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Specific antibody levels in the aqueous humor and serum of two distinct populations of patients with ocular toxoplasmosis

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Abstract

The population of Erechim, Southern Brazil, is characterized by a high incidence of ocular toxoplasmosis, which is presumed to be of acquired origin. We wished to compare the local specific humoral immune response of individuals from this region with that of Swiss patients suffering from the same disease. Paired samples of aqueous humor and serum were withdrawn from 27 Brazilian and 50 Swiss patients presenting consecutively with active ocular toxoplasmosis. The total and specific levels of IgG in each of these were determined. The populations did not differ with respect either to age or sex. The serum levels of total IgG in Brazilian (10.8 g/l) and Swiss patients (11.1 g/l) were similar ($p = 0.499$), but the aqueous humor ones were higher in the former group (95 vs. 20 mg/l; $p = 0.0001$). The systemic and local levels of specific IgG were likewise higher in Brazilian patients [206 i.u. vs. 72 i.u. ($p = 0.001$) and 14 i.u. vs. 4 i.u. ($p = 0.005$), respectively] and the number of individuals without detectable levels of local specific IgG was correspondingly lower (11% vs. 54%; $p = 0.0005$). The Goldmann–Witmer coefficient (an index of local specific antibody production) did not differ between Brazilian and Swiss patients (2.1 vs. 0.08, respectively; $p = 0.107$). Our findings are indicative of a more pronounced uveovascular barrier breakdown in Brazilians than in Swiss patients with active ocular toxoplasmosis. That the systemic and local specific immune response is weaker in Swiss than in Brazilian patients has not been hitherto documented. This finding may reflect differences in the immunological handling of the infection.

Keywords: Ocular toxoplasmosis; Local immune response; Antibody coefficient; Host-specific factors; Population-based studies

Introduction

Ocular toxoplasmosis may account for more than 30% of posterior uveitis cases in western populations (McCannel et al., 1996), and in 95% of these instances the disease has been ascribed a congenital origin

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(Akstein et al., 1982; Hohlfeld et al., 1989; Holland, 2003; Koppe et al., 1986; Perkins, 1973). Up to 80% of congenitally infected individuals manifest signs of ocular recurrence (in association with pre-existing retinal scars) by the age of 20 years (Holland, 2004; McAuley et al., 1994; Wilson et al., 1980).

Acquired ocular toxoplasmosis is a rare condition in Europe and North America (Akstein et al., 1982), but more prevalent in Southern Brazil (incidence:18%), where it seems to be endemic (Glasner et al., 1992). Southern Brazilians have thus been assumed, although not proven, to have a distinct immunologic background. In contrast to toxoplasmosis of congenital origin, the acquired condition has been reported to be typically manifested as a unilateral disease, with a single, active lesion frequently occurring in the absence of pre-existing scars (Montoya and Remington, 1996). However, a few years ago, two prospective studies, involving, respectively, 49 and 154 immunocompetent, non-Brazilian individuals with acquired ocular or neurological toxoplasmosis, revealed one-half of the patients to develop ocular recurrences within 3 years of the initial presentation (Bosch-Driessen and Rothova, 1999; Couvreur and Thulliez, 1996). Hence, the acquired condition can be reactivated (Bosch-Driessen and Rothova, 1999). Moreover, it probably occurs more frequently outside Brazil than was previously supposed (Gilbert et al., 1999). Human (Suzuki et al., 1996) and animal studies (Brown et al., 1995; Gazzinelli et al., 1994) have yielded some evidence of a population-specific background to ocular involvement in toxoplasmosis, but the data are inconclusive (Holland, 2004; Meenken et al., 1995; Nussenblatt et al., 1989). We therefore compared the specific antibody levels in paired samples of aqueous humor and serum derived from a Brazilian population and a European group of patients with active ocular toxoplasmosis. We wished to ascertain whether differences in local or systemic specific antibody levels existed between the two populations.

Materials and methods

In this prospective study, paired samples of aqueous humor and serum were withdrawn in parallel from two collectives of consecutive HIV-negative, immunocompetent patients presenting with active ocular toxoplasmosis at the Departments of Ophthalmology in the Inselspital, Bern, Switzerland ($n = 57$) and in the Clinica Silveira, Erechim, Brazil ($n = 34$). None of the patients had undergone either local or systemic treatment for the disease when the samples were withdrawn. Informed consent was obtained for the analyses, which were approved by the local Ethical Committees in São Paulo and Bern; in the latter case, as part of a routine diagnostic procedure. The Brazilian samples were stored locally at −20 °C and then shipped together on dry ice to Bern, where paired samples were analyzed in parallel. Paired Swiss samples were likewise analyzed in parallel, but within 24 h of collection. Information respecting the age of patients, their history of symptoms, the site of ocular inflammation (which was not quantified) and clinical findings relating to the diagnosis of active ocular toxoplasmosis was documented. Patients with other ocular infections were excluded from the study. The diagnosis of ocular toxoplasmosis was confirmed at a later stage in all patients.

Patients from Erechim

Thirty-four consecutive patients from Erechim, Brazil, presenting with a varying clinical picture, which was nevertheless consistent with active ocular toxoplasmosis, were assigned to this group by two experienced ophthalmologists (A.C. Sobottka Ventura and C. Silveira). Anti-*Toxoplasma* IgG was present within the serum of all patients; anti-*Toxoplasma* IgM was not mandatory for inclusion in this group. Seven patients were subsequently excluded owing either to the lack of informed consent for anterior chamber puncture (two cases) or to an insufficient volume of aqueous humor (five cases). Of the remaining 27 patients, eight manifested no pre-existing scars. According to established criteria, this finding is consistent with a primary ocular disease condition (Bosch-Driessen et al., 2002). The other 19 patients exhibited chorioretinal scars at the time of presentation. This finding is indicative of a recurrent ocular disease condition.

Patients from Bern

Fifty-seven consecutive Caucasian patients from Bern, Switzerland, presenting with a typical clinical picture of recurrent ocular toxoplasmosis, were assigned to this group by a single ophthalmologist (J.G. Garweg). None of these individuals had a history of fever or lymphadenopathy within the 6 months prior to presentation. In all cases, the serological analysis yielded a picture that was compatible with chronic infection, i.e., anti-*Toxoplasma* IgG antibodies were present whereas specific IgM antibodies lay below a detectable level. Nevertheless, seven cases were subsequently excluded, owing either to duplicated inclusion on two separate occasions with consecutive recurrences (four cases), or to the identification of an immunocompromising disease (three cases). A congenital etiology was confirmed at birth in five cases. In the other 45 individuals, the disease origin remained undetermined, although the presence of pre-existing scars was consistent with a recurrent ocular condition.

Analysis of samples

All paired samples of aqueous humor and serum were analysed in parallel for their levels of total IgG, anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM using the same commercial ELISA system (Platelia Toxo®, Sanofi-Diagnostics Pasteur, Marnes la Coquette, France). Aqueous humor samples were first centrifuged (13,000*g*), and the supernatant was used for the antibody analyses. The specific IgG antibody-avidity index was determined using the same ELISA system after treating the samples with 6 M urea. A ratio of non-dissociating antigen-antibody complexes above 0.6 was deemed to represent a chronic infection, whereas one below 0.4 was judged as evidence of a recently acquired one (Lappalainen et al., 1993; Paul, 1999). The Goldmann–Witmer coefficient (C), which is an index of local specific antibody production (comparable to that applied for the determination of intrathecal antibodies) was calculated using the formula: C = [anti-*Toxoplasma* IgG (aqueous humor/serum)]/[total IgG (serum/aqueous humor)].

Statistical evaluation

The statistical analysis was performed using SPSS for Windows Version 11.0 (Chicago, Illinois; USA). Distribution profiles were described in terms of skew and kurtosis using the Shapiro–Wilk-Statistic system. The Mann-Whitney *U*-test was applied to non-parametrical data. Qualitative data were analyzed using the χ^2 test. Specific correlations were calculated using Spearman's rho (*r*) factor. Differences between sets of data were considered to be statistically significant if *p*-values were $\leqslant 0.05$ (on the basis of two-tailed tests). Quantitative data are presented as median values together with the interquartile range [IQR].

Results

At the time of presentation, patients within the Swiss group tended to be younger than those within the Brazilian one (26.7 (IQR = 12.6) years vs. 33.0 (IQR = 19.0) years; $p = 0.065$) and to be more often female [62% vs. 44%; χ^2 test: $p = 0.139$ (Table 1)], but the differences were not significant. The serum levels of total IgG were similar in the Swiss and Brazilian populations [11.1 (IQR = 3.4) g/l vs. 10.8 (IQR = 1.5) g/l; $p = 0.499$], whereas the aqueous humor ones were considerably higher in the latter group [20 (IQR = 30) mg/l vs. 95 (IQR = 195) mg/l; $p = 0.0001$ (Fig. 1)]. Application of Pearson's test revealed a correlation between the total IgG levels in paired samples of serum and aqueous humor derived from Brazilian patients [$r = 0.459$, $p = 0.016$] but not in those obtained from the Swiss group [$r = 0.156$, $p = 0.283$ (Fig. 2)].

The serum levels of specific IgG were higher in the Brazilian than in the Swiss group of patients [206 (IQR = 95) i.u. vs. 72 (IQR = 113) i.u.; $p = 0.001$]; so too were the aqueous humor levels [14.0 (IQR = 138) i.u. vs. 4 (IQR = 14) i.u.; $p = 0.005$ (Fig. 3)]. The proportion of patients without detectable levels of specific IgG in their aqueous humor was correspondingly lower in the Brazilian than in the Swiss group [11% vs. 54%; $p = 0.0005$]. The levels of specific IgG in paired samples of serum and aqueous humor were not correlated in either of the two populations [Brazilian group: $r = 0.194$, $p = 0.333$; Swiss group: $r = 0.061$, $p = 0.675$].

The specific IgG antibody-avidity index for the serum was higher in the Brazilian than in the Swiss population [0.88 (IQR = 0.06) vs. 0.75 (IQR = 0.13); $p = 0.0001$], whereas that for the aqueous humor did not differ between the two groups [0.83 (IQR = 0.16) vs. 0.75 (IQR = 0.17) for Brazilian and Swiss populations, respectively; $p = 0.106$ (Fig. 4)].

The Goldmann–Witmer coefficient, which is an index of local specific antibody production, did not differ significantly between the Brazilian [2.1 (IQR = 7.1)] and Swiss [0.08 (IQR = 17.2)] populations [$p = 0.107$], probably owing to the broad distribution of values.

Table 1. Overview of the most relevant findings relating to each population of patients with active ocular toxoplasmosis

Parameter (measure)	Switzerland $n = 50$	Brazil $n = 27$	*p*-Value
Age (years)	26.7 [12.6]	33.0 [19.0]	0.065°
Sex (% female)	62.0	44.4	0.139*
Serum total IgG (g/l)	11.1 [3.4]	10.8 [1.5]	0.499°
Serum anti-*Toxoplasma* IgG (i.u.)	72 [113]	206 [95]	0.001°
Serum antibody avidity index	0.75 [0.13]	0.88 [0.06]	0.0001°
Aqueous humor total IgG (mg/l)	20 [30]	95 [195]	0.0001°
Aqueous humor anti-*Toxoplasma* IgG (i.u.)	4 [14]	14 [138]	0.005°
Aqueous humor antibody avidity index	0.75 [0.17]	0.83 [0.16]	0.106°
Goldmann–Witmer coefficient	0.08 [17.2]	2.1 [7.1]	0.107°

Median values are represented together with the interquartile range (in parentheses). °Mann–Whitney *U*-test; *χ^2-test; i.u. = international units.

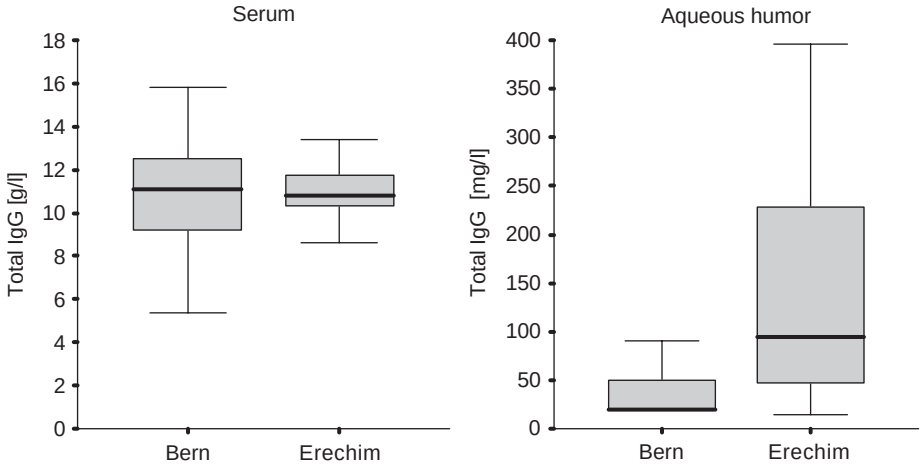

Fig. 1. Levels of total IgG in the serum (left) and aqueous humor (right) of Swiss and Brazilian patients with active ocular toxoplasmosis. Median values (bold horizontal bars) are represented together with the interquartile ranges (shaded areas) and the 95% confidence intervals (vertical ranges).

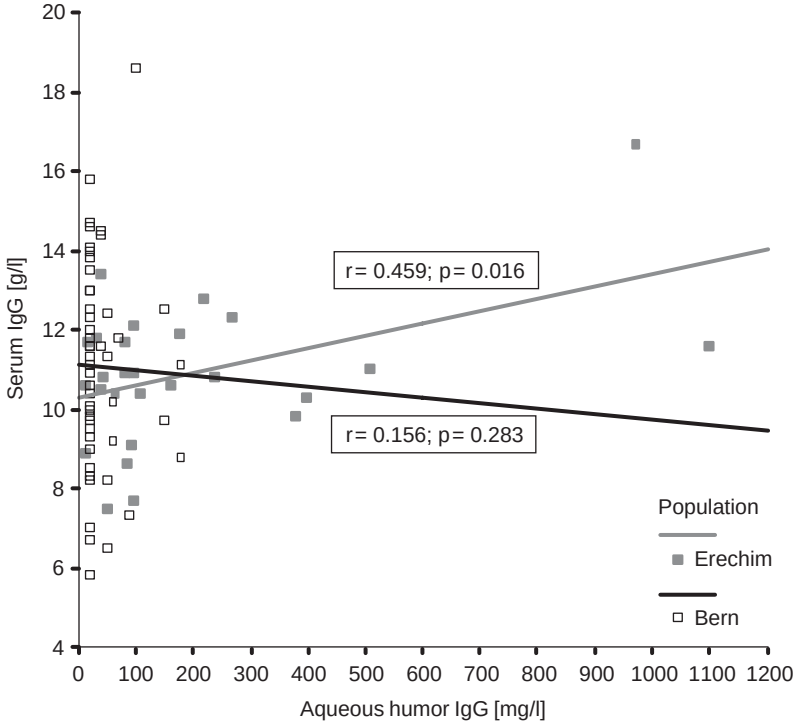

Fig. 2. Relationship between serum and aqueous humor levels of total IgG in Swiss and Brazilian patients with active ocular toxoplasmosis. Application of Pearson's test revealed a correlation between the levels in Brazilian patients but not in Swiss ones.

Discussion

The present study describes differences in the systemic and local humoral immune responses of two populations with the same disease, i.e., ocular toxoplasmosis, but with different socio-economic and geographic backgrounds. In the Brazilian group, the local response paralleled the systemic one, whereas in the Swiss population, the local humoral immune response was not accompanied by a detectable systemic one (Fig. 2). These findings might argue in favor of different disease origins in the two groups, namely, an acquired one (Glasner et al., 1992) in the Brazilian population and a congenital one (Dutton, 1989) in the Swiss group.

Toxoplasmosis of acquired origin may involve the eye during either the acute or the chronic phase of the disease (Ongkosuwito et al., 1999). The absence of specific IgM does not rule out an acquired disease

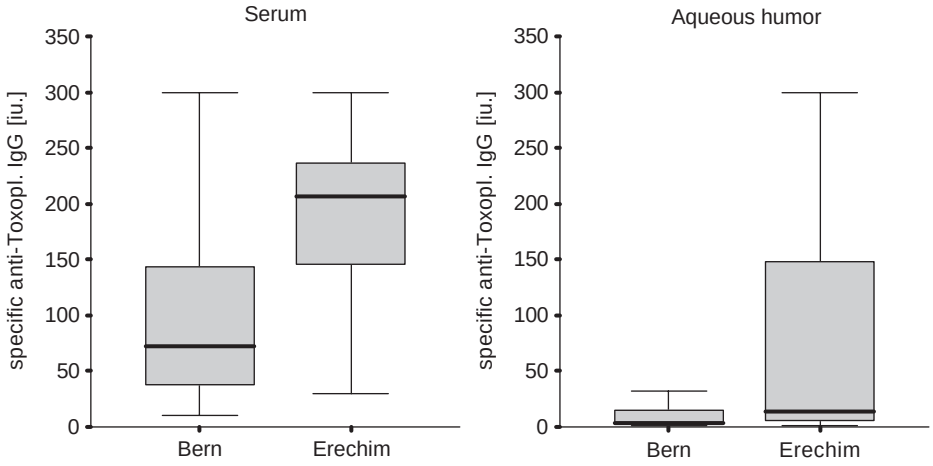

Fig. 3. Levels of specific IgG in the serum (left) and aqueous humor (right) of Swiss and Brazilian patients with active ocular toxoplasmosis. Median values (bold horizontal bars) are represented together with the interquartile ranges (shaded areas) and the 95% confidence intervals (vertical ranges).

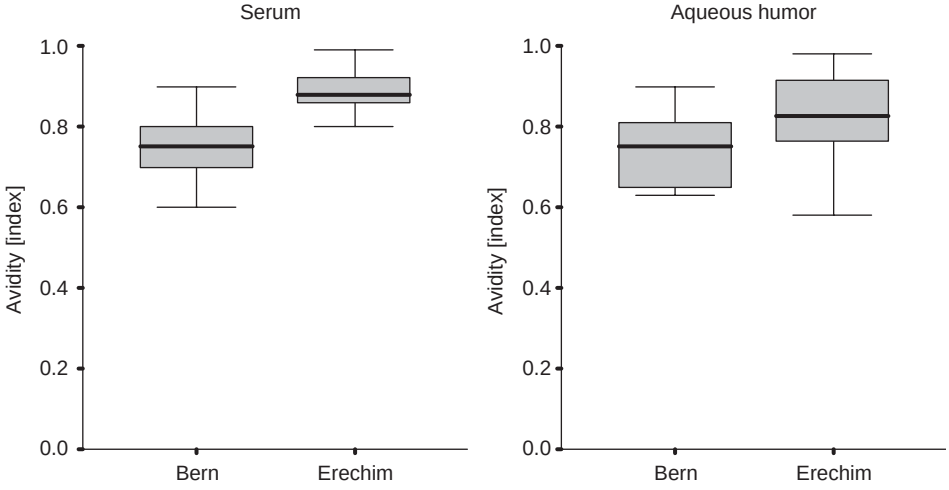

Fig. 4. Antibody-avidity indices for the serum (left) and aqueous humor (right) of Swiss and Brazilian patients with active ocular toxoplasmosis. Median values (bold horizontal bars) are represented together with the interquartile ranges (shaded areas) and the 95% confidence intervals (vertical ranges).

condition (McLeod et al., 1990; Nedeljkovic et al., 1999). The persistence of specific IgM long after the acquisition of the disease is believed to interfere with the interpretation of serological data and to complicate a definitive serological diagnosis of a recently acquired condition (Suzuki et al., 2000, 2001). In many countries, *Toxoplasma* serology has become a routine undertaking during pregnancy only within the last 10–15 years, if at all. Hence, information respecting maternal *Toxoplasma* seroconversion and a congenital origin of the disease is not usually available. And it is generally impossible to distinguish unequivocally between an acquired and a congenital condition on the basis of clinical (Stanford et al., 2002) and laboratory findings (Holliman et al., 1991) at the time of presentation for ocular toxoplasmosis. Hence, we were unable to categorically attribute differences between our two populations to a particular disease origin. Although unexplainable, the observed inter-population differences are nevertheless astonishing. They may be attributable to either host immunological factors or parasitic ones.

Individuals with congenital ocular toxoplasmosis would be expected to manifest clinical signs of this condition earlier than those with the acquired disease (Ongkosuwito et al., 1999; Suzuki et al., 2001; Gilbert and Stanford, 2000). Although the Swiss patients tended to be younger than the Brazilian ones upon first presentation with active ocular toxoplasmosis, the difference in age was not significant. Since the two groups did not include exclusively first clinical

manifestations, this finding is not surprising. Hence, no especial weight can be attached to this particular finding of ours; nor indeed to the absence of significant differences in the distribution of sex and in the serum levels of total IgG between the two populations.

In terms of immunological host factors (Forrester and McMenamin, 1999), our findings relating to the local (i.e., aqueous humor) levels of both total and specific IgG are more important. The local levels of total IgG were considerably higher in the Brazilian than in the Swiss group. This finding indicates that either the mechanisms responsible for maintaining uveovascular barrier integrity or the time elapsing between the onset and detection of the inflammatory response differed in each population (Garweg et al., 2000; Magone and Whitcup, 1999; Vallochi et al., 2002). Evidence for the first tenet is afforded by the existence of a correlation between the levels of total IgG in paired samples of aqueous humor and serum derived from Brazilian patients but the absence of one in the Swiss group (Fig. 2). However, we have no information respecting the exact time intervening between the onset of symptoms and clinical presentation for the active disease in Brazilian patients.

The levels of specific IgG in the serum and aqueous humor of Brazilian patients were much higher than in Swiss ones. This finding may correlate with the severity of the inflammatory response, which is known to be greater in individuals with a darkly pigmented fundus. The result might also reflect lesion size, which tends to be larger in individuals with acquired ocular toxoplasmosis. However, the findings are also compatible with different modes of infection handling (Klaren and Peek, 2001; Vallochi et al., 2001; Yamamoto et al., 2000) and a local or systemic site of infectious activity (Figueroa et al., 2000).

In ocular toxoplasmosis of congenital origin, immunotolerance is to be expected, since the antigens present at birth are but poorly capable of priming specific T-cells and are more likely to be recognized as native (McLeod et al., 1990; Feron et al., 2001; Hara et al., 1996; Roberts and McLeod, 1999), a circumstance which has been suggested also for other congenital infections (Nedeljkovic et al., 1999; Chaye et al., 1992), but has not yet been definitively established (Fatoohi et al., 2003). Differences between the systemic and ocular modes of antigen presentation (i.e., anterior-chamber-associated immune deviation) could likewise contribute to the disparate findings in our two groups (Streilein et al., 2002). On the other hand, the higher serum levels of specific IgG in the Brazilian population could merely reflect constant and chronic antigen stimulation from the environment. On this premise, the higher local levels of specific and total IgG in the Brazilian group could be partially accounted for by a more pronounced breakdown of the uveovascular barrier, with a resulting spillover of specific and non-specific serum antibodies into the aqueous humor. Consistent with this tenet, we observed a positive correlation between the serum and aqueous humor antibody levels in the Brazilian but not in the Swiss group. It is noteworthy that, using the same well-established commercial assay, no specific IgG was detected in the aqueous humor of 54% of the Swiss patients. Immunogenetic factors have been proposed to contribute to the course of the disease (Mack et al., 1999; Freyre et al., 2001; Johnson et al., 2002; Kempf et al., 1999), and these may partially account for the observed inter-population differences. However, since the population of Erechim is composed largely of German immigrants, these immunogenetic factors cannot alone explain the disparate findings.

Another factor that might contribute to the differences between the two groups is the presumed stage at which the parasite was ingested. In tropical countries, the predominant mode of transmission may be the uptake of oocysts from cats, whereas in moderate climates, tissue- or pseudocysts in meat may be the primary source of infection. It could be argued that pseudocysts selectively contain strains with a lower virulence, since otherwise the host would die off (Ruiz and Frenkel, 1980; Frenkel, 1990; Frenkel et al., 2003). However, primary infection would be expected to result in a complete cross-immunity for all strains and stages (Smith and Frenkel, 2003). Ocular toxoplasmosis represents in utmost instances a reactivated stage of the disease, as evidenced by the absence of specific IgM (Garweg et al., 2000). Hence, the primary immune response may be generally of only secondary importance in this condition and particularly in accounting for the local inter-group differences. The specific IgG antibody avidity indices for the serum and aqueous humor were not helpful in a clinical context, since they were higher than 0.7 in both groups (Lappalainen et al., 1993; Paul, 1999; Vinhal et al., 1994), which is consistent with a recurrent disease and our failure to detect specific IgM in either the serum or aqueous humor of either group.

Virulence-associated factors of the involved parasite strains have not yet been demonstrated to play a substantial role in the evolution of ocular toxoplasmosis (Boothroyd and Grigg, 2002; Frenkel and Ambroise-Thomas, 1997; Su et al., 2003), although many atypical varieties of the *Toxoplasma* parasite have been identified in human cases of the disease (Grigg et al., 2001). It is of course conceivable that the recurrences observed in immunocompetent individuals arise, at least partially, from a newly acquired infection with a different *Toxoplasma* strain (Dao et al., 2001). This possibility has not hitherto been considered as a mechanism underlying recurrent ocular toxoplasmosis. But in the future, it should be feasible to ascertain its relevance by means of a PCR genotyping analysis (Grigg et al., 2001). Stage- or virulence-associated factors could thus finally

also account for the differences between individuals infected in Brazil or Switzerland (Grigg et al., 2001; Holland, 2000).

In conclusion, we observed higher levels of specific IgG within the serum and aqueous humor samples of Brazilian patients. The inter-population differences cannot be explained on the basis of our current understanding of the pathophysiology of this infection, either in parasitological or in immunological terms. They might reflect host-specific (Holland, 2004; Gilbert et al., 1999; Mack et al., 1999; Freyre et al., 2001; Johnson et al., 2002; Kempf et al., 1999) and parasite-related (i.e., stage- and virulence-associated) factors (Ruiz and Frenkel, 1980; Frenkel, 1990; Frenkel et al., 2003; Boothroyd and Grigg, 2002; Frenkel and Ambroise-Thomas, 1997; Su et al., 2003; Grigg et al., 2001; Dao et al., 2001), which must now be assessed.

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