Revista da Sociedade Brasileira de Medicina Tropical 45(6):757-760, Nov-Dec, 2012

Short Communication

Genetic polymorphism for *IFNγ +874T/A* in patients with acute toxoplasmosis

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ABSTRACT

Introduction: A single nucleotide polymorphism (SNP) in the gene encoding gamma interferon influences its production and is associated with severity of infectious diseases. This study aimed to evaluate the association of IFNγ+874T/A SNP with duration of disease, morbidity, and development of retinochoroiditis in acute toxoplasmosis. **Methods**: A case-control study was conducted among 30 patients and 90 controls. **Results**: Although statistical associations were not confirmed, A-allele was more common among retinochoroiditis cases and prolonged illness, while T-allele was more frequent in severe disease. **Conclusions:** Despite few cases, the results could indicate a relation between *IFNγ+874T/A* single nucleotide polymorphism and clinical manifestations of toxoplasmosis.

Keywords: Toxoplasmosis. Interferon gamma. Retinochoroiditis.

Acute acquired toxoplasmosis (AAT) provides a wide range of clinical manifestations in immunocompetent individuals. Frequently it is underdiagnosed because of its benign and self-limited aspect[1]. More serious cases are occasionally reported[2, 3] and maybe associated with more virulent strains of *Toxoplasma gondii* (*T. gondii*) or immunodeficiency conditions[1]. The ocular lesion of toxoplasmosis is characterized by necrotizing retinitis or retinochoroiditis (RC) and is the most common cause of posterior uveitis. It is secondary to congenital or acquired disease[1] and appears concomitantly or after the acute episode of infection, with reports of ocular lesions compatible with RC in up to 17.7% of patients infected with *T. gondii*[4]. However, the pathogenesis of this disease is still uncertain, just as there are unclear factors for the emergence of more severe forms[5].

The factors that determine the clinical course and mechanisms of infection by *T. gondii* involve the genetic diversity of the parasite, the individual variation of the host, and the anatomical characteristics of the various sites of infection. The immune response of immunocompetent hosts that display forms of metabolic latency (bradyzoites) grouped into tissue cysts prevents reinfection by the parasite. Macrophages, T lymphocytes, and natural killer cells, in conjunction with cytokines, are the major elements involved in this response. The effector T cells exert their function both by cytotoxicity and by secretion of cytokines, especially IFN-γ. The cell-mediated immunity with resultant production of IL-12 and IFN-γ is essential to control infection by *T. gondii* by restricting the multiplication of the parasite during the acute phase and accelerating the progression to the chronic phase[6]. Variations in genes that encode cytokines interfere with the expression of these molecules and may have an important role in gene regulation in inflammatory response and resistance or susceptibility to infections. Polymorphisms in the promoter region of cytokine genes have been demonstrated to be associated with the development of toxoplasmic retinochoroiditis[7- 9].

IFN-γ is a cytokine that is highly conserved, with few allelic variations in its gene. A single nucleotide polymorphism (SNP) located in the first intron of the human gene for *IFNγ* at the extremity 5' adjacent to the CA repeated region (*IFNγ*+874 T/A polymorphism) influences the secretion of this cytokine. Individuals carrying the A allele are low producers of IFN-γ[10]. Susceptibility to other intracellular pathogens has also been associated withthe variability in the production of IFN-γ related to this SNP[11,12]. A previous study has shown a correlation of *IFNγ+874T/A* polymorphism with ocular toxoplasmosis. The AA genotype showed an increased frequency in individuals with ocular findings suggesting an association with susceptibility to the development of RC[9]. This study aims to evaluate the association between the presence of polymorphism in the gene coding *IFNγ* at position +874T/A among individuals with AAT and compare clinical outcome as time to progress, morbidity, and RC development.

The study involved 30 patients over 18 years old with no history of comorbidities, belonging to the cohort of patients with AAT followed up at the Outpatient Toxoplasmosis Unit at Evandro Chagas Research Institute/FIOCRUZ, Rio de Janeiro, from January 2006 to December 2007. The diagnostic criteria, the clinical laboratory research protocol, and the morbidity scale (classes I, II, and III) are described in a previous publication[5]. Patients were submitted to periodic ophthalmoscopy (fundoscopy) examination for up to three years after the episode of acute toxoplasmosis. The control group consisted of 90 healthy subjects matched by sex and age (the proportion of three controls for each case), with positive IgG for toxoplasmosis and no history of uveitis or lymphadenopathy. All control subjects were submitted to indirect ophthalmoscopy to exclude the presence of retinal scars suggestive

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Received in 17/01/2011
Accepted in 05/05/2011

of RC. The fundoscopy was performed by the same examiner (Curi ALL) in all cases and controls.

DNA was extracted from peripheral blood samples using a commercial method (QIAgen). Amplification reactions of the gene segment coding for IFNγ related to the respective SNP were performed by using the amplification refractory mutation system - polymerase chain reaction (ARMS-PCR) method, and the products were subjected to electrophoresis on agarose gel and visualization under ultra violet (UV) light according to the methodology described in a previous publication[9].

The collected data were analyzed by the software Statistical Package for the Social Sciences (SPSS) (SPSS version 13.0, Chicago, IL, USA). Variables referring to the *IFNγ* polymorphism (position +874), time to clinical progression, and the presence of retinochoroiditis were examined using binary logistic regression, chi-square test, and Fisher's exact test methods. For the scale of morbidity, we used multinomial regression logistics and Spearman correlation. We adopted a statistical significance level of 5%.

The study was approved by the local research ethics committee. All participants were informed verbally and in writing and signed the consent form.

The analysis of the alleles and genotype distribution showed that both the group of symptomatic cases and the controls are in accordance with Hardy-Weinberg balance. There was no association observed between *IFNγ+874 T/A* polymorphism and the presence of symptoms (**Table 1**).

Among the 30 patients with AAT, 16 were males and 14 females, with a mean age of 37.4 years (SD ± 11.8 years). Twenty-four (80%) patients presented prolonged clinical disease (longer than 15 days). The analysis of this variable in response to genotype (AA, AT, and TT) showed no statistically significant results; however, the AA homozygous individuals had a two times larger probability of developing prolonged illness than did the T allele carriers (**Table 2**).

Regarding the scale of morbidity, 7, 15, and 8 individuals were classified into classes I, II, and III, respectively. Individuals homozygous for the T allele (TT) had twice the risk of progression to class III on the scale of morbidity, although this finding was not statistically significant (**Table 2**).

Retinochoroiditis occurred in four of 30 (13.3%) patients with AAT: two patients with genotype AA, one with genotype AT, and one with genotype TT. Although there are not enough patients to support a significant statistical analysis concerning ocular cases, an exercise applying logistic linear regression showed that when the TT genotype was compared to the others (AA + AT), it seemed to produce about 30% protection from the development of retinochoroiditis.

The influence of genetic background on the production of immunoregulatory cytokines in the clinical course and on the severity of disease manifestations has been the object of study in recent years[13, 14]. With regard to toxoplasmosis, resistance to the development of RC was observed in association with specific genotypes of *IL10* (-1082)[8], *IL1A* (-889)[7], and *IFNγ* (+874)[9]. As it is essentially an intracellular pathogen, IFNγ is a key cytokine in the immunopathogenesis of infection by *T. gondii*[15]. There are few studies about the relation of genetic factors to cytokine production in *T. gondii*[7,8,9]. This is the first study in patients with AAT. Although this is a pioneering work on the prospective monitoring of patients with AAT, we performed a cohort study with a convenience sample and limited number of patients, with the potential risk of introducing bias compromising occasional inferences by noninclusion of asymptomatic cases. Although we have not found statistically significant differences in relation to genotypes and alleles of *IFNγ+874 T/A* and association with the studied variables, the results observed point to a possible association between the production of IFN-γ, mediated by the type of SNP, and the clinical manifestations, also mediated by the type of SNP. The lack of statistical significance may be due to the small number of patients with AAT.

Regarding the scale of morbidity, the presence of the A allele might confer protection against the development of clinical symptoms, especially in severe cases (class III), and the TT genotype might be associated with an increased risk for clinical progression to higher morbidity *versus* the other genotypes. Since IFNγ is associated with the activation of macrophages, the destruction of intracellular parasites, and the sequestration of lymphocytes to lymph nodes, individuals with a high production of this cytokine may have a more exaggerated inflammatory response with fever and enlarged lymph nodes but unrelated to the duration of the condition. Even if the individual genetic

TABLE 1 - Distribution of genotypes and alleles for *IFNγ+874 T/A* between cases of acute acquired toxoplasmosis and asymptomatic controls.

	ATT cases n = 30		Controls n = 90				
Genotypes	**n**	**%**	**n**	**%**	**β^2**	**P value**	**OR (95% CI)**
Alleles							
A	38	63.3	109	60.6			
T	22	36.7	71	39.4			
A and T compared					0.146	0.702	1.125 (0.615 - 2.059)
Genotype							
AA	14	46.7	34	37.8			
AT	10	33.3	41	45.5			
TT	6	20.0	15	16.7			
Genotypes compared					1.378	0.502	
AA x AT / TT					0.741	0.389	1.441 (0.626 - 3.319)
TT x AA / AT					0.173	0.677	1.250 (0.436 - 3.581)

IFNγ: interferon-gamma; **OR:** odds ratio; **CI:** confidence interval; **ATT:** acute acquired toxoplasmosis.

TABLE 2 - **Frequency of genotypes and alleles for *IFNγ+874 T/A* in patients (n = 30) with acute acquired toxoplasmosis, and relation to prolonged illness and morbidity scale.**

		Genotypes	Â	P value	Exp (β) (95% CI)
Prolonged disease	Genotype analysis	AA*	–	–	–
(> 15 days)[a]		AT	-0.405	0.712	0.667 (0.077 – 5.749)
		TT	-1.099	0.341	0.333 (0.035 – 3.205)
	A-allele carriers	AA + AT*	–	–	–
	conjugated	TT	-0.916	0.371	0.400 (0.054 – 2.980)
	T-allele carriers	AT + TT*	–	–	–
	conjugated	AA	0.693	0.469	2.000 (0.306 – 13.062)
		Class II			
		AA*	–	–	–
		AT	0.693	0.505	2.000 (0.260 – 15.381)
	Genotype analysis	TT	0.693	0.600	2.000 (0.150 – 26.734)
		Class III			
		AA*	–	–	–
		AT	0.000	1.000	1.000 (0.091 – 11.028)
		TT	0.693	0.624	2.000 (0.125 – 31.975)
		Class II			
Morbidity scale[b,c]		AA + AT*	–	–	–
		TT	0.405	0.747	1.500 (0.127 – 17.667)
	A-allele carriers	Class III			
	conjugated	AA + AT*	–	–	–
		TT	0.693	0.609	2.000 (0.141 – 28.416)
		Class II			
		AT + TT*	–	–	–
	T-allele carriers	AA	-0.693	0.455	0.500 (0.081 – 3.082)
	conjugated	Class III			
		AT + TT*	–	–	–
		AA	-0.288	0.782	0.750 (0.098 – 5.768)

IFNγ: interferon-gamma; **Exp:** experimental; **CI:** confidence interval.*basal parameter, [a]binary logistic regression analysis, [b]multinomial logistic regression analysis, [c]class I as reference category.

variants do not have a role in the clinical progress of the acute phase, the results with respect to RC are consistent with previous studies that have demonstrated the role of host genetic factors in the genesis of ocular injury[9]. Although we have not found statistically significant differences in relation to genotypes and alleles of *IFNγ+874 T/A* we observed the presence of the A allele in most cases that developed RC, which corroborates earlier studies and suggests that individuals with this allele in its homozygous form have a tendency to develop ocular lesions[9].

There is a lack of studies on symptomatic acute toxoplasmosis, thus demanding further researches. Studies with a larger number of patients may clarify whether host genetic factors influence the duration and morbidity of AAT. A case-control study involving a larger sample of RC patients is in course and may contribute to a better understanding of the role of cytokines in the ocular injury of toxoplasmosis infection.

ABSTRACT IN PORTUGUESE

Polimorfismo genético para *IFNγ +874T/A* em pacientes com toxoplasmose aguda

Introdução: Um polimorfismo de nucleotideo único (SNP) no gene codificante para interferon gama influencia a sua produção e pode estar associado à gravidade de diversas doenças infecciosas. O objetivo deste estudo foi avaliar a associação entre SNP para IFNγ+874T/A com a duração da doença, a morbidade e o desenvolvimento de retinocoroidite na toxoplasmose aguda. **Métodos:** Estudo de caso-controle incluindo 30 pacientes e 90 controles. **Resultados:** Apesar da ausência de associação estatística, o alelo A foi mais comum entre os casos com retinocoroidite e doença prolongada e o alelo T nas formas mais severas. **Conclusões:** Os dados encontrados sugerem uma relação entre o polimorfismo de base única em *IFNγ+874T/A* com a morbidade e com o desenvolvimento de retinocoroidite por toxoplasmose.

Palavras-chaves: Toxoplasmose. Interferon-gama. Retinocoroidite.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

IPEC-FIOCRUZ, CNPq, *Diagnósticos da América*, Rio de Janeiro, RJ, Brasil

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