondii*,[3] while such receptors are absent in humans. Furthermore, the number (>20) of murine IFN-inducible immunity-related GTPase (IRG) genes contrasts sharply with the dearth of IRG genes in humans, since a single syntenic truncated (compared to mouse) IRGM copy and IRGC (presumably not playing a key role in immunity) have been described.[4,5] In humans, IRGM has been associated with protection against intracellular bacteria, such as *Mycobacterium tuberculosis*, while some of the IRGM haplotypes are related to Crohn's disease susceptibility. In both diseases, IRGM modulates autophagy.[6,7]

The parasite can reach target tissues either inside an infected cell or free during the phase of parasitemia.[8] *T. gondii* enters the retina through the branches of the retinal temporal vessels. During the infection, all retinal layers can be destroyed and the choroid is secondarily affected. Macrophages, as well as CD4+ and CD8+ T cells, are frequently present in the infected retina.[9] The direct effect of parasite duplication and the release of TNF-α produce the loss of retinochoroidal tissue. As a consequence, a flattened area of the retina will ensue at the site of the original area of retinal inflammation. Such area is known as retinochoroidal scar. Bradyzoites persist at the scar border. An increase in *T. gondii* duplication generates a new area of retinochoroiditis at the border or near the retinochoroidal scar. This event is called reactivation of toxoplasmic retinochoroiditis or RTR.

A significant increase in aqueous humor (AH) IFN-γ during RTR was already shown in humans by the late 1990s. However, this increase is nonspecific for *T. gondii*-induced uveitis, since other intracellular agents—such as some members of the *Herpesviridae* family—can also upregulate the expression of IFN-γ in AH during uveitis.[10] During toxoplasmic retinochoroditis, T-helper (Th) type 2-cytokines are also increased. IL-10 was shown to be upregulated in 63% of patients with active toxoplasmic uveitis.[11] IL-6, a pro-inflammatory molecule, is also increased during RTR.[12] Antibodies against IL-6 were recently shown to decrease parasite load, to inhibit retinal distortion, and to induce downregulation of inflammatory (IL-1ß, IFN-γ, and IL-17) and anti-inflammatory cytokines in a mice model of RTR.[13]

The current study focuses on the search of IFN-γ and other immune mediators during reactivation of RTR of patients from Misiones province, Argentina. This province is located in the northeastern edge of Argentina, bordering the southernmost states of Brazil. That region has been demonstrated to show a high frequency of ocular toxoplasmosis in the general population,[14] among patients,[15] and eye bank donors.[16,17] The aim of this study was to correlate clinical ophthalmological data (e.g., time to resolution, size, and location of the retinochoroidal lesion, inflammation grade) with AH and serum levels of IFN-γ, IL-10, and IL-6.

PATIENTS, MATERIALS, AND METHODS

Patients and Clinical Samples

All patients were examined by M.R. at a secondary level Ophthalmic practice clinic in Oberá, Misiones province. All patients underwent a complete eye examination including visual acuity, anterior biomicroscopy, tonometry, and indirect ophthalmoscopy. Blood and other clinical samples were obtained at the abovementioned clinic. Serum and AH aliquots were kept at −20°C until they were analyzed in the laboratory at UCAMI Research Center.

Patients included in the prospective study showed an RTR episode associated with anti-*T. gondii*-specific IgG.

RTR could be observed in the indirect ophthalmoscopic examination as a raised white-yellowish area in the retina with diffuse borders, near or at the border of a pigmented scar. Patients with IgG antibodies directed against *T. gondii* and inactive scar/s (IS) were also studied as a separate group. Control individuals (CIs) were patients without antibodies against the parasite and without retinochoroidal lesions. We excluded HIV-infected patients, patients with an autoimmune disease undergoing immunosuppressive treatment, and patients with active oncologic disease undergoing chemo- or radiotherapy. AH samples were obtained only from patients with RTR and severe ocular hypertension or from those who met the criteria for intraocular treatment with clindamycin. All *T. gondii*-infected patients received standard oral anti-toxoplasmic treatment with pyrimethamine (25 mg/day), sulfadiazine (2 g/day), meprednisone (40 mg/day), and folinic acid (15 mg every other day).

AH from seven control patients (AH control group) who underwent cataract surgery in the absence of toxoplasmic retinochoroidal lesions was studied for AH IFN-γ as well as the analysis of ILs. Due to significant differences in age between the AH control group and the patients with ocular toxoplasmosis (RTR group and IS group), seven young patients without retinochoroidal lesions and without serum anti-toxoplasmic antibodies were included as CIs for serum comparisons (serum control group). A male predominance in sex distribution was observed in all groups (Table 1).

Clinical Signs Evaluated in Patients with RTR

Visual acuity was evaluated by using Snellen charts at 3 m. Intraocular pressure was measured with a Goldmann applanation tonometer. Readings between

TABLE 1. Data from patients grouped for aqueous humor (AH) and serum analysis comparison.

Group	Age (mean ± SD; median)	Sex (male/total individuals)	Anti-*T. gondii* serology
RTR	30.05 ± 13.91; 27.5	12/18	Positive
Inactive	26.12 ± 8.09; 28.5	5/8	Positive
Serum control	35.57 ± 9.67; 32	4/7	Negative
AH control	61.28 ± 13.61; 63	4/7	Negative

A statistically significant difference ($p < 0.05$) in the age of the AH control group vs ocular toxoplasmosis patients (RTR and inactive scar patients) was recorded. For analytical purposes, the group AH control individuals were included only for AH comparison. For serum comparison, a second group of subjects was included, exhibiting no statistically significant differences regarding the age of patients. A male predominance was observed in the sex distribution of patients and control individuals.

10 and 21 mm/Hg were considered to be normal. Five variables were evaluated during indirect ophthalmoscopy: inflammation grade (vitritis), lesion location, presence of vasculitis and/or papillitis, and size of the retinochoroidal active lesion. For determining the grade of vitritis, we used the NIH standardized clinical classification.[18] The lesion location could be in the macular area, yuxtapapillar, any other area of zone 1 (between the temporal arcades), in retinal zone 2 (between the temporal arcades and the equator of the eye), or zone 3 (outside the equator of the eye). In order to establish the size of the active retinochoroidal lesion, we compared each lesion regarding the diameter of the patient's optic nerve disc and measured the lesion in relative units, regarded as optic disc diameters. We also analyzed temporal variables: elapsed time to resolution of the active retinochoroiditis and the moment of sampling. Time to resolution was recorded as the number of weeks since the patient started the treatment until it got its best-corrected visual acuity (for patients with macular sparing) or the time until the retinochoroidal lesion was flattened and got net borders (macular lesion). Time of sampling corresponded to the number of days from the onset of symptoms to the moment of the AH tap. The number of known RTR episodes was registered from the clinical history of each patient and indicated in the table with all the analyzed results.

Ethics

Ethical approval of the protocol was provided by the Hospital Fernando Barreyro in Posadas city, Misiones province, Argentina. Informed written consent was obtained from all patients and CIs.

ELISA for Determination of Serum IgG Antibodies against *T. Gondii*

The enzyme immunoassay Toxo-test for IgG against *T. gondii* (Wiener, Rosario, Argentina) was used according to the manufacturer's instructions. Serum samples were diluted 1:101 with the indicated diluent and tested as duplicates. Absorbance was measured on a plate reader at 450 nm (Mindray, China). Absorbance values higher than the cut-off calibrator (15 UI/ml) were considered reactive (positive).

ELISA for IFN-γ, IL-10, and IL-6

The enzyme immunoassays for IFN-γ, IL-10, and IL-6 were used according to the manufacturer's instructions (Thermofisher, Pierce Biotechnology, Rockford, IL, USA). Serum samples were run as duplicates, and 50 µl of serum was used for each assay. Due to volume limitations, 30 µl of AH was diluted with 20 µl of standard buffer diluent for each assay. Samples from patients undergoing cataract surgery without retinochoroidal toxoplasmic lesions were used as controls. Absorbance was measured on a microplate reader at 450 nm (Mindray, China) and concentration was determined using a curve fitting statistical software (Graph pad Prism 7.0, La Jolla, CA, USA).

Statistical Analysis

For the analysis between groups, the non-parametric Kruskal–Wallis method was used. The non-parametric Mann–Whitney test was used when the evaluation between two groups involved dichotomous variables. The effect of multiple comparisons was taken into account when considering the statistical significance of the results. Since a limited number of patients were enrolled, the analysis of data with the Kruskal–Wallis test was followed by a *post hoc* Dunn's test; when the Mann–Whitney test was used, Sidak correction for each variable (for an alfa value = 0.1) was used to consider the multiple comparison effect on the statistical significance.

RESULTS

Eighteen patients with reactivation of RTR were included in the study. Eight patients with inactive

scarring lesions were also studied. In addition, AH controls ($n = 7$) and serum controls ($n = 7$) with a similar mean age to those patients with ocular toxoplasmosis were analyzed (Table 1).

Out of the abovementioned 26 patients and 7 serum CIs, 31 serum samples were analyzed for IFN-γ and ILs (1 sample from the active retinochoroiditis group as well as another sample from the IS group were not analyzed due to hemolysis). Serum IFN-γ levels ranged between 2.35 and 6.65 pg/ml (Figure 1a). Mean serum IFN-γ reached 4.65pg/ml in CIs. Patients with active RTR averaged 3.93 pg/ml of IFN-γ in serum, while patients with inactive retinochoroidal scars exhibited 3.37 pg/ml. The difference of IFN-γ levels between CIs and patients with active retinochoroiditis and patients with retinochoroidal scars was statistically significant ($p < 0.05$).

Average AH IFN-γ levels from CIs were 5.64 pg/ml, while such levels from patients with RTR were highly variable with an mean of 84.91 pg/ml (Figure 1b). The difference in AH IFN-γ levels between CIs and patients with RTR was statistically significant ($p < 0.05$).

When comparing serum versus AH IFN-γ levels, a significantly negative correlation was detected for those AH IFN-γ levels ranging from 15 to 135 pg/ml ($r = 0.863$, $p < 0.001$) (Figure 1c). Higher levels of AH IFN-γ and lower levels of serum IFN-γ were associated with a shorter time to resolution ($p < 0.005$). Patients with higher levels of AH IFN-γ also exhibited ocular hypertension as an important clinical sign ($p < 0.05$) (Table 2). Conversely, no significant association was detected between IFN-γ levels and vitritis grade, size, and location of the retinochoroidal lesion, sampling time, and/or number of known retinochoroiditis reactivation episodes.

In patients with RTR, serum levels of IL-10 ranged between 2.8 and 688 pg/ml. Serum IL-10 was increased in five of the patients with RTR and in two patients from the IS group (one with congenital ocular

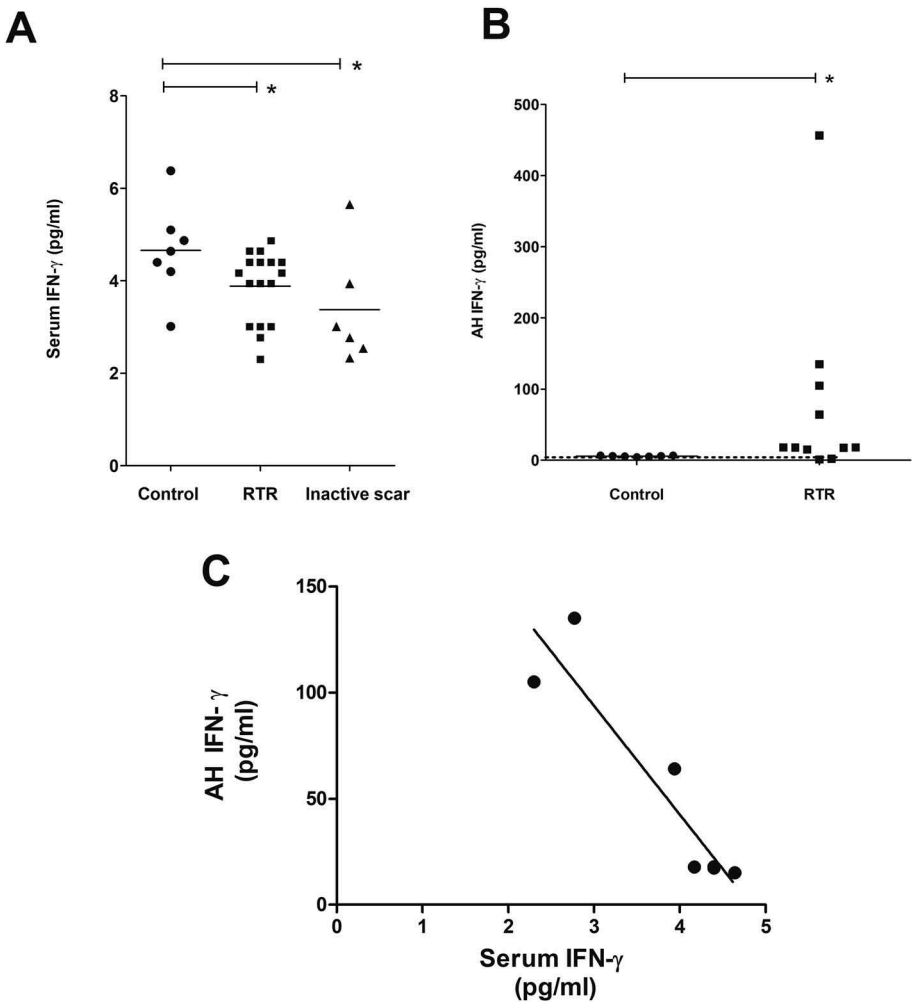

FIGURE 1. (A) Average levels of serum IFN-γ in serum control individuals, patients with RTR, and patients with inactive toxoplasmic scars expressed in pg/ml. (B) Levels of AH IFN-γ in control AH individuals and patients with RTR expressed in pg/ml. (C) Serum IFN-γ vs AH IFN-γ correlation of results from the analysis of paired serum/AH samples obtained during RTR from the RTR patients group. Significant differences between groups are displayed (*$p < 0.05$).

TABLE 2. Data obtained from 18 patients with RTR.

Patient	Sex	Age	Toxo IgG IU/ml	Serum IFN-γ	Serum IL-10	Serum IL-6)	HA IFN-γ	HA IL-10	HA IL-6	HA IFN-γ/ IL-10	Retinitis lesion size	Degree of vitritis	AC Tyndall	Time to resolution	Macular involvement	Clinical observation
1	M	30	149.11	3	2.8	Neg	456	64.7	583.2	7	2.5	3	3	6	No	OH, RV
2	F	64	80.99	2.7	4.2	3.7	135	29.5	33.6	4.5	1.5	2	1	6	No	OH, 6 RTR episodes, EM
3	M	40	135.54	2.3	2.8	Neg	105.6	7.3	26.2	14.4	2	2	3	6	No	OH, RV
4	M	28	156.49	3.9	4.38	3.7	64	7.3	50.7	8.7	2.5	2	2	6	Yes	OH, RV
5	M	38	178.96	4.3	9.12	3.2	17.9	6.6	6	2.7	1.5	2	1	9	Yes	Neutropenia
6	M	14	211.017	4.3	597	2.9	17.9	5.2	4.2	3.4	1	1	0	8	Yes	CBI
7	M	59	161.76	4.16	81.2	2.9	17.7	82.9	60.3	0.2	1.5	3	2	>10	Yes	RD, SBI
8	M	26	182.0*	ND	ND	ND	17.3	6.6	ND	2.6	2	2	1	8	No	Lesions with DDA
9	M	18	134.72	4.6	25	2.9	15	5.6	ND	2.6	1	1	1	8	Yes	SF
10	M	23	1/512*	ND	ND	ND	2	8.7	9.4	0.2	1	3	1	>10	No	ML, cataract
11	F	12	150.64	4.16	4.91	Neg	ND	ND	ND	ND	2	2	1	8	No	–
12	F	32	149.11	3	3.15	Neg	ND	ND	ND	ND	1	1	1	7	No	8 RTR episodes
13	M	20	152.2	3.93	4.38	Neg	ND	ND	ND	ND	2	2	2	8	No	–
14	M	35	128.89	4.86	3.5	3.0	ND	ND	ND	ND	2	2	1	8	No	BRVO
15	F	27	191.92	4.39	688.36	6.02	ND	ND	ND	ND	1.5	2	2	7	Yes	Contralateral RD
16	F	34	125.84	3	7.71	Neg	ND	ND	ND	ND	2	1.5	1	6	No	EM
17	F	22	211.2	7.88	45.21	Neg	ND	ND	ND	ND	1	1	0	8	Yes	–
18	M	19	214.6	4.39	3.15	Neg	ND	ND	ND	ND	1.5	2	2	8	No	RV

Serum and aqueous humor (AH) IFN-γ, IL-10, and IL-6 are expressed as pg/ml. The lesion size is expressed as optic disc diameters. Grade of vitritis and anterior cell (AC) reaction (Tyndall) is expressed according to the NIH classification of vitritis and anterior cell reaction (see "Material and Methods" section). Time to resolution is expressed as weeks. OH: Ocular hypertension; RV: retinal vasculitis; EM: clinically significant epiretinal membrane; RD: retinal detachment; CBI: concomitant bilateral involvement; SBI: successive bilateral involvement; Lesions with DA: lesions with different grade of activity; SF: subretinal fibrosis; BRVO: branch retinal vein occlusion.

"*" Significative differences between groups ($p<0.05$) are displayed as "*"

toxoplasmosis and the other one who had exhibited an RTR episode 6 months before). High levels of serum IL-10 were associated with retinochoroidal lesions localizing in the macular area ($p < 0.005$). AH IL-10 levels ranged between 2.6 and 66.41 pg/ml (Figure2a). A significant difference in AH IL-10 levels in patients with RTR and CIs was detected ($p = 0.017$). Moreover, a significant association between AH IL-10 and inflammation was detected (anterior chamber cells $p < 0.05$ and vitritis $p < 0.005$). We failed to observe any statistically significant association between such IL and sampling time, lesion size, or resolution time.

Patients 7 and 10 exhibited an IFN-γ/IL-10 ratio lower than 1. They also experienced the longest elapsed time to resolution (>12 weeks). Patient 7 failed to respond to various medical treatments during the RTR episode and suffered a retinal detachment as a complication of the long-standing intraocular inflammation. He also experienced a contralateral eye involvement with RTR 3 weeks after the retinal detachment surgery of the former involved eye. Fortunately, he did respond to conventional anti-toxoplasmic treatment (pyrimethamine, sulfadiazine, and meprednisone) after 8 weeks of therapy of the latter eye involvement. Patient 10 was receiving meprednisone (without pyrimethamine nor sulfadiazine) as treatment when he was first examined by MR and the HA sample was obtained.

AH IL-6 levels ranged between 4.26 and 583.25 pg/ml in patients with active retinochoroiditis, in contrast with CIs, who exhibited values below the cut-off (1.7 pg/ml). In serum, IL-6 was only detectable in 3 out of the 10 patients with mean levels of 3.5 pg/ml. A significant association was observed between higher levels of AH IL-6 and anterior chamber cell reaction ($p < 0.05$)

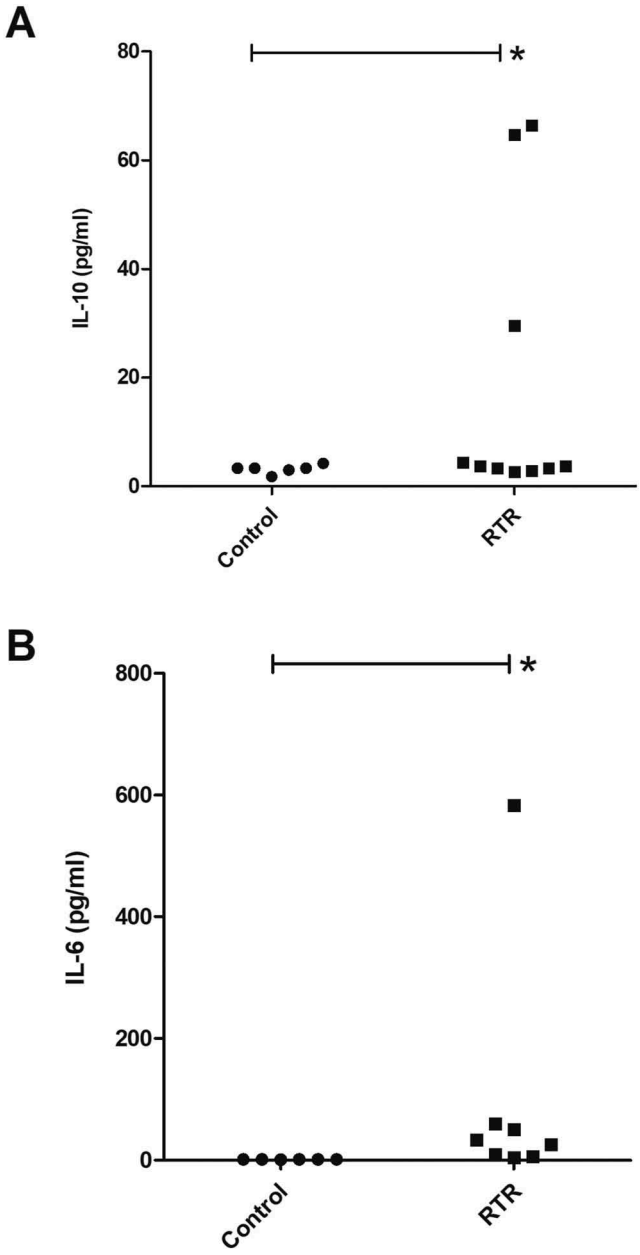

FIGURE 2. (A) Levels of AH IL-10 in control individuals and in patients with RTR expressed in pg/ml. (B) Levels of AH IL-6 in control individuals and in patients with RTR expressed in pg/ml. Significant differences between groups are displayed (*$p < 0.05$).

DISCUSSION

Taking into consideration several *in vitro* studies using dissimilar *Toxoplasma* strains and human cell lineages, it has been proposed that the parasite is controlled by both IFN-γ-dependent and IFN-γ-independent toxoplasmacidal pathways in this host. They respectively lead either to noncanonical or canonical autophagy (killing the parasite or inhibiting its growth), or to host cell death, mediated by pyroptosis of antigen-presenting cells, followed by inflammasome activation and cytotoxicity exerted by CD8+ T cells. In such processes, crucial roles have been established for IL-1β and higher expression of CD40 by human monocytes, after secretion of type II-parasite GRA15 protein promotes NFκB activation. IL-1β can enhance the IL-12-mediated stimulation of IFN-γ production, and CD40–CD40L interaction may activate type II and type III strains of *Toxoplasma* destruction through noncanonical authophagy (without lysosome fusion), while type I strains are restricted by IFN-γ-induced indolamine-2,3-dioxygenase, which degrades L-tryptophan, for which *T. gondii* is auxotrophic. Since nutrient starvation may induce autophagy, it is conceivable that tryptophan scarcity triggers the parasite clearance via noncanonical autophagy as well. Finally, CD8+ T lymphocytes may contribute to parasite elimination in humans, by secretion of pore-forming perforins and granulysin, which destroy the parasitophorous vacuole membrane, allowing granzymes to be introduced into the parasite and to kill it by reactive oxygen

species formation.[19] If the recently described CD4+ T lymphocytes with pathogen-independent memory phenotype do play a Th1 innate role in the human immune response against *T. gondii*, as demonstrated in mice,[21] it deserves to be thoroughly investigated.

The results of the present study show that during RTR, patients exhibit higher IFN-γ levels in AH and lower IFN-γ levels in the serum than the corresponding values observed in CIsat the same timepoint. Eighty percent of the patients with RTR possesses IFN-γ levels ranging from 13 to 450 pg/ml in AH. These results are in concordance with previous reports demonstrating increases in AH IFN-γ during active ocular toxoplasmosis.[10] In mice, high AH IFN-γ levels have been associated with a lower grade of inflammation and fewer retinochoroidal lesions.[22] In a reactivation model of RTR, the intraocular neutralization of an anti-IFN-γ mAb induced a higher intraocular parasite load, the expression of inflammatory cytokines and chemokines, as well as bigger intraocular lesions.[13] In humans, very few studies have analyzed AH IFN-γ during RTR as an attempt to correlate its level with clinical ophthalmologic signs. In 2013, de la Torre et al. showed that patients from France exhibited higher levels of AH IFN-γ and AH IL-17, lower levels of parasite load, and lower grade of inflammation as compared with Colombian patients. The authors suggested that higher levels of IFN-γ induced by *T. gondii* type II strain infecting French patients induce less retinal damage and fewer ocular complications than the Colombian patients infected with non-clonal South-American strains.[12] Our results are in partial agreement with this study, since patients with higher AH IFN-γ levels (1–4) experienced the shorter elapsed time to resolution of the RTR episode with no severe ocular complications. Such patients with higher IFN-γ in AH also showed increased intraocular pressure. Ocular hypertension has already been associated with inflammatory activity in the anterior chamber during ocular toxoplasmosis[23] but not with higher levels of AH IFN-γ. In a subsequent study performed by de la Torre et al. in Colombian patients, analyzing a similar number of AH samples to those described herein (n = 9 vs n = 10), AH IFN-γ levels positively correlated with retinal lesion size and vitritis grade.[24] In patients from Misiones, we also observed a positive correlation between AH IFN-γ levels and retinal lesion size, although it failed to reach statistical significance. Vitritis grade in the Misiones patients positively correlated with higher levels of IL-10 ($p < 0.005$) but not with AH IFN-γ.

Results from the serum of those patients with RTR exhibited a lower average of IFN-γ levels. This result is also in agreement with recently reported data obtained from European patients with ocular toxoplasmosis.[2525] A decrease in serum IFN-γ was also described in *T. gondii* acutely infected pregnant American patients, but not in Colombian pregnant patients under such condition.[20] Interestingly, the lowest significant serum levels of that cytokine were observed in patients with inactive retinochoroidal scars. A recent study demonstrated that peripheral blood mononuclear cell (PBMC) from patients with ocular toxoplasmosis lesions secrete less IFN-γ than chronically infected patients without ocular lesions.[26] A decrease in serum IFN-γ from patients with active and cicatricial RTR is probably a result of a tolerogenic mechanism. After intraocular antigen presentation (ACAID–VCAID), regulatory CD8+ T cells are produced in the spleen in order to decrease the systemic Th-1 immune response[27] and, hence, serum IFN-γ. Similarly, PBMC of patients with congenital ocular toxoplasmosis was shown to produce lower levels of IFN-γ and also higher levels of IL-1 and TNF-α than asymptomatic CIs and acquired ocular toxoplasmosis patients.[28] Although we did not grouped the patients according to their type of infection (acquired vs congenital) due to limited number of studied patients, we cannot rule out the possibility that a higher number of congenital ocular toxoplasmosis patients in the retinochoroidal scar group could have influenced the lower levels of serum IFN-γ of this group.

In the present study, we demonstrate a negative correlation between serum and the AH IFN-γ levels of patients during RTR. If this correlation was confirmed with a larger cohort of patients, the simple analysis of serum IFN-γ could possibly be used as a prognostic marker of the RTR outcome (either for an envisioned elapsed time to resolution or—on the other hand—for bearing in mind the possibility of a severe complication).

Choosing only patients with hypertension or clindamycin treatment could have influenced the results. As the diagnosis was already known in all patients, for ethical reasons, AH tap was performed only in those patients who would benefit from anterior chamber puncture, AH samples were obtained only from patients with RTR with ocular hypertension induced by intraocular inflammation or generated after an intraocular injection with clindamycin. Patients who underwent clindamycin injection were patients who exhibited macular involvement during RTR (patients 4–7 and patient 9). Although clindamycin could have an effect in the dissimilar elapsed time to resolution (patients receiving intraocular treatment in addition to oral treatment could have solved their retinochoroiditis faster), we did not detect such effect.

PCR quantification of *T. gondii* in AH would have been most useful to further investigate the pathogenesis of South American strains, especially regarding the severe pathology observed in these patients. Specifically, it would have shed some light on determining any putative correlation between a higher parasite load and a long resolution time and/or high IFN levels in some patients. However, qPCR could not be performed due to unavailability of enough AH

from the reactivation episode period, since the whole volume had been used for cytokine measurements.

Cytokine- and IL-ratios have been widely used in order to evaluate the type of immune response during an infection or after vaccination. The IFN-γ/IL-10 or IL-2/IL-4 indexes have been used as markers of activity and severity in patients with intracellular parasite infection, such as those with mucosal and visceral leishmaniasis.[29,30] There have not been specific descriptions nor associations between the AH IFN-γ/IL-10 ratio and specific clinical ophthalmologic signs, such as time to resolution during RTR or severe ocular complications. To the best of our knowledge, this is the first study showing that an IFN-γ/IL-10 AH ratio lower than 1 is associated with a longer time to resolution (patients 7 and 10) and serious ocular complications, such as retinal detachment (patient 10). An immunosuppressive AH environment with a low IFN-γ/IL-10 ratio was described in ocular toxoplasmosis patients from Colombia.[24] The low Th-1 response observed in Colombian patients was attributed to specific immune modulation by the South American strains of *T. gondii.*

In Misiones, non-clonal variants of *T. gondii* have been isolated from farm animals of patients with retinochoroiditis.[31] A predominance of strains similar to those isolated in southern Brazil has been reported.[32]

Results obtained in our study indicate that the immune response of patients with RTR against *T. gondii* is heterogeneous. The main difference with the results reported in Colombian patients was the lower level of AH IL-6 in Misiones patients despite the fact that we also detected an association between IL-6 and anterior chamber inflammation grade. The main concordance with the results observed among the Colombian patients was that higher levels of IL-10 were associated with macular lesions. High levels of IL-10 were also detected in peripheral monocytes and neutrophils from Brazilian infants born with retinal scars from congenital toxoplasmosis, which were frequently located in the macular region.[33] When compared to the French patients, some patients from Misiones (such as subjects 1–4) displayed similarly high AH IFN-γ levels, a shorter time to resolution, and absence of severe complications.

One possible explanation for the results obtained in patients from Misiones might be related to the significant Eastern and Central European ancestry in the current complex composition of its population. The aboriginal population of Misiones and the southernmost states of Brazil significantly decreased throughout the eighteenth and nineteenth centuries and was replaced by European immigrants during the twentieth century. In this region, we have demonstrated that patients with Eastern European genealogy have a significant higher frequency of RTR than patients with Spanish genealogy,[34] possibly due to the higher frequency of American aboriginal genetic makeup in the latter group.[35]

We cannot formally rule out the possibility that the divergence of *T. gondii* strains between different regions of South-America could partially account for the abovementioned differences of the patients' immune response against the parasite. Research addressing this issue as well as genetic studies to analyze the molecular ancestry of the patients are currently being performed. These analyses will determine whether any correlation between differences in the molecular markers of the immune response and/or clinical ophthalmologic signs could be attributed to the presence of American aboriginal genes in patients with ocular toxoplasmosis from this region of the world.

In conclusion, the results recorded in our study indicate a mirror (inverted) image between systemic and ocular levels of IFN-γ during RTR. Therefore, we propose that serum IFN-γ levels may be used as a prognostic marker for both time to resolution and severity of any given RTR episode.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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